Evaluation of pesticide residues

for estimation of maximum residue levels and calculation of dietary intake

Training manual

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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Preface

This pesticide residue training manual was developed to introduce trainees to the procedures and practices of residue evaluation in JMPR.

It was first prepared for a training workshop in Budapest in November 2010. It has now been edited and compiled into a single document.

The first part (Chapters 1 to 13) covers the relevant topics. They are illustrated by many examples taken from JMPR Reports and Evaluations.

The Exercises provide the introduction and background suitable for practical exercises by small teams at a training workshop.

The Presentations are PowerPoint versions of lecture topics for the training workshop.

Because pesticide evaluation has developed its own terminology where many acronyms and abbreviations have their own special meaning, at the end of each chapter is a list of those used in the chapter as an encouragement for workshop trainees to become familiar with them.

The "*Participant's evaluation of the course form*" should be used to collect opinions from workshop trainees.

Árpád Ambrus and Denis Hamilton

Addendum, Nov 2011

The trial edition of the Training Manual has been revised in the light of experience at training workshops held in Accra, Bangkok and Sao Paolo in 2011.

Changes include:

- Further exercises have been added. Exercises are now numbered to show their relationships to chapters, e.g. Exercise 10.1 relates to the subject matter of Chapter 10.
- The presentations relevant to the topics of a specific chapter are now included in that chapter. Presentations are now printed in outline format rather than in slide format, with the advantage that text in the outline format is searchable.
- Solutions for exercises, are now included in the Training Manual. This information will be especially helpful for those outside a training course who are studying the Manual by themselves.

In some cases no solution is included (e.g. preparation of summary of a report) because its wording depends on the author and the content is important. In such cases the comments of the participants or the facilitator of the training may provide guidance for improvement.

- Electronic copies of calculators and worksheets are available for use in the training workshops. The calculators are spreadsheets for dietary intake calculation, livestock dietary burden calculation and Kruskal-Wallis test, and the OECD MRL calculator. The worksheets are WORD document blank worksheets used in various exercises.

Árpád Ambrus and Denis Hamilton

Contents

Chapter 1. Introduction and overview of JMPR residue evaluations

Introduction

The need for a JMPR training manual has become apparent in recent times as procedures have become more complex and the interest in the operations of JMPR and the Codex Committee on Pesticide Residues has increased.

Also, FAO has received requests for such a training manual and the FAO Secretary of the JMPR, Ms Yang Yong Zhen initiated a project to produce a JMPR Training Manual in 2010. It would be suitable for use in training workshops and also for study by one-self.

The FAO/WHO Training Manual on Pesticide Specifications, first issued in 2008, was also an inspiration to produce a training manual on pesticide residues.

The first version of the FAO Manual on Pesticide Residues (often referred to as the 'JMPR Manual') was issued in 1997, with the most recent edition in 2009. It is a comprehensive description of the requirements and methods of the FAO Panel of JMPR and is indispensable to its operation. The FAO Manual has also been very useful to national authorities administering pesticide regulations.

Pesticide evaluation has developed its own terminology where words and phrases have their own special meaning which many not always be clear to those outside of the workers specialising in this area. For example, good agricultural practice, hazard, risk, exposure, intake, pre-harvest interval, approved uses, critical GAP, acceptable daily intake, periodic review and residue definition each carries a meaning that includes a long history of debates and discussions about pesticide residues. Someone unfamiliar with the terminology may not be able to make the best use of the JMPR Manual.

People can generally learn more readily from worked examples. The idea of the training manual is to introduce the topics with specific examples. But many aspects cannot be covered by the chosen examples and possibly wrong lessons can be learned from a specific example. We must rely on the JMPR Manual for more comprehensive information.

So, the purpose of the training manual is to introduce the topics and to provide sufficient background for people to appreciate the detailed information in the JMPR Manual. Its focus is on evaluation of pesticide residues data to propose MRLs and to estimate residue concentrations suitable for dietary exposure and risk assessment.

JMPR procedures continue to evolve. Every year changes are recorded in JMPR Reports as general reports. It is a strength of JMPR that it develops the science as issues are foreseen. For example, we have seen many developments in risk assessment in the last 10-15 years.

Spreadsheet models for calculating long-term and short-term dietary intake and livestock dietary burden are now routinely used. The danger is that such models can be used out of context to produce non-valid results. It is essential that users understand what is happening in the calculations and if they make sense in the specific case under examination. The training manual will work through case examples.

There have been suggestions that the training manual may assist people to plan and conduct supervised residue trials. The JMPR Manual already provides information on this subject. However, it is a complex subject and there is really no substitute for working on actual supervised residue trials with an experienced project leader.

The training manual is not designed to train people to conduct supervised residue trials. Neither is it designed to teach pesticide or analytical chemistry or to teach farming practices or food processing.

Persons evaluating pesticide residue data should understand the limitations of guidelines and should be aware of the uncertainties in the data and parameters in the evaluations. Further, they should have a sense of whether the data point or calculated value is intended to be somewhere in the middle of the uncertainty or is pushing towards the 'conservative' end of the range.

The JMPR Manual explains that guidelines should be understood in the context of their origins and they should not be extrapolated too far. There is no reason to expect them to apply more widely than in the situations envisaged at the time they were formulated.

Overview

A pesticide evaluation begins with clear identification of the pesticide and a description of its physical and chemical properties. Knowledge of the physical and chemical properties of a substance is a necessary pre-requisite to understanding its general behaviour in metabolism, analytical methods, formulations and the environment.

Metabolism and environmental fate studies provide the essential data for residue definitions. Metabolism includes the processes of transport or translocation within the organism as well as transformation to metabolites or degradation products. The processes of photolysis on the plant surface are also included. The results of metabolism studies must be interpreted to decide on the likely nature of the residue occurring in feed and food commodities produced from livestock or crops.

Residue sampling and analysis generate the copious data needed for residue studies. Attention to good practices in analysis, understanding the chemical processes involved, checking validation data and using extraction procedures known to extract the residues of interest are all necessary to produce accurate data. Residue samples should be stored under conditions and for durations that are known not to cause changes to the residue concentration or the nature of the residue.

Selection of residue definitions suitable for enforcement and for risk assessment requires the examination of many studies: chemical properties such as isomer composition, hydrolysis and photolysis; metabolism in laboratory animals, livestock and crops; methods of analysis; and toxicity of metabolites. The situation may be further complicated if one pesticide is the metabolite of another or if two pesticides produce a common metabolite.

The central part of the whole process is evaluating supervised trials data to produce MRLs suitable for Codex adoption and STMR and HR values suitable for use in risk

assessments. Many factors affecting residue levels must be considered - application rate, number of applications, formulation and timing and pre-harvest interval. Care is required to extract data accurately from supervised trials studies and to check data validity.

Commodity group MRLs may be proposed where adequate residue data are available and where pesticide residues may be expected on the group, e.g. if there is a registered pesticide use on a crop group that corresponds to the commodity group.

Some residues in food arise from persistent compounds in the environment that were once used as pesticides, e.g. DDT use in agriculture. The residues have no relation to registered uses, so the normal data requirements and evaluation methods are not suitable. Monitoring data are examined and extraneous maximum residue limits (EMRLs) are established, subject to risk assessment, to cover a high percentage of such residues so that trade is not inadvertently interrupted by the extraneous residues.

Spices are very minor crops and mostly do not generate sufficient revenue to pay for residue trials. In certain circumstances, MRLs based on residue monitoring data and subject to risk assessment may now be established on spices or groups of spices.

When raw agricultural commodities are processed into processed foods, e.g. fruit to fruit juice and wheat to bread, the pesticide residues in the raw agricultural commodity may be concentrated, diluted, destroyed or transformed into other compounds. Processing studies determine the nature and concentration of residues during food processing, which permits dietary risk assessments and the setting of MRLs for processed foods when necessary.

Livestock (farm animal) feeding studies can be used to predict the resulting residue levels in meat, milk and eggs from the levels of residue in feed materials. Residues may also arise from direct treatment of livestock for ectoparasites. The residues from both sources must be reconciled in the process of residue evaluation. The results are then used in dietary risk assessments and the setting of MRLs.

In the processes of dietary risk assessment, estimates of residues in food are combined with data on human diets to calculate dietary intakes or exposures for comparison with ADIs (acceptable daily intakes) and ARfDs (acute reference doses). Many of the calculations are completed by spreadsheet, but careful selection of the correct residue levels and food consumption data are needed to produce valid results.

A pesticide residue evaluation is completed when the risk assessment is satisfied and the JMPR can recommend that the estimated maximum residue levels are suitable for establishing maximum residue limits.

The reviewer's task

The reviewer's task is to convert the available experimental data and supporting information into acceptable standards for residues in food and feed commodities.

Residue evaluation relies on the fundamental science chemistry, but combines it with agriculture, animal husbandry, environmental behaviour, risk assessment and regulatory principles to produce conclusions.

It is important to get the science right. Reviewers should bring all of their background experience and knowledge to the process. A team effort, with a wider experience and knowledge than the individual, will generally produce more consistent results.

The reviewer should check the open scientific literature for information on the topic. There may be relevant issues and questions that had not previously occurred to the reviewer and not dealt with in the data submission.

Evaluation documents prepared by the reviewer must be tidy and concise. It is difficult to obtain a clear picture of the data in documents containing unnecessary detail or inconsistent presentation.

Preparation of the evaluation data tables is part of the review process. This is the first point where the reviewer decides that data of doubtful validity either do not enter the summary tables or are entered with a footnote drawing attention to the validity question.

Silence in a data submission about an important point should be an alert for the reviewer, e.g. no information provided whether samples were chopped or unchopped before storage for a residue that is unstable in chopped samples.

Questions of validity, plausibility and authenticity continually arise for the reviewer. Such questions often suggest another look at the information provided or another search through all the associated detail.

Validity

- Have realistic methods been used?
- Have the methods produced acceptable results when tested on relevant substrates at the required residue levels?
- What was used as the reference material for the conjugated residues during tests for analytical recoveries?
- Why has the hydrolysis step been omitted from the analytical method?

Plausibility

- Why is this compound fat-soluble in poultry, but not in goats?
- Is the residue expressed on fresh weight or dry weight?
- In the data submission, why are "percent recoveries" in a freezer storage stability study calculated as "% analytical recovery" at time zero and "% remaining" at subsequent times?

Authenticity

- Are these results real?
- Why is the report silent about the analytical method?
- Why are the 5 analytical recoveries at the LOQ all exactly 100 %?
- Why is only a summary report available?

Frequently, the situation is complex even when all the required studies are available and in good shape. There can be alternative interpretations, and reviewer judgement is required to arrive at a result that is realistic, practical and consistent.

The reviewer's job is well done when the final result is transparently based on scientific procedures and valid methods and data.

PRESENTATION – INTRODUCTION AND OVERVIEW

1. Training on evaluation of pesticide residues

Introduction

2. Objectives of the training workshop

- To introduce the topics with specific examples and to provide sufficient background for people to appreciate the detailed information in the JMPR Manual.
- To show the logic in and critical aspects of the evaluation process.
- To point out the importance of applying basic science and past experience in correctly interpreting experimental data and to avoid reaching non-valid conclusions.
- To assist performing evaluation of pesticide residue data at national level where a comprehensive pesticide registration process is not in place.

3. Background

- The procedures involved in the estimation of maximum residue levels and dietary exposure assessment have become very complex.
- The FAO Manual (first published in 1997 and revised in 2002 and 2009) provides a comprehensive description of the requirements and operation principles of JMPR. It has also been very useful to national authorities administering pesticide regulations.
- However, someone unfamiliar with the terminology may not be able to make the best use of the JMPR Manual.
- The FAO has received requests for preparing a training manual for and providing training in evaluation of pesticide residues.
- The FAO/WHO Training Manual on Pesticide Specifications, first issued in 2008, was also an inspiration to produce a training manual on pesticide residues.

4. Basic principles of JMPR operation

- JMPR procedures continue to evolve. Every year changes are recorded in JMPR Reports as general considerations. It is a strength of JMPR that it develops the science as issues are foreseen.
- The guidelines and methods developed are applicable in the context of their origins and they should not be extrapolated too far. There is no reason to expect them to apply more widely than in the situations envisaged at the time they were formulated.
- The JMPR operates as a team making best use of the different experience and scientific knowledge of its members.

5. Overview of pesticide evaluation process

1) Clear identification of the pesticide and a description of its physical and chemical properties.
2) Metabolism and environmental fate studies provide the essential data to decide on the likely nature of the residue occurring in feed and food commodities produced from livestock or crops, and for defining the residues for enforcement and risk assessment purposes.

3) Sampling and analysis generate the copious data needed for residue studies. Checking the applicability of the procedures is crucial for obtaining valid results.

6. Overview of pesticide evaluation process

4) Selection of residue definitions suitable for enforcement and for risk assessment requires the examination of many studies: chemical properties such as isomer composition, hydrolysis and photolysis; metabolism in laboratory animals, livestock and crops; methods of analysis; and toxicity of metabolites. The situation may be further complicated if one pesticide is the metabolite of another or if two pesticides produce a common metabolite.
5) Selection of appropriate trials for the evaluation is a key task of the evaluator, as it will influence the outcome of the evaluation.

7. Overview of pesticide evaluation process

6) The central part of the whole process is evaluating supervised trials data to produce MRLs suitable for Codex adoption and STMR and HR values suitable for use in risk assessments. Many factors affecting residue levels must be considered - application rate, number of applications, formulation and timing and pre-harvest interval. Care is required to extract data accurately from supervised trials studies and to check data validity.
7) Commodity group MRLs may be proposed where adequate residue data are available and where pesticide residues may be expected on the group, e.g. if there is a registered pesticide use on a crop group that corresponds to the commodity group.

8. Overview of pesticide evaluation process

8) Some residues in food arise from persistent compounds in the environment that were once used as pesticides, e.g. DDT use in agriculture. The residues have no relation to registered uses, so the normal data requirements and evaluation methods are not suitable. Monitoring data are examined and extraneous maximum residue limits (EMRLs) are established, subject to risk assessment, to cover a high percentage of such residues so that trade is not inadvertently interrupted by the extraneous residues.
9) Spices are very minor crops and mostly do not generate sufficient revenue to pay for residue trials. In certain circumstances, MRLs based on residue monitoring data and subject to risk assessment may now be established on spices or groups of spices.

9. Overview of pesticide evaluation process

10) When raw agricultural commodities are processed into processed foods, e.g. fruit to fruit juice and wheat to bread, the pesticide residues in the raw agricultural commodity may be concentrated, diluted, destroyed or transformed into other compounds. Processing studies determine the nature and concentration of residues during food processing, which permits dietary risk assessments and the setting of MRLs for processed foods when necessary.
11) Livestock (farm animal) feeding studies can be used to predict the resulting residue levels in meat, milk and eggs from the levels of residue in feed materials. Residues may also arise from direct treatment of livestock for ectoparasites. The residues from both sources must be reconciled in the process of residue evaluation. The results are then used in dietary risk assessments and the setting of MRLs.

10. Overview of pesticide evaluation process

12) In the processes of dietary risk assessment, estimates of residues in food are combined with data on human diets to calculate dietary intakes or exposures for comparison with ADIs (acceptable daily intakes) and ARfDs (acute reference doses). Many of the calculations are completed by spreadsheet, but careful

selection of the correct residue levels and food consumption data are needed to produce valid results.

13) A pesticide residue evaluation is completed when the risk assessment is satisfied and the JMPR can recommend that the estimated maximum residue levels are suitable for establishing maximum residue limits.

PRESENTATION – THE REVIEWER'S TASK

2. Purpose

- To explain the task of the pesticide residue reviewer in JMPR.

3. The task

The reviewer's task is to convert the available experimental data and supporting information into acceptable standards for residues in food and feed commodities.

4.

Residue evaluation relies on the fundamental science chemistry, but combines it with agriculture, animal husbandry, environmental behaviour, risk assessment and regulatory principles to produce conclusions.

5. Get the science right

- Reviewers should:
  - bring all of their background experience and knowledge to the process.
  - work as a team. A team effort will generally produce more consistent results.
  - check the open scientific literature for information on the topic – perhaps relevant issues and questions are not dealt with in the data submission

6. Evaluation documents

- Reviewers should prepare documents that are tidy and concise.
- Preparation of the evaluation data tables is part of the review process – decide on data validity at this stage.
- Silence in a data submission about an important point should be an alert for the reviewer.

7. Validity, plausibility, authenticity

- **Validity**: are the data and conclusions sound and substantiated?
- **Plausibility**: are the results and conclusions expected from scientific theory and previous experience?
- **Authenticity**: are there doubts about the origin of some of the results?

8. Validity

- Realistic methods
- Method validation data
- Validation data on conjugated residues – what was the reference material?
- Why has the hydrolysis step been omitted from the analytical method?

9. Plausibility

- Why is this compound fat-soluble in poultry, but not in goats?
- Is the residue expressed on fresh weight or dry weight?
- Freezer storage testing – confusion between "% recovery" and "% remaining"?

10. Authenticity

- Are these results real?
- Why is the report silent about the analytical method?
- Why are the 5 analytical recoveries at the LOQ all exactly 100 %?
- Why is only a summary report available?

11. Complexity

- Frequently, the situation is complex even when all the required studies are available and in good shape.
- Alternative interpretations are valid.
- Reviewer judgement is required to arrive at a result that is realistic, practical and consistent.

12.

The reviewer's job is well done when the final result is transparently based on scientific procedures and valid methods and data.

Chapter 2. Identity and Physical and Chemical Properties.

Identity
Physical and chemical properties
Needs of JMPR for physical and chemical properties
Evaluation of physical and chemical property data

The purpose of this chapter is to identify and describe the study material and to describe those properties that are relevant to its behaviour in the environment, livestock, crops, food commodities, food processing and behaviour in methods of analysis.

Relevant sections of JMPR Manual

- Identity 3.2.1
- Physical and chemical properties 3.2.2
- Physical and chemical properties 5.2

Identity

The purpose of the identity section is to identify unambiguously the study material or materials.

The JMPR Manual[1] lists the data requirements for identity in Section 3.2.1.

- ISO common name
- Chemical name
  - (IUPAC)
  - (Chemical Abstracts)
- CAS Registry No.
- CIPAC No.
- Synonyms
- Structural formula
- Molecular formula
- Molecular weight.

Information is readily found at the Compendium of Pesticide Common Names web site[2]. It provides the ISO common name and its status, the IUPAC and CAS systematic names, the CAS Registry Number, the molecular formula and the structural formula.

The CIPAC number is available in CIPAC Handbooks and from the CIPAC website[3].

Producing the correct systematic name, e.g. the preferred IUPAC name, requires specialist knowledge and experience. The rules were changed in 2004[4], so names prepared before then may be inconsistent with the latest position.

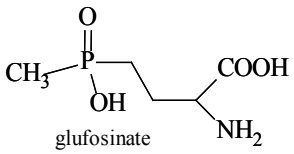
[1] FAO. 2009. Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed. FAO Plant Production and Protection Paper. Second Edition. 197. http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmpr/jmpr-docs/en/
[2] http://www.alanwood.net/pesticides/
[3] http://www.cipac.org/index.htm
[4] Kober R and Bünzli-Trepp U. 2010. IUPAC, Systematic Nomenclature for CIPAC Documentation – an Analysis. Seventh JOINT CIPAC/FAO/WHO Meeting – Symposium (54th CIPAC Meeting and 9th JMPS Meeting) Ljubljana, June 8th 2010. http://www.cipac.org/datepla.htm

Synonyms include trade names and industry code numbers. For example, clofentezine has the trade name "Apollo" and the manufacturer code numbers NC21314, SN 84866 and AE B084866.

It is essential to understand the manufacturer code numbers because the studies to be reviewed rely on the code numbers to describe the compound, its isomers and its transformation products. Isomers, racemates and salts of essentially the one compound may all have different code numbers.

Example glufosinate (JMPR 1998)

Glufosinate	racemate	L-isomer	R-isomer
free acid	AE F035956	AE F057740	AE F090532
ammonium salt	AE F039866	AE F058192	AE F093854
HCl salt	AE F035125	AE F057742	AE F057741



glufosinate

When different isomers or various isomer mixtures are commercially available, accurate identification of the study material is very important. For example, cypermethrin is manufactured as various mixtures of isomers. Identification requires quantitative analysis of isomer contents.

Example cypermethrins

Isomer	cypermethrin CAS 52315-07-8	alpha-cypermethrin CAS 67375-30-8	zeta-cypermethrin CAS 52315-07-8
1R, cis-R	14	-	3
1S, cis-S	14	-	22
1R, cis-S	11	50	22
1S, cis-R	11	50	3
1R, trans-R	14	-	3
1S, trans-S	14	-	22
1R, trans-S	11	-	22
1S, trans-R	11	-	3

CAS numbers

Chemical Abstracts has a very practical approach to assigning CAS Registry numbers.

If a mixture is isolated from a biological source or a partially-purified reaction mixture then it is assigned a number. The mixture is considered as a non-stereospecific substance, thus receiving the Same Registry Number as any other such mixture or substance with possible stereochemistry that is not stated.

The main point to recognize is that the CAS Registry number may not always be a unique identifier.

In the cypermethrin example, zeta-cypermethrin is quoted as included in the cypermethrin CAS Number.

Physical and chemical properties

The terms "physico-chemical properties", "physical-chemical properties" and "physical and chemical properties" are all intended to mean the same thing.

The JMPR requirements are listed in the JMPR Manual[1].

Pure active ingredient

- Appearance
- Vapour pressure (in mPa at stated temperature)
- Melting point
- Octanol-water partition coefficient (at stated pH and temperature)
- Solubility (water and organic solvents at stated temperatures)
- Specific gravity (... g/cm^3 at ...stated temperature)
- Hydrolysis (at stated pH and temperature)
- Photolysis
- Dissociation constant
- Thermal stability

These data are usually available because national governments, before registration, require data on physical and chemical properties of the active ingredient[5], including: vapour pressure; melting point; solubility in water; octanol-water partition coefficient as a function of pH; dissociation constant in water; hydrolysis rate including identification of hydrolysis products; photodegradation in water including identification of photolysis products; and solubility in organic solvents.

The description 'pure active ingredient' is readily understood in simple cases, and genuinely pure active ingredients from whatever source should have the same physical and chemical properties. The different experimental values from different sources should be a reflection of the errors of measurement, not of the true value.

For active ingredients that are mixtures, the composition of pure active ingredient from different sources could be different and physical and chemical properties may not be identical. For example, a nominal 40:60 cis/trans ratio of a pyrethroid compound could have a composition between 50:50 and 30:70 without influencing the purity.

Pure active ingredients that are mixtures, e.g. diastereoisomer mixtures, from different sources may not have the same composition or exactly the same properties.

Needs of JMPR for physical and chemical properties

Knowledge of the physical and chemical properties of a substance is a necessary pre-requisite to understanding its general behaviour in metabolism, analytical methods, formulations and the environment.

Vapour pressure

The vapour pressure of pure compound is needed for:

- understanding diffusion and fumigation in some applications;

[5] OECD. 1994. Data requirements for pesticide registration in OECD member countries: survey results. OECD Environment Monographs N°. 77. Organisation for Economic Co-operation and Development. Paris. http://www.olis.oecd.org/olis/1994doc.nsf/LinkTo/ocde-gd(94)47

- understanding behaviour during food processing and for recognizing possible losses by volatilization;
- understanding analytical methods, GLC traces and potential difficulties in storing residue samples.

Melting point

The melting point of pure compound is needed for:

- a simple practical test of purity of relatively pure materials;
- checking possible extrapolations of vapour pressure (simple extrapolation through a phase change is not valid).

Temperature of decomposition

The temperature of decomposition of pure compound is needed for:

- understanding behaviour in analytical methods, e.g. during gas chromatographic analysis.

Solubility in water

The difference between solubility of the substance in water and the solubility of its salts (or other derivatives) should be noted. A compound that dissociates will be present as a salt or salts when it is dissolved in some buffer solutions; in those cases its measured solubility will include the solubility of the salts.

Some methods for measuring water solubility may not be suitable for organic compounds that dissociate. For example, a reasonably soluble organic compound that dissociates may form a stronger buffer than the buffer solution chosen for the measurement solution.

A very low water solubility may mean stability in the presence of water even though hydrolysis or epimerization occurs readily in an aqueous solvent solution. A compound that hydrolyses or epimerizes in aqueous solution may still be stable in the presence of water in a formulation or in the environment if it has very low water solubility.

Water solubility of the compound is needed for:

- deciding if the compound could be systemic;
- interpreting routes of mammalian excretion;
- understanding environmental behaviour ;
- understanding behaviour in analytical methods.

Octanol : water partition coefficient

Octanol-water partition coefficient of the compound is needed for :

- predicting possible fat solubility;
- predicting behaviour in food processing;
- understanding behaviour in analytical methods.

Dissociation characteristics

Dissociation characteristics of the compound are needed for:

- explaining water solubility as a function of pH;
- explaining P_{ow} as a function of pH;
- understanding behaviour in analytical methods.

Hydrolysis characteristics

Hydrolysis properties of the compound are needed for:

- predicting storage stability in samples;

- predicting changed composition of a mixed compound in the environment.

Measurements should check for epimerization of chiral compounds during hydrolysis studies.

Photolysis characteristics

Photolysis properties of the compound are needed for:

- understanding environmental behaviour;
- taking precautions in analytical methods if sensitive to light.

Measurements should check for epimerization of chiral compounds during photolysis studies.

Evaluation of physical and chemical property data

Checklist for each property

1) Identity of the test substance
2) Purity of the test substance
3) Test method description and test method reference (e.g. OECD method)
4) Test method conditions. Depending on the test, conditions may include temperature range, concentrations in test solution, duration of test, etc.
5) Calculation methods.
6) Units of the reported physical or chemical property and temperature (if the value of the property depends on temperature).
7) Is the reported value of the physical or chemical property consistent with the raw data provided?

Reporting physical and chemical properties – example, clothianidin (JMPR 2010)

Property	Result	References	Guidelines/method
Vapour pressure	purity 99.7% 1.3×10^{-7} mPa at 25°C 3.8×10^{-8} mPa at 20°C (extrapolated)	Morrisey and Kramer, 2000, THP-0026	EEC A4 (effusion method: vapour pressure balance)
Melting point	purity 99.7% 176.8°C	Kamiya and Itoh, 2000, THP-0018	OECD 102 (method not indicated)
Octanol/water partition coefficient	Study 1, purity 99.7% log K_{ow} = 0.7, unbuffered, pH not stated, at 25.0°C	Morrisey and Kramer, 2000, THP-0013	OECD 117 (HPLC-method)
	Study 2, purity 99.7% log K_{ow} = 0.893, pH 4, at 25°C log K_{ow} = 0.905, pH 7, at 25°C log K_{ow} = 0.873, pH 10, at 25°C	O'Connor and Mullee, 2001, THP-0065	EEC A8 (shake flask method + HPLC-UV)
Solubility	Study 1, purity 99.7% 0.327 g/L in water at 20°C	Morrisey and Kramer, 2000, THP-0013	OECD 105 (flask method + HPLC-UV)
	Study 2, purity 99.7% 0.304 g/L in pH 4 buffer (0.01 M potassium hydrogen phthalate) at 20°C 0.340 g/L in pH 10 buffer (0.002 M disodium tetraborate/0.004 M sodium chloride) at 20°C	O'Connor and Mullee, 2001, THP-0065	OECD 105 (flask method + HPLC-UV)

Points to note

- The purity of the test material is stated for each study.
- The temperature is stated for each recorded property.
- An OECD or EEC method is provided in each case.
- Units are clearly stated for each measured value.

Reporting physical and chemical properties – example, cypermethrin (JMPR 2008)

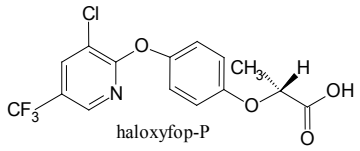
Property	Results		Ref
Melting point (purity 98.3%, cis:trans 37.6:62.4)	41.2-47.3 °C		40/30-D2149 (CYP/C65)
Octanol/water partition coefficient (purity 98.3%, cis:trans 37.6:62.4)	log P_{ow} = 5.3 to 5.6 for the 4 components		40/30-D2149 (CYP/C65)
Hydrolysis rate (radiochem purity 99 %) at 5 μg/L in buffers + acetonitrile, duration 28 days. (Half-lives calculated from table of rate constants.)	pH 3, 25 °C, cis, trans pH 7, 25 °C, cis pH 7, 25 °C, trans pH 11, 25 °C, cis pH 11, 25 °C, trans	<10 % hydrol, 28 days <10 % hydrol, 28 days half-life = 136 days half-life = 38 mins half-life = 23 mins	Takahashi *et al*. 1985a

Points to note

- The purity of the test material is stated for each study.
- Because cypermethrin is a mixture, the composition of the mixture is also stated.
- In the hydrolysis studies where decline was less than 10 % during the duration of the test (28 days), the half-life was not calculated.
- In the hydrolysis studies, half-lives for cis- and trans- isomers were calculated separately.

Example. Haloxyfop-P vapour pressure measurement (JMPR 2009)

In 2009, JMPR reviewed a study where the vapour pressure measurements were made on a test material over the temperature range 59.35 ºC to 103.9 ºC for extrapolation to 25 ˚C. The test material was reported as a white lumpy powder with melting point 107-108 ˚C, i.e. measurements were made on a solid for extrapolation to a solid at 25 ˚C.



haloxyfop-P

However, the melting point of haloxyfop-P is reported as 70.4-74.5 ˚C, while the melting point of haloxyfop is 107 ˚C, so it is likely that the test material was haloxyfop, not haloxyfop-P.

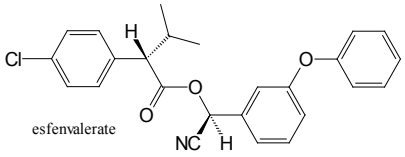
The reviewer should be alert to the identity of the test substance and the validity of the results.

Points to note.

- Vapour pressure measurements were made at temperatures below the melting point of the test substance for extrapolation to 25 ˚C.
- The melting point of the test substance did not match the known melting point of the intended test substance haloxyfop-P, throwing doubt on the identity of the test substance.

Example. Esfenvalerate hydrolysis (JMPR 2002)

Hydrolysis rates were measured for [^{14}C-chlorophenyl]esfenvalerate at concentrations of approximately 50μg/l in sterile aqueous buffers of pH 7 and 9 at 25°C in the dark. Data are summarised in Table 1.



esfenvalerate

The hydrolysis rate of the sum of esfenvalerate and its epimer at pH 7 was too small to be measurable in 28 days. At pH 9 the half-life for the sum of esfenvalerate and its epimer was approx 64 days.

The half-lives for disappearance of esfenvalerate by way of combined hydrolysis and epimerization at pH 7 and 9 at 25 ˚C were 40 days and 25 days respectively. Epimerization was faster than hydrolysis.

Table 1. Hydrolysis testing for esfenvalerate at 25°C in aqueous buffers in the dark.

	% of applied ^{14}C label						
	Incubation period, days						
Compound	0	2	4	7	14	21	28
ESFENVALERATE pH 7							
esfenvalerate	86	82	78	76	68	58	53
[2S,αR] epimer	2.2	5.7	12	17	27	34	38
ESFENVALERATE pH 9							
esfenvalerate	71	48	46	48	41	38	27
[2S,αR] epimer	20	55	53	48	46	49	42

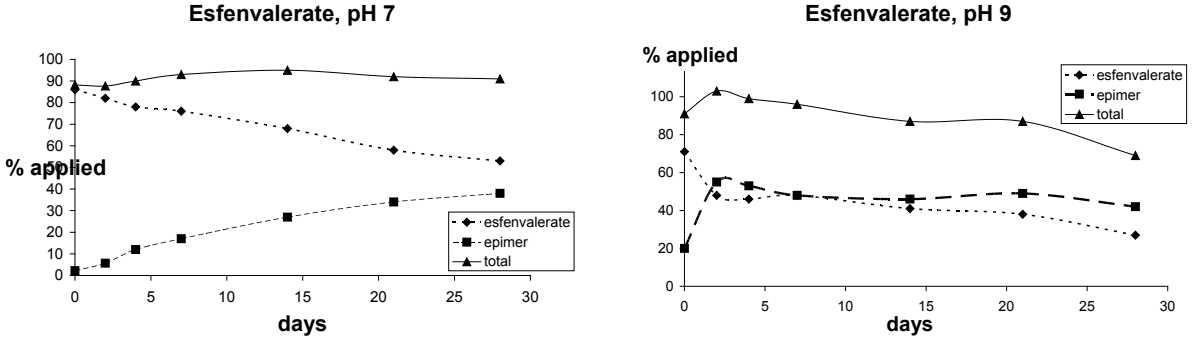


Figure 1. At pH 7, hydrolysis was too slow to measure in 28 days, but epimerization was clearly occurring. At pH 9, hydrolysis was observed in 28 days (decline in total), but epimerization occurred quickly, with apparent equilibrium established between esfenvalerate and its epimer within a few days.

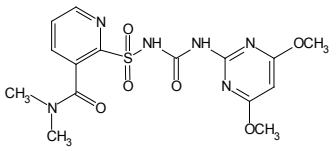
The reviewer should be alert to the potential for epimerization in studies on chiral compounds.

Points to note

- Esfenvalerate is readily subject to epimerization, which occurs more quickly than hydrolysis, if it is brought into aqueous solution.
- Other pyrethroids may behave similarly.

Water solubility – example, nicosulfuron (FAO specifications, 2006)

Nicosulfuron is a sufonylurea herbicide with a pK_a of 4.22.



The usual methods for water solubility may not work for such a compound.

Property	Value and conditions	Purity	Method	Ref
Water	All in buffered solutions at 28 ˚C	97.3 %	US EPA Pesticide	AMR-1333-88

solubilit	370 mg/l at pH 5 (4.6)	Assessment
y	390 mg/l at pH 5 (5.1-5.6)	Guidelines
	9.0 g/l at pH 7 (6.3)	Subdivision
	15.0 g/l at pH 7 (6.6)	D, Deries
	18.0 g/l at pH 9 (7.2)	63-8
	>250 g/l at pH 9 (9)	

Initial buffer solution pHs were 5, 7 and 9. The pH after dissolving the nicosulfuron is shown in parentheses. When pHs were adjusted toward their initial values, more nicosulfuron was dissolved.

When nicosulfuron was dissolved in pH 9 buffer (buffer conc = 0.05M), nicosulfuron reached a concentration of 0.044M (18 g/l) and the pH became 7.2.

The method was not measuring the solubility of nicosulfuron; the method was measuring the solubility of nicosulfuron salts.

Points to note

- Caution should be exercised in choosing methods for water solubility or octanol-water partition coefficient when the compound can form salts.
- Is the intention to measure the properties of the compound or its salts?
- The pH of the saturated solutions should be checked – have the buffer pH values changed?

Abbreviations and acronyms in this chapter

CAS: Chemical Abstracts Service
CIPAC: Collaborative International Pesticides Analytical Council
EEC: European Economic Community
FAO: Food and Agriculture Organization of the United Nations
GLC: gas liquid chromatography
HPLC-UV: high performance liquid chromatography with ultra-violet detection
ISO: International Organization for Standardization
IUPAC: International Union of Pure and Applied Chemistry
JMPR Joint Meeting on Pesticide Residues
JMPS: Joint Meeting on Pesticide Specifications
OECD Organization for Economic Co-operation and Development
P_{ow}: octanol-water partition coefficient
US EPA: United States Environmental Protection Agency

Chapter 3. Livestock and Crop Metabolism

Positions of the ^{14}C label on the study compound.
Livestock metabolism study
Crop metabolism study
Rotational crops
Metabolite names
Metabolic pathways
Presentation – livestock and crop metabolism

The purpose of this chapter is to explain pesticide metabolism studies and how to interpret the results so as to determine the likely nature of the residue occurring in feed and food commodities produced from exposed livestock or crops.

Metabolism in this context includes the processes of transport or translocation within the organism as well as transformation to metabolites or degradation products. The processes of photolysis on the plant surface are also included.

Metabolism studies provide essential data for residue definitions.

Relevant sections of JMPR Manual

- Metabolism..... 3.2.3
- Farm animal metabolism 3.2.3.1
- Plant metabolism..... 3.2.3.2
- Rotational crop studies..... 3.5.2
- Metabolism..... 5.3

In metabolism studies, pesticide is administered to livestock or applied to crops in amounts and for durations of time that could occur in practice when the compound is used for pest control. In the case of livestock, milk and eggs are collected and in due course the animal is slaughtered for collection of meat and offal. In the case of crops, feed and food commodities are harvested after an interval expected under good agricultural practice.

The harvested animal and plant commodities are then examined for content of total residues produced by administration of the compound. Animal excreta and, in some cases, exhaled air are examined for elimination of the residue.

Positions of the ^{14}C label on the study compound.

When a compound undergoes metabolism or other transformation (e.g. hydrolysis or photolysis) the products can be more readily followed and identified when they carry a label. In particular, the label allows observation of the metabolites among all the natural compounds derived from the host plant or animal.

The most common label in such studies is ^{14}C, i.e. a carbon isotope with an atomic weight of 14. It is also radioactive, emitting relatively low-energy β rays.

A compound with one of its ^{12}C atoms replaced by a ^{14}C behaves the same way in chemical and biochemical reactions, but the label permits observation of the fate of the parent compound and its transformation products.

In interpreting metabolism studies, we must remember that we can observe only those products that contain the label. It is important to know precisely where the ^{14}C label is incorporated into the molecule.

The metabolism of fenvalerate in animals illustrates the point that different metabolites are observed for different label positions.

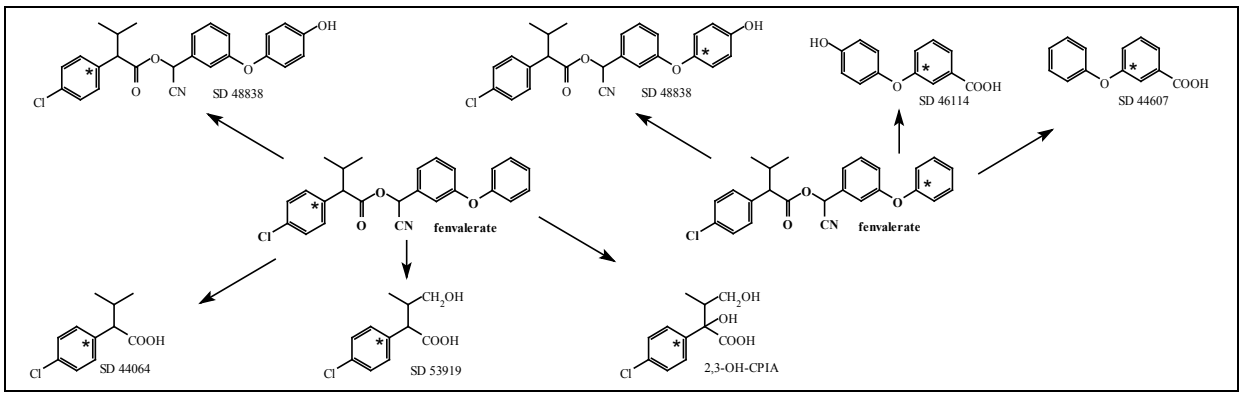


Figure 2. Livestock metabolism of fenvalerate ^{14}C labelled in the chlorophenyl ring or the phenoxyphenyl ring. The asterisk * indicates the position of the label.

When fenvalerate is ^{14}C labelled in the chlorophenyl ring, the metabolites containing that moiety are observable.

When fenvalerate is ^{14}C labelled in the phenoxyphenyl ring, the metabolites containing that moiety are observable.

The metabolite with the intact ester contains both of those moieties, so it is observed in both cases.

When metabolism studies are planned, the ^{14}C label positions must be carefully chosen to cover the fate of the various fragments of the molecule.

The study reviewer should always check the position of the label in the study compound. In some cases the description may be vague or ambiguous.

Example – thiamethoxam (JMPR 2010)

A study on the hydrolysis of a metabolite CGA 322704 described the ^{14}C position as the 2-position, but showed a diagram with the 5-position indicated.

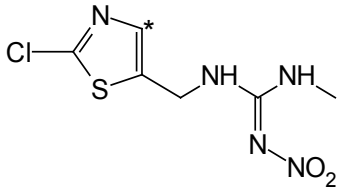


Diagram wrongly showing the ^{14}C label in position 5.

It was found that the text was correct. The ^{14}C label was on position 2, i.e. the carbon between the N and the S.

Example - difenoconazole (JMPR 2007)

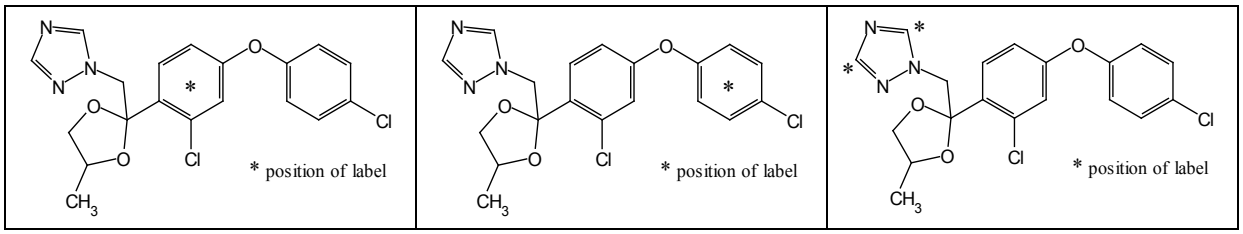


Figure 3. Labelling positions on difenoconazole in metabolism studies.

Difenoconazole was labelled in three different positions to cover the various parts of the molecule in the metabolism studies.

In some studies the position of the label was described as [phenyl-^{14}C]. In this situation, 'phenyl' is ambiguous and could mean either of the phenyl rings.

The reviewer must obtain a precise and unambiguous description of the label position before the results of the study can be correctly interpreted.

Livestock metabolism study

In a typical goat metabolism study, labelled pesticide is orally administered daily via gelatin capsule to lactating goats for 4-15 days. Milk is collected daily and the animals are slaughtered for tissue collection within 24 hours of the final dose.

Checklist

Study material

- compound and position of ^{14}C label
- dose – ? mg/kg bw/day, equivalent to ? ppm in feed dry weight
- method: capsule or mixed into ration
- dosing regime, ? times per day, number of consecutive days

Goats

- body weights, ? kg
- nature of the feed ration
- feed consumption, ? kg feed dry weight per day
- milk production, ? litres or kg per day
- milk collection. ? times per day
- interval between final dose and slaughter for tissue collection

Accountability of administered ^{14}C

- % in excreta, gastro-intestinal tract, housing wash
- % in milk
- % in tissues
- % in expired air (if necessary)
- total % accountability

A similar checklist applies for a typical poultry metabolism study, except that eggs are collected instead of milk.

Samples of the tissues, milk and eggs are subjected to combustion and total ^{14}C analysis, which provides the total radioactive residue (TRR) for each tissue, milk and eggs. Milk may be separated into cream and skim milk and eggs into yolk and white for TRR analysis.

Components of the TRR in each tissue, milk and eggs are then identified or characterized as far as possible.

"***Identified***" means that the structure of the metabolite is fully known.

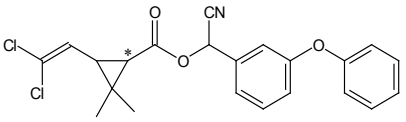
"***Characterized***" means that some properties of the metabolite or metabolites are known, such as polarity (TLC behaviour) and solubility (water soluble and organic solvent soluble). Some metabolites may also be characterized as conjugates.

Conjugate[6]. Molecular species produced in living organisms by covalently linking two chemical moieties from different sources. Example: Conjugate of a pesticide or metabolite with groups such as glutathione, sulfate, or glucuronic acid making it more soluble in water and facilitating its compartmentalization within the cell.

Metabolites and parent compound are extracted from the matrix and then identified by HPLC and TLC separation and comparison with reference compounds. More vigorous extractions and digestions of the matrix are attempted in order to release all of the ^{14}C. The most exhaustive digestions may release ^{14}C in the form of organo-soluble polar compounds, i.e. the ^{14}C that has been incorporated into natural components.

Example – alpha-cypermethrin metabolism in laying hens (JMPR 2008)

Groups of laying hens (8 birds per group) were dosed orally once daily via capsule for 14 consecutive days with [^{14}C-cyclopropyl]alpha-cypermethrin at the equivalent of 18 ppm in the feed. Eggs were collected daily. Birds were slaughtered 22 hours after the final dose for tissue collection.



The TRR in eggs reached an approximate plateau after 7-9 days of dosing.

Parent alpha-cypermethrin was the major identified component of the residue in fat and eggs. Hydroxy-cypermethrin and cis–DCVA were also identified. The numerous components of the residue observed in liver were mostly not identified. The distribution of the residue and the concentrations of identified components are summarised in the table below.

Table. Distribution of ^{14}C residue and metabolites in tissues and eggs of laying hens dosed orally daily for 14 consecutive days with [^{14}C-cyclopropyl]alpha-cypermethrin at the equivalent of 18 ppm in the feed.

Residue component	Concentration, mg/kg, expressed as parent and as %TRR				
	Abdominal fat	Skin with fat	Liver	Muscle	Eggs, day 14
Total ^{14}C residue (TRR)	0.23 mg/kg	0.13 mg/kg	0.30 mg/kg	0.009 mg/kg	0.063 mg/kg
Extracted residue	91 %	93 %	89 %	-	97 %
Unextracted	9.0 %	6.2 %	11 %	-	2.6 %
Alpha-cypermethrin	82 %	78 %	9.2 %		81 %
4'-OH-alpha-cypermethrin	4.3 %	3.0 %	3.3 %		4.3 %
cis-DCVA	1.0 %	3.7 %	30 %		4.3 %

Interpretation of the summary table above.

Total ^{14}C residue (TRR). The TRR in each tissue and in the eggs was measured by combustion analysis. The total ^{14}C was then calculated as mg of alphacypermethrin per

[6]Stephenson GR, Ferris IG, Holland PT and Nordberg M. 2006. IUPAC Glossary of terms relating to pesticides. *Pure Appl. Chem.* 78:2075–2154.

kg of tissue or eggs, e.g. the concentration of ^{14}C in fat was 0.23 mg/kg when calculated as alphacypermethrin.

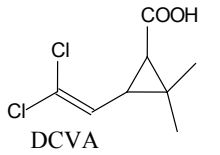
Extracted residue. Percentage of TRR extracted by exhaustive extraction (principally: fat by acetonitrile, liver by acetonitrile and pepsin enzyme, eggs by hexane+tetrahydrofuran).

The extracted residue is available for identification and characterization. The alpha-cypermethrin and the two metabolites shown in the table are part of this extracted residue. The TRR in muscle was too low to measure extractability.

Unextracted. Percentage of TRR remaining after exhaustive extraction.

Alpha-cypermethrin. Levels of alphacypermethrin in the tissues and eggs, expressed as % of TRR. For example, the level of alphacypermethrin in abdominal fat is 82 % of the abdominal fat TRR (0.23 mg/kg). It accounts for most (82 parts of the 91 %) of the extracted residue.

4'-OH-alpha-cypermethrin. Levels of metabolite in the tissues and eggs, expressed as parent compound as % of TRR.



DCVA
MW=209.07

cis-DCVA. Levels of metabolite in the tissues and eggs.
The concentration of a metabolite may be calculated from the %TRR and the TRR concentration.

Conc of cis-DCVA in liver $= 0.30 \times \frac{30}{100} = 0.09$ mg/kg, expressed as alpha-cypermethrin

$= 0.09 \times \frac{209.07}{416.30} = 0.045$ mg/kg, expressed as cis-DCVA.

The MW of cis DCVA is 209.07 and MW of alphacypermethrin is 416.3 g/mole.

Such a calculation is needed when an analytical method for the metabolite is being tested on a tissue sample from the metabolism studies to determine if it provides the same residue concentration.

Crop metabolism study

In a typical plant metabolism study, labelled pesticide is applied to crop plants at a rate equivalent to expected good agricultural practice. Sometimes, a higher rate is applied to facilitate identification of residues. The number of applications and timing are also aligned with expected good agricultural practice. Fruit, grain, foliage or straw, etc are harvested at maturity for TRR analysis and metabolite identification.

Checklist

Study material

- compound and position of ^{14}C label
- formulation
- application rate, ?kg ai/ha
- method of application: foliar spray, soil treatment, seed treatment
- calendar, dates of application

Crop

- variety
- growth stage or age at application
- protected or exposed to sunlight
- growth stage and dates of sampling and harvest

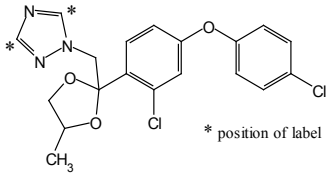
- nature of samples.

Samples of the fruits, grain, foliage or straw, etc are subjected to combustion and total ^{14}C analysis, which provides the total radioactive residue (TRR) for each commodity. Fruit may be processed into juice and pomace, oilseed into oil and meal, etc for TRR analysis.

Components of the TRR in each commodity are then identified or characterized as far as possible from a separate portion of the treated plants by the same procedures as already described for animal commodities.

Example – difenoconazole metabolism in wheat (JMPR 2007)

In a greenhouse metabolism study, spring wheat (variety James) was foliar sprayed 4 times with [^{14}C]triazole labelled difenoconazole formulated as an EC at a rate equivalent to 0.25 kg ai/ha. Wheat was grown in pails of loamy sand, at 15-20 plants per pail. The first difenoconazole application was 43 days post sowing at the early boot stage. Three further applications followed at 7- or 8-day intervals. Mature samples were harvested 29 days after the final application.



Samples were extracted with methanol+water (8+2) and the extracts were cleaned up, subjected to enzyme hydrolysis for release of conjugates, and the constituents were identified and characterized by TLC.

In exposed parts of the plant (tops and stalks) difenoconazole was the major part of the residue. In the unexposed part, i.e. the grain, the composition of the residue was quite different because only the triazole moiety metabolites are mobile within the plant and can translocate to any part. Parent difenoconazole was not identified in the grain.

Table. Distribution of ^{14}C residue in greenhouse wheat plants and grain subjected to 4 foliar applications of [^{14}C]triazole labelled difenoconazole formulated as an EC at a rate equivalent to 0.25 kg ai/ha.

WHEAT METABOLISM Residue component	Concentration, mg/kg expressed as parent, or %TRR		
	Tops 50% mature, 8 days after applic 2	Stalks, mature, 29 days after applic 4	Grain, mature, 29 days after applic 4
Total ^{14}C residue (TRR)	8.7 mg/kg	54 mg/kg	1.4 mg/kg
Extracted residue	88 %	78 %	70 %
Unextracted	10 %	13 %	23 %
Difenoconazole		50 %	
Hydroxy-difenoconazole		1 %	
CGA 205375		5 %	
Hydroxy-CGA 205375		1 %	
Triazolylacetic acid			20 %
1,2,4-triazole			10 %

Interpretation of the summary table above.

Total ^{14}C residue (TRR). The TRR in each commodity was measured by combustion analysis. The total ^{14}C was then calculated as mg of difenoconazole per kg of plant commodity, e.g. the concentration of ^{14}C in grain was 1.4 mg/kg when calculated as difenoconazole.

Extracted residue. Percentage of TRR extracted by exhaustive extraction. The extracted residue is available for identification and characterization.

Unextracted. Percentage of TRR remaining after exhaustive extraction.

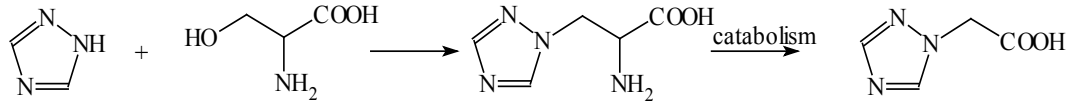
Difenoconazole. Levels of difenoconazole in the plant commodity, expressed as % of TRR.

Metabolites. Levels of identified metabolites in the plant commodity, expressed as parent compound as % of TRR.

Plant metabolism studies provide essential information about translocation of residues from one part of the plant to another.

Parent difenoconazole is not mobile within the plant, so parts of the plant exposed to the foliar spray contain residues of the parent compound, but the grain does not contain residues of difenoconazole.

However, metabolite 1,2,4-triazole and its metabolite triazolylacetic acid are readily translocated within the plant and are transported to the grain.



1,2,4-triazole L-serine

Triazole conjugates with serine to produce triazolylalanine and then triazolylacetic acid. The same situation occurs for other compounds containing the 1,2,4-triazole moiety.

Rotational crops

A pesticide residue study in rotational crops[7] is one where the pesticide is applied to one crop and residues are measured in the following, or rotation, crop planted or sown in the same soil.

If residues are identifiable and measurable in animal feed or food commodities from the rotational crop, residue violations (detected residues with no MRL) could occur. Rotational crop studies provide the information needed by regulatory authorities to allow for this situation when establishing MRLs.

In a **confined rotational crop study**, ^{14}C labelled pesticide is used, which allows the observation of the fate of the labelled material and the identification of soil and plant metabolites. It is confined because radiolabelled material must be controlled and accounted for.

In a **field rotational study**, unlabelled pesticide is used. Information from the confined study with radiolabel determines which crop parts to analyse and which residues should be included in the analysis.

Example of confined rotational crop study design

The [^{14}C]pesticide may be applied directly to bare ground, instead of a first crop. This would be the extreme case where all of the applied pesticide reaches the soil. Rotational crops are usually selected to represent leafy vegetables, root crops and cereals and are sown 30, 120 and 360 days after treatment.

[7] It should be noted that rotational crop studies may be concerned with other matters than detectable residues, e.g. carryover of herbicide from a tolerant crop to a susceptible crop. These studies aim to determine safe re-cropping intervals that avoid damage to the follow crop. Safe re-cropping intervals are provided on the label and become part of GAP.

The rotation crops are grown to full maturity for samples to be taken for analysis. Samples of cereal forage are taken at an earlier growth stage. Soil samples are also taken for analysis. Samples are needed for TRR analysis and metabolite identification.

Day 0	Rotation crop	TSI‡ days	THI✦ days	Sample
Application to bare ground	Leafy veg, e.g. lettuce	30	90	soil, lettuce
		120	180	soil, lettuce
		360	420	soil, lettuce
Application to bare ground	Root veg, e.g. radish	30	90	soil, radish tops, roots
		120	180	soil, radish tops, roots
		360	420	soil, radish tops, roots
Application to bare ground	Cereal, e.g. wheat	30	110	soil, whole plant
			180	soil, straw, grain
		120	200	soil, whole plant
			270	soil, straw, grain
		360	440	soil, whole plant
			510	soil, straw, grain

‡ TSI: interval between treatment on soil and sowing of rotation crop, days.

✦ THI: interval between treatment on soil and harvest of rotation crop (or sampling of soil), days.

Checklist

Study material

- compound and position of ^{14}C label
- application rate, ?kg ai/ha
- calendar, date of application

Soil and crop

- soil type and characteristics
- crop variety
- calendar, dates of sowing and sampling
- nature of samples

The reviewer is seeking

- data on the levels of TRR occurring in rotational crops
- identity and levels of parent and metabolites occurring in rotational crops (is the composition of the residue the same as in direct plant metabolism?).

If identifiable residues occur in sufficient quantities in commodities from the confined studies (see JMPR Manual, section 3.5.2), field rotational crops will be needed to quantify residues likely to occur in practice.

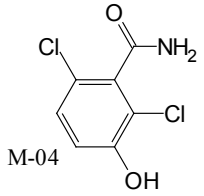
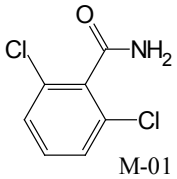
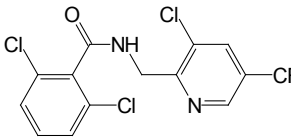
Example – fluopicolide confined rotational crop studies (JMPR 2009)

The metabolism of fluopicolide in confined rotational crops was studied on a sandy loam soil (sand 77%, silt 14%, clay 9.6%, pH 6.2, organic matter 0.81 %). [^{14}C]Fluopicolide was applied to bare soil at a rate of 400 g ai/ha. After plant-back intervals[8] (fallow periods) of 29 days, 133 days and 365 days, crops of lettuce, radish and wheat were sown and grown to maturity.

At the 365-days plant-back interval, the highest TRR levels were in radish tops and wheat straw. Parent fluopicolide was a minor component of the residue (3.8 % radish tops and 7.2 % wheat straw), while metabolites M-01 and M-04 were the major identified residues in wheat straw and radish tops respectively

[8] Plant-back interval: equivalent to TSI, treatment to sowing interval.

Summary of results for [^{14}C]phenyl-labelled fluopicolide from crops sown 365 days after the soil treatment.

		% TRR		
Crop part	TRR, mg/kg	 M-04	 M-01	 fluopicolide
Lettuce	0.53		87 %	2.1 %
Radish tops	1.75		88 %	3.8 %
Radish roots	0.03		61 %	24 %
Wheat forage	0.86	59 %	15 %	4.8 %
Wheat grain	0.05	25 %	18 %	7.3 %
Wheat straw	2.37	28 %	5.1 %	7.2 %

The conclusion from this study and a number of others was that rotational crops may contain low levels of fluopicolide and metabolites.

Metabolite M-01 is also a metabolite of dichlobenil, so was not suitable for inclusion in an enforcement residue definition, but was included for risk assessment.

The levels of residue occurring in a rotational crop situation for leafy vegetables, Brassica vegetables, cereal forage and fodder and root and tuber vegetables were considered when maximum residue levels were estimated.

Points to note

- pesticide residues occurring in rotational crops may be a complex matter requiring many studies to determine the details
- the composition of the residue in a rotational crop may be different from that from direct treatment. For example, parent fluopicolide was the major component of the residue (96 %) in foliar treated lettuce, compared with 2.1 % TRR in lettuce as a rotational crop.
- the results from rotational crop studies should be taken into consideration when maximum residue levels are estimated.
- maximum residue levels to cover rotational crop residues are preferably set on commodity groups

Metabolite names

The IUPAC glossary[6] of terms relating to pesticides defines the names that can be used for describing chemical pesticides.

A **pesticide common name** is the semisystematic (trivial) name of a chemical pesticide. Note: Common names of pesticides are listed by the ISO (International Organization for Standardization).

The **IUPAC name** is the name of a chemical according to the rules of nomenclature of the International Union of Pure and Applied Chemistry (IUPAC).

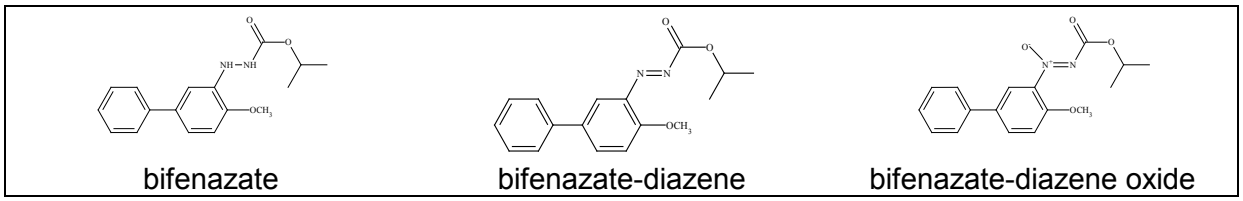
Metabolites do not generally have common names, but code names or trivial names are needed for them in reports and data tables.

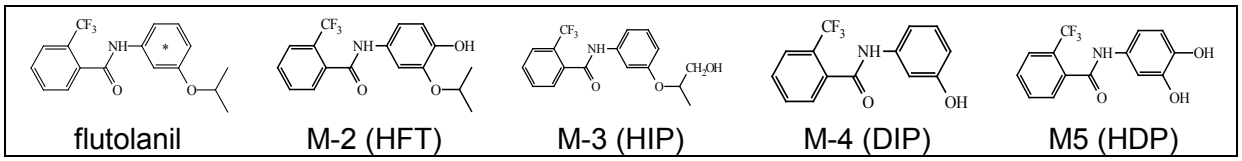
Various names and code numbers have been used for metabolites, including:

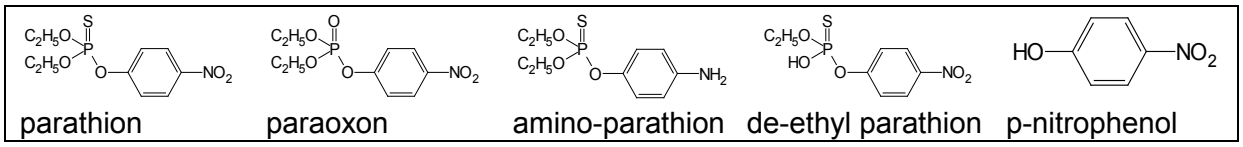
- A simple name, which could be a common name, a simplified systematic name (e.g. triazole) or a pseudo-common name (e.g. hydroxy-difenoconzaole).
- The systematic chemical name – it may be too cumbersome for use in discussion and tables.
- The CAS number – CAS numbers are not available for many metabolites.
- The company code number, e.g. CGA 205375.
- Serial numbers, e.g. metab 1, metab 2, etc, often inconsistent between studies.

Examples

Bifenazate, flutolanil and parathion are common names for pesticides. Names and codes for some of their metabolites are shown below. Some of the names such as bifenazate-diazene and amino parathion provide some suggestion of the metabolite structure, but many others do not.







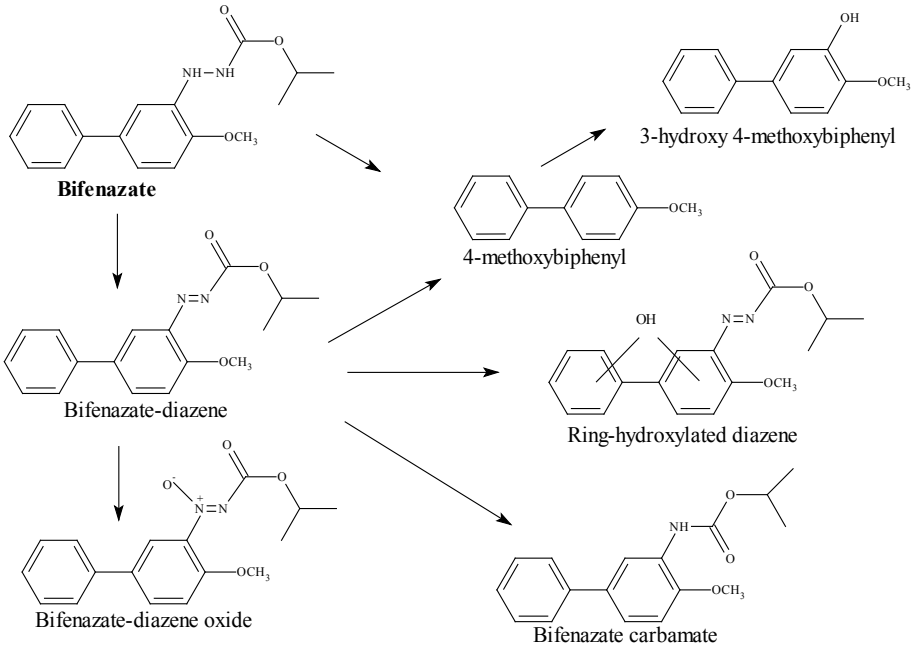
The reviewer should always check that the names and codes for metabolites have been used consistently from one study to the next.

Metabolic pathways

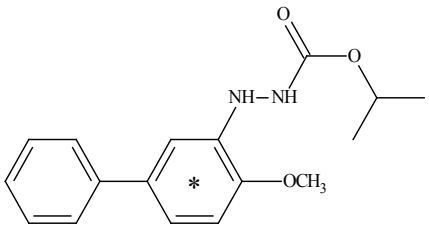
A metabolic pathway is constructed by assembling all of the identified metabolites and searching for plausible metabolism reactions as providing links from the parent compound to primary and later metabolites.

Example – bifenazate plant metabolism

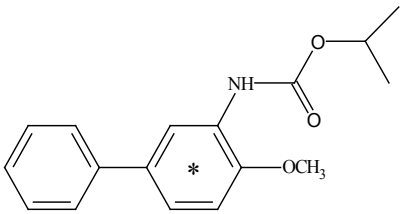
Bifenazate and five identified metabolites are assembled in a plausible metabolic pathway. But other possibilities are easily imagined. For example, it is possible that 3-hydroxy-4-methoxybiphenyl could be generated directly from other metabolites as well as 4-methoxybiphenyl.



Bifenazate carbamate was identified as a minor metabolite in apples after treatment with [^{14}C]bifenazate labelled in the substituted phenyl ring.



bifenazate ^{14}C labelled in the substituted phenyl ring



bifenazate carbamate

It seems an unlikely metabolic process, to break an N-N bond and eliminate an NH from an organic compound.

The bifenazate carbamate was a minor component identified in the mixture of metabolic products. The sponsor expressed the opinion that it may have originated as a small impurity in the labelled bifenazate used in the metabolism study.

The possible presence of impurities in the study material should always be kept in mind when interpreting metabolism studies.

Checklist for essential information to be included in the summary (appraisal) from animal and plant metabolism studies

<u>General for all kind of studies with labelled compounds</u>

1. Selection of ^{14}C or other isotope label position, and unambiguous description of the position by the chemical name of the compound
2. Description of the test system (number of animals, administration of the test compound) and expression of dose level in ppm dry feed, daily feed consumption

Test system, animal metabolism

- number of animals
- administration – oral or external treatment, capsule or feed incorporation, daily dosing for *n* days
- dose –expressed as mg per kg of bodyweight and as ppm of dry feed.

Test system, plant metabolism

- crop and growth stage
- if the test compound is sensitive to photolytic breakdown, note whether the plants were exposed to or protected from direct sunlight.
- administration – foliar, seed or soil treatment; number of applications and timing.
- dose – application rate per plant or tree or per unit area.

3. Type of samples collected and sampling days, intervals between sampling and analyses
   - Intervals between sampling and analyses should be checked and examined for influence on residue stability, but the information should not be included in the Appraisal unless problematic. The Appraisal becomes too cluttered with such background information.

4. Are storage stability test results available where the storage interval exceeds 2 month?
   - See above comment.
5. Animal metabolism.
   - Recovery of radioactivity, material balance.
6. Portion of extractable radioactivity in edible tissues (also milk and eggs for animal metabolism) and potential feed items (plant metabolism) as % of total radioactive residues, TRR, and mg parent compound equivalent/kg sample material;
7. Portion of non-extractable radioactivity in edible tissues (also milk and eggs for animal metabolism) and potential feed items (for plant metabolism) expressed as % TRR.
8. Portion of characterised but not identified radioactivity in edible tissues and potential feed items
9. Identification of metabolites (>10% TRR or >0.05 mg/kg)
   - Identified metabolites. Are all metabolites (>10% TRR or >0.05 mg/kg) identified? Was any metabolite present ≥ 10% not identified?

   All identified metabolites should be described by their systematic chemical names.
10. Characterization of metabolites (< 10% TRR, 0.01- 0.05 mg/kg)
11. Presence of metabolites in conjugated form, extractability of conjugated metabolites
12. Description of major metabolic reactions, transformation of parent compound to various metabolites, and the proposed metabolic pathway.

    Note: The description of major metabolic reactions should be kept brief in the Report. The pathway is for the Evaluation, not the Report.
13. Qualitative comparison of metabolites identified in laboratory animals (rat in tox. studies), farm animals (typically lactating goats and laying hens), identification of metabolites which are present in plants or farm animals but not present in rats.

Animal metabolism studies

1. Radioactivity in faeces, urine, cage wash as % of applied radioactivity
2. Radioactivity in edible tissues as % of applied radioactivity and mg/kg parent equivalent

3. Presence of parent compound and identified major metabolites (> 10% of TRR) expressed as % of total radioactivity and mg/kg in milk, muscle, liver kidney, fat and eggs as appropriate

Plant metabolism studies

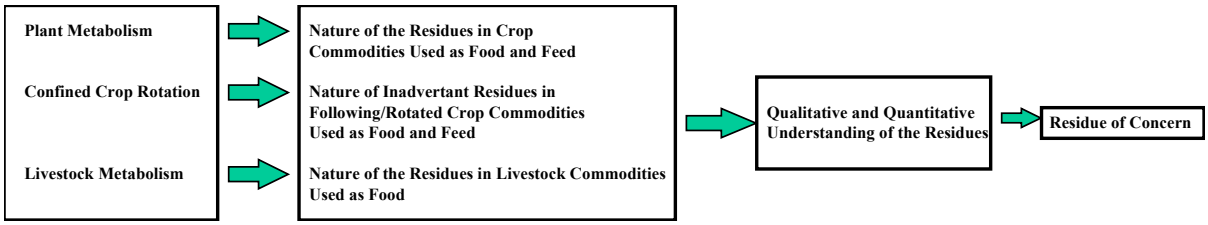
1. Selection of representative crops depending on the targeted use.
2. Identification of the samples taken at full maturity at normal harvest
3. Translocation of the radioactivity within plant tissues. Identification of translocated radioactivity (perhaps only some metabolites translocate from the point of application).
4. Proportion of residues which can be removed by surface wash.
5. Distribution of radioactivity in peel and pulp, or various plant parts (e.g. forage, stover, grains, husk;

Part II. PRESENTATION – LIVESTOCK AND CROP METABOLISM

1. Livestock and Crop Metabolism
2. Objective

- *The purpose of this chapter is to explain pesticide metabolism studies and how to interpret the results so as to determine the likely nature of the residue occurring in feed and food commodities produced from exposed livestock or crops.*
- Metabolism in this context includes the processes of transport or translocation within the organism as well as transformation to metabolites or degradation products. The processes of photolysis on the plant surface are also included.

3.



4. Metabolite names

- The IUPAC glossary of terms relating to pesticides defines the names that can be used for describing chemical pesticides.
- A **pesticide common name** is the semisystematic (trivial) name of a chemical pesticide. Note: Common names of pesticides are listed by the ISO (International Organization for Standardization).
- The **IUPAC name** is the name of a chemical according to the rules of nomenclature of the International Union of Pure and Applied Chemistry (IUPAC).
- Metabolites do not generally have common names, but code names or trivial names are needed for them in reports and data tables. Examples in previous text.

5. Outline

- Positions of the ^{14}C label on the study compound
- Characterization and identification of metabolites
- Livestock metabolism study
- Crop metabolism study
- Rotational crops

6. Conducting metabolism studies

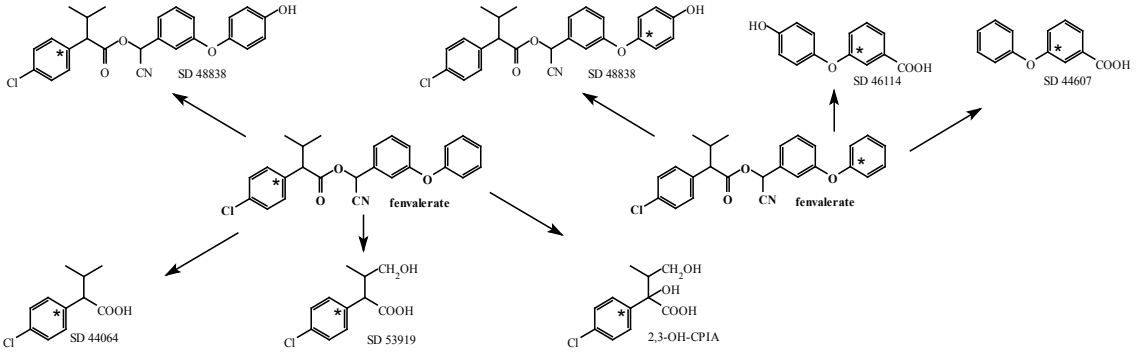
- In metabolism studies, pesticide is administered to livestock or applied to crops in amounts and for durations of time that could occur in practice when the compound is used for pest control.
- In the case of livestock, milk and eggs are collected and in due course the animal is slaughtered for collection of meat and offal.

- In the case of crops, feed and food commodities are harvested after an interval expected under good agricultural practice.
- The harvested animal and plant commodities are then examined for content of total residues and major residue components produced by administration of the compound. Animal excreta and, in some cases, exhaled air are examined for elimination of the residue.

7. Positions of the ^{14}C label on the study compound

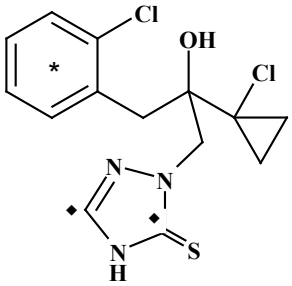
- The label allows observation of the metabolites among all the natural compounds derived from the host plant or animal.
- The most common label in such studies is ^{14}C, i.e. a carbon isotope with an atomic weight of 14. It is also radioactive, emitting relatively low-energy β rays.
- A compound with one of its ^{12}C atoms replaced by a ^{14}C behaves the same way in chemical and biochemical reactions, but the label permits observation of the fate of the parent compound and its transformation products.
- We can observe only those products that contain the label. It is important to know precisely where the ^{14}C label is incorporated into the molecule.

8. Livestock metabolism of fenvalerate

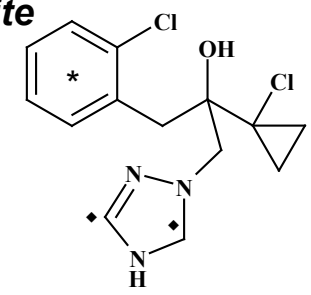


The molecule is labelled in the chlorophenyl ring or the phenoxyphenyl ring, indicated by an asterisk.

9. Multiple label: parent + main metabolite

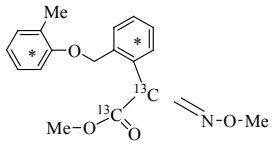


phenyl-UL-^{14}C- and 3,5-triazole-^{14}C-prothioconazole

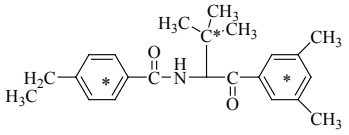


phenyl-UL-^{14}C- and 3,5-triazole-^{14}C- desthio prothioconazole

10. Multilabel positions



Krezoxim-methyl



Tebufenozide

11. Check

- When metabolism studies are planned, the ^{14}C label positions must be carefully chosen to cover the fate of the various fragments of the molecule.
- The study reviewer should always check the position of the label in the study compound. In some cases the description may be vague or ambiguous.

12. Characterization and identification of metabolites

- The desired goal of a metabolism study is the identification and characterization of at least 90% of the total radioactive residues TRR in edible tissues, milk, eggs and in each raw agricultural commodity (RAC) of the treated crop.
- In many cases it may not be possible to identify significant portions of the TRR especially when
  - low total amounts of residue are present,
  - ^{14}C label is incorporated into biomolecules, or
  - the active ingredient is extensively metabolised to numerous low level components.
- In the latter case it is important for the applicants to demonstrate clearly the presence and levels of the components, and if possible, attempt to characterise them.

13.

Relative amount (%)	Concentration (mg/kg)	Required Action
< 10	< 0.01	No action if no toxicological concern
< 10	0.01 – 0.05	Characterize. Only attempt to confirm identity if straightforward, e.g., a reference compound is available or the identification is known from a previous study.
< 10	> 0.05	Characterization/identification needs to be decided on a case-by-case basis taking into account how much has been identified.
> 10	< 0.01	Characterize. Only attempt to confirm identity if straightforward, e.g., a reference compound is available or the identification is known from a previous study.

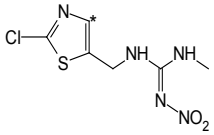
> 10	0.01 – 0.05	Significant attempts to identify should be made especially if needed to establish a pathway, ultimately characterization might be accepted.
> 10	> 0.05	Identify using all possible means.
> 10	> 0.05 unextracted radiolabel	If unextracted radiolabel ≥0.05 mg/kg or 10% of the TRR, release of the radioactivity should be attempted for further identification.

14. Information required for evaluation

The information provided for evaluation should include documentation on the proposed metabolic pathway, including a table with associated chemical structures and names, the quantities of the metabolites in the different parts of the plants (surface, leaves, stems and edible root), in different animal tissues (fat, muscles, kidneys, liver, eggs and milk) and in different soil types. Any postulated intermediates/metabolites should also be indicated in the pathway.

15. Wrong indication of label position

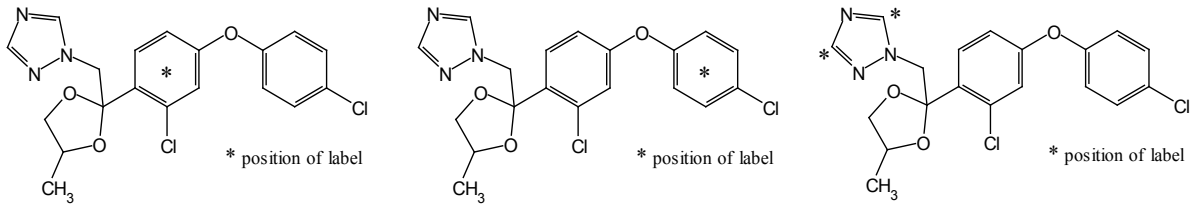
Diagram wrongly showing the ^{14}C label in position 5.



A study on the hydrolysis of a metabolite CGA 322704 described the ^{14}C position as the 2-position, but showed a diagram with the 5-position indicated.

It was found that the text was correct. The ^{14}C label was on position 2, i.e. the carbon between the N and the S.

16. Ambiguous label indication



Difenoconazole was labelled in three different positions to cover the various parts of the molecule in the metabolism studies.

In some studies the position of the label was described as [phenyl-^{14}C]. In this situation, 'phenyl' is ambiguous and could mean either of the phenyl rings.

The reviewer must obtain a precise and unambiguous description of the label position before the results of the study can be correctly interpreted.

17. Livestock metabolism study

In a typical goat metabolism study, labelled pesticide is orally administered daily via gelatin capsule to lactating goats for 4-15 days. Milk is collected daily and the animals are slaughtered for tissue collection within 24 hours of the final dose.

18. Checklist

Study material

- compound and position of ^{14}C label
- dose – ? mg/kg bw/day, equivalent to ? ppm in feed dry weight
- method: capsule or mixed into ration
- dosing regime, ? times per day, number of consecutive days

Goats

- body weights, ? kg
- nature of the feed ration
- feed consumption, ? kg feed dry weight per day
- milk production, ? litres or kg per day
- milk collection. ? times per day
- interval between final dose and slaughter for tissue collection

19. Checklist 2

Accountability of administered ^{14}C

- % in excreta, gastro-intestinal tract, housing wash
- % in milk
- % in tissues
- % in expired air (if necessary)
- total % accountability

A similar checklist applies for a typical poultry metabolism study, except that eggs are collected instead of milk.

20. Analysis of samples

- Samples of the tissues, milk and eggs are subjected to combustion and total ^{14}C analysis, which provides the total radioactive residue (TRR) for each tissue, milk and eggs. Milk may be separated into cream and skim milk and eggs into yolk and white for TRR analysis.
- Components of the TRR in each tissue, milk and eggs are then identified or characterized as far as possible.

"***Identified***" means that the structure of the metabolite is fully known.

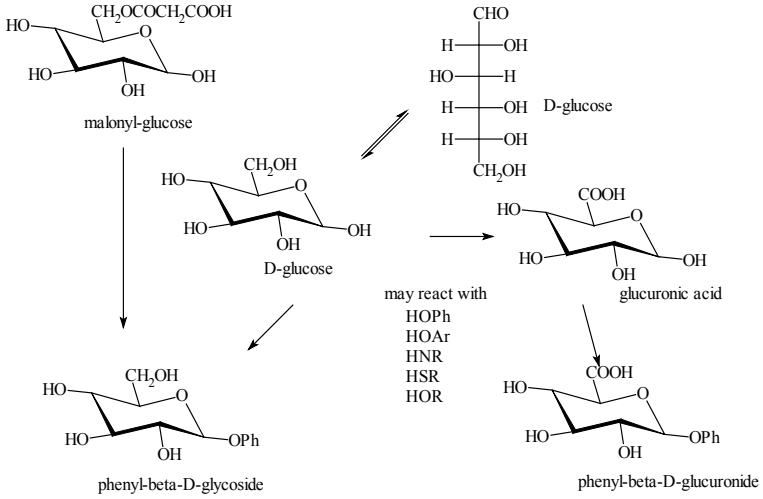
"***Characterized***" means that some properties of the metabolite or metabolites are known, such as polarity (TLC behaviour) and solubility (water soluble and organic solvent soluble). Some metabolites may also be characterized as conjugates.

21. Conjugates

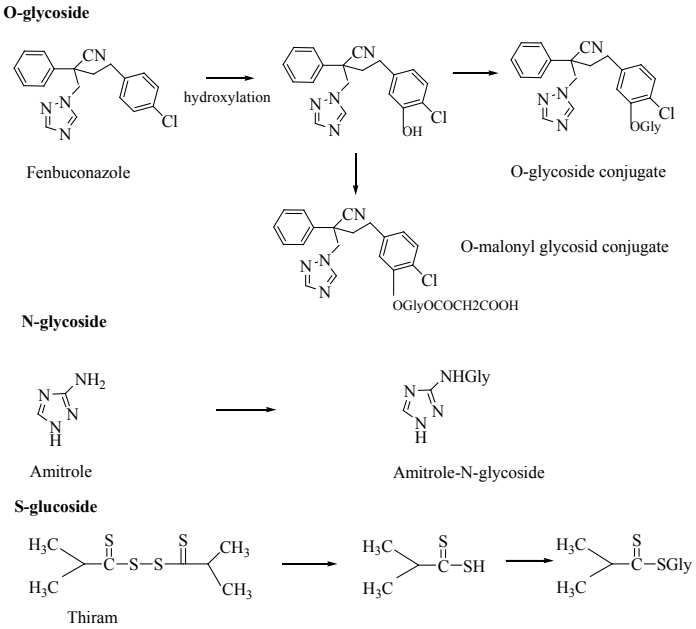
- Molecular species produced in living organisms by covalently linking two chemical moieties from different sources. Example: Conjugate of a pesticide or metabolite with groups such as glutathione, sulfate, or glucuronic acid making it more soluble in water and facilitating its compartmentalization within the cell.
- Metabolites and parent compound are extracted from the matrix and then identified by HPLC and TLC separation and comparison with reference compounds. More

vigorous extractions and digestions of the matrix are attempted in order to release all of the ^{14}C. The most exhaustive digestions may release ^{14}C in the form of organo-soluble polar compounds, i.e. the ^{14}C that has been incorporated into natural components.

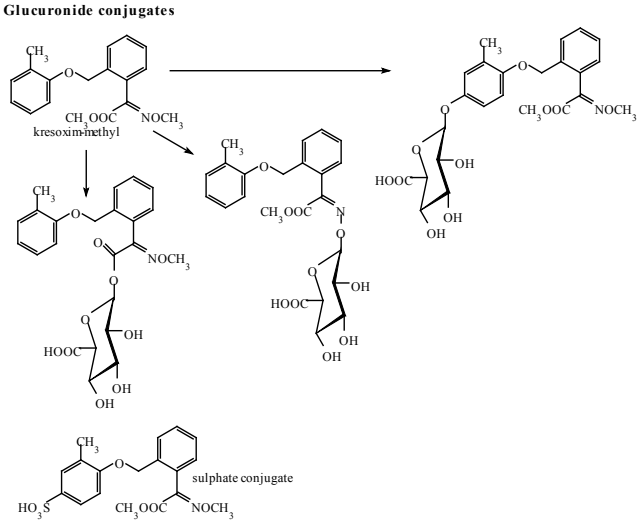
22. Formation of glycoside and glucuronide conjugates



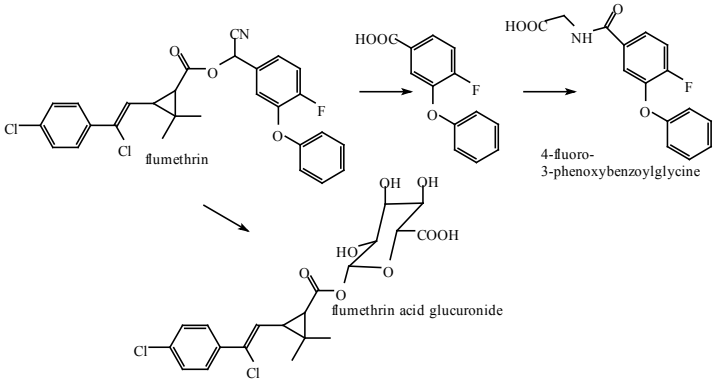
23. Examples for formation of glycoside conjugates



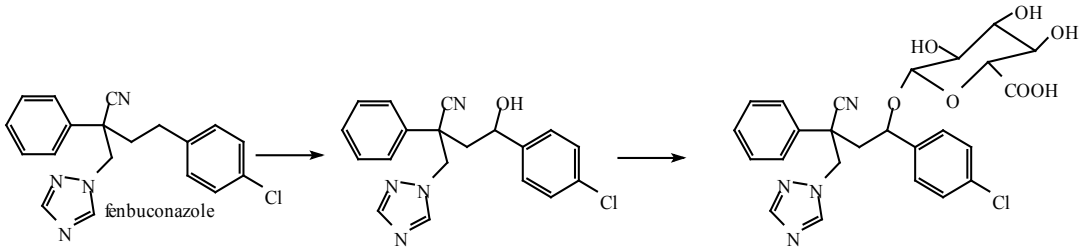
24. Formation of glucuronide and sulphate conjugates



25. Formation of glucuronide and glycine conjugates with carboxyl moiety

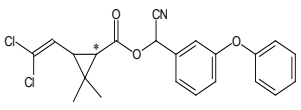


26. Formation of glucuronide conjugate with secondary hydroxyl group



27. Alpha-cypermethrin metabolism in laying hens

Laying hens were dosed orally once daily via capsule for 14 consecutive days with [^{14}C-cyclopropyl]alpha-cypermethrin at the equivalent of 18 ppm in the feed.



Eggs were collected daily. Birds were slaughtered 22 hours

after the final dose for tissue collection.

Parent alpha-cypermethrin was the major identified component of the residue in fat and eggs. Hydroxy-cypermethrin and cis–DCVA were also identified. The numerous components of the residue observed in liver were mostly not identified. The distribution of the residue and the concentrations of identified components are summarised in Table 1

28. Distribution of ^{14}C residue and metabolites in tissues and eggs of laying hens dosed daily with 18 ppm in feed

Table 1

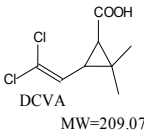
	Concentration, mg/kg, expressed as parent and as %TRR				
Residue component	Abdominal fat	Skin with fat	Liver	Muscle	Eggs, day 14
Total ^{14}C residue (TRR) mg/kg	0.23	0.13	0.30	0.009	0.063
Extracted residue	91 %	93 %	89 %	-	97 %
Unextracted	9.0 %	6.2 %	11 %	-	2.6 %
Alpha-cypermethrin	82 %	78 %	9.2 %		81 %
4'-OH-alpha-cypermethrin	4.3 %	3.0 %	3.3 %		4.3 %
cis-DCVA	1.0 %	3.7 %	30 %		4.3 %

29. Interpretation of the summary table

Total ^{14}C residue (TRR). The TRR in each tissue and in the eggs was measured by combustion analysis. The total ^{14}C was then calculated as mg of alphacypermethrin per kg of tissue or eggs, e.g. the concentration of ^{14}C in fat was 0.23 mg/kg when calculated as alphacypermethrin.

Extracted residue. Percentage of TRR extracted by exhaustive extraction (principally: fat by acetonitrile, liver by acetonitrile and pepsin enzyme, eggs by hexane+tetrahydrofuran). The extracted residue is available for identification and characterization. The TRR in muscle was too low to measure extractability.

30. Calculation of the concentration of metabolites



MW=209.07

The concentration of a metabolite may be calculated from the %TRR and the TRR concentration.

Conc of cis-DCVA in liver $= 0.30 \times \frac{30}{100} = 0.09$ mg/kg, expressed as alpha-cypermethrin

$= 0.09 \times \frac{209.07}{416.30} = 0.045$ mg/kg, expressed as cis-DCVA.

Such a calculation is needed when an analytical method for the metabolite is being tested on a tissue sample from the metabolism studies to determine if it provides the same residue concentration.

31. Crop metabolism study

- In a typical plant metabolism study, labelled pesticide is applied to crop plants at a rate equivalent to expected good agricultural practice. Sometimes a higher rate is

applied to facilitate identification of residues. The number of applications and timing are also aligned with expected good agricultural practice. Fruit, grain, foliage or straw, etc are harvested at maturity for TRR analysis and metabolite identification. There are some exceptions.

- Samples of the fruits, grain, foliage or straw, etc are subjected to combustion and total ^{14}C analysis, which provides the total radioactive residue (TRR) for each commodity. Fruit may be processed into juice and pomace, oilseed into oil and meal, etc for TRR analysis.
- Components of the TRR in each commodity are then identified or characterized from a separate portion of the treated plants as far as possible by the same procedures as already described for animal commodities.
- Plant metabolism studies provide essential information about translocation of residues from one part of the plant to another.

32. Plant metabolism

- Metabolism study should be submitted for each type of crop group for which use is proposed.
- Crops can be considered to belong to one of five categories for crop metabolism studies:
  - *root crops* (root and tuber vegetables, bulb vegetables)
  - *leafy crops* (Brassica vegetables, leafy vegetables, stem vegetables, hops),
  - *fruits* (citrus fruit, pome fruit, stone fruit, berries, grapes, banana, tree nuts, fruiting vegetables, persimmon),
  - *pulses and oilseeds* (legume vegetables, pulses, oilseeds, peanuts, legume fodder crops, cacao beans, coffee beans) and
  - *cereals* (cereals, grass and forage crops).

33. Plant metabolism

- One crop from a group will cover the entire group for purposes of metabolism in those crops within the group.
- In order to extrapolate metabolism of a pesticide to all crop groupings, metabolism studies on a minimum of three representative crops (from the five different crop categories) should be conducted. If the results of these three studies indicate a comparable metabolic route, then additional studies will not be needed.

34. Plant metabolism

- The studies should reflect the intended use pattern of the active ingredient such as foliar, soil/seed, or post-harvest treatments.
- If, for instance, three studies have been conducted using foliar application and at a later date soil application (e.g., seed treatment, granular, or soil drench) is proposed, then an additional study reflecting soil application should be conducted.

35. Plant metabolism

- On the other hand, if different metabolic routes are observed among the representative crops from studies conducted in a similar manner (e.g., foliar spray with similar pre-harvest interval (PHI) and growth stages), further studies should be

conducted for uses on crops in the remaining categories for which use is proposed. Differences in the quantities of metabolites belonging to the same pathway will not trigger the need for additional studies.

- If a use on paddy rice is intended, a metabolism study should be submitted for paddy rice, regardless of other available metabolism studies.

36. Transgenic and non-transgenic crops

- They may metabolize the pesticide differently. Full and detailed information will be required for a transgenic crop with metabolism differences from the non-transgenic crop.
- For genetically modified crops that do not involve the insertion of a gene conveying resistance by means of metabolism, no additional metabolism studies are needed.
- When a gene is inserted that conveys active ingredient resistance due to pesticide metabolism, then a Metabolism in Crops study should be conducted for each crop grouping to which the genetically modified crops belong.
- If one such study shows a similar metabolism to conventional crops, however, no additional studies would be needed. If a different metabolic route is observed, then two additional studies should be conducted.

37. Checklist

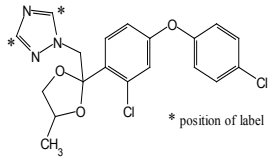
Study material

- compound and position of ^{14}C label
- formulation
- application rate, ?kg ai/ha
- method of application: foliar spray, soil treatment, seed treatment
- calendar, dates of application

Crop

- variety
- growth stage or age at application
- protected or exposed to sunlight
- growth stage and dates of sampling and harvest
- nature of samples.

38. Difenoconazole metabolism in wheat



In a greenhouse, spring wheat was foliar sprayed 4 times with [^{14}C]triazole labelled difenoconazole formulated as an EC at a rate equivalent to 0.25 kg ai/ha.

The first difenoconazole application was 43 days post sowing at the early boot stage. Three further applications followed at 7- or 8-day intervals. Mature samples were harvested 29 days after the final application.

In exposed parts of the plant (tops and stalks) difenoconazole was the major part of the residue. In the unexposed part, i.e. the grain, the composition of the residue was quite

different because only the triazole moiety metabolites are mobile within the plant and can translocate to any part. Parent difenoconazole was not identified in the grain.

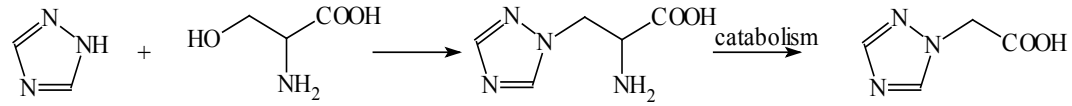
39. Distribution of ^{14}C residue in wheat plants and grain treated with [^{14}C-triazole] difenoconazole

WHEAT METABOLISM Residue component	Concentration, mg/kg expressed as parent, or %TRR		
	Tops 50% mature, 8 days after applic 2	Stalks, mature, 29 days after applic 4	Grain, mature, 29 days after applic 4
Total ^{14}C residue (TRR)	8.7 mg/kg	54 mg/kg	1.4 mg/kg
Extracted residue	88 %	78 %	70 %
Unextracted	10 %	13 %	23 %
Difenoconazole		50 %	
Hydroxy-difenoconazole		1 %	
CGA 205375		5 %	
Hydroxy-CGA 205375		1 %	
Triazolylacetic acid			20 %
1,2,4-triazole			10 %

40. Interpretation of the results

Parent difenoconazole is not mobile within the plant, so parts of the plant exposed to the foliar spray contain residues of the parent compound, but the grain does not contain residues of difenoconazole.

However, metabolite 1,2,4-triazole and its metabolite triazolylacetic acid are readily translocated within the plant and are transported to the grain.



1,2,4-triazole L-serine

Triazole conjugates with serine to produce triazolylalanine and then triazolylacetic acid. The same situation occurs for other compounds containing the 1,2,4-triazole moiety.

41. Residues in rotational crops

A **pesticide residue study in rotational crops** is one where the pesticide is applied to one crop and residues are measured in the following, or rotation, crop planted or sown in the same soil.

It should be noted that rotational crop studies may be concerned with other matters than detectable residues, e.g. *carryover of herbicide* from a tolerant crop to a susceptible crop. These studies aim to determine safe re-cropping intervals that avoid damage to the follow crop. Safe re-cropping intervals are provided on the label and become part of GAP.

If residues are identifiable and measurable in animal feed or food commodities from the rotational crop, residue violations (detected residues with no MRL) could occur.

In a *confined rotational crop study*, ^{14}C labelled pesticide is used, which allows the observation of the fate of the labelled material and the identification of soil and plant metabolites. It is confined because radiolabelled material must be controlled and accounted for. Also: field rotational crop study

In a *field rotational crop study*, unlabelled pesticide is used. Information from the confined study with radiolabel determines which crop parts to analyse and which residues should be included in the analysis.

42. Objectives of rotational crop studies

- Provide an estimate of total radioactive residues (TRR) in the various raw agricultural commodities (RACs) via soil uptake.
- Identify the major components of the terminal residue in the various RACs, thus indicating the components to be analysed for in residue quantification studies (i.e., the residue definition(s) for both risk assessment and enforcement).
- Elucidate the degradation pathway of the active ingredient in rotated crops.
- Provide data to determine appropriate rotational intervals (time from pesticide application to a time when crops can be rotated) and/or rotational crop restrictions based on residue uptake levels.
- Provide information for determining if limited field trials for rotational crops should be performed.

43. Metabolism in rotational crops

- The study should normally be performed using a sandy loam soil that has been treated with the radiolabelled test substance applied at a rate equivalent to the maximum seasonal rate (1X)
- The soil should be treated with radiolabelled pesticide active ingredient, preferably containing formulation ingredients typical of an end use product as applied in the field.

44. Metabolism in rotational crops

- Rotational crops should be representative of each of the following crop groupings: *root and tuber vegetable*, e.g., radish, beets or carrots; *small grain*, e.g., wheat, barley, oats or rye; and *leafy vegetable*, e.g., spinach or lettuce.
- Where possible, crops should include those expected in the rotational schedule on the label, if known.

45. Example of confined rotational crop study design

- The [^{14}C]pesticide may be applied directly to bare ground, instead of a first crop. This would be the extreme case where all of the applied pesticide reaches the soil. Rotational crops are usually selected to represent leafy vegetables, root crops and cereals and are sown 30, 120 and 360 days after treatment.
- The rotation crops are grown to full maturity for samples to be taken for analysis. Samples of cereal forage are taken at an earlier growth stage. Soil samples are also taken for analysis. Samples are needed for TRR analysis and metabolite identification.
- If identifiable residues occur in sufficient quantities in commodities from the confined studies (see JMPR Manual, section 3.5.2), field rotational crops will be needed to quantify residues likely to occur in practice.

46. Design

Day 0	Rotation crop	TSI‡ days	THI✦ days	Sample

Day 0	Rotation crop	TSI‡ days	THI✦ days	Sample
Application to bare ground	Leafy veg, e.g. lettuce	30	90	soil, lettuce
		120	180	soil, lettuce
		360	420	soil, lettuce
Application to bare ground	Root veg, e.g. radish	30	90	soil, radish tops, roots
		120	180	soil, radish tops, roots
		360	420	soil, radish tops, roots
Application to bare ground	Cereal, e.g. wheat	30	110	soil, whole plant
			180	soil, straw, grain
		120	200	soil, whole plant
			270	soil, straw, grain
		360	440	soil, whole plant
			510	soil, straw, grain

‡ TSI: interval between treatment on soil and sowing of rotation crop, days.
✦ THI: interval between treatment on soil and harvest of rotation crop (or sampling of soil), days.

47. Checklist

Study material

- compound and position of ^{14}C label
- application rate, ?kg ai/ha
- calendar, date of application

Soil and crop

- soil type and characteristics
- crop variety
- calendar, dates of sowing and sampling
- nature of samples

The reviewer is seeking

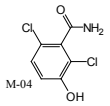
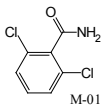
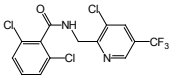
- data on the levels of TRR occurring in rotational crops
- identity and levels of parent and metabolites occurring in rotational crops (is the composition of the residue the same as in direct plant metabolism?).

48. Fluopicolide confined rotational crop studies

- The metabolism of fluopicolide in confined rotational crops was studied on a sandy loam soil (sand 77%, silt 14%, clay 9.6%, pH 6.2, organic matter 0.81 %). [^{14}C]Fluopicolide was applied to bare soil at a rate of 400 g ai/ha. After plant-back intervals (fallow periods) of 29 days, 133 days and 365 days, crops of lettuce, radish and wheat were sown and grown to maturity.
- At the 365-days plant-back interval, the highest TRR levels were in radish tops and wheat straw. Parent fluopicolide was a minor component of the residue (3.8 % radish tops and 7.2 % wheat straw), while metabolites M-01 and M-04 were the major identified residues in wheat straw and radish tops respectively.
- The conclusion from this study and a number of others was that rotational crops may contain low levels of fluopicolide and metabolites.

- Metabolite M-01 is also a metabolite of dichlobenil, so was not suitable for inclusion in an enforcement residue definition, but was included for risk assessment.

49. [^{14}C]phenyl-labelled fluopicolide from crops sown 365 days after the soil treatment.

		% TRR		
Crop part	TRR, mg/kg	 M-04	 M-01	 fluopicolide
Lettuce	0.53		87 %	2.1 %
Radish tops	1.75		88 %	3.8 %
Radish roots	0.03		61 %	24 %
Wheat forage	0.86	59 %	15 %	4.8 %
Wheat grain	0.05	25 %	18 %	7.3 %
Wheat straw	2.37	28 %	5.1 %	7.2 %

50. Points to note

- Pesticide residues occurring in rotational crops may be a complex matter requiring many studies to determine the details;
- The composition of the residue in a rotational crop may be different from that from direct treatment. For example, parent fluopicolide was the major component of the residue (96 %) in foliar treated lettuce, compared with 2.1 % TRR in lettuce as a rotational crop.
- The results from rotational crop studies should be taken into consideration when maximum residue levels are estimated.
- Maximum residue levels to cover rotational crop residues are preferably set on commodity groups

Abbreviations and acronyms in this chapter

CAS:	Chemical Abstracts Service
EC	emulsifiable concentrate
GAP	good agricultural practice
HPLC	high performance liquid chromatography
ISO:	International Organization for Standardization
IUPAC:	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting on Pesticide Residues
MRL	maximum residue limit
THI	treatment to harvest interval (in crop rotation studies)
TLC	thin layer chromatography
TRR	total radioactive residue
TSI	treatment to sowing interval (in crop rotation studies)

Chapter 4. Environmental Fate of Pesticides in Soil, Water and Water-Sediment Systems.

Requirements for environmental fate studies
Planning and conducting the environmental fate studies.
The use of environmental fate study results in the evaluation of residues in food commodities
Presentation – environmental fate

The purpose of this chapter is to explain pesticide environmental fate studies and how to interpret their results for estimation of residue levels in food and feed commodities.

Relevant sections of JMPR Manual

- Metabolism and environmental fate 3.2.3
- Environmental fate in soil, water and water-sediment systems 3.2.3.3
- Plant metabolism (includes rotational crops) 3.2.3.2

Requirements for environmental fate studies

The FAO Panel does not evaluate data on environmental toxicology, but does require studies on environmental fate relevant to the potential for uptake of residues by food and feed crops.

These studies are normally required for all pesticides except those with a specific restricted use, e.g., seed treatment, post-harvest application in storage. The availability of relevant studies is essential for the assessment of the potential for residues in food and feeds.

It should be noted that the studies required are in some cases dependent upon the use pattern (soil, foliar, seed treatment) and that paddy rice presents a unique situation. The data requirements on environmental fate are summarized in the Table below:

Type of study	Type of use and requirement (yes/no/conditional)						Comments
	Foliar	Soil	Plants of root, tuber, bulb, or peanut (at/after pegging)	Seed dressing (including seed potato)	Herbicide (for weeds in crop)	Paddy rice	
Physical and chemical properties	Condi-tional	Condi-tional	Conditional	Conditional	Conditional	Conditional	Only to the extent not provided for the technical material, e.g., hydrolysis and photolysis.
Degradation in soil (aerobic)	No	Yes	Yes	Yes	Yes	No	May be part of confined rotational crop.
Soil photolysis	No	Yes	Yes	Yes	Yes	No	
Degradation in soil (anaerobic)	No	No	No	No	No	No	
Persistence in soil	No	No	No	No	No	No	
Mobility/leaching in soil	No	No	No	No	No	No	

Type of study	Type of use and requirement (yes/no/conditional)						Comments
	Foliar	Soil	Plants of root, tuber, bulb, or peanut (at/after pegging)	Seed dressing (including seed potato)	Herbicide (for weeds in crop)	Paddy rice	
Adsorption by soil types	No	No	No	No	No	No	
Hydrolysis rate and products	Yes	Yes	Yes	Yes	Yes	Yes	Hydrolysis in sterile aqueous buffers. Abiotic epimerization should be provided as appropriate (e.g., pyrethroids)
Photolysis-plant surface	Conditional	No	See foliar	No	No	See foliar	Plant metabolism may suffice. Needed for special cases (e.g., abamectin)
Photolysis-natural pond water	No	No	No	No	No	Conditional	Plant metabolism may be adequate for rice. Useful for GAP involving application to water surface.
Crop uptake and bioavailability (see rotational crops)	No	No	No	No	No	No	
Rotational crops-confined	Yes	Yes	Yes	Yes	Yes	No	Not required where no crop rotation (e.g., orchard crops). Soil and crop should be analysed for radiolabelled residues.
Rotational crops-field	Condi-tional	Condi-tional	Conditional	Conditional	Conditional	No	Requirement conditional on results of confined rotational crop study.
Field dissipation studies	Condi-tional	Condi-tional	Conditional	Conditional	Conditional	No	Requirement conditional on results of confined rotational crop study.
Residue degradation (biodegradability) in water-sediment systems	No	No	No	No	No	Conditional	Metabolism study for paddy rice may be adequate. In other cases, metabolism/degradation needed, e.g., application to pond water.

Planning and conducting the environmental fate studies

The studies are performed with radiolabelled active ingredients. The principles of selecting label position and the general requirements for identification and quantification of metabolites are the same as described in chapter 3.

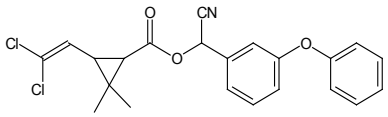
The use of environmental fate study results in the evaluation of residues in food commodities

The results of environmental fate studies assist in the interpretation and understanding of supervised residue trials.

For example, the environmental studies provide answers for such questions as:

- if this compound is used at an early growth stage for potatoes, are residues still present in the soil when the potatoes are harvested?
- if this herbicide is used for weed control around fruit trees, will it persist in the soil and be taken up by the fruit trees?
- are the soil metabolites the same as the plant metabolites?
- for a compound susceptible to photolysis, how does the persistence compare for a soil surface application and a soil incorporated application?
- for a compound susceptible to photolysis, can we expect higher residues from glass-house uses than from field uses?
- are new metabolites formed under the anaerobic sediment conditions during rice growing?

Example – cypermethrin aerobic soil metabolism (JMPR 2008)



When cypermethrin was subject to aerobic soil metabolism in four soils (two sandy loams, a clay loam and a silty clay loam) at 20 °C, the half-lives for disappearance of cypermethrin were in the range of 6 to 24 days. The trans isomers disappeared more quickly than the cis.

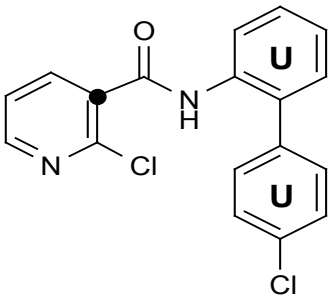
The cis-trans ratio of the test material was 40:60. After 90 or 120 days of exposure, the cis:trans ratio of the remaining cypermethrin was in the range 60:40 to 73:27.

Percentage mineralization (conversion to CO_2) after 90 or 120 days was in the range 37-70 %.

Points to note

- the isomer composition of the residue changed during soil metabolism
- percentage mineralization is a measure of the disappearance of the total residue.

Example – boscalid residue in rotational crops



2006: In a confined rotational crop study, soil was treated directly with [^{14}C]-boscalid labelled in the diphenyl ring or the pyridine ring. Crops of lettuce, radish and wheat were sown into the treated soil at intervals of 30, 120, 270 and 365 days after treatment and were grown to maturity and harvested for analysis.

The major part of the residues was identified as parent. The concentration of boscalid in lettuce leaf ranged from 55.6 – 94.1 % TRR, in radish leaf from 69.4 – 90.2 % TRR, in radish root from 52.6 – 92.8 % TRR and in wheat straw from 50.0 – 87.5 % TRR. In wheat grain the concentration of parent was lower (1.9 – 35.4 % TRR, $\leq$0.028 mg/kg).

Field trials on rotational crop studies weren't submitted prior to this Meeting and couldn't be used for evaluation.

2009: Field dissipation studies were submitted indicating that boscalid did not show a tendency to move into deeper layers of soil and was primarily detected in the top 10 cm soil layer during field dissipation trials (four different soils) of duration up to 12-18 months. Boscalid concentrations declined to half of their initial values in 28 days to 208 days. In all trials a DT_{90} could not be reached within one year after application to bare soil.

In a further study to investigate the soil dissipation of soil newly treated with boscalid and soil treated over several years a much slower dissipation of the active substance was observed in aged soil. DT_{50} values determined under laboratory conditions were estimated with 336 days for new soil and 746 days for aged soil.

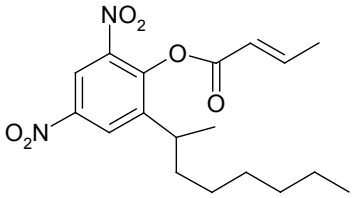
The slow dissipation in soil resulted in significant residues in follow up crops.

Points to note:

- Field trials on rotational crops were not submitted for the first evaluation (only when the JMPR asked for it). Missing data: check what could be the reason.
- Slow degradation, the parent compound is the major residue: it may lead to residues in rotational crops.

Example meptyldinocap – photolysis on leaf and soil surface

^{14}C-Meptyldinocap was applied in an EC formulation post-emergence to squash being at the beginning of blossoming stage. Within 5 minutes of spraying, twelve large squash leaves were covered with aluminium foil to provide dark controls. Two 30 cm square aluminium foil covered boards as well as two 30 cm square pieces of plexiglass (UV transparent) were immediately placed on the treated soil at the end of each row of the squash.



It was found that meptyldinocap photolyzed rapidly and extensively on leaf surfaces, while on soil the photolysis of meptyldinocap was much slower - and less extensive. A large number of photolysis products were formed, which were present in low concentrations.

Point to note: photolysis plays an important role in degradation of active ingredient on the plant surface

Example – abamectin formation of a 'new' photolysis product

Avermectin B_{1a} (^{14}C- and ^{3}H-labelled) was applied to celery in the field at rates from 0.5 to 5 times the proposed commercial rate. After the final application total radioactive residue declined with time, and a greater proportion became unextractable with acetone. Polar degradation products and Δ-8,9-isomer were formed. The use of both ^{14}C- and ^{3}H-labelled avermectin B_{1a} resulted in the same pattern of products, which was also similar to the pattern in the citrus and cotton studies, and photodegradation on glass. Photolytic decomposition products on glass are chromatographically similar to those which occur on the leaves of plants. Glass was chosen for the study because there would be no contamination from plant material.

The photodegradation of avermectin B_{1a} in sterilised aqueous suspension and on soil thin layers was investigated. Labelled avermectin B_{1a} (^{14}C or tritium) was used in the experiments. The half-life for degradation by sunlight in aqueous suspension was 3.5 and 12 hours in duplicate experimental tests, while on soil thin-layers it was 21 hours. The non-polar photo-product was tentatively identified as the Δ-8,9-isomer of avermectin B_{1a}.

The Δ-8,9-isomer was not formed in animal metabolism studies, only on plant surface.

Point to note

The photolysis product is not present in animal metabolism and it is not covered by animal toxicological studies performed with the parent compound. Its toxicity had to be studied separately.

PRESENTATION – ENVIRONMENTAL FATE

1. Chapter 4. Environmental Fate of Pesticides in Soil, Water and Water-Sediment Systems

2. Outline

The purpose of this chapter is to explain pesticide environmental fate studies and how to interpret their results for estimation of residue levels in food and feed commodities.

- Requirements for environmental fate studies
- Planning and conducting the environmental fate studies
- Examples

3. Requirements for environmental fate studies

- Studies are required on environmental fate relevant to the potential for uptake of residues by food and feed crops, depending on the use of pesticides
- Paddy rice presents a unique situation
- Studies are not evaluated, which relate to
  - Environmental toxicology
  - Restricted use (post harvest, seed treatment etc.)

4. Examples for study requirements

Type of study	Type of use and requirement (Y/No/Conditional)					
	Foliar	Soil	Plant of root, tuber, bulb	Seed dressing	Herbi-cide	Paddy rice
Physical and chemical properties	Conditional e.g., hydrolysis and photolysis.					
Aerobic degradation in soil	No	Y	Y	Y	Y	No
Soil photolysis	No	Y	Y	Y	Y	No
Anaerobic degradation in soil	No	No	No	No	No	No
Persistence in soil	No	No	No	No	No	No
Mobility/leaching in soil	No	No	No	No	No	No
Adsorption by soil types	No	No	No	No	No	No

5. Examples for study requirements

Type of study	Type of use and requirement (Y/no/Conditional)					
	Foliar	Soil	Plant, tuber, bulb	Seed dressing	Herbicide	Paddy rice
Hydrolysis rate and products	Hydrolysis in sterile aqueous buffers. Abiotic epimerization should be provided as appropriate (e.g., pyrethroids)					
Photolysis-plant surface	C	No	C	No	No	C
Photolysis-natural pond water	No	No	No	No	No	C
Rotational crops-confined	Y	Y	Y	Y	Y	No
Rotational crops-field	C	C	C	C	C	No
Field dissipation studies	C	C	C	C	C	No
Biodegradability) in water-sediment systems	No	No	No	No	No	C

6. Planning and conducting the environmental fate studies

- The studies are performed with radiolabelled active ingredients. The principles of selecting label position and the general requirements for identification and quantification of metabolites are the same as described in Chapter 3.

7. The use of environmental fate study results in the evaluation of residues in food commodities

The results of environmental fate studies assist in the interpretation and understanding of supervised residue trials.

For example, the environmental studies provide answers for such questions as:

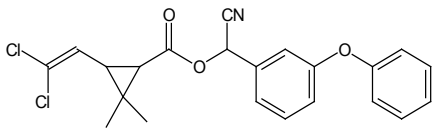
- if this compound is used at an early growth stage for potatoes, are residues still present in the soil when the potatoes are harvested?
- if this herbicide is used for weed control around fruit trees, will it persist in the soil and be taken up by the fruit trees?

8. The use of environmental fate study results in the evaluation of residues in food commodities

For example, the environmental studies provide answers for such questions as:

- are the soil metabolites the same as the plant metabolites?
- for a compound susceptible to photolysis, how does the persistence compare for a soil surface application and a soil incorporated application?
- for a compound susceptible to photolysis, can we expect higher residues from glass-house uses than from field uses?
- are new metabolites formed under the anaerobic sediment conditions during rice growing?

9. Example 1 – cypermethrin aerobic soil metabolism



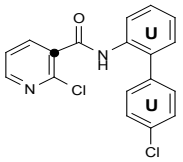
Under aerobic soil degradation at 20 °C, the half-lives of cypermethrin were in the range of 6 to 24 days. The trans isomers disappeared more quickly than the cis.

- The cis-trans ratio of the test material was 40:60. After 90 or 120 days of exposure, the cis:trans ratio of the remaining cypermethrin was in the range 60:40 to 73:27.
- Percentage mineralization (conversion to CO_2) after 90 or 120 days was in the range 37-70 %.

Points to note

- the isomer composition of the residue changed during soil metabolism
- percentage mineralization is a measure of the disappearance of the total residue.

10. Example 2 – boscalid residue in rotational crops



2006: Lettuce, radish and wheat were sown into [^{14}C]-boscalid treated soil at intervals of 30, 120, 270 and 365 days after treatment and were grown to maturity. Parent boscalid was 52.6- 90.25 % of plant tissue TRR.

Field trials on rotational crop studies were not submitted prior to the Meeting

In a field dissipation study, boscalid concentrations declined to half of their initial values in 28 days to 208 days. In all trials a DT_{90} could not be reached within one year after application to bare soil.

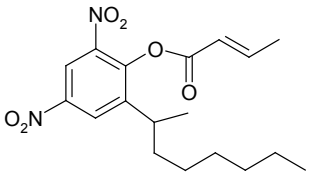
Soil dissipation of the active substance was much slower in aged soil. DT_{50} values determined under laboratory conditions were estimated to be 336 days for new soil and 746 days for aged soil.

The slow dissipation in soil resulted in significant residues in follow up crops.

Points to note:

- Field trials on rotational crops were not submitted for the first evaluation (only when the JMPR asked for it). Missing data: check what could be the reason.
- Slow degradation, the parent compound is the major residue: it may lead to residues in rotational crops.

11. Example 3: meptyldinocap – photolysis on leaf and soil surface



^{14}C-Meptyldinocap was applied in an EC formulation post-emergence to squash. Twelve large squash leaves were covered with aluminium foil to provide dark controls. Two 30 cm square aluminium foil covered boards and two 30 cm square pieces of U.V. transparent plexiglass were immediately placed on the treated soil.

It was found that meptyldinocap photolyzed rapidly and extensively on leaf surfaces, while on soil the photolysis of meptyldinocap was much slower - and less extensive. A large number of photolysis products were formed, which were present in low concentrations.

Point to note:

- Photolysis plays an important role in degradation of active ingredient on plant surface.

12. Example 4 – abamectin formation of a 'new' photolysis product

Avermectin B_{1a} (^{14}C- and ^{3}H-labelled) was applied to celery in the field.

Polar degradation products and Δ-8,9-isomer were formed. The use of both ^{14}C- and ^{3}H-labelled avermectin B_{1a} resulted in the same pattern of products, which was also similar to the pattern in the citrus and cotton studies, and photodegradation on glass.

The half-lives of avermectin B_{1a} (^{14}C or tritium) in sterilised aqueous suspension and on soil thin layers under sunlight were 3.5 – 12 and 21 hours, respectively. The non-polar photo-product was identified as the Δ-8,9-isomer of avermectin B_{1a}.

Point to note:

The photolysis product is not present in animal metabolism and therefore it is not covered by animal toxicological studies performed with the parent compound. Its toxicity had to be studied separately.

Abbreviations and acronyms in this chapter

DT_{50}	dissipation time 50 %. Time required for one-half the initial quantity or concentration of a pesticide to dissipate from a system. (IUPAC definition).
DT_{90}	dissipation time 90 %
EC	emulsifiable concentrate
FAO	Food and Agriculture Organization
JMPR	Joint Meeting on Pesticide Residues
TRR	total radioactive residue
UV	ultraviolet

Chapter 5. Sampling, Sample Preparation and Analysis, Efficiency of Extraction, Stability of Residues

Sampling
Sample preparation and processing
Stability of residues during sample processing and deep-frozen storage
Analytical methods
Extraction efficiency of residue analytical methods
Selectivity of analytical methods
Presentation – sampling and analysis

The purpose of this chapter is to explain the sampling, storage and analysis of food commodities for pesticide residues, the uncertainties associated with the processes of sampling, storage of samples, and analysis, and the necessity of valid data on these processes for the interpretation of all pesticide residue studies.

Relevant sections of JMPR Manual

Sampling

The sampling method and the selection of the objects of sampling depend on the objectives of the study. Utmost attention should be given to the selection of sampling methods and handling (packing, labelling, shipping and storage) of samples.

Take samples from:

Crop metabolism studies:

- inedible peel (e.g. oranges, melons, and bananas) and pulp
- crops consumed at an immature stage, (baby corn or leafy salads) and matured crops
- if more than one use pattern is involved, extra samples need to be taken to reflect, for example, the different PHIs.

Rotational crop studies:

- appropriate plant parts of raw agricultural commodities (RAC) for human food and livestock feed;
- immature and mature crops if harvested as part of normal agricultural practices (forage, hay, straw and grain for cereal crops)

Livestock metabolism studies

- excreta, milk[9] and eggs twice daily (if applicable).
- muscle (loin and flank muscles in ruminant and leg and breast muscle in poultry),
- liver, kidney (ruminants only), and fat (renal, omental and subcutaneous).

[9] For milk the fat fraction should be separated from the aqueous portion by mechanical means and the TRR in each fraction quantified.

Supervised field trials

- from the whole RAC *as it moves in commerce*;
- different plant parts separately: e.g. corn grain (seed), fodder (stover), and forage;

Field surveys and monitoring programmes

- Mature or ready marketed crops (Follow Codex sampling procedure!)

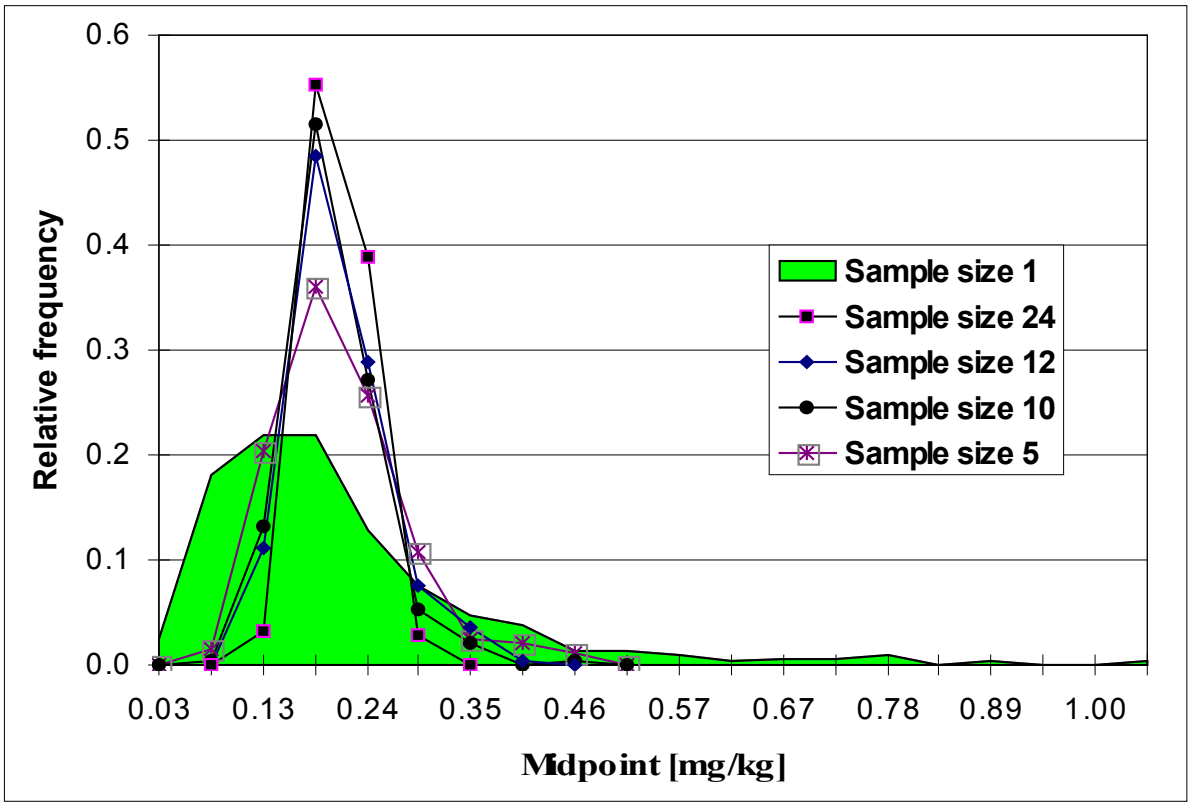
Points to note:

The method of sampling, handling, shipping and storage condition of samples should be described in detail in all studies.

In the case of supervised trials, field surveys and monitoring programmes the information provided should also include the method for selecting the timing of collection of primary samples (sample increments), the number of primary samples in the composite sample and the total weight of the composite sample.

The sampled materials should be prepared (stripped, trimmed or washed) to represent the prevailing commercial practice. The large crops should be packed and shipped to the laboratory intact, and they cannot be cut at the sampling site to reduce the mass of sample.

The sampling uncertainty depends on the variability of residues in/on treated crops and the number of crop units, single increments making up the composite sample.



Distribution of chlorpyrifos-methyl residues in apple samples.

The figure indicates the relationship between the distribution of residues in crop units and the average residues in composite samples. Note the shape of relative frequency curves for different sample sizes.

The minimum sample size to be taken in supervised field trials is given in Appendix 5 of the FAO Manual.

When results are evaluated check:

- sample size and mass in case of supervised field trials and monitoring programmes;
- sample preparation procedures before shipping, shipping conditions and storage time of samples before analysis. (see section on storage stability tests).

Sample preparation and processing

The *sample preparation* procedure is used, if required, to convert the laboratory sample into the analytical sample, by removal of parts (soil, stones, bones, etc.) not to be analysed.

The laboratory samples should be prepared for analysis following the instructions of the Codex Standard on Portion of commodity to which MRLs apply and which is analysed, (FAO Manual Appendix VI)

The *sample processing* includes procedure(s) (e.g. cutting, grinding, mixing) used to make the analytical sample acceptably homogeneous with respect to the analyte distribution, prior to removal of the analytical portion. It must be designed to avoid inducing changes in the concentration of the analyte. See section on stability of residues.

Stability of residues during sample processing and deep-frozen storage

Usually the samples derived from supervised trials are analysed after a long storage period. Therefore the assessment of reliability of residue data obtained requires information on the stability of residues during storage.

Storage stability studies should be designed in such a way that the stability of residues in the stored samples can be definitely determined. *If more than 30% of the residue is lost during storage* before analysis, residues from studies involving similar storage periods may not be valid.

When the analytical method determines a ”total residue”, storage stability studies should include not only the total residue, but also separate analyses of all compounds which may be included in the residue definitions.

The storage stability studies may be performed with representative commodities:

- commodities with high water content;
- commodities with high acid content;
- commodities with high oil content;
- commodities with high protein content;
- commodities with high starch content.

If residues are shown to be stable in all commodities studied, a study on one commodity from each of the five commodity categories is acceptable. In such cases, residues in all other plant commodities would be assumed to be stable for the same duration of time under the same storage conditions.

If MRLs are sought in just one of the five commodity categories, the stability of the test substance in 2–3 diverse commodities within the desired category should be tested.

If supervised trial samples are always analysed within 30 days of their storage in frozen conditions, applicants can omit conducting a freezer storage stability study provided justification is given e.g., basic physical chemical properties data show residues are not volatile or labile.

Some pesticides may rapidly decompose during blending, chopping or grinding of analytical samples. It is indicated by rapid decline of residues applied on the surface of sample materials before processing compared to recovery studies performed with spiking homogenised test portions. Performing the homogenization of the sample materials in the presence of dry ice generally reduces the decomposition of analytes during sample processing.

The results of a study shown below indicates that after one week only 67% of the added residue could be recovered (residue remained), while the recoveries with freshly spiked samples on days 0 and 7 were much higher (procedural recovery). The results suggest that the residues might have decomposed during the initial processing. In such cases, the analyses of test portions at day 1 would help to clarify the cause of decomposition.

Stability of bifenazate residues in lychee following frozen storage at -19.2 to -27ºC

Fortification (mg/kg)	Storage interval (months)	Procedural recovery[1] (%)	Residues in stored fortified samples (mg/kg)[2]	Average uncorrected residues remained (%)	Residues remained [3]
0.10	0	112	0.105, 0.110, 0.115	112	-
	0.25	101	0.061, 0.067, 0.071	67.2	67
	1	76.6	0.041, 0.045, 0.049	45.0	58.7
	2	88.9	0.069, 0.069, 0.218	68.8	77.4
	5	68.6	0.041, 0.042, 0.047	43.9	64.0
	8	54.3	0.021, 0.028, 0.061	37.0	68.2
	10	79.9	0.047, 0.052, 0.060	54.6	68.4

[1] Average recovery obtained from two freshly spike untreated test portions.
[2] As measured
[3] Adjusted for procedural recovery

The following points are to be noted during evaluation of a freezer storage study:

- design of the study - (intended sampling intervals, replication, number of procedural recovery tests)
- storage vessels (size, material, sealed)
- nature of the samples being tested (commodity, unchopped, chopped or homogenised)
- nature of the residue (single compound or mixed)
- incurred or spiked residue (spiking levels)
- procedural recoveries and variability of procedural recoveries
- temperatures of storage (intended and actual record of temperature).

Procedural recoveries (samples spiked and analysed at the time when a stored sample is analysed) should be used to decide on the validity of the batch of analyses. The analytical results for the stored sample should not be adjusted for the procedural recoveries.

Analytical methods

Analytical methods are used to generate the data for estimating dietary exposure, to establish Maximum Residue Limits (MRLs), and to determine processing factors. Analytical methods are also used in enforcement of any MRLs that may be established.

It is important to note that the methods should be able to determine all analytes included in the residue definition for the particular pesticide. The residue definition used for dietary risk assessment purposes may differ from that used for MRL enforcement purposes,

thereby requiring different analytical methods. In the event one analytical method cannot cover all compounds included in a particular residue definition, more than one method may be necessary.

In supervised trials the major residue components should be determined individually as far as technically possible. The use of non-specific methods is generally discouraged.

For enforcement of MRLs the laboratories prefer multi-residue methods, which could include a large number of analytes, as the laboratories generally do not have sufficient capacity to apply individual methods for all compounds possibly present.

The availability of appropriate methods for monitoring purposes should be considered. The method(s) should:

- have the ability to determine all of the likely analytes that may be included in the residue definition (both for risk assessment and enforcement) in the presence of the sample matrix;
- distinguish between individual isomers/analogues when necessary for the conduct of dietary risk assessments;
- be sufficiently selective so that interfering substances never exceed 30% of the limit of quantification (LOQ);
- demonstrate acceptable recovery and repeatability;
- cover all crops and feed items being treated. If significant residues occur, cover processing fractions and drinking water;
- cover all edible animal commodities if animals are likely to consume treated crops.

Enforcement methods should be suitable, where technically possible, to quantify residues at or below 0.01 mg/kg.

The methods used in various studies should be validated to demonstrate that they are fit for the purpose of the study. During the analyses of the samples the performance of the methods should be verified with appropriate quality control tests (e.g. checking concurrent recoveries and the LOQ values).

Details of method validation procedures, including testing the efficiency of extraction and confirmation, the criteria for acceptable performance parameters and format for reporting the method are given for pre- and post-registration studies in the OECD Guidance document on analytical methods[10] and in the Good Laboratory Practice elaborated by the CCPR[11]. There are additional guidelines for method validation which may be applied by the testing laboratories.

The performance characteristic of the analytical methods should include as a minimum:

- recovery values at the reported LOQ and covering the range of residues in treated commodities (average recovery, number of tests and standard deviation of individual recoveries);
- limit of quantification and limit of detection;
- reproducibility of the analytical process;
- efficiency of extraction of methods recommended for enforcement of MRLs.

[10] OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Pesticides Number 39, Series on Testing And Assessment Number 72, 2007

[11] Codex Secretariat (2003) Revised Guidelines on Good Laboratory Practice in Residue Analysis CAC/GL 40 1993, Rev.1-2003, http://www.codexalimentarius.net/download/standards/378/cxg_040e.pdf

The recovery studies may be conducted with representative commodities listed in the Codex Good Laboratory Practice Standard or OECD GL.

Extraction efficiency of residue analytical methods

Extraction efficiency is regarded as key for the development of methods, and data should be provided for the solvents and conditions (temperature, pH, time) typically used. Extraction efficiency may significantly influence the trueness of the analytical results as poor extraction efficiency can be a major source of bias in a method. However, it cannot be checked by traditional recovery studies carried out with samples fortified shortly before analysis.

The rigorous validation of the efficient extraction of all residues included in the residue definition can only be performed with samples that have the analyte(s) incurred through the route by which they would normally reach the sample. This is generally the case in metabolism studies, where the efficiency of extraction can be determined by means of radio-labelled analytes.

Ideally, the commodities of interest from the metabolism and rotational crop studies should be retained for determining the extraction efficiency of the regulatory methods and methods used in supervised field trials and rotational crop studies. The retained commodities should be subjected to the extraction procedures of the analytical methods of interest so the extraction efficiency can be readily determined using radiochemical procedures (combustion analysis, liquid scintillation counting and chromatographic analyses using a radio detector).

Alternatively, comparative extraction efficiency studies including the frequently used extraction solvents, such as acetone + water, ethyl acetate, and acetonitrile, can be conducted on samples from metabolism studies for compounds expected to be included in the residue definition(s). Information should be provided on the efficiency of extraction with the solvents used in relevant regulatory methods.

Example: rounding of recovery data

During an independent laboratory validation of a residue analytical method for thiamethoxam in milk, analytical results were apparently rounded before percentage recoveries were calculated. Recoveries from milk at 0.005 and 0.02 mg/kg were all exactly 100 %.

The rounding would obscure small deviations, e.g. up to 10 % at 0.005 mg/kg, and generally have invalidated the study results.

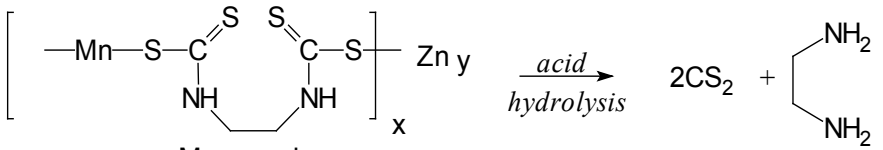
Rounding of data is generally inappropriate except after the final stage of a calculation!

Selectivity of analytical methods

The potential for interference in analytical methods should always be kept in mind.

Example – interference in dithiocarbamate residue analyses by endogenous compounds (JMPR 1993)

Mancozeb and other dithiocarbamate residues are analysed by methods that hydrolyse the dithiocarbamate to carbon disulphide for measurement.



Mancozeb

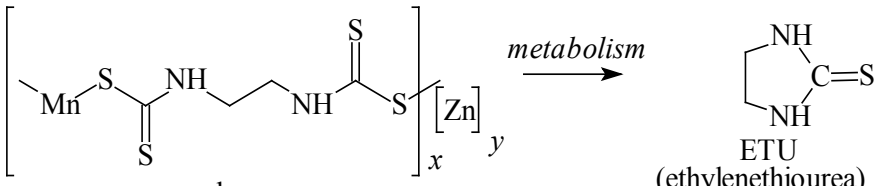
Some plant commodities. such as onions and broccoli contain sulphur compounds that produce carbon disulphide under the acid hydrolysis conditions used in the analytical method. Untreated onions produced CS_2 residues of <0.03 to 0.13 mg/kg, while untreated broccoli produced CS_2 residues from <0.01 to 0.79 mg/kg.

Point to note

- Caution is required in interpretation of dithiocarbamate residue data in situations where endogenous CS_2 might be produced.

Example – interference in ETU residue analyses by ethylenebisdithiocarbamate residues (JMPR 1993)

Ethylenethiourea (ETU) is a metabolite and breakdown product of ethylenebisdithiocarbamates such as mancozeb



mancozeb

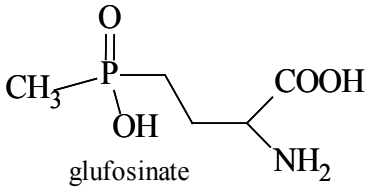
Analysis of low levels of ETU residues in the presence of much higher levels of ethylenebisdithiocarbamates is difficult because some may be converted to ETU during the analysis. Conversion rates of 0.22-8.5 % were reported. The time taken for critical steps should be minimised and other precautions are needed to reduce the conversion.

Point to note

- Additional validation is required in such situations – analysis in the presence of a potentially interfering compound.

Example – interference in analytical recovery testing by a transgenic crop (JMPR 1998)

Glufosinate is a herbicide with uses for weed control in transgenic crops designed for herbicide tolerance. Glufosinate is a racemic compound.



glufosinate

Analytical recoveries for glufosinate residues on transgenic soybean plant material were typically found to be low – around 50-60 %

Transgenic soybean plants convert L-glufosinate (the active isomer) to N-acetyl-glufosinate very rapidly. The low recoveries were caused by a rapid loss of half of the spiked material (the L-isomer) in the extraction bowl.

Point to note

- The low recoveries were not a fault of the analytical method, but an unforeseen loss of 50 % of the reference material at the spiking stage.

PRESENTATION – SAMPLING AND ANALYSIS

1. Chapter 5. Sampling, Sample Preparation and Analysis

2. The purpose of this chapter

To explain the sampling, storage and analysis of food commodities for pesticide residues, the uncertainties associated with the processes of sampling, storage of samples, and analysis and the necessity of valid data on these processes for the interpretation of all pesticide residue studies

3. Outline

- Sampling for various purposes
- Sample preparation and processing
- Stability of residues during sample processing and deep-frozen storage
- Analytical methods

4. Sampling

- Reliable results can only be obtained from samples taken according to the objectives of the study.
- Utmost attention should be given to the selection of sampling methods, handling (packing, labelling, shipping, storage) of samples.
- The study should be designed to assure the integrity of the whole chain of activities.
- The sampling method and the selection of the objects of sampling depend on the purpose of the study.

5. Crop metabolism studies

- In crop metabolism studies, samples of all raw agricultural commodities should be obtained for characterization or identification of residues.
- In commodities with inedible peel such as oranges, melons, and bananas, the distribution of the residue between peel and pulp should be determined.
- For crops that are sometimes consumed at an immature stage, such as baby corn or leafy salads, samples should also be taken of such commodities for analysis.

6. Crop metabolism studies

- Where mature inedible crop parts (e.g., apple leaves, potato foliage) are used to help identify residues, the edible parts must also be sampled and analysed for demonstration of the similarity of metabolic profiles.
- If more than one use pattern is anticipated, extra samples need to be taken to reflect, for example, the different intervals between treatment and sampling.

7. Rotational crop studies

- In rotational crop studies the selected representative rotated crops should be harvested and the appropriate raw agricultural commodities (RAC) for human food and livestock feed plant parts should be sampled.
- Samples should also be collected of selected crops at multiple intervals if both immature and mature crops are normally harvested in the course of usual agricultural practices.
- Samples harvested should include forage, hay, straw and grain for cereal crops; an immature and mature leafy vegetable sample and both the root or tuber and the aerial (leafy) portion of the root crop, even if the leafy portion is not a RAC of the actual root crop planted.

8. Rotational crop studies

- Data from the leafy portion of the root crop and the immature leafy vegetable are needed because the three crops in the study are being used as models to extrapolate to a wide range of food crops.
- In addition, due to the increase in the culinary use of immature greens, an immature leafy vegetable sample is needed. 'Immature leafy vegetable' is defined as the crop stage representing approximately 50 % of the normal time period for the plant to reach full maturity.
- Sampling of the soil is not required, but may be performed depending on the specific objectives of the study.

9. Livestock metabolism studies

- In livestock metabolism studies excreta, milk and eggs should be collected twice daily (if applicable).
- Tissues to be collected should include at least ***muscle*** (loin and flank muscles in ruminant and leg and breast muscle in poultry), ***liver*** (whole organ for the goat and poultry and representative parts of the different lobes of the liver if cattle or swine are used), ***kidney*** (ruminants only), and ***fat*** (renal, omental, subcutaneous).
- The total radioactive residues (TRR) should be quantified for all tissues, excreta, milk, and eggs.
- For ***milk*** the fat fraction should be separated from the aqueous portion by mechanical means and the TRR in each fraction quantified.

10. Sampling

- Supervised field trials
  - from the whole RAC *as it moves in commerce*;
  - different plant parts separately: e.g. corn grain (seed), fodder (stover), and forage;
- Field surveys and monitoring programmes
  - Mature or ready marketed crops
- Codex Alimentarius Commission: 'Recommended method of sampling for the determination of pesticide residues for compliance with MRLs ' should be used.

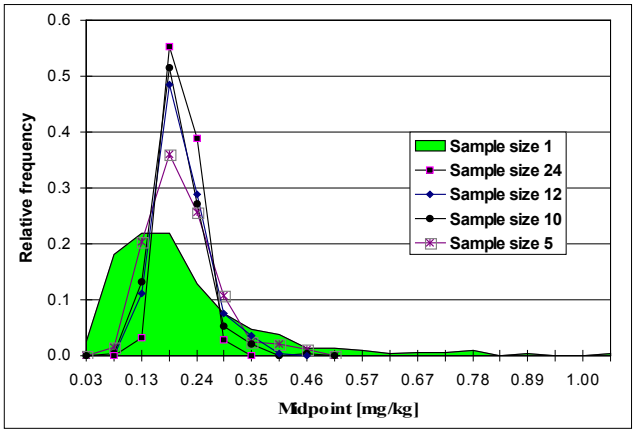
11. Supervised trials, selective field surveys and monitoring programmes

- In selective field surveys and monitoring programmes the Codex standard method of sampling for the determination of pesticide residues for compliance with MRLs should be used.
- The method of sampling and handling storage condition of samples should be described in detail in all types of studies.
- In the case of supervised trials, field surveys and monitoring programmes the information provided should also include the method for selecting the position of primary samples (sample increments), the number of primary samples in the composite sample and the total weight of composite sample.

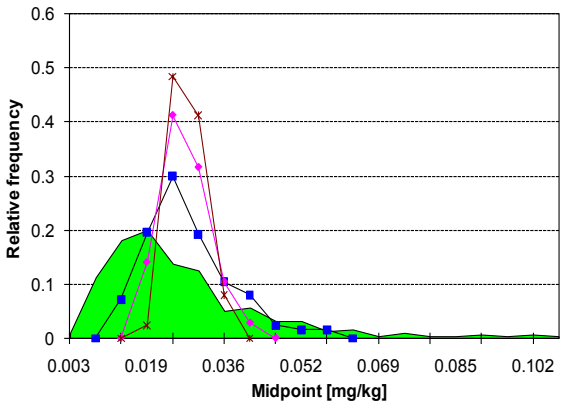
12. Distribution of residues on treated fields

- The pesticide deposit on the treated objects is uneven leading to largely varying residues in/on the crops (CV = 80-100%), and
- inevitably different average residues in composite samples (CV=20-30%) taken from the field.

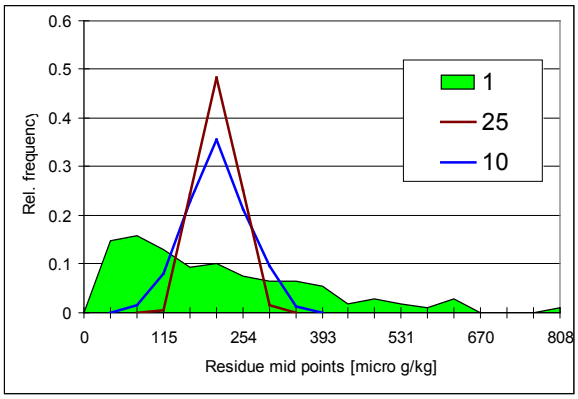
13. Relative frequency distribution of chlorpyrifos residues in apple samples at day 0



14. Relative frequency distribution of chlorpyrifos residues in apple samples at day 14



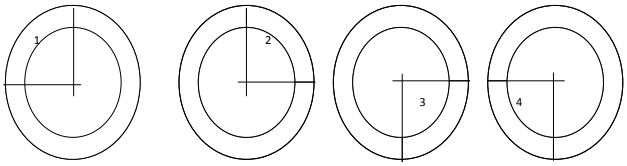
15. Relative frequency distribution of vinclozolin residues in kiwi



16. Typical sampling protocol for fruit trees

- A laboratory sample consists of 24 apples.
- One sample is taken from *each of* 4 adjacent trees representing the standard plot design with four trees according to supervised field trial protocols.

17. Sampling positions for composite sample



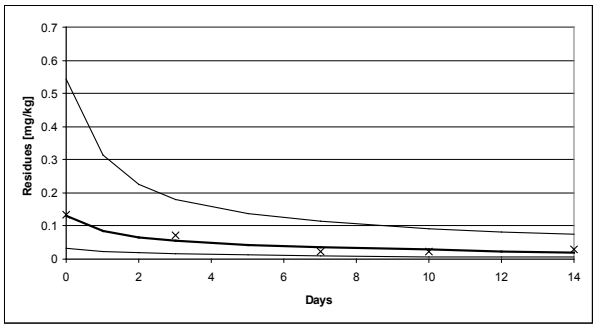
18.

Crop	Place	Sprayer	Plot size m^2	Sample
Mandarin	ES	knapsack	18-195	20-30 f
Orange	ID	knapsack	40	5 kg
Orange	ES	backpack,	68-195	12-16 f
Orange	USA	tractor	?	min 24 f
Papaya	CI	atomizer	36 trees	12 f
Peach	US	airblast	?	?
Peppers	ES	motor sprayer	20-74	1.1-3.4 kg
Peppers	CH	knapsack	6-12	12 f
Potato	UK	small plot	50-120	10-24 kg
Rice	JP	knapsack	24-52	2-2.6 kg
Tomato	ES	motor sprayer	10-120	12 f – 40 kg

19. Decline of residues based on average residues in composite samples

DAT	Sampling site							Ave. R	CV%
	1	2	3	4	5	6	7		
0	0.27	0.13	0.098	0.17	0.10	0.20	0.17	0.164	37
3	0.14	0.072	0.050	0.092	0.066	0.052	0.073	0.078	40
7	0.036	0.024	0.035	0.027	0.034	0.047	0.024	0.032	25
10	0.043	0.024	0.033	0.033	0.026	0.023	0.020	0.029	28
14	0.033	0.028	0.018	0.025	0.019	0.025	0.024	0.025	21

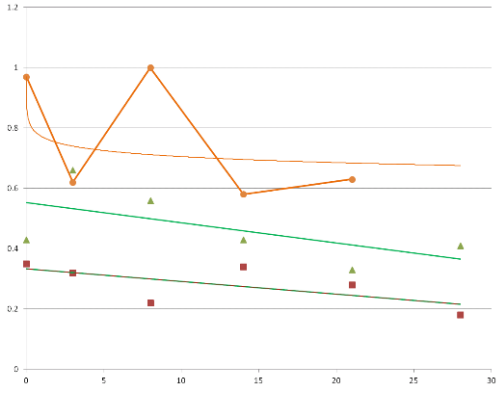
20. Decline of chlorpyrifos methyl residues in apple



21.

Days	Fluopyram (0.25 kg/ka) residues in grape					
0	0.72	0.35	0.43	0.51	0.59	0.97
3	0.61	0.32	0.66	0.3	0.49	0.62
8	0.7	0.22	0.56	0.18	0.6	1.0
14	0.65	0.34	0.43	0.27	0.43	0.58
21	0.63	0.28	0.33	0.22	0.44	0.63
28		0.18	0.41	0.22		

22.



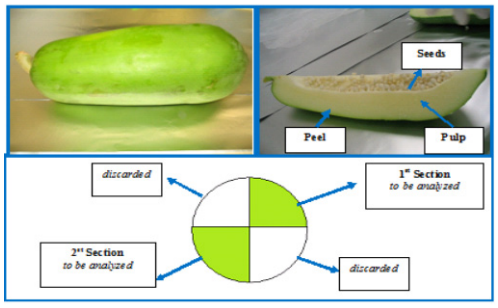
23. Points to note

- The proper selection of method of sampling, handling, shipping and storage condition of samples should be verified.
  - timing of collection of samples,
  - method for selecting primary samples (sample increments),
  - the number of primary samples in the composite sample and the total weight of the composite sample,
  - higher residue may be possible at longer PHI
  - sample preparation procedures before shipping, shipping conditions and storage time of samples before analysis. (see section on storage stability tests).

24. Sample preparation and processing

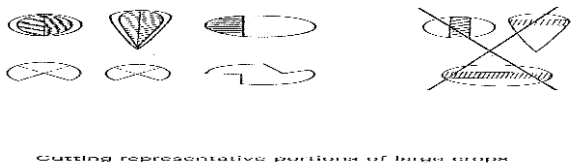
- The *sample preparation* procedure is used, if required, to convert the laboratory sample into the analytical sample, by removal of parts (soil, stones, bones, etc.) not to be analysed.
- The laboratory samples should be prepared for analysis following the instructions of the Codex Standard on Portion of commodity to which MRLs apply and which is analysed, (FAO Manual Appendix VI)
- The *sample processing* includes procedures (e.g. cutting, grinding, mixing) used to make the analytical sample acceptably homogeneous with respect to the analyte distribution, prior to removal of the analytical portion. It must be designed to avoid inducing changes in the concentration of the analyte.

25. Sample size reduction



26. Sample preparation and processing

PREPARATION, HANDLING AND STORAGE OF SAMPLES



Cutting representative portions of large crops

27. Sample processing

- The residue concentration may be a hundred fold different among individual crop units and the residues may also be unevenly distributed between the peel and pulp or within the crop. The particle size distribution in the so-called homogenised sample and the size of test portion will determine the variability of residues in the test portions.
- The smaller the particles are the more uniform the homogenised sample will be.
- ***Contradiction***: rigorous homogenisation may result in decomposition of analytes during sample processing

28. Factors affecting the variability of residues in test portions

According to the Gy's sampling model, the relative standard deviation of the measurand in the test portion (CV_{Sp}) can be calculated as:

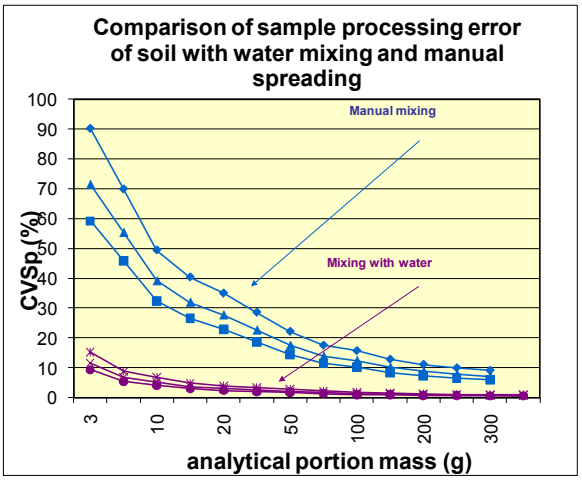
$$CV_{Sp} = Cd^3 \left(\frac{1}{M_{Tp}} - \frac{1}{M_{As}} \right)$$

C : the sampling constant depends on several factors,
d : the 95% upper limit of the particle size distribution,
M_{Tp} : the mass of test portion withdrawn from the homogenised matrix,
M_{As} : the total mass of the homogenised sample material.

29. The Ingamells' sampling constant, Ks

$$K_s = M_{Tp} CV_{Sp}^{\ 2}$$

30.



31. Testing stability of residues during sample processing and deep-frozen storage

- Usually the samples from supervised trials are analysed after a long storage period; therefore the stability of residues during storage must be demonstrated.
- The storage stability studies may be performed with representative commodities

32. Stability of residues during storage and sample processing

- Animal tissues, milk and eggs should be tested for residue storage stability when animal commodity MRLs are needed.
- In the case of studies involving crop commodities, the principles of extrapolation between commodities within specific commodity categories is recommended and the commodity categories are as follows:
  commodities with high water content;
  commodities with high acid content;
  commodities with high oil content;
  commodities with high protein content; and
  commodities with high starch content.

33. Stability of residues during storage and sample processing

- If residues are shown to be stable in all commodities studied, a study on one commodity from each of the five commodity categories is acceptable.
- If uses are sought in just one of the five commodity categories, the stability of the test substance in 2-3 diverse commodities within the desired category should be tested.
- Where sample extracts have been stored for more than 24 hours prior to analysis, the stability of residues should be demonstrated with recovery studies performed under similar conditions.

34. Testing stability of residues

- When the analytical method determines a ”total residue”, storage stability studies should include not only the total residue, but also separate analyses of all compounds which may be included in the residue definitions.
- If supervised trial samples are always analysed within 30 days of their storage in frozen conditions, applicants can omit conducting a freezer storage stability study provided justification is given e.g., basic physical chemical properties data show residues are not volatile or labile.

35. Stability of residues during storage and sample processing

- Acceptable recoveries may be obtained even if a substantial portion of the test material ‘disappeared’ during homogenization.
- Systematic studies, performed with fruits and vegetables applying test substance mixtures containing a stabile and several other compounds with unknown stability, revealed that the decomposition of residues can be substantially reduced or eliminated under cryogenic processing of deep-frozen sample materials

36. Decomposition of residues during sample processing

- Some pesticides may rapidly decompose during blending, chopping or grinding of analytical samples.
- It is indicated by rapid decline of residues applied on the surface of sample materials before processing compared to recovery studies performed with spiking homogenised test portions.

- Performing the homogenization of the sample materials in the presence of dry ice generally reduces the decomposition of analytes during sample processing.
- Known cases: captan, folpet, chlorothalonil, bifenazate, etoxazole, etc.

37. Presentation of storage stability results

Fortification (mg/kg)	Storage interval (months)	Procedural recovery (%)	Residues in stored samples (mg/kg)	Average uncorrected residues remained (%)	*Residues remained* (adjusted for proc recov)
0.10	0	112	0.105, 0.110, 0.115	112	-
	0.25	101	0.061, 0.067, 0.071	67.2	67
	1	76.6	0.041, 0.045, 0.049	45.0	58.7
	2	88.9	0.069, 0.069, 0.218	68.8	77.4
	5	68.6	0.041, 0.042, 0.047	43.9	64.0
	8	54.3	0.021, 0.028, 0.061	37.0	68.2
	10	79.9	0.047, 0.052, 0.060	54.6	68.4

38. Points to note

- design of the study - (intended sampling intervals, replication, number of procedural recovery tests)
- storage vessels (size, material, sealed)
- nature of the samples being tested (commodity, unchopped, chopped or homogenised)
- nature of the residue (single compound or mixed)
- incurred or spiked residue (spiking levels)
- procedural recoveries and variability of procedural recoveries
- temperatures of storage (intended and actual record of temperature).

Procedural recoveries (samples spiked and analysed at the time when a stored sample is analysed) should be used to decide on the validity of the batch of analyses. The analytical results for the stored sample should not be adjusted for the procedural recoveries.

39. Analytical methods

- The methods should be able to determine all analytes included in the residue definition (enforcement, risk assessment).
- More than one method may be necessary.
- In supervised trials the major residue components should be determined individually as far as technically possible. The use of non-specific methods is generally discouraged.

40. Analytical methods

Information should be submitted to the JMPR not only on the analytical principles used in the supervised trials and experiments but also the whole analytical procedure in detail including a precise description of the portion of sample analysed, stability of residues during sample processing, tests to prove the efficiency of extraction, recoveries at various levels, limits of quantification, limits of detection, chromatograms of samples and controls and a description of how the limit of quantification and detection were derived.

41. The methods should:

- have the ability to determine all of the likely analytes that may be included in the residue definition (both for risk assessment and enforcement) in the presence of the sample matrix;
- distinguish between individual isomers or analogues when necessary for the conduct of dietary risk assessments;
- be sufficiently selective so that interfering substances never exceed 30% of the limit of quantification (LOQ);
- demonstrate acceptable recovery and repeatability;
- cover all crops and feed items being treated. If significant residues occur, cover processing fractions and drinking water;
- cover all edible animal commodities if animals are likely to consume treated crops;
- Appropriate LOQ , for enforcement ≤ 0.01 mg/kg.

42. Different requirements of enforcement and risk assessment

- *Enforcement analytical methods* are applied for large numbers of samples: they should be simple and cover a wide range a residues and commodities (multi residue methods)
- Residue analysis for *risk assessment* is normally performed only for cases of dietary concern. The methods should determine all components of toxicological significance.

43. Consequences of complex residue definition for enforcement

- Few samples are analysed for ‘difficult residues’;
- A single residue is compared to a high MRL including several residue components;
- Approximate adjustments may be forced;
- Some laboratories within EU and many laboratories in non-EU countries cannot correctly verify compliance with EU MRLs.
- Consumers may be exposed to higher residues than would be necessary.

44. Validation of methods

- The performance characteristic of the analytical methods should include as a minimum:
  - recovery values at the reported LOQ and covering the range of residues in treated commodities (average recovery, number of tests and standard deviation of individual recoveries);

- limit of quantification and limit of detection;
- reproducibility of the analytical process;
- efficiency of extraction of methods recommended for enforcement of MRLs.

- The recovery studies may be conducted with representative commodities listed in the Codex Good Laboratory Practice Standard or OECD GL.

45. Extraction efficiency of residue analytical methods

- Extraction efficiency may significantly influence the trueness of the analytical results as poor extraction efficiency can be a major source of bias in a method. However, it cannot be checked by traditional recovery studies carried out with samples fortified shortly before analysis.
- Extraction efficiency is regarded as key for the development of methods, and data should be provided for the solvents and conditions (temperature, pH, time) typically used.
- The rigorous validation of the efficient extraction of all residues included in the residue definition can only be performed with samples that have incurred residues.

46. Testing efficiency of extraction

- Ideally, the commodities of interest from the metabolism and confined rotational crop studies should be retained for determining the extraction efficiency of the regulatory methods and methods used in supervised field trials and rotational crop field studies.
- Alternatively, comparative extraction efficiency studies including the frequently used extraction solvents, such as acetone + water, ethyl acetate, and acetonitrile, can be conducted on samples from metabolism studies for compounds expected to be included in the residue definitions.
- Information should be provided on the efficiency of extraction with the solvents used in relevant regulatory methods.

47. Extraction efficiency of residue analytical methods

- In cases where samples from metabolism studies are no longer available for development of a new analytical method, it is possible to "bridge" between two solvent systems.
- Incurred residues obtained, e.g. during supervised field trials, might be extracted using as a first step the solvent system under the conditions applied during the metabolism studies and then, in a second step, by using the solvent under consideration.
- Information on extractability can be obtained by direct comparison of the analytical results.

48.

Substrate	Thiamethoxam, mg/kg		Extraction efficiency %
	Metabolism. analysis	Method AG-675 analysis	

Pear	0.20	0.15-0.18	75-90
Maize fodder	0.047	0.02-0.03	43-64
Cucumber	0.1	0.04-0.05	40-50
Cucumber	0.044	0.02-0.04	45-91
Goat meat	1.0	0.56-0.79	56-79
Goat milk	0.37	0.06-0.09	16-24

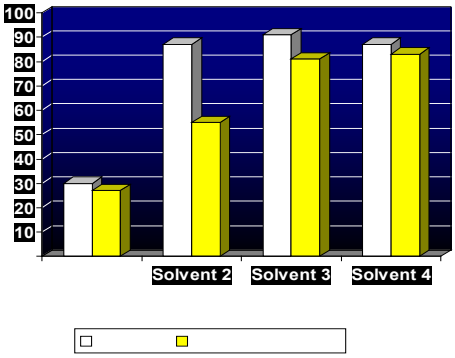
49. "Kovacs Series" of Extraction Solvents - M. H. Kovacs, Jr., Residue Reviews, 97 (1986) 1-17.

1. Organic solvents
2. Water/polar organic solvent mixtures
   - May need to use enzyme or hydrolysis treatments to release conjugates
3. Polar organic solvent/aqueous acidic or alkaline solutions – ambient
4. Polar organic solvent/aqueous acidic or alkaline solutions – boiling

Need to keep going until all residues of concern are recovered or destroyed by the extraction conditions!

Slide provided by Chuck Powley of DuPont

50. Extractability of Incurred Residues



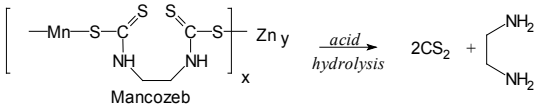
Slide provided by Chuck Powley of DuPont

51. Rounding of values

- During an independent laboratory validation of a residue analytical method for thiamethoxam in milk, analytical results were apparently rounded before percentage recoveries were calculated. Recoveries from milk at 0.005 and 0.02 mg/kg were all exactly 100 %.
- The rounding would obscure small deviations, e.g. up to 10 % at 0.005 mg/kg, and generally have invalidated the study results.
- *Rounding of data is generally inappropriate except after the final stage of a calculation!*

52. Example: Selectivity of analytical methods

Mancozeb and other dithiocarbamate residues are analysed by methods that hydrolyse the dithiocarbamate to carbon disulphide for measurement.



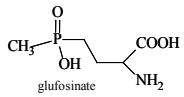
Mancozeb

Some plant commodities. such as onions and broccoli contain sulphur compounds that produce carbon disulphide under the acid hydrolysis conditions used in the analytical method. Untreated onions produced CS_2 residues of <0.03 to 0.13 mg/kg, while untreated broccoli produced CS_2 residues from <0.01 to 0.79 mg/kg.

Point to note: Additional validation is required in such situations – analysis in the presence of a potentially interfering compound.

53. Example – interference in analytical recovery testing by a transgenic crop

Glufosinate is a herbicide with uses for weed control in transgenic crops designed for herbicide tolerance.



glufosinate

Analytical recoveries for glufosinate residues on transgenic soybean plant material were typically found to be low – around 50-60 %.

Transgenic soybean plants convert L-glufosinate (the active isomer) to N-acetyl-glufosinate very rapidly. The low recoveries were caused by a rapid loss of half of the spiked material (the L-isomer) in the extraction bowl.

Point to note: The low recoveries were not a fault of the analytical method, but an unforseen loss of 50 % of the reference material at the spiking stage.

Abbreviations and acronyms in this chapter

CCPR	Codex Committee on Pesticide Residues
ETU	ethylenethiourea
FAO	Food and Agriculture Organization
JMPR	Joint Meeting on Pesticide Residues
LOQ	limit of quantification
MRL	maximum residue limit
OECD	Organization for Economic Co-operation and Development
PHI	pre-harvest interval
RAC	raw agricultural commodity

Chapter 6. Definition of Residues.

Principles of residue definition for enforcement and dietary risk assessment
Examples for various cases
Deciding on fat solubility of residues
Transgenic and non-transgenic crops may metabolize the pesticide differently. Different residue definition proposed by JMPR and JECFA - abamectin
Example for validation of analytical method for determining conjugated metabolites

The purpose of this chapter is to review the general principles of proposing residue definition and to examine the metabolic data and the analytical requirements to be considered, which must also meet the practical requirements of enforcement laboratories and dietary exposure estimations.

Relevant sections of JMPR Manual

- Definition of residues 5.12

Principles of residue definition for enforcement and dietary risk assessment

A **pesticide residue** is any specified substance in food, agricultural commodities, or animal feed resulting from the use of a pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance (Codex Procedural Manual 18th.ed).

Explanatory note: The term “pesticide residue” includes residues from unknown sources, i.e., background residues, as well as those from known uses of the chemical in question.

The **maximum residue limit** MRL is the maximum concentration of a pesticide residue (expressed as mg/kg), recommended by the Codex Alimentarius Commission to be legally permitted in or on food commodities and animal feeds. MRLs are based on GAP data and foods derived from commodities that comply with the respective MRLs are intended to be toxicologically acceptable. (Codex Alimentarius Vol. 2A)

Although metabolites, degradation products and impurities are included in the definition of pesticide residues, this does not necessarily mean that metabolites or degradation products should always be included in the residue definition for enforcement (MRLs) purposes or for estimation of dietary intake (STMR, HR).

The WHO Panel considers and indicates in its evaluations which metabolites are of toxicological significance and should be included in the dietary risk assessment.

The basic requirements for the definition of residues are:

The residue definition **for MRL purposes** should be:

- based on a single compound whenever possible, which is preferably recoverable with multi-residue methods;
- most suitable for *monitoring compliance* with GAP, which enables unambiguous identification of source of residues; therefore including common moiety of pesticides should be avoided;
- the same for all commodities, if possible.

The residue definition ***for dietary intake estimations and risk assessment*** should include compounds of toxicological interest where present in significant concentrations.

The following factors should be considered when proposing or revising a residue definition:

- The composition of the residues found in animal and plant metabolism studies.
- The toxicological properties of metabolites and degradation products (for risk assessment).
- The nature of the residues determined in supervised residue trials.
- The fat-solubility.
- The practicality of regulatory analytical methods.
- Whether metabolites or analytes common to other pesticides are formed.
- Whether a metabolite or a component of one pesticide is registered for use as another pesticide.
- The definitions of residues already established by national governments and long-established and customarily accepted definitions.
- JECFA marker residue definitions already established for compounds that may leave pesticide residues in animal commodities.

The best time for the reconsideration of an existing residue definition is during a periodic review.

Explanatory notes:

The definition of residues for enforcement purposes serves its purpose best if it enables the analyses of large numbers of samples at a reasonable cost with instruments and analytical standards generally available in regulatory laboratories.

Complicated residue definitions typically require single-residue methods, thus lead to lower number of monitoring and enforcement analyses (*vs.* residues that can be analysed using multi-residue methods), as clearly indicated by the results of EU or US monitoring programmes.

Residue methods for incurred conjugated metabolites cannot be validated without labelled compound and having access to specialised laboratories. Analytical standards for them are not readily available. Summary of an extraction efficiency study is given later in this chapter.

The targeted expression of residues with a single compound does not reduce the data requirement. Complete information on the total residue composition and the relative ratio of residue components is needed to determine whether a single compound can be used, and this information is also needed for risk assessment purposes.

In order to enable selection of the most appropriate residue definition, the levels of relevant metabolites should be measured and reported separately from those of the parent compound, but in a way which allows their subsequent combination.

As far as possible the same definition of the residue should apply to all commodities, although there are exceptions.

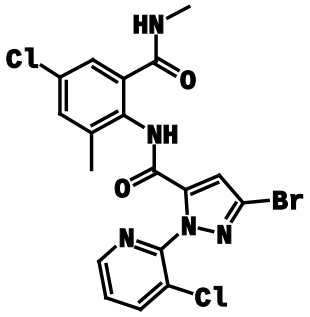
For example, if the major residue in animal commodities is a specific animal metabolite, a definition which includes that metabolite is needed for regulatory monitoring. However, the animal metabolite is not required in the residue definition for crop commodities if it is not

found in the crops. Separate definitions would then be proposed for commodities of plant and animal origin.

The requirements of checking compliance with MRL and exposure assessment of the consumers are sometimes not compatible and, as a compromise, various definitions of residues are possible.

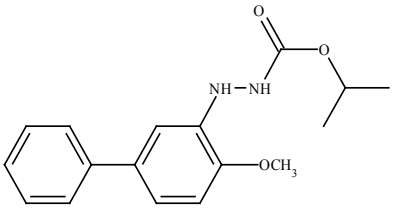
Examples for various cases

Residue definition is the same for plant and animal commodities and includes the parent compound alone: Chlorantraniliprole



Definition of the residue (for compliance with MRL and for estimation of dietary intake) for plants and animal commodities: chlorantraniliprole

Residue definition is the same for plant and animal commodities and includes a metabolite: Bifenazate:

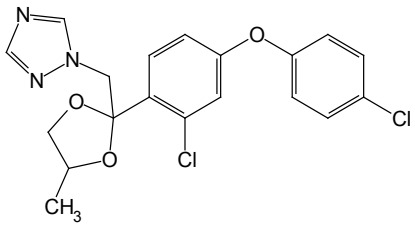


Definition of residue for enforcement and dietary intake calculations as Sum of bifenazate and bifenazate-diazene (diazenecarboxylic acid, 2-(4-methoxy-[1,1'-biphenyl-3-yl] 1-methylethyl ester), expressed as bifenazate;

The residue is fat soluble.

Different residue definition for plant and animal commodities

Different residue definition for plants and animal tissues - Difenoconazole:



Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant commodities: difenoconazole.

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for animal commodities: sum of difenoconazole and 1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1,2,4-triazol)-1-yl-ethanol), expressed as difenoconazole.
The residue is fat soluble.

Expression of residues as measured

Total dithiocarbamates, determined as CS_2 evolved during acid digestion and expressed as mg CS_2 mg/kg.

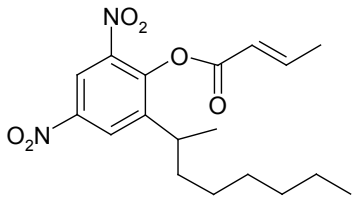
Point to note

The definition of a residue should not normally depend on a particular method of analysis, which means that the definition should not contain the words "determined as". However, in the case of dithiocarbamates it is necessary to describe the residue as ".... determined and expressed as ...." to produce a practical definition for residues.

If compound specific methods will be available (e.g. propineb can now be distinguished from ethylene-bis-dithiocarbamates) the residue definition will be changed during the next periodic review, provided that appropriate residue data will be available.

Expression of residues for single isomers versus mixture of isomers

Meptyldinocap – dinocap



The meptyldinocap is the single isomer of the existing active substance dinocap, which is a mixture of isomers:

Isomers	Meptyldinocap	Dinocap
Meptyldinocap, 2,4-dinitro-6-(1-methylheptyl)phenyl crotonate	98.5 %	22 %
2,6-dinitro-4-(1-methylheptyl)phenyl crotonate	0 %	11 %
2,4-dinitro-6-(1-ethylhexyl)phenyl crotonate[1]	1.5 %	22 %
2,6-dinitro-4-(1-ethylhexyl)phenyl crotonate	0 %	11 %
2,4-dinitro-6-(1-propylpentyl)phenyl crotonate	0 %	22 %
2,6-dinitro-4-(1-propylpentyl)phenyl crotonate	0 %	11 %

The ADI for dinocap is 0.01 mg/kg bw and ARfD is 0.013 mg/kg bw. For meptyldinocap the ADI is 0.02 mg/kg bw and no ARfD is necessary.

The analytical method, which was used in the residue trials, determined meptyldinocap residues as a sum of the parent and corresponding phenol. Multi residue methods based on gas chromatographic and HPLC-MS/MS detection are available for the determination of meptyldinocap alone and have been validated for four representative commodities. Residues deriving from the use of dinocap could be identified based on the presence of 2,6-DNOP isomers (the phenol derivatives) provided that the chromatographic system used has sufficient resolution.

The current residue definition for dinocap is dinocap, *sum of all isomers*.

As meptyldinocap is one isomer of dinocap, it is covered by the current residue definition. Non-selective methods cannot distinguish meptyldinocap from dinocap, but selective methods are available. While meptyldinocap and dinocap are both registered for crop uses, it is preferable, for enforcement purposes, to maintain a single residue definition.

It follows that, at least while dinocap MRLs are maintained, the residue definition for meptyldinocap as "dinocap, sum of all isomers" might be a practical solution.

The ADI of 0.02 mg/kg bw is applicable for the sum of meptyldinocap and its corresponding phenol, when only they are present in the commodities analysed.

The Meeting recommended the following residue definitions that: while dinocap MRLs are maintained, the residue definition for meptyldinocap enforcement purposes should be dinocap, sum of all isomers.

Definition of residue in plant commodities for enforcement purposes: dinocap, sum of isomers.

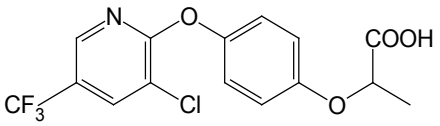
Definition of the residue for dietary exposure assessment: the sum of meptyldinocap and the corresponding phenol, 2,4-DNOP, expressed as the parent meptyldinocap.

A residue definition for animal products is not required as no residue is expected to occur in animal products from the targeted use of meptyldinocap.

Deciding on fat solubility of residues

Haloxyfop – a fat-soluble residue (JMPR, 2009)

Haloxyfop is a selective herbicide for the control of grass weeds in broad-leaf crops. Its residues occur in animal forages and fodders.



It is a carboxylic acid and its log K_{OW} = 0.27 in a pH 7 buffer (probably present as a salt), suggesting non-solubility in fat.

However, haloxyfop residues in animals are largely present as triacylglyceride conjugates incorporated into the fat tissue and lipid fraction of milk and the yolk of eggs.

Because the residue is largely present as fat-soluble conjugates, it was defined as fat-soluble.

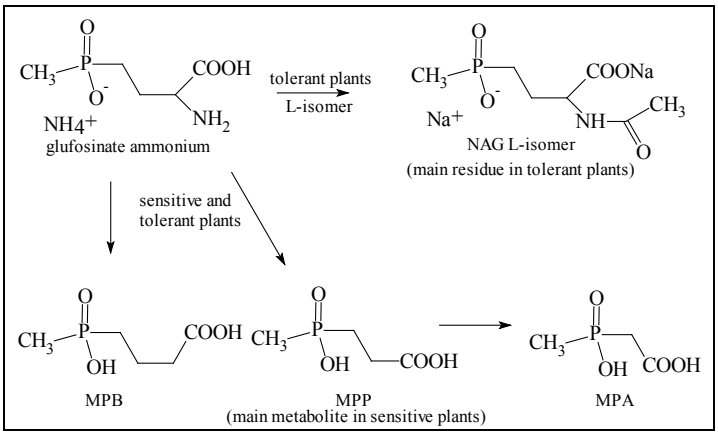
The residue was defined as: sum of haloxyfop (including haloxyfop-P), its esters and conjugates expressed as haloxyfop. The residue is fat soluble.

Transgenic and non-transgenic crops may metabolize the pesticide differently.

The principles for deciding residue definition for compounds in transgenic crops do not change and depend strongly on metabolism and analytical methods. When a commodity produced by a non-transgenic crop cannot be readily distinguished from the transgenic crop commodity, the residue definition should be the same for both. No single approach is applicable to all situations and a case-by-case approach is needed at present.

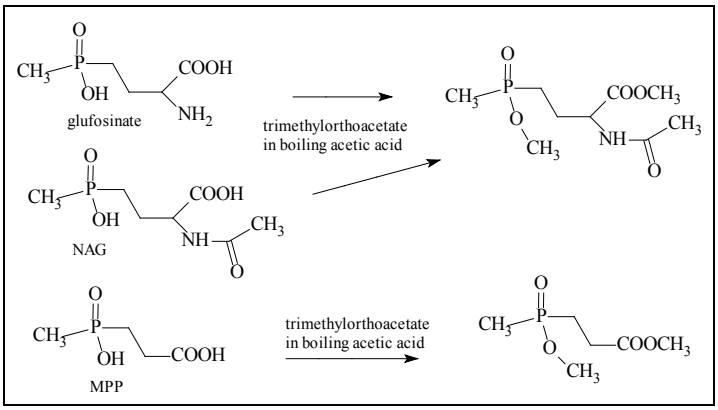
Glufosinate-ammonium – different metabolism in transgenic and non-transgenic crops (JMPR 1998)

Glufosinate-ammonium is a herbicide. Transgenic crops have been developed for tolerance to the herbicide, acting to metabolise it to a herbicidally inactive acetyl derivative (N-acetyl glufosinate, NAG).



Glufosinate-ammonium, plant metabolism.

The residue is subject to a vigorous methylation and acetylation in the GLC analytical method, which converts both the glufosinate and the NAG metabolite to the same acetylated derivative that becomes the GLC analyte.



Methylation and acetylation of the residue in the analytical method.

When glufosinate is used on genetically modified glufosinate-tolerant crops a major part of the residue is N-acetyl-glufosinate (NAG). It should be included in the enforcement residue definition because

- in some cases it is the majority of the residue;
- the same GLC derivative is produced in the analytical method for both glufosinate itself and NAG, so unless the compounds are separated before derivatization they both appear as the GLC peak for their common derivative.

Residue definition

Sum of glufosinate-ammonium, 3-(hydroxy(methyl)phosphinoyl)propionic acid and N-acetyl-glufosinate calculated as glufosinate (free acid).

Point to note

- The residue definition for residues in commodities from transgenic and non-transgenic crops should be the same because the commodities may be indistinguishable.

Different residue definition proposed by JMPR and JECFA - abamectin

Abamectin is the ISO common name for the pesticide, which is a mixture of avermectin B_{1a} (≥80%) and avermectin B_{1b} (≤20%). In sunlight the photoisomer 8,9-*Z* avermectin is produced and becomes part of the residue. It is also described as the *Δ*-8,9 isomer. Avermectin B_{1a} and 8,9-*Z* avermectin B_{1a} produce the same fluorescent compound in the derivatization step of the analytical methods and therefore appear in the one peak in an HPLC chromatogram. Avermectin B_{1b} and its photoisomer 8,9-*Z* avermectin B_{1b} behave in the same way and appear together in a second peak in the chromatogram.

The abamectin residue was defined by 1992 JMPR as:
Sum of avermectin B_{1a}, avermectin B_{1b} and delta-8,9 isomer of avermectin B_{1a}.

The 1997 JMPR noted that the residue definition proposed by JECFA (1997) for liver, kidney and fat from animals subject to veterinary uses with abamectin does not include the 8,9-*Z* isomer *(Δ*-8,9 isomer), because it is not present in animal tissues when abamectin is used directly on the animal. The JECFA residue definition also does not include avermectin B_{1b}, because avermectin B_{1a} was considered the appropriate marker residue.

The JMPR agreed that the wider definition (to include the 8,9-*Z* isomer) was the appropriate one for a laboratory carrying out enforcement or monitoring analyses because the analyst will not know if the residue in the animal originated only from veterinary uses or if animal feed was also a source. In practice, the wider definition accommodates both situations.

Inclusion or exclusion of avermectin B_{1b} from the residue definition is a matter of judgement. In many crop situations B_{1b} is commonly present at approximately 10% of the total residue, so its inclusion or exclusion has little effect on the measured residue. The analytical methods measure B_{1a} and B_{1b} by the same procedure; they appear as two peaks on the same chromatogram, so the analytical data for both components are always available from an analysis and may as well be used. The avermectin B_{1b} residue can be calculated from the avermectin B_{1a} standard curve because the reaction yields and response factors for derivatised B_{1a} and B_{1b} are the same.

Avermectin B_{1b} forms a photoisomer 8,9-*Z* avermectin B_{1b} in the same way as avermectin B_{1a} does. The studies were done with avermectin B_{1a} so when JMPR reviewed the studies in 1992 the possibility of 8,9-*Z* avermectin B_{1b} being produced was not taken into account. In practice the contribution of 8,9-*Z* avermectin B_{1b} to the residue will be small but for the sake of accuracy it should be recognised that the HPLC measurement of avermectin B_{1b} residues includes any 8,9-*Z* avermectin B_{1b}. The JMPR agreed to adjust the residue definition accordingly.

The recommended residue definition for MRLs and STMRs is:
Sum of avermectin B_{1a}, avermectin B_{1b}, 8,9-Z avermectin B_{1a} and 8,9-Z avermectin B_{1b}.

Points to note:

- Different residue definition for veterinary and plant protection use;
- Analytical methods measure B_{1a} and B_{1b} by the same procedure; they appear as two peaks on the same chromatogram,

- Avermectin B_{1b} residues include any 8,9-*Z* avermectin B_{1b}; thus there is no extra work involved in their determination.

Example for validation of analytical method for determining conjugated metabolites

Analytical methods

The methods for animal and plant commodities are similar, both relying on mild alkaline hydrolysis to release haloxyfop acid from haloxyfop conjugates, which are mainly triglycerides in animal fats and oilseed crops.

The methods have been tested with available haloxyfop esters (methyl ester and ethoxyethyl ester).

Analytical methods (JMPR 2009)

Haloxyfop methods rely on an initial extraction and hydrolysis step, usually with methanolic NaOH to release haloxyfop from conjugates. After solvent partition cleanup, the haloxyfop is methylated or butylated ready for GC analysis or further cleanup before the GC analysis. Typically, haloxyfop residues can be measured in most matrices to an LOQ of 0.01-0.05 mg/kg.

None of the methods separates the haloxyfop enantiomers. The methods effectively measure 'total' haloxyfop present as acid, salts, esters and conjugates (esters with natural compounds).

Haloxyfop residues are not suitable for analysis by multiresidue methods because the extraction step is typically also a base-hydrolysis step designed to release haloxyfop from non-polar and polar conjugates found in animal and plant tissues. Such an extraction-hydrolysis step is not suitable for many other pesticides.

Extraction from metabolism study samples (JMPR 2009)

Gardner (1983, GH-C 1625) tested the completeness of extraction of haloxyfop and its conjugates and of their conversion to parent acid using method ACR 83.1 to extract soybean samples available from the previous metabolism study (Yackovich and Miller, 1983, GH-C 1618). Method ACR 83.1 uses overnight shaking of substrate with 0.1M NaOH in 98 % methanol + 2 % water for extraction, which extracted 93 % of the ^{14}C from the soybeans. HPLC produced a single peak matching haloxyfop which accounted for 95 % of the ^{14}C in the extract.

Gardner (1984, GH-C 1709) tested the completeness of extraction of haloxyfop present as the free acid, the methyl ester or as conjugates from milk using method ACR 84.6. The milk was goat milk from a dosing study with [^{14}C]haloxyfop-butyl. Haloxyfop was quantitatively (99-100 %) extracted in the three ether extractions. Completeness of hydrolysis of haloxyfop-methyl and conjugates to haloxyfop acid was checked by measuring the ^{14}C in the benzene washes after hydrolysis (1.6 % of ^{14}C remained) and the ^{14}C remaining in the acidified aqueous solution after benzene extraction (5 % of ^{14}C, representing polar degradation products of hydrolysis). A high percentage of the ^{14}C (91 %) was present in the benzene solution (as haloxyfop acid)ready for further cleanup.

Gardner (1988, 2084-21) drew attention to the potential losses of haloxyfop that may occur during the hydrolysis step of some analytical methods. If haloxyfop is exposed to higher temperatures or longer hydrolysis times than ideal, low recoveries may occur. The condition of 35-40 minutes at 70 ºC was satisfactory.

In some of the validations, a haloxyfop ester was used as the spiking compound to test the hydrolysis step.
Analytical recoveries for spiked haloxyfop esters in various substrates.

Commodity	Spiked compound	Spike conc, mg/kg	n	Mean recov%	Range recov%	Method	Ref
grapes	ethoxy ethyl haloxyfop	0.01-0.10	4	73 %	66-78 %	ERC 84.05	ERC 84.05
oilseed rape	haloxyfop-P-methyl	0.01-10	20	83 %	72-101 %	GRM 04.03	GHE-P-11656
oilseed rape oil	ethoxy ethyl haloxyfop	0.01	7	90 %	70-100 %	ERC 83.20	ERC 83.20
oilseed rape oil	ethoxy ethyl haloxyfop	0.05-1.0	11	89 %	71-102 %	ERC 83.20	ERC 83.20
oilseed rape plants	haloxyfop-P-methyl	0.01-2.0	12	85 %	68-108 %	GRM 04.03	GHE-P-11656
soybeans	haloxyfop-methyl	0.05	2	94 %	90 %, 98 %	ARC 83.1	GH-C 1625
soybeans	haloxyfop-methyl	0.10	1	98 %		ACR 83.1	ACR 83.1.S1
sugar beet cossettes	haloxyfop-ethoxyethyl	0.01	1	120 %		ERC 84.02	GHE-P-1125
sugar beet juice	haloxyfop-ethoxyethyl	0.01	1	96 %		ERC 84.02	GHE-P-1125
sugar beet pulp	haloxyfop-ethoxyethyl	0.025	1	93 %		ERC 84.02	GHE-P-1125

Points to note:

When applied to a plant, the esters of haloxyfop or haloxyfop-P are broken down quickly to release free acid which is readily translocated throughout the plant. The haloxyfop (or haloxyfop-P) becomes conjugated, typically as glycosides (polar metabolites) or as triglycerides (non-polar metabolites), the conjugates often accounting for the major part of the residue.

Little information is available on the completeness of extraction by briefer contact of the substrate with the alkaline extractant. Most of the validations have not included a check on this step. However, some validations have used a haloxyfop ester such as haloxyfop-ethoxyethyl as the spiked analyte, which does check that the extraction conditions quantitatively hydrolyse the spiked ester. Haloxyfop esters are readily hydrolysed, so the release of conjugates by the alkaline extractant with the conditions of the analytical methods *would be generally expected*.

This is the critical phase. Under regulatory laboratory conditions the completeness of hydrolysis of conjugated compound cannot be tested!

Abbreviations and acronyms in this chapter

ADI	acceptable daily intake
ARfD	acute reference dose
DNOP	dinitrooctylphenol
GAP	good agricultural practice
GLC	gas liquid chromatography
HPLC	high performance liquid chromatography
HR	highest residue
JECFA	Joint Expert Committee on Food Additives

JMPR	Joint Meeting on Pesticide Residues
K_{OW}	octanol-water partition coefficient
LOQ	limit of quantification
MRL	maximum residue limit
MS	mass spectrometry
NAG	N-acetyl glufosinate
STMR	supervised trials median residue
WHO	World Health Organization

Chapter 7. Selection of Supervised Trials for Estimation of STMRs, HRs and MRLs Including Application of Statistical Methods.

Use pattern
Supervised trials reflecting the critical GAP
Selection of supervised trials and comparability of supervised trial conditions
Definition of independent supervised trials
Examples for consideration of data populations
Presentation – selection of supervised trials

Relevant sections of JMPR Manual

- Use pattern 3.4
- Residues resulting from supervised trials on crops 3.5
- Results of supervised trials 5.6
- Comparability of supervised trial conditions to GAP 6.2
- Definition of independent supervised residue trials 6.3
- Combining of data populations 6.4

The purpose of this chapter is to review the general principles for selection of supervised trials which provide the basis for estimation of maximum residue levels, supervised trial median values (STMR) and highest residue values (HR).

The objective of establishing MRLs is to provide an objective means to verify that the pesticides were used according to Good Agriculture Practice (GAP)[12]. The nationally authorized safe uses of pesticides are defined by the 'use patterns'. The pesticides may be applied at different dosage rate and time before the harvest within the authorised maximum dosage and over the minimum pre-harvest intervals. The estimated maximum residue levels should cover the residues in or on commodities harvested after the authorised minimum pre-harvest intervals following the repeated applications at the permitted minimum intervals and maximum dosage rate. These conditions are called the 'critical GAP'. The STMR and HR values used for estimation of long- and short-term intakes should correspond to residues deriving from the critical GAP.

Supervised field trials (crop field trials) are conducted to determine pesticide residue levels in or on raw agricultural commodities, including feed items, and should be designed to reflect pesticide use patterns that lead to the highest possible residues within GAP.

As a general precondition, for reliable estimation of maximum residue levels an adequate number of *independent trials* are required reflecting the highest of national maximum GAPs and conducted according to well designed protocols that consider

[12] Good agricultural practice in the use of pesticides (GAP) includes the nationally authorized safe uses of pesticides under actual conditions necessary for effective pest control. It encompasses a range of levels of pesticide applications up to the highest authorized use, applied in a manner which leaves a residue which is the smallest amount practicable.
Authorized safe uses are determined at the national level and include nationally registered or recommended uses, which take into account public and occupational health and environmental safety considerations.
Actual conditions include any stage in the production, storage, transport, distribution of food commodities and animal feed. (CAC, 1995)

geographical distribution and the inclusion of a number of different growing and management practices, and growing seasons.

The selection of supervised trials, which correspond to the critical GAP and suitable for estimation of MRL, STMR and HR values, is one of the most important phases of the evaluation of pesticide residues. It must not be performed automatically as it requires expert judgement in many cases taking into account several factors and the information obtained from the previous trials and relevant scientific studies.

The estimated MRLs can only reflect the maximum residues likely to occur if the residue data used for the estimation are properly selected regardless of whether computerised methods are used or not for assisting the procedure.

The following sections summarise the most important principles without attempting to provide complete guidance which can be applied in all cases. The FAO Manual provides more detailed information. It is emphasised however, that the evaluation process is continuously evolving and new situations may arise which might require different approaches. The JMPR describes the new principles applied in the Reports of the Meetings.

Use pattern

The critical GAP is the set of current registered uses involving the highest rates and shortest PHIs for the same pesticide on the same crop in the same country and the use patterns in the supervised field trials should reflect this condition.

The following GAP information is required:

- Valid copies of current labels must be provided, together with English translations of the relevant sections.
- Information should be provided on the list of individual crops that are included in a crop group indicated on a label.
- Labels reflecting current GAP should be clearly distinguished from 'proposed' labels.
- Summary information on GAP relevant to the submitted supervised trials and current GAP with higher rates or smaller PHIs, etc for the same pesticide on the same crop in the same country should be submitted.

The GAP information should be presented in a systematic manner according to the standardized format(s) given in the FAO Manual. The reported GAP summary should be supported by valid labels. (A proposed label is not acceptable for this purpose.) The summary should not include any information on use that is not given on the label.

Registered uses of XX on YY

Crop	Country	Formulation	Application					PHI, days
			Method	Rate kg ai/ha	Spray conc., kg ai/hl	Number	Interval [a]	
Barley	France			1.5				21
Beans	Greece	WP 800 g/kg	foliar	0.6–1.5	0.1-0.25	3–4		7
Beans	Portugal	WP 800 g/kg	foliar		0.13	1–2		7
Beans, green	Spain	WP 800 g/kg	foliar	1.6	0.16			21
Brassica vegetables	Italy	WP 800 g/kg	foliar	0.35–0.40				10
Lettuce	France	WP 800 g/kg	foliar	0.64				21-41[b]
Lettuce	Israel[3]	WP 800 g/kg	foliar	2.0		weekly		11

[a] in days or weeks [b] summer PHI 21 days, winter PHI 41 days

If GAP information is provided by responsible national regulatory authorities, the above detailed information is required and the submission of the label is desirable.

Supervised trials reflecting the critical GAP

Maximum Residue Limits are generally derived from residue data obtained from supervised trials designed to determine the nature and level of residues resulting from the registered or approved use of the pesticide.

Since the trials are usually conducted before registration is obtained, in many cases the trials are based on the intended use, which is sometimes different from the registered one. Some of the trial data may not be relevant for JMPR evaluations. Note however, that in cases with a limited number of trials at GAP, the results from other supervised trials can provide supporting information, such as residue decline data to indicate rate of concentration decrease or trials with higher rates leading to residues below LOQ.

Residue data should be presented primarily for mature crops at normal harvest. However, where a significant part of the consumable crop is present at the time of application, some residue dissipation studies are required to complement the residue data obtained at normal harvest.

Residue data should be available from trials, preferably carried out in at least two separate years or at least representative of different weather conditions. If uses are authorised in regions with substantially different climatic conditions, trials should also be carried out in each region. Residue data from only one season may be considered sufficient provided that crop field trials are located in a wide range of crop production areas such that a variety of climatic conditions and crop production systems are taken into account.

Residue data for estimation of MRLs should be obtained from independent trials. (see section on independent supervised trials).

Row crops (potatoes, wheat, soya beans, etc.) are typically treated with broadcast sprays for which plot area (length × width) is a key consideration. In contrast, for some crops such as tree nuts, tree fruits, trellised vegetables and vines, the crop height, crown height or tree height, i.e., treated foliage height, should be considered in order to allow crop row volume or tree row volume estimations or rate per unit area calculation as needed. Special consideration may be needed for foliar applications to '*tall' crops,* e.g., orchard and vine crops, hops, greenhouse tomatoes, where flat boom spraying is not common practice and (air assisted) mist blowing equipment is often used. It is important to consider and report both the spray concentration, e.g., kg ai/100 litres, and spray volumes, e.g., litres spray mixture/ha, at the various crop growth stages.

The *formulation tested* in crop field trials should be as close as possible to the commercially available end-use product for the crop or commodity. Data needed to cover *additional formulation types* or classes shall be addressed on a case-by-case basis. Controlled release formulations, e.g., certain microencapsulated products, granular formulations will generally require a complete data set regardless of what data are already available for other formulation types.

Bridging studies may allow using residue data obtained with one of the most common formulation types which are diluted in water prior to application for interpreting residues resulted from other formulation. Such formulations include EC, WP, water dispersible granules (WG), suspension concentrates (SC) (also called flowable concentrates), and

soluble concentrates (SL). A bridging study normally involves a comparison of different formulations or application methods for the purpose of data extrapolation, but may or may not involve side-by-side comparisons.

Adjuvants such as wetting agents, spreader-stickers, non-ionic surfactants, and crop oil concentrates may result in better deposition, penetration, or persistence of pesticide residues in or on the plant. Therefore, for a test substance which has a label allowance for the use of an unspecified adjuvant, crop field trials must include an adjuvant (any locally-available adjuvant), applied according to the label recommendation of the adjuvant. For a test substance which has a label recommendation for the use of a specific adjuvant, crop field trials must include the adjuvant, or another adjuvant with similar properties, applied according to the label recommendation of the adjuvant.

Number of trials

As a general precondition, for reliable estimation of maximum residue levels an adequate number of independent trials are required reflecting the highest of national maximum GAPs and conducted according to well designed protocols that consider geographical distribution and the inclusion of a number of different growing and management practices, and growing seasons.

The JMPR has not specified the minimum number of trials required for estimation of maximum residue levels, high (HR) and supervised trial median residues (STMR). The OECD Working Group on Pesticides elaborated guidance on the minimum number of trials[13] which should be generated for registration of a pesticide in all OECD countries where the target GAP is uniform, i.e., maximum 25% deviation in one of the key parameters. (see Appendix XII of FAO Manual)

Selection of supervised trials and comparability of supervised trial conditions

Firstly, the uniformity or continuity of residue population reflecting GAPs is considered. When there is a large gap in residue values, indicated by a high coefficient of variation of residues in composite samples or other appropriate statistical methods, the presence of different populations may be suspected. In such cases the residue data and trial conditions need more stringent analysis before residue levels for MRL, STMR or HR can be estimated.

Under practical conditions the number of trials which can be performed for a given commodity is limited. A larger data set *representing statistically not different residue populations* provides a more accurate estimation of the selected percentile than a small data set derived from trials representing only one critical GAP. Consequently, where only limited number of trial data are available from a GAP, assumed to lead to the highest magnitude of residues, one approach is to consider those GAPs which may possibly lead to a similar magnitude of residues, and this assumption can be confirmed based on prior experience and with suitable statistical methods. When considering combining different residue data, the distribution of residue data is carefully examined and only those datasets combined which may be expected to arise from the same parent populations, based on comparable GAP. In such cases expert judgement can be assisted with appropriate statistical tests, e.g., Mann-Whitney U-test or Kruskal-Wallis H-test. The calculations are easy using the available Excel template which can be downloaded from:
http://udel.edu/~mcdonald/statkruskalwallis.html.

As usual in statistical tests, if the calculated probability is larger than 0.05 the null hypothesis is accepted and the data sets can be combined.

[13] Draft Revised Guidance Document on Overview of Residue Chemistry Studies (Series on Testing And Assessment No.64) 18 Feb 2009

The JMPR takes into account the following general principles in selecting the residue data population(s) for the estimation of maximum residue levels, STMR and HR values.

Only the results of "supervised trials conducted at the highest nationally recommended, authorized or registered uses", i.e., maximum application rate, maximum number of treatments, minimum pre-harvest interval (PHI), are considered.

If a sufficient number of trials are available, reflecting the maximum GAP of one country or geographical region, the MRL estimates should be based on those residue data alone.

Where prior experience indicate that the agricultural practice and climatic conditions lead to similar residues, the critical GAP of one country can be applied for the evaluation of supervised trials matching this critical GAP but carried out in another country.

Application rate

Dosage rate may be within ± 25% of dosage of critical GAP. Note that the 2010 JMPR decided to take into account the proportionality of residues where applicable. Tolerances on the parameters should be those that would result in ±25% change in the residue concentration, not ±25% changes in the parameters themselves. It is ±25% for application rate because application rate is directly proportional to residue concentration.

When combining field trials for a complete data set for a crop use, this "25% rule" may be applied to any one of the critical GAP components; however it is not acceptable to apply in one direction the rule to more than one *c*GAP component listed here at a time.

The same principle may be applied for judging the equivalency of residue data where a specific formulation type with different active ingredient content was used in the trials, provided that the *c*GAP is not changed significantly as a result, e.g., no more than 25% increase in amount of active ingredient per unit area.

Number of treatments

Consideration of whether the number of applications reported in trials is comparable to the registered maximum number will depend on the persistence of the compound and the interval between applications. Nevertheless, when a large number of applications are made in trials (more than 5 or 6) earlier treatments should not be considered to contribute greatly to the final residue unless the compound is persistent or the treatments are made with unusually short intervals.

Also, treatments from more than about 3 half-lives (obtained from residue decline trials) prior to the final treatment should not make a significant contribution to the final residue.

Example: bifenazate – cane berries

The US GAP specifies one application at maximum 0.56 kg ai/ha with a PHI of 1 day.

Eight supervised trials on cane berries were conducted in the United States and Canada during the 2004-2005 growing season. Six of the trials were on raspberries and two on blackberries. Two applications were made with maximum GAP dosage rate at 29-35 days apart. Residues in samples were collected at day 0 were: 1.4, 1.5, 1.7, 2.2, 2.3, 2.6, 3.3 and 4.6 mg/kg.

The Meeting considered the rate of degradation of bifenazate between 7 and 28 days in grape, apple, pear in supervised trials evaluated by the 2006 JMPR and noted that the half-lives of the compound on grape, apple and pear were about 12.2, 10.9 and 13 days,

respectively. Considering that the residue is mainly on the surface of the fruits, the similarity in the size of grape berries and raspberries, and the comparable rate of decline on several crops, the Meeting assumed that the first treatment performed 29-35 days before the second one did not probably contribute more than 10-15% to the initial residue.

The Meeting estimated maximum residue level, STMR of 7 mg/kg, and 2.25 mg/kg for cane berries.

Point to note

- Some residues might have remained from the first treatment (+); residues decline from day 0 to day 1 (-). As a borderline case the Meeting decided to recommend residue levels.

Definition of independent supervised trials

Judgements are needed on whether trials should be considered sufficiently independent to be treated separately.

As weather (not climate) is usually a major factor in determining the resultant residues for such trials, only one field trial would normally be selected per trial site if multiple plots/trials are conducted in parallel. For trials at the same location there should be convincing evidence that additional trials are providing further independent information on the influence of the range of farming practices on residue levels.

The trials cannot be considered independent if they are carried out at the same location with the same equipment within a growing season. Treatments of different varieties or applying different formulations are generally not sufficient for considering the trials independent. In this situation, the trial producing the highest residues should be used for further assessment.

Replicate field samples, samples from replicate plots or sub or split-plot samples, samples from replicate trials are considered interrelated and the average residues are used for further assessment.

Examples for consideration of data populations

Example 1: boscalid in citrus fruits

In 6 US trials on grapefruit matching GAP, boscalid residues were in whole fruit: 0.10, 0.12, 0.15, 0.15, 0.27 and 0.85 mg/kg. No data were received for the edible portion.

In 5 US trials on lemon matching GAP, boscalid residues were in whole fruit: 0.59, 0.68, 0.74, 0.94 and 1.5 mg/kg. No data were received for the edible portion.

In 13 US trials on oranges matching GAP, boscalid residues were in whole fruit: 0.23, 0.26, 0.30, 0.32, 0.33, 0.35, 0.47, 0.56, 0.64, 0.68, 0.71, 1.2 and 1.4 mg/kg. The residues in pulp were <0.05 (6), 0.05, 0.06, 0.06, 0.09, 0.09, 0.12 and 0.20 mg/kg.

Based on the orange residue data, the Meeting estimated a maximum residue level of 2 mg/kg for citrus fruits. On the basis of the residues in orange pulp, the Meeting estimated an STMR of 0.05 mg/kg.

Points to note:

- Residue distribution in the three commodities
- Are the residue populations different?

- Residues in edible portion.

Example 2: fenpyroximate – citrus fruits

The GAP of fenpyroximate on citrus in USA is a maximum of two foliar applications at a rate of 0.22 kg ai/ha (not exceeding 0.45kg ai/ha per growing season), with a PHI of 14 days.

Residues in oranges (whole fruit) from trials in USA matching critical GAP in rank order were: 0.07, 0.11, 0.18 and 0.28 mg/kg.

Residues in lemons (whole fruit) from trials matching critical GAP in USA in rank order were: 0.17, 0.21 and 0.23 mg/kg.

Residues in grapefruit (whole fruit) from trials matching critical GAP in USA in rank order were: 0.02, 0.04 and 0.09mg/kg.

On the basis of the foliar application in USA, the combined data (whole fruit) in rank order were (n = 10): 0.02, 0.04, 0.07, 0.09, 0.11, 0.17, 0.18, 0.21, 0.23 and 0.28 mg/kg. The Meeting estimated a maximum residue level for the citrus fruit group of 0.5 mg/kg.

Points to note:

- Residue distribution in the four commodities
  - Are the residue populations different?
  - Number of trials
  - Median of individual data sets and the combined data

Example 3: chlorantraniliprole – Brassica vegetables

In trials from Europe on Brassica vegetables complying with the GAP of Spain (35 g ai/ha, PHI 1 day) residues were: cabbage <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, 0.011, 0.012, 0.012, 0.015, 0.018, 0.04, 0.059 and 0.10 mg/kg.

Broccoli: 0.064, 0.10, 0.10, 0.12, 0.14, 0.19 and 0.37 mg/kg.

Cauliflower: <0.01, <0.01, 0.012, 0.019, 0.036, 0.047 and 0.082 mg/kg.

Chlorantraniliprole is registered in Canada for use on Brassica vegetables at 100 g ai/ha, PHI of 3 days and a maximum application per season of 200 g ai/ha.

Residues on broccoli (n=9) complying with the revised Canada GAP were: 0.12, 0.30, 0.32, 0.32, 0.35, 0.38, 0.40, 0.41 and 0.56 mg/kg.

Residues on cabbage (n=10) complying with Canada GAP were: 0.033, 0.066, 0.10, 0.28, 0.29, 0.48, 0.51, 0.64, 0.75 and 1.1 mg/kg.

Residues were highest in the cabbages and this dataset was used for the purposes of estimating a maximum residue level for the Brassica group.

Points to note:

- Residue distribution in the three commodities
- Are the residue populations different
- Data base for estimation of MRLs.

Example 4: thiamethoxam – stone fruits

Spanish GAP for cherries allows the use of thiamethoxam for 2 foliar applications with a spray concentration of 0.0075 kg ai/hl followed by a 7 days PHI.

In 12 cherry trials in France (7), Italy (3) and Spain (2) matching the Spanish GAP, thiamethoxam residues in cherries in rank order were: 0.13, 0.15, 0.16, 0.16, 0.17, 0.19, 0.20, 0.26, 0.31, 0.49, 0.50 and 0.60 mg/kg. Meeting estimated a maximum residue level of 1 mg/kg for thiamethoxam on stone fruits.

Point to note:

- Trials were performed in three countries under similar growing conditions (South Europe) with the same use pattern

PRESENTATION – SELECTION OF SUPERVISED TRIALS

1. Selection of Supervised Trials for Estimation of STMR, HR and MRLs

2. Objectives

- The purpose of this chapter is to review the general principles for selection of supervised trials which provide the basis for estimation of maximum residue levels, supervised trial median residue values (STMR) and highest residue values (HR).
- The objective of establishing MRLs is to provide an objective means to verify that the pesticides were used according to Good Agriculture Practice (GAP)
- MRLs are not safety limits, but the consumption of commodities containing residues at the MRL are considered to be safe.

3. Outline

- Use pattern
- Supervised trials reflecting the critical GAP
- Selection of supervised trials
- Comparability of supervised trial conditions
- Definition of independent supervised trials
- Combination of data populations

4. Use pattern

The nationally authorized safe uses of pesticides are defined by the ‘use patterns’.

- The pesticides may be applied at different dosage rate and time before the harvest within the authorised maximum dosage and over the minimum pre-harvest intervals.
- The ‘critical GAP’ comprises conditions when commodities are harvested after the authorised minimum pre-harvest intervals following the repeated applications at the permitted minimum intervals and maximum dosage rates.
- The maximum residue levels should cover the residues in or on commodities treated according to the critical GAP (cGAP).
- The STMR and HR values used for estimation of long- and short-term intakes should correspond to residues deriving from the critical GAP.

5. Supervised trials

- Supervised field trials (crop field trials) are conducted to determine pesticide residue levels in or on raw agricultural commodities, including feed items, and should be designed to reflect pesticide use patterns that lead to the highest possible residues within GAP.

- For reliable estimation of maximum residue levels an adequate number of independent trials are required reflecting the cGAPs and conducted according to well designed protocols that consider geographical distribution and the inclusion of a number of different growing and management practices, and growing seasons.

6. Selection of supervised trials

- The selection of supervised trials, which correspond to the critical GAP and suitable for estimation of MRL, STMR and HR values, is one of the most important phases of the evaluation of pesticide residues .
- It must not be performed automatically as it requires expert judgement in many cases taking into account several factors and the information obtained from the previous trials and relevant scientific studies.
- The estimated MRLs can only reflect the maximum residues likely to occur if the residue data used for the estimation are properly selected regardless of whether computerised methods are used or not for assisting the procedure.

7. GAP information required

- Valid copies of current labels must be provided, together with English translations of the relevant sections.
- Information should be provided on the list of individual crops that are included in a crop group indicated on a label.
- Labels reflecting current GAP should be clearly distinguished from ‘proposed’ labels.
- Summary information on GAP relevant to the submitted supervised trials and (current GAP with higher rates or smaller PHIs, etc. for the same pesticide on the same crop) should be submitted.

8. Information on USE Pattern

- State the number of treatments per season only if specified on the label.
- Application rate should always be presented in metric units.
- In cases where the indications on the label are given in g/hl or kg/hl (spray concentration), state this spray concentration but do not calculate the kg ai/ha equivalent with the average amount of spray liquid used per hectare.
- The pre-harvest interval (PHI) in days prescribed or recommended and stated on the label - should be presented for the commodities concerned.
- If different PHIs are recommended for the same or similar commodity, e.g. for glasshouse or outdoor grown crops, or in the case of higher dosage rates, the particular circumstances should be clearly indicated.

9. GAP information

- The GAP information should be presented in a systematic manner according to the standardised formats
- The reported GAP summary should be supported by valid labels. Do not include information which is not on a label.

- If GAP information is provided by responsible national regulatory authorities the above detailed information is required and the submission of the label is desirable.

10. Format for presentation of use patterns

Crop	Country	Formulation g/kg	Application		Spray			PHI, days
			Method	Rate kg ai/ha	Conc., kg ai/ hl	No	Interval (days or weeks)	
Barley	France			1.5				21
Beans	Greece	WP 800	foliar		0.13	1–2		7
Beans, green		WP 800	foliar	1.6	0.16			21
Brassica vegetables		WP 800	foliar	0.35–0.40				10
Lettuce		WP 800	foliar	0.64				21-41 Note

Note: summer PHI 21 days, winter PHI 41 days.

11. Criteria for selecting supervised trials

- Trials are usually conducted before registration is obtained; in many cases, the trials are based on the intended use, which is sometimes different from the registered one.
- Typically trials reflecting cGAP should be provided.
- Results from other supervised trials can provide supporting information, such as residue decline data or treatments with higher rates leading to residues below LOQ.
- Residue data are required primarily for mature crops at normal harvest. But residue dissipation studies on consumable crops complement the residue data obtained at normal harvest.

12. Representative trials

- Residue data should be available from independent trials, preferably carried out in at least two separate years or at least representative areas of different weather conditions.
- If uses are authorised in regions with substantially different climatic conditions, trials should also be carried out in each region.
- Residue data from only one season may be considered sufficient provided that crop field trials are located in a wide range of crop production areas such that a variety of climatic conditions and crop production systems are taken into account.

13. Timing of application in supervised trials

- *Application timing* is governed by plant growth stage (e.g., pre-bloom, 50% head emergence, etc.) or as number of days prior to harvest.
- Where a specific PHI is indicated on the label (e.g., “Do not apply this product less than 14 days prior to harvest.”), that specific PHI must be used in the crop field trials

as a component of the cGAP, while the growth stage at application is of minor importance.

- Inversely, there are cases where the growth stage is a critical component of the GAP, (e.g., pre-emergence, at planting, pre-bloom, flag leaf or head emergence, etc.) while the PHI is of secondary importance. In these cases it is important to include as many varieties of the crop as possible in order to evaluate an appropriate range of PHIs (e.g., shorter and longer intervals from planting to maturity in the case of pre-emergence application to an annual crop). Basically in all trials both the growth stage at application (preferably as BBCH code) and PHI should be recorded.

14. Crop characteristics

- *Row crops* (potatoes, wheat, soya beans, etc.) are typically treated with broadcast sprays for which the treated plot area (length × width) is a key consideration.
- In contrast, for some crops such as tree nuts, tree fruits, trellised vegetables and vines, the crop height, crown height or tree height, i.e., treated foliage height, should be considered in order to allow crop row volume or tree row volume estimations or rate per unit area calculation as needed.
- Special consideration may be needed for foliar applications to 'tall' crops, e.g., orchard and vine crops, hops, greenhouse tomatoes, where flat boom spraying is not common practice and (air assisted) mist blowing equipment is often used. It is important to consider and report both the spray concentration, e.g., kg ai/100 litres, and spray volumes, e.g., litres spray mixture/ha, at the various crop growth stages.

15. Comparability of pesticide formulations

- The formulation tested in crop field trials should be as close as possible to the commercially available end-use product for the crop or commodity.
- Controlled release formulations, e.g., certain microencapsulated products, granular formulations will generally require a complete data set regardless of what data are already available for other formulation types.
- Bridging studies may allow using residue data obtained with one of the most common formulation types which are diluted in water prior to application for interpreting residues resulted from other formulation. Such formulations include EC, WP, water dispersible granules (WG), suspension concentrates (SC) (also called flowable concentrates), and soluble concentrates (SL).
- A bridging study normally involves a comparison of different formulations or application methods for the purpose of data extrapolation, but may or may not involve side-by-side comparisons.

16. Bridging trials

- If bridging trials are deemed necessary and a pesticide is used on a wide range of crops, data should be available for at least 3 major crop groups (one crop per crop group), e.g., a leafy crop, a root crop, a tree fruit, a cereal grain, an oilseed with a minimum of 4 trials per crop.
- The trials should be carried out on crops that would be expected to show high levels of residue (often those with applications at or near harvest).

- If a bridging study is conducted and residues are significantly higher with a new formulation or different application method, or the combined residue data set obtained with different formulations would lead to a higher MRL, generation of a complete new data set may be necessary.

17. Other parameters

- *Adjuvants* such as wetting agents, spreader-stickers, non-ionic surfactants, and crop oil concentrates may result in better deposition, penetration, or persistence of pesticide residues in or on the plant.
- Adjuvant should be applied according to the label recommendation: non-specified – specified.
- *Application equipment*: aerial, ground, hand operated, air assisted etc.

18. Application equipment

- *Application of the test substance* may be made with hand-held or commercial equipment as long as the equipment can be calibrated. Hand-held equipment used to make test substance applications in crop field trials should do so in a manner that simulates commercial practice.
- The *maximum label rate* or maximum proposed label rate of the active ingredient with maximum number of applications and minimum re-treatment interval (according to the cGAP) should be used when applying the test substance for crop trials.

19. Number of supervised trials

- For reliable estimation of maximum residue levels an adequate number of independent trials are required reflecting the highest of national maximum GAPs , geographical distribution, different growing and management practices, and growing seasons.
- The JMPR has not specified the minimum number of trials required for estimation of maximum residue levels, high (HR) and supervised trial median residues (STMR).
- Currently there is no international agreement on the minimum number of trials to be provided for the estimation of STMR, HR and MRL.
- The OECD Working Group on Pesticides elaborated guidance on the minimum number of trials which should be generated for registration of a pesticide in all OECD countries where the target GAP is uniform (i.e., maximum 25% deviation in one of the key parameters).

20. Number of supervised trials

- The reduction in the total number of trials within any OECD country or crop production region is compensated for by the total number of crop field trials making up the comprehensive submission data set and the wider geographic distribution of these data.
- To qualify for this comprehensive submission approach, all crop field trials must meet the following criteria:
- (1) Field trials are conducted according to the cGAP (within +/- 25% of the application rate, number of applications or PHI). At least 50% of the trials must be conducted at

or above (within 25%) the cGAP. For this purpose, trials whose intended application rates match the cGAP but actual rates fall up to 10% below the cGAP (e.g., due to the normal variability in preparing spray solutions) are considered acceptable. In addition, for some authorities at least 50% of the trials need to be decline studies.

- (2) The trials span a range of representative crop production practices for each crop including those likely to lead to the highest residues (e.g., irrigated vs. non-irrigated, trellis vs. non-trellis production, fall-planted vs. spring-planted, etc.).

21. Example for calculation of minimum number of trials

Country/Region	USA/CAN	EU	JP	AUS	NZ	Total
Number required by legislation	24	16	2	8	4	54
Number with 40% reduction	14	10	2	5	2	33

22. Number of supervised trials

- Any reduction in the number of crop field trials should be distributed proportionally among the crop production regions
- In no case may the number of trials in a given crop production region be reduced below 2.
- The minimum total number of trials for any crop in a comprehensive submission is eight.
- In addition, the total number of trials to be conducted may not be less than the requirement for any given individual region.

23. Number of supervised trials

- For a comprehensive submission with similar critical GAPs, a minimum of 8 greenhouse trials is needed.
- For such greenhouse trials, the geographic distribution typically is not an issue. However for active ingredients which are susceptible to photodegradation, consideration should be given to locations at different latitudes.
- The number of post-harvest trials on a commodity should be at least four, taking into consideration the application techniques, storage facilities, and packaging materials used. At least three samples should be collected and analysed in studies on bulk and bagged commodities.

24. General principles of selecting the residue data population(s)

- Only the results of supervised trials reflecting cGAP are considered.
- If sufficient number of trials reflecting cGAP are available from one country or geographical region, the MRL estimates should be based on those residue data alone.
- Where prior experience indicates that the agricultural practice and climatic conditions lead to similar residues, the critical GAP of one country can be applied for the

evaluation of supervised trials matching this critical GAP but carried out in another country.

25. Selection of trials for evaluation

- Consider uniformity or continuity of residue population reflecting GAPs. When there is a large gap in residue values, the residue data and trial conditions need more stringent analysis;
- A larger data set *representing statistically not different residue populations* provides a more accurate estimation of the selected percentile than a small data set derived from trials representing only one critical GAP.
- Therefore, those GAPs which may possibly lead to a similar magnitude of residues may also be considered, and residue data may be combined for estimation of residue levels.

26. Combining residue data

- When considering combining different residue data, the distribution of residue data is carefully examined and only those datasets are used which may be expected to arise from the same parent populations, based on comparable GAP.
- This assumption can be confirmed based on prior experience and with suitable statistical methods. Mann-Whitney U-test or Kruskal-Wallis H-test. The calculations are easy using the available Excel template.

27. Example for combination of residue data

Fenpyroximate on citrus fruits the USA GAP is a maximum of two foliar applications at a rate of 0.22 kg ai/ha, with a PHI of 14 days.

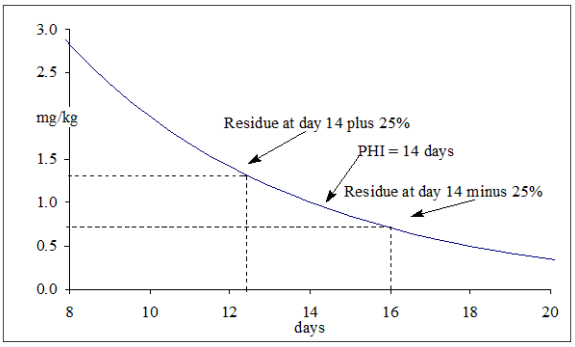
- Residues in oranges (whole fruit) were: 0.07, 0.11, 0.18 and 0.28 mg/kg.
- Residues in lemons (whole fruit) from trials matching critical GAP in USA in rank order were: 0.17, 0.21 and 0.23 mg/kg.
- Residues in grapefruit (whole fruit) from trials matching critical GAP in USA in rank order were: 0.02, 0.04 and 0.09mg/kg.
- Notes:
- 4 orange data would not be sufficient for estimation of residue levels
- The data populations were considered similar (borderline); the JMPR combined the residue data and estimated an MRL of 0.5 mg/kg.
- 3 residue data on lemons and grapefruits are not enough for applying K-W test.

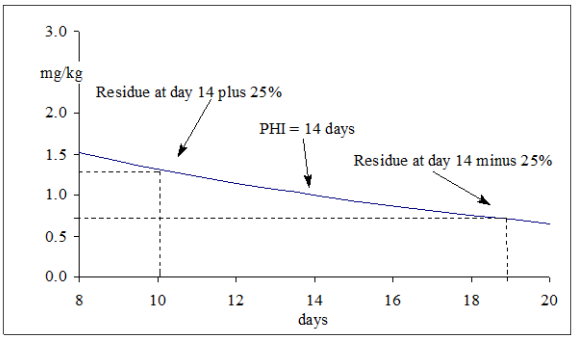
28. General principles of selecting the residue data population(s)

- Dosage rate may be within ± 25% of dosage of cGAP. Note that the 2010 JMPR decided to take into account the proportionality of residues where applicable.
- Tolerances on the parameters should be those that would result in ±25% change in the residue concentration, not ±25% changes in the parameters themselves. It is ±25% for application rate because application rate is directly proportional to residue concentration.

- The latitude of acceptable intervals around the PHI depends on the rate of decline of residues of the compound under evaluation.

29. Allowable latitude around the PHI





30. General principles of selecting the residue data population(s) 2

- The residue concentration may be the basis for judging the equivalency of residue data obtained applying a specific formulation type with different active ingredient contents, provided that the cGAP is not changed significantly e.g. no more than 25% increase in amount of active ingredient per unit area.
- Whether the number of applications reported in trials is comparable to the registered maximum number will depend on the persistence of the compound and the interval between applications.
- Unless the compound is persistent or the treatments are made with unusually short intervals early treatments of 5 or 6 would not normally contribute substantially to the residues at harvest.
- Treatments from more than about 3 half-lives (obtained from residue decline trials) prior to the final treatment should not make a significant contribution to the final residue.

31. Example: bifenazate – cane berries

- The US GAP specifies one application at maximum 0.56 kg ai/ha with a PHI of 1 day.
- Samples from 8 trials were collected at day 0.
- JMPR noted that the half-lives of the compound on grape, apple and pear were about 12.2, 10.9 and 13 days, respectively.
- Considering that the residue is mainly on the surface of the fruits, the similarity in the size of grape berries and raspberries, and the comparable rate of decline on several crops, JMPR assumed that the first treatment performed 29-35 days before the second one did not probably contribute more than 10-15% to the initial residue.

Points to note: some residues might remain from the first treatment (+); residues decline from day 0 to day 1 (-). As a borderline case the JMPR decided to recommend residue levels.

32. Definition of independent supervised trials

- As weather (not climate) is usually a major factor in determining the resultant residues for such trials, only one field trial would normally be selected per trial site if multiple plots or trials are conducted in parallel.
- The trials cannot be considered independent if they are carried out at the same location with the same equipment within a growing season. Treatments of different varieties or applying different formulations are generally not sufficient for considering the trials independent.
- Replicate field samples, samples from replicate plots or sub or split-plot samples, samples from replicate trials are considered interrelated and the average residues are used for further assessment.

33. Examples for consideration of data populations

34. Recommendation is based on one data set

Boscalid residues in citrus fruits from cGAP trials:

- Grapefruits (whole): 0.10, 0.12, 0.15, 0.15, 0.27 and 0.85 mg/kg
- Lemons (whole): 0.59, 0.68, 0.74, 0.94 and 1.5 mg/kg
- Oranges (whole): 0.23, 0.26, 0.30, 0.32, 0.33, 0.35, 0.47, 0.56, 0.64, 0.68, 0.71, 1.2 and 1.4 mg/kg
- Orange pulp: <0.05 (6), 0.05, 0.06, 0.06, 0.09, 0.09, 0.12 and 0.20 mg/kg

Based on the orange residue data, the JMPR estimated a maximum residue level of 2 mg/kg for citrus fruits. On the basis of the residues in orange pulp, the Meeting estimated an STMR of 0.05 mg/kg.

Points to note:

- Residue distribution in the three commodities
- Are the residue populations different?
- Residues in edible portion

35. Trials from different countries of the same geographical location

Thiamethoxam – stone fruits

- Spanish GAP for cherries allows the use of thiamethoxam for 2 foliar applications with a spray concentration of 0.0075 kg ai/hl followed by a 7 days PHI.
- Based on 12 cherry trials in France (7), Italy (3) and Spain (2) matching the Spanish GAP, the Meeting estimated a maximum residue level for thiamethoxam on stone fruits.

Point to note:

- Trials were performed in three countries under similar growing conditions (South Europe) with the same use pattern.

36. Principles of extrapolation of residue data from one crop to another

37. Estimation of group MRLs

- A policy on recommending Group MRLs in wider circumstances should be considered in an attempt to cover more minor uses.
- It is essential to know the metabolism, uptake, distribution of the active substance in question, and definition (expression) of residues in plants.

38. Preconditions for extrapolation of residues

- Extrapolation of residue data for different crops presumes that the following are comparable:
  - conditions of use with regard to the amount of active substance applied,
  - the time of application,
  - the number of applications,
  - the interval between applications,
  - application methods,
  - formulation used, and
  - climatic conditions.

39. JMPR Practice for extrapolation

- Generally, for a group limit to be proposed, residues in the main commodities of the group should not be too different and registered uses should be similar.
- In some cases where the residues on one or a few commodities in the group are quite different from the rest, it may be possible to recommend a limit for "group, except …"

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Crop	Recommendation
Citrus fruit	Oranges and a small citrus to whole group
Tree nuts	Almonds plus one other nut (except coconuts) to whole group
Pome fruit	Apples and pears to whole group
Stone fruit	Peaches, nectarine and cherry or peaches, plum and cherry to whole group
Berries and other small fruit	Any berry and currant to whole group (excluding grapes)

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Crop	Recommendation
Root and tuber vegetables	Potato, carrot and one other root crop to whole group Potato to tuber and corm sub-group Sweet potato or yam to tuber and corm excluding potato sub-group
Bulb vegetables	Onions green and dry to whole group

Fruiting vegetables (non-cucurbits)	Tomato and peppers to whole group
Fruiting vegetables (cucurbits)	Cucumber, melon and other cucurbit to whole group
Brassicas	Cauliflower or broccoli and cabbage and one other Brassica to whole group

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Crop	Recommendation
Leafy vegetables (also see stem vegetables)	Head and leafy lettuce and spinach to leafy vegetables Cos lettuce to leafy Asian vegetables
Herbs	Two leafy herbs to whole group
Legume vegetables (fresh)	Beans green and peas green to whole group
Stem vegetables	Celery to leafy petioles sub group
Pulses	Any dried bean and dried pea to whole group
Oilseeds	Any 3 oilseeds to whole group
Cereals	Rice plus any two other cereals to whole group including rice

Abbreviations and acronyms in this chapter

CAC	Codex Alimentarius Commission
cGAP	critical GAP
EC	emulsifiable concentrate
FAO	Food and Agriculture Organization
GAP	good agricultural practice
HR	highest residue
JMPR	Joint Meeting on Pesticide Residues
LOQ	limit of quantification
MRL	maximum residue limit
OECD	Organization for Economic Co-operation and Development
PHI	preharvest interval
SC	suspension concentrate
SL	soluble concentrate
STMR	supervised trials median residue
WG	water dispersible granules
WHO	World Health Organization
WP	wettable powder

Chapter 8. Evaluation of Supervised Trial Data. Estimation of Maximum Residue Levels and STMR and HR values.

Supervised trials – data variability in a set of trials
Effect of application rate, number of applications, formulation and PHI or growth stage on residue levels
Supervised trials – data extraction and data validity
Are the conditions of a supervised trial in accord with critical GAP?
Evaluation of supervised trials data – no complications situation
Evaluation of supervised trials data when two residue definitions apply
Residues from the use of grain protectants
When residues are essentially zero
Residues in samples from the control plot
MRLs for commodity groups
Minor crops
Statistical methods for estimation of maximum residue levels

The purpose of this chapter is to explain how to evaluate supervised trials data to produce MRLs suitable for Codex adoption and STMR and HR values suitable for use in risk assessments.

Relevant sections of JMPR Manual

Supervised trials – data variability in a set of trials

Supervised trials for estimating maximum residue levels are scientific studies in which pesticides are applied to crops or animals according to specified conditions intended to reflect commercial practice after which harvested crops or tissues of slaughtered animals are analysed for pesticide residues. Usually specified conditions are those which approximate existing or proposed GAP (JMPR Manual).

Supervised trials should be designed to cover the range of practical situations that occur during the production of the crop or the farm animal. A good set of supervised trials will include the different geographic areas of the crop where the pesticide will be used, more than one season if the geographic areas are close together, the crop varieties produced commercially, the cultural practices normally followed and any special application methods as described on the label.

A well-designed set of trials will naturally produce a range of residue data that reflect the range of conditions tested.

"An awareness of the expected variability of residues is necessary. If the data truly reflect the range of conditions, application methods, seasons and cultural practices likely to

be encountered commercially, then considerable variation in the resulting residue levels is expected. Analysis of supervised trials evaluated by the JMPR between 1997 and 2007 revealed that the coefficient of variation of residues between fields can sometimes be over 110%..... It is not a criticism to say that the data are widely spread and variable. If results have been obtained at a number of places over some years they are likely to be a better approximation to commercial practice and will be widely spread." (JMPR Manual).

Information is available on residue levels when a pesticide is used in the same way, but at different sites, by different operators and with different application equipment. "Use in the same way" means following the same label instructions. Examples are recorded in JMPR Residue Evaluations.

Datasets were chosen where at least 8 trials were available (1 residue value per trial), all with the same application rate, same pre-harvest interval (PHI) and same number of applications in the one country. Median values are underlined.

Examples of datasets

Grapes, cyprodinil, France, 0.38-0.50 kg ai/ha, PHI 42-89 days, JMPR 2003: 16 trials: 0.02, 0.05, 0.06, 0.12, 0.16, 0.17, 0.18, <u>0.18</u>, <u>0.24</u>, 0.29, 0.31, 0.33, 0.36, 0.37, 0.44 and 0.78 mg/kg.

Acephate, mandarins, Japan, 0.05 kg ai/hl, PHI 26-60 days, JMPR 2003: 14 trials: 0.38, 0.40, 0.49, 0.68, 0.78, 0.85, <u>0.88</u>, <u>0.98</u>, 1.7, 1.7, 1.8, 1.8, 2.6 and 5.2 mg/kg.

Methamidophos residues, same 14 trials: 0.02, 0.03, 0.06, 0.04, 0.05, 0.08, <u>0.09</u>, <u>0.08</u>, 0.09, 0.14, 0.10, 0.15, 0.25 and 0.26.

Flutolanil, rice, USA, 0.56-0.62 kg ai/ha, PHI 30 days, JMPR 2002; 10 trials: 0.22, 0.25, 0.62, 0.99, <u>1.1</u>, <u>1.3</u>, 1.4, 1.7, 1.7 and 6.2 mg/kg.

Potatoes, chlorpropham, USA, post-harvest 0.015 kg ai/t, JMPR 2001: 19 trials: 8.2 (2), 8.7, 8.9, 9.1, 9.3, 9.4, 9.7, 9.9, <u>11</u> (3), 13, 14 (2), 16 (2), 18 and 23 mg/kg.

The maximum value in the set of trials is typically 3-4 times as high as the median value, but there are examples where the maximum is 10 or more times as high as the median.

The question of "outliers" occurs during residue evaluation. Statistical tests for outliers are not valid if they rely on assumptions of normal or other specific types of distribution. There has to be a clear reason from the conduct of the trial to exclude the data from consideration, e.g. the crop was badly affected by drought or disease and was not of commercial size or quality.

If we look at the data distributions where there are many trials and then imagine only 4 or 5 of those trials with 1 value near the top of the range and the remainder of the 4-5 towards the bottom of the range, the high one will superficially appear as an 'outlier'.

Effect of application rate, number of applications, formulation and PHI or growth stage on residue levels

Effect of application rate on residue levels

The JMPR normally accepts ±25 % in the application rate as being equivalent to the label statement. One would not expect that experimenters (or farmers) would be more accurate than this in practice.

Evidence has accumulated that, in many situations, residue levels are proportional to application rates.

Effect of number of applications on residue levels

Information from residue decline curves can be very helpful in deciding how many applications will influence the residue level at harvest.

For example, if a pesticide is applied at intervals of 10 days and the residue declines with a half-life of 8 days, the residue on day zero of the final application would be influenced by the previous 2 applications (10 and 20 days previously). The application 30 days earlier (more than 3 half-lives) would contribute less than 25 % to the final residue and may be disregarded. Under these conditions, trials with 3 or more applications may be taken as producing the same residues.

Effect of formulation on residue levels

In many situations different formulations would cause no more variation than other factors, and data derived with different formulations would be considered comparable. The most common formulation types which are diluted in water prior to application include EC, WP, water dispersible granules (WG), suspension concentrates (SC) (also called flowable concentrates), and soluble concentrates (SL). Experience from trials demonstrates that these formulations lead to similar residues. (JMPR Manual).

A recent paper[14] compared residues in commodities from the use of different formulations in side-by-side trials and supported the hypothesis that residue concentrations are equivalent from use of the different formulations tested. Comparisons were made between WP and EC; CS capsule suspension and EC, EW emulsion oil in water and EC; WP and SC.

Effect of PHI on residue levels

The pre-harvest interval (PHI) is the time interval between the last application of a pesticide to a crop and harvest. The PHI for many pesticide uses is an important component of GAP and, where appropriate, is part of the directions for use printed on the label.

In evaluating residue data we should accept supervised trials data with PHIs with an allowed variation around the label PHI. The allowance is generally taken as the intervals corresponding with a ± 25 % change in the residue level. The rate of change of residue levels may be obtained from the residue decline curves.

The allowance for PHI data acceptance will be much wider for a stable residue than a rapidly declining one.

Timing of application set by PHI or by a growth stage instruction

The timing of application may be described by a crop growth stage instruction instead of a PHI in some situations. A growth stage instruction is often preferable to a PHI instruction when the time interval is some months, e.g.

- apply to apple trees just prior to blossom
- apply to soybeans up to the 4-leaf stage.

A systematic code for describing the growth stages of crops has been published.[15] It standardises the descriptions, which makes growth stage instructions more understandable.

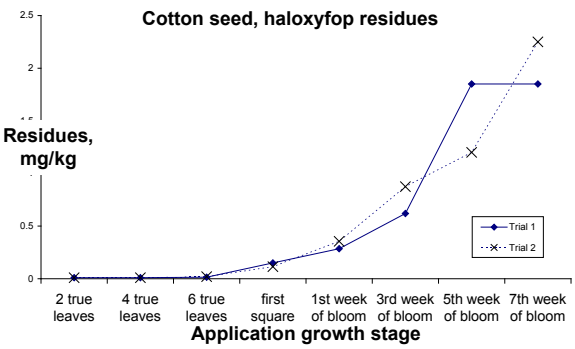
[14] MacLachlan DJ and Hamilton D. 2010. A new tool for the evaluation of crop residue trial data (day zero-plus decline)', *Food Additives & Contaminants*: *Part A,* 27:347-364.

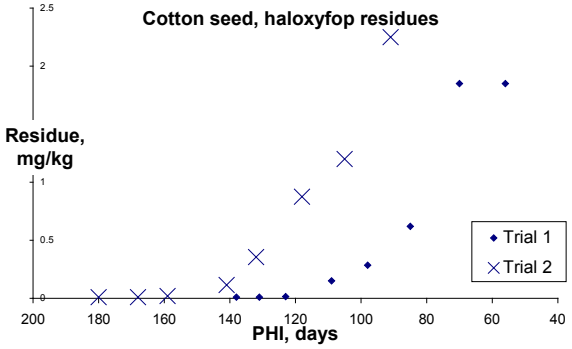
[15] Meier U (ed). 2001. Growth stages of mono-and dicotyledonous plants, BBCH Monograph. 2nd edition. Federal Biological Research Centre for Agriculture and Forestry. Germany.

Example – haloxyfop use patterns and residues (JMPR 2009)

Haloxyfop and haloxyfop-methyl are herbicides, so, when the whole field is sprayed, the main intention is to spray the weeds. When a field crop is at a very early stage, e.g. up to 4 leaves, its percentage field cover is small and it will receive only a small percentage of the dose per unit area. At later growth stages, its percentage field cover will be larger and the crop will receive a larger dose even for the same application rate per unit area. The residue in the harvested commodity will be very much dependent on the crop growth stage at application.

The 2009 JMPR reported the results of trials examining the effect of growth stage at time of application on the haloxyfop residue levels appearing in cotton seed at harvest. In two sets of trials haloxyfop-methyl was applied once to cotton crops at eight different growth stages from 'two true leaves' up to 'seventh week of bloom.' When the residue levels were expressed as a function of growth stage and of interval between treatment and harvest (PHI), the growth stage appeared to be a better predictor of likely residues.





Point to note

- A crop growth stage instruction may be more useful than a PHI instruction for controlling application timing in some cases.

Supervised trials – data extraction and data validity

Most supervised trials are carried out by pesticide companies or under the sponsorship of pesticide companies. The companies must generate and provide such data and all the supporting information as part of the requirements for national pesticide registration.

The companies then may also provide the studies to JMPR when the compound is nominated for review as one step in the process of establishing Codex MRLs.

Construction of the summary data tables is part of the evaluation process.

The evaluator should include only valid data in the evaluation. If a trial is of questionable validity or if vital information is missing, the trial should either not be entered into the evaluation summary tables or if it is entered it should be footnoted with an explanation of the problem and the response from the proponent.

The aim in constructing the summary tables is to produce a summary of valid data. Interpretation is difficult if questionable data are mixed with valid data.

Contents of a supervised trials study

- Title, authors, date of completion or issue, study identification number.
- Study report.

- Abstract or summary.
- Identification and description of test substances.
- Testing facilities – field and laboratory.
- Data tables.

– Field report.
- Location, crop and variety, growing and production conditions, plot sizes, pesticide treatments, application equipment, harvest, nature and size of samples, sample identification, storage conditions, transport to laboratory.
- Calendar of all events.

– Laboratory report.
- Receipt and storage of samples, sample identification, sample preparation, nature of analytical samples, nature of analyte or analytes, extraction and analysis, analytical method, method validation, procedural recoveries, analytical results, expression of analytical results.
- Calendar of sample receipt, sample preparation, extraction, analysis.
- Comments on anomalies or anything affecting the reported results, e.g. poor recoveries, residues in control samples, possible sample identity confusion, adjustment of results for recoveries or losses during storage, methods of expressing the results.

– Annexes
- Chromatograms and calibrations from the sample analyses, laboratory work sheets.
- Field data, field notebooks, weather, irrigation details.
- Study protocols.

Obtaining information from a supervised trials study

A summary table from JMPR provides a checklist of the data to be recorded from supervised trials.

CHERRIES country, year (variety)	Application					PHI	Commodity	Residue, mg/kg				Ref
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no. interval	days		pesticide		metabolite		
								fruit	flesh	fruit	flesh	
France, 2005 (Montmorency)	WG		0.0074 0.0077	1470 +1540	2 7d	0- 0 3 7 10 14	whole fruit and flesh	0.32 0.61 0.34 0.31 0.20 0.13	0.36 0.68 0.38 0.34 0.22 0.14	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02	05-0416 AF/8641/SY/1

Column 1: crop, country, year of the trial, crop variety.

Application: formulation, application rate (kg ai/ha), spray concentration (kg ai/l), spray volume per hectare, number of applications and interval between applications.

PHI: pre-harvest intervals (note that 0- is used for samples taken just prior to the final application).

Commodity: commodity for the reported residue.

Residue: residue concentrations, mg/kg, <0.02 means that the residue is below an LOQ of 0.02 mg/kg.

Residue in samples from control (untreated) plot: data are checked in every case, but entered into the summary table only when residues are equal to or greater than the LOQ.

Ref: the study or report number appearing in the reference list at the end of the evaluation.

Supporting information in the study must also be checked to provide assurance for the data validity.

Checklist for supporting information

- analytical method,
- procedural recoveries;
- sprayer and sprayer calibration
- plot size
- trial design, replicate samples from a single plot or samples from replicate plots;
- field sample size,
- date of harvest
- date of extraction and analysis
- interval of time sample stored in freezer;

One way of checking the supporting information is to record the checks in a spreadsheet.

Crop	Countr	Study	Analyt method	Analyt recov	Sprayer	Plot ze	Sample	Trial design	Sample date	Analyt date	Storage interval days	Storage
barley	USA ID)	02-711	PG 615	65-118% n=8	CO_2 backpack	260 m^2	1.2 kg	unreplicated single plot	31-Jul-02	17-Feb-03	201	OK

Are the conditions of a supervised trial in accord with critical GAP?

Good agricultural practice in the use of pesticides (GAP) includes the nationally authorized safe uses of pesticides under actual conditions necessary for effective pest control. It encompasses a range of levels of pesticide applications up to the highest authorized use, applied in a manner which leaves a residue which is the smallest amount practicable. (JMPR Manual).

The essential GAP is the set of current registered uses involving the highest rates and shortest PHIs for the same pesticide on the same crop in the same country and the use patterns in the supervised field trials should reflect this essential (often referred to as critical) GAP.

JMPR has developed a set of guidelines for evaluating trial data and estimating maximum residue levels, STMR values and HR values.

- Only one data point is taken from each trial.
- Residue data from countries are evaluated against the GAP (Good Agricultural Practice, national registered uses) in the country of the trials or a neighbouring country with similar climate and cultural practices.
- Trial conditions should be comparable with the maximum registered use (critical GAP).
    - Generally, application rates should be within ±25 % of the nominal rate, which includes the probable variation in commercial practice.
    - The influence of number of applications on the residue depends on residue persistence, intervals between applications and the nature of the crop. Residue decline trials provide data on persistence, to help the decision.

  - Decisions on the tolerances for intervals between final treatment and harvest also depend on residue persistence (obtained from residue decline trials). The acceptable range around the official PHI is equivalent to a calculated ±25 % change in residue level.

- Where residues are below LOQ, data from higher application rates may be included.
- Where several values are reported from replicate plots or replicate field samples from a single trial the mean residue is chosen.
- Where two or more trials are carried out at the same location with the same equipment within a growing season, they are generally not recognized as sufficiently different to be described as independent. In this situation, the trial producing the highest residues is selected and the highest residue is chosen.
- Where several values are reported from replicate analyses of the same field sample the mean residue is chosen.
- If a residue level in a trial at the GAP pre-harvest interval is less than another residue at longer PHI, the higher value is chosen.
- Where all trials data are <LOQ, the STMR and HR would be assumed to be at the LOQ unless other evidence suggests residues are "essentially zero". Such evidence may be provided, for example, from the metabolism studies or from trials at exaggerated rates.

Evaluation of supervised trials data – no complications situation

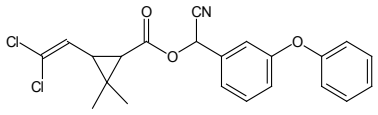
Many cases are simple, with no complications.

- Residue definitions: same for enforcement and dietary risk.
- Commodity: commodity of trade and edible portion are the same.
- Conditions of trials: application rate, PHI, etc agree with critical GAP.
- Location of trials: in the country with suitable GAP.

After the valid trials are summarised in the data tables, the single residue from each trial should be selected and underlined. All of the selected values should then be listed in rank order with the median value underlined.

The dataset is ready for estimation of maximum residue level, STMR and HR values and for suitable statistical calculation as required.

Example – zeta-cypermethrin on pears. JMPR 2008.



US GAP for pome fruit allows the use of zeta-cypermethrin at 0.056 kg ai/ha with a PHI of 14 days.

In 12 US trials matching critical GAP, zeta-cypermethrin residues on pears were: 0.05, 0.05, 0.06, 0.07, 0.24, <u>0.29</u>, <u>0.31</u>, 0.33, 0.39, 0.43, 0.49 and 0.56 mg/kg.

The table shows the summary data for 2 of the 12 trials, one of which is a decline trial (Trial 15).

PEARS	Application				PHI	Commodity	Residue, mg/kg	Ref
country, year (variety)	Form	kg ai/ha	water (l/ha)	no.	days		zeta	
USA (CA), 2001 (Shinko)	EC	0.056	890-920	6	7	pear	0.06 0.07	P-3559 Trial 15
					14	pear	0.06 0.06	
					21	pear	0.06 0.06	
					28	pear	0.05 <u>0.07</u>	

PEARS	Application				PHI	Commodity	Residue, mg/kg	Ref
country, year (variety)	Form	kg ai/ha	water (l/ha)	no.	days		zeta	
USA (WA), 2001 (Bartlett)	EW	0.056	190	6	14	pear	<u>0.29</u>	P-3559 Trial 16

From inspection of the dataset, 0.7 or 1 mg/kg (preferred) would be suitable as a maximum residue level. The draft OECD calculator produces a "mean+4SD = 0.996" and a "3×mean=0.818", also suggesting a 1 mg/kg.

Recommendations for cypermethrin on pears:

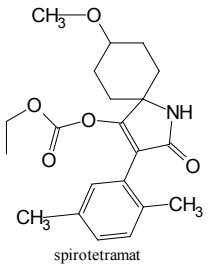
- maximum residue level 1 mg/kg
- STMR 0.30 mg/kg
- HR 0.56 mg/kg

Evaluation of supervised trials data when two residue definitions apply

The data generated with the enforcement residue definition are needed for the estimation of the maximum residue level while data with the risk assessment definition are needed for the STMR and HR.

This means that the samples from supervised residue trials should be analysed in such a way as to cover both situations. Ideally, the parent compound and the relevant metabolites should be reported individually, so that the needs of the different residue definitions can be met.

Example – spirotetramat residues on grapes (JMPR 2008).



spirotetramat

Spirotetramat has different residue definitions for enforcement and dietary intake for plant commodities.

Residue for enforcement, plant commodities: spirotetramat plus spirotetramat enol, expressed as spirotetramat.

Residue for dietary intake, plant commodities: spirotetramat plus the metabolites enol, ketohydroxy, enol glucoside, and monohydroxy, expressed as spirotetramat.

USA and Canada GAP: 0.14 kg ai/ha, 0.22 kg ai/ha/season, with a 7 days PHI.

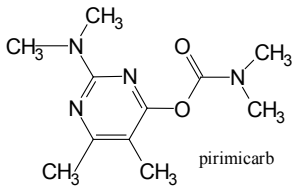
In trials in accord with US GAP, the residues (spirotetramat plus enol, n = 15) in rank order for grapes were: 0.057, 0.14, 0.21, 0.23, 0.24, 0.26, 0.31, 0.32, 0.34, 0.36, 0.44, 0.49, 0.58, 0.62, 1.0 mg/kg. (Dataset with enforcement residue definition).

From inspection of the dataset, 2 mg/kg would be suitable as a maximum residue level. The draft OECD calculator produces a "mean+4SD = 1.30" and a "3×mean=1.119".

Residues in rank order, median underlined, of (spirotetramat plus 4 metabolites, n = 15) on grapes were: 0.11, 0.26, 0.29, 0.32 (2), 0.36, 0.40, <u>0.41</u>, 0.48 (2), 0.55, 0.65, 0.79, 0.85, 1.3 mg/kg. (Dataset with dietary intake risk assessment residue definition).

Conclusions: STMR = 0.41 mg/kg, HR = 1.3 mg/kg, (risk assessment residue definition)
maximum residue level = 2 mg/kg (enforcement residue definition).

Example – pirimicarb residues on citrus (JMPR 2006).



Pirimicarb has different residue definitions for enforcement and dietary intake for plant commodities.

The commodity of trade is whole fruit. The edible portion is the citrus flesh.

Residue for enforcement, plant commodities: pirimicarb.

Residue for dietary intake, plant commodities: sum of pirimicarb, demethyl pirimicarb and demethyl formamido pirimicarb, expressed as pirimicarb.

Maximum residue level to be expressed on whole fruit.
STMR and HR to be expressed on edible portion.

Spanish GAP for citrus: foliar application of 0.05 kg ai/l with 7 days PHI.

In 6 orange trials in accord with Spanish GAP in Italy and Spain, pirimicarb residues in whole fruit were: 0.11, 0.11, 0.25, 0.27, 0.37 and 0.40 mg/kg.

In the same 6 trials, residues (dietary intake residue definition) in orange pulp (edible portion) were: < 0.01 (5) and 0.01 mg/kg.

In 8 mandarin trials in accord with Spanish GAP in Italy and Spain, pirimicarb residues in whole fruit were: 0.35, 0.68, 0.77, 0.87, 1.2, 1.2, 1.8 and 2.2 mg/kg.

In the same 8 trials, residues (dietary intake residue definition) in mandarin pulp (edible portion), median underlined, were: < 0.01, 0.01, 0.01, <u>0.01</u>, <u>0.02</u>, 0.03, 0.04 and 0.08 mg/kg.

A citrus fruits GAP and sufficient residue data suggested a citrus fruits maximum residue level would be in order and it would rely on the mandarin residues, which were higher than the orange residues.

From inspection of the dataset, 3 mg/kg would be suitable as a maximum residue level. The draft OECD calculator produces a "mean+4SD = 3.58" and a "3×mean=3.40".

Conclusions: Citrus fruits - STMR = 0.015 mg/kg, HR = 0.08 mg/kg, maximum residue level = 3 mg/kg.

Points to note.

- The maximum residue level was based on the enforcement residue definition and the whole commodity.
- The STMR and HR, designed for dietary intake estimation, were based on the dietary intake residue definition and the edible portion of the commodity.
- The citrus group estimations were based on mandarin data which appeared to be higher than the orange data. It is not appropriate to combine datasets that appear to be different populations; in this case it would have produced a lower STMR.

Residues from the use of grain protectants

Grain protectants are designed for post-harvest application to cereal grains to protect the grain from stored products insects. These uses are quite different from pre-harvest uses,

where pesticides are applied to the crop and the resulting residues are subject to the vagaries of crop growth and weather conditions.

In post-harvest uses, the pesticide is applied directly to the food commodity at an application rate measured in grams of active ingredient per tonne. If applied evenly and efficiently, the concentration of the pesticide in the grain measured as mg/kg should be close to the application rate in g ai/tonne, an important point when a maximum residue level is to be estimated.

Supervised trials with grain protectants usually involve treatment of the grain and storage in a silo or simulated silo conditions for intervals of time likely to occur in commercial practice, i.e. for 6 months to 1 year. After storage, the grain is processed and converted to food products to determine likely human exposure to the grain protectant.

Example – cypermethrin residues in wheat, postharvest use. JMPR 2009.

Residue definition: cypermethrin.

French GAP: a cypermethrin UL (ultra-low volume) formulation containing cypermethrin and piperonyl butoxide is registered for post-harvest use on cereal grain at a rate of 1.7 g cypermethrin per tonne of grain.

In four trials with wheat treated at 1.7 g ai/tonne and stored for 7 days (2 trials) and 270 days (2 trials), residues were:
day 1: 1.11, 1.17, 1.2, 1.35 mg/kg
day 7: 1.07, 1.3, 1.4 and 1.5 mg/kg.
day 180: 1.3, 0.96 mg/kg
day 270: 1.3, 0.99 mg/kg.

Residues were quite stable in the storage – no measurable decline. The highest residue in each of the 4 trials was: 1.11, 1.35, 1.40, 1.5.

The application rate (1.7 g ai/tonne) was taken into account in the estimation of a maximum residue level of 2 mg/kg. The STMR and HR for wheat were 1.38 and 1.5 mg/kg, respectively.

Processing factors for cypermethrin, available from milling studies on wheat, bran 2.5 and flour 0.35, were applied to the wheat STMR and HR:
flour STMR-P = 1.38×0.35 = 0.48 mg/kg flour HR-P = 1.5×0.35 = 0.53 mg/kg.
bran STMR-P = 1.38×2.5 = 3.45 mg/kg bran HR-P =1.5×2.5 = 3.75 mg/kg

The flour HR-P (0.53 mg/kg) is less than the wheat maximum residue level (2 mg/kg), so a maximum residue level for flour is not necessary.

The bran HR-P (3.75 mg/kg) exceeds the wheat maximum residue level (2 mg/kg), so a maximum residue level for bran is necessary.

The maximum residue level for wheat bran was estimated as 5 mg/kg.

Points to note.

- The maximum residue level for wheat was strongly influenced by the post-harvest application rate.
- Residues were very stable during storage and the highest measured residue at any time during the storage was taken to represent each trial.
- STMR-P and HR-P values were estimated for processed commodities.

- Residues in flour were less than in wheat so a maximum residue level was not required.
- Residues in bran were higher than in wheat, necessitating a maximum residue level for wheat bran.

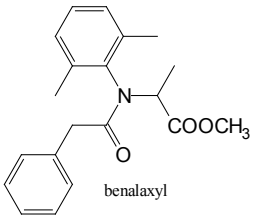
When residues are essentially zero

When residues are not detected or below LOQ in every trial on a particular commodity for a specified use pattern, it may just be that the residues would be measurable with an improved method that could achieve a lower LOQ. For this case, STMR and HR would be estimated as = LOQ.

Alternatively, it could be that residues are "essentially zero" because they dissipated or degraded quickly, or would never reach parts of the crop not directly sprayed. For this case, STMR and HR would be estimated as 0 mg/kg.

Evidence is needed to sustain the "essentially zero residues" case. Evidence from metabolism studies or from supervised trials with exaggerated application rates and still no residues would support the case.

Example – benalaxyl residues in potatoes (JMPR 2009).



Brazilian GAP: 2 foliar applications of benalaxyl at 0.24 kg ai/ha, 7 days PHI.

Brazilian trials: 5 trials in accord with critical GAP and 5 trials at double rate. Benalaxyl residues <LOQ (0.1 mg/kg) in potatoes from all 10 trials.

French and Italian GAP: 4 foliar applications of benalaxyl at 0.24 kg ai/ha, 7 days PHI.

French and Italian trials: 6 trials in accord with critical GAP. Benalaxyl residues <LOQ (0.02 mg/kg) in potatoes from all 6 trials.

The potato metabolism study showed no transfer of radioactivity to the tubers, suggesting that benalaxyl residues are not expected in potatoes.

Estimated maximum residue level: benalaxyl in potato 0.02(*) mg/kg

Estimated STMR and HR of 0 and 0 mg/kg.

Points to note.

- No residues appeared in potato tubers from trials in different places, some at double application rate.
- Benalaxyl was not translocated to tubers in the metabolism study.
- The evidence suggests an "essentially zero" residue situation for benalaxyl in potato, so STMR=HR=0.

Residues in samples from the control plot

Control sample (field) [16]: sample from a field test plot to which no pesticide was applied (a zero rate sample) or which received chemical treatments identical to the test plots except for the test chemical.

[16]Stephenson GR, Ferris IG, Holland PT and Nordberg M. 2006. IUPAC Glossary of terms relating to pesticides. *Pure Appl. Chem.* 78:2075–2154.

Residues may sometimes appear in samples from the control (untreated) plot of a supervised trial. The explanation may be drift from the treated plots, storm runoff from treated plots (for a systemic compound) or sample mislabelling.

The residue evaluator must decide if the trial data are valid if residues occur in the control samples.

If the residue in the control is very low, e.g. close to LOQ, and the residues in the treated samples of interest are much higher, the trial data may be accepted as valid. Otherwise, the affected residue data should generally not be regarded as valid. However, there may be other information available that should also be taken into account.

Examples – supervised trials with residues appearing in samples from control plots

Crop	Application				PHI	Commodity	Residue, mg/kg	Ref
Pesticide	Form	kg ai/ha	kg ai/hl	no.	days			
Black currants Spirodiclofen	SC	0.096	0.0096	1	14 21	fruit	<0.01 0.065 c = 0.08	JMPR 2009
Olives Alpha-cypermethrin	SC	0.015	0.0015	1	0 3 7 14	olives	0.02 0.02 0.04 0.01 c = <0.01, 0.04, 0.01, <0.01	JMPR 2008
Lettuce Pirimicarb	WG	0.25	0.05	2	0 3 7 10	lettuce	5.1 c = 0.03 2.9 c = 0.04 2.7 c = 0.02 2.8 c = 0.01	JMPR 2006 Note: pre-trial contamination of control plots
Brussels sprouts Cyfluthrin	EC	0.050		12	1	heads	0.44 c = 0.01	JMPR 2007
Carrots Difenoconazole	EC	0.13		4	0	root	<0.02 c = 0.19	JMPR 2007 Noted in the study report, perhaps test and control samples switched

c: sample from untreated control plot.

In most of these cases the residues in the control plots made the trials data unacceptable. In the lettuce, although the residues in the controls were much less than in the samples from treated plots, the many detections suggested a general contamination problem. The Brussels sprouts trial was acceptable because the residues were low (at LOQ) and much below the residues in the samples from the treated plots.

MRLs for commodity groups

The JMPR Manual (Section 6.7) explains the many factors and possibilities for recommending commodity group MRLs.

A group MRL can be achieved in the simplest case by satisfying two requirements.

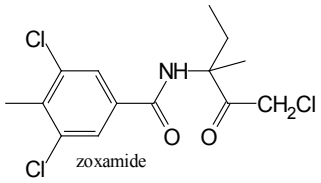
- The pesticide is registered or authorized on the crop group.
- Relevant and adequate residue data are available for at least one major commodity of the group.

An implicit assumption in this statement is that the crop group with the registered uses corresponds with the commodity group with the MRLs.

Commonly adopted commodity group MRLs are for citrus fruits, pome fruits, stone fruits and cucurbit fruiting vegetables. Evaluation is reasonably straight forward because the registered use may specify a crop group that matches a commodity group for the MRL.

Tree nuts are also a commonly adopted commodity group MRL, but the reason for many pesticides is that no residue reaches the kernel, and the MRL can be readily extrapolated to the group.

Example – zoxamide residues in cucurbit fruiting vegetables (JMPR 2009)



zoxamide

US GAP: cucurbits: 8 foliar applications of zoxamide at 0.22 kg ai/ha, 0 days PHI.

Cucumber: 6 US trials in accord with critical GAP: 0.01, 0.02, 0.03, 0.05, 0.12, 0.13 mg/kg.

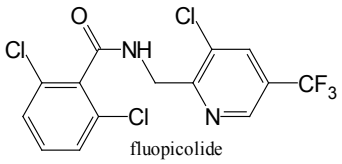
Cantaloupe: 6 US trials in accord with critical GAP: 0.04, 0.06, 0.08, 0.37, 0.44, 0.73 mg/kg.

Summer squash: 5 US trials in accord with critical GAP: 0.08, 0.10, 0.15, 0.19, 0.39 mg/kg.

The cantaloupe had the highest residues, so the cantaloupe data were used to support the group recommendations. An HR is not needed because an ARfD is unnecessary for zoxamide.

Fruiting vegetables, cucurbits:
maximum residue level: 2 mg/kg
STMR: 0.225 mg/kg

Example – fluopicolide residues on fruiting vegetables other than cucurbits (JMPR 2009)



fluopicolide

US GAP: fruiting vegetables: foliar applications of fluopicolide at 0.14 kg ai/ha, max 0.42 kg ai/season, 2 days PHI.

Tomatoes: 12 US trials in accord with critical GAP: 0.05, 0.06, 0.08, 0.10, 0.15, 0.15, 0.17, 0.17, 0.19, 0.19, 0.28 and 0.42 mg/kg.

Sweet peppers: 7 US trials in accord with critical GAP: 0.04, 0.05, 0.09, 0.15, 0.17, 0.19 and 0.57 mg/kg.

Chilli peppers: 3 US trials in accord with critical GAP: 0.10, 0.36 and 0.58 mg/kg.

Combined peppers data: 0.04, 0.05, 0.09, 0.10, 0.15, 0.17, 0.19, 0.36, 0.57 and 0.58 mg/kg.

It was decided to use the combined peppers data to represent the fruiting vegetables group, but to exclude mushrooms and sweet corn.

Fruiting vegetables other than cucurbits (except mushrooms and sweet corn):
maximum residue level: 1 mg/kg
STMR: 0.16 mg/kg
HR 0.58 mg/kg

Points to note:

- If, for some reason, one or two commodities do not fit well into a group MRL, it is possible to propose an MRL for the group except …….
- Mushroom and sweet corn may be seen as vegetables when they are commodities in trade. Clearly, they are not vegetable crops. In the US, the fruiting vegetable crop group is: egg plant, ground cherry, pepino, peppers (bell, chilli, cooking, pimento, sweet), tomatillo, tomato. So, the crop group "fruiting vegetables" does not currently match the commodity group "fruiting vegetables".

Minor crops

A minor use crop may be defined as a crop that is grown on a small area and therefore uses amounts of pesticides that are too small to justify standard pesticide registration[17].

MRLs may be obtained for pesticide residues on commodities from minor crops by:

- inclusion in a commodity group MRL;
- extrapolation from pesticide uses on a relevant major crop;
- evaluation of an adequate data package for the use on a minor crop.

MRLs for commodity groups have been described previously in this chapter.

Examples of extrapolation from uses on a major crop

Extrapolation is possible if the GAP of the minor crop is similar to that of a relevant major crop (e.g. in the same crop grouping).

Examples of JMPR extrapolations

Major crop	Extrapolate to	Basis	Pesticide	Reference
Potato	sweet potato	similar GAP	ethoprophos	JMPR 2004
Raspberry	blackberry, dewberry	not stated	fludioxonil	JMPR 2004
Bulb onion	garlic	same GAP, nil residue	dimethenamid-P	JMPR 2005
Bulb onion	shallot	same GAP, nil residue	dimethenamid-P	JMPR 2005
Cucumber	gherkin	same GAP	fenhexamid	JMPR 2005
Potato	sweet potato	same GAP, nil residue	dimethenamid-P	JMPR 2005
Bulb onion	garlic	'vegetable' GAP	pirimicarb	JMPR 2006
Cucumber	gherkins	no extrapolation[18]	thiacloprid	JMPR 2006
Rapeseed	white mustard	similar GAP	thiacloprid	JMPR 2006
Wheat	triticale	similar GAP	aminopyralid	JMPR 2006
Banana	plantain	same GAP	azoxystrobin	JMPR 2008
Wheat	triticale	similar GAP	azoxystrobin	JMPR 2008
Wheat	triticale	same GAP	lambda-cyhalothrin	JMPR 2008

Points to note

- GAP for the minor crop must be the same as or similar to that of the major crop
- GAP for the minor crop must be valid, e.g. on a registered label.

[17] Stephenson GR, Ferris IG, Holland PT and Nordberg M. 2006. IUPAC Glossary of terms relating to pesticides. *Pure Appl. Chem.* 78:2075–2154.

[18] The different surface area-to-mass ratios mean that higher residues are expected in gherkins than in cucumbers, so extrapolation is not possible.

Examples of minimum data packages for the minor crop

JMPR accepts that three supervised residue trials at critical GAP are the minimum for a minor crop. It should be noted that the trials must be fully documented and valid and must be supported by valid GAP information.

Examples of minor-crop decisions by JMPR

Crop	Pesticide	Number of available trials at critical GAP	Decision	Reference
Chilli peppers	bifenazate	3 + support from bell pepper data	Minor crop – sufficient for MRL	JMPR 2006
Hops	bifenazate	3	Minor crop – sufficient for MRL	JMPR 2006
Celeriac	difenoconazole	3	Minor crop – sufficient for MRL	JMPR 2007
Chilli peppers	difenoconazole	2, also 2 at double rate	Minor crop – insufficient, require 3 trials	JMPR 2007
Cranberry	carbaryl	4	Minor crop – sufficient for MRL	JMPR 2007
Artichoke, globe	azoxystrobin	3	Minor crop – sufficient for MRL	JMPR 2008
Carambola	cypermethrin	5	Minor crop – sufficient for MRL	JMPR 2008
Pistachio	azoxystrobin	3	Minor crop – sufficient for MRL	JMPR 2008
Pomegranate	imidacloprid	3	Minor crop – sufficient for MRL	JMPR 2008

Points to note

- 3 valid trials at critical GAP with full documentation (trial design, application records, analytical method and recoveries, etc) are necessary
- GAP for the minor crop must be valid, e.g. on a registered label.

Statistical methods for estimation of maximum residue levels

Statistical methods for calculating MRLs directly from the results of supervised residue trials have been available for a number of years.

The task of calculating MRLs from the results of residue trials is formidable:

- sets of residue trials are generally not designed for statistical calculation,
- a dataset is unlikely to be a random representative sample of crop varieties, application equipment, geographical locations and weather conditions that occur in commercial practice;
- the number of data points is limited, necessitating extrapolation beyond the range of observations;
- data distributions are generally unknown.

Consequently, the situation is evolving with new methods and modifications to old methods appearing each year.

JMPR has used the NAFTA calculator (described in the JMPR Manual) in recent years but has also recently tested a version of an OECD calculator (highest of 'mean+4SD', '3×mean', 'highest residue').

JMPR uses the calculators as tools in estimating maximum residue levels. Other information, such as residues on related commodities, residues from other application rates, expected residues from the application rate, etc., are taken into account in the expert judgement process.

Only limited information is currently available about the uncertainty of the calculator results.

The calculated MRLs from the different calculators are often similar, but may be quite different in particular cases. The differences may reflect the uncertainty in the calculated results.

Table. JMPR 2009. Selected supervised trials supporting MRL recommendations – a comparison of NAFTA and OECD MRL calculations. The reported calculations are unrounded.

Pesticide	Commodity	Data	n	JMPR MRL	NAFTA	OECD
Buprofezin	strawberries	0.09, 0.15, 0.39, 0.44, 0.55, 0.85, 1.24	7	3 strawberries	3.19	2.14
Chlorpyrifos-methyl	peaches	<0.01, <0.01, 0.01, 0.01, 0.02, 0.02, 0.02, 0.02, 0.02, 0.06, 0.07, 0.08, 0.17, 0.23	14	0.5 stone fruits	0.117	0.323
Chlorpyrifos-methyl	peppers	0.03, 0.03, 0.04, 0.04, 0.06, 0.06, 0.14, 0.16, 0.16, 0.52, 0.72	11	1 peppers	0.372	1.093
Fenbuconazole	almond hulls	0.10, 0.13, 0.45, 0.51, 0.77	5	3 almond hulls	2.407	1.513
Fluopicolide	celery	0.16, 0.76, 1.0, 1.4, 5.2, 6.7, 14	7	20 celery	10.15	24.1
Fluopicolide	head cabbage	0.31, 0.36, 0.61, 1.2, 1.9, 2.3, 3.9	7	7 head cabbage	8.7	6.72
Fluopicolide	onions	0.01, 0.05, 0.05, 0.07, 0.08, 0.11, 0.58	7	1 onions	0.5075	0.929
Haloxyfop	beans (dry)	0.01, 0.06, 0.07, 0.08, 0.08, 0.21, 0.32, 0.39, 0.42, 0.86, 1.5, 1.5, 1.8, 2.0	14	3 beans (dry)	2.0709	3.557
Haloxyfop	rapeseed	<0.01, <0.01, <0.01, <0.05, <0.05, <0.05, <0.05, <0.05, <0.05, <0.05, 0.07, 0.10, 0.11, 0.33, 0.37, 0.42, 0.43, 0.57, 0.62, 1.1, 1.5, 1.9	22	3 rape seed	5.18	2.42
Metaflumizone	Chinese cabbage	<0.10, <0.10, 0.76, 0.77, 1.4, 1.6	6	6 Chinese cabbage	5.9689	3.307

Pesticide	Commodity	Data	n	JMPR MRL	NAFTA	OECD
Prothioconazole	wheat straw, barley straw	<0.05, 0.12, 0.15, 0.17, 0.19, 0.21, 0.22, 0.23, 0.27, 0.36, 0.41, 0.57, 0.61, 0.67, 0.85, 0.89, 0.89, 0.92, 1.3, 1.4, 1.4, 1.6, 1.7	23	4 cereal straws	4.493	2.742

Ideally, an MRL calculator should provide an estimate that lies between the 95th and 99th percentile values of the residue population of interest and it should provide an uncertainty on the calculated result.

Further developments are continuing, but consistent accuracy is not possible on the generally small numbers of trials available in a dataset.

Abbreviations and acronyms in this chapter

CS	capsule suspension
EC	emulsifiable concentrate
EW	emulsion, oil in water
GAP	good agricultural practice
HR	highest residue
HR-P	highest residue in a processed commodity calculated by multiplying the HR of the raw agricultural commodity by the corresponding processing factor.
JMPR	Joint Meeting on Pesticide Residues
LOQ	limit of quantification
MRL	maximum residue limit
NAFTA	North American Free Trade Agreement
OECD	Organization for Economic Co-operation and Development
PHI	pre-harvest interval
SC	suspension concentrate
SD	standard deviation
SL	soluble concentrate
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor
UL	ultra-low volume liquid
WG	water dispersible granules
WP	wettable powder

Chapter 9. Evaluation of Monitoring Data for Estimation of MRLs for Spices and Extraneous Maximum Residue Limits (EMRLs).

Estimation of pesticide residue levels in or on spices
Estimation of maximum residue levels for pesticides in or on dry chilli peppers
Data requirements for estimation of extraneous maximum residue levels (EMRL)
Presentation – evaluation of monitoring data for spices and EMRLs

The purpose of this chapter is to explain how residue monitoring data may be used to estimate maximum residue levels for spices and to estimate EMRLs for environmental contaminants that were previously used as pesticides.

Relevant sections of JMPR Manual

- Submission of information for estimation of MRLs of pesticide residues in/on spices 3.6
- Data requirements for EMRL estimation 3.13
- Results of national monitoring programs.....5.9
- Special considerations for dried chilli peppers.....6.9.2
- Estimation of maximum residue levels based on monitoring data 6.11

Estimation of pesticide residue levels in or on spices

Spices are typically grown at small scale, often together with other crops such as fruits and vegetables. Only a few are produced at commercial scale. Pesticides are rarely registered specifically on spices. The farmers use similar pesticides as are applied on other crops.

In view of the economic importance of spices for some countries and their generally negligible dietary consumption, the CCPR decided to establish MRLs for pesticide residues in or on spices based on monitoring data. Accordingly, the JMPR considered the specific nature of monitoring data and elaborated the principles for estimating residue levels for pesticides residues in or on spices.

There are principal differences from residue data deriving from monitoring programmes and supervised field trials:

- The origin and treatment history of the lots sampled are not known;
- The sampled commodity may be composed of the produce of several small fields;
- The residues in spice samples are generally determined with multi-residue procedures with relatively high LOQ values.

Selection criteria of residue data for evaluation:

- Only pesticides already in the Codex system are considered.
- Pesticides with national registrations on any commodity in either the exporting or importing country are included.
- Persistent pesticides that have been banned or no longer approved for use in crop protection (e.g. DDT, aldrin, heptachlor, endrin, etc.) are excluded.

- Only residues on the spices in the modified Group 028 are considered. For example, data for herbs, dried onions, chillies, etc. are excluded.
- As poppy seed (SO 0698), mustard seeds (SO 0090) and sesame seed (SO 0700) are used as major food ingredients in several countries, they are not considered among spices.
- All residue data are considered; no data point is excluded as an outlier.
- Residue values reported as "0" are replaced with <LOQ.
- Only those data sets are considered which include at least 59 data points (satisfying the 95th percentile coverage with 95% confidence), preferably for more than one sub-group of spices. An exception is the case where all reported residue values are below the LOQ: maximum residue levels corresponding to the highest reported LOQ are recommended based on fewer data points.
- Pesticide residues assumed to be resulting from post-harvest applications are excluded. (They should be regulated by national governments as any other use of pesticides).

Principles of evaluation of spice monitoring data:

- In cases where all data are non-detectable and different LOQ values are reported for a particular pesticide by the different data sources, the maximum residue level is proposed at the highest LOQ provided for the pesticide.
- Since there is no evidence for nil residues the median residue is calculated with the values corresponding to the reported LOQ levels. The high residue is considered to be equal to the highest reported LOQ. A residue value indicated with (*) does not necessarily mean that residues may not occur in detectable amount where a more sensitive method is used.
- The distributions of residues are scattered or skewed at the higher end. No distribution fitting seems to be appropriate. Consequently, a distribution-free statistics is used, based on binominal probability calculation, in estimating the maximum residue level covering the 95th percentile of the population with 95% confidence level.
    - Provided that random samples are taken, this provision means, that the estimated maximum residue level will encompass at least 95% of the residues with 95% probability (in 95% of the cases). To satisfy this requirement a minimum of 59 samples are required. It should be noted that the minimum sample size of 59 provides 95% assurance for finding at least one residue value above the 95th percentile of the residue population in the sampled object. We do not know, however, that how many of the measured values are above the 95th percentile, and what percentile (95.1th, 99th or 99.9th) of the highest residue represents. The 95th percentile of the sample does not necessarily represent the 95th percentile of the residue population in the sampled commodity.
    - Where more than 72 samples contain detectable residues, the upper 95% confidence limit of the 95th percentile of the residue population can also be calculated.
    - Where the data set contains larger number of detectable residues, some of the high residues are above the upper confidence limit of the 95th percentile of the residue population and they can be disregarded for estimation of MRLs. (see Figure 1.)
- A substantial proportion of random monitoring samples do not contain detectable residues indicating that probably the sampled lots were not treated with or exposed

to the given pesticide. Therefore, the median residue values are derived from the detected residues. The chronic intake is calculated from the residue data on that commodity which gave the largest contribution to the intake and the percentage of the treated proportion of that particular commodity group. (It is unlikely that all sampled commodities were treated with the pesticides included in the multi-residue screening procedure, therefore the proportion of commodities treated with or exposed to a given pesticide was calculated from the ratio of samples containing detectable residues and total number of samples analysed.)

- The estimated high residue and median residue values can be used similarly to the HR and STMR values obtained from supervised trials for the estimation of short-term and long-term intake of residues.

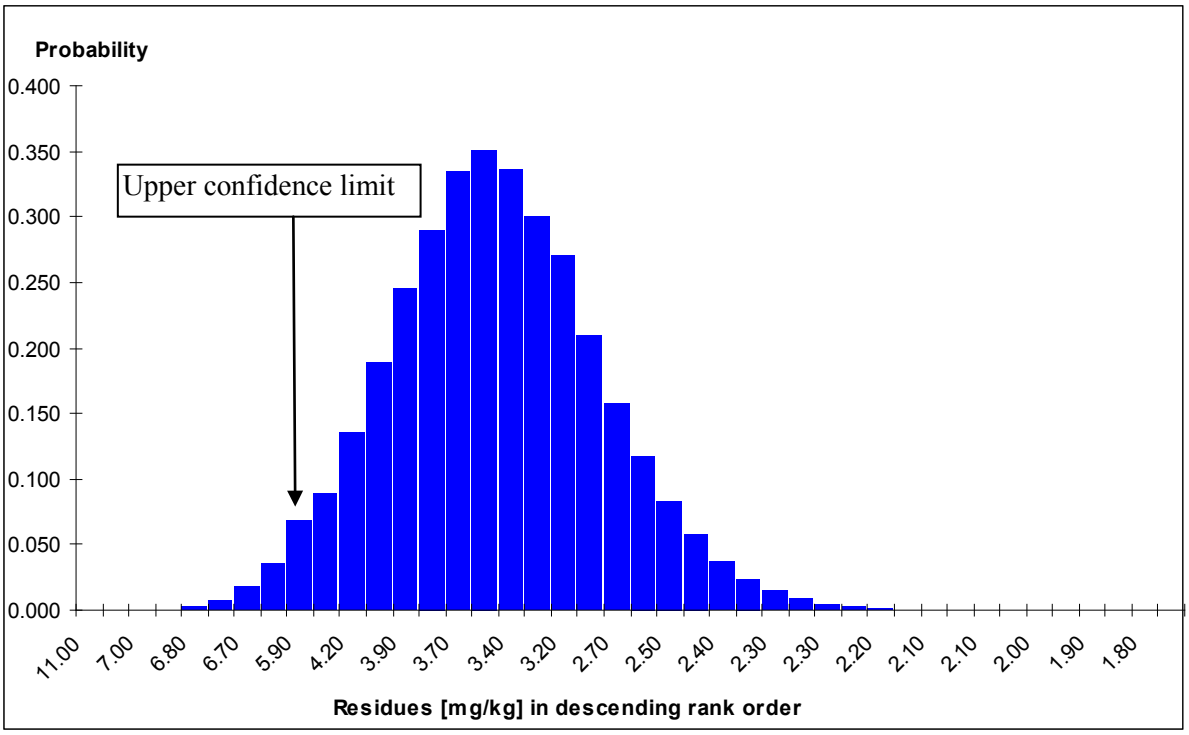


Figure 1. Upper 95% confidence limit for the 95th percentile of residues (Number of samples = 343).

Selective field surveys

Selective field surveys are an alternative approach to generate residue data to support the elaboration of MRLs for spices, as monitoring results have limited use in estimating maximum residue levels mainly because of the lack of information on the pesticide treatment history of the sampled commodity.

In a selective field survey, samples are taken from fields where the crop is grown, treated directly or indirectly with pesticides, and harvested according to the local agricultural practice. The essential feature of the selective field survey is that all pesticide applications, the growth stage of the crop and post-harvest treatment of spices are recorded and are attached to the sampling report. This allows the laboratory to identify for analysis all pesticides applied, in addition to environmental contaminants such as organochlorine pesticides, which may be taken up from soil.

For MRL estimation the selective field survey is a better data source as the pesticides used are known rather than pesticide monitoring data involving the testing for pesticide residues in samples of unknown origin.

Estimation of maximum residue levels for pesticides in or on dry chilli peppers

As a general practice, chilli peppers are traded according to colour. They are not normally harvested at maturity. Harvest practices differ from country to country and even farmer to farmer, but usually chillies are harvested when the colour is at its optimum on the industry scale which is almost always much later than peak ripeness. It is now common practice for farmers to leave the fruit on the plant to be dried in the sun, thus saving dehydration costs.

In establishing the existing Codex MRLs for fresh peppers, it was assumed, on the basis of GAP, that samples were taken when the fruits were mature. This is not the harvest point for the peppers that are dried and consumed as spices. Estimating dehydration factors based on the fruit at harvest does not reflect the common cultural practices described.

Based on the available data on water content of peppers and dried chilli peppers, the JMPR estimated a concentration factor of 10 and 7 for converting bell pepper and fresh chilli pepper residues to residues in dried chilli pepper powder, respectively. Consequently, when residue data are available on peppers, the residues in dried chilli peppers are estimated by multiplying the residues in peppers by 10. However where the trials were performed on chilli peppers, the residues detected in fresh chilli peppers are multiplied by 7 for obtaining residues in dried chilli peppers.

Example – chlorpyrifos-methyl in dried chilli peppers (JMPR 2009)

The Meeting estimated a maximum residue level of 1 mg/kg, an HR of 0.72 mg/kg and an STMR of 0.06 mg/kg for chlorpyrifos-methyl in peppers.

Using the default dehydration factor of 10 to extrapolate from peppers to dried chilli peppers, the Meeting estimated a maximum residue level of 10 mg/kg (based on a highest residue of 7.2 mg/kg) and an STMR of 0.6 mg/kg for chlorpyrifos-methyl in Peppers, chilli dried.

Point to note.

- Sweet pepper data were used to support a maximum residue level, STMR and HR for 'peppers' and then with a factor of 10 to produce suitable values for dried chilli peppers.

Example – buprofezin in dried chilli peppers (JMPR 2009)

The residues of buprofezin in non-bell peppers (i.e. chilli peppers) from trials in accordance with the maximum US GAP for fruiting vegetables other than cucurbits were: 0.17, 0.54 and 1.1 mg/kg.

Data from bell peppers and non-bell peppers were combined for a peppers MRL of 2 mg/kg, an STMR of 0.33 and an HR of 1.1 mg/kg.

On the basis of the STMR and HR for peppers and the default dehydration factor of 7, an STMR and HR for chilli peppers (dry) were calculated to be 2.31 and 7.7 mg/kg, respectively. Based on the HR, the Meeting recommended a maximum residue level for chilli peppers (dry) at 10 mg/kg.

Point to note.

- Chilli pepper data were used to support a maximum residue level, STMR and HR for 'peppers' and then with a factor of 7 to produce suitable values for dried chilli peppers.

Estimation of extraneous maximum residue levels (EMRL)

The extraneous residue refers to a pesticide residue arising from environmental sources (including former agricultural uses) other than the use of a pesticide directly or indirectly on the commodity. EMRLs are estimated from residue data generated in food monitoring programmes.

Ideally, all geographically representative monitoring data for extraneous residues should be evaluated to cover international trade. The data should include the nil residue results (and the LOQs).

The JMPR evaluates the monitoring data in terms of the expected violation rate if a specified EMRL is selected. Violation rates of 0.5 to 1 % or greater are generally unacceptable in trade.

In the evaluation of DDT residues in meat in 2000, JMPR estimated residue levels corresponding to violation rates of 0.1, 0.2 and 0.5 % and suggested that CCPR should choose the acceptable violation rate (a risk management decision).

PRESENTATION – EVALUATION OF MONITORING DATA FOR SPICES AND EMRLs

1. Evaluation of monitoring data for estimation of residue levels for spices

2. Objectives

The purpose of this chapter is to explain how residue monitoring data may be used to estimate maximum residue levels for spices and to estimate EMRLs for environmental contaminants that were previously used as pesticides.

3. Outline

- Estimation of pesticide residue levels in or on spices
  - Selection criteria of residue data for evaluation
  - Principles of evaluation of spice monitoring data
  - Selective field surveys
- Estimation of maximum residue levels for pesticides in or on dry chilli peppers
- Data requirements for estimation of extraneous maximum residue levels (EMRLs)

4. Estimation of pesticide residue levels in or on spices

- Special conditions for growing spices:
- Spices are typically grown at a small scale, often together with other crops such as fruits and vegetables.
- Only a few are produced at commercial scale.
- Pesticides are rarely registered specifically on spices. The farmers use similar pesticides as are applied on other crops.

5. Principal differences between monitoring data and supervised field trials

- The origin and treatment history of the lots sampled are not known;
- The sampled commodity may be composed of the produce of several small fields;
- The residues in spice samples are generally determined with multi-residue procedures with relatively high LOQ values.

6. Selection criteria of residue data in or on spices for evaluation - 1

- Only pesticides already in the Codex system are considered.
- Pesticides with national registrations on any commodity in either the exporting or importing country are included.
- Persistent pesticides that have been banned or no longer approved for use in crop protection (e.g. DDT, aldrin, heptachlor, endrin, etc.) are excluded.

- Only residues on the spices in the modified Group 028 are considered. For example, data for herbs, dried onions, chillies, etc. are excluded.
- As poppy seed (SO 0698), mustard seeds (SO 0090) and sesame seed (SO 0700) are used as major food ingredients in several countries, they are not considered among spices.

7. Selection criteria of residue data in or on spices for evaluation - 2.

- All residue data are considered; no data point is excluded as an outlier.
- Residue values reported as "0" are replaced with <LOQ.
- Only those data sets are considered which include at least 59 data points (satisfying the 95th percentile coverage with 95% confidence), preferably for more than one sub-group of spices. An exception is the case where all reported residue values are below the LOQ: maximum residue levels corresponding to the highest reported LOQ are recommended based on fewer data points.
- Pesticide residues assumed to be resulting from post-harvest applications are excluded. (They should be regulated by national governments as any other use of pesticides)

8. Principles of evaluation of spice monitoring data

- In cases where all residues are non-detectable and different LOQ values are reported for a particular pesticide by the different data sources, the maximum residue level is proposed at the highest LOQ provided for the pesticide.
- Since there is no evidence for nil residues the median residue is calculated with the values corresponding to the reported LOQ levels. The high residue is considered to be equal to the highest reported LOQ. A residue value indicated with (*) does not necessarily mean that residues may not occur in detectable amounts where a more sensitive method is used.

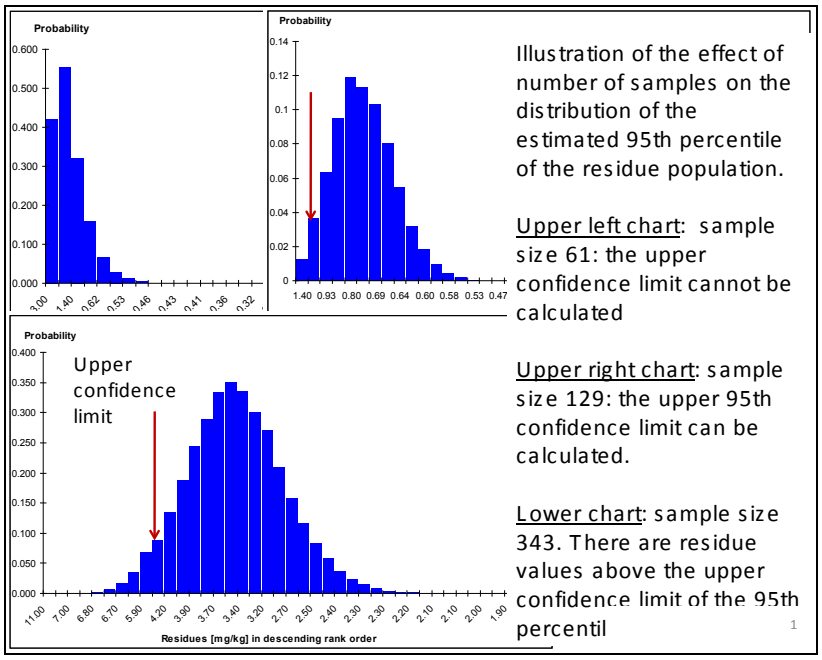
9. Principles of evaluation of spice monitoring data

- The distributions of residues are scattered or skewed at the higher end. No distribution fitting seems to be appropriate. Consequently, a distribution-free statistics is used, based on binominal probability calculation, for estimating the maximum residue level covering the 95th percentile of the population with 95% confidence level.
- Provided that random samples are taken, this provision means, that at least one residue value will be above the 95th percentile of the residue population in the sampled object.
- Thus the estimated maximum residue level will encompass at least 95% of the residues with 95% probability (in 95% of the cases).
- We do not know, however, how many of the measured values are above the 95th percentile, and what percentile (95.1st, 99th or 99.9th) of the highest residue represents.

10. Principles of evaluation of spice monitoring data

- The 95th percentile of the sample does not necessarily represent the 95th percentile of the residue population in the sampled commodity.
- Where more than 72 samples contain detectable residues, the upper 95% confidence limit of the 95th percentile of the residue population can also be calculated.
- Where the data set contains a larger number of detectable residues, some of the high residues are above the upper confidence limit of the 95th percentile of the residue population and they can be disregarded for estimation of MRLs.

11.



12. Principles of estimation of consumers' exposure from residues in spices

- The revised sub-groups of spices (A28) do not always correspond with the consumption figures used for intake calculations. Therefore the calculations ere carried out with the combined g/day amounts listed for spices in the GEMS Food tables.
- A substantial proportion of random monitoring samples do not contain detectable residues. In these cases the sampled lots were probably not treated with or exposed to the given pesticide.
- Therefore, the median residue values are derived from the detected residues.
- The highest residue observed is taken as HR. (It may be higher than the estimated maximum residue level!)

13. Principles of evaluation of spice monitoring data

- The estimated high residue and median residue values can be used similarly to the HR and STMR values obtained from supervised trials for the estimation of short-term and long-term intake of residues.

- The chronic intake is calculated from the residue data on that commodity from the corresponding subgroup which gave the largest contribution to the intake and the calculated factor of the treated proportion of that particular commodity group.
- A factor is calculated for the proportion of treated commodities from the ratio of number of samples containing detectable residues and non-detectable residues. The median residue was adjusted with the factor as one of the recommended procedures for refining intake calculations.

14. Example for estimation of diazinon residue levels in spices

Detectable diazinon residues were found (upper tail is shown):

- *Anise samples* (69 out of 667): x, x, 0.82, 0.88, 0.9, 1.1, 1.1, 1.2, 1.3, 1.8, 1.8, 2.1, 2.7, 3.5, 3.6 mg/kg.
- *Fennel seeds* (31 out of 734): 0.26, 0.45, 0.59, 0.65, 0.72, 0.76, 0.77, 1.2, 1.7, mg/kg
- *Celery and cumin seeds*: 0.1, 0.1, 0.14, 0.29 mg/kg

The Meeting estimated 5 mg/kg maximum residue level, 3.6 mg/kg high residue and 0.19 mg/kg median residue level (based on anise seed residue data) for seed sub-group.

15. Example for estimation of diazinon residue levels in spices

The Meeting estimated 0.5 mg/kg maximum residue level, 0.26 mg/kg high residue and 0.05 mg/kg median residue level for *roots/rhizome subgroup*. (data not shown)

No recommendation could be made for bark, buds, and aril subgroups

The Meeting recommended to use 3.6 mg/kg high residue for the calculation of short term intake, and 0.19 mg/kg median residue, and a factor of 0.1 (~69/667) to take into account the proportion of samples containing detectable residues in chronic intake calculation.

16. Preconditions for obtaining reliable estimates for residues in spices

- MRL should cover pesticide residues likely to result from uses on spices.
- The LOQ should be sufficiently low (preferably ≤ 0.01 mg/kg) for excluding application of non-detected pesticides.
- The identity of detected residues should be confirmed.
- Samples should be taken from single lots as far as possible.
- Relatively large numbers of samples should be analysed over several years.

17. Selective field surveys

- Selective field surveys are an alternative approach to generate residue data to support the elaboration of MRLs for spices, as several limitations of monitoring data are eliminated.
- In a selective field survey, samples are taken from fields where the crop is grown, treated directly or indirectly with pesticides, and harvested according to the local agricultural practice.

- The essential feature of the selective field survey is that all pesticide applications, the growth stage of the crop and post-harvest treatment of spices are known and recorded on the sampling report.
- Main advantage: the laboratories know what residues they should look for, and the samples represent single lots.

18. Estimation of maximum residue levels for pesticides in or on dry chilli peppers

- Chillies are harvested when the colour is at its optimum on the industry scale which is almost always much later than peak ripeness.
- MRLs for residues in fresh peppers are based on mature crops. This is not the harvest point for the peppers that are dried and consumed as spices.
- Based on the available data on water content of peppers and dried chilli peppers, the JMPR estimated concentration factors of 10 and 7 for converting bell pepper and fresh chilli pepper residues to residues in dried chilli pepper powder, respectively.

19. Example1: chlorpyrifos-methyl in dried chilli peppers

The Meeting estimated a maximum residue level of 1 mg/kg, an HR of 0.72 mg/kg and an STMR of 0.06 mg/kg for chlorpyrifos-methyl in peppers.

- Using the default dehydration factor of 10 to extrapolate from peppers to dried chilli peppers, the Meeting estimated a maximum residue level of 10 mg/kg (based on a highest residue of 7.2 mg/kg) and an STMR of 0.6 mg/kg for chlorpyrifos-methyl in peppers, chili dried.

Point to note.

- Sweet pepper data were used to support a maximum residue level, STMR and HR for 'peppers' and then with a factor of 10 to produce suitable values for dried chilli peppers.

20. Example 2: buprofezin in dried chilli peppers

- The residues of buprofezin in non-bell peppers (i.e. chili peppers) from trials in accordance with the maximum US GAP for fruiting vegetables other than cucurbits were: 0.17, 0.54 and 1.1 mg/kg.
- Data from bell peppers and non-bell peppers were combined for a peppers MRL of 2 mg/kg, an STMR of 0.33 and an HR of 1.1 mg/kg.
- On the basis of the STMR and HR for peppers and the *default dehydration factor* of 7, an STMR and HR for chili peppers (dry) were calculated to be 2.31 and 7.7 mg/kg, respectively. Based on the HR, the Meeting recommended a maximum residue level for chilli peppers (dry) at 10 mg/kg.
- Point to note: Chili pepper data were used to support a maximum residue level, STMR and HR for 'peppers' and then with a factor of 7 to produce suitable values for dried chilli peppers.

21. Estimation of extraneous maximum residue levels (EMRL)

- The extraneous residue refers to a pesticide residue arising from environmental sources (including former agricultural uses) other than the use of a pesticide directly or indirectly on the commodity. EMRLs are estimated from residue data generated in food monitoring programmes.
- Include all geographically representative monitoring data.
- Include the nil residue results (and reported LOQs).

22. Estimation of extraneous maximum residue levels (EMRL)

- JMPR evaluates the monitoring data in terms of the expected violation rate if a specified EMRL is selected.
- Violation rates of 0.5 to 1 % or greater are generally unacceptable in trade.
- DDT residues in meat. JMPR (2000) estimated residue levels corresponding to violation rates of 0.1, 0.2 and 0.5 %.
- CCPR to choose the acceptable violation rate, a risk management decision.

Abbreviations and acronyms in this chapter

CCPR	Codex Committee on Pesticide Residues
EMRL	extraneous maximum residue limit
GAP	good agricultural practice
HR	highest residue
JMPR	Joint Meeting on Pesticide Residues
LOQ	limit of quantification
MRL	maximum residue limit
STMR	supervised trials median residue

Chapter 10. Fate of Residues in Storage and Processing.

Fate of residues during storage
Nature of the residue after food processing
Physical and chemical properties to assist interpretation
Example, cotton seed processing
Example, processing factors and STMR-P values
Example, ETU produced from mancozeb
Presentation – food storage and processing

The purpose of this chapter is to explain the interpretation of studies on the fate of pesticide residues during food processing and to explain when an MRL is needed for a processed commodity.

Relevant sections of JMPR Manual

- Fate of residues in food storage and processing.....3.7
- Processing studies.....5.8
- Processed commodities.....6.9

Fate of residues during storage

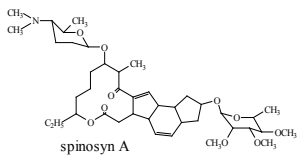
Some commodities, such as cereal grains, are stored for long periods after harvest and before consumption. Post-harvest treatment with grain protectants is needed to control insect attack on the grain.

Storage studies with treated grain to determine the persistence of the grain protectants are needed to indicate the interval of protection against insects and the level of insecticide expected in the wheat when it is milled.

When the grain is milled, "fresh residues" (residues on recently treated grain) may not behave the same as "aged residues" (residues after a long storage interval), so it is important to have processing studies on treated grain after suitable storage intervals.

Example, spinosad as a grain protectant on cereal grains (JMPR 2004)

In a series of trials in USA, cereal grains were treated with spinosad at a target rate of 1 g ai/t and stored at ambient temperature. Grain samples were taken periodically up to 11 months for analysis.



Residues in the grain immediately after treatment ranged from 43% to 91% of the target application rate, reflecting the efficiency of application. Residue levels declined very little during the storage period.

Grain	Laboratory-scale, with 14-23 kg grain treated in each trial Spinosad residue, mg/kg				
	0 month	3 months	6 months	11 months	High residue
Barley	<u>0.69</u>	0.50			0.69
Barley	<u>0.91</u>	0.81			0.91
Barley	0.72	<u>0.86</u>			0.86
Maize	0.57	<u>0.59</u>			0.59
Maize	<u>0.45</u>	0.41			0.45
Maize	0.54	<u>0.58</u>	0.42	0.50	0.58

Grain	Laboratory-scale, with 14-23 kg grain treated in each trial Spinosad residue, mg/kg				
	0 month	3 months	6 months	11 months	High residue
Maize	0.59	0.90	0.66	0.58	0.90
Maize	0.63	0.54			0.63
Oats	0.47	0.33			0.47
Oats	0.67	0.69			0.69
Oats	0.69	0.63			0.69
Rice	0.48	0.67			0.67
Rice	0.73	0.63	0.68	0.93	0.93
Rice	0.75	0.78	0.75	0.91	0.91
Wheat	0.43	0.34			0.43
Wheat	0.81	0.63			0.81
Wheat	0.73	0.75	0.56	0.59	0.75
Wheat	0.61	0.72	0.79	0.48	0.79
Wheat	0.70	0.62			0.70

In two larger scale trials on wheat and maize the moving grain was spray-treated as it entered the augur, and the amounts treated were 9.9 t of maize and 30.9 t of wheat.

Grain	Spinosad residue, mg/kg			
	0 month	3 months	6 months	11 months
Maize, 9.9 t, at 1.6 g ai/t	1.4	0.77	0.77	0.50
Wheat, 30.9 t, at 1.2 g ai/t	0.92	0.74	0.95 ‡	0.71

‡ This wheat, from the 6 months storage interval, was milled – see below.

Points to note

1) The concentration of pesticide in the grain (mg/kg) was less than expected from the intended application rate (g ai/t), which generally occurs with post-harvest application.
2) The measured residues from one storage interval to the next were too variable to observe a residue decline, if any.
3) The highest residue found at any time after treatment was selected for maximum residue level assessment.
4) The GAP application rate of 1 g ai/t would also be taken into account when the maximum residue level was estimated.

Example – milling of stored wheat, spinosad residues (JMPR 2004)

Spinosad residues were mostly on the outside of the wheat grain. Consequently, when treated stored wheat (stored 6 months) was cleaned and milled, high residues were found in the aspirated grain fraction (grain dust).

Spinosad residues in stored wheat and processed fractions from a milling trial in USA.
Processing factors = residue levels in processed commodities ÷ residue levels in grain.

Commodity	Spinosad residues, mg/kg	Processing factor
Wheat grain	0.95	
aspirated grain fraction	302	317
bran	0.92	0.97
middlings	0.29	0.30
shorts	1.05	1.2
germ	0.68	0.72
flour	0.33	0.34
gluten	1.2	1.3
starch	0.007	0.0074

Point to note

- Residues in the flour were much lower than in the grain. Residues in bran were approximately the same as in the grain.

Nature of the residue after food processing

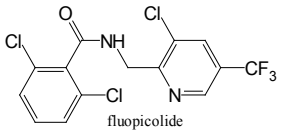
It is not practical to use ^{14}C labelled compounds in food processing studies simulating commercial practice to trace the fate of the compound during the process.

To enable the fate to be studied in the laboratory, hydrolysis conditions of temperature, pH and duration have been chosen to represent the processes of pasteurisation, baking, brewing, boiling and sterilisation.

The results of the laboratory studies suggest whether the compound is stable or if the processed commodities should be analysed for decomposition products.

Example – fluopicolide is stable during food processing (JMPR, 2009)

The degradation of fluopicolide was tested under simulated processing conditions. Buffer solutions at pH values of 4, 5 and 6 were fortified with [^{14}C]fluopicolide and subjected to hydrolysis conditions simulating pasteurisation, baking, brewing or boiling and sterilisation. The results are summarised in the table.



fluopicolide

pH	Temperature	Incubation time	Simulated process	Mean % applied ^{14}C determined as fluopicolide
pH 4	90 ˚C	20 min	pasteurisation	99 %
pH 5	100 ˚C	60 min	baking, brewing or boiling	104 %
pH 6	120 ˚C	20 min	sterilisation	100 %

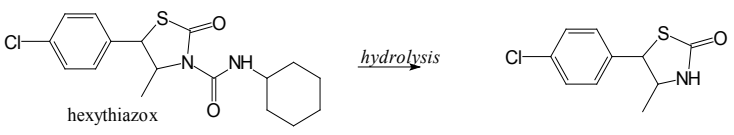
Fluopicolide was stable under these processing conditions, remaining unchanged.

Example – hexythiazox is stable during pasteurisation and boiling, but not during sterilisation (JMPR, 2009)

The degradation of hexythiazox was tested under simulated processing conditions. Buffer solutions at pH values of 4, 5 and 6 were fortified with [^{14}C]hexythiazox and subjected to hydrolysis conditions simulating pasteurisation, baking, brewing or boiling and sterilisation. The results are summarised in the table.

pH	Temp	Incubation time	Simulated process	Result
pH 4	90 ˚C	20 min	pasteurisation	89 % ^{14}C remains as hexythiazox 6 % ^{14}C identified as hydrol products
pH 5	100 ˚C	60 min	baking, brewing or boiling	99 % ^{14}C remains as hexythiazox
pH 6	120 ˚C	20 min	sterilisation	53 % ^{14}C remains as hexythiazox 54 % ^{14}C identified as hydrol products

Hexythiazox was stable during the pasteurisation and boiling processes, but not during sterilisation, where



hexythiazox

approximately half was hydrolysed.

Physical and chemical properties to assist interpretation

The physical and chemical properties of the study compound are useful in predicting and interpreting the fate of the compound during processing.

A water-soluble compound will preferentially partition to the juice phase in the production of a clear fruit juice, while a water-insoluble compound will preferentially partition to the pomace phase.

A fat-soluble compound will preferentially partition to the oil phase in the production of vegetable oils, while a fat-insoluble compound will more likely remain in the oilseed meal.

The residues of a compound that exists mostly on the surface of grain or fruit are more likely to be reduced during the cleaning phase than the residues of a compound distributed within the grain or fruit.

The yield of a processed commodity from the raw agricultural commodity determines the maximum theoretical processing factor.

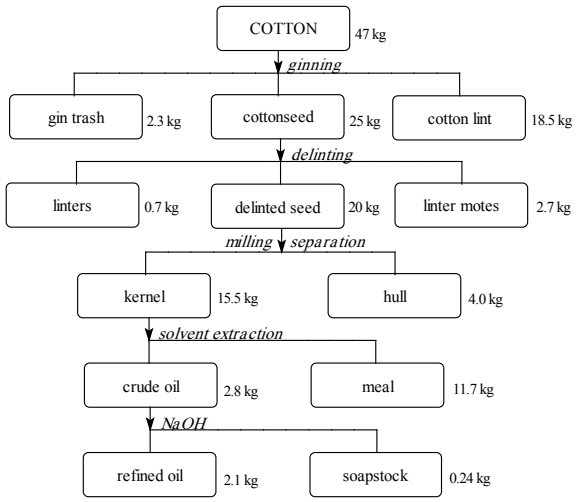
For example, the maximum theoretical processing factor for a fat-soluble compound in sunflower seed oil from sunflower seeds containing 45 % oil is 100 ÷ 45 = 2.2. The maximum theoretical processing factor for a fat-soluble compound in maize oil from maize containing 5 % oil is 100 ÷ 5 = 20.

If an alkaline solution is used to assist the cleaning or peeling process for fruits, the residue levels of compounds susceptible to alkaline hydrolysis should decrease.

Example, cotton seed processing

Flow diagram of the food process to assist understanding of the place of each product – example: pyriproxyfen on cotton seed (JMPR, 1999).

Cotton was treated with pyriproxyfen at an exaggerated application rate and harvested 28 days after the final application for processing. Processing is summarised in the figure. Pyriproxyfen residues of 0.10 mg/kg in cotton seed produced residues of 0.02 mg/kg in the crude and refined oil, but no residues (<0.01 mg/kg) in the meal.



Commodity	Pyriproxyfen, mg/kg
Cotton seed	0.10
Solvent extracted meal	<0.01
Cotton hulls	<0.01
Crude oil	0.02
Refined oil	0.02

Residues in cotton seed and processing fractions from cotton treated with an exaggerated application rate, 0.25 + 0.37 + 0.37 kg ai/ha (5×label rate) and harvested 28 days after the final application for processing in USA.

The diagram clearly illustrates where the commodities occur in the process and the relative weights assist with understanding the process.

Calculated processing factors for pyriproxyfen in cotton seed

Cotton seed to meal = $\frac{<0.01}{0.10} = <0.1$

Cotton seed to hulls = $\frac{<0.01}{0.10} = <0.1$

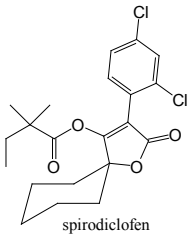
Cotton seed to crude oil = $\frac{0.02}{0.10} = 0.2$

Cotton seed to refined oil = $\frac{0.02}{0.10} = 0.2$

Example, processing factors and STMR-P values

Estimation of STMR-P values: example spirodiclofen (JMPR 2009)

Processing factors were calculated from residue levels measured in the processing studies. Where residues in the processed commodity were below LOQ, processing factors were calculated from the LOQ and the residue in the raw agricultural commodity (RAC) and were reported with a 'less than' (<) sign.



spirodiclofen

Processing factor $= \frac{LOQ}{Residue_{RAC}}$

Commodity	Processing factors	Processing factor (median or best estimate)		STMR of RAC, mg/kg	STMR-P, mg/kg *NOTE 5*
Orange juice	0.05	0.05		0.13	0.13×0.05 = 0.0065
Apple juice	<0.02 (2), <0.71 (3)	<0.02	*NOTE 1*	0.20	0.20×0.02 = 0.004
Apple pomace (dry)	16, 17, 21	17	*NOTE 2*	0.20	0.20×17 = 3.4

Dried apple	<0.02, 0.16	0.09	*NOTE 3*	0.20	0.20×0.09 = 0.018
Grape juice	<0.006, 0.0081, <0.54 (3)	0.0081	*NOTE 4*	0.063	0.063×0.0081 = 0.00051

Points to note

NOTE 1: Residues in apple juice were all below LOQ, which means that the calculated factors depend on the measured residues in the RAC (apples). In this case, the best estimate is derived from the apples with the highest residue, i.e. the lowest processing factor.

NOTE 2: Apple pomace (dry) – the median is the best estimate where the values are close and all the residues were at LOQ or higher.

NOTE 3: Dried apple – the factor calculated from an LOQ is taken into account because it shows that, in this trial, a higher proportion of residues disappeared than in the other trial.

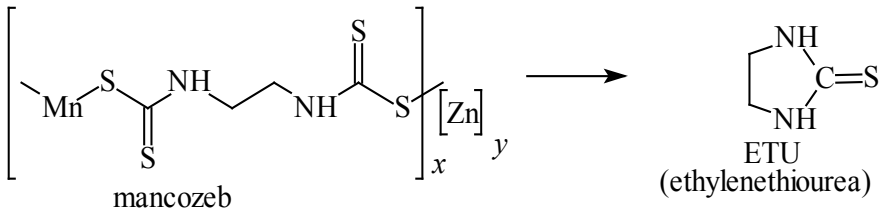
NOTE 4: Grape juice – the factor calculated from the trial where residues were measurable in the processed commodity is the best estimate.

NOTE 5: The STMR-P of the processed commodity is calculated from the STMR of the RAC and the best estimate of processing factor.

Example, ETU produced from mancozeb

Residues generated during processing (JMPR 1993)

Ethylenethiourea (ETU) is produced from ethylenebisdithiocarbamates such as mancozeb during food processing operations such as boiling. ETU is also a metabolite and may be present in the raw agricultural commodity.



mancozeb

ETU
(ethylenethiourea)

The processing factor concept does not apply to residues produced during processing. The concept assumes that the residues of a compound in the processed commodity originate only from the same compound in the raw agricultural commodity (RAC).

Commodity	Dithiocarbamate residues, expressed as CS_2 mg/kg		ETU residues, mg/kg	
	Treatment 1	Treatment 2	Treatment 1	Treatment 2
Raw grapes	21 17	49 36	0.01 0.01	0.28 0.35
Dry pomace	12 14	20 18	0.20 0.21	1.3 0.90
Thick juice	2.4 2.6	1.4 1.2	0.08 0.08	4.3 4.3
Clear juice	<0.1 <0.1	<0.1 <0.1	0.19 0.23	2.4 2.6
Pasteurised juice	<0.1 <0.1	<0.1 <0.1	0.08 0.09	0.93 0.90
	PROCESSING FACTORS		PERCENTAGE YIELD	
Dry pomace	0.68	0.45	1.7 %	3.8 %
Thick juice	0.13	0.031	0.68 %	15 %
Clear juice	<0.005	<0.002	1.8 %	8.7 %
Pasteurised juice	<0.005	<0.002	0.72%	3.2 %

A percentage yield of ETU in the processed commodity may be calculated from its two origins in the raw agricultural commodity.

$$\text{Percentage yield of ETU} = \frac{100 \times ETU_{ProcCom}}{ETU_{RAC} + 0.67 \times DITH_{RAC}}$$

The 0.67 is a molecular weight adjustment that recognizes that each mancozeb unit can produce 2 molecules of CS_2 or 1 molecule of ETU.

It should be noted that accurate analysis of ETU in the presence of high concentrations of ethylenebisdithiocarbamates is difficult, because conversion to ETU may occur during analysis. Estimated conversion rates of 0.22-8.5 % were reported (JMPR 1993).

PRESENTATION – FOOD STORAGE AND PROCESSING

1. Fate of Pesticide Residues during Food Processing and Storage

2. The need for data on pesticide residues in processed foods

- Dietary exposure estimates are refined for
  - raw agricultural commodities (RACs) that are always processed before consumption, e.g. wheat.
  - raw agricultural commodities that may be consumed directly, e.g. apples, or after processing, e.g. apple juice.
- MRLs are needed for processed commodities where the residue levels are higher than the MRLs of the raw agricultural commodities.

Questions

1) Examples where higher residue levels are expected in the processed food than in the RAC?
2) Why not MRLs for all processed foods?

3. Food processes

- Food preparation, e.g. cleaning and peeling.
- Cooking.
- Juicing.
- Brewing and vinification.
- Canning.
- Milling and baking.
- Oil production.
- Drying.

4. Outline

- commodities that are processed;
- definitions;
- the nature of the residue;
- effect on the residue level - the processing factor;
- food storage;
- examples of food processes and processing factor;
- pre-registration risk assessment

5. Commodities that are subject to food processing

- Always – wheat

- Sometimes – oranges

- Household operations – washing, cleaning and cooking
- Whole fruit – edible portion of bananas
- Commercial processes

6. Definition (JMPR Manual)

For the purposes of the Codex Alimentarius, the term "*primary food commodity*" means the product in or nearly in its natural state intended for processing into food for sale to the consumer or as a food without further processing.

The term "*raw agricultural commodity (RAC)*" means the same as "primary food commodity."

7. Definition (JMPR Manual)

For the purposes of the Codex Alimentarius, the term "*processed food*" means the product, resulting from the application of physical, chemical or biological processes to a "primary food commodity" intended for direct sale to the consumer, for direct use as an ingredient in the manufacture of food or for further processing.

8. Definition (JMPR Manual)

The *processing factor* for a specified pesticide residue, commodity and food process is the residue level in the processed product divided by the residue level in the starting commodity, usually a raw agricultural commodity.

9. Nature of the residue

Hydrolysis studies with the radiolabelled pesticide in the absence of crop matrix are used as the model for degradation of the compound during processing.

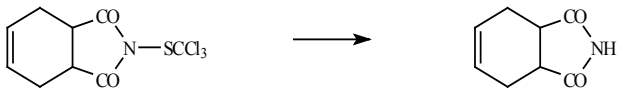
10. Hydrolysis studies

- ^{14}C labelled pesticide is subjected to hydrolysis conditions at selected temperatures and pH values for suitable times. Products of hydrolysis are identified.
- The conditions are chosen to represent various food processes.

Temperature	Time, min	pH	Processes represented
90ºC	20	4	pasteurisation
100ºC	60	5	baking, brewing, boiling
120ºC	20	6	sterilisation

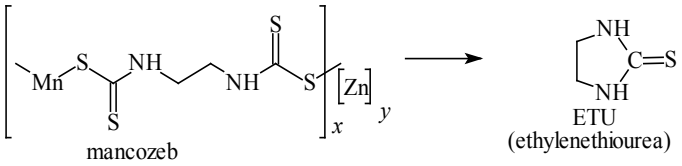
Timme and Walz-Tylla, 2004

11. Captan hydrolysis



captan THPI

12. Mancozeb hydrolysis



13. Processing factor

$$\text{Processing factor} = \frac{\text{residue level [mg/kg] in processed product}}{\text{residue level [mg/kg] in RAC}}$$

Alternative terms:
"*concentration factor*" when residue levels increase, and
"*reduction factor*" (inverse of processing factor) when residue levels decrease.

14. Processing factor

- Processing factor > 1 for some processes

Examples
- Oil-soluble compounds with oil extraction
- Residues in bran
- Drying of fruit

15. Cereal storage

Wheat at 30ºC
- Dichlorvos: half-life 2 months
- deltamethrin, fenvalerate, permethrin: very stable during storage.

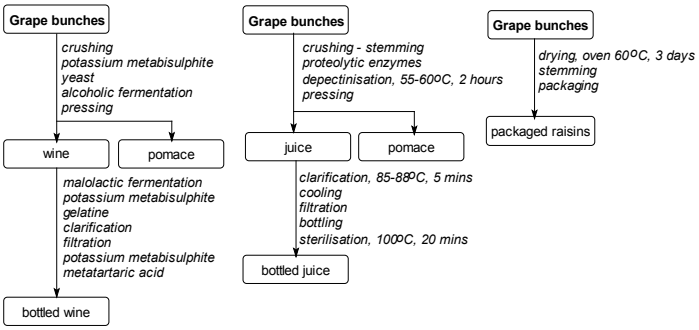
16. Diphenylamine on apples at 2ºC

- Slow penetration into the apple during storage
- Metabolism to hydroxy conjugates
- Transfer from treated to untreated fruit in the same cold store.

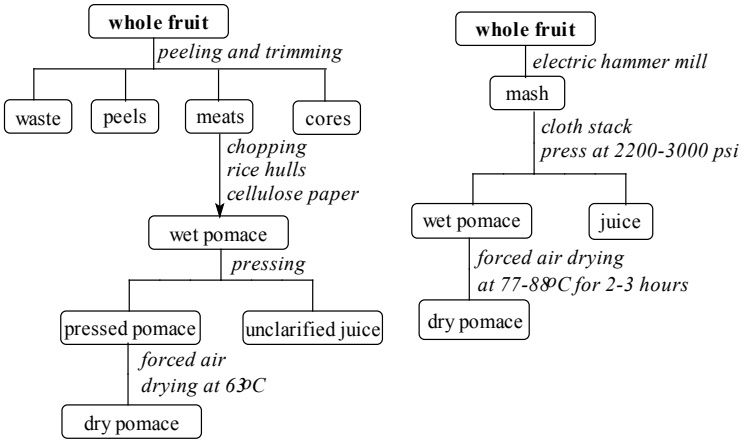
17. Does the simulated process follow the conditions of the commercial process?

- Examine the *flow diagram* of the process and the process conditions.
- Note that *livestock feed materials* (e.g. meals and pomaces) are produced as well as processed food.
- Residues in feed materials may find their way into meat, milk and eggs.
- Flow diagram examples.

18. Grape processing



19. Apple processing

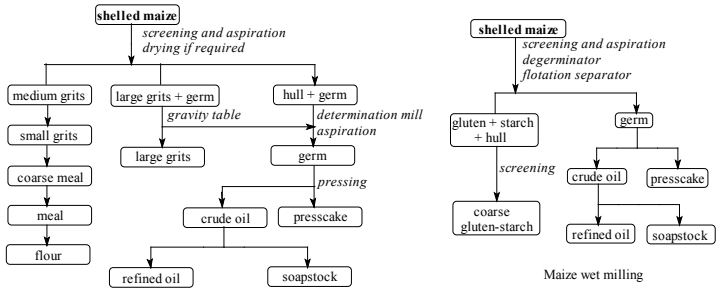


Apple processing, EP-AP-2017

Apple processing, EP-AP-2018

Variations in the process may produce variations of processing factors.

20. Maize processing



Maize dry milling

Maize wet milling

21. Processing factor examples

Pesticide	RAC	Processed commodity	Processing factors	Median or best estimate
Pirimicarb	tomato	juice	0.50 0.62 0.70 0.86 1.54	0.70
Pirimicarb	tomato	puree	0.62 0.64 1.49 2.19 2.33	1.49
Bifenazate	grapes	raisins	0.36 3.2	3.2
Thiamethoxam	coffee beans	roasted coffee	<0.14 <0.14 <0.17 <0.20 <0.20 <0.20 <0.25 <0.25 <0.25 <0.33 <0.33 <0.50	<0.14

Uncertainty in estimation of processing factors? Variability in processing factors?

22. Processing factor examples

Pesticide	Raw agricultural commodity	Processed commodity	Processing factor
Carbaryl	apple	juice	0.36
Deltamethrin	apple	juice	<0.09
Imidacloprid	citrus fruit	juice	0.28
Imidacloprid	cottonseed	oil	<0.09
Propargite	cottonseed	oil	0.20
Propargite	grapes	raisins	1.6

23. Processing factor examples

Pesticide	Raw agricultural commodity	Processed commodity	Processing factor
Bifenthrin	wheat	wheat bran	3.15
Bifenthrin	wheat	white flour	0.31
Novaluron	plum	plum, dried	3.1
Thiamethoxam	cotton seed	meal	0.27
Thiamethoxam	cotton seed	oil	<0.02
Pirimicarb	tomato	juice	0.70
Boscalid	canola	refined oil	1.29

24. Estimation of HR-P and STMR-P - cypermethrin

- Cypermethrin has a registered use for post-harvest treatment of cereal grains as a grain protectant.
- Wheat.
  - STMR: 1.38 mg/kg.
  - HR: 1.5 mg/kg.
  - MRL 2 mg/kg.
- Processing factors
  - wheat → flour 0.35
  - wheat → bran 2.5

25. Estimation of HR-P and STMR-P – cypermethrin

	Wheat flour	Wheat bran
Processing factor	0.35	2.5
STMR-P	1.38 × 0.35 = **0.48**	1.38 × 2.5 = **3.45**
HR-P	1.5 × 0.35 = **0.53**	1.5 × 2.5 = **3.75**

Wheat bran: estimate maximum residue level 5 mg/kg.

Wheat flour: highest residue is less than wheat MRL (2 mg/kg). Therefore a separate MRL for wheat flour is not necessary.

HR-P values are needed because, although flour and bran are bulked and blended commodities, post-harvest treatment may be after bulking and blending.

26. Estimation of STMR-P - thiamethoxam

- Thiamethoxam is registered for foliar use on pome fruits.
- Pome fruits.
  - STMR: 0.07 mg/kg.
  - HR: 0.15 mg/kg.
  - MRL: 0.3 mg/kg.
- Processing factor
  - apple → apple juice 0.93

27. Estimation of STMR-P – thiamethoxam

	Apple juice
Processing factor	0.93
STMR-P	$0.07 \times 0.93 =$ **0.065**

Apple juice: STMR-P is less than pome fruit MRL (0.3 mg/kg). Therefore a separate MRL for apple juice is not necessary.

An HR-P is not necessary for apple juice because the juice is a bulked and blended commodity with the RAC sourced from a number of farms and the thiamethoxam use was on-farm.

28. Consumer risk assessment

- Procedures are available for STMR-P and HR-P estimation.
- Dietary data are available for many processed foods.
- Dietary intake estimation of residues in processed foods is integrated with the dietary intake estimation for unprocessed foods in the IEDI and IESTI spreadsheets.

Abbreviations and acronyms in this chapter

ETU	ethylenethiourea
GAP	good agricultural practice
HR	highest residue
HR-P	highest residue in a processed commodity calculated by multiplying the HR of the raw agricultural commodity by the corresponding processing factor.
IEDI	international estimated daily intake
IESTI	international estimated short-term intake
JMPR	Joint Meeting on Pesticide Residues
LOQ	limit of quantification

RAC	raw agricultural commodity
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor.

Chapter 11. Pesticide Residues in Livestock. Exposure through Animal Feed. Direct Animal Treatments

Livestock feeding studies
Livestock dietary burden of residues
Integration of dietary burden data with livestock feeding data
External treatment of livestock with pesticides
Reconciliation of MRL recommendations from direct treatment and residues in feed
Presentation – residues in livestock

The purpose of this chapter is to explain how livestock (farm animal) feeding studies can be used to predict the resulting residue levels in meat, milk and eggs from the levels of residue in feed materials. Residues may also arise from direct treatment of livestock for ectoparasites. The residues from both sources must be reconciled in the process of residue evaluation.

Relevant sections of JMPR Manual

- Farm animal feeding and external animal treatments.....3.9
- MRLs and STMR values for commodities of animal origin 6.12

In livestock feeding studies, livestock are dosed daily with pesticide at levels that could occur in their diet and higher for some weeks. Milk or eggs are collected throughout the dosing period and in due course the dosed livestock are slaughtered for collection of meat and offal.

The collected animal commodities are then analysed for pesticide residues to establish the relationship between residues in the livestock diet and residues in the meat, milk or eggs.

Livestock feeding studies

The aim of livestock feeding studies is to find the levels of residue likely to result in animal tissues, milk and eggs from repeated daily dosing of the livestock over a few weeks. The equivalent feeding levels should be close to expected residue level burdens from feed commodities.

Milk from dairy cows and eggs from poultry are collected daily. It is important for studies to continue at least until plateau residue levels are reached in milk and eggs.

Fat-soluble pesticides may not be distributed at equal concentrations in the various fat depots in a cow. Fat depots should be sampled and analysed separately (not as a composite) for fat-soluble compounds. MRLs will be established to allow for the highest residue occurring in a fat sample.

For a persistent residue it is also very useful to determine the rate of depletion when the dosing ceases. This is the depuration phase, where additional cattle or birds usually at the highest dose are maintained for periods of 1-3 days up to 1-2 weeks after the final dose. Residues in milk or eggs are monitored and tissues from cattle or birds slaughtered at intervals after the final dose are collected for analysis.

Dairy cow feeding study checklist

Study material

- compound and purity
- doses – ? mg/kg bw/day, equivalent to ? ppm in feed dry weight

- method: capsule or mixed into ration
- dosing regime, ? times per day, number of consecutive days

Animals

- breed
- number of animals in each dosing group
- number of groups, typically 3 dosing groups and control group
- body weights, ? kg
- feed consumption, ? kg feed dry weight per day
- milk production, ? litres or kg per day

Procedures

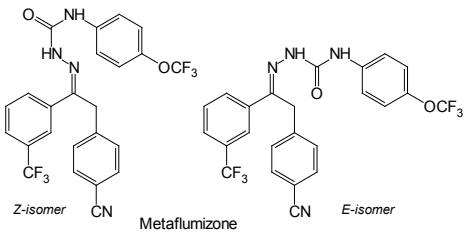
- nature of the feed ration
- milk collection. ? times per day
- milk compositing
- interval between final dose and slaughter for tissue collection
- list of tissues collected, noting different types of fat and muscle
- fat types kept separate or composited for analysis
- separation of cream from milk for separate analysis.

Laying hen feeding study checklist

In principle, the checklist follows that of the dairy cows, with egg production instead of milk production.

Example - laying hens dosed with metaflumizone (JMPR 2009).

Groups of laying white leghorn hens were dosed via gelatin capsule with metaflumizone at the equivalent of 0.1 ppm, 0.3 ppm and 0.9 ppm in the dry-weight feed for 55 days. Eggs were collected twice daily. Birds were slaughtered within 24 hours of the final dose for tissue collection. Some birds from the higher dose group were maintained through a depuration phase for 3, 7, 10, 14, 17 and 27 days after the final dose.



Metaflumizone is a fat-soluble residue.

Table 2. Residues in tissues of birds slaughtered within 24 hours of the final dose.

Tissue	Metaflumizone, mg/kg		
	0.1 ppm in diet	0.3 ppm in diet	0.9 ppm in diet
Muscle	<0.02 (3), 0.021	0.021, 0.024, 0.026, 0.031	0.040, 0.046, 0.051, 0.057
Liver	0.029, 0.030, 0.032, 0.033	0.081, 0.089, 0.096, 0.114	0.161, 0.217, 0.264, 0.298
Fat	0.297, 0.303, 0.327, 0.338	0.921, 1.045, 1.051, 1.245	2.649, 2.737, 3.396, 3.493

Transfer factors may be calculated for the metaflumizone residues in the tissues.

$$\text{Transfer factor} = \frac{Residue_{tissue}}{Conc_{feed}}$$

A reasonably consistent transfer factor across feeding levels suggests that residues are proportional to feeding level and adds to the confidence in interpolation within the feeding levels and extrapolating down to zero and also slightly above the highest level.

For liver and fat, the variability of calculated transfer factors between feeding levels is of the same order as variability within a feeding level. For muscle, where the residues are low (close to LOQ), proportionality or non-proportionality is not so clear.

Table 3. Transfer factors for metaflumizone in laying hens.

Feeding level	Muscle		Liver		Fat	
	Residue, mg/kg	*Transfer factor*	Residue, mg/kg	*Transfer factor*	Residue, mg/kg	*Transfer factor*
0.1 ppm	<0.02		0.029	*0.29*	0.297	*2.97*
0.1 ppm	<0.02		0.030	*0.30*	0.303	*3.03*
0.1 ppm	<0.02		0.032	*0.32*	0.327	*3.27*
0.1 ppm	0.021	*0.21 (omit)*	0.033	*0.33*	0.338	*3.38*
0.3 ppm	0.021	*0.070*	0.081	*0.27*	0.921	*3.07*
0.3 ppm	0.024	*0.080*	0.089	*0.30*	1.045	*3.48*
0.3 ppm	0.026	*0.087*	0.096	*0.32*	1.051	*3.50*
0.3 ppm	0.031	*0.103*	0.114	*0.38*	1.245	*4.15*
0.9 ppm	0.040	*0.044*	0.161	*0.18*	2.649	*2.94*
0.9 ppm	0.046	*0.051*	0.217	*0.24*	2.737	*3.04*
0.9 ppm	0.051	*0.057*	0.264	*0.29*	3.396	*3.77*
0.9 ppm	0.057	*0.063*	0.298	*0.33*	3.493	*3.88*
Mean		*0.069*		*0.296*		*3.37*

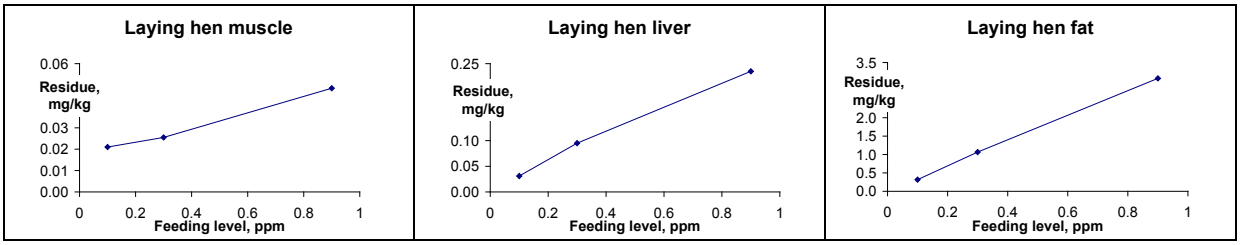


Figure 4. Plots of metaflumizone residues in tissues as a function of feeding levels. Proportionality is suggested for liver and fat.

A plot of residue concentrations in eggs as a function of study day is shown in Figure 2. Residues in eggs have apparently reached a plateau after approximately 21 days, but the large variations in residues at the highest feeding level obscure the situation.

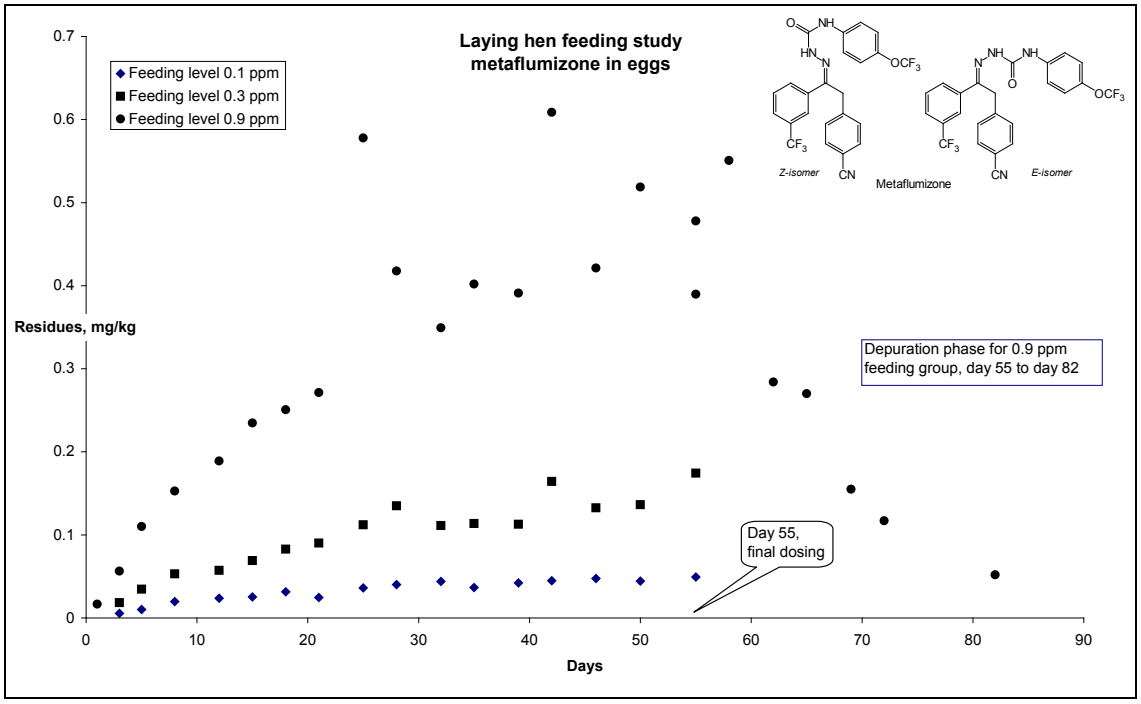
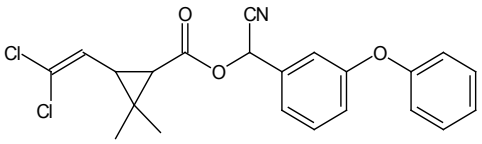


Figure 5. Metaflumizone residues in eggs as a function of study day for three feeding levels. Residue levels declined from day 55 onwards in the depuration phase for the highest feeding level group.

Example - lactating dairy cows dosed with alpha-cypermethrin (JMPR 2008).



Groups of 3 lactating Holstein dairy cows were dosed once daily via gelatin capsule with alpha-cypermethrin at nominal 4 ppm (1×), 12 ppm (3×) and 40 ppm (10×) in the dry-weight diet for 28 consecutive days. Milk was collected on 14 occasions for analysis. On day 29 within 24 hours of the final dose, animals were slaughtered for tissue collection: liver, kidney, omental fat and muscle.

Alpha-cypermethrin is a fat-soluble residue.

Tissue	Alpha-cypermethrin, mg/kg		
	4 ppm in diet	12 ppm in diet	40 ppm in diet
Kidney	<0.05 (3)	<0.05 (3)	<0.05 (3)
Liver	<0.05 (3)	<0.05 (3)	<0.05 (3)
Muscle	<0.05 (3)	<0.05 (3)	<0.05 (3)
Fat, omental	<0.05, 0.058, 0.064	0.16, 0.14, 0.18	0.89, 0.42, 1.01

Residues were below LOQ (0.05 mg/kg) in kidney, liver and muscle even at the highest dose, equivalent to 40 ppm in the dry weight diet. Measurable residue levels were observed at all doses in the omental fat.

Transfer factors may be calculated for the alpha-cypermethrin residues in fat.

$$\text{Transfer factor} = \frac{Residue_{fat}}{Conc_{feed}}$$

Alpha-cypermethrin in feed	Residue in fat, mg/kg	Transfer factor
4 ppm	0.058	0.015
4 ppm	0.064	0.016
12 ppm	0.16	0.013
12 ppm	0.14	0.012
12 ppm	0.18	0.015
40 ppm	0.89	0.022
40 ppm	0.42	0.011
40 ppm	1.01	0.025

The transfer factors are consistent throughout the dosing range, with allowance for the typical variation observed between animals within the one dosing group. The results suggest a proportional relationship between alpha-cypermethrin residues in the animal fat and in the animal diet over the test range. The proportionality could reasonably be assumed down to zero dose and slightly above the 40 ppm.

Livestock dietary burden of residues

Livestock are exposed to pesticide residues through their diet.

In the Codex Commodity Classification primary animal feeds fall into three main groups.
AL. Legume animal feeds, e.g. alfalfa fodder, pea hay and peanut forage.
AS AF. Straw, fodder and forage of cereal grains, e.g. barley straw and fodder and maize forage.
AM AV. Miscellaneous fodder and forage crops, e.g. fodder beet, turnip leaves or tops, sugar cane fodder and almond hulls

Food processing produces by-products that are also used extensively as animal feeds.
CM. Milled cereal products, e.g. wheat bran.
AB. By-products of fruit and vegetable processing, e.g. apple pomace and sugar beet pulp.
SM. Miscellaneous secondary food commodities of plant origin, e.g. cotton seed meal.

Some human foods that are also used as animal feeds.
GC. Cereal grains, e.g. wheat and maize.
VR. Root vegetables, e.g. potato culls.
VB. Brassica vegetables, e.g. cabbage.

After supervised residue trials and food processing studies have been evaluated for a pesticide, a table of STMR, high residue and MRLs is prepared. Those commodities that are used as livestock feeds are selected for dietary burden calculations.

The calculation of the dietary burden in livestock relies on the diets listed in OECD Feed Table 2009 (available from the FAO website: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmpr/jmpr-docs/en/).

Calculation from high residue, STMR (some bulk commodities) and STMR-P values provides the levels in feed suitable for estimating MRLs, while calculation from STMR and STMR-P values for feed is suitable for estimating STMR values for animal commodities.

For the calculation, residues are expressed on dry weight.

Procedure
Choose the feed commodities that will produce the highest residues in the animal dietary burden, noting the limits in the diet for each commodity and for commodity groups. The final total diet is not to exceed 100 %.

The limits within commodity groups are best illustrated by an example. The maximum percentage of the diet for each feed commodity must include the percentages already assigned to other feeds of the same group.

Example – cypermethrin on cereal grains, calculation for dietary burden

Beef cattle

Commodity	Commod group	Residue mg/kg	Basis	% Dry matter	Residue mg/kg dw	*Diet content (%)*				Residue contribution (ppm)			
						US-CAN	*EU*	*AU*	*JP*	US-CAN	EU	AU	JP
Barley grain	GC	0.04	STMR	88	0.040	*50*	*70*	*80*	*70*	0.02	0.03	0.03	0.03
Corn, field, grain	GC	0.01	STMR	88	0.011	*80*	*80*	*80*	*75*	0.01	0.01	0.01	0.01
Oats	GC	0.02	STMR	89	0.022		*40*	*80*	*55*		0.01	0.02	0.01
Rice	GC	0.57	STMR	88	0.648	*20*		*40*		0.13		0.26	
Wheat grain	GC	0.01	STMR	89	0.011	*20*	*40*	*80*	*25*	0.00	0.01	0.01	0.00

Sort the group in descending order of residue expressed on dry weight and then assign the diet content percentage in that order of priority.

Beef cattle

Commodity	Commod group	Residue mg/kg	Basis	% Dry matter	Residue mg/kg dw	*Diet content (%)*				Residue contribution (ppm)			
						US-CAN	*EU*	*AU*	*JP*	US-CAN	EU	AU	JP
Rice	GC	0.57	STMR	88	0.648	*20*		*40*		0.13		0.26	
Barley grain	GC	0.04	STMR	88	0.040	*50*	*70*	*80*	*70*	0.02	0.03	0.03	0.03
Oats	GC	0.02	STMR	89	0.022		*40*	*80*	*55*		0.01	0.02	0.01
Corn, field, grain	GC	0.01	STMR	88	0.011	*80*	*80*	*80*	*75*	0.01	0.01	0.01	0.01
Wheat grain	GC	0.01	STMR	89	0.011	*20*	*40*	*80*	*25*	0.00	0.01	0.01	0.00

US-Can animal diet. Rice is assigned 20 %. Barley is allowed 50 %, but 20 % of the group is already assigned, so 50-20 %=30 % is assigned to barley. Field corn grain is allowed 80 %, but 50 % of the group is already assigned, so 30 % is assigned to corn. Wheat is allowed 20 %, which is less than the 80 % already assigned to the group, so wheat has no contribution.

EU animal diet. Barley grain is assigned 70 %. Oats and wheat grain are not allowed more than 70 %, so are deleted. Field corn grain is allowed 80 %, but 70 % of the group is already assigned, so 10 % is allocated to field corn grain.

Australia animal diet. Rice grain is assigned 40 %. Barley grain is allowed 80 %, but with 40 % of the group already assigned, 40 % is assigned to barley grain. Oats, corn and wheat are not allowed more than the 80 % already allocated for the group, so are deleted.

Japan animal diet. Barley grain is assigned 70 %. Oats and wheat grain are not allowed more than 70 %, so are deleted. Field corn grain is allowed 75 %, but 70 % of the group is already assigned, so 5 % is allocated to field corn grain.

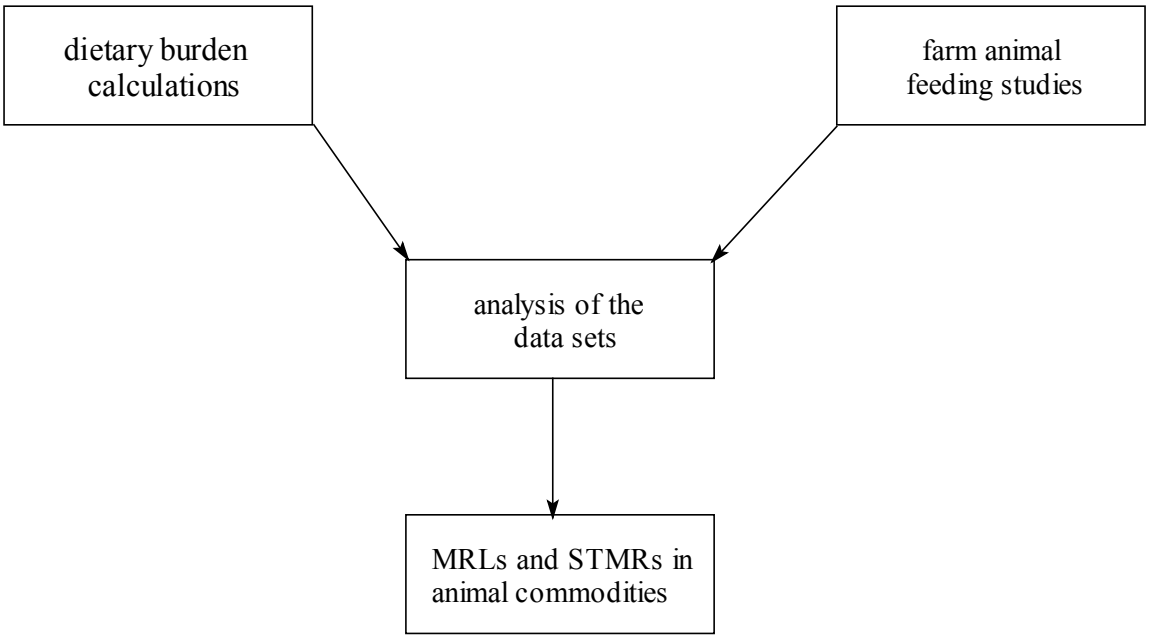
The table now becomes:

Beef cattle

Commodity	Commod group	Residue mg/kg	Basis	% Dry matter	Residue dw mg/kg	*Diet content (%)*				Residue contribution (ppm)			
						US-CAN	*EU*	*AU*	*JP*	US-CAN	EU	AU	JP
Rice	GC	0.57	STMR	88	0.648	*20*		*40*		0.13		0.26	
Barley grain	GC	0.04	STMR	88	0.040	*30*	*70*	*40*	*70*	0.01	0.03	0.02	0.03
Oats	GC	0.02	STMR	89	0.022								
Corn, field, grain	GC	0.01	STMR	88	0.011	*30*	*10*		*5*	0.00	0.00		0.00
Wheat grain	GC	0.01	STMR	89	0.011								

Once all of the group allocations are completed, all of the feed items are sorted in descending order of residue expressed on dry weight, and final assignments are made in that priority order until 100 % total diet is reached.

Integration of dietary burden data with livestock feeding data



The next step is to apply the relevant livestock dietary burden to the results of the feeding studies to predict likely residues in meat, milk and eggs.

The JMPR has decided when to use median residues or high residues and when to use average or high-animal values from the feeding studies. The many possibilities are explained in the JMPR Manual.

The following example shows how the selected dietary burdens for cattle were applied to an alpha-cypermethrin feeding study to estimate STMR and high residue values for cypermethrin residues in fat.

A similar process is followed for the other animal commodities.

Example - estimated maximum and mean dietary burdens of livestock, cypermethrin

The results of all of the dietary burden calculations are summarised for a decision on which ones to use for maximum residue level and STMR estimations. (Note: in 2008, when cypermethrin was evaluated, the livestock feed tables did not include data from Japan.)

		Livestock dietary burden, cypermethrin, ppm of dry matter diet		
		US-Canada	Europe	Australia
Max	beef cattle	20.7	24.4	**31.4** [a]
	dairy cattle	13.8	17.1	**21.6** [c]
	poultry - broiler	0.16	0.05	0.35
	poultry - layer	0.16	**2.2** [e]	0.35
Mean	beef cattle	7.9	8.3	**11.3** [b]
	dairy cattle	5.3	7.6	**8.3** [d]
	poultry - broiler	0.16	0.05	0.35
	poultry - layer	0.16	**0.66** [f]	0.35

[a] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat.
[b] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.
[c] Highest maximum dairy cattle dietary burden suitable for MRL estimates for milk.
[d] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.
[e] Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs.
[f] Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs.

The selected dietary burdens are then applied to the results of livestock feeding studies to determine the residues in the livestock commodities.

For the cattle tissues, the maximum burden for beef or dairy cattle is applied to the highest individual animal residues in the feeding studies to estimate the high residues in tissues.

The mean dietary burden is applied to the mean (of the animal study group) residues in the feeding studies to estimate STMR values for residues in the tissues.

In the cypermethrin example the 31.4 ppm and the 11.3 ppm dietary burdens are used for HR and STMR estimations respectively (Figure 3).

The 31.4 ppm produced a high residue of 0.76 mg/kg in the fat.

The 11.3 ppm produced an STMR value of 0.15 mg/kg for the fat.

Similar procedures are followed for the other tissues and milk and for residues in poultry.

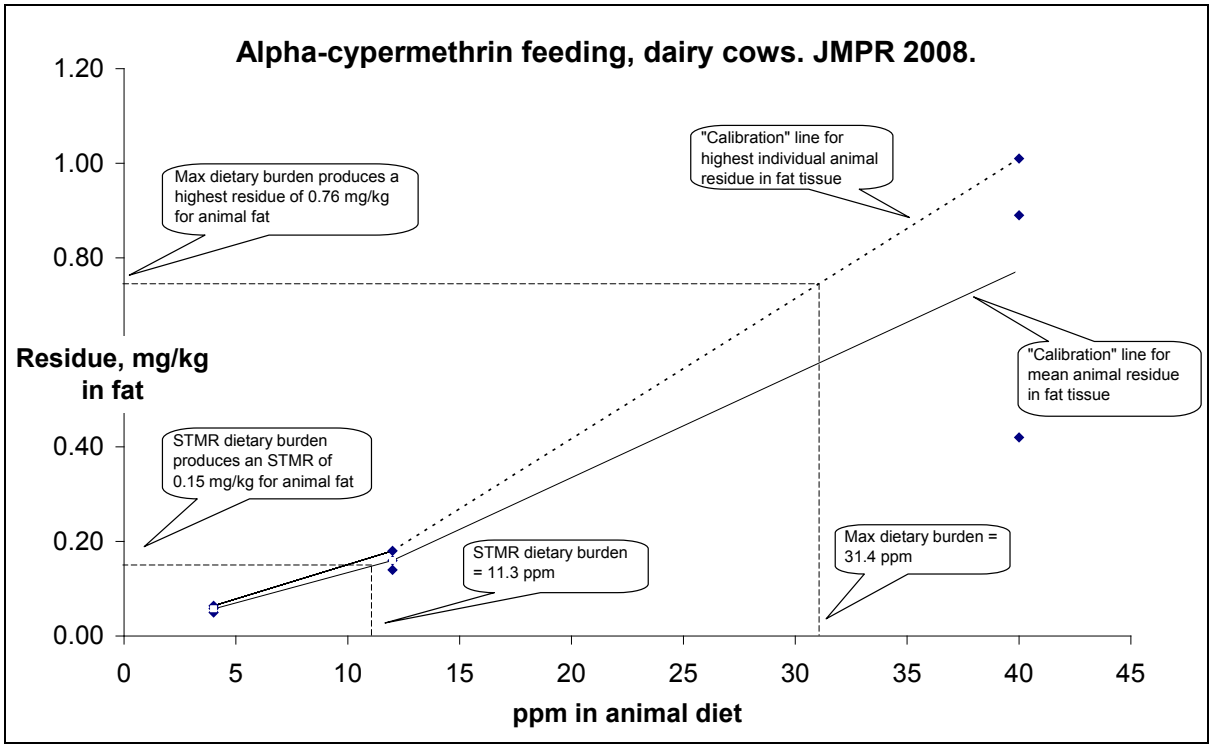


Figure 6. Alpha-cypermethrin residues in beef fat as a function of feeding level resulting from a dairy cow feeding study. Studies on the cypermethrins were generally considered mutually supportive and could be considered together.

External treatment of livestock with pesticides

Pesticides are used directly on livestock for control of lice, flies, ticks and other pests.

The treatment may produce residues in meat, milk or eggs. However, control of the situation and the timing is more under the control of the stock owner than is possible with residues in animal feed materials.

The permitted methods of use, the doses and the timing should be described clearly on the registered label.

As with supervised trials on crops, supervised trials with pesticide uses on livestock should focus on doses, methods and timing within the label instructions likely to produce the highest residues. This is because the data will be used for estimation of maximum residue levels.

Ectoparasiticides are needed in sheep production especially for lice and blow-fly control.

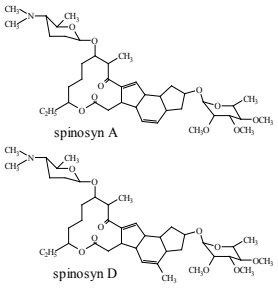
A number of use patterns are possible.

- Sheep production cycle
  - off-shears
  - short-wool
  - long-wool
- Application method
  - plunge dip
  - spray or shower dip
  - jetting
  - pour-on

- Specific situations
  - flystrike dressing
  - wound dressing

When the directions for use combine sheep at different stages of the production cycle with the various application methods, the number of possibilities to be all covered by supervised trials is unworkable. Supervised trial conditions should be selected carefully on the basis of experience to obtain the maximum likely residues.

Example - spinosad uses on sheep and cattle. (JMPR 2001, 2004).



Supervised trials data were available from Australia for the uses of spinosad on sheep in a plunge dip, by jetting application and by application of an aerosol formulation to flystrike wounds.

The flystrike treatment produced higher residues in the tissues than the other treatments.

Resulting maximum residue levels.

MM 0822	Sheep meat	0.3 (fat)
MO 0822	Sheep, edible offal of	0.1 mg/kg

Supervised trials were available from USA for the direct application of spinosad to cattle by spraying or pour-on.

Resulting maximum residue levels.

MM 0812	Cattle meat	3 (fat)
MO 1280	Cattle kidney	1 mg/kg
MO 1281	Cattle liver	2 mg/kg

A cattle dietary burden for spinosad and a dairy cow feeding study resulted in the following maximum residue level recommendations

MM 0095	Meat (mammalian)	2 (fat)
MO 0105	Edible offal (mammalian)	0.5 mg/kg

Reconciliation of MRL recommendations from direct treatment and residues in feed

Three points need to be kept in mind in the reconciliation to reach suitable MRL recommendations.

1) The MRL recommendations must be high enough to cover residues arising from the various legitimate uses.

2) Residues arising from a direct use registration should occur only in the species listed on the product label, i.e. a registered use on sheep cannot legitimately produce residues in cattle. MRLs based on direct uses should be MRLs for the commodities from the species listed on the registration.

3) Residues in an animal feed may produce residues in any species consuming the feed, so MRLs for animal commodities based on residues in feed should relate to the broad commodity groups of mammalian and poultry.

In the spinosad example, residues in sheep commodities from direct uses are lower than residues in mammalian commodities from feed intake, so it is not necessary to recommend sheep commodity MRLs separately from mammalian commodity MRLs.

However, residues in cattle commodities are higher from the direct treatment than from feed intake, so it is necessary to recommend separate cattle MRLs.

This means that the mammalian commodity MRL recommendations need to exclude cattle commodities and include the qualification *"except cattle"*.

After the process of reconciliation, the MRL recommendations become:

MM 0812	Cattle meat	3 (fat)
MO 1280	Cattle kidney	1 mg/kg
MO 1281	Cattle liver	2 mg/kg
MM 0095	Meat (mammalian) [except cattle]	2 (fat)
MO 0105	Edible offal (mammalian) [except cattle]	0.5 mg/kg

The estimation of STMRs and HRs follows the same procedure.

A similar approach is followed when JECFA recommends MRLs for veterinary uses of compounds that are used as pesticides. A reconciliation is needed so that Codex has consistent MRLs for commodities in trade, whether the MRLs are based on veterinary or pesticide uses or both.

PRESENTATION – RESIDUES IN LIVESTOCK

1. Residues in livestock.

2. Sources of residues in livestock

Pesticide residues may occur in meat, milk and eggs as a result of residues in feed materials.

Residues may also arise from direct treatment of livestock for ectoparasites.

The residues from both sources must be reconciled in the process of residue evaluation.

3. Primary animal feeds

Legume animal feeds – Codex Code AL
- alfalfa fodder
- pea hay
- peanut forage

Straw, fodder and forage of cereal grains – Codex Code AS and AF
- barley straw and fodder
- maize forage

Miscellaneous fodder and forage crops – Codex Code AM and AV
- fodder beet
- turnip leaves or tops

4. Dry-weight basis

MRLs for animal feeds should be set and expressed on a "dry-weight" basis.

A “dry-weight” basis implies that the commodity is analysed for pesticide residues as received, that the moisture content is determined, preferably by a standard method for use on the relevant commodity, and that the residue content is then calculated as if it were wholly contained in the dry matter.

5. Processed commodities used for animal feed

Milled cereal products - Codex Code CM
- wheat bran
- rice hulls

By-products of fruit and vegetable processing – Codex Code AB
- apple pomace
- sugar beet pulp.

Miscellaneous secondary food commodities of plant origin – Codex Code SM
- cotton seed meal
- soybean hulls.

6. Food commodities used as animal feeds

Root vegetables – Codex Code VR
- potato culls

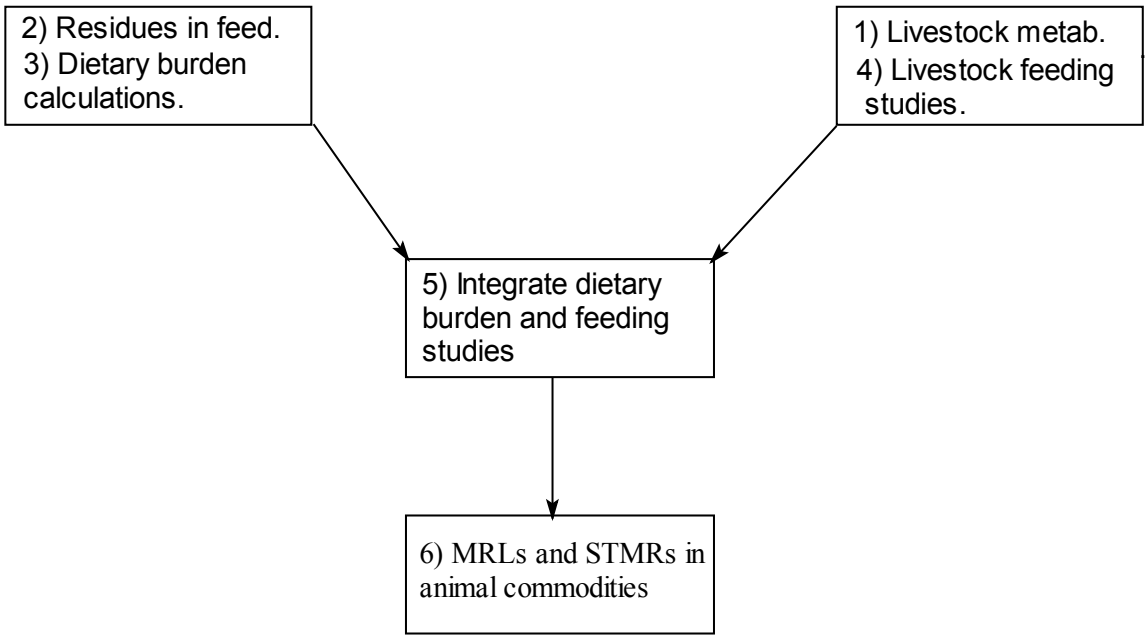
Pulses - Codex Code VD

- dry beans

Cereal grains - Codex Code GC

- maize

7. The evaluation process



8. The evaluation process 1

Step 1. Livestock metabolism – determine the composition of the residue in livestock tissues, milk and eggs.
Step 2. Determine the levels of residue occurring in animal feed materials as a result of the pesticide use following (GAP) and as by-products from food processing.
Step 3. Calculate the livestock dietary burden from residue levels and livestock diets.

9. The process 2

Step 4. Livestock feeding studies – determine the levels of residue in livestock tissues and milk as a function of residue levels in the animal diet.
Step 5. Integrate the dietary burden and the results of the livestock feeding studies to estimate residue levels in animal commodities.
Step 6. Estimate MRLs, STMRs and HRs for animal commodities.

10. Direct animal treatment

Examine supervised trials where the pesticide was used on livestock according to a registered label.

Estimate animal commodity STMR, HR and MRL values that relate to the registered use.

11. Reconciliation

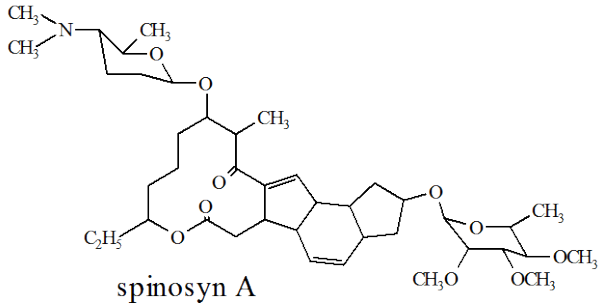
Reconcile the estimates from the two sources of residues.

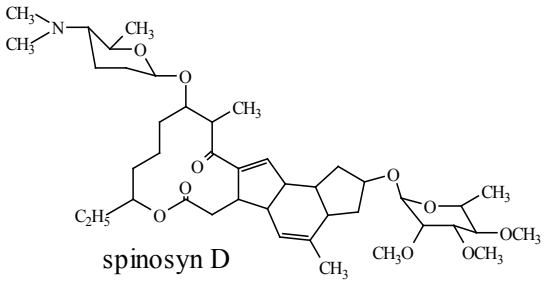
The final MRL and intake estimates should be based on the source of the higher residues.

Note that the direct use will often relate to a single species, while residues from feed may produce residues in the broad groups, mammalian livestock and poultry.

12. Worked example

Select spinosad





13. Step 1. Livestock metabolism

The metabolism suggests a residue definition and provides data on fat-solubility.

Spinosad.
Residue definition: sum of spinosyn A and spinosyn D (enforcement and dietary intake).
The residue is fat soluble.

14. Step 2. Supervised trials and food processing

Supervised trials results

Commodity	Group	STMR mg/kg	High residue mg/kg
Maize forage	AF AS	.70 dw note[19]	.1 dw
Maize fodder	AF AS	.46 dw	.1 dw
Wheat straw and fodder, dry	AF AS	.215 dw	.83 dw
Cereal grains (Po) note[20]	GC	.70	.95 note[21]
Almond hulls	AM	.56	.1

15. Step 2. Supervised trials and food processing

Processed commodities

Commodity	Group	STMR-P
Apple pomace wet	AB	0.064
Citrus pulp	AB	0.12
Cotton seed hulls	SM	0.002

[19] dw = dry weight
[20] The calculation requires data entry for individual commodities.
[21] A post-harvest treatment may occur after blending and bulking, so we need the high residue.

Cotton seed meal	SM	0.0017

16. Step 2. Supervised trials and food processing

Convert national descriptions to OECD Document descriptions. Examples

Fodder beet =	Beet, mangel fodder
Peanut fodder =	Peanut hay
Rape seed meal =	Canola meal
Maize =	Field corn grain
Maize fodder =	Field corn stover

17. Step 3. Livestock dietary burden

Information required

- residue levels in feed materials
- livestock diets (OECD Feed Table 2009 (available from the FAO website: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmpr/jmpr-docs/en/).)
- automated dietary burden calculator spreadsheet

18. Dietary burden calculation

Data to enter

Commodity group	Commodity	Highest residue	STMR or STMR-P	Basis for max	% dry matter
AM/AV	Almond hulls		0.56	STMR	90
AB	Apple pomace, dry		0.064	STMR	40
GC	Barley Po	0.95	0.70	HR	88
AB	Citrus pulp, dry		0.12	STMR	91
AM/AV	Cotton gin byproducts		0.002	HR	90
SM	Cotton meal		0.0017	STMR	89
GC	Maize Po	0.95	0.70	HR	88
AF/AS	Maize fodder	2.1	0.46	HR	100
AF/AS	Maize forage	3.1	0.70	HR	100
CM/CF	Maize milled byproducts		0.13	STMR	85
GC	Millet Po	0.95	0.70	HR	88
GC	Oats Po	0.95	0.70	HR	89
GC	Rice Po	0.95	0.70	HR	88
CM/CF	Rice hulls		2.0	STMR	90
GC	Rye Po	0.95	0.70	HR	88
GC	Sorghum Po	0.95	0.70	HR	86
GC	Triticale Po	0.95	0.70	HR	89
GC	Wheat Po	0.95	0.70	HR	89
CM/CF	Wheat milled byproducts		1.4	STMR	88
AF/AS	Wheat straw and fodder, dry	0.83	0.215	HR	88

Notes:

1) Cereals with residues from post-harvest uses (Po) require data entries for highest residue and STMR. In the "Basis" column, "STMR" is replaced by "HR".
2) Where residues are already expressed on dry weight, 100 should be entered into "% dry matter" column.

19. Dietary burden calculation

BEEF CATTLE												MAX
Commodity	CC	Residue (mg/kg)	Basis	DM	Residue dw (mg/kg)	*Diet content (%)*				Residue Contribution (ppm)			
						US-	*EU*	*AU*	*JP*	US-	EU	AU	JP
Maize forage	AF/AS	3.1	HR	100	3.10	*15*	*80*	*80*		0.47	2.48	2.48	
Rice hulls	CM/CF	2	STMR	90	2.22			*5*				0.11	
Wheat milled bypdts	CM/CF	1.4	STMR	88	1.59	*40*	*20*	*15*	*55*	0.64	0.32	0.24	0.87
Sorghum grain	GC	0.95	HR	86	1.10	*40*			*35*	0.44			0.39
Barley grain	GC	0.95	HR	88	1.08	*5*			*10*	0.05			0.11
Total						*100*	*100*	*100*	*100*	**1.60**	**2.80**	**2.83**	**1.37**

Section of the spreadsheet.
Highest max dietary burden for beef cattle is 2.83 ppm in the dry matter diet, based on the Australian standard animal diet.

20. Dietary burden calculation

		US-Can	EU	Aust	Japan
Max	Beef	1.60	2.80	**2.83**	1.37
	Dairy				
	Broiler				
	Layer				
Mean	Beef	1.12	1.04	1.16	**1.24**
	Dairy				
	Broiler				
	Layer				

Summary table.
Highest max dietary burden for beef cattle is 2.83 ppm in the dry matter diet, based on the Australian standard animal diet.
The mean dietary burden for beef cattle is 1.24 ppm, based on the Japanese standard animal diet.

21. Step 4. Livestock feeding studies

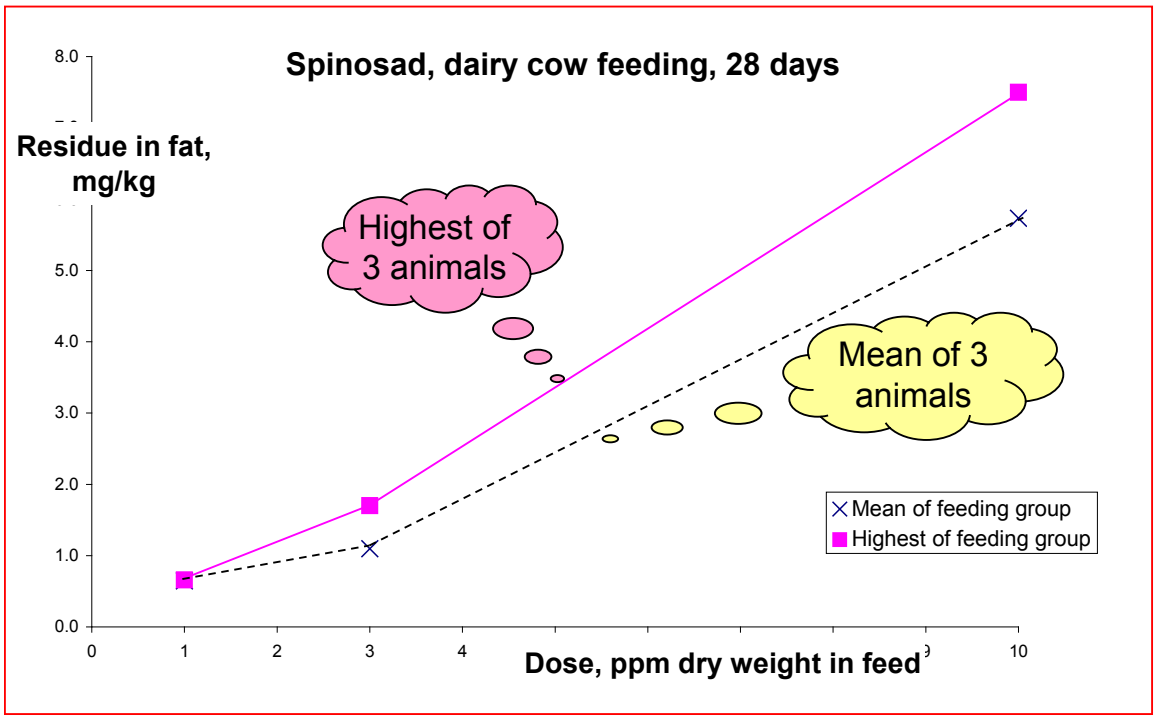
Dairy cows (groups of 3) were dosed daily orally with spinosad at 1, 3 and 10 ppm in dry weight diet for 28 days.
Residues were measured regularly in the milk.
After slaughter on day 28, residues were measured in muscle, kidney, liver and fat.

22. Step 4. Livestock feeding studies

Dose, ppm	Tissue	Residues (3 animals)	Mean	Highest
1	fat	0.62 0.66 0.66	0.65	0.66
3	fat	0.81 0.78 1.7	1.1	1.7
10	fat	6.1 3.6 7.5	5.7	7.5

Similar data are available for the other tissues – liver, kidney and muscle.

23. Residues in fat – relation to dose



24. Step 5. Integrate the dietary burden and the results of the livestock feeding studies

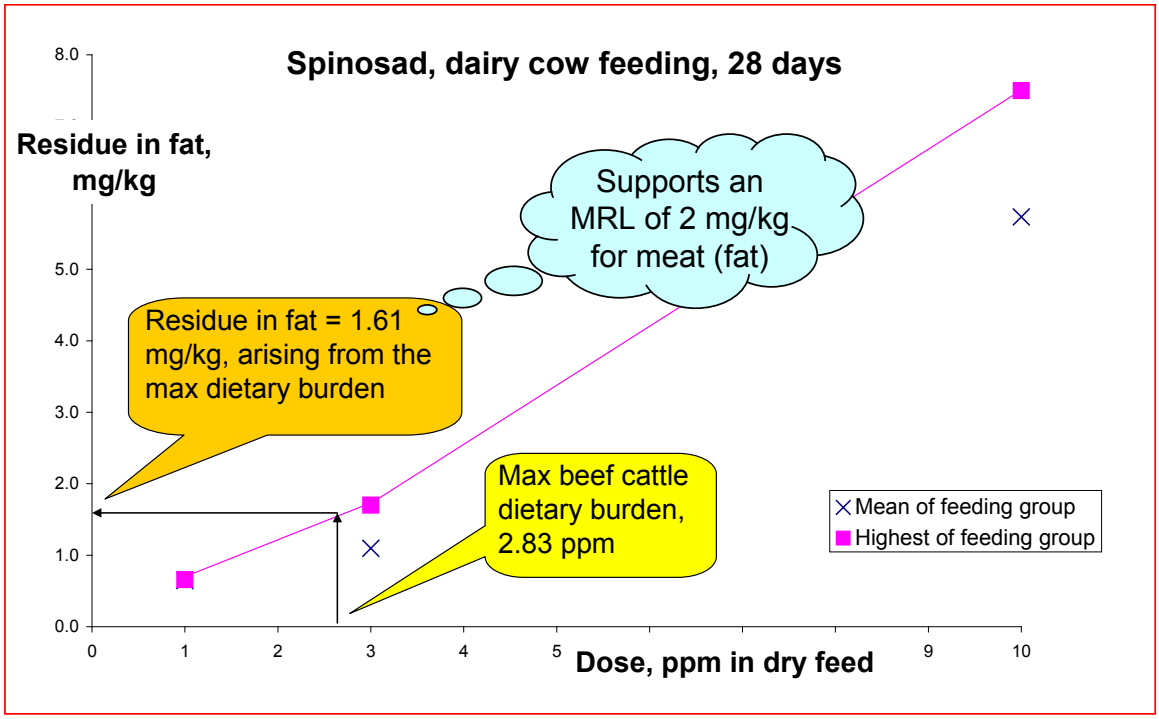
Highest likely residues

- Combine the maximum dietary burden with the highest individual animal residues in the feeding studies.

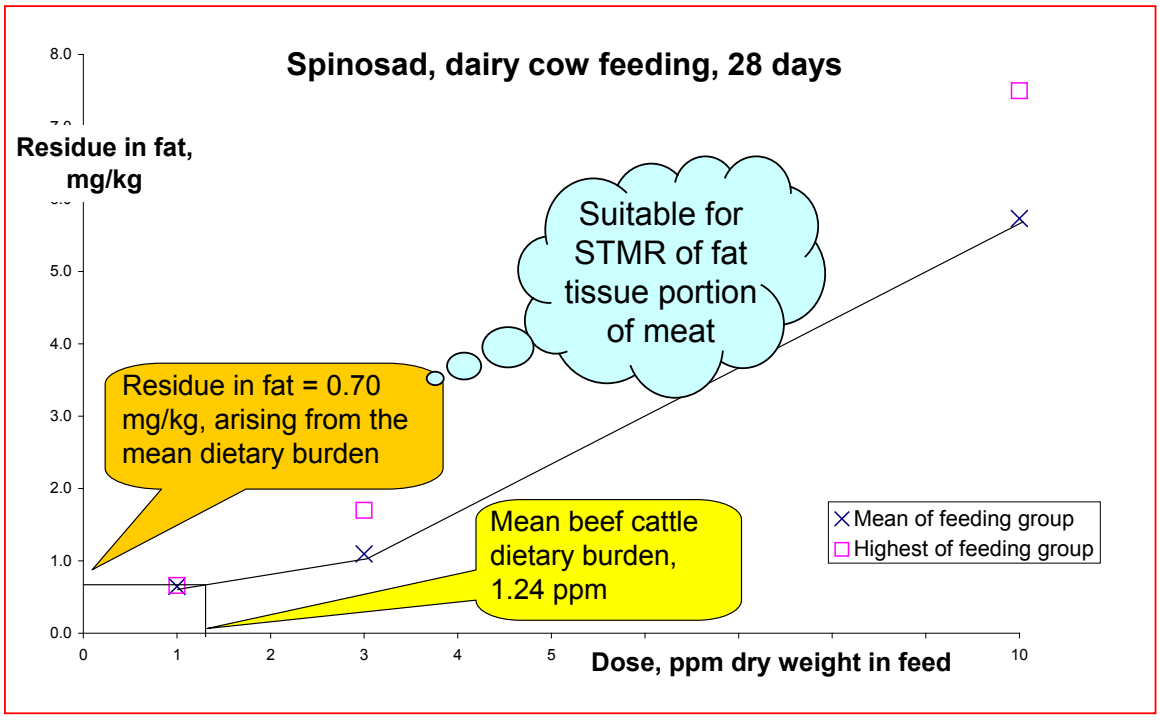
STMR values

- Combine the mean dietary burden with mean animal residues in the feeding studies.

25. Residue in tissue resulting from max dietary burden



26. Residue in tissue resulting from mean dietary burden



27. Residues in tissues

Similar processes are followed for each of the tissues

- Muscle
- Liver
- Kidney
- Fat

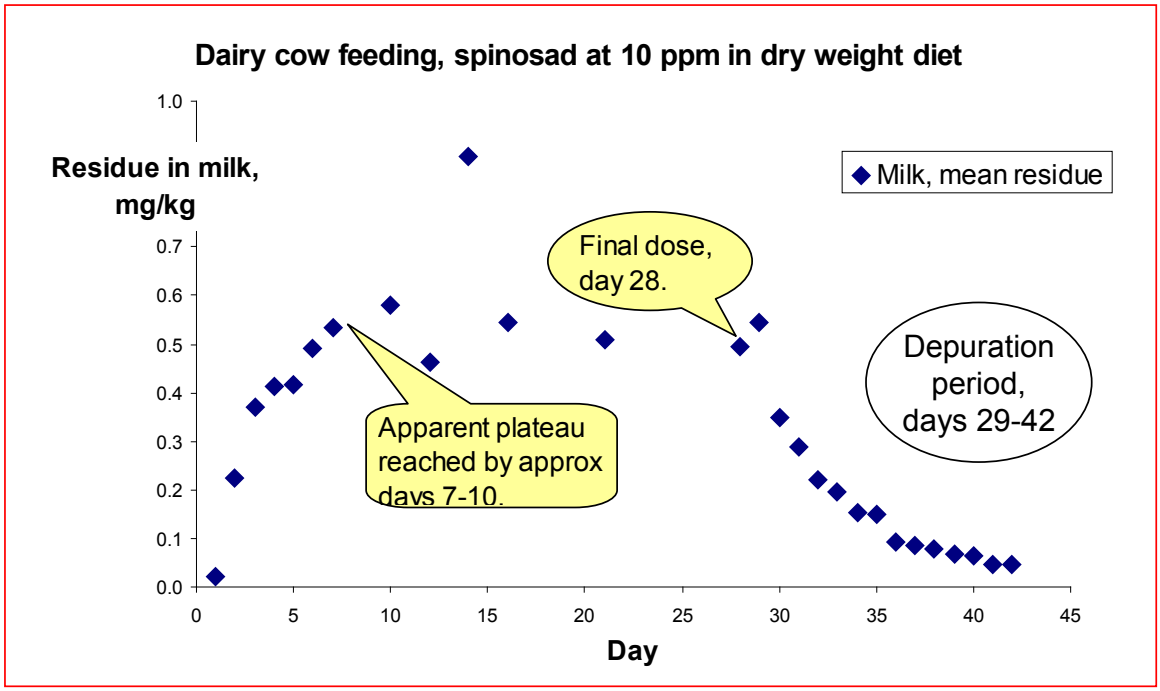
28. Residues in milk

Residue levels in milk should increase during early dosing until an approximate plateau level is established.

Residue levels during the plateau are used in the STMR and MRL estimates.

Some studies include a depuration period, where the dosing is discontinued and the disappearance of residues from the milk is observed.

29. Residues in milk



30. Tissues, milk and eggs

Procedures similar to those described for fat are followed for the other tissues: muscle, liver and kidney.
Because milk is bulked, the mean value is used in the calculations. For fat-soluble compounds, the concentrations in milk fat are also estimated.
For poultry studies, similar procedures are followed to estimate STMR and highest residues in muscle, fat, liver and eggs.

31. Step 6. Estimate MRLs, STMRs and HRs for animal commodities

If data are available for cattle, the JMPR policy is to estimate MRLs, STMRs and HRs for the groups

- Meat (from mammals ……..
- Edible offal (Mammalian)
- Milks

If data are available for chickens, similar policy

- Poultry meat
- Poultry, Edible offal of
- Eggs

32. Spinosad, direct treatment of livestock

In USA, beef and dairy cattle may be treated directly with a pour-on formulation. Animals may also be sprayed.
In Australia, sheep may be treated directly with spinosad at different production stages and by different methods

- Jetting – long wool
- Plunge dip, 2-6 weeks after shearing.
- Shower dip, 2-6 weeks after shearing.
- Wound dressing

33. Reconciliation of residues from feed and direct uses

The direct spinosad uses on cattle produced higher residues than from feed.
The direct spinosad uses on sheep produced residues below those from feed.
The end result:

- MRLs for cattle meat and offal (to cover the direct use)
- MRLs for mammalian, except cattle, meat and offal (to cover residues from feed and the direct uses on sheep)

34. Spinosad MRLs

MRL accommodates external animal treatment.

- MO 1280 Cattle kidney 1 mg/kg
- MO 1281 Cattle liver 2 mg/kg
- MM 0812 Cattle meat 3 (fat) mg/kg
- ML 0812 Cattle milk 1 mg/kg

Other animal commodity MRLs

- MO 0105 Edible offal (mammalian) [except cattle] 0.5 mg/kg
- MM 0095 Meat (from mammals other than marine mammals) [except cattle] 2 (fat) mg/kg

35. Pesticide residues in livestock

When feed is the source of residue

- Metabolism → residue definition
- Residue levels in feed materials
- Livestock dietary burden
- Livestock feeding studies
- Calculate levels in tissues from dietary burden and feeding studies
- Estimate MRLs, STMRs and HRs for animal commodities.

36. Pesticide residues in livestock

When direct use is the source of the residue

- Registered uses
- Residue trials with conditions matching registered uses generate residue data
- Estimate levels in tissues

MRL recommendations to accommodate the various sources of residues from registered uses on crops and animals.

Abbreviations and acronyms in this chapter

AB	by-products, used for animal feeding purposes, derived from fruit and vegetable processing
AF	forage of cereal grains and grasses
AL	legume animal feeds
AM	miscellaneous fodder crops
AS	straw, fodder (dry) and hay of cereal grains and other grass-like plants
AV	miscellaneous forage crops
CM	milled cereal products
GC	cereal grains
JECFA	Joint Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
MRL	maximum residue limit
OECD	Organization for Economic Co-operation and Development
SM	miscellaneous secondary food commodities of plant origin
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor
VB	Brassica (cole or cabbage) vegetables, head cabbage, flowerhead brassicas
VR	root and tuber vegetables

Chapter 12. Expression of Maximum Residue Limits (MRLs).

General principles
Expression of MRLs at or about the limit of quantification (LOQ)
Numerical expression of MRLs

The purpose of this chapter is to explain the conventions for expressing MRLs.

Relevant sections of JMPR Manual

- Expression of maximum residue limits (MRLs).....6.13

General principles

The estimated maximum residue levels and recommended residue limits are expressed in mg residue (as defined)/kg commodity. The portion of commodity to which Codex MRLs apply is given in Codex Alimentarius Vol. 2 (extracted in Appendix VI).

The residues are expressed on fresh-weight basis as they enter international trade (as received by the laboratory) in most commodities, with the exception of animal feeds.

There are some special cases:

MRLs for **animal feeds** are recommended on a dry-weight basis, because of the great variation of their moisture content. For estimation of maximum residue levels the moisture content of the feed item should be known. Alternatively, as a worst case, it can be assumed that the residues were expressed in the trial reports on fresh weight basis, or the data may not be suitable for estimation of maximum reside levels.

For meat, the MRLs for fat soluble pesticides are expressed on the fat (trimmable fat or fat tissue expressed on the lipid content). This is indicated in brackets (fat) after the residue value.

For those commodities where the adhering fat is insufficient to provide a suitable sample, the whole meat commodity (without bone) is analysed and the MRL applies to the whole commodity.

For all other pesticides and residues in liver and kidney the MRLs apply to the whole commodity as it moves in trade. For eggs, the portion of the commodity to which the MRL applies (and which is analysed) is whole egg whites and yolks combined after removal of shell.

For milk, since 2004, where possible, two maximum residue levels are estimated for fat soluble pesticides: one for whole milk and one for milk fat. When needed, maximum residue levels for milk products (e.g. cheese) can then be calculated from the two values, by taking into account the fat content of the milk product and the contribution from the non-fat fraction.

Based on the decision of the 2008 CCPR, a footnote is inserted to indicate where MRLs are established for both milk fat and whole milk: “for monitoring and regulatory purposes, whole milk is to be analysed and the result compared to the MRL for whole milk”.

For compounds that are not fat-soluble, MRLs are expressed on the whole milk.

MRLs based on **direct animal treatment** are footnoted “the MRL accommodates external animal treatment”.

MRLs reflecting special uses or conditions are also distinguished by letters after the limit: Currently the following cases are distinguished by the letters indicated below:

E	The MRL is based on extraneous residues
Po	The MRL accommodates post-harvest treatment of the commodity
PoP	The MRL for the processed commodity accommodates post-harvest treatment of the primary commodity

Expression of MRLs at or about the limit of quantification (LOQ)

The LOQ is the lowest concentration of a compound that can be determined in a commodity with an acceptable degree of certainty.

The JMPR generally recommends maximum residue limits for non-detected residues at a level which can be achieved in regulatory laboratories. Such levels are reported as residues at or about the LOQ* and indicated with an asterisk after the number.

An MRL so identified does not always necessarily imply that residues of the pesticides do not occur in that commodity. The application of a more sensitive or specific method may reveal detectable residues in some commodities. Where metabolism studies or other information indicates that no residue is expected in a commodity, the STMR is considered to be zero and this value is used in the intake calculations.

Setting and enforcing MRLs for residues occurring at or about the LOQ of analytical procedures may require different approaches depending on the composition and definition of the residues. It is emphasized that all available relevant information should be carefully considered ensuring that an MRL established at a level equivalent to a practical LOQ of the individual residue components will fully accommodate the levels of these components which could occur in commodities following treatment according to GAP.

As in cases of detectable residues, the definition of residues at or about the LOQ may also include a single residue component, e.g., fenpropimorph in sugar beet, or several residues components, e.g., aldicarb, its sulphoxide and its sulphone expressed as aldicarb in peanut oil, bentazone, 6-hydroxy bentazone and 8-hydroxy bentazone expressed as bentazone in soya bean; and fenthion, its oxygen analogue and their sulphoxides and sulphones expressed as fenthion in potato.

In cases where several metabolites are included in the definition of the residue two basic situations can be distinguished.

1) The residue components are, or may be converted to, a single compound or analyte by the analytical method, e.g., fenthion. The total residue is measured as a single compound and expressed as the parent compound, i.e., fenthion oxygen analogue sulphone is measured and expressed as fenthion. The MRL is set and enforced on the basis of the total measured residue. After the conversion of all the residue components a single compound is determined, the MRL can be simply enforced either at or above the LOQ. This situation is similar to other cases where the residue is defined as a single compound.

2) The residue components are determined separately by the method. The concentrations of measurable residues are adjusted (or not) for molecular weight and summed, and their sum is used for estimating the maximum residue level.

When residues are undetectable in a commodity an MRL based on the sum of the LOQs of the individual residue components may not be appropriate for enforcement purposes. The best option should be selected on a case-by case basis taking into account the relative ratio of metabolites for which the metabolism studies may provide the best information.

The problem is best illustrated with an example. The residues of bentazone in plant commodities are defined as the sum of bentazone, 6-hydroxybentazone and 8-hydroxybentazone, expressed as bentazone. The LOQs reported in supervised trials for each of the three components were generally 0.02 mg/kg, but the practical LOQs were regarded as 0.05 mg/kg for regulatory purposes. If an MRL for bentazone was set as the sum of the practical LOQs of the three components of the residue, it would have to be established at 0.2 mg/kg (3 times the practical limit of determination to incorporate all three residue components and round it to the next whole number). In this case, any one of the residue components could be present at 0.2 mg/kg, or all of the three at 0.06 mg/kg, without exceeding the MRL. Consequently, individual residue components could be respectively 10 and 3 times those which should arise from the recommended use of the compound but would be within the MRL. Similarly, if the sum of the LOQs achieved in the supervised trials was considered, an MRL of 0.1 mg/kg would be needed, which would still allow 5 times the residue that would arise from treatments complying with GAP.

Numerical expression of MRLs

In order to fully reflect the impact of the statistical calculation methods, the JMPR concluded that the scaling steps traditionally applied (0.01, 0.02, 0.03, 0.05, 0.07, 0.1, 0.2, 0.3, 0.5, 0.7, 1, 2, 3, 5, 7, 10, 15, 20, 25, 30, 40, and 50 mg/kg can be replaced with a more detailed scale where the statistical tools are successfully used.

The MRLs are always expressed with whole numbers and 0 is not added after the decimal point as it would provide a false impression on the precision (uncertainty) of the estimation process.

The recently approved OECD calculator applies the following rounding schemes (some values are extracted for illustration):

MRL classes (numerical values)	
MRL estimate ≥	**Proposed MRL**
0.000001	0.01
0.0105	0.015
0.0155	0.02
0.021	0.03
0.091	0.1
0.105	0.15
0.155	0.2
0.21	0.3
0.91	1
1.05	1.5
1.55	2
2.1	3
9.1	10
10.5	15
15.5	20
21	30
91	100
105	150
155	200
210	300
910	1000

PRESENTATION – EXPRESSION OF MAXIMUM RESIDUE LIMITS

1. Expression of Maximum Residue Limits

2. Objective

The purpose of this chapter is to explain the conventions for expressing MRLs.

3. Outline

- General principles
- Expression of MRLs at or about the limit of quantification (LOQ)
- Numerical expression of MRLs

4. Expression of MRLs

- The estimated maximum residue levels and recommended residue limits are expressed in mg residue (as defined)/kg commodity.
- The portion of commodity to which Codex MRLs apply is given in Codex Alimentarius Vol. 2 (extracted in Appendix VI of FAO Manual).
- The residues are expressed on fresh-weight basis as they enter international trade (as received by the laboratory) in most commodities, with the exception of animal feeds.

5. MRLs for animal feeds

- MRLs for animal feeds are recommended on a dry-weight basis, because of the great variation of their moisture content.
- For estimation of maximum residue levels the moisture content of the feed item should be known.
- Alternatively, as a worst case, it can be assumed that the residues were expressed in the trial reports on fresh weight basis, or the data might not be suitable for estimation of maximum reside levels.

6. MRLs for residues in meat

- MRLs *for fat soluble pesticides* are expressed on the fat (trimmable fat or fat tissue expressed on the lipid content). This is indicated in brackets (fat) after the residue value.
- For those commodities where the adhering fat is insufficient to provide a suitable sample, the whole meat commodity (without bone) is analysed and the MRL applies to the whole commodity.
- For all other pesticides and residues in liver, kidney and eggs the MRLs apply to the whole commodity as it moves in trade.

7. MRLs for residues in milk

- Since 2004, where possible, two maximum residue levels are estimated for *fat soluble pesticides*: one for whole milk and one for milk fat.
- When needed, maximum residue levels for processed milk products (e.g. cheese) can then be calculated from the two MRL values and the fat contents of the milk products.
- Based on the decision of the 2008 CCPR, a footnote is inserted to indicate where MRLs are established for both milk fat and whole milk: “for monitoring and regulatory purposes, whole milk is to be analysed and the result compared to the MRL for whole milk”.
- For compounds that are not fat-soluble, MRLs are expressed on the whole milk.

8. MRLs for special cases

MRLs based on *direct animal treatment* are footnoted “the MRL accommodates external animal treatment”.

MRLs reflecting special uses or conditions are also distinguished by letters after the limit: Currently the following cases are distinguished by the letters

E	The MRL is based on extraneous residues.
Po	The MRL accommodates post-harvest treatment of the commodity.
PoP	The MRL for the processed commodity accommodates post-harvest treatment of the primary commodity.

9. MRLs at or about LOQ

- The JMPR generally recommends maximum residue limits for non-detected residues at a level which can be achieved in regulatory laboratories. Such levels are reported as residues at or about the LOQ* and indicated with an asterisk after the number.
- An MRL so identified does not always necessarily imply that residues of the pesticides do not occur in that commodity. The application of a more sensitive or specific method may reveal detectable residues in some commodities.
- Where metabolism studies or other information indicates that no residue is expected in a commodity, the STMR is considered to be zero and this value is used in the intake calculations.

10. MRLs at or about LOQ

- Setting and enforcing MRLs for residues occurring at or about the LOQ of analytical procedures may require different approaches depending on the composition and definition of the residues.
- All available relevant information should be carefully considered ensuring that an MRL established at a level equivalent to a practical LOQ of the individual residue components will fully accommodate the levels of these components which could occur in commodities following treatment according to GAP.
- The definition of residues at or about the LOQ may also include a single residue component, or several residue components, e.g., aldicarb, its sulphoxide and its sulphone expressed as aldicarb in peanut oil, bentazone, 6-hydroxy bentazone and

8-hydroxy bentazone expressed as bentazone in soya bean; and fenthion, its oxygen analogue and their sulphoxides and sulphones expressed as fenthion in potato.

11. Inclusion of several metabolites

a) The residue components are, or may be converted to, a single compound or analyte by the analytical method, e.g., fenthion, its oxygen analogue and their sulphoxides and sulphones expressed as fenthion.
b) The residue components are determined separately by the method. The concentrations of measurable residues are adjusted (or not) for molecular weight and summed, and their sum is used for estimating the maximum residue level.

When residues are undetectable in a commodity an MRL based on the *sum of the LOQs of the individual residue components may not be appropriate* for enforcement purposes.

12. Example: MRL based on sum of residues

- The residues of bentazone in plant commodities are defined as the sum of bentazone, 6-hydroxybentazone and 8-hydroxybentazone, expressed as bentazone.
- The LOQs reported in supervised trials for each of the three components were generally 0.02 mg/kg, but the practical LOQs were regarded as 0.05 mg/kg for regulatory purposes.
- If an MRL for bentazone was set as the sum of the practical LOQs of the three components of the residue, it would have to be established at 0.2 mg/kg (rounded value of 3 times the practical limit of determination).
- In this case, any one of the residue components could be present at 0.2 mg/kg, or all of the three at 0.06 mg/kg, without exceeding the MRL.

13. Numerical expression of MRLs

- The MRLs are always expressed with whole numbers and 0 is not added after the decimal point as it would provide a false impression on the precision (uncertainty) of the estimation process.
- The recently approved OECD calculator applies the following rounding schemes: 0.01, 0.015, 0.02, ...0.1, 0.15, 0.2, ...15, 20, ...100, 150, 200 ... mg/kg

Abbreviations and acronyms in this chapter

CCPR	Codex Committee on Pesticide Residues
EMRL	extraneous maximum residue limit
GAP	good agricultural practice
JMPR	Joint Meeting on Pesticide Residues
LOQ	limit of quantification
MRL	maximum residue limit
OECD	Organization for Economic Co-operation and Development
Po	post-harvest treatment
PoP	processed commodity from post-harvest treated raw agricultural commodity
STMR	supervised trials median residue

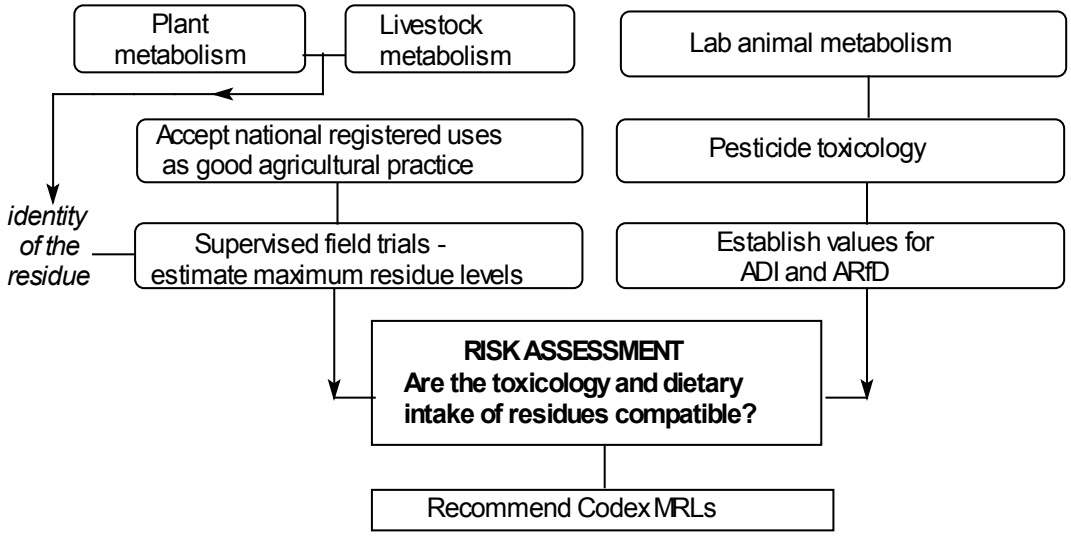
Chapter 13. Estimation of Dietary Intake

Chronic intake – IEDI calculations
Short-term intake – IESTI calculations
IESTI and alternative GAP (good agricultural practice)
The dietary risk assessment statement

The purpose of this chapter is to explain how to combine the estimates of residues in food with data on human diets in order to calculate dietary intakes or exposures, a necessary part of the risk assessment.

Relevant sections of JMPR Manual

- Long-term dietary intake 7.2
- Short-term dietary intake.....7.3
- Acute reference dose.....7.4
- IESTI tables 7.5
- When JMPR estimates of dietary intake exceed the ADI or ARfD 7.6



The risk assessment process is a vital part of pesticide residue evaluation.

JMPR processes have evolved and formalised. The exposure side of risk assessment depends on IEDI and IESTI calculations, which rely on residue data and human diets. The components of the residue are expressed in a residue definition designed for risk assessment.

IEDI, International estimated daily intake. The IEDI is a prediction of the long-term daily intake of a pesticide residue on the basis of the assumptions of average daily food consumption per person and median residues from supervised trials, allowing for residues in the edible portion of a commodity and including residue components defined by the JMPR for estimation of dietary intake. The IEDI is expressed in milligrams of residue per person. (JMPR Manual).

IESTI International estimated short-term intake. The IESTI is a prediction of the short-term intake of a pesticide residue on the basis of the assumptions of high daily food consumption per person and highest residues from supervised trials, allowing for residues in the edible portion of a commodity and including residue components defined by the JMPR for estimation of dietary intake. The IESTI is expressed in milligrams of residue per kg body weight. (JMPR Manual).

Both of the expressions 'dietary intake' and 'dietary exposure' are intended to have the same meaning.

The formula for calculating dietary intake of residues is very simple.

$$\textit{Dietary intake} = \frac{\textit{concentration of residue in the food (mg/kg)} \times \textit{food consumption (kg)}}{\textit{body weight (kg)}}$$

However, the values selected for these three parameters depend on the situation. Also, for IEDI calculations, i.e. long-term intake calculations, intakes from all foods are added. For IESTI calculations, where large portions of food are considered, each food is considered separately.

JMPR uses spreadsheets for these calculations and the spreadsheets carry the dietary information and calculate intakes according to the accepted formulae.

Chronic intake - IEDI calculations

Reliable estimates of long-term dietary intake of pesticide residues are needed for assessment of chronic risk. The intake is compared with the acceptable daily intake (ADI), derived from whole-of-life animal feeding experiments. The ADI definition includes the phrase "daily intake which, during an entire lifetime, appears to be without appreciable risk to the health of the consumer."

Historically, chronic intakes were calculated from the MRL and dietary information. The idea was that if such an exaggerated calculation gave a result less than the ADI, there was no further requirement. If the calculated intake exceeded the ADI then refinements would be included to produce more accurate intake estimates.

In practice, such a system of repeated refinements was less than ideal at the Codex level because meetings were held only once per year.

The practice now is to make use of all available data[22] at the first evaluation and to produce the best possible estimates of intake.

For example, it is not possible to produce a commodity with residues consistently at the MRL. Pesticide residues vary widely even when treatments are precisely the same. Therefore if the highest residue cannot exceed the MRL, the typical residue must be much less than the MRL.

Residue data are now usually available on the edible portions of food commodities with the residues including those metabolites considered necessary for risk assessment.

[22] Hamilton DJ, Holland PT, Ohlin B, Murray WJ, Ambrus A, De Baptista GC and Kovacicová J. 1997. Optimum use of available residue data in the estimation of dietary intake of pesticides. *Pure & Applied Chemistry*, 69:1373-1410.

For dietary intake purposes, residues should be assumed to be at the LOQ when the median residue is less than the LOQ except when evidence suggests that the residues are essentially zero.

Residue data are also available on processed foods such as fruit juices, flour and bread, vegetable oils and wine. All of these available data should be included in the IEDI calculations.

The IEDI spreadsheet

a	b	c	d	e	f	g	h	i	j	k	l
				A		B		C		D	
Codex Code	Name	STMR or STMR-P mg/kg	diet correction factor	diet	intake	diet	intake	diet	intake	diet	intake
002	POME FRUIT		-	-	-	-	-	-	-	-	-
FP 0009	Pome fruit (incl apple juice)		1	0.5	-	84.1	-	21.9	-	45.2	-
FP 0009	Pome fruit (excl apple juice)		1	0.5	-	79.9	-	21.8	-	43.6	-
FP 0226	Apple (incl juice)		1	0.3	-	60.5	-	18.5	-	39.9	-
FP 0226	Apple (excl juice)		1	0.3	-	56.3	-	18.4	-	38.3	-
JF 0226	Apple juice		1	0.0	-	2.8	-	0.1	-	1.1	-
FP 0230	Pear		1	0.1	-	22.3	-	2.8	-	4.8	-
FP 0231	Quince		1	0.1	-	1.3	-	0.6	-	0.5	-

The pome fruit section of the IEDI spreadsheet is presented for discussion

Columns a, b: identity of the commodity. For the purposes of chronic intake, the consumption data for apples includes the consumption of raw apples as well as apples as an ingredient in any other food, e.g. apple juice, apple pies, preserved apples, etc. If residue data are available for apple juice, then adjustment is needed for the apple – use the apple (excluding apple juice) row for the apple entry.

Column c: data entry for the residues, STMR for raw commodities, STMR-P for processed commodities.

Column d: a factor may be needed in some calculations.

Columns e, g, i and k: diets A, B, C, D, the first 4 of 13 cluster diets. The spreadsheet explains which country is in which cluster. The numbers in the diet columns are food consumption of the particular food in grams per person per day.

Columns f, h, j and l: intakes are calculated by multiplying the STMR (mg/kg) in column c by the diets in grams per person per day. Daily intakes are recorded as µg/person.

All of the calculated intakes in column f are added to produce the total intake for diet A. The total intake, as µg/person is then divided by the ADI (multiplied by bodyweight) and expressed as % of ADI. The same procedures are followed for the other 12 diets.

Allowing for processed foods in IEDI calculations

When a raw agricultural commodity is subjected to food processing, pesticide residues may be lost or redistributed among the processed fractions.

Pesticide residue data are usually available for large scale food processes such as milling of wheat, fruit juice production, vegetable oil production, beer brewing and wine making. STMR-P and HR-P values may then be estimated for the processed commodities.

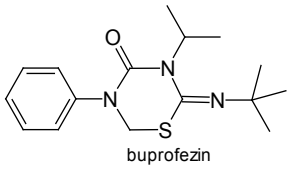
HR-P, highest residue – processed: the HR-P is the highest residue in a processed commodity calculated by multiplying the HR of the raw agricultural commodity by the corresponding processing factor. (JMPR Manual).

STMR-P, supervised trials median residue – processed: the STMR-P is the expected residue in a processed commodity calculated by multiplying the STMR of the raw agricultural commodity by the corresponding processing factor. (JMPR Manual).

When residues do not exceed the LOQ in the processed commodity, the processing factor will be expressed with a 'less-than (<)' sign to indicate that it has been calculated with the LOQ of an analytical method instead of a measured residue.

The 'less-than' sign is not transferred to the STMR-P when it is calculated from the STMR of the raw agricultural commodity.

Example - processing of tomatoes, buprofezin residues (JMPR 2008).



buprofezin

The buprofezin STMR for tomatoes was 0.24 mg/kg.

Tomato paste, processing factor 0.9	0.9×0.24	STMR-P 0.22 mg/kg.
Tomato juice, processing factor 0.22	0.22×0.24	STMR-P 0.053 mg/kg.
Peeled tomato, processing factor 0.17	0.17×0.24	STMR-P 0.041 mg/kg.

When the STMR-P values are used in the IEDI calculation, the consumption data for the raw agricultural commodity must be reduced by an amount accounted to the processed product.

In the present example, tomato consumption is divided among raw agricultural commodity and three processed commodities.

In diet M, the consumption of all tomato products is equivalent to 103.0 g of raw agricultural commodity (RAC) per day. But the table gives consumption of RAC (alone) as 27.3 g/day.

Section of the IEDI spreadsheet.

	STMR-P	Diet G		Diet H		Diet I		Diet J		Diet K		Diet L		Diet M	
Commodity	mg/kg	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake
Tomato (including juice, paste, peeled)		23.5		31.7		15.0		16.2		35.6		9.9		**103.0**	
Tomato (excl juice, excl paste, excl peeled)	0.24	22.8	5.5	4.1	1.0	12.3	3.0	1.8	0.4	32.8	7.9	0.4	0.1	**27.3**	6.6
Tomato juice	0.053	0.0	0.0	0.8	0.0	0.1	0.0	7.2	0.4	0.0	0.0	2.4	0.1	45.2	2.4
Tomato, paste	0.22	0.1	0.0	2.1	0.5	0.6	0.1	0.4	0.1	0.6	0.1	1.4	0.3	1.2	0.3
Tomato, peeled	0.041	0.2	0.0	14.5	0.6	0.2	0.0	0.0	0.0	0.3	0.0	0.8	0.0	1.2	0.0

Tomato (excluding juice, paste, peeled)	27.3 g/day	equiv RAC 27.3 g/day
Tomato juice	45.2 g/day (×1.25)	equiv RAC 56.5 g/day
Tomato paste	1.2 g/day (×4)	equiv RAC 4.8 g/day
Tomato, peeled	1.2 g/day (×1.25)	equiv RAC 1.5 g/day
TOTAL		equiv RAC 90.1 g/day[23]

A footnote to the table provides information on the yield of processed commodities and the factors for calculating the grams of RAC that produced a gram of processed product.

[23] Note that the total of 90.1 g/day does not match the total of 103 g/day. Possibly data have originated from different sources or different factors were used in the calculations. Users should be alert for anomalies in such tables compiled from many sources.

For tomatoes, 1.25 g of tomatoes produced 1 g of tomato juice or 1 g of peeled tomatoes, while 4 g of tomatoes produced 1 g of tomato paste.

The current spreadsheet already makes the calculations for tomatoes. However, if a dietary intake calculation is required for a processed commodity not already included, adjustment of the total commodity consumption must be made when the processed commodity is included.

Processing usually reduces or eliminates some of the pesticide residue. In the example with buprofezin on tomatoes, intake can be calculated assuming all tomatoes are consumed as RAC (top row) or with processing as shown in the table by adding the four rows of RAC + processed (bottom row).

	STMR	Diet G	Diet H	Diet I	Diet J	Diet K	Diet L	Diet M
Commodity	mg/kg	diet intake	diet intake	diet intake	diet intake	diet intake	diet intake	diet intake
Tomato total, no processing	0.24	5.6	7.6	3.6	3.9	8.5	2.4	24.7
Tomato, RAC + juice, paste and peeled		5.5	2.1	3.1	0.9	8.0	0.5	9.3

Points to note

- The weights of RAC tomato to produce unit weights of juice, paste and peeled tomato are 1.25, 4 and 1.25 respectively. In a previous spreadsheet (2005), these three weights were 1.06, 6.4 and 1.0 respectively. This provides a little perspective on the variability of such values used in national systems and the uncertainty of the derived values used internationally.
- In diets H, J, L and M, where a majority of tomato is consumed in processed form, the estimated intake is much reduced when the intake calculation is refined by including the processing information.
- Most calculated long term intakes are less when processing data are included, because food processing usually removes some of the residue. The first processing step is likely to be a cleaning step, which may remove much of the surface residue.

Short-term intake - IESTI calculations

The idea behind the IESTI calculation is that residue levels may be quite variable within a single lot of a food commodity all from the same farm with the same pesticide treatments. Individual units of a fruit or a vegetable may have a higher or lower residue level than the average for the lot.

As well as the variability of residue level, consumption per person may also be quite different from day to day.

The IESTI calculation assumes that the high consumption day corresponds with the high residue lot and a fruit or vegetable unit has a residue three times as high as the average for the lot. If more than one unit is consumed, the remaining units are assumed to contain a residue equal to the lot average.

IESTI calculations – sometimes HR, sometimes STMR

HR, highest residue. The HR is the highest residue level (expressed as mg/kg) in a composite sample of the edible portion of a food commodity when a pesticide has been used according to maximum GAP conditions. The HR is estimated as the highest of the residue values (one from each trial) from supervised trials conducted according to maximum GAP

conditions, and includes residue components defined by the JMPR for estimation of dietary intake. (JMPR Manual).

STMR, supervised trials median residue. The STMR is the expected residue level (expressed as mg/kg) in the edible portion of a food commodity when a pesticide has been used according to maximum GAP conditions. The STMR is estimated as the median of the residue values (one from each trial) from supervised trials conducted according to maximum GAP conditions.

The HR is used in IESTI calculations for fruit and vegetables and other foods that reach the consumer without processing and without blending and bulking.

Examples: apples, cabbages, potatoes and meat.

The STMR or STMR-P is used in IESTI calculations where processing or blending and bulking is likely to produce a food product that originates from more than one farm and with different pesticide treatments.

Examples: cereal grain food products, pulses, vegetable oils, fruit juices and milk.

Exceptions should be noted. The HR-P should be used for products such as dried grapes and tinned pineapple, where the original fruits are still discrete units after the process. The HR-P should also be used for post-harvest treatments of cereal grains because the treatment may be on a very large scale and may occur after the bulking of product from many farms.

The IESTI spreadsheet

a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q
Code	Commodity	STMR	HR	Country	Body Weight (kg)	Large portion g/kg bw/day	Large portion, g/person	Unit	Country	% Edible portion	Unit weight, edible portion, g	Variability factor	Case	IESTI µg/kgbw/day	% ARfD rounded	Preset
FS 0247	Peach			SAF	55.7	12.30	685	110	FRA	90%	99	3	2a	ND	-	
FS 0247	Peach			SAF	55.7	12.30	685	150	JPN	100%	150	3	2a	ND	-	
FS 0247	Peach			SAF	55.7	12.30	685	122	UNK	90%	110	3	2a	ND	-	
FS 0247	Peach			SAF	55.7	12.30	685	98	USA	87%	85	3	2a	ND	-	
FS 0247	Peach			SAF	55.7	12.30	685	141	SWE	76%	107	3	2a	ND	-	
FS 0247	Peach			SAF	55.7	12.30	685	140	BEL	90%	126	3	2a	ND	-	

The section of the IESTI spreadsheet for peaches is selected as an example.

Columns a, b: identity of the commodity

Columns c, d: data entry columns, STMR or HR. For peaches, HR is the correct entry.

Column e: the country with the biggest large-portion weight for the nominated commodity, in this case, South Africa.

Columns f, g, h: South Africa has a large-portion consumption of 685 g/person for peaches for the general population (55.7 kg body weight). For the purpose of IESTI calculation, 'large-portion' is the 97.5th percentile consumption value for eaters of that food in one day.

Columns i, j: the most popular or most common unit weights for peaches in the countries identified in column j.

Columns k, l: the percentage edible portion of the commodity as described by the country in column j and the calculated % edible portion.

Column m: the variability factor used in the IESTI calculation. The variability factor of 3 means that the residue in the high-residue unit is 3× the average residue in the lot.

Column n: the case of the calculation. The spreadsheet determines the case from the parameters of the commodity being considered. The 4 cases are fully explained in the JMPR Manual, pages 128-130.

Column o: the calculated IESTI value.

Column p: the IESTI expressed as a percentage of the acute reference dose and rounded.

Column q: if column n shows 'ND' and columns e, f, g and h have entries for large portion diet, column q requires a 'Case 1' or 'Case 3' entry to complete the calculation. Also, the spreadsheet selection of case in column n may be over-ridden by entering 'Case 1' or 'Case 3' as required in column q.

For peaches, there are 6 possible rows for data entry of the HR value.

Originally, the selection was based on the unit weight data provided by the country where the relevant trials were carried out. In practice, that was not always possible.

Now, with the automated spreadsheets available, it is common practice to enter the data in all the available rows.

'Case 1' or 'Case 3' decisions in the IESTI spreadsheet

For some commodities in the IESTI table, no calculation will occur until the user chooses 'Case 1' or 'Case 3' in column q.

A 'Case 1' choice will require an entry in the HR column (column d). A 'Case 3' choice requires an STMR (or STMR-P) entry in column c.

When the residue in a composite sample reflects the residue in a meal-sized portion of the commodity (unit weight less than 25 g), it is Case 1.

When the commodity goes through bulking and blending (commodity from a number of farms) and is destined for processing, it is Case 3. (See JMPR Manual, pages 128-130 for an explanation of the cases in IESTI calculations).

Examples from the IESTI table requiring an entry in column q to obtain a calculation.

a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q
Code	Commodity	STMR	HR	Country	Body weight (kg)	Large portion g/kg bw/day	Large portion, g/person	Unit weight, g	Country	% edible portion	Unit weight, edible portion, g	Variability factor	Case	IESTI µg/kg bw/day	% ARfD rounded	Preset
VP 0541	Soya bean (immature seeds)			Thai	53.5	2.41	129	-	-	-	ND	ND	ND	ND	-	
VP 0063	Peas (pods & immature seeds)			JPN	52.6	1.19	63	-	-	-	ND	ND	ND	ND	-	
FI 0343	Litchi			Thai	53.5	4.93	264	-	-	-	ND	ND	ND	ND	-	
VA 0389	Spring onion			THAI	53.5	1.33	71	-	-	-	ND	ND	ND	ND	-	
VL 0845	Mustard greens			USA	65.0	3.50	228	-	-	-	ND	ND	ND	ND	-	
VL 0470	Corn salad			FRA	52.2	1.60	84	-	-	-	ND	ND	ND	ND	-	

IESTI calculations for commodity groups

When sufficient data are available JMPR prefers to recommend a commodity group MRL. Associated with the group MRL are the group STMR and HR.

An IESTI calculation is associated with a particular food, not a group of foods. So, the IESTI calculation must be applied to important commodities within the group. The choice of commodities is largely dictated by the availability of specific dietary data for the specific commodities.

If a group MRL is recommended for stone fruits, IESTIs may be calculated for cherries, plums, apricots, nectarines and peaches.

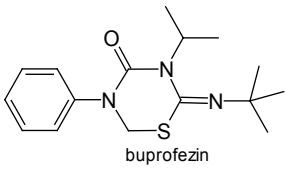
If a group MRL is recommended for cucurbit fruiting vegetables, IESTIs may be calculated for cucumber, gherkin, melons (except watermelon), summer squash, watermelon and winter squash.

If a group MRL is recommended for Brassica vegetables, IESTIs may be calculated for broccoli, Brussels sprouts, head cabbage, cauliflower and kohlrabi.

Example - buprofezin, cucurbit fruiting vegetables. IESTI (JMPR 2009).

In this case the same HR was used for the whole group, whether the fruit had edible peel or not.

Residue data were available for cucumbers, cantaloupes and summer squash (n is number of trials).



buprofezin

Cucumbers, n=10,	STMR = 0.03 mg/kg,	HR = 0.30 mg/kg
Cantaloupes, n=10,	STMR (whole fruit) = 0.195 mg/kg,	HR (whole fruit) = 0.41 mg/kg
Summer squash, n=10,	STMR = 0.04 mg/kg	HR = 0.11 mg/kg

The cantaloupe data, as the highest, were taken to represent residues for the commodity group, cucurbit fruiting vegetables.

A group MRL of 0.7 mg/kg was recommended for cucurbit fruiting vegetables.

The recommended HR was 0.41 mg/kg for cucurbit fruiting vegetables.

The HR (0.41 mg/kg) was used in IESTI calculations for: cucumber, gherkin, melons (except watermelon), summer squash, watermelon and winter squash.

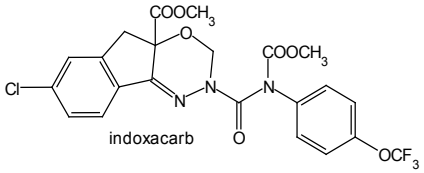
Points to note

- The group MRL, STMR and HR were based on data from one commodity, the one producing the highest residue.
- The group HR (0.41 mg/kg) was used for cucumbers and summer squash even though sufficient data were available to support the individual HR values.
- IESTI calculations were made for cucumber, gherkin, melons (except watermelon), summer squash, watermelon and winter squash. These are the members of the cucurbits commodity group where suitable consumption data were available in the IESTI spreadsheets:

Example - indoxacarb, cucurbit fruiting vegetables. IESTI (JMPR 2009).

In this case, although there was a group MRL, separate HRs were used for cucurbit fruits depending on peel edibility.

Residue data were available for cucumbers, melons and summer squash (n is number of trials).



indoxacarb

Cucumbers, USA, n=10,	HR = 0.07 mg/kg
Cantaloupe melons, USA, n=11,	HR (whole fruit) = 0.39 mg/kg
Summer squash, USA, n=12,	HR = 0.12 mg/kg
Cucumber, Europe, n=13	HR = 0.10 mg/kg
Melons, Europe, n=18	HR (whole fruit) = 0.09 mg/kg
Melons, Europe, n=18	HR (melon pulp) = <0.02 mg/kg

Cantaloupe melons, with the highest residues in whole fruit, were taken to represent the commodity group, cucurbit fruiting vegetables with a recommended HR of 39 mg/kg.

A group MRL of 0.5 mg/kg was recommended for cucurbit fruiting vegetables.

The recommended HR was 0.39 mg/kg for cucurbit fruiting vegetables with edible peel. Based on the melon pulp data, an HR of 0.02 mg/kg was recommended for cucurbits with inedible peel.

The HR (0.39 mg/kg) was used in IESTI calculations for: cucumber, gherkin and summer squash.

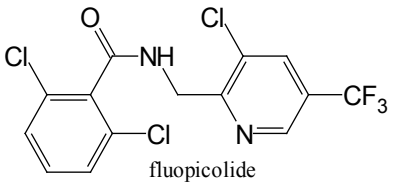
The HR (0.02 mg/kg) was used in IESTI calculations for: melons[24] (except watermelon), watermelon and winter squash.

Points to note

- The group MRL was based on data from one commodity, cantaloupe melons, the one producing the highest residues.
- The cantaloupe melon HR (whole fruit) of 0.39 mg/kg was used for the cucurbit fruiting vegetables with edible peel: cucumber, gherkin and summer squash.
- An HR of 0.02 mg/kg, based on melon pulp data, was used for cucurbit fruiting vegetables with inedible peel.
- Separating cucurbits into two groups for dietary intake calculations may be seen as a refinement of the previous case where they were kept together as one group.

Example - fluopicolide, leafy vegetables, IESTI (JMPR 2009).

Residue data were available for lettuce and spinach (n is number of trials).



fluopicolide

Lettuce, Europe, n = 8	HR = 4.9 mg/kg
Head lettuce, USA, n= 7,	HR = 7.2 mg/kg
Leaf lettuce, USA, n= 7,	HR = 12 mg/kg
Spinach, USA, n= 7,	HR = 17 mg/kg

[24] Note error in Annex 4 of 2009 JMPR Report: 0.2 mg/kg instead of 0.02 mg/kg.

Spinach, with the highest residues, was taken to represent the commodity group, leafy vegetables with a recommended HR of 17 mg/kg.

A group MRL of 30 mg/kg was recommended for leafy vegetables.

IESTI calculations must be applied to individual foods. IESTI calculations with HR = 17 mg/kg were made for members of the commodity group where data were available in the IESTI spreadsheets: chard, chicory leaves, Chinese cabbage, corn salad, cos lettuce, endive, garden cress, head lettuce, kale, leaf lettuce, mustard greens, purslane, spinach, turnip greens and watercress.

Point to note

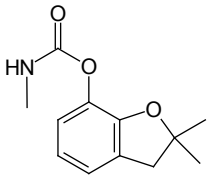
– The HR for spinach, representing leafy vegetables, was used in the IESTI calculations for each of the individual leafy vegetables where suitable consumption data were available.

IESTI calculation. A low ARfD may require low detection limits

Especially careful attention should be paid to the capabilities of the residue analytical methods for a compound with a low ARfD. Calculations will reveal the required likely detection limits for each commodity of interest.

Example - carbofuran, bananas, IESTI (JMPR 2009).

When carbofuran was used in the production of bananas, residues were not detected in the bananas. Various analytical methods had been used and JMPR estimated an HR of 0.02 mg/kg for carbofuran in bananas based on the limit of detection of methods in use.



carbofuran

Calculated IESTI values for carbofuran in bananas for the general population and for children were 80 % and 150 % of the ARfD (0.001 mg/kg bw) respectively.

It would have been preferable if data were generated by an analytical method with a lower detection limit.

Point to note

– During the design phase of the trial, it is possible to calculate the required LOQ for an analytical method to determine a dividing line between residue levels that would be acceptable or not acceptable.

IESTI and alternative GAP (good agricultural practice)

When JMPR evaluates residue data from supervised trials to estimate maximum residue levels, the critical GAP that generates the highest residues is examined first because an MRL that covers the residues from this critical GAP will cover residues from others also.

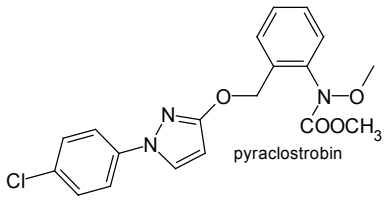
If the estimated short-term intake for a pesticide residue exceeds the acute reference dose (ARfD), the associated maximum residue level is not suitable for establishing a maximum residue limit. However, other uses if they generate acceptable residues, should not be deprived of an MRL

If an alternative critical GAP is available, it should be examined for its resulting residues and the possibility of supporting an MRL.

Example – pyraclostrobin residues on lettuce, alternative GAP (JMPR 2006)

Pyraclostrobin use on head lettuce:

- USA GAP: 4 foliar applications of 0.12-0.23 kg ai/ha, with a 0 day PHI.
- 6 US trials, HR = 19.7 mg/kg.



pyraclostrobin

Pyraclostrobin use on lettuce

- European GAP: 2 foliar applications at 0.1 kg ai/ha, with a 14 days PHI.
- 8 European glasshouse trials, HR = 0.81 mg/kg.

IESTI calculations for the HR of 19.7 mg/kg for head lettuce produced 390 % and 810% of ARfD for the general population and for children respectively.

IESTI calculations for the HR of 0.81 mg/kg for head lettuce produced 20 % and 30% of ARfD for the general population and for children respectively.

Extract from the IESTI spreadsheets.

Code	Commodity	**HR** mg/kg	Country	Body weight (kg)	Large portion, g/person	Unit weight, g	Country	% edible portion	Unit weight, edible portion, g	Variability factor	Case	IESTI µg/kg bw/day	**% ARfD rounded**
General population													
VL 0482	Lettuce, head	**19.7**	USA	65.0	213	539	USA	95%	512	3	2b	193.26	**390%**
VL 0482	Lettuce, head	**19.7**	USA	65.0	213	450	BEL	80%	360	3	2b	193.26	**390%**
VL 0482	Lettuce, head	**0.81**	USA	65.0	213	539	USA	95%	512	3	2b	7.95	**20%**
VL 0482	Lettuce, head	**0.81**	USA	65.0	213	450	BEL	80%	360	3	2b	7.95	**20%**
Children													
VL 0482	Lettuce, head	**19.7**	Thai	17.1	117	539	USA	95%	512	3	2b	403.65	**810%**
VL 0482	Lettuce, head	**19.7**	Thai	17.1	117	450	BEL	80%	360	3	2b	403.65	**810%**
VL 0482	Lettuce, head	**0.81**	Thai	17.1	117	539	USA	95%	512	3	2b	16.60	**30%**
VL 0482	Lettuce, head	**0.81**	Thai	17.1	117	450	BEL	80%	360	3	2b	16.60	**30%**

An estimated maximum residue level of 2 mg/kg for pyraclostrobin on head lettuce, based on trials with an HR = 0.81 mg/kg, was then recommended for use as an MRL.

Points to note

- This is an example of prospective alternative GAP. Two quite different use patterns were available. When the use pattern producing the higher residues did not meet the IESTI test, the alternative use pattern (alternative GAP) was evaluated.
- MRL recommendations were based on the alternative GAP.

The dietary risk assessment statement

The statement provides very brief summaries of the results of IEDI and IESTI calculations. Standard wording should be followed where possible (JMPR Manual, pages 236-239).

Attention should be drawn to any unusual situation or difficulty in the risk assessment.

Phorate. Short-term intake, JMPR 2009.

Attention was drawn to two different ways of cooking potatoes, but both resulting in essentially the same IESTI.

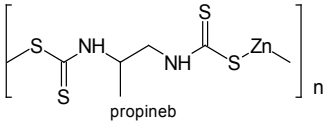
The IESTI for phorate was calculated for potatoes, both by using the HR for potatoes, microwaved with peel, and for French fries, the latter based on new consumption data. The IESTI represented 70% of the ARfD (0.003 mg/kg bw) for the general population (both for potatoes, microwaved with peel, and for French fries) and 170% and 180% of the ARfD for children, from consumption of potatoes, microwaved with peel, and French fries, respectively.

Fenitrothion. Long-term intake, JMPR 2004.

Calculated IEDI values exceeded the ADI. It was pointed out that more processing data on cereal grains might resolve the situation

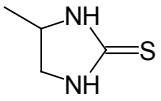
The Meeting noted that the calculations of long-term intake were conservative, as they did not take into account the reduction in residue levels obtained by processing cereal grains, except for processing of wheat, barley and rice. The Meeting extrapolated processing data on wheat to rye. Information on processing of barley (uses besides beer), maize, millet and sorghum would be particularly useful for refining the intake calculations.

Propineb. Long-term and short-term intake, JMPR 2004



propineb

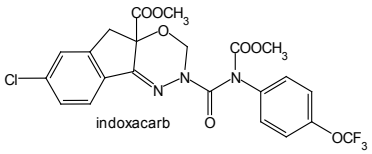
Propylene thiourea (PTU) is a known toxic metabolite of propineb. Its residues may occur in company with residues of propineb. A brief explanation was provided on how this mixture was dealt with in the risk assessment.



PTU

The Meeting considered how best to approach the dietary risk assessment of mixed residues of propineb and propylene thiourea and decided that an appropriately conservative approach would be to calculate the sum of the residues after scaling the propylene thiourea residues to account for the difference in toxicity. The relevant factors for long-term and short-term intake were derived from the ratios of the ADI and ARfD values for propineb and propylene thiourea, which are 2.3 and 3.3, respectively. Dietary intake estimates for the residues, adjusted for potency and combined, were compared with the ADI and interim ARfD for propineb.

Indoxacarb. Short-term intake, JMPR 2005

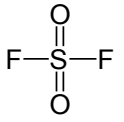


indoxacarb

Attention was drawn to missing dietary information on leaf lettuce, which prevented the completion of a risk assessment.

The IESTI of indoxacarb calculated on the basis of the recommendations made by the JMPR represented 0–130% of the ARfD (0.1 mg/kg bw) for children and 0-50 % for the general population. The IESTI for head cabbage for children was 130% of the ARfD. It should be noted that unit weight data are not available for leaf lettuce in the GEMS/Food data base. Availability of a realistic unit weight would improve the estimate of short-term intake.

Sulfuryl fluoride. Long-term intake. JMPR 2005



sulfuryl fluoride

Fluoride is a metabolite of sulfuryl fluoride and the use of sulfuryl fluoride as a grain fumigant leaves a fluoride residue in the grain. This residual fluoride should be subject to a risk assessment in company with fluoride in food from other sources.

The Meeting concluded that the dietary intake of fluoride associated with the use of sulfuryl fluoride as a fumigant (range of 7–15 mg/person/day across the five GEMS/Food regional diets) should be included in an overall assessment of fluoride from all sources. Upper levels for fluoride intakes have been proposed by a number of organizations. The dietary risk assessment for fluoride from fumigant use needs to be considered in light of the overall exposure to fluoride from other sources and FAO and WHO are requested to further investigate how this issue can be addressed at an international level.

Abbreviations and acronyms in this chapter

ADI	acceptable daily intake
ARfD	acute reference dose
GAP	good agricultural practice
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
HR	highest residue
HR-P	highest residue in a processed commodity calculated by multiplying the HR of the raw agricultural commodity by the corresponding processing factor.
IEDI	international estimated daily intake
IESTI	international estimated short-term intake
JMPR	Joint Meeting on Pesticide Residues
LOQ	limit of quantification
MRL	maximum residue limit
PTU	propylene thiourea
RAC	raw agricultural commodity
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor

PART II. EXERCISES[25]

Exercise 2.1. Identity and Physical and Chemical Properties

See also Chapter 2

IDENTITY

1. Identity and physical and chemical properties exercise

2. The aim

The aim of this exercise is to explain the review of identity information and physical and chemical properties for a JMPR evaluation.

3. Identity of test substance

Aim:

To identify a test substance unambiguously.

4. Procedure

Step 1. Check the common name, systematic names, CAS registry number, formula and structure at the Compendium of Pesticide Common Names
http://www.alanwood.net/pesticides/

Step 2. Check the CIPAC number at the CIPAC site - http://www.cipac.org/

Step 3. Check if specifications have been issued for the compound
http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmps/ps/ps-new/en/
http://www.who.int/whopes/quality/newspecif/en/

Step 4. If the test substance or compound is a mixture of isomers, check that the composition is clearly defined

5. Data submissions normally include:

- ISO common name
- Chemical name
  (IUPAC)
  (Chemical Abstracts)
- CAS Registry. No.
- CIPAC No.
- Synonyms

[25] The first digit in the number of exercises refers to the chapter number

- Structural formula
- Molecular formula
- Molecular weight

6. The exercise

Prepare identity information for:

1) alphacypermethrin
2) azoxystrobin
3) chlorothalonil

PHYSICAL AND CHEMICAL PROPERTIES OF TEST SUBSTANCE

7. Physical and chemical properties of test substance

Aim:

—To estimate physical and chemical properties from test data.

8. Hydrolysis rates

9. Fenvalerate hydrolysis rates (JMPR 2000)

Hydrolysis rates were measured for [^{14}C]fenvalerate at concentrations of approximately 50 µg/L in sterile aqueous buffers at pH 5, 7 and 9 at 25 ˚C in the dark. The estimated half-life was 80 days at pH 9.

pH	Fenvalerate as % of applied ^{14}C						
	Incubation periods						
	0 days	2 days	4 days	7 days	14 days	21 days	28 days
pH 5	*86*	*83*	*82*	*86*	*89*	*77*	*93*
pH 7	*83*	*83*	*85*	*88*	*101*	*87*	*79*
pH 9	*96*	*90*	*90*	*91*	*90*	*79*	*72*

10. Theory

For a first-order reaction, $C = C_0 \times e^{-kt}$ ..... (1)

C: concentration at time t
C_0: concentration at time 0
k: rate constant
t: time

$$ln(C) = ln(C_0) - kt \quad (2)$$

A plot of ln(C) as a function of t should produce a straight line with slope of –k.

At the half-life (t_{half}), $C = 0.5 \times C_0$

$$t_{half} = \frac{ln(0.5)}{-k} \quad (3)$$

A plot of ln(C) against time (days) should produce a straight line with a slope (-k) that can be converted to a half-life (days) using equation 3.

11 The exercise

With an Excel spreadsheet:

- convert concentrations to ln(C) values
- plot ln(C) as a function of time in days (use an xy scatter plot)
- insert a trendline (choose linear, options: display equation, display R squared)
- record the slope, -k. 'k' is the rate constant with units of $days^{-1}$
- calculate the half-life using equation (3)
- interpret the results in terms of uncertainty.

12. The question

What are the half-lives of fenvalerate in sterile aqueous solutions at pH 5, 7 and 9 at 25 ˚C in the dark?

13. Vapour pressures

14. Vapour pressures of test substance

temp °C	vp, Pa
80.5	0.00046
85.6	0.00075
90.7	0.00075
95.8	0.0015
100.8	0.0050
105.7	0.0090
115.9	0.0091
120.8	0.0278
130.9	0.0730
135.9	0.1573
150.9	0.4486
171.1	0.9205

15. Extrapolation of vapour pressure measurements at higher temperatures to 25 ˚C.

Theory

For a substance where the latent heat of vaporization is not a function of temperature,

$$\ln(VP) = \frac{a}{T} + b$$

VP: vapour pressure
T: absolute temperature (= t ºC + 273)
a, b: constants

16. The exercise

With an Excel spreadsheet:

- convert vapour pressures to ln(VP) values

- convert temperatures to 1/T values
- plot ln(VP) as a function of 1/T (use an xy scatter plot)
- insert a trendline (choose linear, options: display equation, display R squared)

17. The exercise (2)

Record the equation of the form

$$\ln(VP) = \frac{a}{T} + b$$

Calculate the vapour pressure at 25 ˚C (T = 298).

18. The question

- What is the vapour pressure of the test substance at 25 ˚C?

19. Questions?

Abbreviations and acronyms in this exercise

CAS:	Chemical Abstracts Service
CIPAC:	Collaborative International Pesticides Analytical Council
FAO:	Food and Agriculture Organization of the United Nations
ISO:	International Organization for Standardization
IUPAC:	International Union of Pure and Applied Chemistry
T:	absolute temperature

Exercise 3.1: Summary of metabolism of F64 in goat[26]

Introduction

F64 is a systemic fungicide with protective, curative, eradicative activity. It is mainly used on cereals (barley, oats, wheat, rye, triticale), pulses (bean and pea), oil seed rape and groundnut (peanut). It may be applied for seed dressing and foliar spray typically at 125-200 g a.i./ha rate.

Its metabolism was studied in goat, wheat, peanuts, and sugar beet plants applying the [phenyl-UL-^{14}C]-F64 referred to as phenyl-label, and [3,5-triazole-^{14}C]-labelled F64 referred to as triazole-label. In addition the metabolism of its major metabolite [phenyl-UL-^{14}C]- and [3,5-triazole-^{14}C]-labelled F64M1 was studied in goat.

Structural formula/ labelling position of test substances have been given but not copied here due to confidential nature of the report. The codes of the active substance and its metabolites are used only in these exercises.

The list of abbreviations and symbols are given on page 5. It is applicable for exercises 3.1-3.4

Tasks:

(a) Validate the study conditions concerning:
- Study material, test system, application conditions of test material, sampling and analysis, etc.
- Identification and characterization of metabolites
- Completeness of information provided in the summary of the study.

(b) Identify major residue components to be considered for definition of residues

(c) Compare the results of metabolism studies with the parent compound (F64) and its main plan metabolite (F64M1).

Summary of metabolism of F64 in goat

The kinetic behaviour and the metabolism of the fungicide F64 was investigated in the lactating goat. The test compound uniformly labelled with carbon-14 in the benzene ring of the molecule was administered in a tragacanth suspension to one lactating goat (39.0 kg body weight at first dosing). The oral target dose of 10 mg/kg body weight was given on three consecutive days at time intervals of 24 hours. Radioactivity was measured at different sampling intervals in excreta, plasma and milk, and at sacrifice in the edible tissues kidney, liver, muscle and fat. The milk and edible tissues were analysed for parent compound and metabolites by extraction, chromatographic separation techniques and spectroscopic methods.

Until sacrifice (53 hours after the first administration), the excretion amounted to about 66.6% of the radioactivity totally administered, a portion of 42.4% was eliminated with urine and 24.2% with faeces. An extremely low amount (0.02% of the total dose) was secreted with the milk.

[26] The material presented in this section is taken with the permission of the manufacturer from confidential reports.

The urinary excretion rate was relatively high: About 15.9% and 17.4% of the dose administered in total was eliminated with the urine within 24 hours after the first and the second administration, respectively,
The value for the total clearance amounted to CL = 11.3 mL per min and kg body weight as calculated from plasma curve analysis from a two compartment disposition model assuming a complete absorption process.

At sacrifice, 53 hours after the first administration, the compound-related residue in the edible tissues and organs was calculated or estimated to be about 0.96% of the total dose. Based on these values, the recovery amounted to about 67.6%.

The absorption process of the compound-related radioactivity administered in a 0.5% tragacanth suspension was characterised by a very fast onset (lag-time t_{lag} of about 7 min) followed by a short half-life of absorption t_a of about 14 min. The radioactivity concentrations in the plasma showed a distinct maximum with a measured peak level of 1.7 µg/mL one hour after the first administration, corresponding to only 17% of the equidistribution concentration. The radioactivity was monophasically eliminated from the plasma with a half-life of 5.3 h. Based on the analysis of the concentration-time plot in plasma, a low value of 8.2 hours was obtained for the mean residence time (MRT) (the weighting of concentrations vs. time). At the end of the observation period, the plasma concentration had declined by a factor of 17 to a value of 0.10 µg/mL.

At sacrifice (53 hours after the first administration), the relatively highest equivalent concentration was measured in the kidneys (6.762 µg/g wet tissue), followed by that obtained for the liver (6.092 µg/g). The concentrations in kidneys and liver were followed in decreasing order by those obtained for the omental fat (0.171 µg/g), perirenal fat (0.162 µg/g), subcutaneous fat (0.149 µg/g), flank muscle (0.106 µg/g), loin muscle (0.100 µg/g), round muscle (0.084 µg/g), and milk at sacrifice (0.061 µg/mL).

For elucidation of metabolism, the parent compound and the metabolites were extracted from milk and the edible tissues and purified by applying chromatographic techniques (HPLC and HPTLC). Metabolite identification was based on co-chromatography with authentic reference compounds or on spectroscopic evidence (HPLC/MS/MS and partly by HRMS and NMR). The quantification of all metabolites was conducted by integrating the ^{14}C-signals in the HPLC-chromatograms of the extracts.

In summary, the following amounts of the active substance and the metabolites - expressed as % of the total radioactive residue (% of TRR) and as residue concentration (equivalent concentration [µg/g]) - were found in milk and in edible tissues of the lactating goat:

	Milk		Liver		Muscle		Kidney		Fat	
TRR [pg/g] (combustion analysis)		**0.037**		**6.092**		**0.088**		**6.762**		**0.169**
Compound (F64)	**% of TRR**	**equiv. conc. [µg/g]**	**% of TRR**	**equiv. conc. [µg/g]**	**% of TRR**	**equiv. conc. [µg/g]**	**% of TRR**	**equiv. conc. [µg/g]**	**% of TRR**	**equiv. conc. [µg/g]**
polar metabolic group#										
fied polar met.	10.12*	0.004*	6.44`	0.392`						
cterised polar met.	31.30	0.012	10.78	0.657	12.01	0.011	7.13	0.482	4.25	0.007
other ident. compounds										
4-hydroxy-glucuronide			2.39	0.146	2.05	0.002	4.01	0.271	2.46	0.004
hydroxy-glucuronide			5.05	0.307	5.42	0.005	7.44	0.503	3.17	0.005
0- or S-glucuronide 3-hydroxy-desthio	11.96	0.004	10.02	0.610	14.80	0.013	34.32	2.321	10.09	0.017
4-hydroxy-F64M1			1.52	0.092						
N-glucuronide	1.27	0.000	2.80	0.170	1.14	0.001	2.64	0.179	0.80	0.001
4-hydroxy	2.10	0.001	11.21	0.683	4.94	0.004	3.10	0.210	3.61	0.006
F64M1	2.83	0.001	1.24	0.076	2.95	0.003	1.29	0.087	18.98	0.032
parent tomp. (F64)	0.89	0.000	12.94	0.788	13.37	0.012	17.97	1.215	13.31	0.022
ident. metabolites	19.05	0.007	47.15	2.87	44.69	0.040	70.78	4.786	52.41	0.089
charact. metab.	7.64	0.003	2.99	0.182					4.23	0.007
sum identified	**29.18**	**0.011**	**53.59**	**3.265**	**44.69**	**0.040**	**70.78**	**4.786**	**52.41**	**0.089**
sum characterised	**38.94**	**0.015**	**13.77**	**0.839**	**12.01**	**0.011**	**7.13**	**0.482**	**8.48**	**0.014**
solids	17.59	0.007	16.72	1.018	16.46	0.015	2.45	0.166	22.52	0.038
not analysed	14.29	0.005	15.92	0.970	26.85	0.024	19.65	1.329_	16.58	0.028
balance	**100.00**	**0.037**	**100.00**	**6.092**	100.00	0.088	100.00	6.762	100.00	0.169

polar metabolic group contained different polar glucuronides, conjugates and other metabolites; for identified metabolites see * and +.

* sum of 6 identified metabolites:
dihydroxy-diene F64M1
4-hydroxy - F64M1--glucuronide
dihydroxy-desthio F64M1-glucuronide
hydroxymethoxy-F64M1-glucuronide.
– F64-dihydroxy-diene
F64M1-glucuronide

\+ sum of 2 identified metabolites:
- F64M1-dihydroxy-dienyl-glucur.
- F64M1-dihydroxy-diene

Characterisation and identification of the more polar metabolites detected in varying amounts in milk and all edible tissues was performed by comparing the HPLC retention times of the unknown metabolites with those of identified urine metabolites. The metabolites that were used as reference compounds were isolated from goat urine in this study and in a corresponding goat study, in which F64M1 was used as the parent compound (see 3.2). The comparison showed that the majority of the polar metabolites in milk and edible tissues could be assigned to different conjugates with glucuronic acid. In addition, dihydroxy-dienes of F64 and F64M1 were also detected. To confirm the assignments, the milk sample (which was used as an example) was treated with boiling acid in order to cleave the glucuronides and other conjugates and to restore the aromatic structure which was broken in the dienes.

For the glucuronides, it could be shown that they were transformed nearly completely to the corresponding aglycons, which were identified by their retention times. Thus, elucidation of the aglycons was used as an additional confirmation for the identity of the glucuronides.
An increase in the concentrations of the hydroxyl-F64- and hydroxyF64M1 isomers after acid treatment (due to transformation of the dienes) was not obvious, but since only low concentrations of the dienes were present this was not unexpected.

The sum of all identified and characterised metabolites approximately represented the following percentages of the radioactive residue: 68% in milk, 67% in liver, 57% in muscle, 78% in kidney and 61% in fat. Due to the necessity of different clean-up steps during sample preparation there were small losses of radioactivity. However, since residue levels in milk, muscle and fat were very low, this resulted in a high percentage loss of radioactivity. The extraction efficiencies, corresponding to the first extraction step were as follows: approx. 77% for milk, approx. 90% for liver, approx. 90% for muscle, approx. 112% for kidney and approx. 81% for fat.

The metabolic pathway of F64 in lactating goat was characterised by the following main reactions:

- Conjugation of the unchanged parent compound with glucuronic acid forming an S-glucuronide (most likely) or an O-glucuronide.
- Glucuronidation of the triazole-thione nitrogen atom of the parent compound resulting in F64-N-glucuronide.
- Hydroxylation of the parent compound forming 4-hydroxy-F64 and a further hydroxy isomer, followed by conjugation with glucuronic acid. The position of the conjugation could not be accurately determined by the spectroscopic methods used.
- Elimination of sulphur to form the desthio metabolite F64M1.
- Further hydroxylation of the chlorophenyl moiety led to 3- F64M1 and 4-hydroxy-F64M1 and was followed by conjugation with glucuronic acid.
- There was also some evidence for the occurrence of dihydroxylated desthio isomers, formed as intermediates before conjugation with glucuronic acid.
- Oxidation of the chlorophenyl moiety of the parent compound or of the metabolite F64M1 led partly to de-aromatisation. As a consequence, several diene-moieties were detected. To some extent, this was followed by conjugation with glucuronic acid.

Based on these results, the authors believe that the metabolism of F64 in lactating goat is adequately understood.

List of abbreviations and symbols used in the text

ai	active ingredient
as	active substance
Bq	Becquerel
bw	body weight
°C	degree Celsius (centigrade)
CH3CN	acetonitrile
CH3OH	methano!
Ci	Curie
cm	centimetre
C.V.	coefficient of variation in percent

d	day
D	diastereomer
EtOH	ethanoi
g	gram
dpm	disintegrations per minute
GLP	good laboratory practice
h	hour(s)
HPLC	high performance liquid chromatography
HPTLC	high performance thin layer chromatography
HRMS	high resolution mass spectroscopy
I	isorner
i.d.	internal diameter
kBq	kilo Becquerel
kg	kilogram
L	litre
LOD	limit of detection
LOQ	limit of quantification
LSC	liquid scintillation counting
m	metre
M	molar
MBq	Mega Becquerel
mCi	milli Curie
fjCi	micro Curie
µg	microgram
mg	milligram
min	minute(s)
ml	millilitre
mm	millimetre
MS	mass spectrometry
nm	nano metre
NMR	nuclear magnetic resonance spectroscopy
n.d.	not determined (< LOD)
n.q.	not quantified *(*< LOQ)
no.	number
pH	pH-value

Exercise 3.2: Preparation of summary of report on the metabolism of F64 in goat[27]

Tasks for self study

1. Read the extract of complete study report first and prepare the summary of the report taking into account the checklist (see lecture Livestock and crop metabolism) for essential information.
2. Compare your summary with that given in Exercise 3.1

Extract from the complete study report:

The list of abbreviations and symbols are given in exercise 3.1.

Introduction

The test compound F64 is a new systemic broad-spectrum fungicide showing very good efficacy against a wide range of fungal diseases in many crops, especially in cereals.

Due to the target crops the compound and/or its metabolites may occur in the feed commodities of farm animals, may be absorbed from the intestinal tract into the systemic circulation, and may be present as contaminants in the edible tissues of those animals.

For the investigations concerning absorption, distribution, excretion and metabolism in the lactating goat as a model for ruminants reported herewith, the test compound was used which was uniformly labelled with carbon-14 in the benzene ring of the molecule.

The purpose of the experiments under consideration was to gain information on absorption, distribution, and excretion of total radioactivity and to identify and quantify - to the extent possible - the metabolites of F64 in milk, edible tissues and organs after oral administration of three doses of 10 mg/kg body weight on three consecutive days in time intervals of 24 hours. The dose regimen was selected with reference to the corresponding recommendations of the EPA Residue Chemistry Test Guidelines OPPTS 860.1300, the PMRA Ref. DACO6.2, and to the corresponding Council Directive 91/414/EEC amended by the Commission Directive 96/68/EC.

Materials and methods Chemicals

Non-labelled test compound

The non-labelled parent compound was used for the radio-dilution of the labelled compound and as reference material.

Company develop. name:
Chemical name (IUPAC):
Chemical name (CAS):
CAS Reg. No.: Empirical formula:
CAS-No.:
Molar mass:
Batch-no.:

[27] The material presented in this section is taken with the permission of the manufacturer from confidential reports.

Certified purity: 99.9%
Appearance:
Storage of the solid compound: in a refrigerator at 0 - 10 °C
Safety precautions:
Expiry date:
Last check on purity:
Date of certificate:

Radio-labelled test compound
Identity:[Phenyl-UL-^{14}C]-F64
Structural formula and labelling position: has been given but not copied here

Batch code-no.:	Lot no. 1210611 Purity Control Protocol: THS 4754
Specific radioactivity: 35.45 Ci/mole	3.81 MBq/mg = 228600000 dpm/mg = 103 µCi/mg =
Radiochemical purity:	>99% by radio-HPLC; LiChrospher 60 RP-Select B®; particle size: 5 µm, column size:125 x 4 mm; flow: 1.5 mL/min; gradient: aqueous H_3PO_4 (0.2% weight) for 5 min, then within 30 min to 100% acetonitrile.
Chemical purity:	>98% by HPLC with UV-detector at 210 nm; conditions as described above
Storage:	in a freezer at ca. -18 °C
Date of certificate:	April 22, 1998

The results of the rat metabolism study showed that the labelling position in the molecule was stable with respect to a possible degradation to C-1 fragments exhaled as $^{14}CO_2$. The excretion with the expired air amounted to 0.06% of the administrated dose (2 mg [phenyl-UL-^{14}C]F64/kg bw to male rats) within 48 hours after oral administration.

Chemicals and Reference compounds

Ali solvents and reagents were obtained from commercial suppliers and were used without additional purification. Water was obtained from a Milli-Q water purification system (Millipore Corporation, Bedford, USA). The XAD 7 (40 g or 80 g) solid phase extraction cartridges were obtained from Sigma-Aldrich (Deisenhofen, Germany). The buffer used (pH 3) was obtained from Riedel-de Haén (Seelze, Germany). AlL reference compounds were assayed for authenticity (laboratory Dr. Bornatsch, XX, YY, Germany).

Test system

Animal

Species/Strain: Lactating goat (Capra *hircus),* "Bunte Deutsche Edelziege"

Breeder/Source; Ziegenzuchtverband Baden-Württemberg e.V.,
Heinrich Baumann-Str. 1 - 3, D-70190 Stuttgart/FRG
Number: 1; animal no. 547

Age: About 30 months

Body weights: Weight of the goat at the first dosage: 39.0 kg; weight at sacrifice: 36.8 kg
Identification: Individual cage cards; skin markings

Acclimation: Six days to laboratory conditions

Rationale: Recognised by the registration authorities as model species for pesticide metabolism and residue studies with ruminants.

Animal accommodation

Conditions: Air-conditioned rooms (rooms 211 and 212)

temperature:	20 ± 1 °C
relative humidity:	57 ± 6%
light:	18 hours of illumination
air change:	10 - 15 times per hour.

Cage: During the period of acclimation, the animal was kept in a raised stall with a metal grid as base and straw and hay as bedding. One day prior to the start of the study and during the whole duration of the test, the animal was kept in an electro-polished stainless steel metabolism cage for farm animals (goat, sheep, pig), which allowed for an almost separate and quantitative collection of urine and feces, supplied by E. Becker & Co. GmbH "EBECO", Castrop-Rauxel/FRG. The cage was equipped with a variable restraining device.

Diet: During the whole residence time, the goats were fed with hay and ruminant feed (feed no. 18, additional feed for sheep, supplied by Höveler Kraftfutterwerke, D-40764 Langenfeld-Immigrath/ FRG.). This feed was not a certified diet, i.e. it was not checked for contaminants according to current standards.

Quantity: About 2000 g ruminant feed per day plus apples. In addition, hay was offered *ad libitum.*

Water: Tap water *ad libitum;*

Water specification in accordance to the local drinking water regulations.

Study design

The purpose of the present lactating goat study was to elucidate absorption, distribution, excretion and metabolism of [phenyl-UL-^{14}CJ] F64 after repeated (3x) oral administration. In order to facilitate metabolite characterisation and identification, the animal (test no.1, animal no. 547) was sacrificed 53 hours after the first administration (5 hours after the last dose), i.e. at a time of a relatively high residue level in the edible tissues and organs. The concentration-time course of total radioactivity in the plasma was followed after the first administration to gain information on the biokinetic behaviour of the total compound-related residues. Blank samples of excreta and milk were collected from the animal during the acclimation period prior to administration.
Blank samples of tissues and organs were taken from an untreated companion goat used in another lactating goat metabolism study (study M 41819041, test no. 0, animal no. 841). They served as background samples for the determination of the quantification limits of the radioactivity measurement, and also as biological material for the elaboration of adequate processing procedures in the metabolism part of this study.

Dose level

The labelled material was administered to the goat (animal-no.: 547) by oral intubation of the test substance as the pere compound in 0.5% aqueous tragacanth suspension, one dose per day at a target dose level of 10 mg/kg body weight. The goat received the three oral doses on 3 consecutive days in time intervals of 24 hours.
Based upon the experimentally determined daily feed consumption during the test of 4.1% of body weight, this dose level corresponds to the exaggerated concentration of 246 mg/kg (ppm) in the feed commodity.

Preparation of the compound for administration

The labelled compound was delivered in solid form. In order to prepare a stock solution, the whole amount was dissolved in 25 mL acetonitrile. This solution was calibrated by radioactivity measurement. The total amount of radioactivity was 15951.91 µCi (3.54 x $10^{}$ dpm or 590.22 MBq) corresponding to 154.92 mg compound. The radioactivity concentration was 638.08 µCi/mL (1.42 x 10^{9} dpm/mL or 23.61 MBq/mL) corresponding to ca. 6.2 mg/mL. The specific radioactivity was 102.97 pCi/mg (2.286 x 10^{8} dpm/mg or 3.81 MBq/mg).

An amount of 48 mg of the compound corresponding to 7.75 mL (4944 µCi or 1.10 x 10^{10} dpm or 182.93 MBq) of the acetonitrile stock solution was pipetted into each of 3 Erlenmeyer flasks and radio-diluted with 9 parts (432 mg) of the authentic unlabelled compound (batch-no. M00175) to give 480 mg of the radio-diluted compound with a specific radioactivity of 10.3 µCi/mg (2.29 x $10^{}$ dpm/mg or 0.381 MBq/mg; corresponding to 3.55 µCi/mole).
In order to prepare the three administration suspensions the solvent was removed by a nitrogen gas stream at room temperature. The compound was suspended in 48 mL of a 0.5% aqueous tragacanth suspension on an ultrasonic water bath for 15 min at about 50 °C and stirred on a magnetic stirrer until administration. The target concentration of the compound in suspension was 10 mg/mL with 103 µCi/mL or 2.29 x 10^{8} dpm/mL. The three suspensions were freshly prepared immediately prior to each administration. The radioactivity concentration of each suspension was calibrated by liquid scintillation counting. The animal was dosed related to its individual body weight. The administration volume was 1.0 mL/kg body weight.

Stability of the ^{14}C-labelled test compound in tragacanth suspension

The ^{14}C-labelled parent compound proved to be stabile in the 0.5% aqueous tragacanth suspensions for at least 4 h after administration at room temperature as shown by radio-HPLC analysis. The evaluation of the chromatogram revealed a radiochemical purity of 99.4%.

Administration procedure

The oral administration was performed by intubation using a 50 mL disposable perfusion syringe attached to a Teflon®-perfusion tube (outer diameter: 0.3 cm, inner diameter: 0.2 cm, length: 85 cm, supplied by Labokron, Sinsheim/FRG). This tube was passed through a second thicker, flexible plastic stomach tube (inner diameter: 0.8 cm, length: 80 cm; outside lubricated with corn oil) that had been inserted into the rumen at first.

A volume of 39 mL suspension was administered to the goat by intubation through the pertusion tube, directly followed by a volume of 50 mL 0.5% aqueous tragacanth that was administered through the same Teflon® tube in order to rinse the remainder of the suspension into the rumen.

At last, this tube was removed from the rumen and rinsed with 50 mL acetonitrile into an Erlenmeyer flask in order to determine the amount that was not administered. The goat received the following amounts of radioactivity or compound:

Radioactivity [dpm]					daily dose, [mg/kg]
1. administr.	2. administr.	administr.	sum	mean	mean
9081376200	9116874000	779000	7029200	576400	

Related to a body weight of 39 kg the mean actual daily dose was 10.1 mg/kg. The radioactivity amount that was not administered amounted in total to 26190600 dpm corresponding to 0.1 mg.
The radioactivity values of the amount actually administered served as a reference for the percentage calculation of total radioactivity in the biological samples. The dose level was tolerated without any observable toxicological sign.

Sacrifice

The goat was weighed and sacrificed 53 hours following the first dosage. The animal was anaesthetised by an intravenous dose of about 2 mg/kg Rompun® and 5 mg/kg Ketavet®, then sacrificed by an intravenous dose of about 10 mL per animal of the sacrificing agent "T 61" (Hoechst AG, Frankfurt-Hoechst/FRG), and exsanguinated by cannulating the jugular veins.

Sampling procedure

Blood

Micro-samples of blood were taken from the ear veins of the goat at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 24 hours after the first administration. The blood was collected in heparinised capillaries. In order to obtain the plasma fraction, the capillaries were centrifuged at about 12.000 x g for 10 min using a hematocrit. The plasma samples (mean weight: 45 mg) were weighed and prepared for liquid scintillation counting.

Milk

The goat was milked in the morning immediately prior to each administration, about 8 hours later in the afternoon, and directly before sacrifice (time schedule: 8, 24, 32, 48 and 53 hours after the first administration). The mik weights were recorded. One aliquot was taken from each fraction, processed for liquid scintillation counting and measured in duplicate. The remaining milk was directly extracted or stored at about -18 °C for metabolite analysis.

Urine

The urine fractions were collected as quantitatively as possible under dry-ice cooling in intervals of 24 hours after the first and second administration and at 5 hours after the third administration (at sacrifice). The collection vessel was changed and the collection funnel was rinsed with deionised water into the urine vessel at the end of each collection period. One aliquot of the urine was taken from all fractions. After having recorded the total volumes, the samples were prepared for liquid scintillation counting and measured in duplicate. The remaining urine amounts were stored at about -18 °C for optional analysis of metabolites.

Faeces

The faeces fractions were collected as quantitatively as possible at room temperature in intervals of 24 hours after the first and second administration, i.e. immediately before the next dosage, and at 5 hours after the last administration (at sacrifice). The collecting grid was cleaned prior to each administration. No samples of the rinsing water were taken for radioactivity measurement. The faeces fractions were freeze-dried and homogenised. After having recorded the total dry weight, one aliquot of each fraction was prepared and combusted in triplicate. The absorbed $^{14}CO_2$ was measured by liquid scintillation counting techniques. The remainder was stored at room temperature for optional analysis of metabolites.

Organs/tissues

The following organs and tissues were dissected:

- liver without bile bladder, kidneys,
- three different types of muscle (round, flank, loin), and
- three different types of fat (perirenal, omental, subcutaneous).

After recording the weights, the organs or tissue samples were transferred into ice-cooled vessels.

Liver, kidneys and muscle samples were passed 4 to 5 times through mincing machines in half-frozen state. The fat samples were also disintegrated. The equipment of the mincing machines were carefully cleaned prior to a new sample. One sample of the resulting tissue pulp was weighed, freeze-dried, weighed again, and prepared for tissue combustion followed by liquid scintillation counting of the $^{14}CO_2$ absorbed. As in the case of faeces, three sub-samples were prepared from each sample for combustion and radioassayed.

In parallel, the organs or tissues were prepared for metabolite analysis in wet state. These samples were stored at about -18 °C.

Non-radioactive biological material

Blank samples of excreta and milk were collected from the animal (animal no. 547) used for the present study during the acclimation period prior to administration. Blank samples of organs and tissues were taken from an untreated companion goat used in another lactating goat metabolism study (study no. M 41819041, test no. 0, animal no. 841). These samples served as background samples for the determination of the limits of quantification of the radioactivity measurement, and also as biological material for the elaboration of adequate processing procedures in the metabolism part of this study.

Measurement of radioactivity in liquid and solid samples

Measurement of liquid samples using liquid scintillators

of sample	**le volume**	**of lator**	**Scintillator volume [mL]**
		safe A*	2
		safe A*	7
a-microsamples	04	safe A*	7
solution		szint 401	2
nistr. suspensions		szint 401	2
g of the intub. tube		safe A*	2

* Quicksafe A +5% water

Liquid scintillation counter(s):
Beckman LS 6500, Quench correction using the "H-number"
Philips PW 4700, Quench correction using the "ESCR-number"
LKB Rack Beta 1219 Spectral, Quench correction using the "SQP(E)-number"

H-number: The inflection point of the external-standard-spectrum is used to correct for the quench.
ESCR: External standard channels ratio.
SQP(E): The end point of the external-standard-spectrum is used to correct for the quench.

Measurement of solid samples using liquid scintillators

Samples of the freeze-dried and homogenised tissues and organs are weighed and combusted in an oxygen atmosphere using the following equipment:

Oxidizer 307 (Packard Instruments) to combust solid samples with weights of 10 to 500 mg. CO_2-binding reagent: Carbosorb (8 mL). Scintillator: Permafluor E+ (10 mL), as recommended by Packard Instruments.

Test material: Organs or tissues and faeces

Liquid scintillation counter(s):
Philips PW 4700, Quench correction using the "ESCR-number"

ESCR: External standard channels ratio

Calculations

Results from scintillation counters were rounded to integer numbers, averaged for repeated measurements and the average values rounded to integer dpm values. These values were used for further calculations.
Calculations in tables and appendices were performed mainly using the Microsoft Excel® software. The data were not restricted to a certain number of digits for calculation. Numbers in the tables and appendices section of this report are printed as rounded values (two to four digits). Use of these rounded values for calculations may therefore give results differing slightly from those calculated using Excel. The fundamental calculations were based on the LSC results, expressed in disintegrations per minute (dpm-values). The amount of the radioactivity of each aliquot shown in the tables or appendices was normally the arithmetic mean (integer) from 3 measurements (liquid samples) or 2 - 3 combustion values (solid samples). For all steps during the preparation of samples or isolation procedures the recovery was corrected to 100%. The actual experimental recoveries for all individual operations are given in the corresponding figures.

The equivalent concentrations C related to the parent compound were calculated in accordance with the following equation:

$$C[\mu g\ equiv./g\ wet\ materias l] = \frac{\frac{dpm}{g\ (dry\ material)}\ x\ D_f}{spec.radioact.\left[dpm/\mu g\right]}$$

dpm/g dry material: after background correction (i.e. correction for the radioactivity of the blank samples).
D_f: freeze-drying factor; this factor is equal to 1 in the case of liquid samples.

spec. radioact.: specific radioactivity of the test compound after radio-dilution with the authentic non-labelled test compound

Limits of quantification

In addition to the samples containing the compound-related radioactivity, blank samples were prepared from milk, tissues or organs and excreta as mentioned above. The threshold value Cumit was defined by a drop in the net counting rate of the radioactivity containing sample to the counting rate that was determined for the corresponding blank sample.

Based on the "background" radioactivity of the blank samples and on the specific radioactivity of the radio-diluted test compound the following limits of quantification were calculated:

Organ / tissue / biological material	Limit of quantification [µg/g or µg/mL wet material]
Liver	0.002
Kidney	0.001
Round muscle	0.002
Flank muscle	0.001
Loin muscle	0.003
Perirenal fat	0.009
Subcutaneous fat	0.006
Omental fat	0.005
Milk	0.001
Urine	0.001
Faeces	0.010 µg/g dry material

<u>High performance thin laver chromatography (HPTLC)</u>

For HPTLC, pre-coated HPTLC glass plates from Merck (Darmstadt, Germany), with a size of 10 x 20 cm were used. The absorbent was silica $60F_{254}$. The plates were pre-conditioned with ammoniumhydroxide and developed over a distance of approx. 7 cm with method AMD2 in an instrument for automatic multiple development (Camag, Muttenz, Switzerland).

AMD2: methanol (solvent 1) / dichloromethane (solvent 3)

Run No.	preconditioned	Solvent1 [Vol%]	Solvent 3 a]	Running distance [mm]
1	yes	100	0	15
2	yes	100	0	15
3	yes	100	0	15
4	yes	100	0	15
5	yes	100	0	15
6	yes	80	20	18
7	yes	70	30	21
8	yes	60	40	24
9	yes	50	50	27
10	yes	40	60	30
11	yes	30	70	33
12	yes	20	80	36
13	yes	20	80	41
14	yes	20	80	46
15	yes	20	80	51
16	yes	20	80	56
17	yes	20	80	61
18	yes	10	90	66
19	yes	0	100	69
20	yes	0	100	72
21	yes	0	100	75
22	yes	0	100	78
23	yes	0	100	81

The samples were applied using a Linomat IV — automated application device (Camag, Muttenz, Switzerland). The TLC-spots or lanes were visualised under a UV-lamp set at 254 nm by quenching the fluorescence emitted by the indicator $_{F254.}$ The radioactive zones were detected by radioluminography. The imaging data were transferred with BAS

Reader Software (Fuji, Japan) to an appropriate computer and evaluated by data conversion with "TINA" — software (Raytest, Straubenhardt, Germany).

HPLC/MS determination

The chromatographic conditions for the MS experiments are given below. A radioactivity detector (Ramona 90, Raytest, Straubenhardt, Germany) was coupled via a flow splitter between HPLC instrument (Hewlett Packard, Waldbronn, Germany) and mass spectrometer.

Sample	Column and Flow	Solvent	Gradient
KOE052OA, KOE0520B, K0E0832B, K0E0833A, K0E08336, 33E, 33F	Column: LiChrospher 60 RP Select B (VDS b), Dimensions: 250 x 2 mm Particle Size: 5pm Flow: 0.2 ml/min Split Ratio: 25 :175 [MS : (UV + 14C)]	A: 0.1 %acetic acid in water; B: 0.1% acetic acid in acetonitrile	0-1 min 5% B, at 25 min 95% B, nin 95% B
H0220898, H0240898, KOE0516A, K0E0516C, 161, 516J, KOE0811, KOE0812, KOE0813, KOE0817, K0E0819, KOE0820A, KOE0828, KOE0829, KOE0830A	Column: LiChrospher 60 RP Select 8 (VDS Optilab), Dimensions: 250 x 2 mm Particle Size: 5pm Flow: 0.2 ml/min Split Ratio: 40 :160 [MS : (UV + 14C)]	A: 1% acetic acid in water; C: acetonitrile	0-1 min 5% C, at 25 min 95% C, at 35 min 95% C

NMR-spectroscopy

The 300 MHz NMR-spectra were recorded on a BRUKER DPX 300 instrument, 600 MHz NMR-spectra on a BRUKER DMX 600 instrument. Sample ID's and solvents (supplier: Merck, Wilmad or Sigma Aldrich) are given in the spectra headers.

Isolation and purification of metabolites

Biological material used for metabolism investigations

The milk fractions obtained after the first, second and third administration were weighed and radioassayed. Subsamples of each fraction (about 50%) were withdrawn and combined for metabolite analysis (Appendix 1).

The samples of liver and kidneys, the three types of fat and the three types of muscle remaining after the first combustion were combined per tissue (Appendix 2). Thereby, the portions of perirenal, subcutaneous and omental fat, as well as the samples of round, loin and flank muscle were prepared as a composite sample. Ali samples were thoroughly homogenised and stored at about -18 °C until metabolism investigations.

The urine fractions were collected as quantitatively as possible. Aliquots were prepared for liquid scintillation counting and measured in duplicate. The remaining urine amounts were stored at about -18 °C for optional analysis of metabolites. The urine fraction collected 53 h after the first administration was used for isolation and identification of metabolites by mass spectroscopy.

Extraction and sample processing

Milk

Six aliquots of 200 mL of the combined milk sample were extracted three times with methanol using an ultrasonic bath. The extracts were combined and concentrated. The concentrated extract was diluted with 50 mL buffer solution (pH 3) and submitted to a clean-up step using an XAD 7 column (40 g). The eluate was collected. After rinsing with approx. 200 mL water complete drainage of the liquid was allowed. The radioactive adsorbed compounds were eluted with methanol. The methanolic eluate was evaporated to dryness and redissolved in a small amount of methanol/water. This sample was used for HPLC profiling. A flow chart of the extraction and the clean-up procedure is presented in Scheme 1.
The extraction steps including the radioactive balance of the combined milk sample (experiment KOE0505) are described in detail in Appendix 3.

Liver (first and third extraction), kidneys (first and second extraction) and muscle composite

Aliquots of liver (first and third extraction), kidney (first and second extraction) and muscle composite were extracted as described below for the liver sample.

For the first extraction of liver an aliquot of approx. 78 g was used. The liver sample was extracted three times with an acetonitrile/water mixture (8:2 v/v) and two times with an acetonitrile/water mixture (5:5 v/v). 1 g cysteinhydrochloride was added per litre water. The first three extracts were combined and evaporated to approx. 50 mL. The two following acetonitrile/water extracts were discarded because of low radioactivity levels. The remainder of the first extraction steps was diluted with 50 mL acetonitrile and partitioned against n-hexane (2x 100 mL). The hexane phase was evaporated to dryness and redissolved in 3 mL methanol for HPTLC analysis. The acetonitrile phase was concentrated to approx. 50 mL and dissolved in 50 mL buffer solution (pH 3) for a further clean-up step using an XAD 7 column (40 g). The sample was applied to the pre-conditioned column (methanol; water; buffer solution (pH 3)) and the effluent was collected. Then, the column was rinsed with approx. 50 mL buffer solution and 100 mL water; the resulting effluent was collected. Elution of the radioactive residues was done with methanol (2x 100 mL). The methanol eluates were combined and evaporated to dryness and dissolved in a small portion of methanol. This sample was used for co-chromatography with reference compounds and for HPLC profiling.

A flow chart of the extraction and the clean-up procedure of the liver sample is presented as an example in Scheme 2. All other tissues used for metabolic profiling with exception of the second liver aliquot and the fat composite - were processed according to this scheme. Solvent and XAD column volumes were adapted to the amount of the individual sample aliquots.

The sample preparation steps including the radioactive balances of the first and third extraction of the liver sample (experiments KOE0507 and K0E0521) are described in detail in Appendix 4 and in Appendix 6. A second aliquot of liver was extracted for isolation of metabolites (experiment K0E0518); the extraction is described in paragraph 3.8.2.3. The sample preparation steps including the radioactive balances for the muscle

composite (experiment KOE0509) and the kidney samples (experiments KOE0508 and KOE0520) are described in detail in Appendix 5, Appendix 8 and Appendix 9. For the different samples the volumes of solvents used for the extraction and dissolving steps were adjusted to the corresponding sample weights.

Fat composite and second liver aliquot

Homogenised perirenal, subcutaneous and omental fat was prepared as a combined sample for extraction procedures and analytical analysis. The sample preparation steps for the combined fat sample are described below as an example. The second aliquot of liver was processed according to this procedure.
The pooled sample was extracted three times with an acetonitrile/water mixture (8:2 v/v) and two times with an acetonitrile/water mixture (5:5 v/v). 1 g cysteinhydro-chloride was added per litre water. Ali extracts were combined and evaporated to approx. 50 mL. The aqueous remainder was diluted with approx. 200 mL methanol and partitioned against n-hexane (2x 200 mL). The hexane phase was discarded after LS-measurement. The methanol phase was concentrated to approx. 100 mL and dissolved in 100 mL buffer solution (pH 3) for a further clean-up step using an XAD 7 column (40 g). The sample was applied to the pre-conditioned column (methanol; water; buffer solution of pH 3), the column rinsed with approx. 100 mL buffer solution and the effluent was collected. Then, the column was rinsed with approx. 100 mL water and the resulting wash was collected. Elution of the retained radioactive residues was done with methanol (2x 100 mL). The methanol eluates were combined and evaporated to dryness and dissolved in a small portion of methanol/water. This sample was used for HPLC profiling.

The sample preparation steps including the radioactive balance of the fat composite (experiment KOE0512) are described in detail in Appendix 10. The sample preparation steps of the second liver aliquot (experiment KOE0518) are described in Appendix 5. The second liver aliquot was used to perform co-chromatography with reference compounds isolated from another goat study using F64M1 as parent compound (study M91819091

<u>Quantification, isolation and identification of metabolites</u>

In the presented study metabolites were isolated from urine samples and the second kidney extract, respectively. Isolation and purification was done by HPLC using methods JAU4 and JAU6. The purified fractions were subjected to HPLC/MS analysis and furthermore to NMR analysis, if the amount of the isolated compound was sufficient.

For identification purposes, HPLC and HPTLC co-chromatography was carried out using aliquots of the extracts, non-labelled and labelled reference compounds as well as mixtures of extracts with the respective reference compounds. Most of the reference compounds were isolated and identified by spectroscopic methods in the course of the present goat study or corresponding metabolism studies [1], [3], [4].

For metabolic profiling, RP-HPLC method JAU6 was used exclusively. Co-chromatography with respective reference compounds was carried out using the RP-HPLC methods FAU6, SXX1 and SXX3. Methods SXX1 and SXX3 were modifications of method FAU6. Method SXX1 has been predominantly used in the goat metabolism study with F64M1 as parent compound (study M91819091) [4]. Thus, comparisons of the retention times of unknown compounds with F64M1 metabolites were done mainly using this method. Method SXX3 was used only for the comparison of the retention of several liver metabolites with that of the wheat metabolite F64-sulfonic acid.
The independent HPTLC method AMD2 was used for confirmatory purposes.

Milk

The metabolic profile in milk was measured with method F64.6 within 3 months after sacrifice of the goat using the extract of the first sample preparation (experiment K0E0505). Metabolites in the extract of milk were identified by comparison of the chromatogram with that of the liver profile. Additionally, the profile was compared to the more polar metabolites isolated during the course of the goat metabolism study of F64M1. HPTLC analysis was performed for confirmatory purposes. Furthermore, the extract was treated with boiling acid to cleave glucuronic acid conjugates. An identification of the formed aglycons was done by comparing the retention time with corresponding reference compounds.

Liver

The first metabolic profile in liver was measured with HPLC method JAU6 within 3 months after sacrifice of the goat using the extract of the first sample preparation (experiment K0E0507). The metabolites were identified by HPLC and HPTLC co-chromatography using reference compounds. HPTLC was used for confirmatory purposes. The chromatogram of the profile of the first extraction was integrated and used for the quantitative evaluation of the radioactivity.

A further extraction (experiment KOE0518) was done to provide enough extract for co-chromatography with F64M1 metabolites using HPLC methods F64.6 and SXX1. The extract of the third sample preparation (experiment KOE0521) was used for co-chromatography with F64-sulfonic acid using HPLC methods SXX1 and SXX3.

Muscle

The metabolic profile in muscle was measured with method JAU6 within 3 months after sacrifice using the extract of the first sample preparation (experiment K0E0509). Metabolites in the extract of muscle were identified by comparing the chromatogram with that of the liver profile. Additionally, HPTLC co-chromatography was performed for confirmatory purposes. Based on these results, the chromatogram of the muscle profile was integrated (Figure 7).

Kidney

The metabolic profile in kidney was measured with method JAU6 within 3 months after sacrifice using the extract of the first sample preparation (experiment K0E0508). Most of the metabolites in the extract of kidney were identified by comparing the chromatogram with that of the liver profile. From a further extraction (experiment KOE0520), the more polar glucuronic acid conjugates were isolated by subsequent purification steps using micro-preparative HPLC (methods JAU6 and JAU4). The purified metabolites were identified by HPLC/MS and partly by NMR and were used as reference compounds for co-chromatography. Confirmation of the major metabolites identified by HPLC was achieved using the independent HPTLC method AMD2. Based on these results, the chromatogram of the kidney profile of the first sample preparation was integrated (Figure 8).

Fat composite

The metabolic profile in fat was measured with method JAU6 within 3 months after sacrifice using the extract of the first sample preparation (experiment K0E0512). The metabolites in the extract of fat were identified by comparison of the chromatogram with that of the liver profile. Additionally, HPTLC co-chromatography was performed for confirmatory purposes.

RESULTS AND DISCUSSION

Absorption and excretion of radioactivity

The recovery of radioactivity and the excretion pattern of the lactating goat after administration of a daily dose of 10 mg [phenyl-UL-^{14}C]F64 per kg body weight on three consecutive days is presented in Table 1 (*not included*).

Until sacrifice (53 hours after the first administration), the excretion amounted to about 66.6% of the radioactivity totally administered, a portion of about 42.4% was excreted with the urine and about 24.2% with the faeces. An extremely low amount (about 0.02% of the total dose) was secreted with the milk.
The urinary excretion rate was relatively high: About 15.9% and 17.4% of the dose administered in total was eliminated with the urine within 24 hours after the first and the second administration, respectively.
The value for the total clearance amounted to CL = 11.3 mL per min and kg body weight as calculated from plasma curve analysis from a two compartment disposition model assuming a complete absorption process.
At sacrifice, 53 hours after the first administration, the compound-related residue in the edible tissues and organs was calculated or estimated to be about 0.96% of the total dose.

Based on these values, the recovery amounted to about 67.6%.

Due to the relatively short survival period after the last dosage the missing amount (about one third of the total dose) was not measured in the excreta. Taking into account the low quantities of radioactivity which were determined or estimated in body muscle and fat, the main portion of the missing amount could be present in the contents of the gastrointestinal tract at sacrifice.

Concerning the percentage fraction absorbed from the gastrointestinal tract, no exact data could be obtained, but in context with the findings of the relatively high amount which was excreted with the urine and the significant concentrations determined in liver and kidney it is plausible to assume that each of the oral doses was almost completely absorbed prior to excretion.

The absorption process was characterised by a very fast onset (lag-time t,a_9 of about 7 min) followed by a short half-life *of* absorption (t_a) *of* about 14 minutes.

Concentration-time-course of radioactivity in the plasma

The radioactivity concentrations in the plasma were followed in dependence on time after the first administration (Table 2 and Figure 3, *not included*), in order to determine the plasma peak level and the kinetic behaviour.

The radioactivity concentrations in the plasma after the first administration showed a distinct maximum with a measured peak leve) of 1.70 µg/mL at 1 hour after dosage, corresponding to only 17% of the equidistribution concentration of 10 µg/mL.

The radioactivity was monophasically eliminated from the plasma with a half-life of 5.3 h as obtained by the computer-assisted two compartment disposition model curve analysis. This half-life was very short with respect to the observation period of 24 hours. At this time, the plasma concentration had declined by a factor of ca. 17 to a value of 0.10 µg/mL.

Based on the analysis of the concentration-time-course in plasma, a low value of MRT = 8.2 hours was obtained for the mean residence time (the concentrations weighted vs. time).

The calculation of the biokinetic characteristics was done computer-assisted using the

software "TOPFIT" [2].

Radioactivity level in the milk

The radioactivity level measured in the milk samples and the amounts of milk are recorded in Table 3 (*not included)*.

Equivalent concentrations of 0.042 µg/mL and 0.071 µg/mL were measured in the milk at 8 hours after the first and second dosage, respectively, whereby the second value represented the relatively highest concentration measured during the whole test period. The values declined within the time period from 8 to 24 hours after the first and second administration to values of 0.020 µg/mL and 0.026 µg/mL, respectively. This finding indicates that there is no risk of a significant bioaccumulation of compound-related residues in the milk after repeated dosage. The equivalent concentrations in the milk were lower by a factor of 17 (8 h after the first dosage) and **5** (24 h after the first dosage) when compared with the radioactivity level of the plasma.

In terms of amounts, an extremely low fraction of 0.02% of the dose administered in total was found in the milk during the whole test period.

4.4 Residual radioactivity in the dissected tissues and organs

The radioactivity levels measured in the samples of the edible organs and tissues as well as their respective weights are presented in Table 4 and in Figure 4.

At sacrifice (53 hours after the first administration), the relatively highest equivalent concentration was measured in the kidneys (6.762 µg/g wet tissue), followed by that obtained for the liver (6.092 µg/g). This result reflects the significance of these organs for excretion andlor metabolism of the compound. These concentrations corresponded to 0.07% (kidneys) and 0.44% (liver) of the total dose.

The concentrations in kidneys and liver were followed in decreasing order by those obtained for the omental fat (0.172 µg/g), perirenal fat (0.162 µg/g), subcutaneous fat (0.149 pg/g), fiank muscle (0.106 µg/g), loin muscle (0.100 µg/g) and round muscle (0.084 µg/g).

In terms of amounts, the radioactivity concentration of the total body fat corresponded to about 0.18% of the dose totally administered assuming a value of 12% of body weight for this tissue.

Assuming a value of 30% of body weight for total body muscle the total compoundrelated residue in this tissue amounted to about 0.27% of the radioactivity totally administered.

Structure elucidation and identification of metabolites

Structure elucidation was done by HPLCIMS/MS and in some cases additionally by NMR spectroscopy. All relevant metabolites were identified, whereby the position of the conjugation of several glucuronic acid conjugates was not always clearly assigned. Although ^{1}H-NMR spectroscopy allowed the identification of F64-N-glucuronide, a differentiation between F64-S- and F64-O-glucuronide was not possible. However, considering the behaviour of the reference compound F64M1glucuronide and the isolated metabolite F64-glucuronide during add treatment it was concluded that the isolated glucuronide was more likely to be F64-S-glucuronide.

Sometimes, also no unambiguous assignments concerning the position of hydroxylation and the conjugation of glucuronide conjugates of mono- and dihydroxy compounds were possible. Apart from the different possibilities for conjugation when different hydroxy groups were present in the molecule, multiple conjugation could also have been involved.

Exercise 3.3: Validation of goat metabolism study with F64M1[28]

Tasks:

(d) Validate the study conditions concerning:
- Study material, test system, application conditions of test material, sampling and analysis, etc.
- Identification and characterization of metabolites
- Completeness of information provided in the summary of the study.

(e) Identify major residue components to be considered for definition of residues

(f) Compare the results of metabolism studies with the active substance (F64) and its main plan metabolite (F64M1)

The list of abbreviations and symbols are given in exercise 3.1.

Explanation

The test compound F64M1is a prominent metabolite of the new broad spectrum fungicide F64M in cereal forage, hay and straw.

Therefore, the test compound may be consumed with the feed by farm animals and may be absorbed from the intestinal tract into the systemic circulation. The test compound and its metabolites may be present as contaminants in the edible tissues of those animals.

For the investigations concerning absorption, distribution, excretion and metabolism in the lactating goat as a model for ruminants reported herewith, the test compound was used which was uniformly labelled with carbon-14 in the benzene ring of the molecule.

The purpose of the experiments under consideration was to gain information on absorption, distribution, and excretion of total radioactivity and to identify and quantify - to the extent possible- the metabolites of F64M1 in milk, edible tissues and organs after oral administration of three doses of 10 mg/kg body weight on three consecutive days in time intervals of 24 hours. The dose regimen was selected with reference to the corresponding recommendations of the EPA Residue Chemistry Test Guidelines OPPTS 860.1300, the PMRA Ref. DACO6.2, and to the corresponding Council Directive 91/414/EEC amended by the Commission Directive 96/68/EC.

Summary of results

The test compound uniformly labelled with carbon-14 in the benzene ring of the molecule was administered in a tragacanth suspension to one lactating goat at the oral target dose level of 10 mg/kg body weight on three consecutive days in time intervals of 24 hours corresponding to 195 ppm in the feed commodity. Radioactivity was measured in the excreta, plasma and milk at different sampling intervals, and in the edible tissues kidney, liver, muscle and fat at sacrifice. The milk and edible tissues were analysed for

[28] The material presented in this section is taken with the kind permission of the manufacturer from confidential reports.

parent compound F64M1 and metabolites by extraction, chromatographic separation techniques and spectroscopic methods.

Until sacrifice (53 hours after the first administration), the excretion amounted to about 73.9% of the radioactivity totally administered, a portion of 53.1 % was excreted with the urine and 20.7% with the faeces. An extremely low amount (0.05% of the total dose) was secreted with the milk.

The urinary excretion rate was high: About 21% and 23% of the dose administered in total was eliminated with the urine within 24 hours after the first and the second administration, respectively.
The value for the total plasma clearance amounted to CL = 9.8 mL per min and kg body weight as calculated from plasma curve analysis using a two compartment disposition model assuming a complete absorption process.

At sacrifice, 53 hours after the first administration, the compound-related residue in the edible tissues and organs was calculated or estimated to be about 1.9% of the total dose.

The absorption process of the compound-related radioactivity administered in a 0.5% tragacanth suspension was characterized by a very fast onset (lag-time t_{lag} of about 6 min) followed by a short half-life of absorption t_a of about 31 min. The radioactivity concentrations in the plasma showed a distinct maximum with a measured peak level of 2.0 µg/mL at 2 hour after the first administration. The radioactivity was monophasically eliminated from the plasma with a half-life of 8.3 hours. Based on the analysis of the concentration-time plot in plasma, a low value of MRT = 10 hours was obtained for the mean residence time (MRT) (the weighting of concentrations vs. time). At the end of the observation period, the plasma concentration had declined by a factor of ca. 14 to a value of 0.14 µg/mL.

At sacrifice (53 hours after the first administration), the relatively highest equivalent concentrations were measured in kidney (18.975 µg/g wet tissue) and liver (18.421 µg/g). The concentrations in rnilk at sacrifice as well as in the different types of muscle and fat were significantly lower in the narrow range between 0.2 µg/g and 0.3 µglg: milk 0.286 µg/mL, round muscle 0.276 µg/g, omental fat 0.239 µg/g, subcutaneous fat 0.233 µg/g, flank and loin muscle 0.232 µg/g and perirenal fat 0.215 µg/g.

Elucidation of metabolism of F64M1 in goat was based on different steps: First of all, all metabolites detected in the urine sample collected 24 h after the first application were isolated, purified and identified. Structure elucidation was done by PLC/MS/MS and NMR spectroscopy. The identified components were used as reference compounds in the further course of the study.

Since the metabolic pattern in the urine sample was quite complex, an additional way for the characterization of the metabolites or metabolic groups of minor metabolites was performed: The urine sample was treated with boiling hydrochloric acid. The aim of the hydrolysis was to cleave conjugates and to convert non-aromatic compounds into aromatic compounds with known structures. In fact, besides some minor components, five relevant compounds (two isomers of dihydroxy-F64M1, two isomers of hydroxy-F64M1 and the parent compound F64M1) were formed by the treatment. The isomers of dihydroxy-F64M1 and hydroxyl-F64M1 were isolated, identified by spectroscopic methods and were used as reference compounds, as well. Additionally, each isolated fraction of the urine sample was treated with an enzyme mixture containing β-glucuronidase and arylsulfatase. The HPLC chromatograms of the treated and the untreated fractions were compared. The detection of cleavage products was an unambiguous evidence for the presence of glucuronides or sulfate conjugates.

In a next step, parent compound and metabolites were extracted from the different matrices (milk, liver, kidney, muscle and fat) and purified before chromatographic

analysis (HPLC and HPTLC). Identification of metabolites was done in the main part by co-chromatography using the urine metabolites identified in the first step as reference compounds or using reference compounds isolated in other studies. In some cases, metabolites were identified by comparing the retention behaviour of the metabolite of interest and of a reference compound using two independent chromatographic methods with different selectivity.
The metabolic patterns of the goat matrices were of a similar complexity as the pattern of the urine sample. Therefore, all extracts were additionally treated with boiling hydrochloric acid. Under the conditions chosen, glucuronides and other conjugates were cleaved and the compounds with diene structure were converted into metabolites with known aromatic structure. As in the urine sample, a simplified metabolic pattern resulted. The five relevant compounds formed, were used for additional confirmation of the assignments made in the profiles before the acid treatment. Based on the additional information of the hydrolysis, a large number of minor metabolites (partly summarized in metabolic groups) could be traced back to a few basic structures. A point to consider was that the metabolite F64M1-glucuronide was cleaved only partly during acidic hydrolysis and was still detected after the treatment.

Quantification of all relevant metabolites was conducted by integrating the ^{14}C-signals in the HPLC-chromatograms of the extracts used for metabolic profiling.
The amounts of the F64M1 and the metabolites - expressed as % of the total radioactive residue (% of TRR) and as residue concentration (equivalent concentration [µg/g]) - found in milk and in edible tissues of the lactating goat are given in Table 1.

The sum of all identified and characterized metabolites represented approximately the following percentages of the total radioactive residue: 89% in milk, 71% in liver, 86% in kidney, 77% in muscle and 84% in fat. The extraction efficiencies, corresponding to the first extraction step (3 to 4 extractions with a mixture of acetonitrile/water or for the milk sample, 3 extractions with methanol, respectively) were as follows: approx. 94% for milk, approx. 81 % for liver, approx. 97% for kidney, approx. 82% for muscle and approx. 87% for fat.

The metabolic pathway of F64M1 in lactating goat was characterized by the following main reactions:

- Conjugation of the parent compound with glucuronic acid resulted in the metabolite F64M1-glucuronide.
- Hydroxylation of the parent compound resulted for the main part in the formation of the isomers 3-hydroxy-F64M1 and 4-hydroxy-F64M1. Conjugation with glucuronic acid followed partly.
- Further hydroxylation of the chlorohydroxyphenyl moiety led to 4,5-dihydroxy-F64M1and another dihydroxy- F64M1 isomer. Conjugation with glucuronic acid followed partly. According to NMR spectroscopy, conjugation with glucuronic acid could take place at each of the aromatic hydroxy groups – an unambiguous assignment to the position of conjugation was not possible.
- Oxidation of the chlorophenyl moiety of the parent compound F64M1 led partly to de-aromatisation. As a consequence, F64M1-dihydroxy-dienes were detected. To some extent, the oxidation was followed by conjugation with glucuronic acid.
- There was also some evidence for the occurrence of hydroxy-methoxyF64M1, formed as intermediate before conjugation with glucuronic acid. Sulfate conjugates of hydroxy-desthio-F64M1, dihydroxy-desthio- F64M1 and hydroxy-methoxy- F64M1 were detected exclusively in the milk sample.

Based on these results, the authors believe that the metabolism of F64M1in lactating goat is adequately understood.

Table 1: Summary of the concentration of substances found in goat tissues

	Milk		**Liver**		**Kidney**		**Muscle**		**Fat**	
TRR [µglg] **(combustion analysis)**		**0.286**		**18.421**		**18.975**		**0.266**		**0.231**
Compound (F64M1-)	% of TRR	equiv. conc. [µglg]	% of TRR	equiv. conc. [µglg]	% of TRR	equiv. conc. [µglg]	% of TRR	equiv. conc. [µglg]	% of TRR	equiv. conc [µglg].
F64M2(glucuronide)	2.35	0.007	1.98	0.366	7.28	1.382	8.17	0.022	7.83	0.018
F64M3-(glucuronide)	3.01	0.009	3.81	0.703	13.75	2.610	12.77	0.034	15.02	0.035
F64M2	2.44	0.007	1.16#	*0.213*	*1.62*	0.307	3.64	0.010	4.31	*0.010*
F64M3	*3.04*	*0.009*					7.11	0.019		
-dihydroxy-F64M1_ glucuronide* (							*5.88*	*0.016*		
Mixture of dihydroxy- and hydroxyl F64M1 glucuronide*	*2.63*	*0.008*	*2.74*	*0.504*	*4.92*	0.933			*5.30*	*0.012*
-4-hydroxy-F64M1 glucuronide	*5.11#*	*0.015*	*2.77#*	*0.511*	7.32#	1.388	5.84	*0.016*	4.68	*0.011*
-hydroxy-methoxy-F64M1-glucuronide							*5.20*	*0.014*		
F64M1-glucuronide	6.22	0.018			24.07	4.567	3.57'+	0.009	4.17##	0.010
dihydroxy-F64M1(11)*	*1.56*	*0.004*	2.15[1]	0.396			1.72'	0.005	5.36'	0.012
4,5-dihydroxy-F64M1	*1.38*	*0.004*	4.76xx	0.878			2.80	0.007		
3-hydroxy-F64M1			0.96	0.178	1.22	0.231	4.80	0.013		
4-hydroxy-F64M1			8.37	1.542	4.06	0.770	3.03	0.008	14.55	0.034
sulfate conjugates**	44.03	0.126								
F64M1			31.18	5.744	7.66	1.454	1.76	0.005	13.88	0.032
sum identified	**58.06**	**0.166**	**53.23**	**9.805**	**58.04**	11.013	**49.37**	**0.131**	**60.81**	**0.141**
sum tentatively iididident.	***13.71***	***0.039***	***6.66***	***1.227***	***13.85***	***2.628***	***16.91***	***0.045***	***14.29***	***0.033***
sum characterized	**16.74**	**0.048**	**10.99**	**2.024**	**14.50**	**2.750**	**10.73**	**0.029**	**9.04**	**0.021**
subtotal	**88.51**	**0.253**	**70.88**	**13.057**	**86.38**	**16.392**	**77.01**	**0.205**	**84.14**	**0.195**
solids	5.88	0.017	18.45	3.398	2.80	0.531	11.16	0.030	11.45	0.026
not analysed	5.61	0.016	10.67	1.965	10.82	2.053	11.83	0.031	4.41	0.010
balance	**100.00**	0.286	**100.00**	**18.421**	**1100.00**	**18.97511**	**100.00**	**0.266**	**1100.00**	**0.231**

D: diastereomer I: isomer

* according to NMR spectroscopy, 3,4-; 5,6- and 3,6-position of the hydroxy groups was possible

** sulfate conjugates of dihydroxy-F64M1, hydroxy-methoxy-F64M1 and hydroxy-F64M1

\# co-elution with F64M1-4,5-dihydroxy-dienyl-glucuronide is possible

co-elution with F64M1-hydroxy-methoxy-desthio is possible

co-elution with F64M1-alpha-hydroxy-desthio is possible

co-elution with F64M1dihydroxy-desthio-gluc. and hydroxy-F64M1-gluc. is possible

\## co-elution with F64M1-glucuronide is plausible

Residues of metabolites identified tentatively only are printed in *italics.*

Exercise 3.4: Metabolism of F64 in spring wheat[29]

Use patterns of the compound in spring wheat ranges from 100 g a. i/ha to 200 g a.i./ha with 1 or 2 applications, in winter wheat 2 -3 applications with 100-200 g. The product is applied in EC 250 or EC 450 formulations.

The metabolism of F64 was investigated using [phenyl-UL-^{14}C]- F64 referred to as phenyl-label, and [3,5-triazole-^{14}C]-labelled F64 referred to as triazole-label.

The objective of this study was to determine the overall fate of [phenyl-UL^{14}C]-F64 (formulated as EC 250) and the terminal residues in spring wheat following two foliar applications at the growth stages tillering and flowering. The total amount applied corresponded to the maximum annual field rate of 400 g a.i./ha.

The list of abbreviations and symbols are given on page 5 on exercises 3.1.

Tasks:

(a) Validate the study conditions concerning:
- Study material, test system, application conditions of test material, sampling and analysis, etc.
- Identification and characterization of metabolites
- Completeness of information provided in the summary of the study.

(b) Identify major residue components to be considered for definition of residues

For self study:
1 Read the extract of complete study report first and prepare the summary of the report taking into account the checklist (section 3.1) for essential information.
2 Compare your summary with that given in Exercise 3.1
3 Compare the metabolism of the active substance (F64) and its main plan metabolite (F64M1) in spring wheat

Solution:

Use the checklist provided in the lecture on metabolism studies. Compare your answers given in the solutions for exercises 3.1 and 3.3

[29] The material presented in this section is taken with the permission of the manufacturer from a confidential report.

3.4.1 Summary of metabolism of F64 in spring wheat

In this study the metabolic fate of the fungicide F64 was investigated after two spray applications in spring wheat. Wheat was treated at growth stages tillering (code 32-37 according to BBCH) and full flowering (BBCH code 65) with [phenyl-UL-^{14}C]F64 250EC; each treatment at a rate of 200g active ingredient (a.i.)/ha, corresponding to the maximum annual field rate recommended for wheat.

Total radioactive residue (TRR) levels in forage, hay, straw and grain were 10.45, 8.90, 26.74 and 0.08 mg/kg (a.i. equivalents), respectively. Identified metabolites in the raw agricultural commodities (RACs) accounted for 73.1 % of the TRR in forage, 64.7 % of the TRR in hay, 66.2 % of the TRR in straw and 33.7 % of the TRR in grain.

Unidentified metabolites were at least characterised based on their extraction and partition behaviour. In addition hydrolysis experiments with dioxan/HC1 were performed for hay and straw.

Besides single metabolites of both aqueous phases and the whole aqueous phase 2 (obtained after partitioning with dichloromethane and n-butanol) were characterised after enzymatic hydrolysis yielding hydroxy-F64M1-isomers as aglycons.

While unidentified residues represented only 3.6 % of the TRR in forage, 3.1 % in straw and 2.0 % in hay (the latter after hydrolysis with dioxan/HC19:1), 31.6 % could not be extracted or solubilised out of the grain matrix by conventional or by exhaustive (ASE) extraction methods. Therefore, in a second experiment, the solids 1 of grain were treated with the enzyme *diastase,* which solubilised 14.7 % of the TRR, but still leaving 17.5 % of the TRR unextracted. The approach hydrolysing solids with dioxan/HCI (9:1) left no nonextractable residues in grain.

The active ingredient (a.i.) F64 was intensively metabolised. The proposed metabolic reactions involved:

* oxidation and loss of sulphur, resulting in the main metabolite F64M1
* hydroxylation of the chlorobenzyl methylene C-atom and hydroxylation of the chlorobenzyl ring at position 3, 4 and 6 of F64M1
* conjugation of all these hydroxylated metabolites and to a minimal extend of F64M1

As minor reactions of degradation occurred:

* exchange of sulphur against oxygen
* elimination of the triazol moiety and conjugation of the benzylpropyldiol

Hence major metabolites, identified in the different RACs, included F64M, as main metabolite and its derivatives alpha-hydroxy-F64M1 and at least two isomers (3- and 4-) hydroxyl F64M1. Two other metabolites of F64 were identified as F64 sulfonic acid and F64-triazolinone. The percentages of TRR and mg/kg equivalents a.i. of these major metabolites are given in the table below.

nt compound/ bolite	orage		hay		straw		grain	
	kg		kg		kg		%	kg
arent compound)	3.3	0.35	2.6	0.24.	3.7	0.98	1.0	<0.01
1	35.4	3.70	18.5	1.64	22.3	5.95	15.9	0.014
- F64M1	4.5	0.47	9.4	0.83	5.8	1.56	2.8	<0.01
- F64M1	2.4	0.25	8.5	0.75	2.9	0.76	<1.0*	<0.01
- F64M1	1.2	0.13	6.7	0.60	2.7	0.72	<1.0*	<0.01
6-OH- F64M1	1.1	0.12	1.2	0.11	1.2	0.32	n.d.	<0.01
riazolinone	6.9	0.71	5.1	0.46	6.1	1.64	1.3	<0.01
ulfonic acid	7.1	0.75	3.3	0.29	8.4	2.24	n.d.	n.d.
cosides of the OH-desthio isomers	8.6	0.91	2.6	0.24	7.3	1.96	8.4	<0.01

n.d.: not detected; *trace amounts; no discrimination between isomers was possible

3.4.2 Study of the F64 metabolism in spring wheat

(selected parts of the complete report)

Test facilities

The spring wheat plants were grown in the vegetation area (building 6682) of the Institute for Metabolism Research and Residue Analysis.

Non-radiolabelled test compound

The non-radiolabelled F64 used as reference compound and for dilution of the radiolabelled substance was obtained from xxxx:

Company develop. name: F64
Chemical name:
CAS-No.:
Batches no.:
Empirical formula:
Chemical purity: 99.8 %
Identity: ^{1}H-NMR spectrum
Molar mass: 344.3 g/mole
Aggregation state: colourless powder
Date of certificates: Jan. 07, 1997; Jan 22, 1997, respectively

Radiolabelled test compound

The radiolabelled parent test compound [phenyl-UL-^{14}C]-F64 was synthesised by XY in the Isotope laboratory of YY, Germany. The structural formula and the position of the radiolabel (*) are shown below (*not included*):

Chemical identity: [phenyl-UL-^{14}C]F64
Origin:
Storage no.:
Specific radioactivity: 2.97 MBq/mg (80.3 µCi/mg)
Consistency: solid, vacuum dried

Purity check:

LiChrospher 60 RP-select B, 5µm, 125 x 4 mm, flow: 1.5 ml/min., 5 min. 0.2 % H_3PO_4 followed by a linear gradient to 100 % acetonitrile in 30 min.,

Radiochemical purity: > 99 % by radio – HPLC
Chemical purity: > 99 % by HPLC(UV at 210 nm)
Date of certificate: May 05, 1997

The active ingredient was formulated in the laboratory Dr. W. Ecker. Further details are given below:

Appiication no.	**1 (May 27, 1997)**	**2 (June 13, 1997)**
Sample ID - no.	ECW 11160-A	ECW 11160-B
Type of formulation	EC 250 (liquid)	EC 250 (liquid)
Amount of formulation	88.07 mg	88.07 mg
Concentration of a.i. in the formulation	25.0 %	25.0 %
Specific radioactivity (a.i.)	2.97 MBq/mg (80.3 µCi/mg)	2.97 MBq/mg (80.3 µCi/mg)
Total radioactivity	65.39 MBq	65.39 MBq
Radiochem. purity of a.i. used for the formulation (ID no.)	> 99 % (HPLC) (THS 4529)	> 99 % (HPLC) (THS 4529)

The active ingredient was mixed with the blank formulation using a ball mill. The conditions necessary to ensure that the formulation corresponded to the commercially available formulation were determined in preliminary experiments. The radiochemical purity of the formulated a.i. was determined by HPLC (lab. Dr. Ecker). The identity of the active ingredient used for the formulation (ECW11160A+B) was confirmed by MS – and ^{1}HNMR spectroscopy.

Test systems

Planting containers and soil

The standardised planting containers (surface area 1 m^2) were filled a sandy loam soil. At the begin of the study, the container was labelled with the study number and with a radioactivity symbol.

Plant

Species: Spring wheat *(Triticum aestivum)*
Cultivar: Kadett

Wheat was sown in 5 rows, seeds placed in shallow furrows at approximately 1 cm intervals corresponding to 480 seeds/m^2. The furrows were covered after sowing with a thin layer (0.5 cm) of soil.

Details of the plant development, plant protection and fertilisation measures were recorded as were climatic details and environmental conditions (Appendix IV).

The description of the developmental stages of the wheat plants were taken from the extended BBCH scale

Methods

Application of the test compound

Formulated [phenyl-UL-^{14}C]F64 was prepared and applied to wheat plants at beginning tillering (growth stage 32 of the BBCH code) and at full flowering (BBCH 65). The amount of F64 to be applied was based on a field application rate of 200 g a.i./ha for each application plus a 10 % excess compensating losses during application. Just before application, the formulated test compound (ECW 11160-A or B, respectively; 22.0 mg a.i.; 65.39 MBq, each) was diluted with distilled water (Milli - Q) to make up the specifc spray mixtures. For application a computer controlled track sprayer with a fiat fan nozzle was used. After spraying, the plastic protection was removed and rinsed with methanol as was the spray equipment. As a result of the radioactivity measured in the wash solutions, the actually applied radioactivity was calculated as given below:

cation no.	**y 27, 1997)**	**e 13, 1997)**
le ID code	01 CA	05CA
of formulation	0 (liquid)	0 (liquid)
nt of a.i.	ng	ng
fic radioactivity (a.i.)	MBq/mg	MBq/mg
radioactivity applied	MBq	MBq
nt of water used for application	l	l

A small aliquot of each application solution was used to confirm the identity and check the stability of the test substance before and after (washing solutions) application using HPLC-chromatography.

Sampling, harvest, processing and storage

Forage (early hay stage)

In order to collect plant material, having obtained two applications as proposed in agricultural practice, a forage sample was first sampled 6 days after the second application (June 19, 1997). This still green plant material corresponded to growth stage 69 (early hay stage) of the BBCH code. Wheat plants were removed by cutting each plant off at soil surface level. The harvested plants were combined, cut into approximately l cm pieces, and weighed. The plants were homogenised by freezing the pieces in liquid nitrogen and grinding the frozen material using an Ultra-Turrax T 50 (Janke und Kunkel). Aliquots of 10 up to 112 g plant material were stored in a freezer at -20 °C.

Hay

A hay sample was collected 26 days after the second application (July 9, 1997) at early dough state (BBCH 83). Plants were sampled and stored as described for forage (4.2.1).

Straw and Grain

Mature ears were harvested 48 days after the second application (July 31, 1997) by cutting them from the stalks using scissors. The remaining straw was cut at soil surface level and processed as described for forage (4.2.1).

Seeds were picked by hand, weighed, frozen in liquid nitrogen and homogenised with the Ultra Turrax T50. The remaining chaff was combined with the straw sample and processed as described above.

Extraction and fractionation

For each extraction 1 mg cysteine-hydrochloride /ml solvent was added to prevent oxidative decomposition of F64 during extraction procedures and while concentrating samples to small volumes at 35 °C under vacuum using a rotary evaporator. To the dichloromethane phases, small volumes of acetonitrile (ca. 15 ml) were additionally added before concentration. Before analysis, the samples were centrifuged to remove precipitated material (i.e. cysteine-hydrochloride). Storage conditions of extracts were ca. + 4°C (refrigerators) and ca. - 20°C in freezers for longtime storage. The identity of the samples was guaranteed by a special code number (i.e. PO4004ES).

Exhaustive extraction of solids 1 (remaining after the conventional extraction) was performed using accelerated solvent extraction (ASE).

A first extraction experiment including subsequent TLC-analysis for a profiling of the raw extracts was performed with small aliquots of the four RACs (ID codes starting with: PO4002..) within a month after each sampling date. A comparison of the metabolic pattern of these raw extracts is depicted in Figure 1. These extracts were also used for storage stability investigations.

Forage

For the *metabolism experiment* an aliquot (20 g) of the homogenised (see 4.2.1) wheat forage was extracted 3 x with acetonitrile/water (80/20 v/v; each extraction ca. 100 ml) with the Ultra Turrax homogenisor. The extracts were obtained by vacuum filtration of the homogenates through a filter (type: black ribbon, Schleicher und Schuell, Germany) topped with 10 g

Celite® (Merck, Darmstadt, Germany). A 10 ml aliquot of the combined extracts (PO4008EF) was kept as retain sample and for direct investigation by chromatographic methods.

Exhaustive extraction of solids 1 applying ASE (Accelerated Solvent Extraction):

A representative aliquot of solids 1 including Celite® (Merck, Darmstadt, Germany) were filled into 33 ml ASE extraction cells and extracted two times at 50 and 100 °C (two cycles, each) with an ASE 200 extractor (Dionex, Idstein, Germany).

The combined acetonitrile/water extracts (300m1, PO4008EF) were concentrated under vacuum (35 °C) to the aqueous remainder (61 ml).

This aqueous remainder was partitioned with dichloromethane (3 x 60 ml) leaving the aqueous phase (60 ml, PO4008HF). The dichloromethane solution was concentrated yielding the concentrated dichloromehtane phase (PO4008IF).

The filtered solids 1 were air dried. Triplicate aliquots of each phase were radioassayed by LSC. Five aliqouts of the solids 1 were combusted and trapped $^{14}CO_2$ was radiaoassayed as well.

The extraction procedure is depicted in Figure 2 (*not included*). Quantitative details are given in Appendix V.

Hay

The extraction scheme used for hay is shown in Figure 3 (*not included*). A 20 g aliquot of the hay homogenate was macerated 3x with the Ultra Turrax applying acetonitrile/ water (80/20 v/v; ca. 250 ml for each extraction).

Exhaustive extraction using ASE was done with premixed solids 1 (2:1 with Celite®)

The ASE extracts were combined with the crude acetonitrile extracts concentrated to the

aqueous remainder and partitioned with dichloromethane; following the processing procedure as described for forage.

Acidic hydrolysis of solids 2 using dioxan/HCl (PO4006CH)

An aliquot of 5g of solids 2 was further extracted using dioxan/2N HC1 9:1 (v:v, 45 ml) under reflux for 2 hours. The suspension was filtered by suction and the remaining residues were washed with 60 ml of water. The concentrated solutions were radioassayed by LSC and chromatographed on silica-TLC. Five aliquots of the remaining solids 3 were lyophylised and combusted measuring radioactivity as trapped $^{14}CO_2$ in a scintillation cocktail.

Quantitative data are given in Appendix VII (*not included*).

Straw

A 20 g aliquot of the homogenised straw sample (4.2.3) was extracted 3 times with 200 ml acetonitrile/water (80/20 v/v).

Exhaustive extraction using ASE was done with premixed solids 1 (2:1 with Celite®) and the extraction conditions applied as described for hay. The ASE extracts were combined with the crude acetonitrile extracts concentrated to the aqueous remainder and partitioned with dichloromethane; following the processing procedure as described for forage.

Acidic hydrolysis of solids 2 using dioxan/HCl

An aliquot of 5g of solids 2 was further extracted using dioxan/2N HC1 9:1 (v:v, 45 ml) under reflux for 2 hours. The suspension was filtered by suction and the remaining residues were washed with 60 ml of water. The concentrated solutions (sample IDs for dioxan/HCl: PO4037CS and for aqueous phase: PO4037DS) were radioassayed by LSC and chromatographed on silica-TLC. Aliquots of the remaining solids 3 (PO4037DS) were lyophylised and combusted measuring radioactivity as trapped $^{14}CO_2$.

For isolation and identification of metabolites a second extraction was performed using 200 g homogenised wheat straw. The straw was soaked overnight in water (refrigerator + 4 °C) and then extracted 4 x with 80 % acetonitrile and then processed as described for forage. The aqueous remainder was first partitioned into dichloromethane (3 times, yielding after concentration the organic phase I; PO4004HS) and then in a second step in nbutanol (3 times, yielding the organic phase II, PO4004KS) leaving the aqueous phase 2 (PO4004JS).

Grain

The extraction scheme used for grain is shown in Figure 5; quantitative data is given in Appendix XII. A 50 g aliquot (PO4001CG) of the grain homogenate (4.2.3) was extracted 3 x with a mixture of acetonitrile/water (80/20 v/v; each extraction performed with 150 ml) following the extraction procedure described for forage.

The combined filtrates were concentrated to the aqueous remainder (81 ml) and partitioned with dichloromethane (3 x 80 ml) resulting in a concentrated organic fraction PO4009IG and the corresponding aqueous phase PO4009JG.

The filtered solids 1 were air dried and radioassayed (PO4009DG).

Exhaustive extraction by ASE

An aliquot of 22 g of the solids 1 (PO4009DG) was mixed with 11 g of Celite® (Merck, Darmstadt, Germany) and submitted to exhaustive extraction by ASE applying the extraction conditions as described for hay.

Again remaining solids 2 (PO401OCG) were air dried and radioactivity was measured after combustion by LSC.

Enzymatic digestion using the enzyme Diastase (a-Amylase)

A third aliquot (5 g) of solids 1 was extracted by a repeated enzymatic hydrolysis procedure applying the enzyme *diastase.* Diastase (100mg, Merck no. *3604)* was dissolved in 55 ml citrate/NaOH-buffer (pH 6, Fixanal; Riedel de Haen, no. 38745) containing 10 mg NaN_3, incubated with the solids, and stirred for nine days in a closed glass bulb at room temperature. At days 2, 3, 4, 7 and 9 the suspension was filtered by suction and the dissolved amount of former solid residue 1 was determined by weighing the remaining solids. The undissolved residue was again incubated with freshly prepared enzyme solution. The procedure was terminated after 5 extraction repetitions, the remaining solids 3 having decreased to 0.81 g, which were completely combusted (PO4012BG).

The aqueous extracts (buffer solutions) were combined (PO4012AG) and aliquots of the solubles and the solids 3 were taken for radioactivity measurement.

Acidic hydrolysis of solids 1 using dioxan/HCl (PO4009DG)

A second aliquot of 5g of solids 1 PO4009DG was further extracted using dioxan/2N HCl 9:1 (v:v, 44 ml) under reflux for 2 hours (PO4011AG). The suspension was filtered by suction and the remaining residues could be dissolved in water. Therefore no solids remained after this hydrolysis (PO4011BG).

Analyses of radioactive residues

Measurement of radioactivity

The measurement of the radioactivity in the various samples was carried out by liquid scintillation counting (LSC). For this purpose, the volume of the entire solution was determined and the radioactivity of an aliquot of the solution was measured in triplicate. Extracted solid samples containing radioactivity were combusted in an oxygen atmosphere using a "Harvey OX 500" oxidiser (Zinsser, Germany); the released "CO_2 was trapped in an alkaline scintillation cocktail and radioassayed by LSC

Results and Discussion

Determination of the total radioactive residues (TRR)

The total radioactive residues (TRRs) in forage, hay, straw and grain of wheat were determined by summation of the extracted radioactivity in the acetonitrile/water extracts plus radioactivity remaining in the solids 1 and were expressed as mg/kg parent compound equivalents (Table II).

Wheat forage (early hay stage) collected 65 days after sowing (6 days after the second application) had a total radioactive residue (TRR) of 10.45 mg/kg (Table III, Appendix V). Hay collected 79 days after sowing and 26 days post-treatment had a TRR of 8.90 mg/kg (Table V, Appendix VII). Straw and grain were harvested 95 days after sowing and 48 days after the second treatment had TRRs of 26.74 mg/kg and 0.08 mg/kg, respectively (Tables VII + IX, Appendices IX + XII). (Tables are *not included*)

These values reveal that radioactivity in grain —the commodity for human consumption- was rather low compared to other plant parts (RACs) taken.

Extraction, distribution and quantitation of radioactivity

The different plant samples were successively extracted with a mixture of acetonitrile/water (80/20 v/v) at room temperature (conventional extraction), followed by exhaustive extraction using accelerated solvent extraction (ASE) at 50 and 100 °C (each with 2 cycles). To all solutions cysteine hydrochloride was added in excess as an SH - group protecting reagent. Following extraction, radioactivity was partitioned with organic solvents to facilitate the characterisation of metabolites and was quantified in appropriate chromatographic systems. The metabolite patterns proved to be quite identical in all commodities.

The distribution of the radioactivity and the TRRs are summarised in Tables III, V, VII and IX (*not included*).

Metabolite identification

Metabolites of [phenyl-UL-^{14}C]-F64 were isolated from wheat straw using silica gel columns and micropreparative HPLC (sample IDs PO40..S; for extraction scheme, see Figures 16a + b). The metabolites were identified by TLC-cochromatography with authentic reference compounds and by comparison of their HPLC retention times to those of known reference compounds. Structures of these isolated metabolites of straw were confirmed by LC/MS and LC/MS/MS experiments and —if possible- by NMR.

As metabolic patterns in the four RACs were nearly identical, metabolites in forage, hay and grain samples were identified by comparison of their chromatographic characteristics to either authentic reference compounds or the compounds isolated and identified from straw or by co-chromatography of relevant extracts using radio-TLC and HPLC with radioactivity detectors.

Conclusion

The metabolism of the fungicide **F64** was investigated in spring wheat after two spray applications (each at 200 g a.i. / ha). The total radioactive residues (TRRs) in forage, hay, straw and grain amounted to 10.45, 8.90, 26.74 and 0.08 mg/kg parent compound equivalents, respectively. The vast majority of the TRR (forage: 96.5 %; hay: 98.0 %; straw: 96.9 % and grain: 67.5 %) was extracted. For grain incubation with the enzyme *diastase* solubilised another 14.7 % of the TRR.

The parent compound [phenyl-UL-^{14}C]F64 was intensively metabolised in spring wheat. While extensive, the degradation was well defined. It was proposed, that the sulphur was oxidised (sulfonic acid) and eliminated, resulting in the main metabolite F64M1. This metabolite was further hydroxylated in the chlorophenyl ring to the 3-, 4-, and 6-Hydroxy-F64M1 isomers, which were further transformed to different conjugates. Similarly, alpha-hydroxy-F64M1 was formed by hydroxylation of the chlorobenzyl methylene group. Hence hydroxylation followed by conjugation with glucose and subsequent storage of the conjugates as well as higher conjugation was a major metabolic route within the plant.

To a smaller extend the F64 triazolinone was formed by oxidation of the parent compound.

As a third route of metabolisation seen in straw and hay only, benzylpropyldiol, a metabolite of F64, which has lost its triazole moiety and the correspondent glucoside were detected. Probably benzylpropyldiol was taken up by the roots and was translocated as glucoside into the shoots.

For identification and quantitation of the metabolites radio-TLC and radio-HPLC was used. The structure of the major metabolite F64M1, and its derivatives *alpha-,* 3-, and 4-hydroxy-F64M1, respectively, were confirmed by LC/MS, LC/MS/MS and NMR

experiments. LC/MS/MS experiments were also done for the respective B-D-glucosides of these hydroxy-desthio metabolites.

In addition two metabolites of F64 (F64 sulfonic acid and F64 triazolinone) were also identified by spectroscopic methods.

On the basis of the nature and amount of metabolites found in the respective extracts a metabolic pathway was proposed.

Exercise 5.1 Checking the efficiency of extraction

Samples with incurred ^{14}C residues from the metabolism studies were re-analysed by a 'regulatory method'. The results obtained were

Substrate	Metabolism ref	Residue measured, mg/kg	
		Metabolism analysis	'Regulatory' method
Pear	A98041, 198-96	0.20	0.15-0.18
Maize grain 3/	PSA41PR2, 19/97	0.006	<0.01
Maize fodder	PSA41PR2, 19/97	0.047	0.02-0.03
Cucumber	A98048, 282-95	0.10	0.04-0.05
Cucumber	A98048, 282-95	0.044	0.02-0.04
Goat meat	AM03027	1.0	0.56-0.79
Goat milk	AM03027	0.37	0.06-0.09

Task:

1. Calculate the efficiency of extraction
2. Evaluate the results and consider if the method is suitable for regulatory purposes
3. How the residue data obtained from supervised trials can be used for risk assessment purposes.

Exercise 5.2: Evaluation of supervised trials conditions in papaya: Checking validity of sampling and sample handling procedures.

Introduction

Supervised trials were conducted with the pesticide active substance 'acar' in the United States.

The US GAP for tropical fruits is summarized hereunder.

Crop	Country	Formulation (g ai/l or g ai/kg)	Application				PHI Days
			kg ai/ha	Water, l/ha	kg ai/hl	No.	
Tropical fruits[3]	USA	480 SC/ 500 WP	0.40-0.56	468	0.09- 0.12	1	1

[3]: Guava, Lychee, Papaya, Star apple, Black sapote, Mango, Sapodilla, Canistel, Mamey, Longan, Spanish lime, Rambutan, Pulasan, Fejioa, Jaboticaba, Wax jambu, Starfruit, Passionfruit, Acerola

Tasks:

- Taking into account the basic requirements for sampling sample processing and analysis validate the procedures applied

Extract of Trial report on papaya[30]

1. **Study Site Information** Each field trial consisted of one untreated control plot and one treated plot. Treated plot sizes ranged from 924 to 1344 ft^2. Common cultural practices were followed to maintain the crop, and additional maintenance pesticides and fertilizers were used at the sites to produce a commercial quality crop. Trial site conditions and use pattern data are summarized in Table 1 and Table.2, respectively. Appendix 1 provides additional information about the sites.

2. **Sample Handling and Preparation**

At each trial, samples of mature to half ripe papaya were collected from each plot 1 day after the final application (duplicate samples were collected in HI). Each fruit was cut into fractions (1/8 to 1/2) to reduce sample size. Each sample weighed at least 4 lb and was collected in a manner to assure a representative, impartial sample. Samples were stored frozen within 6 hours of collection. All samples were shipped frozen via Federal Express or Airborne Express to the analytical laboratory, for extraction and analysis.
Upon arrival at the analytical laboratory, the samples were assigned unique sample numbers and stored frozen at -30.4 to -15.8 °C until sample preparation. The samples were smashed

[30] This part of the report was used as an exercise with the permission of the data owner. Appendices of the report are not copied here.

into smaller pieces and homogenized with dry ice in a food processor, and returned to frozen storage until extraction for analysis.

3. Analytical Method

Samples were analyzed for combined residues of acar and acar metabolite using the working method, "Determination of Combined acar and acar metabolite Residues in Papaya". Minor modifications were made to improve the performance of the method. (See page 11 of the analytical summary report in Appendix 2; a copy of the method can be found in Appendix III of Appendix VI of the analytical summary report.)

Briefly, residues of acar and acar metabolite were extracted from homogenized papaya by blending twice with 100 mL extraction solvent. The extracts were combined and filtered into a 250-mL vacuum flask and transferred to a 250-mL volumetric flask. The volume of the sample was brought to 250 mL with acetonitrile. A 50-mL aliquot of the sample was partitioned twice with hexane. The acetonitrile phase was then conditioned with methylene chloride and 2% aqueous sodium sulfate. The methylene phase from the partition was evaporated to near dryness, and the extract was diluted with sample dilution solvent. After an incubation period of at least 2 hours, the samples were analyzed for acar by reversed-phase HPLC with oxidative coulometric electrochemical detection. The ascorbic acid added to the sample ensured that residues of acar metabolite were converted to acar and were, therefore, in the oxidative mode.
The lowest level of method validation (LLMV) in this study was 0.01 ppm for each analyte. Based on recoveries of samples fortified at the LLMV, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 0.12 ppm and 0.37 ppm, respectively, for acar. For those fortified with acar metabolite, the LOD and LOQ were calculated as 0.0012 ppm and 0.0037 ppm, respectively.
Treated samples were analyzed within 1 day of extraction. Analytical sets typically consisted of bracketing calibration standards, unfortified controls, fortified controls, reagent blanks, and treated samples. The analytical standard solutions were stored frozen at between -20 and -8 C. Appendix 4 contains OECD GLP Certificates of Analysis for the analytical reference standards.

The samples were analysed within 8-11 days after sampling.

Summary of recovery of acar and its metabolites in papaya

Analyte	Fortification (mg/kg)	No. of tests (n)	Recoveries (%)	Mean (%)	RSD (%)
Acar	0.01	3	70.4, 70.8, 70.2	70.5	0.43
	0.1	3	94.7, 95.3, 93.2	94.4	1.2
	1	3	102, 97.9, 103	101	2.8
	0.01- 1	**9**	**70.2- 103**	**88.6**	**16**
Acar metabolite	0.01	3	91.5, 92.9, 81.6	88.7	6.9
	0.1	3	70.1, 70.3, 74.1	71.5	3.1
	1	3	80.2, 78.9, 78.1	79.1	1.4
	0.01- 1	**9**	**70.1- 92.9**	**79.7**	**10**

Table 1 Trial Site Conditions

Trial ID (City, State)	Trial Start Year	Soil Characteristics				Meteorological Data	
		Type	%OM	pH	C (meq/100 g)	Overall Monthly Rainfall Rangem (in.)	Overall mperature Range (°F)
03-FL19 (Homestead, FL)	2003	Krome very Gravelly Loam	3–10	7.4–8.4		76 (May) 31 (Jun)	-90.8 (May) -91.4 (Jun)
03-HI01 (Haleiwa, HI)	2003	Silty clay	2.74	5.3		.40 (May) .60 (Jun)	5–97.18 (May) –9L51 (Jun)
03-HIO2 (Keaau, HI)	2003	Extremely stony muck	7.29	4.8		–1.16 (Jun) –1.08 (Jul)	4–88.56 (Jun) 4–87.22 (Jul)

Irrigation was used to supplement as needed. Temperature and precipitation data were reported to be within normal parameters at all trial Bites.

Table 2 Study Use Pattern

			Application					
Trial ID (City, State)	Trial Start Year	EP[1]	Method/ Timing	GPA[2]	e (lb ai/A)	RTI[3] (days)	Total Rate (Ib ai/A)	Tank Mix Adjuvants
03-FL19 (Homestead, FL)	2003	Acarmite® 50WP	Foliar directed/ Fruiting, 22 days prior to harvest	149.71		—		PLYACO
			Foliar directed/ Fruiting, 1 day prior to harvest	148.27		21	1.02	PLYACO
03-HI01 (Haleiwa, HI)	2003	Acarmite® 50WP	Foliar directed/ Fruiting, 22 days prior to harvest	50.67		—		Latron B-1956
			Foliar directed/ Fruiting, 1 day prior to harvest	50.43		21	1.02	Latron B-1956
03-HIO2 (Keaau, HI)	2003	Acarmite® 50WP	Foliar directed/ Fruiting, 23 days prior to harvest	102.87	0.52	—		Latron B-1956
			Foliar directed/ Fruiting, 1 day prior to harvest	103.14	0.52	22	1.04	Latron B-1956

EP = End use product.

GPA = Gallons per acre.

RTI = Retreatment interval.

Only apPlicable for cotton commodities.

RESULTS AND DISCUSSION

Three papaya trials were conducted in Florida and Hawaii, representing NAFTA growing region 13. The number of trials and geographic representation are adequate for papaya. The papayas were grown in very gravely loam, silty clay, and extremely stony muck; no unusual environmental conditions were reported in any of the trials.

The trials were conducted to collect data on the residues of acar, the active ingredient in Acarmite® 50WP. Two foliar applications of approximately 0.5 lb ai/A each were made 21 to 22 days apart and timed so that samples could be collected 1 day after the final application. A total of approximately 1.0 lb ai/A was applied to each treated plot.

Control samples were fortified separately with acar and acar metabolite and analyzed concurrently with field-treated samples. Method validation was waived because the analytical facility provided an OECD GLP-compliant report of their validation study, which is included in Appendix 2. Table C.1 summarizes the recovery data. Residues below the LLMV were observed in each control sample, and recoveries were corrected for background if the residues were greater than half the LLMV (> 0.005 ppm). Fortification levels ranged from 0.01 to 2.0 ppm. Recoveries of samples fortified with acar at the LLMV ranged from 93.06 to 118.2%, with an average recovery of 107 ± 10% (n = 6). The average recovery of all acar fortifications was 102 ± 11% (n = 9). For acar metabolite, recoveries of samples fortified at the LLMV ranged from 111.2 to 119.2%, with an average recovery of 115 ± 3% (n = 6). The average recovery of all acar metabolite fortifications was 100 ± 18% (n = 11).
The LOD and LOQ were statistically calculated as 0.12 ppm and 0.37 ppm, respectively, for acar and as 0.0012 ppm and 0.0037 ppm, respectively, for acar metabolite (see Appendix 5). Chromatograms, shown in Appendix 4 of the analytical summary report (Appendix 2), show no interference. Two six-point standard curves were generated each time an analysis set was run; the correlation coefficient was always ≥ 0.993.
The maximum storage interval for field-treated samples in this study was 10 days, as shown in Table C.2. No storage stability was required for this study.

Detailed results are given in field data summaries

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-FL19

Field Research Director (FRD): J. H. Cool

Field Personnel: O.S. Sipson

TEST SUBSTANCE RECORDS (Separate page for each formulation or lot no.)	
Test Substance (Name on Container Label)/Batch or Lot No.: Acarmite® 50WP/HC1G15P074	
Source: XXResearch Laboratories,	
Date Received: 07 Jan 2003	Expiration Date[1]: 14 Feb 2004
Spray Additives (Adjuvants) Used: PLYAC®	
Storage Location: Pesticide Shed, Bldg. 8245 IR-4 Lock Up, University of FL, TREC 18905 SW 280	
Storage Temperature Range (from receipt of test substance to last application): Approx. 40—90°F	

TRIAL SITE INFORMATION			
Test Site (Name, Street, Town, State): Block 8, University of FL, TREC, 18905 SW 280 St., Homestead, Dade County, FL 33031			
Soil Texture/Type: Krome very Gravelly Loam	%Sand: Not reported	%Silt: Not reported	%Clay: 15—20
	%Organic Matter: 3—10		Soil pH: 7.4—8.4
Crop Variety: Red Lady papaya			
Field Planting Date (Seeded or Transplanted X) or Age of Established Crop : 29 Apr 2002			
Row Width: 12 ft	Plant Spacing: 7 ft	No. Rows_/Trees X per Plot: 16	
Control Plot Dimensions: 12 ft x 112 ft		Treated Plot Dimensions: 12 ft x 112 ft	

1 As determined by the registrant or characterization laboratory.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-FL19

Maintenance Fertilizers and Pesticides applied during the year(s) of the field trial (Product/Date)	
4-0-8 (8 gal 32 oz/2.8 A, 02 Jan 2003; 03 Jan' 2003; 06 Jan 2003; 10 Jan 2003; 13 Jan 2003; 16 Jan 2003; 17 Jan 2003; 21 Jan 2003; 27 Jan 2003; 17 Feb 2003; 19 Feb 2003; 24 Feb 2003)	4-0-8 (2 gal/2.8 A, 06 Jan 2003; 14 Jan 2003; 28 Jan 2003; 09 Apr 2003; 22 Apr 2003; 22 May 2003)
4-0-8 (17 gal/2.8 A, 11 Feb 2003; 12 Feb 2003; 21 Feb 2003; 28 Feb 2003; 07 Mar 2003;11 Mar 2003)	4-0-8 (1 gal 34 oz/2.8 A, 28 Apr 2003; 01 May 2003; 05 May 2003; 12 May 2003)
4-0-8 (2 gal 68 oz/2.8 A, 22 May 2003)	4-0-8 (1 gal 116 oz/2.8 A, 23 May 2003; 27 May 2003; 2 Jun 2003, 9 Jun 2003)
Acarmite (8 oz/100 gal, 02 Jan 2003)	Dithane (22 oz/100 gal, 06 Jan 2003; 17 Feb 2003)
Abound (8 oz/100 gal, 21 Jan 2003)	Vendex (15 oz/100 gal, 29 Jan 2003)
Bravo (48 oz/100 gal, 03 Feb 2003)	Pounce (9 oz/100 gal, 14 Feb 2003)
Dithane M-45 (2.5 oz/10 gal, 15 Apr 2003)	Pounce (0.75 oz/10 gal, 15 Apr 2003)
Acarmite (0.75 oz/l0 gal, 22 Apr 2003; 27 May 2003)	Abound (0.75 oz/10 gal, 22 Apr 2003; 27 May 2003)
Bravo (3 oz/10 gal, 02 May 2003)	Vendex (1.5 oz/10 gal, 02 May 2003)
Pounce (11 oz/100 gal, 21 May 2003)	Dithane M-45 (35 oz/100 gal, 21 May 2003)

FIELD DATA SVMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-FL19

APPLICATI~N RECORDS (Separate page for each calibration/application}		
Application 1 Date: 19 May 2003	Output Calibration/Re-Check Date: 19 May 2003	Days between Applications: NA[1]
Application Equipment Type: Tractor-mounted sprayer		Propellant: Pump
Type of Application: Foliar directed		
No. Nozzles/Outlets: 7	Nozzle Spacing (inches}: NA	Screen Mesh: 150, #3 Medium
Nozzle Brand/Type/Size: T-Jet 11502/Flat Fan/Stainless Steel		Spray Swath Width: NA
Treated Area: 1344 ft^2	Delivery Rate[2]: 149.71 GPA	
Test Substance: Acarmite®50WP		Batch/Lot No.: HC 1 G15P074
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water): 22,710 mL (6.0 gal) Formulated Product: 18.7 g Additives (Adjuvants): 5.0 mL Total Mix Volume: *22,715* mL		

Protocol Rate	lb ai/A	Actual Applied Rate lb ai/A
Treatment No. 02	0.5	0.51(1.03X)

Crop Growth Stage: Fruiting	Crop Height: 7—S ft
Wind Speed and Direction: 3.l mph/SE	Air Temperature: 88 °F
First Rain after Application: Date: 20 May 2003	Amount (inches): 0.21
Time after Application of First Rain (Days or Hours): 1 day	
First Irrigation after Application: Date: NR[5]	Amount (inches): NR
Irrigation Type: Drip	after Application of First Irrigation (Days or Hours): 3 days .a week, as needed
Were any phytotoxic effects seen? Yes No X Unrecorded	
Description of the severity and/or symptoms of any phytotoxic effects: NA	

[1] NA = Not applicable

a Gallons of water delivered per acre, as determined by the Study Director using actual application data.

[3] Note: Treatment No. O1 is the untreated control.

[4] Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

s NR = Not reported

FIELD DATA SUMMARY

PesticidelCrop/Field ID No: Acar/Papaya/08270.03-FL19

APPLICATION REC4RDS (Separate page for each calibration/application)		
Application 2 Date: 09 Jun 2003	Output Calibration/Re-Check Date: 09 Jun 2003	Days between Applications: 21
Application Equipment Type: Tractor-mounted sprayer		Propellant: Pump
Type of Application: Foliar directed		
No. Nozzles/Outlets: 7	Nozzle Spacing (inches): NA[1]	Screen Mesh: 150, #3 Medium
Nozzle Brand/Type/Size: T-Jet 11502/Flat Fan/Stainless Steel		Spray Swath Width: NA
Treated Area: 1344 ft^2	Delivery Rate[2]: 148.27 GPA	
Test Substance: Acaamite®50WP		Batch/Lot No.: HC 1 G 1 SP074
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water): 22,710 mL (6.0 gal) Formulated Product: 18.7 g Additives (Adjuvants): 5.0 mL Total Mix Volume: 22,715 mL		
Protocol Rate lb ai/A Treatment No. 02	0.5	Actual Applied Rate lb ai/A a 0.51(1.02X)
Crop Growth Stage: Fruiting		Crop Height: 7—8 ft
Wind Speed and Direction: 1.2 mph/SE		Air Temperature: 85 °F
First Rain after Application: Date: 09 Jun 2003		Amount (inches): 0.25
Time after Application of First Rain (Days or Hours): 6 hours		
First Irrigation after Application: Date: NR[5]		Amount (inches): NR
Irrigation Type: Drip Irrigation	Time after Application of First Irrigation (Days or Hours): 3 days a week, as needed	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

[1] NA = Not applicable

[2] Gallons of water delivered per acre, as determined by the Study Director using actual application data. [3]

Note: Treatment No. O1 is the untreated control.

a Based on sprayer output and applicator pass Limes. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

[5] NR = Not reported

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-FL19

SAMPLE COLLECTION/STORAGE (separate page for each crop fraction/sampling date)		
Harvest Date: 10 Jun 2003	Sampling Date: 10 Jun 2003	PHI: 1 day
Description of Crop Stage/Fraction at Harvest: Mature green to half ripe papayas		
Harvesting Equipment: Gloved hands, 70% alcohol, baskets, coolers		
Procedures Utilized in Harvesting: TRT 01 then TRT 02. Fruit was hand picked from both sides of the row, taking fruit from high, low, inside, outside, exposed, and shielded areas of 14 trees, avoiding two trees at row ends. Samples consisted of at least 12 fruits and weighed a minimum of 4 lb.		
Modifications after Harvest (e.g., Trimming, Cleaning, Cutting, Drying, Compositing): Fruit was cut into 1 /8 fractions to reduce sample weight.		
Holding and Transport of Samples from Field to Freezer (or between Field and Shipment): Samples were put in bags and placed in the freezer.		
Maximum Elapsed Time from Treated Sample Collection to Frozen Storage: 1 hour, 10 minutes		
Freezer Temperature Range(s) (prior to shipment): Approx. -17 to -5 °F (TRT 01) Approx. -12 to -5 °F (TRT 02)		
Shipped: Frozen (Packed in Dry Ice) X	Fresh (Packed in Dry Ice)	Fresh (not in Dry Ice)
Name of Carrier: Federal Express		Shipment Date: 1 1 Jun 2003

METEOROLOGICAL INFORMATION

Were any unusual weather events noted for this test site during the field trial? Yes No X

Description of any unusual weather occurrences: None indicated

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-HI01

Field Research Director (FRD): Michael Kawate University of Hawaii

Field Personnel: Ms. J. Cho, James Kamar

TEST SUBSTANCE RECORDS (Separate page for each formulation or lot no.)	
Test Substance (Name on Container Label)/Batch or Lot No.: Acarmite® 50WP/HC 1 G 15P074	
Source: XX Research Laboratories,	
Date Received: 07 Feb 2003	Expiration Date[1]: 14 Feb 2004
Spray Additives (Adjuvants) Used: Latron B-1956	
Storage Location: Pesticide Storage Room 310, University of Hawaii;	
Storage Temperature Range (from receipt of test substance to last application): Approx. 70 °F (07–10 Feb 2003); Approx. 17.76–26.53 °C (10 Feb–05 Jun 2003)	

TRIAL SITE INFORMATION			
Test Site (Name, Street, Town, State): Matsuda-Fukuyama Farm, Inc. on Opaeula Rd., 59-715 Maulukua Rd., Haleiwa, HI 96712			
Soil Texture/Type: Silty clay	%Sand: 0—20	%Silt: 40—60	%Clay: 40—60
	%Organic Matter: 2.74		Soil pH: 5.3
Crop Variety: Gold papaya			
Planting Date (Seeded or Transplanted) or Age of Established Crop X :1.5 years			
Row Width: 11 ft	Plant Spacing: 7 ft	No. Rows_/Trees X per Plot: 14	
Control Plot Dimensions: 11 ft x 98 ft		Treated Plot Dimensions: 11 ft x 98 ft	

Maintenance Fertilizers and Pesticides applied during the year(s) of the felel trial (Product/Date)	
Sulfur (4.5 lb/A, 10 May 2003; 31 May 2003; 21 Jun 2003)	Dithane DF (1.7 lb/A, 10 May 2003)
Latron B-1956 (8 oz/100 gal, 10 May 2003; 31 May 2003; 21 Jun 2003)	Kocide (1.5 lb/A, 31 May 2003; 21 Jun 2003)

[1] As determined by the registrant or characterization laboratory.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-HI01

APPLICATION RECORDS (Separate page for each calibration/application)		
Application 1 Date: 15 May 2003	Output Calibration/Re-Check Date: 15 May 2003	Days between Applications: NA[1]
Application Equipment Type: Backpack mistblower		Propellant: CO_2
Type of Application: Foliar directed		
No. Nozzles/Outlets: 1	Nozzle Spacing (inches): NA	Screen Mesh: None
Nozzle Brand/Type/Size: No nozzle attachment used		Spray Swath Width: NA
Treated Area: 1078 ft^2	Delivery Rate[2]: 50.67 GPA	
Test Substance: Acarmite® 50WP		Batch/Lot No.: HC1G1.5P074
Tank Mix Amounts Trt.: Treatment No. 02 Carrier (Water): 5578 mL Formulated Product: 13.46 g Additives (Adjuvants): 7.0 mL Total Mix Volume: 5585 mL		
Protocol Rate [lb ai/A]		Actual Applied Rate [lb ai/A1[4]
Treatment No. 02 0.5		0.51 (1.02X)
Crop Growth Stage: Fruiting		Crop Height: 11 ft
Wind Speed and Direction: 5–15 mph/NE		Air Temperature: 78 °F
First Rain after Application: Date: 04 Jun 2003		Amount (inches): 0.04
Time after Application of First Rain (Days or Hours): 20.28 days		
First Irrigation after Application: Date: 16 May 2003		Amount (inches): 0.35
Irrigation Type: trickle	Time after Application of First Irrigation (Days or Hours):20.42 hr	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

NA = Not applicable

Gallons of waer delivered per acre, as determined by the Study Director using actual application data. Note: Treatment No. 01 is the untreated control.

Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-HI01

APPLICATION RECORDS (Separate page for each calibration/application)		
Application 2 Date: 05 Jun 2003	Output Calibration/Re-Check Date: 05 Jun 2003	Days between Applications: 21
Application Equipment Type: Backpack mistblower		Propellant: CO_2
Type of Application: Foliar directed		
No. Nozzles/Outlets: 1	Nozzle Spacing (inches): NA[1]	Screen Mesh: None
Nozzle Brand/Type/Size: No nozzle attachment used		Spray Swath Width: NA
Treated Area: 1078 ft^2	Delivery Rate[2]: 50.43 GPA	
Test Substance: Acarmite® 50WP		Batch/Lot No.: HC 1 G 15P074
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water):*5578* mL Formulated Product:13.46 g Additives (Adjuvants):7.0 ml Total Mix Volume:5585 mL		
	Protocol Rate	Actual Applied Rate
	[lb ai/A[	jlb ai/A][4]
Treatment No. 02	0.5	0.51 (1.01X)
Crop Growth Stage: Fruiting		Crop Height: 1 1 ft
Wind Speed and Direction: 0 mph		Air Temperature: 85 °F
First Rain after Application:	Date: 06 Jun 2003	Amount (inches): 0.04
Time after Application of First Rain (Days or Hours): 1 day		
First Irrigation after Application:	Date: 06 Jun 2003	Amount (inches): 0.35
Irrigation Type: trickle	Time after Application of First Irrigation (Days or Hours):20.92 hr	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

NA = Not applicable

Gallons of water delivered per acre, as determined by the Study Director using actual application data. Note: Treatment No. 01 is the untreated control.

Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-HI01

SAMPLE COLLECTION/STORAGE (separate page for each crop fraction/sampling date)		
Harvest Date: 6 Jun 2003	Sampling Date: 06 Jun 2003	PHI: 1 day
Description of Crop Stage/Fraction at Harvest: Mature green to half ripe papayas		
Harvesting Equipment: Gloved hands		
Procedures Utilized in Harvesting: TRT 01 then TRT 02. Fruit was hand picked from different areas of the fruit column of 12 trees, avoiding one tree at the end of each row. Samples consisted of 12 fruits and weighed a minimum of 4 lb.		
Modifications after Harvest (e.g., Trimming, Cleaning, Cutting, Drying, Compositing): Fruit was cut into 1 /4 fractions to reduce sample weight.		
Holding and Transport of Samples from Field to Freezer (or between Field and Shipment): Sample bags were placed in coolers and transported to the freezer.		
Maximum Elapsed Time from Treated Sample Collection to Frozen Storage: 3 hours, 15 minutes		
Freezer Temperature Range(s) (prior to shipment): Approx. -24 to -11 °F		
Shipped: Frozen (Packed in Dry Ice) X	Fresh (Packed in Dry Ice)	Fresh (not in Dry Ice)
Name of Carrier: Airborne Express		Shipment Date: 09 Jun 2003

METEOROLOGICAL INFORMATION

Were any unusual weather events noted for this test site during the field trial? Yes _____ No_ X

Description of any unusual weather occurrences: None indicated

FIELD DATA SUMMARY

Pesticide/Crop/Fie1d ID No: Acar/Papaya/08270.03-HI02

Fie1d Research Director: (FRD): M.K. White University of Hawaii, Honolulu

Other Field Personnel: Ms. J. Chou, James Kamar

TEST SUBSTANCE REC~RDS Se arate a e for each formulation or lot no.	
Test Substance Name on Container Label /Batch or Lot No.: Acarmite®50WP/HC1G15P074	
Source: XX Research Laboratories,	
Date Received: 07 Feb 2003	Expiration Date[1]: 14 Feb 2004
Spray Additives Adjuvants Used: Latron B-1956	
Storage Location: Pesticide Storage Room 310, University of Hawaii (07—10 Feb 2003);	
Storage Temperature Range (from receipt of test substance to last application): Approx. 70 °F (07—10 Feb 2003 ; Approx. 18.3—32.42 °C 10 Feb—OS Jun 2003	

TRIAL SITE INFORMATION			
Test Site (Name, Street, Town, State): Diamond Head Papaya Co. Ltd.,16-309A Volcano Rd., Keaau, HI 96749			
Soil Texture/Type: Extremely stony muck	% Sanad: NA[2]	% Silt: NA	% Clay: NA
	% Organic Matter: 7.29		Soil H: 4.8
Crop Variety : Kaoho papaya			
Field Planting Date Seeded or Transplanted or A e of Established Crop X : 2 years			
Row Width: 11 ft	Plant Spacing : 6 ft	No. Rows /Trees X er Plot: 14	
Control Plot Dimensions: 11 ft x 84 ft		Treated Plot Dimensions: 11 ft x 84 ft	

As determined by the registrant or characterization laboratory. NA = Not applicable

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-HIO2

Maintenance Fertilizers and Pesticides applied during the year(s) of the field trial (Product/Date)	
Sulfur (2 lb/100 gal, 24 Jun 2003; 09 Jul 2003)	Manzate DF (2 11)/100 gal, 24 Jun 2003; 09 Jul 2003)
Latron B-1956 (5 oz/100 gal, 24 Jun 2003;09 Jul 2003)	Basic Copper (21b/gal, 24 Jun 2003; 09 Jul 2003)
Gramoxone (3 qt/100 gal, 01 Jul 2003)	Fertilizer 14-14-14 (300 lb/A, 18 Jul 2003)

Pesticide/Crop/Field ID No: Acar/Papaya/08270.03-HIO2

APPLICATION RECORDS (Separate page for each calibration/application)		
Application 1 Date: 25 Jun 2003	Output Calibration/Re-Check Date: 25 Jun 2003	Days between Applications: NA[1]
Application Equipment Type: Backpack mistblower		Propellant: CO_2
Type of Application: Foliar directed		
No. Nozzles/Outlets: 1	Nozzle Spacing (inches): NA	Screen Mesh: None
Nozzle Brand/Type/Size: No nozzle attachment used		Spray Swath Width: NA
Treated Area: 924 ft~	Delivery Rate[2]: 102.87 GPA	
Test Substance: Acarmite®50WP		Batch/Lot No.: HC 1 G 15P074
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water): 9594 mL Formulated Product: 11.54 g Additives (Adjuvants): 12.0 mL Total Mix Volume: 9606 mL		
Protocol Rate lb ai/A Treatment No. 02 0.5		Actual Applied Rate lb ai/A a 0.52 (1.03X)
Crop Growth Stage: Fruiting		Crop Height:12—14 ft
Wind Speed and Direction: 0—5 mph/NE		Air Temperature: 82 °F
First Rain after Application: Date: 26 Jun 2003		Amount (inches): 0.04
Time after Application of First Rain (Days or Hours): 21.67 hours		
First Irrigation after Application: Date: NA		Amount (inches): NA
Irrigation Type: None	Time after Application of First Irrigation (Days or Hours): NA	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

[1] NA = Not applicable

2 Gallons of water delivered per acre, as determined by the Study Director using actual application data.

[3] Note, Treatment No. O1 is the untreated control.

a Based on sprayer output and applicator pass Limes. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

FIELD DATA SUMMARY

Pesticide/Crop/F1eld ID No: Acar/Papaya/08270.03-HIO2

APPLICATI4N REC~RDS (Separate page for each calibration/application)		
Application 2 Date: 17 Ju12003	Output Calibration/Re-Check Date: 17 Ju12003	Days between Applications: 22
Application Equipment Type: Backpack mistblower		Propellant: None
Type of Application: Foliar directed		
No. Nozzles/Outlets: 1	Nozzle Spacing (inches): NA[1]	Screen Mesh: None
Nozzle Brand/Type/Size: No nozzle attachment used		Spray Swath Width: NA
Treated Area: 924 ft^2	Delivery Rate[2]: 103.14 GPA	
Test Substance: Acarmite®50WP		Batch/Lot No.: HC1G15P074
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water): 9594 mL Formulated Product: 11.54 g Additives (Adjuvants): 12.0 mL Total Mix Volume: 9606 mL		
Protocol Rate lb ai/A Treatment No. 02	0.5	Actual Aplied Rate lb ai/A [4] 0.52 (1.03X)
Crop Growth Stage: Fruiting		Crop Height: 12—14 ft
Wind Speed and Direction: 0—5 mph/NE		Air Temperature: 85 °F
First Rain after Application: Date: NA		Amount (inches): NA
Time after Application of First Rain (Days or Hours): NA		
First Irrigation after Application: Date: NA		Amount (inches): NA
Irrigation Type: None	Time after Application of First Irrigation (Days or Hours): NA	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

[1] NA = Not applicable

[2] Gallons of water delivered per acre, as determined by the Study Director using actual application data.

[3] Note: Treatment No. Ol is the untreated control.

[4] Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Papayal08270.03-HIO2

SAMPLE COLLECTION/STORAGE (separate page for each crop fraction/sampling date)		
Harvest Date: 18 Jul 2003	Sampling Date: 18 Ju12003	PHI: 1 day
Description of Crop Stage/Fraction at Harvest: Mature green to half ripe stage papayas		
Harvesting Equipment: Gloved hands		
Procedures Utilized in Harvesting: TRT 01 then TRT 02. Fruit was hand picked from different areas of the fruit column of 12 trees, avoiding one tree at the end of each row. Samples consisted of 12 fruits and weighed a minimum of 4 lb.		
Modifications after Harvest (e.g., Trimming, Cleaning, Cutting, Drying, Compositing): Fruit was cut into 1 /2 fractions to reduce sample weight.		
Holding and Transport of Samples from Field to Freezer (or between Field and Shipment): Sample bags were placed in coolers and transported to the freezers.		
Maximum Elapsed Time from Treated Sample Collection to Frozen Storage: 5 hours		
Freezer Temperature Range(s) (prior to shipment): Approx. -27 to -13 °F		
Shipped: Frozen (Packed in Dry Ice) X	Fresh (Packed in Dry Ice)	Fresh (not in Dry Ice)
Name of Carrier: Airborne Express		Shipment Date: 21 Jul 2003

METEOROLOGICAL INFORMATION

Were any unusual weather events noted for this test site during the field trial? Yes No X

Description of any unusual weather occurrences: None indicated

Exercise 6.1 Definition of residues of happyplant (F64)

Tasks:

1. Summarise the metabolism pathways and select the relevant residue components in food and feed items taking into account the information on the metabolism of F64 in goat and spring wheat, as well as the metabolism of the main metabolite F64M1 of the active substance F64 in goat.
2. Consider the concentration of residues in fat and tissues as well as in milk and milk cream in order to decide on the fat solubility of residues of interest,
3. Taking into account the significance of residue components and the analytical methods available for their determination, prepare the recommendation for residue definition for enforcement and risk assessment purposes in plant and animal commodities.

Exercise 6.2 Expression of sum of residues included in residue definition and rounding of residue values

Supervised trial results were reported in rice as follow:

Acephate	Methamidophos	Sum 1	Sum 2
0.036	<0.05		
0.065	<0.01		
0.69	0.38		
0.09	0.05		
0.04	0.021		
0.1	0.046		
0.042	<0.025		
<0.025	<0.025		

1. Report the results in the appraisal for estimation of maximum residue levels, STMR and HR values
2. Calculate the sum of the residues expressed as acephate for

2.1 long term risk assessment:

ADI: acephate 0.03 mg/kg bw/day; methamidophos: 0.004 mg/kg bw/day

Sum 1=acephate mg/kg+7.5* methamidophos mg/kg

2.2 Short term risk assessment

ARfD: acephate 0.1 mg/kg bw/day; methamidophos: 0.01 mg/kg bw/day

Sum 2= acephate mg/kg+10* methamidophos mg/kg

Note that the factors of 7.5 and 10 are derived from the ratios of ADI and ARfD values of acephate and methamidophos

3. Report the residues for calculation of long term and short-term intake
4. Calculate the median residue for acephate and methamidophos separately and for the Sum 1 and sum 2 residues.

Exercise 7.1. Summarising GAP Information

See also Chapter 7

1. Summarising GAP information

Exercise GAP-summary

2. The aim

- The aim of this exercise is to interpret the directions-for-use on registered labels and to convert the information to systematic summaries to be used in evaluation of pesticide residue trials.
- GAP information in a systematic form is needed for ready comparison with the conditions of supervised residue trials.

3. Examine each label

- formulation type;
- active ingredient concentration;
- crops treated;
- product application rate;
- product spray concentration;
- number of applications;
- intervals between applications;
- pre-harvest interval (PHI).

4. Calculate

- active ingredient application rate;
- active ingredient spray concentration.

5. Add footnotes

- growth stage instructions on treatment timing;
- timing for livestock grazing;
- restrictions on spray volumes;
- list of crops in a crop group.

6. The labels

The labels are based on the words from real labels, but are for fictitious products and a fictitious active ingredient.

Labels (or English translations) are available from:

Australia	France	Spain
Belgium	Germany	Switzerland
Brazil	Italy	UK
Central America	Poland	USA

7. The exercise

- Extract information from the labels and fill in the summary GAP tables.
- Back in plenary

Questions?
Specific problems?
Apparently missing data?

Note for planning the exercise

The following crops appear on a number of labels (3 or more): apples, bananas, broccoli, Brussels sprouts, cabbage, carrots, cauliflower, celery, cucumber, grapes, peach, pears, potato, sugar beet, tomato and wheat.

Match the time allowed for the exercise with the number of crops selected for the GAP summary.

Supporting information

Portions of labels for the fictitious pesticide happychloronid are provided (in English) for a number of countries.

Use the worksheets for this exercise to assist in summarising the relevant GAP information from the labels.

Abbreviations and acronyms in this exercise

BBCH	Biologische Bundesanstalt, Bundessortenamt and CHemical industry
CAS	Chemical Abstracts Service
EC	emulsifiable concentrate
FS	suspension concentrate for seed treatment
GAP	good agricultural practice
GS	growth stage
LV	low volume
PHI	pre-harvest interval
SC	suspension concentrate
WG	water dispersible granules

Australia

CAUTION
KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING

FAORONIC EC

FOLIAR *FUNGICIDE*
Controls target spot of tomatoes, leaf blight of carrots, leaf spot diseases of bananas and husk spot of Macadamias

Active constituent250 g/L happychloronid

DIRECTIONS FOR USE

Restraint:

DO NOT apply more than 6 applications of this product per season. The effect of FAORONIC EC could be diminished if rain falls within 2 hours of application.

Crop	Disease	Rate per hectare	Withholding period	Critical comments
Bananas	Yellow Sigatoka, Black Sigatoka	GROUND APPLICATION: 400 mL + 3 L of water-miscible oil in a convenient volume of water AERIAL APPLICATION: 400 mL + 3 L of water-miscible oil in minimum 30 L water	1 day	For use in Qld, NSW and NT.
Carrots	Leaf blight	300 mL or 500 mL	7 days	For use in all States. Apply the 300 mL rate at 7 day intervals and the 500 mL rate at 10 to 14 day intervals
Macadamia nuts	Husk spot	50 mL/100 L water	-	For use in Qld, NSW and NT. Commence application at nut set and continue until late December at 3-4 week intervals.
Potatoes	Target spot, early blight	300 mL or 500 mL	7 days	For use in all States. Apply the 300 mL rate at 7 day intervals and the 500 mL rate at 10 to 14 day intervals
Tomatoes	Target spot	300 mL or 500 mL	3 days	For use in all States. Apply the 300 mL rate at 7 day intervals and the 500 mL rate at 10 day intervals

WITHHOLDING PERIODS:	
Bananas:	*Do not harvest for 1 day after application.*
Potatoes, carrots:	*Do not harvest for 7 days after application.*
Tomatoes:	*Do not harvest for 3 days after application.*
Macadamias:	*Not required when used as directed.*

Australia

CAUTION
KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING

Fuligocide FS

FUNGICIDE SEED TREATMENT
For the control or suppression of seedling diseases in barley and wheat.

Active constituent120 g/L happychloronid

DIRECTIONS FOR USE

Crop	Disease	Rate mL/100 kg seed	Critical comments
Barley	Covered smut	100	Apply diluted with water to clean and healthy seed before sowing. Complete coverage is essential. Rhizoctonia control: Use the highest rate (280 mL per 100 kg seed) in paddocks with a history of Rhizoctonia root rot and where minimum tillage is used.
	Loose smut, net blotch, Pythium root rot	120	
	Rhizoctonia root rot	280	
Wheat	Common bunt, flag smut	100	
	Loose smut, Pythium root rot	120	
	Rhizoctonia root rot	280	

APPLICATION

Apply FULIGOCIDE FS as a water based slurry using standard slurry treatment equipment that provides uniform seed coverage. For best results, FULIGOCIDE FS should be used to treat only undamaged seed of high viability.

WITHHOLDING PERIODS:

Barley and Wheat:
HARVEST: Not required when used as directed
GRAZING: Do not graze or cut for stock food for 6 weeks after sowing treated grain.

Australia

CAUTION
KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING

Mucidicide 100 WG

FOLIAR FUNGICIDE

Active constituent100 g/kg happychloronid

DIRECTIONS FOR USE

Restraint:
DO NOT apply more than 6 applications of this product per season. The effect of MUCIDICIDE 100 WG could be diminished if rain falls within 2 hours of application.

Crop	Disease	Rate	Critical comments
Apples, pears	Apple scab and pear scab	Dilute spraying 35 g alone or 25 g + full recommended rate (tank mix) of a registered protectant Scab fungicide per 100 L	Commence fungicide spray program at green tip stage with an approved fungicide. Apply MUCIDICIDE 100 WG from spurburst. Repeat applications at 7 to 10 day intervals until full petal fall. Use the high rate (35 g) if applying MUCIDICIDE 100 WG alone or at the low rate (25 g) only in mixture with a registered protectant Scab fungicide. Apply no more than 4 applications of MUCIDICIDE 100 WG alone. After 4 applications, apply MUCIDICIDE 100 WG only as a tank mix with a protectant Scab fungicide. See instructions below. After petal fall apply MUCIDICIDE 100 WG only as a tank mix with a protectant Scab fungicide.
		Dilute spraying 25 g + full recommended rate (tank mix) of a registered protectant Scab fungicide per 100 L	PETAL FALL ONWARDS Apply the tank mixture of MUCIDICIDE 100 WG plus a registered protectant Scab fungicide at 14 to 21 day intervals as required, depending on weather conditions and disease incidence.

WITHHOLDING PERIOD:

Apples and Pears: DO NOT HARVEST FOR 4 WEEKS AFTER APPLICATION.

Belgium

FAORONIC

Registration no.

Fungicide used on apple trees, pear trees, carrots, beets, broccoli, cabbages, cauliflower, Brussels sprouts Chinese cabbage, paksoi, asparagus, grapes, celery, and ornamentals.

Active ingredient 250 g/l of happychloronid
Formulation Emulsifiable Concentrate (EC)

Directions for use

Apple trees and pear trees

Control of powdery mildew and scab.
Spray every 10 days starting from first warning.
Rate: 100 ml/ha hedge (150 ml/ha of standard orchard), in mixture with a traditional fungicide with contact activity such as captan or a dithiocarbamate in order to avoid resistance.

Carrots

Control of powdery mildew and blight. Start applications when the first symptoms appear. Maximum of 3 applications. Rate: 0.5 l/ha.

Asparagus

Control rust. Spray after harvest. Rate: 0.5 l/ha.

Cauliflower, broccoli, cabbages and Brussels sprouts

Control of dark leaf spot.
Start applications when the first symptoms appear. Maximum 2 applications. Rate: 0.5 l/ha.

Chinese cabbage and paksoi

Use when first symptoms appear to control leaf spot.
Rate: 0.5 l/ha. 1-2 applications with a 14 day interval.

Celery

Control late blight. Rate: 0.5 l/ha.
Celery: 1-3 applications with 14 day intervals.

Beets

Control of powdery mildew, rust and leaf spot. Spray when first symptoms appear.
Rate: 0.5 l/ha.

Grapes

Control of powdery mildew and black-rot.
Treat at the time of a risk of infection.
Rate: 0.12 l/ha.

Safety interval (time period that must elapse between last application and harvest):

Apple, pear, carrots, cauliflower, broccoli, Chinese cabbage, paksoi and celery: 14 days.

Headed beets , cabbages (red, white, ox heart and of Savoy) and Brussels sprout: 21 days.

Brazil

FAORONIC®

Composition:
Active ingredient: HAPPYCHLORONID: 25% w/v (250 g/L)
Inert ingredients (total): 75% w/v

Class: Systemic fungicide.

Type of formulation: Emulsifiable concentrate.

Batch or lot nº:
Date of manufacture:
Expiration date:

INSTRUCTIONS FOR USE:
FAORONIC is a systemic fungicide recommended for control of the following diseases in the crops below:

CROP	DISEASE	DOSE OF PRODUCT	NUMBER AND TIMING
APPLE	Apple scab Powdery mildew	14 mL / 100L water	Treatment should start at green tip. Reapply whenever there are symptoms of re-infection in the crop. Apply FAORONIC at most 8 times per year
AVOCADO	Scab Anthracnose	20 mL / 100L water	Start applications at full blossom; reapply at intervals of 14 days until fruit are around 5 cm in diameter. Apply FAORONIC at most 4 times per year.
BANANA	Sigatoka (yellow)	0.2 L/ha	FAORONIC may be used at any time recommended for the treatment of yellow Sigatoka, at average intervals of 30 days or black Sigatoka at intervals of 14-21 days. Apply FAORONIC at most 5 times per year.
	Sigatoka (black)	0.4 L/ha	
BEAN	Rust	0.3 L/ha	Start applications when the first signs of the disease appear. Repeat every 14–15 days. Apply FAORONIC at most 3 times per season.
CARROT	Leaf blight	0.60 L/ha	Start spraying as soon as the first symptoms of disease occur. Repeat applications every 7 days. Apply FAORONIC at most 8 times per season.
CAULIFLOWER	Alternaria	20 mL / 100L water	Start applications on appearance of the first symptoms; reapply every 7 days. Apply FAORONIC at most 5 times per season.
CUCUMBER	Powdery mildew	10 mL / 100L water	Start applications when the first signs of the disease appear. Repeat every 10 days. Apply FAORONIC at most 5 times per season.

EGGPLANT	Blight	30 mL / 100L water	Start applications when the first signs of the disease appear. Repeat every 7 days whenever climatic conditions are favorable to the disease. Apply FAORONIC at most 6 times per season.
GARLIC	Purple blotch	0.5 L/ha	Start applications when the first signs of the disease appear. Repeat every 7 days whenever climatic conditions are favorable to the disease. Apply FAORONIC at most 6 times per season.
GRAPE	Anthracnose	8 mL / 100L water	Start applications when the plants are in full bloom. Repeat applications at intervals of 14 days whenever conditions are favorable for the diseases. Apply FAORONIC at most 6 times per year.
	Powdery mildew	12 mL / 100L water	
	Leaf blight	12 mL / 100L water	
MANGO	Powdery mildew	20 mL / 100L water	Start applications just before the flowers open, reapplying every 14 days and continuing until small fruits have formed. Apply FAORONIC at most 3 times per year.
	Anthracnose	50 mL / 100L water	
PAPAYA	Black spot	30 mL/ 100L water	Start applications when the fruit starts to form; reapply every 7–10 days. Apply FAORONIC at most 4 times per year.
POTATO	Early blight Black spot	0.3 L/ha	Treatment should begin at the first signs of disease. Reapply whenever there are signs of reinfection of the crop, up to a maximum of 4 applications.
RICE	Brown spot	0.3 L/ha	Apply once immediately after appearance of the first symptoms.
STRAWBERRY	Common leaf spot	40 mL/ 100L water	Begin treatment at first symptoms. Repeat applications every 14 days. Apply FAORONIC at most 6 times per season.
SUMMER SQUASH	Powdery mildew	14 mL/ 100L water	Begin treatment when the first signs of the disease appear. Repeat every 10 days. Apply FAORONIC at most 4 times per season.
TOMATO (VINE	Early blight Black spot	50 mL/ 100L water	Start spraying on appearance of the first symptoms. Repeat every 7 days. Apply FAORONIC at most 3 times per season.

Volumes of spray mixture recommended for ground spraying:

Volume L/ha	Crop
100–200	Bean, rice, strawberry.
200–400	Carrot, cauliflower, eggplant, garlic.
200–800	Cucumber, grape, papaya, tomato (vine).
500–1000	Avocado, banana, mango.

800–1500	Apple.

Parameters for aerial application
Airborne spraying with FAORONIC on crops of rice and banana should only be done at low volume (LV).

Application volume=>_ Banana: 15 L/ha.
Flight height => 2–4 m above target
For more efficient treatment in the case of banana crops, the use of mineral oil is recommended as spray vehicle.
Recommended dose of FAORONIC + 5 L mineral oil + 220 ml leaf surfactant. Make up to a volume of 15 L with water.
Do not use FAORONIC mixed only with oil.

Safety interval (time period that must elapse between the last application and harvest):

Cucumber: ..... 1 day
Eggplant, summer squash: .....3 days
Apple: .....5 days
Banana, mango, potato, strawberry: .....7 days
Avocado, cauliflower, garlic, papaya, tomato: .....14 days
Carrot: .....15 days
Grape: .....21 days
Bean: .....25 days
Rice: .....45 days

Central America

MUCIDICIDE® 25 EC

SYSTEMIC FUNGICIDE
HAPPYCHLORONID

DENSITY: 1.01 g/cm^3 at 25°C
SOLVENT: PINE OIL

PRODUCT CHARACTERISTICS:
MUCIDICIDE® 25 EC is a systemic fungicide for control of foliar diseases on bananas and plantains.

DISEASES TO CONTROL

Black sigatoka
Yellow sigatoka

RECOMMENDED RATES:
0.4 L/ha in each application (100 g of active ingredient/ha).

TIMING AND APPLICATION INTERVAL:
Integrate MUCIDICIDE® 25 EC in Sigatoka management programs.

Concentrate applications of MUCIDICIDE® 25 EC in the rainy season or high-pressure disease period. For best results, apply the first MUCIDICIDE® 25 EC spray at the beginning of the rainy season.

Observe application intervals of 15-20 days. Make up to 8 applications of MUCIDICIDE® 25 EC in a season.

PRE-HARVEST INTERVAL:
There are no restrictions.

RE-ENTRY PERIOD:
There are no restrictions.

CROP TOLERANCE:
When used as recommended, MUCIDICIDE® 25 EC is well tolerated by all crops.

APPLICATION EQUIPMENT:

MUCIDICIDE® 25 EC can be applied with fixed wing aircraft or helicopter, using micronairs or nozzles. In order to achieve a good and uniform spray deposit, calibrate the aircraft before use with regard to forward speed, effective swath width and flow rate.
After application or at the end of the day thoroughly clean the application and protective equipment with clean water. Clean also the filters, impermeable gloves and hat or cap.

Switzerland

FAORONIC ®

Fungicide to control cereal leaf and ear diseases and sugar beet foliar diseases.

Active ingredient: 23.7% of happychloronid (250 g/l)
Formulation: Emulsifiable Concentrate EC.

Activity

WHEAT: Powdery mildew, brown rust, yellow rust, leaf blotch and leaf and ear spot.
RYE: Brown rust.
SUGAR BEETS: Cercospora leaf spot, powdery mildew, rust.

Directions for use

Application
Dose and timing

WHEAT : 0.5 l/ha, only one application from BBCH31 to 61.
Powdery mildew: when more than 30% of the 3 last leaves show symptoms.
Brown rust: on less sensitive varieties: when more than 20% of the 3 upper leaves show symptoms. on sensitive varieties: at the beginning of the attack.
Yellow rust: at the beginning of the attack.
Leaf and ear spot: in potential areas and on sensitive varieties.

RYE: 0.5 l/ha, only one application from BBCH39 to 61.

SUGAR Beet: 0.4 l/ha from beginning of the diseases - in general only one treatment. If in situation of high disease pressure, repeat the application.

Switzerland

MUCIDICIDE®

Highly active fungicide against sclerotinia on oil seed rape and phoma on sunflower.

Active ingredient: 62.5 g/l of happychloronid
Formulation: Suspension Concentrate (SC).

Mode of action
Happychloronid is an active ingredient with local systemic activity

MUCIDICIDE can be used preventively by stopping the penetration of fungi, or curatively by stopping the fungal development.

Direction for use

OIL SEED RAPE: against sclerotinia. 2 l/ha : 1 application between beginning of flowering and full flowering.

SUNFLOWER: against phoma. 2 l/ha : 1 application at appearance of first inflorescence.

Germany

FAORONIC®

Description of formulation: *Emulsion concentrate containing 250 g/l (24.4% by weight) Happychloronid*

Information on correct use

Waiting periods:

Cucumbers: .....3 days.
Carrots, drumhead cabbage, Savoy cabbage, Brussels sprouts, cauliflower, broccoli, bulb vegetables: .....21 days.
Sugar beet, forage turnips: .....28 days.

Brussels sprouts Powdery mildew	0.4 l/ha When infestation commences. A maximum of three applications, at intervals of 14 to 21 days
Bulb vegetables Leaf blotch, purple blotch	0.4 l/ha in 400 to 600 l water/ha When infestation commences. A maximum of three applications at intervals of 7 to 14 days
Carrots Leaf blight, black rot, powdery mildew, leaf spot	0.4 l/ha. When infestation commences. A maximum of three applications, at intervals of 14 to 21 days
Cauliflower, broccoli Dark leaf spot, stem canker, ring spot.	0.4 l/ha When infestation commences. A maximum of three applications, at intervals of 14 to 21 days
Cucumber (greenhouse) Powdery mildew, fungal leaf spot pathogens	- plant height below 120 cm : 0.4-0.6 l/ha - plant height above 120 cm: 0.8 l/ha When infestation commences. A maximum of three applications, at intervals of 5 to 14 days
Cucumber (open land) Powdery mildew), fungal leaf spot pathogens	0.4 l/ha When infestation commences. A maximum of three applications at intervals of 5 to 14 days
Drumhead cabbage, savoy cabbage, Brussels sprouts. Dark leaf spot, stem canker.	0.4 l/ha When infestation commences. A maximum of three applications, at intervals of 14 to 21 days
Sugar beet, forage turnip Powdery mildew	0.4 l/ha When infestation commences. A maximum of two applications.

Resistance management

In the event of repeated application of this product or products from the same resistance management group, a reduction in efficacy may result. To prevent the development of resistance, the product should be used on an alternating basis with products from other resistance management groups.
If premature loss of efficacy has occurred, despite FAORONIC being applied in the recommended manner, further treatment with fungicides from a different group is advised.

Spain

FAORONIC 25 EC Fungicide

Systemic fungicide for foliar application, in concentrated emulsifiable form (EC) for the control of rusts, Alternaria, scab, and blight in different horticultural and ornamental crops, potato, fruit trees, olive trees and beets.

Composition: 25% w/v. Happychloronid (250 g/l)
Naphtha petroleum solvent No. CAS: 64742-94-5
Polyglycol ether

Manufacturing Date/Batch:

Characteristics

FAORONIC 25 EC is a systemic fungicide with preventive and curative action. The active substance, happychloronid, has a local systemic action.

Authorised applications, dose and instructions for use

Always apply as a normal foliar spray. It is recommended that application be commenced on a preventive basis, or as soon as the first symptoms of the disease appear.

Garlic (against rusts and Alternaria): 500 cc/ha, 3-4 applications at intervals of 7-14 days.
Celery (against Septoria): 300-500 cc/ha, 4 applications with intervals of 7-14 days.
Asparagus (against rusts and Alternaria): 500 cc/ha, 3 applications with intervals of 14-21 days.
Lettuce (against Alternaria): 500 cc/ha, 3 applications with intervals of 10-14 days.
Apple and pear trees: (against scab), 20 cc/100 l water. 3-5 applications at intervals of 7-10 days, from the swelling of the buds up to the point at which the fruit is 1 cm in diameter; after this stage, apply at intervals of 12-18 days; ***(against rusts and Septoria)*** 20 cc/100 l water. If volumes of less than 1500 l/ha of the mix are used for adult plants, the product must be applied in a dose of 300 cc/ha with 3-5 applications at intervals of 14 days.
Loquat (against scab): 10-20 cc/100 l water, 5 applications with intervals of 14-21 days.
Olive (against blight): 60 cc/ 100 l water, if the product is used in isolation. 10-20 cc/100 l water, if the product is applied in a tank mixed with CUPROCOL (150-175 cc/100 l water). 1-2 applications, exclusively in spring, with an interval of 14-21 days.
Potato (against Alternaria): 800 cc/ha, 3-4 applications at intervals of 12 days.
Sugar beet (against Cercospora): 300-500 cc/ha, 1-3 applications, at intervals of 3-4 weeks.
Tomato (against Alternaria): 50-64 cc/100 l water (in extensive tomato crops, apply 500-800 cc/ha). 2-4 applications at intervals of 7-10 days.

Waiting periods:

7 days in tomato; 14 days in celery, fruit trees with seeds, lettuce and loquats: and 30 days for other crops.

France

FAORONIC®

Multicrop fungicide

Vine: against powdery mildew and black rot
Apple tree and pear tree: against scab
Peach tree, apricot tree: against powdery mildew
Vegetable growing: against early blight, powdery mildew and rust.

250 g/l of happychloronid - emulsifiable concentrate

Crop and disease	Dose	Time and frequency of treatment	Waiting period before harvesting
Vine: powdery mildew, black rot	0.12 l/ha	Every two weeks as a preventive measure. Do not exceed 3 applications per year.	
Apple trees, pear trees, quince trees, Japanese pear: scab	0.015 l/hl	Every 10 days. Do not exceed 3 applications per year.	30 days
Peach, apricot trees: powdery mildew	0.02 l/hl	Every 12-14 days as a preventive measure. Do not exceed 3 applications per year.	14 days
Carrot: powdery mildew, early blight	0.5 l/ha	Do not exceed 3 applications per year.	14 days
Cabbage, Brussels sprouts: early blight	0.5 l/ha	Do not exceed 3 applications per year.	21 days
Cauliflower: early blight	0.5 l/ha	Do not exceed 3 applications per year.	14 days
Tomato: early blight, fruit rot	0.5 l/ha	Do not exceed 3 applications per year.	20 days
Stalk celery: leaf spot	0.5 l/ha	Do not exceed 3 applications per year.	14 days

APPLE TREE, PEAR TREE, QUINCE AND JAPANESE PEAR

Avoid the practice of treating one row out of two.
DOSE: 0.015 litre/hl
If a spray volume of less than 1000 litres per hectare is to be applied, the dose of FAORONIC applied ought to be 0.15 litre/hectare.

PEACH TREE, APRICOT TREE

FAORONIC is applied in 2 to 3 applications at intervals of 12 to 14 days from the stage of 7-8 mm in diameter up to the stage where the stone has hardened.
For peach trees: 2 applications are recommended:
- 20% of white or pink buds
- 20% of open flowers

For apricot trees, there should be 3 applications:
- stage D (corolla visible)
- full flowering
- petal fall, or 7 days after the 2nd application.

If a spray volume of less than 1000 litres per hectare is to be applied, the dose of FAORONIC applied ought to be 0.2 litre/hectare.

TOMATO

TREATMENT INTERVALS: generally 14 days
EARLY BLIGHT AND FRUIT ROT ON TOMATOES FOR CANNING
First application: at the end of flowering.

Second application: first ripe fruits stage.
Third application: 10 days after the second application. This will be the final treatment in the case of harvesting with 85% ripe fruit.
Fourth application: 10 days after the third application in the case of harvesting with 99% ripe fruit.

As with all tomatoes supplied to industry, fruit treated with FAORONIC must be subjected to compulsory washing prior to processing.

CARROT

TIME AND FREQUENCY OF TREATMENT: FAORONIC is applied on a preventive basis as of the first agricultural warnings.

CAULIFLOWER

TIME AND FREQUENCY OF TREATMENT: FAORONIC is applied on a preventive basis as of the first agricultural warnings. Spring and summer cauliflower: repeat the treatment every 14 days. Autumn and winter cauliflower: repeat the treatment every 2 to 4 weeks.

Italy

FAORONIC® 25 EC

Emulsifiable concentrate
Composition

100 g of product contains:
pure happychloronid 23.9 g (250 g/l) and coformulants

Characteristics

FAORONIC 25 EC is a happychloronid based fungicide, with long lasting and curative action.

Dosages and instructions for use

Crop	Pathogen	Dose	Indications for use
Sugar beet	Cercospora beticola	0.3 l/ha	Maximum 3 treatments 14-21 days apart.
Apple, pear	Scab, powdery mildew. Mixture with a coverage product is advised	15 ml per hl	Maximum 4 treatments
Potato, celery, carrot, cauliflower	Rust, powdery mildew	0.4-0.5 l/ha	3 -4 preventive treatments 7-14 days apart according to the severity of the disease.
Tomato	Rust, powdery mildew	0.4-0.5 l/ha	3 -4 preventive treatments 7-14 days apart according to the severity of the disease.
Cucumber	Powdery mildew	0.5 l/ha	3 -4 preventive treatments 10-14 days apart.
Peach	Blister	20-30 ml per hectolitre	2-3 treatments at bud break
	Monilia		1-2 treatments pre-harvest

Whenever low volume apparatus is used, use the same dose of product per hectare as would be distributed with normal volume apparatus.

Compatibility warning: in the event of mixture with other formulations the longest waiting period must be applied. The precautionary regulations for the most toxic products must also be observed. Should intoxication occur, inform the doctor of the mixture made up.

Suspend treatment 21 days prior to harvest for sugar beet and celery; 14 days prior to harvest for apple, pear, potato and cauliflower; 7 days for other crops.

Do not apply by aeroplane.

Poland

FAORONIC 250 EC

Fungicide in form of emulsifiable concentrate, for use in orchards and ornamental crops against diseases.

Active substance content:

Happychloronid: 250 g in 1 litre product.

APPLICATION RANGE, TIMING AND RATES

ORCHARDS

Apple, pear.

Recommended rate: 200 ml/ha.
Apply up to 120 hours after infection. To eradicate scab spots it is recommended to apply FAORONIC 250 EC twice in 5 days interval. Apply from pink flower bud, not more than 2-3 times in the season.

Apple.

Recommended rate: 200 ml/ha.
Apply from pink bud phase every 7-14 days, not more than 2-3 times in the season.

WAITING PERIOD (period from last application to the harvest of crop intended for consumption): - apple, pear - 14 days

UK

Faoronic

An emulsifiable concentrate containing 250 g/l (24.2% w/w) happychloronid

A fungicide with contact and systemic activity against a wide range of diseases of wheat, oilseed rape, Brussels sprouts, cabbage, broccoli and cauliflower.

STORE IN A COOL, DRY PLACE
Batch No.

Crop	Maximum individual dose (litres product per hectare)	Maximum total dose (litres product per hectare per crop)	Latest timing of application.
Wheat	0.3	0.3	Before early milk stage
Oilseed rape	0.5	1.0	End of flowering
Brussels sprouts	0.3	0.9	21 days before harvest
Cabbage	0.3	0.9	21 days before harvest
Broccoli	0.3	0.9	21 days before harvest
Cauliflower	0.3	0.9	21 days before harvest

A minimum interval of 14 days must be observed between applications to cauliflower and broccoli.

CROP SPECIFIC INFORMATION

Winter Wheat

Faoronic should be applied at any time from ear fully emerged stage of the crop to before grain early milk-ripe stage (GS 59-71). Only one application should be made to any one crop.

Oilseed Rape

For control of leaf spot, a 2 spray program of 0.25 litres Faoronic per ha may be used, starting in the autumn.
For effective control of pod spot, spray 0.5 litres Faoronic per ha at the end of flowering.

Brussels Sprouts, Cabbage, Broccoli and Cauliflower

A 3 spray program is recommended commencing at the first sign of disease and repeated at 14-21 day intervals. A minimum interval of 14 days must be observed between applications to cauliflower and broccoli.

Spray Volume

Wheat 200 litres water per hectare.
Oilseed rape 200 litres water per hectare.
Brussels sprouts, cabbage, broccoli and cauliflower 400 litres water per hectare.

USA

Mucidicide WG

FUNGICIDE

For use in horticulture.

Active constituent20 % happychloronid

DIRECTIONS FOR USE

Crop	Amount MUCIDICIDE WG per acre	Minimum gallons per acre ground application	Total number of sprays per season	Harvest days after application (PHI days)
Cucurbit vegetables Note[1]	0.3-0.5 lbs	50	3	3
Tomatoes Note[2] Fruiting vegetables Note[3]	0.6 lbs	50	2	3
Grapes	0.75 lbs	50	1	14
Hops	0.75-1.5 lbs	50	1	14
Pome fruits Note[4]	0.75-1.0 lbs	50	2	7
Stone fruit: nectarines, peaches, plums, prunes	0.75-1.0 lbs	50	3	5

Notes

1. CUCURBIT VEGETABLES: crop group includes chayote, Chinese waxgourd, citron melon, cucumber, edible gourd, gherkin, momordica species, muskmelon (includes cantaloupe and honeydew), pumpkin, summer squash:, winter squash, watermelon.
2. TOMATOES: Use only on plants that will produce tomatoes greater than 1 inch in diameter when mature.
3. FRUITING VEGETABLES: crop group includes egg plant, ground cherry, pepino, peppers (bell, chili, cooking, pimento, sweet), tomatillo, tomato.
4. POME FRUITS: crop group includes apples, crabapples, pears, quince, loquat, Mayhaw, oriental pear.

Worksheets

GAP Summary Table – happychloronid foliar sprays.

Country	Crop	Formulation and Concentration		Max product application rate or product spray concentration	Max application rate kg ai/ha	Spray concentration kg ai/hl	No. of applications	Interval between applications, days	PHI, days
Australia	Apples	WG	100 g/kg	35 g/hl	-	0.0035	6	7-10 Note[31] 14-21 Note[32]	28
Australia	Bananas	EC	250 g/l	400 ml/ha	0.10	-	6		1

GAP Summary Table – happychloronid seed treatments.

Crop	Country	Formulation and concentration	Max product application rate per 100 kg seed	Max application rate, g ai per 100 kg seed	Use instructions and notes

[31] Before petal fall.
[32] After petal fall

Crop	Country	Formulation and concentration		Max product application rate per 100 kg seed	Max application rate, g ai per 100 kg seed	Use instructions and notes

Exercise 7.2 Evaluation of bridging trials

Four broccoli trials matching the foliar GAP conditions were side-by-side trials providing bridging data for the use of WG and SL formulations. The residues measured in duplicate composite samples were:

	SL, mg/kg	WG, mg/kg
Broccoli	0.34, 0.37	0.49, 0.44
Broccoli	0.01, 0.01	0.02, 0.02
Broccoli	0.38, 0.41	0.32, 0.34
Broccoli	0.02, 0.04	0.03, 0.02

Tasks:

1. Evaluate the results and decide:
   (a) if the application of WG and SL formulations results in similar or different reside levels.
   (b) whether the trial data can be considered independent or not
2. Select the residues suitable for estimation of maximum residue values

Exercise 7.3 Evaluation of azoxystrobin residue in stone fruits

Supervised trials with azoxystrobin used as a foliar treatment on stone fruits (cherry, peach, and plum) were conducted in the USA.

The GAP of the USA for stone fruit specifies 0.28 kg ai/ha with maximal seasonal application of 1.7 kg ai/ha (6 applications at 7-14 day intervals) and a PHI of 0 days.

Seven trials on sweet *cherry* were conducted at the GAP rate with 8 applications. Azoxystrobin residues in cherry, in ranked order, were (n = 7): 0.20, 0.42 (2), 0.45, 0.50, 0.98, and 1.0 mg/kg.

Fourteen trials on *peach* were conducted at the GAP rate with 8 applications. Azoxystrobin residues in peach, in ranked order, were (n = 14): 0.28, 0.38, 0.41, 0.60, 0.64, 0.72 (2), 0.73, 0.74, 0.83, 0.84, 0.86, 0.89, 0.94, and 1.4 mg/kg.

Eight trials on *plum* were conducted at the GAP rate with 8 applications. Azoxystrobin residues in plum, in ranked order, were (8): 0.02, 0.09, 0.24 (2), 0.25, 0.30, 0.37, and 0.42 mg/kg.

Task:

1. Based on the principles described in the lecture on 'Evaluation of supervised trial data. Estimation of maximum residue levels and STMR and HR values' evaluate the trial results corresponding to US GAP.
2. Test applying the Kruskal-Wallis test whether the residue populations are statistically significantly different or not.
3. Decide whether you estimate maximum residue levels, HR and STMR values for cherry peach and plum or recommend a group MRL.
4. Provide the estimated values.

Exercise 7.4 Checking validity of report on supervised trial with triazophos in rice

The report was submitted by a national Government describing a trial which was conducted by a university. Report on method validation was also submitted, but not included in the exercise.

Task:

1. Check the content of the report for completeness of essential information and clarity.
2. Identify missing or ambiguous information.
3. Decide if the report meets basic requirements and can be accepted or not

REPORT ON PESTICIDE RESIDUE TRIAL. PART A. FIELD REPORT **page 1**

1. RESPONSIBILITY

1 YEAR	2008	3 Company or Organization Name and Address	Institute of Pesticide and Environmental Toxicology Z University XX XX
2.Trial identity or number	200814/A-01-02		
4. Person (s) responsible for (include signature)	a. Trial design XY1 b. Application XY2 c. Sampling XY2 d. Analysis XY3		

2. IDENTITY OF TRIAL

5 Active ingredient (s) (common name)	6 Class of pesticide or agricultural use	7 Trade name (s) or Code number (s)	8 Formulation		
			Type	Conc'n in SI units	Comm/ Exper'l
Triazophos	Insecticide	-	EC	200 g/l	Comm

CROP/COMMODITY location

9. Type	Rice	12 Country / Region	
10 Variety/ Cultivar	Songjing No7	13 Site or Map ref. (include address)	Hx City, P1 Province
11 Codex commodity classification	GC 0649		

14 Pests / Diseases	Chilo suppressalis, paddyborer

REPORT ON PESTICIDE RESIDUE TRIAL. PART A. FIELD REPORT. page 2

3. GENERAL INFORMATION ON THE TRIAL.	Trial identity or number	200814/A-01-02
15 Crop production System or lay-out. e.g. commercial orchard glasshouse; crop planting date; age of crop; guard rows; SOIL TYPE	Commercial field Guard row : 4 rows of paddy Soil type : red sandy soil	

Plot data

16 plot dimensions in International units	6 x 5m	19 Crop spacing	20 x 25 cm
17 Number of plots per treatment (replicates)	3	20 Number of plants per plot (if relevant)	
18 Number of control plots	3	21 Number of rows per plot (if relevant)	

22 Previous year's pesticide treatment	- -
23 Other pesticides applied to the plot (Rates and times) during trial.	
24 Cultural treatments e.g. irrigation, fertilisers	Irrigation : Irrigation Fertilizer : chemical
25 Summary of climatic conditions. e.g. temperature (°C) rainfall wind sunlight (attach details if available)	

Month	.Max.Temp (℃)	.Min.Temp (℃)	Aver.Temp. (℃)	Rainfall (mm)	Rainy days (days)
7	32.5	18.2	24.4	92.9	11.0
8	33.1	15.4	23.2	46.1	5.0
9	20.1	8.5	14.1	44.8	6.0

REPORT ON PESTICIDE RESIDUE TRIAL. PART A. FIELD REPORT. page 3

4. APPLICATION DATA.

Trial identity or number	200814/A-01-02

26 Method/Equipment Type of application e.g. spray to run-off, band, overall. volume applied	Knapsack sprayer	
27 Dose rate	0.45kg a.i./ha	
28 Dilution or spray Conc'n in SI units	0.075 kg a.i./hl	Spray volume 600 l/ha
29 Numbers of applications	3	
30 Dates of applications	6 Aug 2008 16 Aug 2008 26 Aug 2008	
31 Growth state at last treatment *	Heading	

* Internationally recognized scales if available

5. SAMPLING

32 Control and treated			
33 Sampled part of crop	Rice	34 Growth stage t sampling	mature
35 Method of Sampling	Simple random sampling Random up to 2 kgs.		
36 No of samples per plot	1	38 Sample Weight and treatment	Sampling from primary sample about 800-1000grams to chop and weigh 200 grams for analysis.
37 No of units in Primary sample	2 kgs.		

39 dates

sampling	16/9	23/9			-
freezing	-	-			-
receipt in laboratory	19/9	26/9			-

40 intervals (days)

Last treatment/sampling	21	28				-	
sampling/freezing	-	-			-	-	-
sampling/receipt in laboratory	3	3			-	-	-

REPORT ON PESTECIDES RESIDUE TRIAL. PART B. ANALYTICAL REPORT

Person (s) responsible for the analysis Mr. XX3

IDENTITY OF SAMPLE (Please type or use BLOCK capitals)

Crop Commodity	Rice	Sample identity or number	200814/A-01-02
Pesticide (s) used on samples	Triazophos		

CONDITION AND TREATMENT OF SAMPLE (S)

Date (s) of receipt in laboratory	19/9,26/9	Date (s) of analysis	11/10,15/10
Sample treatment	Sampling from primary sample about 800-1000 grams to chop and weigh 200 grams for analysis.		
Method of storage and condition of sample (s)	Freezer –20□C		
Portion of sample (s) to be analysed	Rice		

ANALYSIS

Method of analysis (or reference) and/or modifications	• Weigh20.0 g of smashed rice sample in the 250 ml borosilicate centrifuge bottle. Three replicates are prepared for every sample. • Label according to label detailed in Registry Log Book. • Add 50mL of acetonitrile, than mix it by homogenizer for about 2 minutes • Filter to 100mL cylinder with stopper • Add 5g NaCl and 8g MgSO4 to cylinder • Cap the cylinder and shake the cylinder for about 2 minutes • Put it aside for about 20 minutes or until a clear solution is obtainable. • Decant 25 ml of supernatant into 100 ml pear-shaped flask. • Concentrate to 1-2mL by evaporator at 40 ℃ water bath • Carry out silica gel clean-up to the extract.
Extraction: Clean-up	Column clean up using silica gel sorbent • Pack the column with 5g silica gel • Wet the silica gel with 30 ml of petroleum ether :ethyl acetate(1:1v/v). Do not let the sorbent dry. • Discard the wash solution. • Place a 100ml pear-shaped flask at the bottom of the column, which acts as collection flask for clean-up extract. • Load extrator into the coloum. • Wash the silica gel with 40 ml of petroleum ether :ethyl acetate(1:1v/v). • Reduce the volume of the eluate to almost dryness by a gentle stream of nitrogen and make up to 1.0 ml with acetone. • Keep the purified extract in freezer (< 0 ^{o}C) prior to GC determination.
Method of determination and expression of residue	Residue determination in GC-FPD (Agilent 6890 Series) using HP-5 column cross-linked with phenyl methyl silicone with phase ratio of 250 (film thickness 0.25 µm, i. d. 0.25 mm & length 30 m). Carrier gas Nitrogen was set at 2.0 ml/minute. Make up gas, nitrogen was set at 50-60 ml/minute. Sample injection volume was 2 µl. The temperature for injector and detector were set at 230 °C and 250 °C, respectively. Temperature programming: Initial temperature of 80 °C was held for 1 min. It was increased to 180 °C at the rate of 50 °C/minute and held for 1min,than increased to 240 °C at the rate of 10 °C/minute. The latter temperature was maintained for 7 minutes. Residue was expressed in mg/kg.

Recoveries	85.6 % (0.05 mg/kg), 99.7 % (1.0 mg/kg)
Limit of determination	0.01 mg/kg

RESULTS

Dosage Rate	0.45 kg a.i./ha (0.075 kg a.i/hl)
Interval (Treatment to sampling)	21and 28 days
Residue* (Not corrected for recovery or control)	0.598 and 0.513mg/kg
Control (including standard deviation)	ND for all

ND = not detected

Other information e.g. stability of residues under storage conditions:0.05mg/kg ,20-60days,0.042-0.051mg/kg

*give mean values range and number of analyses

Exercise 7.5: Evaluation of supervised trials conditions in *Lychee*

Introduction

Supervised trials were conducted with the pesticide 'acar' in the United States. The major metabolite of the active substance is converted back to the parent compound and the sum of the residues is measured and reported.

The US GAP for tropical fruits is summarized hereunder.

Crop	Country	Formulation (g ai/l or g ai/kg)	Application				PHI Days
			kg ai/ha	Water, l/ha	kg ai/hl	No.	
Tropical fruits[3]	USA	480 SC/ 500 WP	0.40-0.56	468	0.09- 0.12	1	1

[3]: Guava, Lychee, Papaya, Star apple, Black sapote, Mango, Sapodilla, Canistel, Mamey, Longan, Spanish lime, Rambutan, Pulasan, Fejioa, Jaboticaba, Wax jambu, Starfruit, Passionfruit, Acerola

Tasks:

Taking into consideration the checklist for evaluation and reporting the summary of supervised trials:

- Validate the study conditions (study material, test system, compliance with GAP, application conditions, sampling and analysis, etc.)
- Identification of independent trials
- Summarise the residues in tabulated form
- Select residue data suitable for estimation of maximum residue levels

Extract of Trial report on lychee[33]

EXPERIMENTAL DESIGN

The purpose of this study was to collect and analyze treated and untreated residue samples from appropriate field sites according to the application parameters requested to provide the sponsor with residue chemistry data to support a pesticide tolerance.

The U.S. EPA OPPTS 860 Series Guidelines recommend that one residue trial be conducted on lychee. Three trials were conducted for this study in Florida (EPA Region 13).

- **Study Site Information**

Each field trial consisted of one untreated control plot and one treated plot. Treated plots contained 12 trees each. Common cultural practices were followed to maintain the crop, and additional maintenance pesticides and fertilizers were used at the sites to produce a commercial quality crop. Trial site conditions and use pattern data are summarized in Table .1 and Table 2, respectively. Appendix 1 provides additional information about the sites.

[33] This part of the report was used as an exercise with the permission of the data owner. Appendices of the report are not copied here.

Table 1 Trial Site Conditions

Trial ID (City, State)	Trial Start Year	Soil Characteristics			
		Type	%OM	pH	CEC (meq/100 g)
04-FL33 (Homestead, FL)	2004	Loam	3–10	7.4–8.4	Not reported
04-FL34 (Homestead, FL)	2004	Loam	3–10	7.4–8.4	Not reported
04-FL35 (Homestead, FL)	2004	Loam	3–10	7.4–8.4	Not reported

At each of the three trials, temperature and precipitation data were reported to be within normal parameters.

Table 2 Study Use Pattern

			Application						
Trial ID (City, State)	Trial Start Year	EP[1]	Method/ Timing	GPA[2]	Rate (lb ai/A)	RTI[3] (days)	Total Rate (lb ai/A)	Tank Mix Adjuvants	Harvest Procedures[4]
04-FL33 (Homestead, FL)	2004	Acarmite® SOWS	Foliar directed/Fruiting/ 21 days prior to harvest	126.1 1	0.50	—		Ultra-Fine Oil	
			Foliar directed/Fruiting/ 1 day prior to harvest	127.54	0.51	20	1.01	Ultra-Fine Oil	—
04-FL34 (Homestead, FL)	2004	Acarmite® SOWS	Foliar directed/Fruiting/ 22 days prior to harvest	128.32	0.51	—		Ultra-Fine Oil	
			Foliar directed/Fruiting/ 1 day prior to harvest	127.11	0.51	21	1.02	Ultra-Fine Oil	-
04-FL35 (Homestead, FL)	2004	Acarmite® SOWS	Foliar directed/Fruiting/ 22 days prior to harvest	127.43	0.51	—		Ultra-Fine Oil	
			Foliar directed/Fruiting/ 1 day prior to harvest	128.42	0.51	21	1.02	Ultra-Fine Oil	—

- **Sample Handling and Preparation**

At each trial, duplicate samples of commercially mature lychees were collected from each plot 1 day after the final application in a manner to assure a representative, impartial sample. Each sample initially weighed at least 6.75 lb. Samples were stored frozen within 35 minutes of collection. The weight of each frozen sample was later reduced to approximately 4.5 lb by impartially choosing fruit and placing it into a different bag. Samples were returned to frozen storage and shipped frozen via Federal Express to Crompton Co., Ontario, Canada, for extraction and analysis.
Upon arrival at the analytical laboratory, the samples were assigned individual identification numbers and stored frozen at temperatures between -28.9 and -19.9 °C until sample preparation. The samples were smashed with a hammer, homogenized with dry ice, and returned to frozen storage until extraction for analysis.

- **Analytical Methodology**

Samples were analyzed for combined residues of acar and D3598 using the working method, "Determination of Combined Acar and D3598 Residues in Lychee". No modifications were made to improve the performance of the method. This working method is based on the Ricerca, Inc. method "Analytical Method for the Analysis of D2341 and D3598 Residues in Apples and Citrus". A copy of the working method can be found in Appendix V of the Analytical Summary Report in Appendix 2. (not copied here)

Briefly, residues of acar and D3598 were extracted from homogenized lychee by blending twice with 100 mL acetonitrile and acetic acid (0.1%) solution. The extracts were combined and filtered, and the volume was brought up to 250 mL with acetonitrile. A 50-mL aliquot of the sample was partitioned with methylene chloride and 2% aqueous sodium sulfate. The methylene chloride phase from the partition was evaporated to just dryness. The residue was taken up in acetonitrile and cleaned up using an amino propyl extraction column. The resulting eluant was evaporated to just dryness and the extract diluted with sample dilution solvent (HPLC mobile phase containing ascorbic acid [0.1%]). After at least 2 hours of incubation, the samples were analyzed for acar by reversed-phase HPLC with oxidative coulometric electrochemical detection. The ascorbic acid added to the sample ensured that residues of D3598 were converted to acar and were, therefore, in the oxidative mode.
The lowest level of method validation (LLMV) in this study was 0.01 ppm for each analyte. Based on recoveries of samples fortified at the LLMV, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 0.002 ppm and 0.0059 ppm, respectively, for acar and as 0.001 ppm and 0.0041 ppm, respectively, for D3598.
Treated samples were analyzed within 1 day of extraction. Analytical sets typically consisted of calibration standards, unfortified controls, fortified controls, and treated samples. The analytical standard solutions were stored frozen at -15 to -10 °C. Appendix 4 contains OECD GLP Certificates of Analysis for the analytical reference standards.(not copied)

Table 1: Summary of method validation data performed with acar and metabolite in fruits and vegetables

Analyte	Fortification (mg/kg)	No. of tests (n)	Recoveries (%)	Mean (%)	RSD (%)
Lychee					
Acar	0.01	6	75.8[1], 76.0[1], 81.3[1], 102, 117, 110	93.8	20
	0.1	3	98.9, 95.0, 95.2	96.4	2.3
	1	3	92.1, 91.7, 92.6	92.2	0.44
	0.01- 1	**12**	**75.8-117**	**94**	**13**
Acar metabolite	0.01	6	83.6[1], 77.1[1], 89.1[1],	99.2	18

Analyte	Fortification (mg/kg)	No. of tests (n)	Recoveries (%)	Mean (%)	RSD (%)
			117.3, 113.6, 114.7		
	0.1	3	84.2[1], 85.6[1], 83.2[1]	84.3	1.4
	1	3	77.3[1], 80.2[1], 77.1[1]	78.2	2.2
	0.01- 1	**12**	**77.1- 117.3**	**90.2**	**17**
	0.1	3	70.1, 70.3, 74.1	71.5	3.1
	1	3	80.2, 78.9, 78.1	79.1	1.4
	0.01- 1	**9**	**70.1- 92.9**	**79.7**	**10**

[1] Corrected for average residue detected in the corresponding control samples.

[2] For the acar sample set (0.01 first three results, and at 0.5), the control samples had an average residue of 0.003 mg/kg. Since this is less than half of the LOQ (of 0.01 mg/kg), all recovery results, averages and % RSD values for this set are uncorrected. For the acar (0.01 last three results), acar metabolite (0.01, 0.5) sample set, the control samples had an average residue of 0.006 mg/kg. Since this is greater than half of the LOQ (of 0.01 mg/kg), all recovery results, averages and %RSD values for this set are corrected for this residue.

The stability of residues of acar and its metabolite in lychee samples under frozen conditions was investigated (GRL-12272). Untreated un-homogenized samples of lychee fruit were fortified with acar at 0.1 mg/kg. Samples were placed in frozen storage at -19.2 to -27ºC and analyzed at storage intervals of 0, 0.25, 1, 2, 5, 8, and 10 months. At each sampling period, an untreated control was freshly fortified with acar at 0.1 mg/kg and analyzed at the same time as the stored samples with the validated method described in the previous section. The reported LOQ is 0.01 mg/kg for each analyte in/on lychee. Three freshly fortified samples of lychee fruit were analyzed at 0 time.

Table 2: Stability of acar residues in lychee following frozen storage at -19.2 to -27ºC

Fortification (mg/kg)	**Storage interval (months)**	**Procedural recovery[1] (%)**	**Residues in stored fortified samples (mg/kg)[2]**	**Average uncorrected residues remained (%)**	**Residues remained [3]**
0.10	0	112	0.105, 0.110, 0.115	112	-
	0.25	101	0.061, 0.067, 0.071	67.2	67
	1	76.6	0.041, 0.045, 0.049	45.0	58.7
	2	88.9	0.069, 0.069, 0.218[4]	68.8	77.4
	5	68.6	0.041, 0.042, 0.047	43.9	64.0
	8	54.3	0.021, 0.028, 0.061	37.0	68.2
	10	79.9	0.047, 0.052, 0.060	54.6	68.4

[1] Average recovery obtained from two freshly spike untreated test portions.

[2] All residues with the exception of 0-day interval were corrected for apparent residues (0.01 to 0.06 mg/kg).

[3] Corrected for procedural recovery

[4] The report indicated that a sample preparation error was suspected with this sample and therefore its result was not used to determine the mean recovered residues and recoveries.

The results indicate that average acar residue (corrected for apparent residues in untreated samples) remaining after one week storage were significantly lower than the day 0 residues (t-test, equal variances). The average procedural recoveries and the residues measured in stored test portions showed relatively high variation. After one week, the degradation of acar became much slower and after correction for procedural recoveries (54-79.9%) it was around 70% of the day 0 residues.

RESULTS AND DISCUSSION

Three lychee trials were conducted in Florida representing NAFTA growing region 13. The number of trials is adequate for lychee. The lychees were grown in loam. At each trial, environmental conditions were within normal parameters.

At each trial, two applications of approximately 0.5 lb ai/A each were made, for a total of approximately 1.0 lb ai/A. The initial applications were made at the fruiting stage; the second applications were made 20 to 21 days later and timed so that commercially mature lychee could be collected 1 day after the final application.

Method validation was performed as a separate study (see Volume 4 of this submission). In that study, control samples were fortified separately with acar and D3598 at levels ranging from 0.01 to 1 ppm. In this study, concurrent recoveries were obtained for control samples fortified at levels ranging from 0.01 to 1 ppm. Since treated samples yielded residues >1.0 ppm, method validation at 3 ppm was performed. Method validation recoveries of samples fortified with 3 ppm acar ranged from 89.4 to 104%, while method validation recoveries of samples fortified with 3 ppm D3598 ranged from 74.7 to 76.5%.

Recoveries of samples fortified with acar at the LLMV ranged from 75.8 to 119% with an average recovery of 103± 17% (n = 12). The average recovery of all acar fortifications was 89.4 ± 20.1% (n = 34). For D3598, recoveries of samples fortified at the LLMV ranged from 77.1 to 118%, with an average recovery of 107 ± 15.5% (n = 12). The average recovery of all D3598 fortifications was 94.9 ± 17.8% (n = 22). The LOD and LOQ were statistically calculated as 0.002 ppm and 0.0059 ppm, respectively, for acar and as 0.001 ppm and 0.0041 ppm, respectively, for D3598. Chromatograms are shown in Appendix 2. A six-point standard curve was generated each time an analysis set was run; r^{22} was always ≥ 0.999.
The maximum storage interval for field-treated samples in this study was 302 days. Since sample analysis for this study did not occur within the 30 days required by the protocol, a freezer stability study was performed outside of this study phase.
Residues ranged from 1.545 to 2.594 ppm acar equivalents (see Table 3). The highest residues were observed in samples from the 04-FL34 trial (2.553 ppm and 2.594 ppm), representing the highest average residue of 2.574 ppm).

The details of field trials are reported in the field trial summaries.
Table 6: Residues of acar resulting from supervised trials on lychee carried out with 500 WP formulations in USA in 2004

Corp/Location,	Application					PHI	Residue		Reference/
	kg ai/ha	l/ha	kg ai/hl	No.	RTI	days	mg/kg		Field ID
US GAP: 500 WP/ 480 SC, at 0.40-0.56 kg ai/ha (0.09-0.12 kg ai/hl, number of applications 1, PHI 1 day									
Mauritius	0.56	1188	0.05	2	20	1	2.0	2.9[1]	PR 08268
Homestead, FL	0.57						1.9	2.8[1]	04-FL33
Mauritius	0.57	1193	0.05	2	21	1	2.6	3.6[1]	PR 08268
Homestead, FL	0.57						2.6	3.7[1]	04-FL34
Mauritius	0.57	1193	0.05	2	21	1	2.3	3.3[1]	PR 08268
Homestead, FL	0.57						1.5	2.2[1]	04-FL35

RTI: retreatment interval
1./ The residues reported are corrected for the loss during storage.

FIELD DATA SUMMARIES

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL33
Field Research Director (FRD): J.P. Cool Address of FRD:
Other Field Personnel: Osvany Rodriguez

TEST SUBSTANCE RECORDS (Separate page for each formalation or lot no.)	
Test Substance (Name on Container Label)/Batch or Lot No.: Acarmite® 50WS/BA3K13P008	
Source: Crompton Corporation, 74 Amity Road, Bethany, CT 06524-3406	
Date Received: 18 Feb 2004	Expiration Date[1]: 12 Jan 2006
Spray Additives (Adjuvants) Used: Ultra-Fine Oil	
Storage Location: University of Florida,	
Storage Temperature Range (from receipt of test substance to last application): Approx. 48—92 °F	

TRIAL SITE INFORMATION			
Test Site (Name, Street, Town, State): Block 7, Tropical Research and Education Center (TREC), Institute *of* Food and Agricultural Sciences, University *of* Florida, 18905 S.W. 280 Street, Homestead, Dade County, FL 33031-3314			
Soil Texture/Type: Loam	%Sand: Not reported	%Silt: Not reported	%Clay: 15—20
	%Organic Matter: 3—10		Soil pH: 7.4—8.4
Crop Variety: Mauritius lychee			
Field Planting Date (Seeded or Transplanted) or Age of Established Crop X : 1998			
Row Width: 25 *ft*	Plant Spacing: 15 *ft*	No. Rows_ /Trees X per Plot: 18 (TRT 01) 12 (TRT 02)	
Control Plot Dimensions: 75 ft x 90 *ft*		Treated Plot Dimensions: 50 ft x 90 ft	

[1]

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL33 (Unless otherwise specified, amounts were applied to 4.13 acres.)

Maintenance Fertilizers and Pesticides applied during the year(s) of the field trial (Product/Date)	
Intrepid (41 oz, 02 Feb 2004; 05 Feb 2004)	Confirm (41 oz, 02 Feb 2004)
8-3-9 (3–5 lb/tree, 11 Feb 2004)	Minors #1(NR[1],12 Feb 2004; 03 Mar 2004; 30 April 2004; 04 May 2004; 22 June 2004)
Manzate (81b, 24 Feb 2004; 30 Mar 2004; 07 Apr 2004; 28 Apr 2004; 12 May 2004)	Switch (35 oz, 02 Mar 2004; 16 Mar 2004; 23 Mar 2004; 14 Apr 2004; 12 May 2004)
Ferragro (6 qt, 03 Mar 2004)	Dynagro (75 oz, 03 Mar 2004)
Tech. Mag (12 lb, 03 Mar 2004)	Sequestrene 138 (1–2 gal/tree, 08 Mar 2004)
Admire 4E (1–2 gal/tree, 27 Mar 2004)	Spintor (35 oz, 06 Apr 2004)
8-3-9 (3 lb/tree, 14 Apr 2004; 03 Jun 2004)	
Abound (30 oz, 04 May 2004)	Plyac (6 oz, 04 May 2004)
8-3-9 (4–5 lb/tree,14 May 2004)	Sequestrene #2 (24 May 2004; 14 June 2004)
Sequestrene #3 (25 May 2004, 15 June 2004)	Supercide 25WP (6 oz, 03 Jun 2004)

[1] NR = Not reported.

FIELD DATA SIJMMARY

Pesticide/Crop/Field ID No: Aca/Lychee/08268.04-FL33

APPLICATION RECORDS (Separate page for each calibration/application)		
Application 1 Date: 18 May 2004	Output Calibration/Re-Check Date: 17 May 2004	Days between Applications: NA[1]
Application Equipment Type: Tractor-mounted airblast sprayer		Propellant: Pump
Type of Application: Foliar directed		
No. Nozzles/Outlets: 13	Nozzle Spacing (inches): NA	Screen Mesh; 150
Nozzle Brand/Type/Size: T-Jet/Stainless Steel Flat Fan/l 1502		Spray Swath Width: NA
Treated area 4500 ft^2	Delivery Rate[2]: 126.11 GPA	
Test Substance: Acarmite®SOWS		Batch/Lot No.: BA3K13P008
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water): 60,560 mL (16.0 gal) Formulated Product: 58 g Additives (Adjuvants): 152 mL Total Mix Volume: 60,712 mL		
Protocol Rate lb ai/A Treatment No. 02 0.5		Actual Ap~lied Rate lb ai/A [4] 0.50 (1.01X)
Crop Growth Stage: Fruiting		Crop Height: 10—12 ft
Wind Speed and Direction: 4.3 mph/SE		Air Temperature: 89 °F
First Rain after Application: Date: 04 Jun 2004		Amount (inches): 0.03
Time after Application of First Rain (Days or Hours): 17 days		
First Irrigation after Application: Date: 19 May 2004		Amount: 26 gal/tree
Irrigation Type: Micro irrigation under tree	Time after Application of First Irrigation (Days or Hours): 1 day	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

[1] NA = Not applicable.

a Gallons of water delivered per acre, as determined using actual application data.

[3] Note: Treatment No. O1 is the untreated control.

[4] Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL33

APPLICATION RECORDS (Separate page for each calibration/application)		
Application 2 Date: 07 Jun 2004	Output Calibration/Re-Check Date: 07 Jun 2004	Days between Applications: 20
Application Equipment Type: Tractor-mounted airblast sprayer		Propellant: Pump
Type of Application: Foliar directed		
No. Nozzles/Outlets: 13	Nozzle Spacing (inches): NA'	Screen Mesh: 150
Nozzle Brand/Type/Size: T-Jet/Stainless Steel Flat Fan/l 1502		Spray Swath Width: NA
Treated Area: 4500 ft^2	Delivery Rate[2]: 127.54 GPA	
Test Substance: Acarmite®SOWS		Batch/Lot No.: BA3K13P008
Tank Mix Amounts Trt.:	Treatment No. 02 [3] Carrier (Water): Formulated Product: 58 g Additives (Adjuvants): Total Mix Volume:	 60,560 mL (16.0 gal) 152 mL 60,712 mL
Protocol Rate lb ai/A Treatment No. 02	0.5	Actual A~plied Rate lb ai/A a 0.51(1.02X)
Crop Growth Stage: Fruiting		Crop Height: 10—12 ft
Wind Speed .and Direction: 1.8 mph/SE		Air Temperature: 89 °F
First Rain after Application:	Date: 08 Jun 2004	Amount (inches): 0.34
Time after Application of First Rain (Days or Hours): 1 day		
First Irrigation after Application:	Date: 07 Jun 2004	Amount: 26 gal/tree
Irrigation Type: Micro irrigation under tree	Time after Application of First Irrigation (Days or Hours): < 1 day	
Were any phytotoxic effects seen? Yes	No X Unrecorded	
Description of the severity and/or symptoms of any phytotoxic effects: NA		

' NA = Not Applicable

a Gallons of water delivered per acre, as determined using actual application data.

3 Note: Treatment No. O1 is the untreated control.

a Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

FIELD DATA SUMMARY

PesticidelCrop/Field ID No: Acar/Lychee108268.04-FL33

SAMPLE COLLECTION/STORAGE (separate page for each crop fraction/sampling date)		
Harvest Date: 08 Jun 2004	Sampling Date: 08 Jun 2004	PHI: 1 day
Description of Crop Stage/Fraction at Harvest: Commercially matere lychee		
Harvesting Equipment: Gloved hands and hand pruners		
Procedures Utilized in Harvesting: TRT O1 then TRT 02. A minimum of 80 fruit per sample was picked from 12 trees for TRT Ol and 8 trees for TRT 02 from all sides of the rows, avoiding 1 tree at each row end, to obtain a minimum sample size of 6.75 lb.		
Modifications after Harvest (e.g., Trimming, Cleaning, Cutting, Drying, Compositing): The weight of each frozen sample was fater reduced to 4.5 ib by randomly choosing fruit and piacing it into the sample bag until a weight of 4.5 lb was reached.		
Holding and Transport of Samples from Field to Freezer (or between Field and Shipment): Samples were hand-clipped into bags and transported in coolers with jeli packs to the freezers.		
Maximum Elapsed Time from Treated Sample Collection to Frozen Storage: 5 minutes		
Freezer Temperatere Range(s) (prior to shipment): Approx. -15 to -1 °F (TRT 01) Approx. -19 to -7 °F (TRT 02)		
Shipped: Frozen, Packed in Dry Ice X	Fresh, Packed in Dry Ice	Fresh (not in Dry Ice)
Name of Carrier: Federal Express		Shipment Date: 22 Jun 2004

METEOROLOGICAL INFORMATION

Were any unusual weather events noted for this test site during the field trial? Yes No X

Description of any unusual weather occurrences: None indicated.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL34
Field Research Director (FRD): J.P. Cool UF-IFAS-TREC 18905 S.W
Other Field Personnel: O.V Simpson

TEST SUBSTANCE RECORDS Se arate a e for each formulabon or lot no.	
Test Substance Name on Container Label /Batch or Lot No.: Acarmite®SOWS/BA3K13P008	
Source: Crom ton Co oration, 74 Ami Road, Bethan , CT 06524-3406	
Date Receíved: 18 Feb 2004	Ex iration Date[1]: 12 Jan 2006
S ra Additives Ad uvants Used: Ultra-Fine Oil	
Storage Location: Pesticide Building, University of Florida,	
Storage Temperature Range from receipt of test substance to last application : Approx. 48—92 °F	

TRIAL SITE INFORMATION			
Test Site (Name, Street, Town, State): Block 7, Tropical Research and Education Center (TREC), Institute of Food and Agricultural Sciences, University of Fiorida,18905 S.W. 280 Street, Homestead, Dade Coun , FL 33031-3314			
Soil Texture/Type: Loam	% Sand Not reported	%Silt: Not reported	%C lay: 15—20
	% Organic Matter: 3—10		Soil H: 7.4—8.4
Crop Variety:Mauritius lychee			
Field Planting Date Seeded or Transplanted or Age of Established Crop X :1998			
Row Width: 25 ft	Plant Spacing: 15 ft	No. Rows_ 1Trees X per Plot: 18 (TRT O 1) 12 TRT 02	
Control Plot Dimensions: 75 ft x 90 ft		Treated Plot Dimensions: 50 ft x 90 ft	

[1] As determined by the registrant or characterization laboratory.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL34

(Unless otherwise specified, amounts were applied to 4.13 acres.)

Maintenance Fertilizers and Pesticides applied during the year(s) of the field trial (Product/Date)	
Intrepid (41 oz, 02 Feb 2004; 05 Feb 2004)	Confirm (41 oz, 02 Feb 2004)
8-3-9 (3–5 lb/tree, 11 Feb 2004)	Minors #1(NR[1],12 Feb 2004; 03 Mar 2004; 30 April 2004; 04 May 2004; 22 June 2004)
Manzate (81b, 24 Feb 2004; 30 Mar 2004; 07 Apr 2004; 28 Apr 2004; 12 May 2004)	Switch (35 oz, 02 Mar 2004; 16 Mar 2004; 23 Mar 2004; 14 Apr 2004; 12 May 2004)
Ferragro (6 qt, 03 Mar 2004)	Dynagro (75 oz, 03 Mar 2004)
Tech. Mag (12 lb, 03 Mar 2004)	Sequestrene 138 (1–2 gal/tree, 08 Mar 2004)
Admire 4E (1–2 gal/tree, 27 Mar 2004)	Spintor (35 oz, 06 Apr 2004)
8-3-9 (3 lb/tree,14 Apr 2004; 03 Jun 2004)	
Abound (30 oz, 04 May 2004)	Plyac (6 oz, 04 May 2004)
8-3-9 (4–5 lb/tree,14 May 2004)	Sequestrene #2 (24 May 2004; 14 June 2004)
Sequestrene #3 (25 May 2004, 15 June 2004)	Supercide 25WP (6 oz, 03 Jun 2004)

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL34

APPLICATION RECORDS (Separate page for Bach calibration/application)		
Application 1 Date: 19 May 2004	Output Calibration/Re-Check Date: 19 May 2004	Days between Applications: NA[1]
Application Equipment Type: Tractor-mounted airblast sprayer		Propellant: Pump
Type of Application: Foliar directed		
No. Nozzles/Outlets: 13	Nozzle Spacing (inches): NA	Screen Mesh: 150
Nozzle Brand/Type/Size: T-Jet/Stainless Steel Fiat Fan/11502		Spray Swath Width: NA
Treated Area: 4500 ft^2		Delivery Rate[2]: 128.32 GPA
Test Substance: Acarmite® 50WS		Batch/Lot No.: BA3K13P008
Tank Mix Amounts, Trt.: Carrier (Water): Formulated Product: Additives (Adjuvants): Total Mix Volume:	Treatment No. 02 [3] 60,560 mL (16.0 gal) 58 g 152 mL 60,712 mL	
	Protocol Rate [lb ai/A]	Actual Applied Rate jlb ai/A][4]
Treatment No. 020.5	0.51	0.51 (1.02X)
Crop Growth Stage: Fruiting		Crop Height: 10–12 ft
Wind Speed and Direction: 3.8 mph/SE		Air Temperature: 89 °F
First Rain after Application:	Date: 04 Jun 2004	Amount (inches): 0.03
Time after Application of First Rain (Days or Hours): 16 days		
First Irrigation after Application:	Date: 19 May 2004	Amount: 26 gal/tree
Irrigation Type: Micro irrigation under tree	Time after Application of First Irrigation (Days or Hours):	1 day
Were any phytotoxic effects seen? Yes	No X	Unrecorded
Description of the severity and/or symptoms of any phytotoxic effects: NA		

1 NA = Not applicable.

2 Gallons of wftter delivered per acre, as determined using actual application data.

3. Note: Treatment No. 01 is the untreated control.

4. Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL34

APPLICATION RECORDS (Separate page for each calibration/application)		
Application 2 Date: 09 Jun 2004	Output Calibration/Re-Check Date: 09 Jun 2004	Days between Applications: 21
Application Equipment Type: Tractor-mounted airblast sprayer		Propellant: Pump
Type of Application: Foliar directed		
No. Nozzles/Outlets: 13	Nozzle Spacing (inches): NA[1]	Screen Mesh: 150
Nozzle Brand/Type/Size: T-Jet/Stainless Steel Fiat Fanll 1502		Spray Swath Width: NA
Treated Area: 4500 ft^2	Delivery Rate[2]: 127.11 GPA	
Test Substance: Acarmite® 50WS		Batch/Lot No.: BA3K13P008
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water): 60,560 mL (16.0 gal) Formulated Product: 58 g Additives (Adjuvants): 152 mL Total Mix Volume: 60,712 mL		
	Protocol Rate jlb ai/A]	Actual Applied Rate jlb ai/A][4]
Treatment No. 02	0.5	0.51(1.01X)
Crop Growth Stage: Fruiting		Crop Height: 10–12 ft
Wind Speed and Direction: 1.2 mph/SE		Air Temperature: 82 °F
First Rain after Application: Date: 10 Jun 2004		Amount (inches): 0.11
Time after Application of First Rain (Days or Hours): 1 day		
First Irrigation after Application: Date: 09 Jun 2004		Amount: 26 gal/tree
Irrigation Type: Under tree micro irrigation	Time after Application of First Irrigation (Days or Hours): 1 day	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

' NA = Not Applicable.

Gallons of water delivered per acre, as determined using actual application data.

Note: Treatment No. 01 is the untreated control.

Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

2 3

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL34

SAMPLE COLLECTION/STORAGE (separate page for each crop fraction/sampling date)		
Harvest Date: 10 Jun 2004	Sampling Date: 10 Jun 2004	PHI: 1 day
Description of Crop Stage/Fraction at Harvest: Commercially mature Lychee		
Harvesting Equipment: Gloved hands and clippers		
Procedures Utilized in Harvesting: TRT 01 then TRT 02. A minimum of 80 fruit per sample was randomly picked from high, low, exposed, and sheltered areas of 8 trees, avoiding 1 tree at each row end, to obtain a minimum sample size of 8.01b.		
Modifications after Harvest (e.g., Trimming, Cleaning, Cutting, Drying, Compositing): The weight of each frozen sample was later reduced to 4.51b by randomly choosing fruit and piacing it into the sample bag until a weight of 4.5 lb was reached.		
Holding and Transport of Samples from Field to Freezer (or between Field and Shipment): Samples were hand-clipped into bags and transported in coolers with jeli packs to the freezers.		
Maximum Elapsed Time from Treated Sample Collection to Frozen Storage: 15 minutes		
Freezer Temperature Range(s) (prior to shipment): Approx. -15 to -7°F (TRT 01) Approx. -19 to -7 °F (TRT 02)		
Shipped: Frozen, Packed in Dry Ice X	Fresh, Packed in Dry Ice	Fresh (not in Dry Ice)
Name of Carrier: Federal Express		Shipment Date: 22 Jun 2004

METEOROLOGICAL INFORMATION

Were any unusual weather events noted for this test site during the field trial? Yes _____ No X

Description of any unusual weather occurrences: None indicated.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL35
Field Research Director (FRD): J.P. Cool
Field Personnel: O.S. Simpson

TEST SUBSTANCE RECORDS (Separate page for each formulation or lot no.)	
Test Substance (Name on Container Label)/Batch or Lot No.: Acarmite® 50WSBA3K13P008	
Source: Crompton Corporation, 74 Amity Road, Bethany, CT 06524-3406	
Date Received: 18 Feb 2004	Expiration Date[1]: 12 Jan 2006
Spray Additives (Adjuvants) Used: Ultra-Fine Oil	
Storage Location: Pesticide Building, University of Florida, Tropical Research and Education Center (TREC),	
Storage Temperature Range (from receipt of test substance to last application): Approx. 48-92 °F	

TRIAL SITE INFORMATION			
Test Site (Name, Street, Town, State): Block 7, Tropical Research and Education Center (TREC), Institute of Food and Agricultural Sciences, University of Florida,			
Soil Texture/Type: Loam	%Sand: Not reported	%Silt: Not reported	%Clay: 15—20
	%Organic Matter: 3—10		Soil pH: 7.4—8.4
Crop Variety: Mauritius lychee			
Field Planting Date (Seeded or Transplanted) or Age of Established Crop X : 1998			
Row Width: 25 ft	Plant Spacing: 15 ft	No. Rows /Trees X per Plot: 18 (TRT 01) 12 (TRT 02)	
Control Plot Dimensions: 75 ft x 90 ft		Treated Plot Dimensions: 50 ft x 90 ft	

[1] As determined by the registrant or characterization laboratory.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL35 (Ihless otherwise specif ed, amounts were applied to 4.13 acres.)

Maintenance Fertilizers and Pesticides applied during the year(s) of the field trial (Product/Date)	
Intrepid (41 oz, 02 Feb 2004; 05 Feb 2004)	Confirm (41 oz, 02 Feb 2004)
8-3-9 (3–5 lb/tree,11 Feb 2004)	Minors #1(NR',12 Feb 2004; 03 Mar 2004; 30 April 2004; 04 May 2004; 22 June 2004)
Manzate (81b, 24 Feb 2004; 30 Mar 2004; 07 Apr 2004; 28 Apr 2004; 12 May 2004)	Switch (35 oz, 02 Mar 2004; 16 Mar 2004; 23 Mar 2004; 14 Apr 2004; 12 May 2004)
Ferragro (6 qt, 03 Mar 2004)	Dynagro (75 oz, 03 Mar 2004)
Tech. Mag (121b, 03 Mar 2004)	Sequestrene 138 (1–2 gal/tree, 08 Mar 2004)
Admire 4E (1–2 gal/tree, 27 Mar 2004)	Spintor (35 oz, 06 Apr 2004)
8-3-9 (3 lb/tree, 14 Apr 2004; 03 Jun 2004)	
Abound (30 oz, 04 May 2004)	Plyac (6 oz, 04 May 2004)
8-3-9 (4–5 lb/tree,14 May 2004)	Sequestrene #2 (24 May 2004; 14 June 2004)
Sequestrene #3 (25 May 2004, 15 June 2004)	Supercide 25WP (6 oz, 03 Jun 2004)

NR = Not reported.

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL35

APPLICATION RECORDS (Separate page for each calibration/application)		
Application 1 Date: 24 May 2004	Output Calibration/Re-Check Date: 24 May 2004	Days between Applications: NA'
Application Equipment Type: Tractor-mounted airblast sprayer		Propellant: Pump
Type of Application: Foliar directed		
No. Nozzles/Outlets: 13	Nozzle Spacing (inches): NA	Screen Mesh: 150
Nozzle Brand/Type/Size: T-Jet/Stainless Steel Fiat Fan/11502		Spray Swath Width: NA
Treated Area: 4500 ft^2	Delivery Rate[2]: 127.43 GPA	
Test Substance: Acarmite® 50WS		Batch/Lot No.: BA3K13P008
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water): 60,560 mL (16.0 gal) Formulated Product: 58 g Additives (Adjuvants): 152 mL Total Mix Volume: 60,712 mL		
	Protocol Rate [ib ai/A]	Actual Applied Rate jlb ai/A][4]
Treatment No. 02	0.5	0.51 (1.02X)
Crop Growth Stage: Fruiting		Crop Height: 10-12 ft
Wind Speed and Direction: 1.9 mphlSE		Air Temperature: 81 °F
First Rain after Application: Date: 04 Jun 2004		Amount (inches): 0.03
Time after Application of First Rain (Days or Hours): 1 1 days		
First Irrigation after Application: Date: 24 May 2004		Amount: 26 gal/tree
Irrigation Type: Under tree micro irrigation	Time after Application of First Irrigation (Days or Hours): <1 day	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

NA = Not applicable.

Gallons of water delivered per acre, as determined using actual application data.

Note: Treatment No. 01 is the untreated control.

Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

2 3 4

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL35

APPLICATION RECORDS (Separate page for each calibration/application)		
Application 2 Date: 14 Jun 2004	Output Calibration/Re-Check Date: 14 Jun 2004	Days between Applications: 21
Application Equipment Type: Tractor-mounted airblast sprayer		Propellant: Pump
Type of Application: Foliar directed		
No. Nozzles/Outlets: 13	Nozzle Spacing (inches): NA[1]	Screen Mesh: 150
Nozzle Brand/Type/Size: T-Jet/Stainless Steel Fiat Fan/l 1502		Spray Swath Width: NA
Treated Area: 4500 ft^2	Delivery Rate[2]: 128.42 GPA	
Test Substance: Acarmite® 50WS		Batch/Lot No.: BA3K13P008
Tank Mix Amounts Trt.: Treatment No. 02 [3] Carrier (Water): 60,560 mL (16.0 gal) Formulated Product: 58 g Additives (Adjuvants): 152 mL Total Mix Volume: 60,712 mL		
Protocol Rate		Actual Applied Rate
lb ai/A1		ib ai/A][4]
Treatment No. 02	0.5	0.51 (1.02X)
Crop Growth Stage: Fruiting		Crop Height: 10–12 ft
Wind Speed and Direction: 1.2 mphlSE		Air Temperature: 75 °F
First Rain after Application: Date: NA		Amount (inches): NA
Time after Application of First Rain (Days or Hours): NA		
First Irrigation after Application: Date: 14 Jun 2004		Amount: 26 gal/tree
Irrigation Type: Under tree micro irrigation	Time after Application of First Irrigation (Days or Hours): <1 day	
Were any phytotoxic effects seen? Yes No X Unrecorded		
Description of the severity and/or symptoms of any phytotoxic effects: NA		

NA = Not Applicable

Gallons of water delivered per acre, as determined using actual application data.

Note: Treatment No. 01 is the untreated control.

Based on sprayer output and applicator pass times. The value in parentheses is the Actual Applied Rate divided by the Protocol Rate.

2 3 4

FIELD DATA SUMMARY

Pesticide/Crop/Field ID No: Acar/Lychee/08268.04-FL35

SAMPLE COLLECTION/STORAGE (separate page for each crop fraction/sampling date)		
Harvest Date: 15 Jun 2004	Sampling Date: 15 Jun 2004	PHI: 1 day
Description of Crop Stage/Fraction at Harvest: Commercially mature lychee		
Harvesting Equipment: Gloved hands and clippers		
Procedures Utilized in Harvesting: TRT 01 then TRT 02. A minimum of 80 fruit per sample was randomly picked from high, low, exposed, and sheltered areas of 8 trees, avoiding 1 tree at each row end, to obtain a minimum sample size of 7.5 lb.		
Modifications after Harvest (e.g., Trimming, Cleaning, Cutting, Drying, Compositing): The weight of each frozen sample was later reduced to 4.51b by randomly choosing fruit and placing it into the sample bag until a weight of 4.5 Ib was reached.		
Holding and Transport of Samples from Field to Freezer (or between Field and Shipment): Samples were hand-clipped into bags and transported in coolers with jell packs to the freezers.		
Maximum Elapsed Time from Treated Sample Collection to Frozen Storage: 35 minutes		
Freezer Temperature Range(s) (prior to shipment): Approx. -15 to -10°F (TRT 01) Approx. -19 to -7 °F (TRT 02)		
Shipped: Frozen, Packed in Dry Ice X	Fresh, Packed in Dry Ice	Fresh (not in Dry Ice)
Name of Carrier: Federal Express		Shipment Date: 22 Jun 2004

METEOROLOGICAL INFORMATION

Were any unusual weather events noted for this test site during the field trial? Yes _____ No X

Description of any unusual weather occurrences: None indicated.

Exercise 7.6: Evaluation of supervised trials conditions in *papaya*

Introduction

Supervised trials were conducted with the pesticide active substance 'acar' in the United States.

The US GAP for tropical fruits is summarized hereunder.

Crop	Country	Formulation (g ai/l or g ai/kg)	Application[1]				PHI Days
			kg ai/ha	Water, l/ha	kg ai/hl	No.	
Tropical fruits[2]	USA	4 SC/ 500 WS	0.40- 0.56	234[3]	0.09- 0.12	1	1

[1]: The metrical units are given for convenience

[2]: Guava, Lychee, Papaya, Star apple, Black sapote, Mango, Sapodilla, Canistel, Mamey, Longan, Spanish lime, Rambutan, Pulasan, Fejioa, Jaboticaba, Wax jambu, Starfruit, Passionfruit, Acerola

[3]: Minimum amount of water

Tasks:

Taking into consideration the basic requirements of sampling sample processing and analysis evaluate the implementation of supervised trials in papaya.

- Validate the study conditions (study material, test system, compliance with GAP, application conditions,
- Identify independent trials
- Summarise the residues in tabulated form
- Select residue data suitable for estimation of maximum residue levels

The background information is given in exercised 5.2

Exercise 8.1. Evaluation of data from supervised residue trials and processing studies – Estimation of maximum residue levels

See also Chapter 8

1. Residue evaluation exercise

Pome fruits

2. The aim

The aim of this exercise is to explain the residue evaluation process, including deciding if supervised residue trials data are valid and selecting suitable data for MRL estimation and risk assessment.

3. Aim: to recommend maximum residue levels, STMRs, HRs

- Step 1. Which residue data are valid and fully supported by essential information?
- Step 2. Which trials match GAP?
- Step 3. Propose MRLs, STMRs, HRs for raw agricultural commodities.
- Step 4. Which processing trials are valid?
- Step 5. Derive processing factors, MRLs and STMR-Ps for processed commodities.

4. Step 1. Which residue data are valid and fully supported by essential information?

Residue trials checklist

- trials data
  - country
  - crop
  - crop variety
  - application conditions
  - pre-harvest interval
  - commodity analysed
  - residue expressed as residue definition
  -

5. Step 1. Which residue data are valid and fully supported by essential information?

Residue trials checklist (continued)

- analytical method used in the trials
- % recoveries
- residues in samples from control plots
- if residue in control plot is detected, is the trial still OK?
- sprayer
- plot size
- field sample size
- trial design
- interval of time sample is in freezer
- is it OK?

6. ZAPPACARB

- Fictitious pesticide and fictitious data
- Data represent a typical situation
- Typical problems

7. Freezer storage

Storage interval	Procedural recov %	Zappacarb mg/kg	Storage interval	Procedural recov %	Zappacarb, mg/kg
Homogenized apples, fortified at 0.1 mg/kg zappacarb stored at freezer temperature -24 °C to -20 °C.			**Homogenized apples**, fortified at 0.1 mg/kg zappacarb- stored at freezer temperature -24 °C to -20 °C.		
0	94% 92% 97% 109%		0	95% 96% 95% 97%	
7 days	91% 95%	0.088 0.090	7 days	94% 91%	0.101 0.095
14 days	72% 69%	0.063 0.064	14 days	70% 78%	0.076 0.074
21 days	68% 68% **1/**	0.052 0.046	21 days	63% 67% 1/	0.056 0.056
28days	73% 77%	0.060 0.066	28days	81% 81%	0.076 0.074
41days	67% 67% **1/**	0.059 0.059	41days	68% 72%	0.059 0.056
70 days	91% 88%	0.064 0.062	70 days	86% 79%	0.060 0.066
106 days	87% 88%	0.047 0.047	106 days	77% 81%	0.048 0.051
182 days	89% 91%	0.043 0.043	182 days	79% 71%	0.043 0.048
		30% decline in 106 days			**30% decline in 97 days**

1/ Procedural recovery <70% - disregard the associated data.

8. Freezer storage

Storage interval	Procedural recov %	Zappacarb mg/kg	Storage interval	Procedural recov %	Zappacarb, mg/kg
Apples, fortified **on the skin surface** at 0.1 mg/kg zappacarb stored at freezer temperature -24 °C to -20 °C.			Apples, fortified **on the skin surface** at 0.1 mg/kg zappacarb- stored at freezer temperature -24 °C to -20 °C.		
0	88% 93% 88% 90%		0	77% 76% 73% 76%	
14 days	81% 92%	0.085 0.089	14 days	81% 83%	0.079 0.095
28 days	94% 88%	0.076 0.087	28 days	79% 79%	0.084 0.072
56 days	88% 84%	0.095 0.083	56 days	89% 90%	0.074 0.071
127 days	92% 87%	0.090 0.093	127 days	90% 83%	0.077 0.054
224 days	94% 92%	0.095 0.085	224 days	86% 80%	0.076 0.086

Residues appear to be stable on the skin surface for at least 224 days

9. Residue definition

- Enforcement (MRL)
  zappacarb
- Risk assessment (dietary intake)
  zappacarb

10. Trials data 1

APPLE country, year (variety)	Application					PHI days	Commodity	Zappacarb, mg/kg 1/ 2/	Ref
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.				
USA (NY) 1998, Idared	500 WP	2.8		470	1	7	whole fruit	1.3	TRIAL C107 STUDY 419
USA (WA) 1998, Red Delicious	500 WP	2.8		460	1	7	whole fruit	2.0	TRIAL J107 STUDY 419
USA (NY) 1998, Monroe	500 WP	0.56	0.12	470	1	7 14 21	whole fruit	0.058 0.014 0.014	STUDY 346 TRIAL 104
USA (PA) 1998, Red Delicious	500 WP	0.56	0.12	480	1	7 14 21	whole fruit	0.58 c 0.01 0.36 0.084	STUDY 346 TRIAL 5

Checklist

- country
- crop
- crop variety
- application conditions
- pre-harvest interval
- commodity analysed
- residue expressed as residue definition
- control plot

11. Trials data 2

crop	country	study	analyt method	recov %	sprayer	plot sq m	field sample size	trial design	sample date	analysis date	storage interval days
apple	USA (GA)	STUDY 346 TRIAL 105	HPLC7	72-120 (n=46)	tractor-mounted airblast	557	?	unreplicated single plot	24-Aug-98	9-Mar-99	197
apple	USA (OR)	STUDY 346 TRIAL 4	HPLC7	72-120 (n=46)	tractor-mounted airblast	178	?	unreplicated single plot	26-Aug-98	17-Jan-99	144
apple	USA (OR)	STUDY 346 TRIAL 20	HPLC7	72-120 (n=46)	tractor-mounted airblast	178	?	unreplicated single plot	29-Aug-98	13-Jan-99	137
apple	USA (MI)	STUDY 346 TRIAL 97	HPLC7	72-120 (n=46)	tractor-mounted airblast	268	?	unreplicated single plot	3-Sep-98	14-Jan-99	133
apple	USA (MI)	STUDY 346 TRIAL 98	HPLC7	72-120 (n=46)	tractor-mounted airblast	381	?	unreplicated single plot	3-Sep-98	14-Jan-99	133

Checklist

- analytical recoveries
- sprayer
- plot size
- field sample size
- trial design
- interval of time sample is in freezer

12. Trials data 3

pears	USA (PA)	STUDY L18 TRIAL L13	HPLC7	102-123%	airblast sprayer	18 trees	5 lb	unreplicated single plot	21-Jul-98	03-Dec-99	500
pears	USA (CA)	STUDY L18 TRIAL P106	HPLC7	102-123%	airblast sprayer	16 trees	5 lb	unreplicated single plot	29-Jul-98	05-Dec-99	494
pears	USA (WA)	STUDY L18 TRIAL J108	HPLC7	102-123%	airblast sprayer	24 trees	5 lb	unreplicated single plot	3-Aug-98	25-Nov-99	479

Checklist

- Interval of time sample was in freezer is much longer than duration of storage test on apples (224 days)
- Is it acceptable?

13. Step 2. Which trials match GAP?

Crop	Country	Application						
		Form	Type	Rate kg ai/ha	Conc kg ai/hl	Min spray vol, l/ha	Max number	PHI days
Pome fruit	USA	500 WP	foliar	0.42-0.56		450	1	7

APPLE country, year (variety)	Application					PHI days	Commodity	Zappacarb, mg/kg 1/ 2/	Ref
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.				
USA (NY) 1998, Idared	500 WP	2.8		470	1	7	whole fruit	1.3	TRIAL C107
USA (WA) 1998, Red Delicious	500 WP	2.8		460	1	7	whole fruit	2.0	TRIAL J107
USA (NY) 1998, Monroe	500 WP	0.56	0.12	470	1	7 14 21	whole fruit	0.058 0.014 0.014	STUDY 346
USA (PA) 1998, Red Delicious	500 WP	0.56	0.12	480	1	7 14 21	whole fruit	0.58 c 0.01 0.36 0.084	STUDY 346

14. Step 2. Which trials match GAP?

Crop	Country	Application						
		Form	Type	Rate kg ai/ha	Conc kg ai/hl	Min spray vol, l/ha	Max number	PHI days
Pome fruit	USA	500 WP	foliar	0.42-0.56		450	1	7

APPLE country, year (variety)	Application					PHI days	Commodity	Zappacarb, mg/kg 1/ 2/
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.			
USA (MI) 1998, Empire	500 WP	0.56	0.12	460	1	7 14 21	whole fruit	0.15 c 0.019 0.16 0.10

- Residues in control plot
- Residues on day 14 higher than at day 7.

15. Residue Interpretation Table

Crop	Country	Form	Use pattern				Trial	Residues
			kg ai/ha	kg ai/hl	No of appl	PHI days		mg/kg
Pome fruit	US GAP	WP	0.56		1	7		
Apple	US trial	WP	0.56	0.12	1	7	Trial 104	0.058
Apple	US trial	WP	0.56	0.12	1	7	Trial 5	0.58
Apple	US trial	WP	0.56	0.12	1	7	Trial 12	0.20 (0.13) 1/

- Insert the GAP in the first row.
- Insert trial information, 1 trial in each row.
- Include only those trials that match the GAP.
- The final column then contains the valid residue data supporting an MRL related to the stated GAP.

16. Step 3. Propose maximum residue levels, STMRs, HRs

- Apples: list valid data in rank order, median underlined.
- Pears: list valid data in rank order, median underlined.
- Pome fruit: decide if apple and pear data can be combined for pome fruit.
- Recommendations: maximum residue levels, STMRs, HRs.

17 Step 4. Which processing trials are valid?

APPLE	Application					PHI	Commodity	Zappacarb	Ref
country, year (variety)	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.	days		mg/kg 1/ 2/	
USA (NY) 1998, Idared	500 WP	2.8		470	1	7 7	whole fruit whole fruit washed fruit juice wet pomace	1.3 3/ 0.89 4/ 0.63 0.20 c 0.14 1.6	TRIAL C107
USA (WA) 1998, Red Delicious	500 WP	2.8		460	1	7 7	whole fruit whole fruit washed fruit juice wet pomace	2.0 3/ 2.1 4/ 1.8 0.22 c 0.14 3.6	TRIAL J107

1/ mean of duplicate samples.
2/ c control juice from untreated apples. On a second analysis of these juice samples, no residue was detected (<0.005 mg/kg).
3/ field samples taken at the same time as the fruit for processing.
4/ sampled at the processing laboratory.

- Exaggerated application rate is OK for processing trials
- Residues in apples and juice.
  Processing factors = 0.2/0.89 = 0.23 and
  0.22/2.1 = 0.10
  (Median = 0.17)

18. Step 5. Derive processing factors, MRLs and STMR-Ps for processed commodities.

Processing factor apples to juice = 0.17.

Residues in juice (processed commodity) are lower than residues in apples (raw commodity). Therefore an MRL in juice is not required.

Processing factor for juice X STMR for apples or pome fruits gives an STMR-P for apple juice.

19. Questions?

Evaluation of zappacarb[34] residues on pome fruit – available data

Data on zappacarb – a carbamate insecticide

Characteristic	Value
Physico-chemical characteristics of the pure active ingredient	
zappacarb molecular weight	257
water solubility at 25 ºC	95 mg/l
Log K_{ow}	2.5
hydrolysis, half-life at 25 ºC	pH 4 >60 days; pH 7 = 34 days; pH 9 = 1.2 days
photolysis	stable to photolysis
Active ingredient content of TC, minimum	
zappacarb	940 g/kg
Formulation characteristics	
formulation types	WG, GR, SC, WP
Tox assessment	
ADI	0-0.01 mg/kg bw
ARfD	0.04 mg/kg bw/day

Zappacarb

Zappacarb is an insecticide which controls insects either by direct contact or through contact with foliar residues.
Zappacarb is not systemic in action; therefore complete coverage of both upper and lower leaf surfaces is necessary for effective control.

Analytical method summary

Apples and citrus
Analyte: ,zappacarb,
HPLC-UV, Method HPLC6, LOQ:,0.01 mg/kg
Description,Residues are extracted twice from homogenized matrix with acetonitrile + acetic acid. An aliquot of filtered extract is partitioned with aqueous sodium sulphate and dichloromethane. The organic phase, which contains the residues, is dried and evaporated to near dryness. The residue is taken up in HPLC mobile phase for analysis.

Method HPLC7 is a later version of HPLC6.

Freezer storage data summary

Storage stability data are recorded in the tables unadjusted for concurrent procedural recoveries. If the concurrent procedural recoveries were outside of the 70-120% range the data from that sampling occasion were not taken into account.

[34] Zappacarb is a fictitious pesticide with fictitious data. The data represent a typical residue situation, with typical problems to be solved.

Table 4. Freezer storage stability data for zappacarb spiked into matrices of apples.

Storage interval	Procedural recov %	Zappacarb mg/kg	Storage interval	Procedural recov %	Zappacarb, mg/kg
Homogenized apples, fortified at 0.1 mg/kg zappacarb stored at freezer temperature -24 °C to -20 °C.			Homogenized apples, fortified at 0.1 mg/kg zappacarb- stored at freezer temperature -24 °C to -20 °C.		
0	94% 92% 97% 109%		0	95% 96% 95% 97%	
7 days	91% 95%	0.088 0.090	7 days	94% 91%	0.101 0.095
14 days	72% 69%	0.063 0.064	14 days	70% 78%	0.076 0.074
21 days	68% 68% 1/	0.052 0.046	21 days	63% 67% 1/	0.056 0.056
28days	73% 77%	0.060 0.066	28days	81% 81%	0.076 0.074
41days	67% 67% 1/	0.059 0.059	41days	68% 72%	0.059 0.056
70 days	91% 88%	0.064 0.062	70 days	86% 79%	0.060 0.066
106 days	87% 88%	0.047 0.047	106 days	77% 81%	0.048 0.051
182 days	89% 91%	0.043 0.043	182 days	79% 71%	0.043 0.048
		30% decline in 106 days			30% decline in 97 days
Apples, fortified on the skin surface at 0.1 mg/kg zappacarb stored at freezer temperature -24 °C to -20 °C.			Apples, fortified on the skin surface at 0.1 mg/kg zappacarb- stored at freezer temperature -24 °C to -20 °C.		
0	88% 93% 88% 90%		0	77% 76% 73% 76%	
14 days	81% 92%	0.085 0.089	14 days	81% 83%	0.079 0.095
28 days	94% 88%	0.076 0.087	28 days	79% 79%	0.084 0.072
56 days	88% 84%	0.095 0.083	56 days	89% 90%	0.074 0.071
127 days	92% 87%	0.090 0.093	127 days	90% 83%	0.077 0.054
224 days	94% 92%	0.095 0.085	224 days	86% 80%	0.076 0.086

1/ Procedural recovery <70% - disregard the associated data.

Residue definition

Definition of the residue (for compliance with the MRL and for estimation of dietary intake: zappacarb.
The residue is not fat soluble.

GAP – use pattern

Table 5. Registered field uses of zappacarb in Australia, Japan and USA. Labels for the following uses were available to the Meeting.

Crop	Country	Application						
		Form	Type	Rate kg ai/ha	Conc kg ai/hl	Min spray vol, l/ha	Max number	PHI days
Apple	Australia	480 SC	foliar		0.031	1000	1	7
Apple	Japan	200 SC	foliar	0.27-1.4			1	7
Pear	Australia	480 SC	foliar		0.031	1000	1	7
Pear	Japan	200 SC	foliar	0.27-1.4			1	1
Pome fruit[35]	USA	500 WP	foliar	0.42-0.56		450	1	7

Supervised Trials

Although trials included control plots, no control data are recorded in the tables except where residues in control samples exceeded the LOQ.

Residue data are recorded unadjusted for % recovery.

Apples Table 3
Pears Table 4

[35] Pome fruit include apple, crabapple, pear, quince.

Table 6. Zappacarb residues in apples resulting from supervised trials in USA and Japan.

APPLE	Application					PHI	Commodity	Zappacarb, mg/kg	Ref
country, year (variety)	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.	days		1/ 2/	
USA (NY) 1998, Idared	500 WP	2.8		470	1	7	whole fruit	1.3	TRIAL C107 STUDY 419
USA (WA) 1998, Red Delicious	500 WP	2.8		460	1	7	whole fruit	2.0	TRIAL J107 STUDY 419
USA (NY) 1998, Monroe	500 WP	0.56	0.12	470	1	7 14 21	whole fruit	0.058 0.014 0.014	STUDY 346 TRIAL 104
USA (PA) 1998, Red Delicious	500 WP	0.56	0.12	480	1	7 14 21	whole fruit	0.58 c 0.01 0.36 0.084	STUDY 346 TRIAL 5
USA (PA) 1998, Law Rome	500 WP	0.56	0.12	480	1	7 14 21	whole fruit	0.13 0.20 0.074	STUDY 346 TRIAL 12
USA (GA) 1998, Golden Delicious	500 WP	0.55	0.12	470	1	7 14 21	whole fruit	0.16 0.052 0.086	STUDY 346 TRIAL 105
USA (MI) 1998, Empire	500 WP	0.56	0.12	460	1	7 14 21	whole fruit	0.15 c 0.019 0.16 0.10	STUDY 346 TRIAL 97
USA (MI) 1998, Red Max	500 WP	0.56	0.12	480	1	7 14 21	whole fruit	0.22 0.20 0.11	STUDY 346 TRIAL 98
USA (CO) 1998, Golden Delicious	500 WP	0.56	0.12	460	1	7 14 21	whole fruit	0.23 c 0.068 0.20 0.016	STUDY 346 TRIAL 101
USA (CA) 1998, Golden Delicious	500 WP	0.58	0.12	500	1	7 14 21	whole fruit	0.18 0.17 0.11	STUDY 346 TRIAL S105
USA (WA) 1998, Red Delicious	500 WP	0.55	0.12	460	1	7 14 21	whole fruit	0.18 0.15 0.072	STUDY 346 TRIAL J102
USA (WA) 1998, Red Delicious	500 WP	0.56	0.12	470	1	7 14 21	whole fruit	0.37 0.15 0.17	STUDY 346 TRIAL J103
USA (OR) 1998, Jonagold	500 WP	0.54	0.12	470	1	7 14 21	whole fruit	0.17 c 0.01 0.13 0.078	STUDY 346 TRIAL 4
USA (OR) 1998, Gala	500 WP	0.55	0.12	470	1	7 14 21	whole fruit	0.049 0.017 0.024	STUDY 346 TRIAL 20
USA (NY) 1998, Empire	500 WP	0.56	0.12	470	1	3 7 14 20 30	whole fruit	0.11 0.19 0.13 0.13 0.15	STUDY 346 TRIAL 103
USA (WA) 1998, Red Delicious	500 WP	0.56	0.12	470	1	3 7 14 21 28	whole fruit	0.48 0.38 0.36 0.25 0.22	STUDY 346 TRIAL 8105
Japan, 1997 (Fuji)	200 SC	1.2	0.02	6000	4	7 14 21 28	whole fruit	0.28 0.43 0.11 0.13	Report No 6A
Japan, 1997 (Fuji)	200 SC	1.2	0.02	6000	4	7 14 21 30	whole fruit	0.62 0.32 0.13 0.09	Report No 6No

APPLE country, year (variety)	Application					PHI days	Commodity	Zappacarb, mg/kg 1/ 2/	Ref
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.				
Japan, 2003 (Tsugaru)	200 SC	1.2	0.02	6000	1	1 3 7	whole fruit	0.57 0.32 0.24	Report No 24Ie
Japan, 2003 (Tsugaru)	200 SC	1.0	0.02	5000	1	1 3 7	whole fruit	0.82 0.39 0.26	Report No 24

1/ higher of duplicate field samples (USA trials)
2/ c: sample from control plot.

Table 7. Zappacarb residues in pears resulting from supervised trials in USA and Japan.

PEAR country, year (variety)	Application					PHI days	Commodity	Zappacarb, mg/kg 1/ 2/ 3/	Ref
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.				
USA (NY) 1998, Bartlett	500 WP	0.55	0.12	470	1	7 14 21	whole fruit	0.10 0.036 0.025	STUDY L18 TRIAL L8
USA (PA) 1998, Bartlett	500 WP	0.56	0.12	480	1	7 14 21	whole fruit	0.24 0.077 0.11	STUDY L18 TRIAL L13
USA (CA) 1998, Bartlett	500 WP	0.55	0.13	440	1	7 14 21	whole fruit	0.14 0.034 0.025	STUDY L18 TRIAL P106
USA (CA) 1998, Bartlett	500 WP	0.56	0.12	470	1	7 14 21	whole fruit	0.076 0.13 0.082	STUDY L18 TRIAL P107
USA (WA) 1998, Bartlett	500 WP	0.54	0.12	450	1	7 14 21	whole fruit	0.16 0.12 c 0.014 0.12	STUDY L18 TRIAL J108
USA (WA) 1998, D'Anjou	500 WP	0.55	0.12	480	1	7 14 21	whole fruit	0.094 0.056 c 0.01 0.074	STUDY L18 TRIAL J109
USA (OR) 1998, Red Clapp	500 WP	0.55	0.12	460	1	7 14 21	whole fruit	0.097 c 0.01 0.095 0.043	STUDY L18 TRIAL J110
USA (WA) 1998, D'Anjou	500 WP	0.53	0.12	450	1	7 14 21	whole fruit	0.29 0.19 0.099	STUDY L18 TRIAL 121
Japan, 1998 (Housui)	200 SC	1.2	0.02	6000	4	7 14 21 28	whole fruit	0.45 0.36 0.11 0.12	Report No. 7 Nagano
Japan, 1998 (Kousui)	200 SC	1.2	0.02	6000	4	7 14 21 28	whole fruit	0.44 0.31 0.09 0.06	Report No. P7O
Japan, 2000 (Kousui)	200 SC	1.2	0.02	6000	1	1 3 7	whole fruit	0.42 0.26 0.32	Report No. P14N1
Japan, 2000 (Kousui)	200 SC	1.2	0.02	6000	1	1 3 7	whole fruit	0.82 0.90 0.57	Report No. P14N2
Japan, 2001 (Kousui)	200 SC	0.80	0.02	4000	1	1 3 7	whole fruit	0.54 0.34 0.28	Report No. P19F
Japan, 2001 (Housui)	200 SC	0.40	0.02	2000	1	1 3 7	whole fruit	0.32 0.26 0.18	Report No. P19S

PEAR country, year (variety)	Application					PHI days	Commodity	Zappacarb, mg/kg 1/ 2/ 3/	Ref
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.				
Japan, 2001 (Kousui)	200 SC	0.7	0.02	3500	1	1 3 7	whole fruit	0.56 0.50 0.15	Report No. P19I
Japan, 2001 (Kousui)	200 SC	1.0	0.02	5000	1	1 3 7	whole fruit	0.10 0.24 0.11	Report No. P19T

1/ higher of duplicate field samples (USA trials)
2/ c: sample from control plot.
3/ Pear samples from the 8 US trials recorded in this table spent 15-16 months in frozen storage between harvest and analysis.

Food processing studies

Zappacarb was stable when subjected to hydrolysis under conditions representing food processes.

Researchers (STUDY 419) processed apples from two zappacarb field trials with exaggerated (×5) application rates into juice and wet pomace in a small-scale process. The process was suitable for 20-50 kg of apples. Apples were washed and then ground in a hammer-mill and the wet mash was collected into clothstacks on a hydraulic press. The stack was pressed at 2200-3000 psi for a minimum of 5 minutes and juice was collected. The wet pomace cake within the bags was sampled as wet pomace. Residues were measured on whole fruit, juice and wet pomace (Table 5).

Table 8. Zappacarb residues in apple juice and wet pomace from processing trials in USA (STUDY 419).

APPLE country, year (variety)	Application					PHI days	Commodity	Zappacarb mg/kg 1/ 2/	Ref
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.				
USA (NY) 1998, Idared	500 WP	2.8		470	1	7 7	whole fruit whole fruit washed fruit juice wet pomace	1.3 3/ 0.89 4/ 0.63 0.20 c 0.14 1.6	TRIAL C107 STUDY 419
USA (WA) 1998, Red Delicious	500 WP	2.8		460	1	7 7	whole fruit whole fruit washed fruit juice wet pomace	2.0 3/ 2.1 4/ 1.8 0.22 c 0.14 3.6	TRIAL J107 STUDY 419

1/ mean of duplicate samples.
2/ c control juice from untreated apples. On a second analysis of these juice samples, no residue was detected (<0.005 mg/kg).
3/ field samples taken at the same time as the fruit for processing.
4/ sampled at the processing laboratory.

Summary of field data supporting supervised trials

crop	country	study	analyt method	recov %	sprayer	plot sq m	field sample size	trial design	sample date	analysis date	storage interval days
apple	USA (GA)	STUDY 346 TRIAL 105	HPLC7	72-120 (n=46)	tractor-mounted airblast	557	?	unreplicated single plot	24-Aug-98	9-Mar-99	197
apple	USA (OR)	STUDY 346 TRIAL 4	HPLC7	72-120 (n=46)	tractor-mounted airblast	178	?	unreplicated single plot	26-Aug-98	17-Jan-99	144
apple	USA (OR)	STUDY 346 TRIAL 20	HPLC7	72-120 (n=46)	tractor-mounted airblast	178	?	unreplicated single plot	29-Aug-98	13-Jan-99	137
apple	USA (MI)	STUDY 346 TRIAL 97	HPLC7	72-120 (n=46)	tractor-mounted airblast	268	?	unreplicated single plot	3-Sep-98	14-Jan-99	133
apple	USA (MI)	STUDY 346 TRIAL 98	HPLC7	72-120 (n=46)	tractor-mounted airblast	381	?	unreplicated single plot	3-Sep-98	14-Jan-99	133
apple	USA (CO)	STUDY 346 TRIAL 101	HPLC7	72-120 (n=46)	tractor-mounted airblast	535	24 fruit	unreplicated single plot	2-Sep-98	19-Jan-99	139
apple	USA (WA)	STUDY 346 TRIAL 8105	HPLC7	72-120 (n=46)	tractor-mounted airblast	190	?	unreplicated single plot	4-Sep-98	19-Jan-99	137
apple	USA (NY)	STUDY 346 TRIAL 104	HPLC7	72-120 (n=46)	tractor-mounted airblast	476	?	unreplicated single plot	7-Sep-98	19-Feb-99	165
apple	USA (WA)	STUDY 346 TRIAL J103	HPLC7	72-120 (n=46)	tractor-mounted airblast	491	24 fruit	unreplicated single plot	8-Sep-98	21-Jan-99	135
apple	USA (PA)	STUDY 346 TRIAL 5	HPLC7	72-120 (n=46)	tractor-mounted airblast	502	24 fruit	unreplicated single plot	11-Sep-98	10-Feb-99	152
apple	USA (NY)	STUDY 346 TRIAL 103	HPLC7	72-120 (n=46)	tractor-mounted airblast	401	?	unreplicated single plot	13-Sep-98	22-Mar-99	190
apple	USA (CA)	STUDY 346 TRIAL S105	HPLC7	72-120 (n=46)	tractor-mounted airblast	334	24 fruit	unreplicated single plot	16-Sep-98	25-Feb-99	162
apple	USA (WA)	STUDY 346 TRIAL J102	HPLC7	72-120 (n=46)	tractor-mounted airblast	321	24 fruit	unreplicated single plot	23-Sep-98	09-Mar-99	167
apple	USA (PA)	STUDY 346 TRIAL 12	HPLC7	72-120 (n=46)	tractor-mounted airblast	502	24 fruit	unreplicated single plot	24-Sep-98	05-Mar-99	162
apple	USA (WA)	TRIAL J107 STUDY 419	HPLC7	74-111 (n=27)	tractor-mounted airblast	268	100 lb	unreplicated single plot	8-Oct-98	04-Jun-99	239
apple	USA (NY)	TRIAL C107 STUDY 419	HPLC7	74-111 (n=27)	tractor-mounted airblast	297	100 lb	unreplicated single plot	10-Oct-98	05-Jun-99	238
apple	Japan	REPORT 6A	?	?	CO2 powered backpack	8 trees	3 kg	unreplicated single plot	28-Sep-97	?	?
apple	Japan	REPORT 6NO	?	?	CO2 powered backpack	8 trees	3 kg	unreplicated single plot	28-Sep-97	?	?
apple	Japan	REPORT 24IE	HPLC6	84-115%	CO2 powered backpack	8 trees	2 kg	unreplicated single plot	6-Oct-03	29-Oct-03	23
apple	Japan	REPORT 24	HPLC6	84-115%	CO2 powered backpack	8 trees	2 kg	unreplicated single plot	6-Oct-03	29-Oct-03	23
pears	USA (PA)	STUDY L18 TRIAL L13	HPLC7	102-123%	airblast sprayer	18 trees	5 lb	unreplicated single plot	21-Jul-98	03-Dec-99	500
pears	USA (CA)	STUDY L18 TRIAL P106	HPLC7	102-123%	airblast sprayer	16 trees	5 lb	unreplicated single plot	29-Jul-98	05-Dec-99	494
pears	USA (WA)	STUDY L18 TRIAL J108	HPLC7	102-123%	airblast sprayer	24 trees	5 lb	unreplicated single plot	3-Aug-98	25-Nov-99	479
pears	USA (OR)	STUDY L18 TRIAL J110	HPLC7	102-123%	airblast sprayer	16 trees	5 lb	unreplicated single plot	6-Aug-98	30-Nov-99	481
pears	USA (CA)	STUDY L18 TRIAL P107	HPLC7	102-123%	airblast sprayer	16 trees	5 lb	unreplicated single plot	09-Aug-98	25-Nov-99	473
pears	USA (WA)	STUDY L18 TRIAL 121	HPLC7	102-123%	airblast sprayer	18 trees	5 lb	unreplicated single plot	12-Aug-98	29-Nov-99	474

crop	country	study	analyt method	recov %	sprayer	plot sq m	field sample size	trial design	sample date	analysis date	storage interval days
pears	USA (NY)	STUDY L18 TRIAL L8	HPLC7	102-123%	airblast sprayer	16 trees	5 lb	unreplicated single plot	13-Aug-98	01-Dec-99	475
pears	USA (WA)	STUDY L18 TRIAL J109	HPLC7	102-123%	airblast sprayer	16 trees	5 lb	unreplicated single plot	18-Aug-98	01-Dec-99	470
pears	Japan	REPORT 7NAGANO	HPLC6	94-102	backpack airblast sprayer	55	2 kg	unreplicated single plot	12-Sept-98	30-Oct-98	48
pears	Japan	REPORT P7	HPLC6	94-102	backpack airblast sprayer	63	2 kg	unreplicated single plot	12-Sept-98	30-Oct-98	48
pears	Japan	REPORT P14N1	HPLC6	83-97	backpack airblast sprayer	77	12 fruit	unreplicated single plot	7-Sep-00	14-Nov-00	68
pears	Japan	REPORT P14N2	HPLC6	83-97	backpack airblast sprayer	41	12 fruit	unreplicated single plot	7-Sep-00	14-Nov-00	68
pears	Japan	REPORT P19F	HPLC6	82-110	motorised knapsack	50	12 fruit	unreplicated single plot	?	?	?
pears	Japan	REPORT P19S	HPLC6	82-110	motorised knapsack	63	12 fruit	unreplicated single plot	?	?	?
pears	Japan	REPORT P19I	HPLC6	82-110	motorised knapsack	36	12 fruit	unreplicated single plot	?	?	?
pears	Japan	REPORT P19T	HPLC6	82-110	motorised knapsack	56	2 kg	unreplicated single plot	?	?	?

Note 1: all fruit, where storage data were available, were stored whole (unchopped) in the freezer.

Note 2: all sprayers were calibrated.

Evaluation of zappacarb residues on pome fruit
Worksheets

Supervised trials, validation checks

Trial	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?
TRIAL C107	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	74-111 (n=27)	<LOQ	238 days ✓	**yes**
TRIAL J107	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	74-111 (n=27)	<LOQ	239 days ✓	**yes**
TRIAL 104	apple	✓	✓	✓	**?**	whole fruit	✓	HPLC7	✓	72-120 (n=46)	<LOQ	165 days ✓	**yes**
TRIAL 5	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	72-120 (n=46)	c = 0.01	152 days ✓	**yes**

Trial	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?

Trial	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?

Trial	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?

Residue interpretation table

Residue interpretation table for zappacarb residues on apples. GAP and trial conditions are compared for treatments considered valid for MRL and STMR estimation.

Crop	Country	Form	Use pattern				Trial	Residues mg/kg
			kg ai/ha	kg ai/hl	No of appl	PHI days		
Pome fruit	US GAP	WP	0.56		1	7		
Apple	US trial	WP	0.56	0.12	1	7	Trial 104	0.058
Apple	US trial	WP	0.56	0.12	1	7	Trial 5	0.58
Apple	US trial	WP	0.56	0.12	1	7	Trial 12	0.20 (0.13) 1/
Apple	Japan GAP	SC	1.4		1	7		
Apple	Japan trial	SC	1.2	0.02	1	7	No 24Ie	0.24
Apple	Japan trial	SC	1.0	0.02	1	7	No 24	0.26

1/ The residue on day 14 (0.20 mg/kg) exceeded the residue on day 7 (0.13 mg/kg).

Summary of residue data selected for STMR, HR and maximum residue level estimation (rank order, median underlined):

Recommendations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR, mg/kg	HR, mg/kg
FP 0226	Apple			
FP 0230	Pear			
FP 0009	Pome fruits			

GAP on which the estimations are based:

Food processing

Raw agricultural commodity (RAC)	Processed commodity	Calculated processing factors.	Median or best estimate
Apples	Washed apples		
	Wet pomace		
	Apple Juice		

Raw agricultural commodity (RAC)	Processed commodity	Processing factor (PF)	Raw commodity		Processed commodity	
			STMR	HR	STMR-P =STMR×PF	HR-P 1/ =HR×PF
Apples	Washed apples					
	Wet pomace					
	Apple Juice					

The HR-P calculation is relevant only where commodity units maintain their integrity through the process.

Recommendations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR-P, mg/kg	HR-P, mg/kg
AB 0226	Apple pomace dry			
JF 0226	Apple juice			

Abbreviations and acronyms in this exercise

AB	Codex commodity group, by-products, used for animal feeding purposes, derived from fruit and vegetable processing
ADI	acceptable daily intake
ARfD	acute reference dose
FP	Codex commodity group, pome fruits

GAP	good agricultural practice
GR	granules
HPLC	high performance liquid chromatography
HPLC-UV	high performance liquid chromatography with ultra-violet absorbance detection
HR	highest residue
JF	Codex commodity group, fruit juices
LOQ	limit of quantification
MRL	maximum residue limit
PF	processing factor
PHI	pre-harvest interval
RAC	raw agricultural commodity
SC	suspension concentrate
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor
WG	water dispersible granules
WP	wettable powder

Exercise 8.2. Evaluation of data from supervised residue trials and processing studies – Estimation of dietary intake

See also Chapter 8

1. Residue evaluation exercise

Tomatoes

2. The aim

- The aim of this exercise is to explain the selection of suitable data for dietary exposure assessment and how to integrate data from raw and processed commodities into that assessment, and also to explain dietary exposure calculations.

3. Aim: to recommend maximum residue levels, STMRs, HRs

- **Step 1**. Which residue data are valid and fully supported by essential information?
- **Step 2**. Which trials match GAP?
- **Step 3**. Propose MRLs, STMRs, HRs for raw agricultural commodities.
- **Step 4**. Which processing trials are valid?
- **Step 5**. Derive processing factors, MRLs and STMR-Ps for processed commodities.
- **Step 6**. Check intakes with IEDI, IESTI spreadsheets.

4. Step 1. Which residue data are valid and fully supported by essential information?

Residue trials checklist

- trials data
- country
- crop
- crop variety
- application conditions
- pre-harvest interval
- commodity analysed
- residue expressed as residue definition

5. Step 1. Which residue data are valid and fully supported by essential information?

Residue trials checklist (continued)

- analytical method used in the trials
- % recoveries
- residues in samples from control plots
- if residue in control plot is detected, is the trial still OK?
- sprayer
- plot size

- field sample size
- trial design
- interval of time sample is in freezer
- is it OK?

6. HAPPYFOS

- Fictitious pesticide and fictitious data
- Metabolite is happyfos oxon
- Data represent a typical situation
- Typical problems

7. Freezer storage

Table 9. Freezer storage stability data for **field-incurred** happyfos residues in macerated tomatoes.

Storage interval	Procedural recov %	**Happyfos** mg/kg	Storage interval	Procedural recov %	**Happyfos oxon**, mg/kg
Homogenized tomatoes, stored at freezer temperature approx -20 °C.			Homogenized tomatoes, stored at freezer temperature approx -20 °C.		
0	109 %	3.8, 3.7	0	70 %	0.51, 0.53
237 days	93 %	3.3, 3.6	237 days	88 %	0.55, 0.63
268 days	93 %	3.8, 3.5	268 days	91 %	0.63, 0.60
387 days	105 %	3.9, 4.1	387 days	101 %	0.67, 0.72

8. Residue definition

- Enforcement (MRL)
  happyfos

- Risk assessment (dietary intake)
  sum of happyfos and happyfos oxon, expressed as happyfos.

9. Trials data 1

TOMATO country, year (variety)	Application Form	kg ai/ha	kg ai/hl	water (l/ha)	no.	PHI days	Commodity	Residues, mg/kg **happyfos**	happyfos **oxon**	Ref
Italy, 1999 (Alican)	WG	1.0	0.1	1000	2	14	tomato	0.14	0.05	R11099I
						21		0.11	0.04	
Italy, 1999 (Red Setter)	WG	1.0	0.1	1000	2	14	tomato	0.08	0.03	R81099I
						21		0.07	0.03	

Checklist

- country
- crop
- crop variety
- application conditions
- pre-harvest interval

- commodity analysed
- residues of happyfos and its oxon

10. Trials data 2

tomato	Italy, 1999	R11099I	B5150	71-99% n=14	hand gun with nozzle	30 m^2	14 fruits	unreplic single plot	21-Oct-99	10-Feb-00	112 days
tomato	Italy, 1999	R81099I	B5150	71-99% n=14	hand carried boom	45 m^2	24 fruit	unreplic single plot	6-Aug-99	15-Feb-00	193 days

Checklist

- analytical recoveries
- sprayer
- plot size
- field sample size
- trial design
- interval of time sample is in freezer

11. Step 2. Which trials match GAP?

Crop	Country	Application						
		Form	Type	Rate kg ai/ha	**Conc** kg ai/hl	Min spray vol, l/ha	Max number	**PHI** days
Tomatoes	Spain	WG	foliar		0.038-0.11			14

TOMATO country, year (variety)	Application					PHI days	Commodity	Residues, mg/kg	
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.			happyfos	happyfos oxon
Spain, 1995 (Roma)	WG	1.1	**0.13**	1000	3	**14**	tomato	0.05	0.03

12. Residue Interpretation Table

Crop	Country	Form	Use pattern				Study	Residues, mg/kg		
			kg ai/ha	kg ai/hl	No of appl	PHI days		happyfos	happyfos oxon	happyfos + oxon expressed as happyfos
Tomatoes	Spanish **GAP**	WG		0.11		14				
Tomatoes	Spanish **trial**	WG	1.1	0.13	3	14	10PS4510	**0.05**	**0.03**	0.08

- Insert the GAP in the first row.
- Insert trial information, 1 trial in each row.
- Include only those trials that match the GAP.
- The final columns then contain the valid residue data supporting an MRL related to the stated GAP.

13. Step 3. Propose maximum residue levels, STMRs, HRs

- Tomatoes: list valid happyfos data in rank order, for maximum residue level estimation.
- : list valid data for happyfos + oxon (expressed as happyfos) in rank order, median underlined for STMR and HR estimation.

14. Step 4. Which processing trials are valid?

Checklist, as for supervised trials

15. Step 5. Derive processing factors, MRLs and STMR-Ps for processed commodities.

Raw agricultural commodity (RAC)	Processed commodity	**happyfos + oxon** expressed as happyfos mg/kg		Calculated processing factors.	Median or best estimate
		2117-T37P	D2760-NMT		
Tomatoes		1.48	1.41		
	Washed tomatoes	1.91	0.36	1.3, 0.26	**1.3**

- Use **risk assessment residue definition**
- If the trials appear to be different processes**, a median is inappropriate.**

16. Step 6. Intake assessment IEDI.

- Spreadsheet: IEDI_calculation14_FAO.xlt
- Data to be entered.
    - Compound: HAPPYFOS
    - ADI = 0.03 mg/kg bw
    - Tomato STMR:
    - Tomato paste STMR:
    - Tomato juice STMR
    - Tomato canned STMR.
- Because tomato processing data are available for the 3 processed commodities, we should enter the tomato STMR using “Tomato (excl juice, excl paste, excl canned)”.

17. Step 6. Intake assessment IESTI.

- Spreadsheet: IESTI_calculation11_FAO.xls
- Data to be entered
    - Compound: HAPPYFOS
    - ARfD = 0.1 mg/kg bw
    - Tomato HR:

18. Questions?

Evaluation of happyfos[36] residues on tomatoes – available data

Data on happyfos – an organic phosphate ester insecticide

Characteristic	Value
Physico-chemical characteristics of the pure active ingredient	
happyfos molecular weight	330
water solubility at 25 ºC	4 mg/l
Log K_{ow}	3.0
hydrolysis, half-life at 25 ºC	pH 5 17 days; pH 7 = 120 days; pH 9 = 0.5 days
photolysis	stable to photolysis
vapour pressure	1.3×10^{-4} Pa at 25 °C
Active ingredient content of TC, minimum	
happyfos	930 g/kg
Formulation characteristics	
formulation types	WG, GR, WP
Tox assessment	
ADI	0-0.03 mg/kg bw
ARfD	0.1 mg/kg bw/day

HAPPYFOS

Happyfos is an insecticide which controls insects either by direct contact or through feeding. It is not systemic.

Analytical method summary

Fruits, vegetables

Analytes:	happyfos and happyfos oxon	GLC-FPD	Method B5150
LOQ:	0.01 mg/kg		
Description	Residues are extracted twice from homogenized matrix with ethyl acetate. The extract is dried with sodium sulphate and evaporated to dryness. Cleanup is effected through a silica gel column. After evaporation of the eluting solvent, the residue is taken up in ethyl acetate for GLC-FPD (phosphorus mode) analysis. Happyfos and its oxon require different GLC conditions and are analysed in separate GLC runs.		

Happyfos oxon is poorly recovered in multiresidue methods.

Freezer storage data summary

Storage stability data are recorded in the tables unadjusted for concurrent procedural recoveries. If the concurrent procedural recoveries were outside of the 70-120% range the data from that sampling occasion were not taken into account.

Table 10. Freezer storage stability data for field-incurred happyfos residues in macerated tomatoes.

Storage interval	Procedural recov %	Happyfos mg/kg	Storage interval	Procedural recov %	Happyfos oxon, mg/kg
Homogenized tomatoes, stored at freezer temperature approx -20 °C.			Homogenized tomatoes, stored at freezer temperature approx -20 °C.		
0	109 %	3.8, 3.7	0	70 %	0.51, 0.53
237 days	93 %	3.3, 3.6	237 days	88 %	0.55, 0.63
268 days	93 %	3.8, 3.5	268 days	91 %	0.63, 0.60
387 days	105 %	3.9, 4.1	387 days	101 %	0.67, 0.72

[36] Happyfos is a fictitious pesticide with fictitious data. The data represent a typical residue situation, with typical problems to be solved.

Table 11. Freezer storage stability data for processed tomato products with field-incurred happyfos residues.

Storage interval	Canned tomatoes		Tomato puree		Tomato juice	
	happyfos	happyfos oxon	happyfos	happyfos oxon	happyfos	happyfos oxon
0	0.61, 0.50	0.28, 0.23	0.65, 0.53	0.26, 0.24	0.40, 0.37	0.22, 0.20
4 weeks	0.54, 0.49	0.26, 0.19	0.46, 0.51	0.17, 0.23	0.51, 0.43	0.23, 0.24
13 weeks	0.50, 0.51	0.24, 0.25	0.78, 0.73	0.25, 0.28	0.59, 0.79	0.24, 0.29

Residue definition

Definition of the residue for compliance with the MRL: happyfos.

Definition of the residue for estimation of dietary intake: sum of happyfos and happyfos oxon, expressed as happyfos.

The residue is fat soluble.

Note that the molecular weight of happyfos oxon is 314.

GAP – use pattern

Table 12. Registered field uses of happyfos on tomatoes.

Crop	Country	Application						
		Form	Type	Rate kg ai/ha	Conc kg ai/hl	Min spray vol, l/ha	Max number	PHI days
Tomatoes	Spain	WG	foliar		0.038-0.11			14
Tomatoes	Portugal	WG	foliar		0.075			21
Tomatoes	France	WG	foliar		0.075			3
Tomatoes	Italy	WP	foliar		0.034-0.05			21
Tomatoes	Cyprus	WG	foliar		0.056-0.075			15

SUPERVISED TRIALS

Although trials included control plots, no control data are recorded in the tables except where residues in control samples exceeded the LOQ.

Residue data are recorded unadjusted for % recovery.

Table 13. Happyfos residues in tomatoes resulting from supervised trials in Europe.

TOMATO country, year (variety)	Application Form	kg ai/ha	kg ai/hl	water (l/ha)	no.	PHI days	Commodity	Residues, mg/kg happyfos	happyfos oxon	Ref
Italy, 1999 (Alican)	WG	1.0	0.1	1000	2	14	tomato	0.14	0.05	R11099I
						21		0.11	0.04	
Italy, 1999 (Red Setter)	WG	1.0	0.1	1000	2	14	tomato	0.08	0.03	R81099I
						21		0.07	0.03	
France, 1996 (FY55)	WG	0.76	0.075	1000	3	0	tomato	0.73	0.10	4841-T
						7		0.40	0.13	
						10		0.37	0.15	
						14		0.33	0.15	
Spain, 1995 (Roma)	WG	1.1	0.13	1000	3	14	tomato	0.05	0.03	10PS4510
Spain, 1999 (Valenciano)	WG	1.0	0.1	1000	2	14	tomato	0.18	0.11	R63099S
						21		0.12	0.06	
Spain, 1999 (Valentine)	WG	1.0	0.1	1000	2	14	tomato	0.08	0.05	R74099S
						21		0.04	0.02	
Cyprus, 1994 (Alican)	WP	1.5	0.1	1500	2	3	tomato	0.43	0.03	4260-TMN
						7		0.67	0.05	
						15		0.38	0.06	
Cyprus, 1994 (Alican)	WP	1.5	0.1	1500	2	3	tomato	0.70	0.06	5361-CY
						7		0.85	0.08	
						15		0.65	0.12	
France, 1989 (Campbell 28)	WG	0.84	0.1	800	4	7	tomato	0.53	0.17	1521-TA37
						14		0.40	0.15	

Food processing studies

Happyfos was stable when subjected to hydrolysis under conditions representing the food processes of baking, brewing, boiling and sterilization. Partial hydrolysis occurred under pasteurization conditions (pH 4, 90 ºC for 20 minutes).

In a processing study in USA (2117-T37P), field-treated tomatoes were processed in a laboratory according to commercial practice. Tomatoes were washed to remove dirt and debris, then dipped in water containing chlorine and rinsed. Tomatoes were crushed, heated and screened to remove skins and seeds. The juice was canned, sealed and cooked for 10 minutes in boiling water.
Whole washed tomatoes were treated with boiling water to remove the skin. They were then sealed in cans and cooked for 30 minutes. Tomato paste and purée were prepared by concentrating the juice before canning and cooking.

In the second study (D2760-NMT), the first step involved soaking tomatoes in 0.5 % NaOH at 54 ºC before blanching and crushing. The remainder of the process was similar to the first one.

Table 14. Happyfos residues in tomatoes and tomato products from processing trials.

TOMATO country, year (variety)	Application Form	kg ai/ha	kg ai/hl	water (l/ha)	no.	PHI days	Commodity	Residues, mg/kg happyfos	happyfos oxon	Ref
USA (CA), 1988 (204C)	?	1.1	0.4		8	3	tomatoes	1.4	0.08	2117-T37P
							washed	1.8	0.1	
							canned	0.54	0.04	
							paste	5.6	0.43	
							purée	2.5	0.17	
							juice	1.3	0.08	
							wet pomace	0.84	0.04	
							dry pomace 1/	1.4	0.09	
							paste		c 0.03	

TOMATO country, year (variety)	Application					PHI days	Commodity	Residues, mg/kg		Ref
	Form	kg ai/ha	kg ai/hl	water (l/ha)	no.			happyfos	happyfos oxon	
USA (CA), 1993 (UC 82B)	?	1.1	0.4		6	3	tomatoes	1.3	0.10	D2760-NMT
							washed	0.30	0.06	
							paste	1.2	0.37	
							purée	0.75	0.26	
							juice	0.35	0.09	
							wet pomace	0.24	0.06	
							dry pomace 2/	0.38	0.05	

c: processed sample from control plot.
1/ Wet pomace 36 % dry matter, dry pomace 95 % dry matter.
2/ Wet pomace 23 % dry matter, dry pomace 93 % dry matter.

Summary of field data supporting supervised trials

crop	country	study	analyt method	recov %	sprayer	plot sq m	field sample size	trial design	sample date	analysis date	storage interval days
tomato	Italy, 1999	R11099I	B5150	71-99% n=14	hand gun with nozzle	30	14 fruits	unreplic single plot	21-Oct-99	10-Feb-00	112
tomato	Italy, 1999	R81099I	B5150	71-99% n=14	hand carried boom	45	24 fruit	unreplic single plot	6-Aug-99	15-Feb-00	193
tomato	France, 1996	4841-T	B5150	87-102% n=10	motorised knapsack	30	30 fruits	unreplic single plot	4-Oct-96	16-Mar-97	163
tomato	Spain, 1995	10PS4510	B5150	87-102% n=10	hand gun with nozzle	36	30 fruits	unreplic single plot	4-Oct-95	16-Mar-96	164
tomato	Spain, 1999	R63099S	B5150	82-105% n=10	hand gun with nozzle	30	15 fruit	unreplic single plot	31-Jul-99	28-Jan-00	181
tomato	Spain, 1999	R74099S	B5150	82-105% n=10	hand gun with nozzle	22	15 fruit	unreplic single plot	28-Jul-99	27-Jan-00	183
tomato	Cyprus, 1994	4260-TMN	B5150	72-102% n=17	motorised knapsack	26	12 fruit	unreplic single plot	15-Oct-94	26-Jan-95	103
tomato	Cyprus, 1994	5361-CY	B5150	72-102% n=17	motorised knapsack	48	12 fruit	unreplic single plot	5-Aug-94	15-Feb-95	194
tomato	France, 1989	1521-TA37	B5150	82-105% n=10	motorised knapsack	36	15 fruit	unreplic single plot	29-Sep-89	25-Jan-90	118
tomato	USA, 1988	2117-T37P	B5150	72-106% n=12	CO_2 sprayer with 6 nozzle boom	237	500 lb	unreplic single plot	11-Sep-88	13-Nov-88	63
tomato	USA, 1993	D2760-NMT	B5150	78-122% n=23	tractor-mounted CO_2 sprayer with 6-nozzle boom	474	300 lb	unreplic single plot	13-Aug-93	25-Oct-93	73

Note 1: all sprayers were calibrated.
Note2: Recovery ranges include happyfos and happyfos oxon.

Evaluation of happyfos residues on tomatoes
Worksheets

Supervised trials, validation checks

Study	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?
R110991	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	71-99% (n=14)	<LOQ	112 days ✓	**yes**

Residue interpretation table

Residue interpretation table for happyfos residues on tomatoes. GAP and trial conditions are compared for treatments considered valid for MRL, STMR and HR estimation.

Crop	Country	Form	Use pattern				Study	Residues, mg/kg		
			kg ai/ha	kg ai/hl	No of appl	PHI days		happyfos	happyfos oxon	happyfos + oxon expressed as happyfos
Tomatoes	Spanish GAP	WG		0.11		14				
Tomatoes	Spanish trial	WG	1.1	0.13	3	14	10PS4510	0.05	0.03	0.08

Happyfos + happyfos oxon, expressed as happyfos
= happyfos residue + (330/314) × happyfos oxon residue

Summary of residue data selected for STMR, HR and maximum residue level estimation (rank order, median underlined):

Recommendations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR, mg/kg	HR, mg/kg
VO 0448	Tomato			

GAP on which the estimations are based:

Food processing

Raw agricultural commodity (RAC)	Processed commodity	happyfos + oxon expressed as happyfos mg/kg		Calculated processing factors.	Median or best estimate
		2117-T37P	D2760-NMT		

Raw agricultural commodity (RAC)	Processed commodity	happyfos + oxon expressed as happyfos mg/kg		Calculated processing factors.	Median or best estimate
Tomatoes		1.48	1.41		
	Washed tomatoes	1.91	0.36	1.3, 0.26	1.3
	Tomato paste				
	Tomato puree				
	Tomato juice				
	Canned tomato				

Raw agricultural commodity (RAC)	Processed commodity	Processing factor (PF)	Raw commodity		Processed commodity	
			STMR	HR	STMR-P = STMR×PF	HR-P 1/ = HR×PF
Tomatoes	Tomato paste					
	Tomato puree					
	Tomato juice					
	Canned tomato					

The HR-P calculation is relevant only where commodity units maintain their integrity through the process.

Recommendations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR-P, mg/kg	HR-P, mg/kg
	Tomato juice			
	Tomato paste			
	Tomato puree			
	Canned tomato			

Other commodities to include in the dietary intake calculations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
FP 0226	Apple	2	0.42	1.3
FB 0269	Grapes	0.2	0.02	0.09
MM 0095	Meat from mammals other than marine mammals:	3 (fat)	0.04 (muscle) 0.95 (fat)	0.1 (muscle) 2.2 (fat)
TN 0085	Tree nuts	0.2	0.03	0.11
VC 0432	Watermelon	1	0.02	0.02
	Wine		0.005	

Dietary intake calculations

Read Chapter 12 for an understanding of dietary intake calculations.

Understanding the IEDI calculation

There are 13 diets. Choose Diet B and Diet F for this exercise.

		Grams per person per day	
Codex Code	Name	Diet B	Diet F
FP 0226	Apple	60.5	39.4
FB 0269	Grapes	128.5	44.0
MM 0095	Meat 20% as fat	23.3	26.3
MM 0095	Meat 80% as muscle	93.2	105.0
VO 0448	Tomato	185.0	40.9
JF 0448	Tomato juice	0.5	15.2
	Tomato paste	1.3	4.5
	Tomato peeled	0.4	3.2
TN 0085	Tree nuts	21.5	10.2
VC 0432	Watermelon	43.1	6.0
	Wine	76.8	25.6

Body weight for Diet B and Diet G = 60 kg.

Factors are required for calculating the grams of RAC (raw agricultural commodity) that produced a gram of processed product.

For tomatoes, 1.25 g of tomatoes produced 1 g of tomato juice or 1 g of peeled tomatoes, while 4 g of tomatoes produced 1 g of tomato paste.

For grapes, 1.4 g grapes produced 1 gram wine.

IEDI calculation on RACs

Codex Code	Name	STMR or STMR-P mg/kg	B diet	B intake	F diet	F intake
FP 0226	Apple		60.5	-	39.4	-
FB 0269	Grapes		128.5	-	44.0	-
MM 0095	Meat 20% as fat		23.3		26.3	-
MM 0095	Meat 80% as muscle		93.2		105.0	-
VO 0448	Tomato		185.0		40.9	
TN 0085	Tree nuts		21.5		10.2	-
VC 0432	Watermelon		43.1		6.0	-
	TOTAL µg/person per day					

IEDI calculation on RACs and processed commodities.

Subtract the consumption of processed commodity (adjusted for production factor) from the consumption of RAC (raw agricultural commodity).

Codex Code	Name	STMR or STMR-P mg/kg	B diet	B intake	F diet	F intake
FP 0226	Apple		60.5	-	39.4	-
FB 0269	Grapes		128.5	-	44.0	-
MM 0095	Meat 20% as fat		23.3		26.3	-
MM 0095	Meat 80% as muscle		93.2		105.0	-
VO 0448	Tomato		185.0		40.9	
JF 0448	Tomato juice		0.5		15.2	
	Tomato paste		1.3		4.5	
	Tomato peeled		0.4		3.2	
TN 0085	Tree nuts		21.5		10.2	-
VC 0432	Watermelon		43.1		6.0	-
	Wine		76.8	-	25.6	-
	TOTAL µg/person per day					

Calculate intake as % of ADI.

Express ADI as µg per person: ADI (mg/kg bw) $\times$ 60 $\times$ 1000

Express estimated intake (µg/person per day) as % of ADI.

Understanding the IESTI calculations

Read Section 7.3 (pages 127-130) of the JMPR Manual.

Estimates for each food commodity are made separately.

Brief summary

LP:	large portion consumed in 1 day.	Units: kg.
U:	unit weight of whole commodity.	Units: kg.
U_c:	unit weight of edible portion.	Units: kg.
v:	variability factor, default value 3.	Unitless.
bw:	bodyweight.	Units: kg.
HR:	highest residue in edible portion.	Units: mg/kg.
IESTI:	estimated intake.	Units: mg/kg bw per day.
STMR-P:	STMR for processed commodity	Units: mg/kg

Case 1: residue in the composite sample reflects the residue in a meal-sized portion; unit weight is below 25 g.

$$IESTI = \frac{LP \times HR}{bw}$$

Examples: almonds, pecans, raisins, cherries, meat.

Case 2: the meal-sized portion or a single fruit or vegetable unit might have a higher residue than the composite.

Case 2a

$$IESTI = \frac{U_C \times HR \times v + (LP - U_c) \times HR}{bw}$$

Examples: apples, grapes (unit is a bunch), carrots, tomatoes.

Case 2b: only one or part of one unit is consumed.

$$IESTI = \frac{LP \times HR \times v}{bw}$$

Examples: watermelons, pineapples, head cabbages.

Case 3: in commodities subject to bulking and blending, the STMR-P for processed commodities (or STMR for unprocessed commodities subject to bulking and blending) represents the likely highest residue.

$$IESTI = \frac{LP \times STMR - P}{bw}$$

Examples: wine, tomato juice, wheat.

Parameters for calculating IESTI values directly from the IESTI formulae.

	Total population				Children			
	bw, kg	LP, kg	U, kg	U_c, kg	bw, kg	LP, kg	U, kg	U_c, kg
Apple	65	1.348	0.11	0.10	15	0.679	0.11	0.10
Grapes	67	0.513	0.125	0.118	19	0.342	0.125	0.118
Meat 20% as fat	67	0.104			19	0.052		
Meat 80% as muscle	67	0.417			19	0.208		
Tomato	52.2	0.387	0.105	0.102	18.9	0.215	0.105	0.102

Tomato juice	no data							
Tomato paste	no data							
Tomato peeled	no data							
Tree nuts	52.6	0.107			19	0.028		
Watermelon	65	1.939	4.518	2.078	19	1.473	4.518	2.078
Wine	52.2	1.006			18.9	0.089		

Calculate IESTI values

			Total population		Children	
	STMR or STMR-P	HR or HR-P	Case	IESTI, µg/kg bw per day	Case	IESTI, µg/kg bw per day
Apple						
Grapes						
Meat 20% as fat						
Meat 80% as muscle						
Tomato						
Tree nuts						
Watermelon						
Wine						

Compare calculated IESTI values with ARfD.

Spreadsheet calculations for IEDI and IESTI

IEDI calculation

Spreadsheet: IEDI_calculation14_FAO.xlt

Data to be entered.

Compound: HAPPYFOS
ADI = 0.03 mg/kg bw
STMRs from page 12

Because tomato processing data are available for the 3 processed commodities, we should enter the tomato STMR using “Tomato (excl juice, excl paste, excl peeled)”.

Results: Intakes =.xx% to xx% for the 13 diets.

IESTI calculation

Spreadsheet: IESTI_calculation11_FAO.xlt

Data to be entered
Compound: HAPPYFOS
ARfD = 0.1 mg/kg bw
HR and STMR values from page 12.

Results: Intakes = xxxx % for adults and xxxx % for children.

Abbreviations and acronyms in this exercise

ADI	acceptable daily intake
ARfD	acute reference dose
GAP	good agricultural practice
GLC-FPD	gas-liquid chromatography with flame-photometric detection
GR	granules
HR	highest residue
HR-P	highest residue in a processed commodity calculated by multiplying the HR of the raw agricultural commodity by the corresponding processing factor.
IEDI:	international estimated daily intake
IESTI	international estimated short-term intake
LOQ	limit of quantification
MRL	maximum residue limit
PF	processing factor
PHI	pre-harvest interval
RAC	raw agricultural commodity
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor
TC	technical material
WG	water dispersible granules
WP	wettable powder

Exercise 8.3: Evaluation of residues derived from supervised trials in *passion fruits*[37]

A national use pattern in Brazil permits up to four foliar applications of difenoconazole EC 25 (250 g/l) on passion fruit at a rate of 5 g ai/hl or between 0.01 and 0.04 kg ai/ha with a PHI of 14 days.

In four Brazilian trials the applications were performed within GAP (1 treatment with -25% dosage rate) and the samples taken at 7 days after the treatment contained residues below the LOQ of 0.01 mg/kg with one exception (0.04 mg/kg).

Where the trials were conducted at 2.5-5 times max GAP kg ai/ha rate the residues in all samples taken at 7 or 14 days were below the limit of quantification (0.01-0.05 mg/kg).

Task: *Evaluate the residue data and estimate maximum residue levels, HR and STMR values*

Difenoconazole residues in passion fruit from supervised residue trials in Brazil

Location crop variety	Application					Residue (mg/kg)	Reference
	Formulation	Rate (g ai/ha)	Conc. (g ai/hl)	Interval (days)	PHI (days)		
GAP in Brazil: 10-40 g ai/ha, spray concentration: 5 g ai/hl, up to 4 times at 15 days, PHI: 14 days							
Morretes – PR not stated	EC 125 g/L	30	-	NA	0 1 3 5 7	<0.01 0.01 <0.01 <0.01 ND	M08078 Trial M08078-DMO F: A13703G- 10304
Uberlandia – MG not stated	EC 125 g/L	30	-	NA	0 1 3 5 7	0.01 0.01 <0.01 <0.01 <0.01	M08078 Trial: M08078-JJB F: A13703G- 10304
Piedade - SP not stated	EC 125 g/L	30	-	NA	0 1 3 5 7	0.08 0.07 0.04 0.04 0.04	M08078 Trial: M08078-LZF1 F: A13703G- 10304
Santa Amelia – PR not stated	EC 125 g/L	30	-	NA	0 1 3 5 7	0.02 0.03 <0.01 <0.01 <0.01	M08078 Trial: M08078-JJB F: A13703G- 10304
São Paulo Amarelo	EC 250 g/L	100	10	-	14	<0.01	FHF 017B Trial: FHF 017B X 14 F:A7402T-10007
São Paulo Amarelo	EC 250 g/L	200	20	-	7	<0.01	FHF017B Trial: FHF017B2X7 F:A7402T-10007

[37] The trial data were taken from the 2010 JMPR Evaluation

Location crop variety	Application					Residue (mg/kg)	Reference
	Formulation	Rate (g ai/ha)	Conc. (g ai/hl)	Interval (days)	PHI (days)		
São Paulo Amarelo	EC 250 g/L	200	20	-	14	<0.01	FHF017B Trial: FHF017B2X14 F:A7402T-10007
São Paulo Amarelo	EC 250 g/L	100 (x4)	10 (x4)	7 - 9 days	0 3 7 10 14	<0.05 <0.05 <0.05 <0.05 <0.05	FHF 017/98 Trial: FHF 017B 2X14 F:A7402T-10008
São Paulo Amarelo	EC 250 g/L	200 (x4)	20 (x4)	7 - 8 days	14	<0.05	FHF 017/98 F:A7402T-10008
São Paulo Azedo	EC 250 g/L	100 (x4)	10 (x4)	7 days	0 3 7 10 14	<0.02 <0.02 <0.02 <0.02 <0.02	M00164 Trial:M00164 F:A7402T-10009
São Paulo Azedo	EC 250 g/L	200 (x4)	20 (x4)	7 days	0 3 7 10 14	0.38 <0.02 <0.02 <0.02 <0.02	M00164 Trial: M00164 F:A7402T-10009

Exercise 8.4 Preparing a protocol for a supervised residue trial

See also Chapter 8

1. Preparing a protocol for a supervised residue trial

2. The aim

- The aim of this exercise is to explain how to prepare a protocol for a supervised residue trial.

3. Protocol for a supervised residue trial

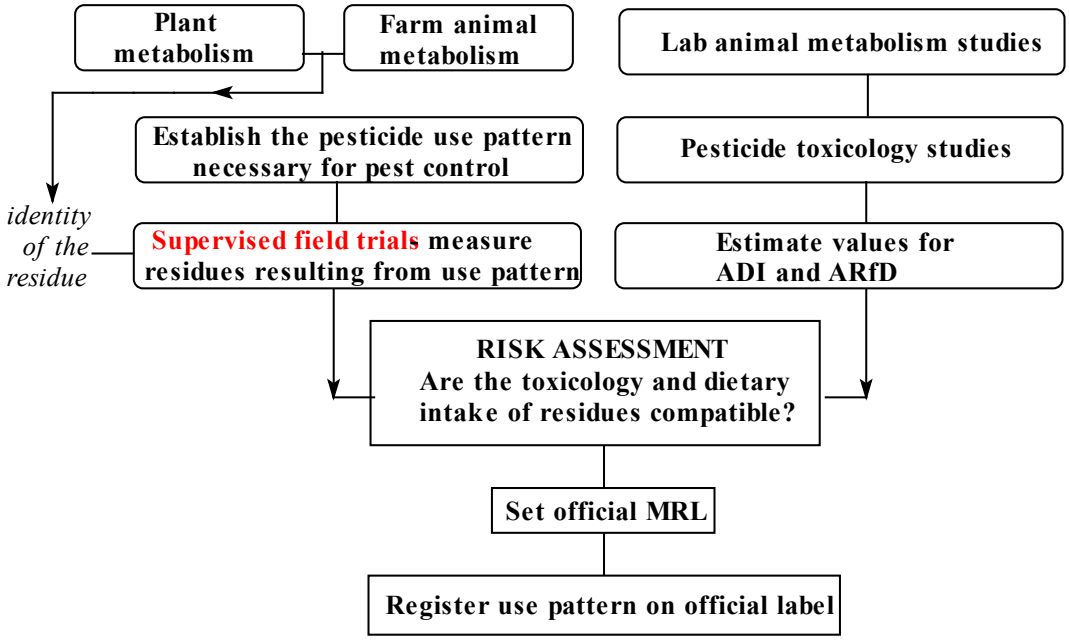
- Aim

To use a protocol template to plan a supervised residue trial.

4. Background

- Supervised residue trials provide the link between the uses of a pesticide and the residue levels expected in the harvested commodity.

5. Position of supervised trials in the process



6. A protocol for supervised trials

- A well prepared protocol ensures that all personnel working on the trial know what is expected of them and when the phases of the trial begin and end.
- The protocol is a good starting point when the final report is to be prepared.
- The study objectives should be clearly expressed.
- Preparing the final report (data submission) is recognized as a separate task.
- This protocol is designed for field trials. A protocol for post-harvest uses would be different in some sections.

7. Information to assemble

- Residue definition
- Use pattern, from efficacy studies
- Capability to analyse samples
- Suitable analytical method
- Laboratory experience and capability

8. Nature of the information

- The information in the protocols should be specific.
- Phrases such as "where applicable" and "as relevant" are alerts that the information is too vague.
- In a protocol it is necessary to imagine what is going to happen and then to describe the required actions to be taken. The reader should be left in little doubt what is to be done.

9. The exercise

- Choose a pesticide use on a minor crop of interest to you.
- Prepare a protocol for 3 supervised trials that will generate data to support a submission to a Registration Authority for an MRL.

10. The process

- **Step 1**. Decide on a pesticide use pattern and crop that require residue data to support an MRL.
- **Step 2.** Use the protocol template as a checklist for assembly of information.
- **Step 3**. Complete the template as far as possible.
- **Step 4.** Prepare a list of actions needed to complete the protocol.

11. Questions?

Abbreviations and acronyms in this exercise

ARfD:	acute reference dose
ADI:	acceptable daily intake
AOAC:	AOAC
CAS:	Chemical Abstracts Service
CIPAC:	Collaborative International Pesticides Analytical Council
EC	emulsifiable concentrate
FAO:	Food and Agriculture Organization of the United Nations
MRL	maximum residue limit
MSDS:	material safety data sheet

PROTOCOL

SUPERVISED RESIDUE TRIALS FOR [*insert* PESTICIDE COMMON NAME] ON [*insert* CROP]

Name of organization

Study Director
[*insert* NAME, ADDRESS]

Study Number: *unique identifying number*
File:

Full address

pages Protocol date

CONTENTS

GENERAL CONSIDERATIONS

Personnel and responsibilities

Study director:	
Laboratory operations:	
Field operations:	
Data submission:	
Protocol planning:	

Study objectives

The objective of the study is to provide valid [*insert* PESTICIDE] residue data for field treatment of [*insert* CROP] to the Registration Authority so that an MRL for [*insert* RESIDUE] on [*insert* COMMODITY] can be established.

Field portion of the study

The objective is to generate samples of [*insert* COMMODITY] treated with [*insert* PESTICIDE] according to the approved or registered use or proposed registered use under controlled commercial conditions and to document the field data.

Laboratory portion of the study

The objective is to analyse the samples of [*insert* COMMODITY] and document the laboratory data.

Data submission

The objective is to assemble the documentation and prepare the case for submission to the Registration Authority.

Number of trials

[*insert* NUMBER] trials will be conducted.
The trial identification numbers are:
[*insert* UNIQUE IDENTIFICATION NUMBER] for trial 1
[*insert* UNIQUE IDENTIFICATION NUMBER] FOR TRIAL 2, etc
The sites are:

Address (*Site A*). Trial (*unique ident number*)
Address (*Site B*). Trial (*unique ident number*)
Address (*Site C*). Trial (*unique ident number*)

Communication of variations to the agreed protocol

After the protocol is agreed, variations may be made by agreement with the study director. Variations will be in writing and will describe the changes required and will state the effect on the project validity and results.

Confidentiality (note[38])

The work will be considered confidential until it has been evaluated by the Registration Authority. The work will then be published in a scientific journal under joint authorship.

[38] Decide at the protocol stage which information will be confidential and which will become public knowledge. For example, a paragraph such as that provided might be included in the protocol.

Copy of all correspondence

Copies of all correspondence and attachments will be sent to the study director, who will maintain a comprehensive file for the project.

Fund code

The work is being done under the sponsorship of [*insert* SPONSOR'S NAME AND ADDRESS].
Fund code: [*insert* FUND CODE]

Statistical methods (note[39])

No statistical methods are required.

Storage of raw data

At the completion of the study a copy of all notebooks, charts, worksheets, correspondence and other documents will be archived in the files of scientific data held at [*insert* NAME AND ADDRESS OF DOCUMENT REGISTRY].

Registration Authority Guidelines

The Study Director will use the Registration Authority Guidelines relevant to the conduct of the trial(s) at the planning stage and subsequently. They will be made available to personnel involved. The relevant Registration Authority Guidelines will be identified in the final report.

FIELD PORTION OF STUDY

Field notebooks

Field personnel will maintain a separate notebook for each trial. Each page will be numbered and headed with the trial number, date of the entry and name of the person making the notebook entry. Entries will be made in black pen or at least in a colour which photocopies clearly. Copies of all notebook pages will be included in the compiled submission to the Registration Authority.

Locations of test sites

The trials will be conducted at [*insert* ADDRESS OF TRIAL 1] and [*insert* ADDRESS OF TRIAL 2], etc
The officer in charge of field operations will obtain prior written agreement from the owners of the crops.

Justification for selection of test sites

The test sites (and crop varieties) must represent the crop to be treated commercially by the proposed use.

Spraying equipment

The equipment to be used in the trials must be fully described. It should simulate application by commercial equipment.

Calibration of spraying equipment

On each day of application the spray equipment will be cleaned and calibrated with water. All calibration data will be recorded.

[39] Decide at the protocol stage which statistical methods will be used. If none is required, say so.

Arrangements with crop owner(note[40])

The owner of the crop will be paid a fair price for the lost production as a result of the trial.

Test substance - active ingredient and formulation

Identify the active ingredient by common name, systematic name and CAS number.

Describe the formulation by name, type (e.g., EC), brand, batch number and date of manufacture or formulation.

A material safety data sheet (MSDS) shall accompany the formulation. Personnel shall follow safety instructions and guidelines on the MSDS.

Field personnel will take a sample of formulation (approximately 100 ml) into a clean glass bottle for subsequent analysis and will record the date of sampling. The sample will be labelled (see section "Labelling of samples") and will be sent to the officer in charge of laboratory operations at [*insert* NAME AND ADDRESS OF CO-OPERATING LABORATORY]. The formulation sample must NOT be in the same package or container as spray samples or residue samples.

Test substance - storage

The formulation used in the trials will be stored in an approved pesticide store. Field personnel will record the conditions (temperature) of storage. The use of the test substance shall be recorded. The records shall be stored with the raw data.

Use pattern - approved or label instructions

Record the current approved or label instructions. Describe proposed changes to approved or label instructions.

Use pattern proposed for trials

Describe the use pattern in the trials so that it may be readily compared with the registered or proposed label use pattern.

Experimental design

- number of trials at each test site.
- number of replications in each trial.
- number of plots (treated and untreated) in each trial or replication.
- plot size
- treatments

Calendar

Describe the anticipated calendar of treatment and sampling at each site.

Treated crop

Field personnel will record:

- variety
- grade or quality

[40] If the trial is on a private farm, decide at the protocol stage the arrangements with the farmer. Will the farmer be permitted to sell the treated produce? Will the farmer be paid for the lost production if the produce is not permitted to be sold? For example, a paragraph such as that provided might be included in the protocol.

Spray mixture

Field personnel will prepare the spray as accurately as possible to contain [*insert* CONCENTRATION] mg active ingredient per litre.

Field personnel will take two samples of well stirred or agitated spray into clean glass bottles (500 mL). One sample will be taken just prior to treatment and one just after. Samples will be labelled (see section "Labelling of samples") and will be sent to the officer in charge of laboratory operations. Spray samples must NOT be in the same package or container as residue samples.

Field personnel will take a sample (1 litre) of the water used for preparation of the spray. The sample will be labelled (see section "Labelling of samples") and will be sent to the officer in charge of laboratory operations. The water sample may accompany the dip or spray samples.

Field personnel will record:

- additives or other components of the spray solution
- method of preparing the spray solution
- exact measured volumes of formulation and water used to prepare the spray
- time of day the spray was prepared and the time elapsing until treatment.
- ambient temperature and humidity

To ensure that the commercial spray in the trial is prepared at the correct concentration it is advisable to have samples of spray analysed from an earlier run. If the concentration was not close to the required [*insert* CONCENTRATION] mg/L it would suggest that volumes of tank, etc, need more careful measurement.

Application conditions

All applications will be made within 2 hours of mixing the spray solution and according to agreed instructions and under typical conditions for the crop. Do not apply if rainfall is expected within 4 hours.

Application technique

All applications will be typical [foliar, soil, directed] application for the crop. [Foliage, fruit, soil] should be treated evenly and thoroughly. Avoid excessive run-off and drift. Directed application should be described according to a label (or proposed label) instruction.

Application rate

The amount of spray solution used on a plot will be recorded by difference (before and after spraying). The exact application rate will be recorded.

Previous pesticide uses on crop

Field personnel will record previous uses of all pesticides on the crop in the current season.

Labelling of samples

Formulation

- unique sample number
- trial number
- date of collection
- person collecting sample
- description of sample (formulation, batch)

Spray solutions

- unique sample number
- trial number
- date of collection
- person collecting sample
- description of sample (spray, prior or post treatment, expected concentration)

Residue samples

- unique sample number
- trial number
- date of collection
- person collecting sample
- description of sample

Water samples

- unique sample number
- trial number
- date of collection
- person collecting sample
- description of sample (water for producing spray)

Sample containers

Formulation:	glass bottle or jar with secure screw-cap lid.
Spray solution:	glass bottle with secure screw-cap lid
Residue samples:	[*insert* PROPOSED CONTAINERS, E.G. DOUBLE PLASTIC BAGS]
Water:	glass bottle with secure lid or stopper.

Field personnel will obtain suitable sample containers and labels from [*insert* APPROVED CONTAINER SOURCE].

Residue samples - field study

Field personnel will take [*insert* NUMBER] field control (no treatment) [*insert* COMMODITY] samples and [*insert* NUMBER] field treated samples. Each sample will be at least 2 kg and will be packed in a [*insert* PROPOSED CONTAINER, E.G. DOUBLE PLASTIC BAGS] container.

Samples will be labelled (see section “Labelling of samples”) and will be sent to the officer in charge of laboratory operations. Residue samples must NOT be in the same package or container as spray samples.

Despatch of samples to laboratory

Samples will be labelled (see section “Labelling of samples”) and will be sent to the officer in charge of laboratory operations. Field officers sending the samples to the laboratory must notify the officer in charge of laboratory operations when the samples are sent and when they are expected to arrive at the laboratory. Field officers should also notify the laboratory of the nature and the number of samples being sent. Samples must be despatched in good time to be received by the laboratory in business hours. Crop samples must NOT be in the same package or container as dip or spray samples. [*Insert* TEMPERATURE AND PACKING CONDITIONS NEEDED FOR INTEGRITY OF COMMODITY AND RESIDUE DURING TRANSPORT TO LABORATORY].

Storage conditions, dates and times for commodities

Field personnel will record the dates for:

- harvest
- treatment (date and time).

- packing (date and time).
- dispatch to laboratory (date and time).

[*Insert* COMMODITY] samples will be transported to the laboratory by [*insert* MODE OF TRANSPORT] in [*insert* COOL or FROZEN] condition in insulated containers.

Weather records

Record the weather conditions at the test sites during the trials.

Quality assurance

Describe the procedures and inspections of operations planned to ensure the reliability and validity of field operations.

LABORATORY PORTION OF STUDY

Laboratory notebooks

Laboratory personnel will maintain a separate notebook for each trial. Each page will be numbered and headed with the trial number, date of the entry and name of the person making the notebook entry. All relevant worksheets and instrument charts will be annotated the same way. Entries will be made in black pen or at least in a colour which photocopies clearly. Copies of all notebook pages, work sheets and instrument charts will be included in the compiled submission to the Registration Authority.

Sample identification list

Laboratory personnel will produce a table showing for each sample in the project the site, the trial identification, field number, laboratory number, and sample description (type of sample, date of treatment and date of sampling).

Residue samples - laboratory study

Residue samples will be analysed for [*insert* REQUIRED RESIDUE DEFINITION] (define precisely the pesticides and metabolites to be included in the analyses).
Analytical results (unadjusted for % recoveries) will show

1. [*Insert* PESTICIDE] defined residues on [*insert* COMMODITY] on a fresh weight basis
2. [*Insert* PESTICIDE] defined residues on [*insert* COMMODITY] on a dry weight basis (if needed)
3. etc

Analyses

State what portion(s) of commodity are to be analysed and how residue results are to be expressed, e.g. on a whole commodity basis, on edible portion basis, etc.

All residue samples will be analysed for [*insert* REQUIRED RESIDUE DEFINITION].

Each laboratory sample will be analysed in duplicate. This means that two sub-samples will be taken for analysis after a laboratory sample has been prepared.

The formulation will be analysed for [*insert* PESTICIDE COMMON NAME] content.

Spray solutions will be analysed for [*insert* PESTICIDE COMMON NAME] content.

The water will be characterised according to a standard water analysis, which will include pH and hardness.

Analytical methods

Residue samples will be analysed by method [*insert* METHOD TITLE AND IDENTIFYING NUMBER].

Spray solutions will be analysed by method [*insert* METHOD TITLE AND IDENTIFYING NUMBER]. Formulation samples will be preferably analysed by an AOAC or CIPAC method [*insert* METHOD TITLE AND IDENTIFYING NUMBER].

Analytical method validation

Validation data will be available for linearity of calibration, repeatability, limit of quantification, limit of detection and recoveries. The response of untreated (control) samples will be checked.

A limit of quantification of [*insert* REQUIRED LIMIT OF QUANTIFICATION] mg/kg will be achieved on a fresh weight basis.

Procedural recovery samples will be run with each batch of analyses.

Storage conditions, dates and times for samples

Laboratory personnel will record the date and time for:

- Sample receipt in laboratory
- Sample preparation
- Sample analysis. (Also record the number of days freezer storage before analysis).

Laboratory personnel will record the storage conditions of samples pending analysis (temperature, sample container, sample chopped or unchopped).

If samples have to be kept more than 2 weeks in freezer storage pending analysis information will be sought on the freezer storage stability of [*insert* PESTICIDE NAME] residues. If such information is not available it may be necessary to conduct a freezer storage stability study.

Anticipated analyses for one trial

Nature of sample	Treatment	Replicate		Replicate	
		Plot	Plot	Plot	Plot
Formulation Spray					
Water					
Fruit		duplicate analyses	duplicate analyses		

Laboratory capacity

The officer in charge of laboratory operations will ensure that the laboratory workload is compatible with the sample preparation, analyses and documentation required for the trial.

Quality assurance

Describe the procedures and inspections of operations planned to ensure the reliability and validity of laboratory operations.

DOCUMENTATION OF DATA SUBMISSION

A submission to the Registration Authority will be prepared according to the current guidelines. The main document will consist of a proposal and supporting data for [*insert* PESTICIDE NAME] MRLs, the laboratory report and the field report. Copies of all other documents relating to the project will be included in attachments.

Exercise 9.1. Estimation of an EMRL

See also Chapter 9

The aim of this exercise is to estimate a maximum residue level from residue monitoring data, suitable for use as EMRL (extraneous maximum residue limit).

The extraneous residue refers to a pesticide residue arising from environmental sources (including former agricultural uses) other than the use of a pesticide directly or indirectly on the commodity. EMRLs are estimated from residue data generated in food monitoring programmes.

Ideally, all geographically representative monitoring data for extraneous residues should be evaluated to cover international trade. The data should include the nil residue results (and the LOQs).

JMPR evaluates the monitoring data in terms of the expected violation rate[41] if a specified EMRL is selected. Violation rates of 0.5 to 1 % or greater are generally unacceptable in trade.

The task

Estimate the violation rates if an EMRL was established at 0.1, 0.5, 1, 2 or 5 mg/kg for each set of data.

Determine the critical data set. Determine that the critical data set is sufficient and is not selected data from a specific region of DDT usage.

Estimate a suitable EMRL for DDT residues in:

MM 0095 Meat (from mammals other than marine mammals) (fat)

DDT monitoring data for meat (fat)

DDT monitoring data for mammalian meat (fat) were reported by JMPR 1996[42].

JMPR (1996) received national residue survey data on animal products from Australia, Germany, New Zealand, Norway, Thailand and USA.

The presentation of the data differed from country to country, and the layouts in the Tables are consequently different. With the exception of the Australian data, all the residues are expressed as the sum of *p*,*p'*-DDT, *o*,*p'*-DDT, *p*,*p'*-DDE and *p*,*p'*-TDE (*p*,*p'*-DDD), in conformity with the Codex definition. In the Australian survey, the residues of DDT, DDE and TDE were reported separately.

[41] Violation rate: the incidence of residues exceeding the MRL or EMRL. Violation rate is usually expressed as a percentage. In the present exercise, the violation rate is the percentage of monitoring samples where the residue concentration exceeds a selected value that may be proposed as an EMRL.

[42] FAO. 1996. DDT. Pesticide residues in food. Evaluations 1996. Part I – Residues. FAO Plant Production and Protection Paper. 142:151-174.

Table 1. Residues of DDT in meat in Australia. 1989-1994.

Commodity	Compound	No. of samples	No. of residue-free samples	No. of samples with trace only	No. of samples with residues, mg/kg, in ranges				
					0.1-1	1.1-2.5	2.6-5	5.1-10	>10
Beef (fat)	DDT	39854	39730	60	61	1	1		1
	DDE	39854	37149	1283	1394	24	3	1	
	TDE	39854	39752	47	53	2			
Sheep (fat)	DDT	29270	29169	59	41				
	DDE	29270	25604	1336	2314	13			
	TDE	29270	29208	33	28	1			
Porcine (fat)	DDT	15900	15761	62	74	2		1	
	DDE	15900	15257	427	210	5	1		
	TDE	15900	15814	44	40	2			

Table 2. Residues of DDT in meat in Germany, 1993.

Commodity	No. of samples	No. of samples with DDT residues, mg/kg, in range												max mg/kg
		<0.001	0.001	0.002-0.01	0.011-0.015	0.016-0.02	0.021-0.05	0.051-0.1	0.11-0.2	0.21-0.5	0.6-1	1.1-2	2.1-5	
Meat[43] (fat)	777	128		87	54	102	230	119	39	17	1			0.5
Sheep meat (fat)	87	6		2	4	14	18	11	24	6	1	1		1.01

Table 3. Residues of DDT in meat in New Zealand, 1990-1994.

Commodity	No. of samples analysed	No. positive (≥0.02 mg/kg)	No. of samples with residues, mg/kg fat, in range					DDT max, mg/kg
			0.02-0.5	0.51-1.0	1.01-2.0	2.1-5.0	>5	
Lambs	965	534	491	25	16	2		3.7
Adult sheep	548	277	250	15	8	4		2.6
Adult bovine	739	319	304	11	5			1.4
Suckling calves	1211	857	768	58	21	9	1	5.2
Pigs	925	507	487	10	6	3	1	6.2

Table 4. Residues of DDT in meat from lambs in New Zealand from a region with a known DDT history, 1992-1993.

Commodity	No. of samples analysed	No. positive (≥0.02 mg/kg)	No. of samples with residues, mg/kg fat, in range					DDT max, mg/kg
			0.02-0.5	0.51-1.0	1.01-2.0	2.1-5.0	>5	
Lambs	403	396	183	82	60	58	13	13

Table 5. Residues of DDT in meat in Norway, 1990-1994.

Commodity	No. of samples	No. of samples with DDT residues (mg/kg fat)	
		<0.02	0.02-0.5
Bovine (fat)	537	536	1
Pigs (fat)	537	536	1
Sheep (fat)	149	149	
Moose (fat)	169	169	

Table 6. Residues of DDT in meat in Thailand, 1993 and 1994.

Commodity	No. of samples	No. of samples with DDT residues, mg/kg fat, in range				
		<0.01	0.01-0.05	0.06-0.1	0.11-0.5	0.51-1
Cattle meat, 1993	30	2	23	2	3	
Cattle meat, 1994	123	2	94	16	11	
Pig meat, 1993	65	1	48	10	6	
Pig meat, 1994	157	1	129	19	8	

[43] Except sheep.

Table 7. Residues of DDT in meat in USA. 1991-1994.

Animal	No. of samples	No. of samples with DDT residues, mg/kg fat, in range							
		0.01-0.1	0.11-0.2	0.21-0.3	0.31-0.5	0.51-1.0	1.01-2.5	2.51-5	>5.0
Cattle, 1991	4650	58	20	8	6	4	2		
Sheep, 1991	347	2	1	3					
Hogs, 1991	643	5	1	1		1	1	1	
Cattle, 1992	1546	67	38	11	3	5	1	1	
Sheep, 1992	342	15	11	4	7	4	1		
Hogs, 1992	3604	51	25	16	12	6	2	1	2
Cattle, 1993	4032	138	82	32	25	10	6		
Sheep, 1993	1107	61	37	15	7	4	2		
Hogs, 1993	1488	22	12	10	5	2	1		1
		0.04-0.1 mg/kg							
Cattle, 1994	3955	151	66	39	31	7	2	1	1
Pigs, 1994	1457	57	27	14	8	3	1		1
Sheep and goats, 1994	900	91	55	27	15	18	2		

Worksheet

Calculate percentage of samples exceeding selected DDT residue concentrations of 0.1, 0.5, 1, 2 or 5 mg/kg.

Country	Commodity	No. of samples	Percentage of samples exceeding DDT residue, mg/kg				
			0.1 mg/kg	0.5 mg/kg	1 mg/kg	2 mg/kg	5 mg/kg
Australia 1989-94	beef (fat)	39854	3.6		0.073		0.005
Australia 1989-94	sheep (fat)	29270					
Australia 1989-94							
Germany, 1993							
Germany, 1993							
New Zealand, 1990-94							
New Zealand, 1990-94							

Exercise 10.1. Evaluation of food processing data

See also Chapter 10

1. Evaluation of food processing data

2. The aim

The aim of this exercise is to estimate processing factors from the residue data resulting from food processing trials.

The processing factors will then be used to estimate STMR-P values for processed commodities.

3. Calculate processing factors

Commodity	Residues mg/kg	Processing factor
Apples apple juice	0.25 0.15	0.60
Apples apple juice	0.02 <0.01	<0.05

Processing factor =

$$\frac{\text{residue level [mg/kg] in processed product}}{\text{residue level [mg/kg] in RAC}}$$

4. Estimate processing factor

Processed commodity	Calculated processing factors	Median or best estimate
Cotton seed meal	0.15, 0.20, 0.27, <0.3, 0.49	0.27

Cotton seed oil, refined	<0.02, <0.08, <0.09, <0.20, <0.33 **Note**[44]	<0.02
Tomato paste	0.8, 4.1 **Note**[45]	4.1

5. Estimate STMR-Ps, HR-Ps for processed commodities.

- In a large commercial process where the RAC originates from many farms, bulking and blending occur.
- Therefore, a residue equivalent to the HR is highly unlikely in a batch for processing and the processing factor is generally applied to the STMR.
- It is appropriate to use the HR for canned tomatoes because the individual tomato comes through the process. **Question**[46]

6. Estimate STMR-Ps, HR-Ps for processed commodities.

Raw agricultural commodity (RAC)	Processed commodity	Processing factor (PF)	Raw commodity		Processed commodity	
			STMR, mg/kg	HR, mg/kg	STMR-P =STMR ×PF, mg/kg	HR-P =HR xPF, mg/kg
Tomatoes	Tomato paste	4.1	0.28		1.1	
	Tomato puree	1.8	0.28		0.50	
	Tomato juice	0.93	0.28		0.26	
	Canned tomato	0.39	0.28	0.76	0.11	0.30

7. Data for evaluation

1 Pirimicarb residues in apples.
2 Pirimicarb residues in tomatoes.
3 Trifloxystrobin residues in grapes.
4 Hexythiazox residues in oranges.

[44] No residues in oil above LOQ. The calculated factors just reflect the starting concentration in the cotton seed. Best estimate is the one with highest starting concentration
[45] Perhaps 2 different processes. The mid-point may not represent either one.
[46] Are there other situations where it would be correct to use the HR?

8. The exercise

1. Calculate the processing factor for each processed commodity in each trial.
2. Make the best estimate of processing factor from the experimentally derived values.
3. Use the processing factors and the STMR of the RAC to produce an STMR-P value for each processed food or feed commodity.
4. Also produce HR-P values for processed commodities where needed.

9. The exercise

- Back in plenary
  - Your results
  - Comments and observations
  - Uncertainties in the estimates?
  - Questions?
  - Specific problems
  - What did you learn?

FOOD PROCESSING DATA

Data from processing trials

Table 1. Pirimicarb residues in apples and processed commodities resulting from trials in Italy and France.

APPLES	Application		PHI	Commodity	Residues	Ref
country, year (variety)	kg ai/hl	no.	days		mg/kg	
Italy, 2000 (Red Chief)	0.05	2	7	apples wet pomace dry pomace apple juice	0.06 0.10 0.33 0.03	IT20-00-S391
France, 2003 (Golden)	0.0375	2	7	apples dry pomace apple juice	0.08 0.40 0.06	AF/7359/SY/1
France, 2003 (Golden)	0.0375	2	7	apples dry pomace apple juice	0.08 0.44 0.06	AF/7359/SY/2

APPLES	Application		PHI	Commodity	Residues	Ref
country, year (variety)	kg ai/hl	no.	days		mg/kg	
France, 2003 (Golden)	0.0375	2	7	apples dry pomace apple juice	0.05 0.38 0.05	AF/7359/SY/3

Table 2. Pirimicarb residues in tomatoes and processed commodities resulting from trials in Italy and France.

TOMATOES	Application		PHI	Commodity	Residues	Ref
country, year (variety)	kg ai/hl	no.	days		mg/kg	
Italy, 1997 (Red River)	0.05	2	3	tomatoes tomato juice tomatoes canned	0.13 0.08 0.02	IT33-97-E379
France, 2003 (Quest)	0.10	2	3	tomatoes tomato juice tomatoes canned	0.43 0.37 0.39	AF/7363/SY/1
France, 2003 (Quest)	0.10	2	3	tomatoes tomato juice tomatoes canned	0.37 0.57 0.51	AF/7363/SY/2
France, 2003 (Quest)	0.10	2	3	tomatoes tomato juice tomatoes canned	0.47 0.33 0.51	AF/7363/SY/3
France, 2003 (Quest)	0.10	2	3	tomatoes tomato juice tomatoes canned	0.56 0.28 0.37	AF/7363/SY/4

Table 3. Trifloxystrobin residues in grapes and processed commodities resulting from trials in Europe.

GRAPES	Application			PHI	Commodity	Residues	Ref
country, year	kg ai/ha	kg ai/hl	no.	days		mg/kg	
Germany, 1996	0.35-0.39	0.047-0.094	8	35	berries wine	1.01 <0.02	gr01396

GRAPES	Application			PHI	Commodity	Residues	Ref
country, year	kg ai/ha	kg ai/hl	no.	days		mg/kg	
Germany, 1996	0.34-0.38	0.044-0.075	8	35	berries wine	0.37 <0.02	gr01496
Germany, 1997	0.19	0.023-0.047	8	36	berries wine	0.71 <0.02	gr45597
Germany, 1997	0.19	0.023-0.047	8	36	berries wine	0.66 <0.02	gr46597
Germany, 1995	0.19-0.22	0.023-0.047	8	41	berries wine	0.44 <0.02	CGD03
Switzerland, 1995	0.19	0.013	8	42	berries wine	0.22 0.05	2035/95
Switzerland, 1995	0.19	0.013	8	42	berries wine	0.58 0.17	2036/95
Germany, 1995	0.19-0.20	0.02	8	41	berries wine	1.01 <0.02	951047008
Germany, 1996	0.18-0.19	0.024-0.047	8	35	berries wine	1.23 <0.02	gr01196
Germany, 1996	0.15-0.21	0.022-0.038	8	35	berries wine	0.35 <0.02	gr01296
France, 1996	0.19	0.17-0.19	8	35	berries wine	0.64 0.03	FRA-DE17
France, 1996	0.19	0.094	8	36	berries wine	0.94 0.10	FRA-KJ58
Switzerland, 1998	0.20	0.05	4	46	berries wine	0.22 <0.02	SWZ-98-3-211.051
Switzerland, 1998	0.20	0.05	4	46	berries wine	0.15 <0.02	SWZ-98-3-211.052
Switzerland, 1998	0.20	0.02	4	42	berries wine	0.13 <0.02	SWZ-98-3-211.060

GRAPES	Application			PHI	Commodity	Residues	Ref
country, year	kg ai/ha	kg ai/hl	no.	days		mg/kg	
Switzerland, 1998	0.20	0.033	4	50	berries wine	0.25 0.04	SWZ-98-3-211.061
Italy, 1996	0.19	0.021	8	35	berries wine	0.16 <0.02	ITA-2084-96
Italy, 1996	0.19	0.027	8	35	berries wine	1.36 0.10	ITA-2085-96

Table 4. Hexythiazox residues in oranges and processed commodities resulting from supervised trials in USA, Italy and Spain.

ORANGES	Application			PHI	Commodity	Residues	Ref
country, year	kg ai/ha	kg ai/hl	no.	days		mg/kg	
USA (CA), 2006 (Valencia)	1.05	0.056	1	28	whole fruits juice pulp, dried citrus oil	0.29 <0.02 0.78 60	TCI-06-142
USA (CA), 2006 (Valencia)	1.05	0.056	1	28	whole fruits juice pulp, dried citrus oil	0.44 <0.02 0.76 32	TCI-06-142-01
Italy, 2002 (Navel 115)	0.8	0.02	2	14	whole fruit marmalade juice	0.67 0.18 0.15	A2058 IT2
Spain, 2002 (New Holl)	0.8	0.02	2	14	whole fruit marmalade juice	0.44 0.06 0.13	A2058 PA2
Spain, 2002 (Navel New Gold)	0.8	0.02	2	13	whole fruit marmalade raw juice dry pomace final juice	0.85 0.09 0.33 2.4 0.22	A2058 ES2

STMR and HR values

Trifloxystrobin	FB 0269 Grapes	STMR 0.15 mg/kg
Pirimicarb	FP 0009 Pome fruits	STMR 0.18 mg/kg
Pirimicarb	VO 0050 Fruiting vegetables other than cucurbits	STMR 0.105 mg/kg
Pirimicarb	VO 0050 Fruiting vegetables other than cucurbits	HR 0.25 mg/kg
Hexythiazox	FC 0001 Citrus fruits (edible portion)	STMR 0.077 mg/kg
Hexythiazox	FC 0001 Citrus fruits (whole fruit)	median 0.11 mg/kg

MRL values

Trifloxystrobin	FB 0269 Grapes	3 mg/kg
Pirimicarb	FP 0009 Pome fruits	1 mg/kg
Pirimicarb	VO 0050 Fruiting vegetables other than cucurbits	0.5 mg/kg
Hexythiazox	FC 0001 Citrus fruits	0.5 mg/kg

WORKSHEETS

1. Calculate the processing factor for each processed commodity in each trial.

Pirimicarb residues in apples

APPLES country, year (variety)	Commodity	Residues mg/kg	Processing factors	Ref
Italy, 2000 (Red Chief)	apples	0.06		IT20-00-S391
	wet pomace	0.10	1.67	
	dry pomace	0.33	5.5	
	apple juice	0.03	0.50	
France, 2003 (Golden)	apples			
	dry pomace			
	apple juice			

Pirimicarb residues in tomatoes

TOMATOES country, year (variety)	Commodity	Residues mg/kg	Processing factors	Ref
Italy, 1997 (Red River)	tomatoes	0.13		IT33-97-E379
	tomato juice	0.08	0.62	
	tomatoes canned	0.02	0.15	
France, 2003 (Quest)	tomatoes			
	tomato juice			
	tomatoes canned			

Trifloxystrobin residues in grapes.

GRAPES country, year	Commodity	Residues mg/kg	Processing factors	Ref
Germany, 1996	berries wine	1.01 <0.02	<0.02	gr01396
Germany, 1996	berries wine			

Hexythiazox residues in oranges.

ORANGES country, year	Commodity	Residues mg/kg	Processing factors	Ref
USA (CA), 2006 (Valencia)	whole fruits juice pulp, dried citrus oil	0.29 <0.02 0.78 60	<0.069 2.69 207	TCI-06-142

2. Make the best estimate of processing factor from the experimentally derived values.

RAC	Processed commodity	Processing factors	Median or best estimate
PIRIMICARB			
Apples	apple pomace, dry		
	apple juice		
Tomato	tomato juice		
	tomato, canned		
TRIFLOXYSTROBIN			
Grapes	wine		
HEXYTHIAZOX			
Oranges	juice		
	dried pomace (dried pulp)		
	citrus oil		
	marmalade		

3. Use the processing factors and the STMR of the RAC to produce an STMR-P value for each processed food or feed commodity.

RAC	STMR	HR	Processed commodity	Processing factor	STMR-P	HR-P
PIRIMICARB						
Apples	0.18[47]		apple pomace, dry			
Apples	0.18[47]		apple juice			
Tomatoes	0.105[48]		tomato juice			
Tomatoes	0.105[48]	0.25[49]	canned tomato			
TRIFLOXYSTROBIN						
Grapes	0.15[50]		wine			

[47] Pirimicarb FP 0009 Pome fruits STMR 0.18 mg/kg
[48] Pirimicarb VO 0050 Fruiting vegetables other than cucurbits STMR 0.105 mg/kg.
[49] Pirimicarb VO 0050 Fruiting vegetables other than cucurbits HR 0.25 mg/kg.
[50] Trifloxystrobin FB 0269 Grapes STMR 0.15mg/kg.

RAC	STMR	HR	Processed commodity	Processing factor	STMR-P	HR-P
HEXYTHIAZOX						
Oranges	0.11[51]		orange juice			
Oranges	0.11[51]		citrus pulp, dry			

NOTE: The STMR for citrus fruits applies to the edible portion (citrus flesh), but the processing factor refers to the raw agricultural commodity (RAC). Therefore, the STMR-P for citrus processed commodities is calculated from the median RAC value, not the STMR.

[51] Hexythiazox FC 0001 Citrus fruits (whole fruit) median 0.11 mg/kg

Exercise 11.1 Calculation of livestock dietary burden.

See also Chapter 11

1. Calculation of livestock dietary burden

2. The aim

- The aim of this exercise is to explain the use of the automated dietary burden calculator spreadsheet.

- The exercise involves
  Selection of residue data on animal feed commodities
  Calculation of dietary burden from residue data and standard livestock diets

3. Residue levels in feed materials.

- The values are obtained from the evaluation of supervised trials and food processing trials.
- Information required
  STMRs and high residues for feed materials
  STMRs and high residues on food items used as feed materials
  STMR-Ps on processed commodities.

4. Expressed on dry weight

- The calculations are made on residues expressed on dry weight.
  i.e. the residue content is expressed as if it were wholly contained in the dry matter.
- The spreadsheet assumes standard % dry matter for each commodity.
- If the residue in a commodity is already expressed on dry weight, the % dry matter in the spreadsheet should be amended to 100.

5. Calculate livestock dietary burden

- Information required
  residue levels in feed materials

livestock diets (OECD Feed Table 2009 (available from the FAO website: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmpr/jmpr-docs/en/).)
automated dietary burden calculator spreadsheet.

Note 1. Livestock diet data are currently available from US-Canada, EU, Australia and Japan.

Note 2. The calculator spreadsheet contains the OECD Feed Table 2009.

6. Dietary burden calculator performs these tasks

- Selects the commodities within the diet that lead to the highest burden of residues.
- Allows for commodity group constraints.
- Generates summary tables.

7. Cypermethrin residues

Cypermethrin residue data are available for:
food commodities (e.g. cereal grains);
animal feed commodities (e.g. straw and fodders); and
processed commodities (e.g. grape pomace).

8. Residue data

Commodity	Commodity group NOTE 1	STMR or STMR-P mg/kg	high residue mg/kg	% dry matter
Alfalfa fodder	L	11.5	20	100
Alfalfa forage	L	3.65	11	
Barley forage	S AF	0.39	1.4	
Barley grain	C	0.035	NOTE 2	

NOTE 1. Commodity groups, e.g.
AL: legume animal feed
AS AF: forage, straw, fodder (dry) and hay of cereal grains and other grass-like plants
GC: cereal grains
NOTE 2. High residue not necessary for cereal grains because the grain from many farms is bulked and blended.

9. The spreadsheet procedure

Open *BASIC_TAB* and enter highest residues, STMRs and STMR-Ps for the required commodities.
Open *Summary Sheet*, where calculated results are available for maximum and mean for each of beef cattle, dairy cattle, poultry broiler and poultry layer.

10. The calculated results

Summarise dietary burden calculations in the table provided.

		US-Canada	EU	Australia	Japan
Max	beef				
	dairy				
	broiler				
	layer				
Mean	beef				
	dairy				
	broiler				
	layer				

11. The calculated results

- Select the dietary burdens for integration with results of livestock feeding studies.

	Maximum	Mean
Beef, for residues in tissues		
Dairy, for residues in milk		
Poultry, for residues in tissues		
Poultry, for residues in eggs		

12. Questions?

Calculation of livestock dietary burden.

Information required

1) Residue levels in feed materials

2) Livestock diets (OECD Feed Table 2009 (available from the FAO website: http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmpr/jmpr-docs/en/).)

3) Automated dietary burden calculator spreadsheet (contains the OECD Feed Table data).

Cypermethrin residue levels in feed materials.

Residue data are available for food commodities (e.g. cereal grains), animal feed commodities (e.g. straw and fodders) and processed commodities (e.g. grape pomace).

The data for some feed commodities are already expressed on the dry weight, indicated in the final column of the table as 100 % dry matter.

Commodity	Commodity group	STMR or STMR-P mg/kg	high residue mg/kg	% dry matter
Alfalfa fodder	AL	11.5	20	100
Alfalfa forage	AL	3.65	11	
Barley forage	AS AF	0.39	1.4	
Barley grain	GC	0.035		
Barley straw	AS AF	3.6	6.9	100
Bean forage (green)	AL	0.71	2.1	
Beans (dry)	VD	0.05		100
Beet, sugar tops	AV	1.5	8.3	100
Cabbage heads leaves	VB	0.02	0.65	
Carrot culls	VR	0.01	0.01	
Grape pomace, dry	AB	0.032		100
Maize	GC	0.01		
Maize fodder	AS AF	3.6	6.9	100
Maize forage	AS AF	0.05	0.1	
Oat straw	AS AF	3.6	6.9	100
Oats	GC	0.02		
Pea hay or Pea fodder (dry)	AL	0.42	1.1	100
Pea straw	AL	0.42	1.1	100
Pea vines	AL	0.45	2.1	
Peas (dry)	VD	0.05		100
Rice grain	GC	0.57		
Rice straw and fodder, dry	AS AF	3.6	6.9	100

Commodity	Commodity group	STMR or STMR-P mg/kg	high residue mg/kg	% dry matter
Soya bean (dry)	VD	0.05		
Sugar beet leaves or tops	AV	1.5	8.3	100
Wheat	GC	0.01		
Wheat forage	AS AF	0.38	1.4	
Wheat milled (bran)	CM	0.024		
Wheat straw and fodder, dry	AS AF	3.6	6.9	100

The task is to:

1. enter the data into the automated dietary burden spreadsheet calculator;
2. summarise the results for maximum and mean burdens for beef cattle, dairy cattle, poultry broiler and poultry layer for each of the four livestock diets;
3. select the highest of the maximum and mean burdens for the next step in the evaluation process, integration with results of livestock feeding studies

Automated dietary burden spreadsheet used by JMPR 2011

(2011Animal_burden_09.26.xls).

Procedure.

- Open *BASIC_TAB* and enter highest residues, STMRs and STMR-Ps for the required commodities.
- Open *Summary Sheet*.
  The results are immediately available for maximum and mean for each of beef cattle, dairy cattle, poultry broiler and poultry layer.

Summarise the results in the following tables.

Summary of dietary burden calculations

		US-Canada	EU	Australia	Japan
Max	beef				
	dairy				
	broiler				
	layer				
Mean	beef				
	dairy				
	broiler				
	layer				

Select dietary burdens for integration with results of livestock feeding studies.

	Maximum	Mean
Beef, for residues in tissues		
Dairy, for residues in milk		
Poultry, for residues in tissues		
Poultry, for residues in eggs		

Abbreviations and acronyms in this exercise

AB	Codex commodity group, by-products, used for animal feeding purposes, derived from fruit and vegetable processing
AF	Codex commodity group, forage of cereal grains and grasses
AL	Codex commodity group, legume animal feeds
AS	Codex commodity group: straw, fodder (dry) and hay of cereal grains and other grass-like plants
AV	Codex commodity group, miscellaneous fodder and forage crops (forage)
CM	Codex commodity group, milled cereal products
FAO	Food and Agriculture Organization of the United Nations
GC	Codex commodity group, cereal grains
HR	highest residue
MRL	maximum residue limit
OECD	Organization for Economic Co-operation and Development
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor
VB	Codex commodity group, Brassica (cole or cabbage) vegetables, head cabbages, flowerhead cabbages
VD	Codex commodity group, pulses
VR	Codex commodity group, root and tuber vegetables

Exercise 11.2. Evaluation of livestock feeding studies.

See also Chapter 11

1. Evaluation of livestock feeding studies.

2. The aim

- The aim of this exercise is to explain the interpretation of livestock feeding studies and the integration of the feeding results with dietary burden to produce STMRs, HRs and MRLs for animal commodities.

3. The exercise involves

Summarising the feeding studies and finding the relationships between residue levels (in tissues and milk) and dosing levels.
Estimating residue levels in tissues and milk that would occur when the livestock consume diets with residue levels equivalent to the dietary burdens.
Converting those estimated residue levels to STMRs, HRs and MRLs.

4. Available information 1.

The pesticide is bifenthrin

- Results of metabolism studies.

Definition of the residue (for compliance with the MRL for plant and animal commodities and for estimation of dietary intake for plant and animal commodities): bifenthrin (sum of isomers). The residue is fat soluble.

5. Available information 2.

Dietary burden for bifenthrin

	Livestock dietary burden, ppm of dry matter diet	
	max	mean
Beef cattle	8.26	3.35
Dairy cattle	7.41	3.21

6. Available information 3.

Residue data (individual animal) from feeding studies on lactating dairy cows.

- Residues of bifenthrin in tissues from lactating Holstein dairy cows dosed for 28 days with bifenthrin at 5, 15 and 50 ppm in the dry weight diet.
- Residues of bifenthrin in milk from lactating Holstein dairy cows dosed for 28 days with bifenthrin at 5, 15 and 50 ppm in the dry weight diet.
- Residues of bifenthrin in milk fat from lactating Holstein dairy cows dosed for 28 days with bifenthrin at the equivalent of 5 and 50 ppm in the dry weight diet.

7. Evaluation procedure

Step 1. Which feeding study data are valid and fully supported by essential information?
Step 2. When did residues in milk and milk fat reach a plateau level?
Step 3. What are the relationships between residues in tissues and feeding levels?
Step 4. What residue levels are predicted in cattle tissues, milk and milk fat when cattle consume diets with residue levels equivalent to the dietary burdens.
Step 5. Estimate STMRs, HRs and MRLs for animal commodities.

8. Step 1. Valid and fully supported data

Which feeding study data are valid and fully supported by essential information?
Checklist

country;
animal breed;
number of animals in each feeding group;
animal weights and changes during the study;
feed consumption, expressed on dry weight;
duration and mechanism of dosing, e.g. daily oral capsule for 28 days;
milk collection and timing, milk production;
for fat-soluble compounds, milk fat to be mechanically separated and analysed;

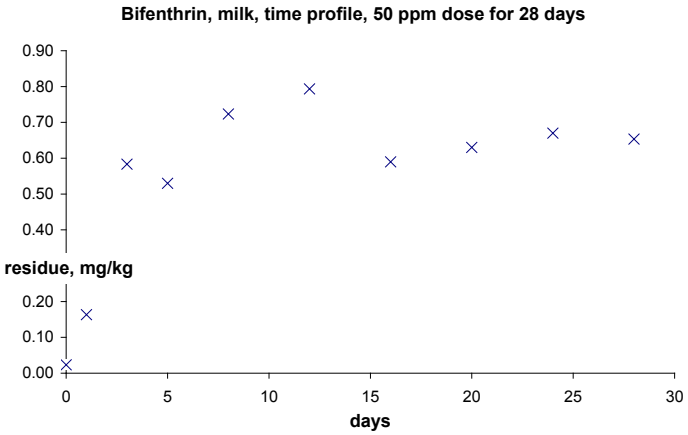
9. Step 1. Checklist (continued)

tissues collected and timing;
commodities analysed;
residue expressed as residue definition;
analytical method;
% recoveries;
residues in samples from control group;
interval of time sample is in freezer and freezer conditions - is it OK?

10. Step 2. Time profile for residues in milk

The plateau for residue levels (mean of the group) in milk has apparently been reached after approximately 3-5 days of dosing.

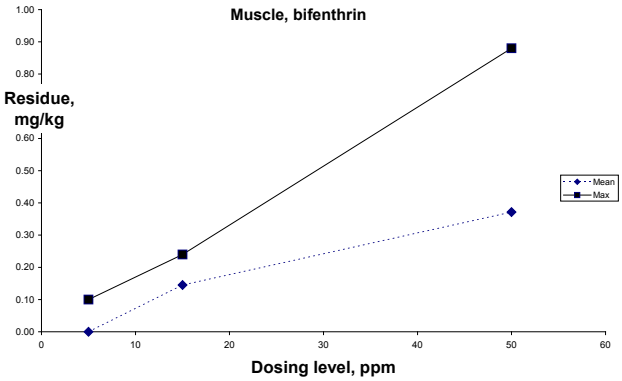
The mean residues in milk may then be calculated as the mean from day 3 to day 28.



11. Step 3. Relationships between residues in tissues and feeding levels

A plot of residues in the tissue (mean and maximum of the group) as a function of feeding level is helpful for interpretation.

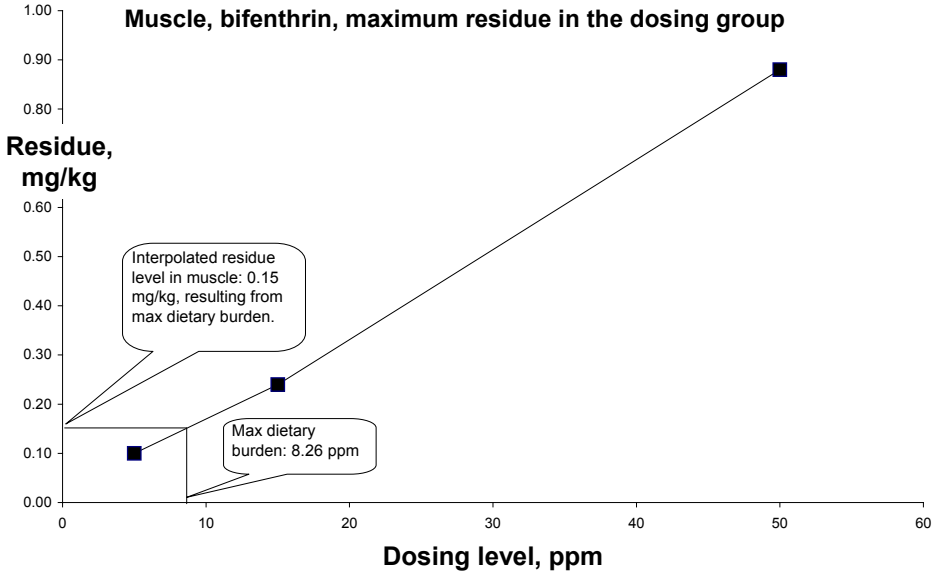
Are residues consistent with dosing levels?



12. Step 4. Integrate the dietary burden values into the feeding study relationships

- Tissues
  - Integrate maximum dietary burden with maximum residue in tissues of individual animals in the dosing groups to estimate HR and MRL.
  - Integrate mean dietary burden with mean residue in tissues of animals in the dosing groups to estimate STMR.
- Milk
  - Integrate maximum dietary burden with mean residue in milk of the dosing groups to estimate MRL.
  - Integrate mean dietary burden with mean residue in milk of the dosing groups to estimate STMR.

13. Step 4. Estimate residue levels by interpolation



14. Interpreting "<LOQ" values

Is the value close to LOQ or is it essentially zero?

Context may sometimes assist with the interpretation.

Example 1

	5 ppm dose	15 ppm dose	50 ppm dose
Liver	<0.1 <0.1	<0.1 <0.1	<0.1 <0.1

At 50 ppm dosing, residues are below LOQ. At 5 ppm and 15 ppm, residues are expected to be closer to zero than to LOQ.

15. Interpreting "<LOQ" values 2

Example 2

	5 ppm dose	15 ppm dose	50 ppm dose
Muscle, adductor	<0.1 <0.1	<0.1 <0.1	0.11 0.23
Muscle, pectoral	<0.1 <0.1	0.15 0.24	0.33 0.88
Muscle, cardial	<0.1 <0.1	0.11 0.17	0.27 0.41

Residues in muscle are quite variable between animals and at different sites within the one animal.
This suggests that, at the 5 ppm dosing, mean residues are likely to be well below LOQ.
In this situation it may be preferable to assess a 3 ppm dietary burden against the 15 ppm dosing level.

16. Step 5. Estimate STMRs, HRs and MRLs for animal commodities.

Recommendations table

CCN	Commodity	Recommended MRL, mg/kg	STMR, mg/kg	HR, mg/kg
	meat			
	offal			
	milk			
	milk fat			

17. Questions?

DAIRY COW FEEDING STUDIES – RESIDUE DATA

Table 1. Residues of bifenthrin in tissues from lactating Holstein dairy cows dosed for 28 days with bifenthrin at 5, 15 and 50 ppm in the dry weight diet. Data are available for 2 animals per feeding group.

Tissue	Bifenthrin residues, mg/kg		
	5 ppm dose	15 ppm dose	50 ppm dose
Muscle, adductor	<0.1 <0.1	<0.1 <0.1	0.11 0.23
Muscle, pectoral	<0.1 <0.1	0.15 0.24	0.33 0.88
Muscle, cardial	<0.1 <0.1	0.11 0.17	0.27 0.41
Liver	<0.1 <0.1	<0.1 <0.1	<0.1 <0.1
Kidney	<0.1 <0.1	0.18 0.19	0.44 0.49
Fat, subcutaneous	0.25 0.74	0.68 0.92	2.0 2.7
Fat, peritoneal	0.77 1.7	1.5 2.2	3.3 5.8

Table 2. Residues of bifenthrin in milk from lactating Holstein dairy cows dosed for 28 days with bifenthrin at 5, 15 and 50 ppm in the dry weight diet. Data are available for 3 animals per feeding group. (Same study as in Table 1).

Milk	Bifenthrin residues, mg/kg		
Study day	5 ppm dose	15 ppm dose	50 ppm dose
0	<0.01 (3)	<0.02 (3)	0.02 0.02 0.03
1	0.03 0.04 0.09		0.12 0.030 0.34
3	0.05 0.05 0.13	0.08 0.11 0.15	0.48 0.59 0.68
5	0.06 0.08 0.16		0.47 0.49 0.63
8		0.16 0.22 0.14	0.62 0.75 0.80
12	0.04 0.04 0.10		0.55 0.83 1.00
16		0.11 0.15 0.16	0.43 0.66 0.68
20	0.07 0.07 0.14		0.44 0.70 0.75
24		0.14 0.16 0.24	0.54 0.73 0.74
28	0.05 0.07 0.12		0.53 0.63 0.80

Table 3. Residues of bifenthrin in milk fat from lactating Holstein dairy cows dosed for 28 days with bifenthrin at the equivalent of 5 and 50 ppm in the dry weight diet. Data are available for 3 animals per feeding group. (Different study from that in Tables 1 and 2).

Milk fat	Bifenthrin residues, mg/kg	
Study day	5 ppm dose	50 ppm dose
0	<0.2 (3)	<0.2 (3)
3	0.72 0.78 0.97	7.8 8.8 9.6
8	0.64 0.67 1.6	7.8 8.9 10.2
16	0.54 0.62 1.2	8.0 8.2 10.1
24	0.35 0.70 1.1	7.4 8.6 9.4
28	0.48 0.50 0.61	8.0 9.4 10

Data validity

Checklist

- country;
- animal breed;
- number of animals in each feeding group;
- animal weights and changes during the study;
- feed consumption, expressed on dry weight;

- duration and mechanism of dosing, e.g. daily oral capsule for 28 days;
- milk collection and timing, milk production;
- for fat-soluble compounds, milk fat to be mechanically separated and analysed;
- tissues collected and timing;
- commodities analysed;
- residue expressed as residue definition;
- analytical method;
- % recoveries;
- residues in samples from control group;
- interval of time sample is in freezer and freezer conditions - is it OK?

For the purposes of the exercise, accept that data validity has been checked and that the supporting information is sufficient.

Residue definition

(for compliance with the MRL for plant and animal commodities and for estimation of dietary intake for plant and animal commodities): bifenthrin (sum of isomers). The residue is fat soluble.

Cattle dietary burden for bifenthrin

	Livestock dietary burden, ppm of dry matter diet	
	max	mean
Beef cattle	8.26 Note [a]	3.35 Note [b]
Dairy cattle	7.41 Note [c]	3.21 Note [d]

Note [a] Highest maximum beef or dairy cattle burden suitable for MRL estimates for mammalian meat
Note [b] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.
Note [c] Highest maximum dairy cattle dietary burden suitable for MRL estimates for milk.
Note [d] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

Worksheets for estimation of residue levels in tissues and milk that relate to the dietary burdens - interpolation

Feeding study values		Max dietary burden	Calculated by interpolation	
Dosing, conc in dry wt feed, ppm	**Highest residues**, mg/kg	Conc in dry wt feed, ppm	Residue (mg/kg) corresponding to dietary burden	
Muscle tissue				
5	<0.1 Note[52]	8.26	0.146	HR for muscle
15	0.24			
Kidney tissue				
Liver tissue				
Fat tissue				

[52] At 5 ppm dosing, residues in muscle were all <0.1 mg/kg. Accept that 0.1 mg/kg is the highest.

Example of interpolation calculation for highest residue in muscle.

$$residue = residue1 + \frac{(Dburden - dose1)(residue2 - residue1)}{(dose2 - dose1)}$$

$$0.146 = 0.1 + \frac{(8.26 - 5)(0.24 - 0.1)}{(15 - 5)}$$

Feeding study values		Mean dietary burden	Calculated by interpolation	
Dosing, conc in dry wt feed, ppm	**Mean residues**, mg/kg	Conc in dry wt feed, ppm	Residue (mg/kg) corresponding to dietary burden	
Muscle tissue				
Kidney tissue				
Liver tissue				
Fat tissue				STMR for fat

Feeding study values		Dietary burden	Calculated by interpolation	
Dosing, conc in dry wt feed, ppm	**Mean residues**, mg/kg	Conc in dry wt feed, ppm	Residue (mg/kg) corresponding to dietary burden	
Milk				
5 15	0.083 0.152	7.41 max	0.100	supports MRL for milk
Milk				
		3.21 mean		
Milk fat				
		7.41 max		
Milk fat				
		3.21 mean		

Recommendations table.

Estimate values for *mrl*, *stmr* and *hr* in the table.

CCN	Commodity	Recommended MRL, mg/kg	STMR, mg/kg	HR, mg/kg
MM 0095	Meat (from mammals other than marine mammals)	*mrl* (fat)	*stmr* fat *stmr* muscle	*hr* fat *hr* muscle
MO 0105	Edible offal (Mammalian)	*mrl*	*stmr*	*hr*
ML 0106	Milks	*mrl*	*stmr*	
FM 0183	Milk fats	*mrl*	*stmr*	

Abbreviations and acronyms in this exercise

CCN	Codex Commodity Number
HR	highest residue
LOQ	limit of quantification
MRL	maximum residue limit
ppm	parts per million
STMR	supervised trials median residue

Exercise 13.1. IEDI and IESTI Calculations for dietary intake

See also Chapter 13

1. Dietary intake calculations

2. The aim

The aim of this exercise is to gain experience with the IEDI and IESTI spreadsheets. Estimating long-term and short-term dietary exposures (or intakes) for pesticide residues in food is a critical part of the evaluation process.

3. IEDI spreadsheet

IEDI_calculation14_FAO.xlt

- Open the spreadsheet
- Enable macros – yes
- Save as "Compound~IEDI_calculation14_FAO.xls"
- Open tab "GEMS_Food_diet" for data entry.

4. IEDI spreadsheet

Data entry

- Compound name and Codex Number
- ADI
- STMR values, from supervised trials
- STMR-P values, from STMR values and processing factors

To find the correct commodity, it is sometimes convenient to search for the Codex Commodity Number, e.g. VC 0424

5. Processed commodities

8 possibilities for grapes, because dietary data are available for 3 processed commodities
Grape (incl dried, incl juice, incl wine)
Grape (excl dried, excl juice, excl wine)
Grape (excl dried, excl juice, incl wine)
Grape (excl dried, incl juice, incl wine)
Grape (excl dried, incl juice, excl wine)

Grape (incl dried, excl juice, excl wine)	
Grape (incl dried, incl juice, excl wine)	NOTE 1
Grape (incl dried, excl juice, incl wine)	

NOTE 1. Processing data available for wine, but not for dried or juice. Choose this one for the grape STMR and then enter the STMR-P for wine into the cell for wine.

6. IEDI calculation

- When data entry is complete
- Go to 'Tools', 'Macro', 'Calculate'.
- The IEDI spreadsheet generates a "Final_table" with the results for the 13 diets.

7. IESTI spreadsheet

IESTI_calculation11_FAO.xlt

- Open the spreadsheet
- Enable macros – yes
- Save as "Compound~IESTI_calculation11_FAO.xls"
- Open tab "General_population" for data entry.

8. IESTI spreadsheet

Data entry

- Compound name and Codex Number
- ARfD
- HR values, from supervised trials
- STMR-P values, from STMR values and processing factors
- HR-P values, if processing does not cause bulking and blending

9. IESTI spreadsheet

Data entry

- Substitute individual food commodities for commodity group recommendations
    - Citrus fruit HR requires entries for lemon, mandarin, orange, grapefruit

10. IESTI spreadsheet

Data entry

- Multiple entries are required to cover the range of possibilities
    - 6 entries are needed for peach to include calculations with unit weight data from France, Japan, UK, USA, Sweden and Belgium.

11. IESTI calculation

- When data entry is complete
- Go to 'Tools', 'Macro', 'Calculation'.
- The IESTI spreadsheet generates two tables, with calculated IESTI values and % ARfD for each food for:
    - general population. Final_table_gen_pop.
    - children. Final_table_children.

12. The exercise

- Each team is to complete an IEDI and an IESTI spreadsheet.
- Back in plenary
    - Comments and observations
    - Uncertainties in the results?
    - Questions?
    - Specific problems
    - What did you learn?

DIETARY INTAKE CALCULATIONS

The aim of this exercise is to gain experience with the IEDI and IESTI spreadsheets.

Estimating long-term and short-term dietary exposures (or intakes) for pesticide residues in food is a critical part of the evaluation process.

IEDI calculation

Spreadsheet: *IEDI_calculation14_FAO.xlt*

Data to be entered.

Compound: Name and Codex Number.

ADI: [units must be mg/kg bw]
Residue data STMRs from recommendations tables.

Results: Intakes =.xx% to xx% for the 13 diets.

IESTI calculation

Spreadsheet: *IESTI_calculation11_FAO.xlt*

Data to be entered
Compound: Name and Codex Number.
ARfD: [units must be mg/kg bw]
Residue data HRs and STMRs from recommendations tables.

Results: Intakes = xxxx % for adults and xxxx % for children.

Suggested team allocations for exercise

	Long-term intake	Short-term intake
Team 1	bifenazate	cyromazine
Team 2	mandipropamid	fluopicolide
Team 3	spirodiclofen	bifenthrin

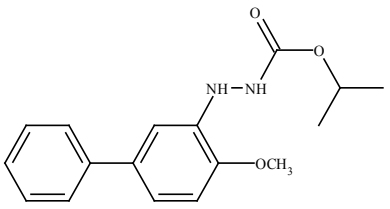
The exercise

1. Complete an IEDI spreadsheet.
2. What are the long-term intakes in comparison to the ADI for the 13 diets?
3. Complete an IESTI spreadsheet.
4. Make a list of food commodities where short-term intake exceeds ARfD.
5. What advice could be given about these cases?

DATA FOR EVALUATION

Bifenazate (219)

ADI: 0–0.01 mg/kg bw
ARfD: unnecessary



Definition of the residue (for compliance with the MRL and for estimation of dietary intake): Sum of bifenazate

and bifenazate diazene (diazenecarboxylic acid, 2-(4-methoxy-[1,1'-biphenyl-3-yl] 1-methylethyl ester), expressed as bifenazate. The residue is fat soluble.

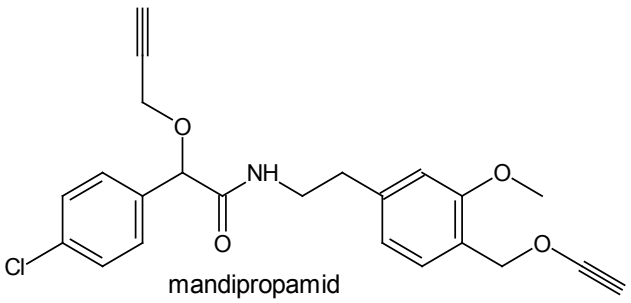
CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg
AM 0660	Almond hulls	10	5.0
SO 0691	Cotton seed	0.3	0.01
DF 0269	Dried grapes (= currants, raisins, sultanas)	2	0.59
MO 0105	Edible offal (mammalian)	0.01*	0.01
PE 0112	Eggs	0.01*	0
VC 0045	Fruiting vegetables, cucurbits	0.5	0.04
FB 0269	Grapes	0.7	0.185
DH 1100	Hops, dry	20	7.8
MM 0095	Meat (from mammals other than marine mammals)	0.05 (fat)	0.01 muscle 0.01 fat
FM 0813	Milk fats	0.05	0.01
ML 0106	Milks	0.01*	0.01
HH 0738	Mints	40	12.9
VO 0444	Peppers, Chili	3	1.1
VO 0445	Peppers, Sweet (including Pimento or pimiento)	2	0.235
FP 0009	Pome fruits	0.7	0.175
PM 0110	Poultry meat	0.01* (fat)	0 muscle 0 fat
PO 0111	Poultry, Edible offal of	0.01*	0
FS 0012	Stone fruits	2	0.34
FB 0275	Strawberry	2	0.63
VO 0448	Tomato	0.5	0.095
TN 0085	Tree nuts	0.2	0.03
JF 0226	Apple juice		0.030
	Apple pomace, wet		0.32
	Cotton seed hulls		0.0023
	Cotton seed meal		0.00004
OR 0691	Cotton seed refined oil		0.00004
DF 0014	Plum, dried (prunes)		0.02
JF 0269	Grape juice		0.020
	Tomato paste		0.13
	Tomato puree		0.53

* at or about limit of quantification.

Mandipropamid (231)

ADI: 0–0.2 mg/kg bw
ARfD: Unnecessary

Definition of the residue (for compliance with the MRL and for estimation of dietary intake for plant and animal commodities): mandipropamid.



mandipropamid

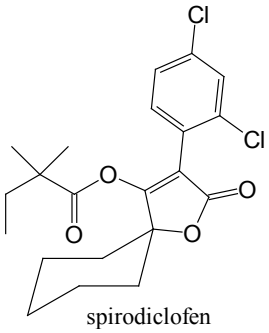
CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg

CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg
VB 0400	Broccoli	2	0.435
VB 0041	Cabbages, Head	3	0.01
VS 0624	Celery	20	2.70
HS 0444	Peppers, chili (dried)	10	0.84
VC 0424	Cucumber	0.2	0.02
FB 0269	Grapes	2	0.51
DF 0269	Dried grapes (= Currants, Raisins, Sultanas)	5	1.68
	Wine		0.366
JF 0269	Grape, juice		0.14
VL 0053	Leafy vegetables	25	5.65
VC 0046	Melons, except Watermelon	0.5	0.115
VA 0385	Onion, Bulb	0.1	0.01
VO 0051	Peppers	1	0.12
VR 0589	Potatoes	0.01*	0.01
VA 0389	Spring onion	7	0.48
VC 0431	Squash, summer	0.2	0.04
VO 0448	Tomato	0.3	0.06
JF 0448	Tomato juice		0.059
	Tomato puree		0.068
	Canned tomatoes		0.022

* at or about limit of quantification.

Spirodiclofen (237)

ADI: 0–0.01 mg/kg bw
ARfD: Unnecessary



spirodiclofen

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant commodities: spirodiclofen.
Definition of the residue for compliance with the MRL for animal commodities: spirodiclofen.
Definition of the residue for estimation of dietary intake for animal commodities: the sum of spirodiclofen and spirodiclofenenol, expressed as spirodiclofen.
The residue is fat-soluble.

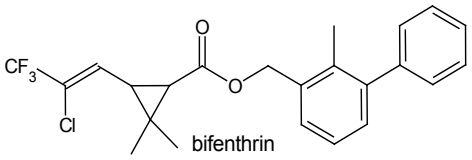
CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg
AM 0660	Almond hulls	15	3.5
AB 0226	Apple pomace, dry	4	3.4
FC 0001	Citrus fruits	0.4	0.13 [b] 0.02 [c]
SB 0716	Coffee beans	0.03 *	0.03
VC 0424	Cucumber	0.07	0.03
FB 0021	Currants, Black, Red, White	1	0.040
DF 0269	Dried grapes (= Currants, Raisins and Sultanas)	0.3 a	0.13
MO 0105	Edible offal (Mammalian)	0.05 *	0
FB 0269	Grapes	0.2	0.059
VC 0425	Gherkin	0.07	0.03

CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg
DH 1100	Hops, dry	40	11
ML 0106	Milks	0.004 *	0
MM 0095	Meat (from mammals other than marine mammals)	0.01 * (fat)	0
FI 0350	Papaya	0.03 *	0.03
VO 0445	Peppers, Sweet (including pimento or pimiento)	0.2	0.08
DF 0014	Plums, dried (prunes)		0.79
FP 0009	Pome fruits	0.8	0.20
FS 0012	Stone fruits	2	0.315
FB 0275	Strawberry	2	0.0615
VO 0448	Tomato	0.5	0.08
TN 0085	Tree nuts	0.05	0.0155
JC 0001	Citrus juice		0.0065
JF 0226	Apple juice		0.004
DF 0226	Apples, dried		0.018
JF 0269	Grape juice		0.00051
DF 0014	Plum, dried (prunes)		0.79
-	Wine		0.018
	Beer (from hops)		0.011

* at or about limit of quantification.
Notes: [a] dry weight basis. [b]whole fruit. [c]edible portion

Bifenthrin (178)

ADI 0.01 mg/kg bw
ARfD 0.01 mg/kg bw

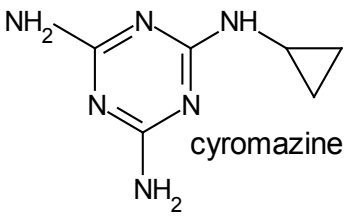


Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant and animal commodities: bifenthrin (sum of isomers).
The residue is fat soluble.

CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
FI 0327	Banana	0.1	0.01	0.01
FB 0264	Blackberries	1	0.29	0.51
VB 0040	Brassica vegetables	0.4	0.115	0.19
FC 0001	Citrus fruits	0.05	0.05	0.05
VO 0440	Egg plant	0.3	0.05	0.10
MM 0095	Meat (from mammals other than marine mammals)	3 (fat)	0.59 (fat) 0.07 (muscle)	1.9 (fat) 0.104 (muscle)
ML 0106	Milks	0.2	0.053	
VL 0485	Mustard greens	4	1.16	2.1
VO 0051	Peppers	0.5	0.14	0.31
	Peppers chilli dry	5	1.4	
VD 0070	Pulses	0.3	0.05	
FB 0272	Raspberries, Red, Black	1	0.29	0.51

CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
VR 0075	Root and tuber vegetables	0.05	0.05	0.05
FB 0275	Strawberry	3	0.46	2.3
DT 1114	Tea, Green, Black (black, fermented and dried)	30	5.2	
VO 0448	Tomato	0.3	0.06	0.15
TN 0085	Tree nuts	0.05	0.05	0.05

Cyromazine (169)



cyromazine

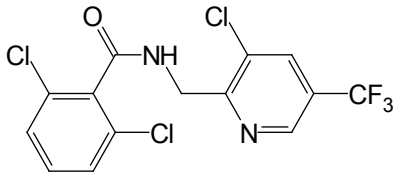
ADI: 0–0.06 mg/kg bw
ARfD: 0.1 mg/kg bw

Definition of residues for compliance with MRL and for estimation of dietary intake for plants and animal commodities: cyromazine.

CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
VS 0620	Artichoke, globe	3	1.0	1.3
VD 0071	Beans (dry)	3	1.0	
VB 0400	Broccoli	1	0.15	0.51
VB 0041	Cabbages, head	10	0.26	6.1
VS 0624	Celery	4	0.58	2.3
VC 0424	Cucumber	2	0.48	1.3
PE 0112	Eggs	0.3	0.07	0.16
VO 0050	Fruiting vegetables, other than cucurbits (Note 2)	1	0.16	0.58
VL 0482	Lettuce, head	4	0.34	2
VP 0534	Lima beans, young pods and or immature beans.	1	0.23	0.58
MM 0095	Meat (from mammals other than marine mammals)	0.3	0.01	0.20
VC 0046	Melons, except Watermelon	0.5	0.04	0.19
VO 0450	Mushroom	7	2.2	4.2
VL 0485	Mustard greens	10	2.7	7.4
VA 0385	Onion, bulb	0.1	0.05	0.07
PO 0111	Poultry, edible offal	0.2	0.065	0.08
VL 0502	Spinach	10	2.0	6.1
VA 0389	Spring onion	3	0.345	1.7
JF 0448	Tomato juice		0.12	

(Note2) Except mushrooms and sweet corn-on-the-cob

Fluopicolide (235)



ADI: 0–0.08 mg/kg bw
ARfD: 0.6 mg/kg bw (women of childbearing age)
ARfD: unnecessary for other groups of the population.

Definition of the residue (for compliance with the MRL) for plant and animal commodities: fluopicolide. Definition of the residue (for estimation of dietary intake) for plant and animal commodities: fluopicolide and 2,6-dichlorobenzamide measured separately.
The residue is fat-soluble.

CCN	Commodity	MRL, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
VB 0402	Brussels sprouts	0.2	0.04	0.13
VB 0041	Cabbages, Head	7	1.2	4
VS 0624	Celery	20	1.4	14
HS 0444	Peppers Chilli, dried	7	0.91	7
PE 0112	Eggs	0.01 *	0	0
VB 0042	Flowerhead Brassicas (includes Broccoli: Broccoli, Chinese and Cauliflower)	2	0.385	0.69
VC 0045	Fruiting vegetables, Cucurbits	0.5	0.07 ,	0.3
VO 0050	Fruiting vegetables, other than Cucurbits (except mushrooms and sweet corn)	1	0.16	0.58
FB 0269	Grapes	2	0.38	1.2
DF 0269	Dried grapes (= currants, Raisins and Sultanas)	10	2.47	7.8
VL 0053	Leafy vegetables	30	8.6	17
MM 0095	Meat (from mammals other than marine mammals)	0.01 *(fat)	0	0
VA 0385	Onion, Bulb	1	0.07	0.58
VA 0387	Onion, Welsh	10	2.1	4.5
JF 0448	Tomato juice		0.048	
	White wine		0.16	
	Red wine		0.12	

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* : at or about the limit of quantification.

WORKSHEETS

Summary of IEDI calculations

Pesticide: ADI =
Residue definition:

Diet	A	B	C	D	E	F
Total intake (µg/person)=						
Bodyweight per region (kg bw) =						
ADI (µg/person)=						
%ADI=						
Rounded %ADI=						

Diet	G	H	I	J	K	L	M
Total intake (µg/person)=							
Bodyweight per region (kg bw) =							
ADI (µg/person)=							
%ADI=							
Rounded %ADI=							

Summary of IESTI calculations

Pesticide: ARfD =
Residue definition:

Pesticide	Population	IESTI as %ARfD		
		Range	List where IESTI>100 % ARfD	
			food	IESTI as %ARfD
Cyromazine	general population			
Cyromazine	children up to 6 years			

PART III. SOLUTIONS FOR EXERCISES

Solutions for Exercise 2.1. Identity and physical and chemical properties

IDENTITY

Identity

1) alpha-cypermethrin

ISO common name: alpha-cypermethrin

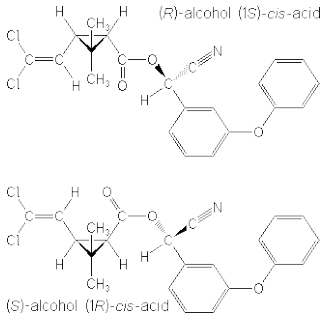
Chemical name:

(IUPAC): racemate comprising (*R*)-α-cyano-3-phenoxybenzyl (1*S*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

or

racemate comprising (*R*)-α-cyano-3-phenoxybenzyl (1*S*)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (*S*)-α-cyano-3-phenoxybenzyl (1*R*)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

(Chemical Abstracts): (*R*)-cyano(3-phenoxyphenyl)methyl (1*S*,3*S*)-rel-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate



CAS Registry No.: 67375-30-8

CIPAC No.: 454

Synonyms: alphamethrin (rejected common name), alfoxylate

Structural formula:

Molecular formula: $C_{22}H_{19}Cl_2NO_3$

Molecular weight: 416

2) azoxystrobin

ISO common name: azoxystrobin

Chemical name:

(IUPAC): methyl (2E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate

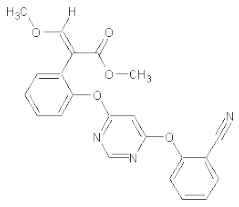
(Chemical Abstracts): methyl (αE)-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy]-α-(methoxymethylene)benzeneacetate

CAS Registry. No. 131860-33-8

CIPAC No.: 571

Synonyms:

Structural formula:



Molecular formula: $C_{22}H_{17}N_3O_5$

Molecular weight: 403

3) chlorothalonil

ISO common name: chlorothalonil

Chemical name:

(IUPAC): tetrachloroisophthalonitrile

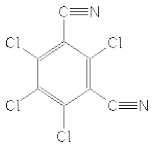
(Chemical Abstracts): 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile

CAS Registry No.: 1897-45-6

CIPAC No.: 288

Synonyms: TPN (JMAF)

Structural formula:



Molecular formula: $C_8Cl_4N_2$

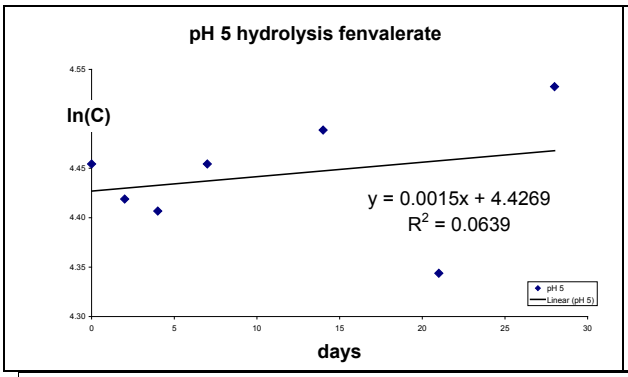
Molecular weight: 266

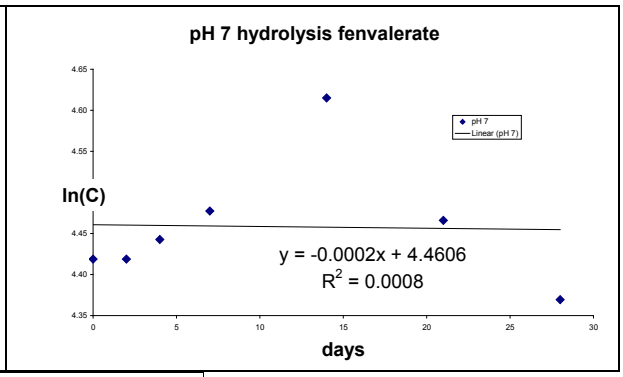
Physical and chemical properties

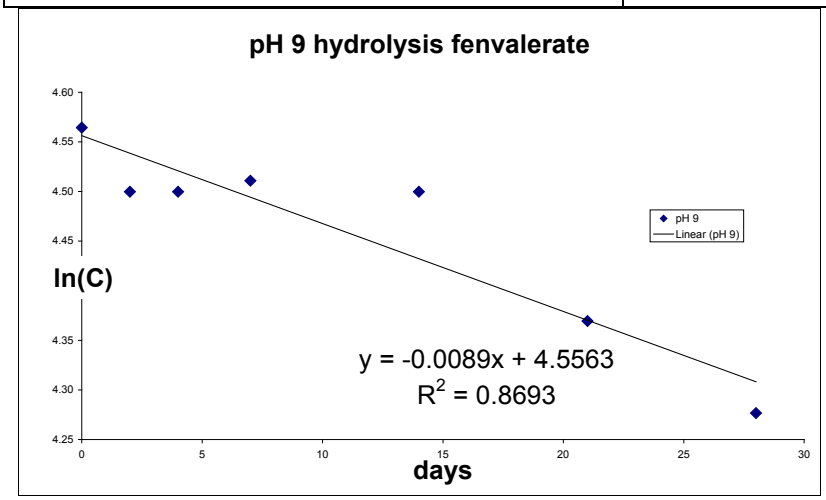
Fenvalerate hydrolysis rates (JMPR 2000)

Hydrolysis rates were measured for [^{14}C]fenvalerate at concentrations of approximately 50 µg/L in sterile aqueous buffers at pH 5, 7 and 9 at 25 ˚C in the dark. The estimated half-life was 80 days at pH 9.

Plot ln(C) as a function of time.







For pH 5 and pH 7, the data are too variable and the hydrolysis rate is too slow to be observed in 28 days.

For pH 9, the rate constant k = 0.0089 days^{-1}.

$$\text{Half-life} = \frac{ln(0.5)}{-0.0089} = \frac{-0.6931}{-0.0089} = 78\ days$$

- interpret the results in terms of uncertainty.

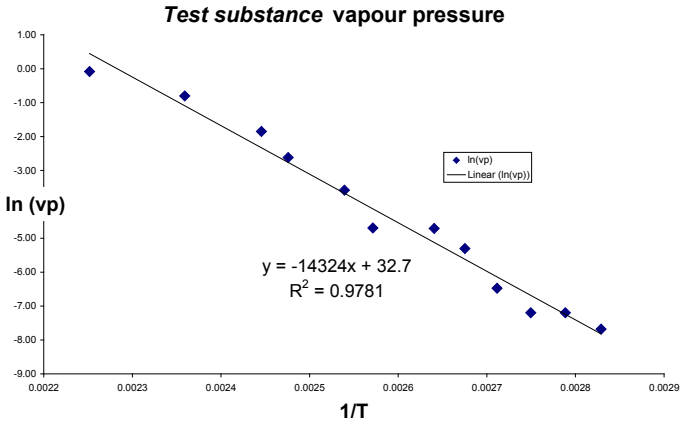
For pH 5 and 7, it is difficult to be sure that any hydrolysis has taken place. However, the variability in the data would prevent the observation of small changes, if they had occurred.

At pH 9, the calculated half-life of 78 days is essentially the same as the 80 days previously recorded.

We may gain some idea of the uncertainty in the estimate by making the calculations with ±5 % error (possible analytical error) on the concentration measured on day 28. The ±5 % range on 72 % is 68.4-75.6 %. The calculated half-lives from this ±5 % on the one point is 68 days to 91 days. It suggests that a value of 78 days is claiming too much precision. "Approximately 80 days" is a better expression of the half-life.

Extrapolation of vapour pressure measurements at higher temperatures to 25 ˚C.

Vapour pressure measurements have been made from 80 ˚C to 170 ˚C on a pesticide that is a liquid at 25 ˚C.



$$ln(VP) = \frac{-14324}{T} + 32.7$$

Vapour pressure at 25ºC = 2.1×10^{-7} Pa

Solution for exercise 3.1 Summary of metabolism of F64 in goat

The content of the summary should be considered based on the essential information included in the checklist given in the lecture on metabolism studies.

General for all kind of studies with labelled compounds

1. Selection of ^{14}C or other isotope label position, and unambiguous description of the position by the chemical name of the compound.
   Due to the agreement on confidential treatment of the content of the report this information was not provided in the exercise. However in the original report it was provided.
2. Description of the test system (number of animals, administration of the test compound) and expression of dose level in ppm dry feed, daily feed consumption

Test system, animal metabolism

- number of animals
- administration – oral or external treatment, capsule or feed incorporation, daily dosing for *n* days
- dose –expressed as mg per kg of bodyweight and as ppm of dry feed.

Included in the first 2 paragraphs of the report

3. Type of samples collected and sampling days, intervals between sampling and analyses
   1st para
   - Intervals between sampling and analyses should be checked and examined for influence on residue stability, but the information should not be included in the Appraisal unless problematic. The Appraisal becomes too cluttered with such background information.
     Not included

4. Are storage stability test results available where the storage interval exceeds 2 month?
   - See above comment.
   - Yes, but the samples were analysed within 3 months without storage stability information
5. Animal metabolism.
   - Recovery of radioactivity, material balance.

   4th para
6. Portion of extractable radioactivity in edible tissues (also milk and eggs for animal metabolism) and potential feed items (plant metabolism) as % of total radioactive residues, TRR, and mg parent compound equivalent/kg sample material;
   Yes
7. Portion of non-extractable radioactivity in edible tissues (also milk and eggs for animal metabolism) and potential feed items (for plant metabolism) expressed as % TRR.
   See table

8. Portion of characterised but not identified radioactivity in edible tissues and potential feed items

See table

9. Identification of metabolites (>10% TRR or >0.05 mg/kg)
   - Identified metabolites. Are all metabolites (>10% TRR or >0.05 mg/kg) identified?
   - Yes
   - Was any metabolite present ≥ 10% not identified?

   No
   - All identified metabolites should be described by their systematic chemical names. Due to the agreement on confidential treatment of the content of the report this information was not provided in the exercise. However in the original report it was provided.

10. Characterization of metabolites (< 10% TRR, 0.01- 0.05 mg/kg)
    Yes
11. Presence of metabolites in conjugated form, extractability of conjugated metabolites
    Yes
12. Description of major metabolic reactions, transformation of parent compound to various metabolites, and the proposed metabolic pathway.
    Metabolic reactions are described. Metabolic pathway was not provided due to the agreement on confidential treatment of the content of the report. However in the original report it was provided.

13. Note: The description of major metabolic reactions should be kept brief in the Report. The pathway is for the Evaluation, not the Report.
    Summary complies with this requirement

14. 13. Qualitative comparison of metabolites identified in laboratory animals (rat in tox. studies), farm animals (typically lactating goats and laying hens), identification of metabolites which are present in plants or farm animals but not present in rats
    No.

<u>Animal metabolism studies</u>

1. Radioactivity in faeces, urine, cage wash as % of applied radioactivity
2. Radioactivity in edible tissues as % of applied radioactivity and mg/kg parent equivalent
3. Presence of parent compound and identified major metabolites (> 10% of TRR) expressed as % of total radioactivity and mg/kg in milk, muscle, liver kidney, fat and eggs as appropriate

The above information has been provided. The summary report comprises 4 pages thus it is concise and informative.

Exercise 3.2: Preparation of summary of report on the metabolism of F64 in goat

The content and wording of a summary of a report depends on the author. A good summary report may be prepared with slightly different contents. Therefore no solution is provided.
However, the content is important. The comments of the other participants or the facilitator of the training may provide guidance for improvement.

Solution for Exercise 3.3: Validation of goat metabolism study with F64M1

Tasks:

(g) Validate the study conditions concerning:
- Study material, test system, application conditions of test material, sampling and analysis, etc.
- Identification and characterization of metabolites
- Completeness of information provided in the summary of the study.

(h) Identify major residue components to be considered for definition of residues

(i) Compare the results of metabolism studies with the active substance (F64) and its main plan metabolite (F64M1)

Use the checklist given in the lecture on metabolism as a guide:

The information provided in the summary report is underlined in the checklist.

(a) Validation of the study

General for all kind of studies with labelled compounds

1. Selection of ^{14}C or other isotope label position, and unambiguous description of the position by the chemical name of the compound.
   Due to the agreement on confidential treatment of the content of the report this information was not provided in the exercise. However in the original report it was provided.
2. Description of the test system (number of animals, administration of the test compound) and expression of dose level in mg/kg bw and ppm dry feed, daily feed consumption.
   Note, that the way of expressing the dose is not precise

   Included in the first 2 paragraphs of the report

3. Type of samples collected and sampling days, intervals between sampling and analyses
   1st para
   - Intervals between sampling and analyses should be checked and examined for influence on residue stability, but the information should not be included in the Appraisal unless problematic. The Appraisal becomes too cluttered with such background information.
     Not included

4. Are storage stability test results available where the storage interval exceeds 2 months?
   - See above comment.
   - Yes, but the samples were analysed within 3 months without storage stability information
5. Recovery of radioactivity, material balance.
6. Portion of extractable radioactivity in edible tissues (also milk and eggs for animal metabolism) as % of total radioactive residues, TRR, and mg parent compound equivalent/kg sample material;
   Yes
7. Portion of non-extractable radioactivity in edible tissues (also milk and eggs for animal metabolism) and potential feed items (for plant metabolism) expressed as % TRR.
   See table
8. Portion of characterised but not identified radioactivity in edible tissues and potential feed items
   See table

9. Identification of metabolites (>10% TRR or >0.05 mg/kg)
   - Identified metabolites. Are all metabolites (>10% TRR or >0.05 mg/kg) identified?
   - Yes
   - Was any metabolite present ≥ 10% not identified?

No

   - All identified metabolites should be described by their systematic chemical names.
     Due to the agreement on confidential treatment of the content of the report this information was not provided in the exercise. However in the original report it was provided.

10. Characterization of metabolites (< 10% TRR, 0.01- 0.05 mg/kg)

Yes

11. Presence of metabolites in conjugated form, extractability of conjugated metabolites

Yes

12. Description of major metabolic reactions, transformation of parent compound to various metabolites, and the proposed metabolic pathway.
    Metabolic reactions are described. Metabolic pathway was not provided due to the agreement on confidential treatment of the content of the report. However in the original report it was provided.

13. Note: The description of major metabolic reactions should be kept brief in the Report. The pathway is for the Evaluation, not the Report.

Summary complies with this requirement

14. 13. Qualitative comparison of metabolites identified in laboratory animals (rat in tox. studies), farm animals (typically lactating goats and laying hens), identification of metabolites which are present in plants or farm animals but not present in rats

No.

<u>Specific for animal metabolism studies</u>

1. Radioactivity in faeces, urine, cage wash as % of applied radioactivity NO
2. Radioactivity in edible tissues as % of applied radioactivity and mg/kg parent equivalent YES
3. Presence of parent compound and identified major metabolites (> 10% of TRR) expressed as % of total radioactivity and mg/kg in milk, muscle, liver kidney, fat and eggs as appropriate YES

The above information has been provided. The summary report comprises 4 pages thus it is concise and informative. Though several important data were not included, the information provided is sufficient to assess the metabolic behaviour or the major metabolite of F64.

(b) The major residue components which could be considered for the definition of residue are:
The majority of the metabolites containing one or two hydroxyl groups are present in conjugated forms.
F64M2 and F64M3 are also present in free form in milk and muscle
The F64M1 is present in free form in liver, kidney and fat and at much lower concentration in muscles. It was not detectable in milk.

(c) The F64M1 and some of the metabolites are also present in goat tissues and milk following the administration of the active substance F64, but the majority of metabolites formed after the administration of F64 and F64M1 are qualitatively and quantitatively different.

Solution for exercise 5.1 Checking the efficiency of extraction

1. Calculate the average efficiency of extraction

Substrate	Results, mg/kg		Extraction efficiency		
	Metabolism analysis	Regulatory analysis 2/	Replicate measurements		Average
Pear	0.2	0.15-0.18	0.75	0.90	0.825
Maize fodder	0.047	0.02-0.03	0.43	0.64	0.535
Cucumber	0.1	0.04-0.05	0.40	0.50	0.450
Cucumber	0.044	0.02-0.04	0.45	0.91	0.680
Goat meat	1	0.56-0.79	0.56	0.79	0.675
Goat milk	0.37	0.06-0.09	0.16	0.24	0.200

Conclusions

1. The results suggest that the method is marginally acceptable for the analyses of plant commodities of high water content (average efficiency is 62%) provided that very similar extraction procedure was used for the analysis of samples from supervised trials.
2. It is not possible to fully evaluate the results based on the measured residues alone. Collection of available information on the analysis conditions and sample history would be needed
   (a) reproducibility of analysis and sample processing with regulatory method and 'metabolite ' method,
   (b) what was the recovery from freshly spiked samples; were the reported values adjusted with the analytical recovery;
   (c) time elapsed between the analyses carried out within the metabolism study and reanalysis of samples;
   (d) storage conditions of the samples
3. If the information collected in step 2 confirms that the results are reliable and more rigorous extraction procedure would not be practical under the conditions of regulatory analyses then the method could be used for enforcement purposes, as it would give similar results as obtained in supervised trials.
4. However, the underestimation of residues might be taken into account in assessing the consumers exposure

Solution for Exercise 5.2: Evaluation of supervised trials conditions in *papaya*

Task:

Taking into account the basic requirements for sampling sample processing and analysis validate the procedures applied

Solution

Sampling:

The procedure applied

'Fruit was hand picked from both sides of the row, taking fruit from high, low, inside, outside, exposed, and shielded areas of 14 trees, avoiding two trees at row ends. Samples consisted of at least 12 fruits and weighed a minimum of 4 lb'.

is written in sufficient detail, and it is in line with general sampling requirements. Fruit was taken from different positions of the tree. The number of fruits and the total weight of the sample met the minimum requirements.

However, *the "*fruit was cut into 1 /8 fractions to reduce sample weight' at the site before placing them to shipping bag. The cut fruit was cooled (temperature unspecified) and 3 hour 15 minutes elapsed between sampling and freezing the samples.

The analyses of samples were carried out within a short period of time with properly validated method. The method performance was verified with concurrent recoveries.

Note that there may be a problem with validation of the analytical method. The statement on LODs and LOQs is unrelated to data in the accompanying table. It is puzzling how LOQs of the two compounds could be different in this situation.

Page 2: "The lowest level of method validation (LLMV) in this study was 0.01 ppm for each analyte. Based on recoveries of samples fortified at the LLMV, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 0.12 ppm and 0.37 ppm, respectively, for acar. For those fortified with acar metabolite, the LOD and LOQ were calculated as 0.0012 ppm and 0.0037 ppm, respectively."

Conclusion:

As the cross contamination of the samples and getting the peel in contact with the pulp may cause substantial change in the residue level, such procedure is not permitted and clearly indicated in the protocols for sampling (See FAO GI and Codex Standard on sampling for pesticide residues (CXG_033e[1]).

Consequently the residue data obtained in these trials are not reliable and cannot be used for estimation of maximum residue levels.

Solution for Exercise 6.1 Definition of residues of happyplant (F64)

As usual in such cases, there are several possibilities for summarising the essential information and different experts may reach different conclusions. The definition of residues is one of the topics of the JMPR Meetings in which consensus could be reached only after lengthy discussions and arguments by the members of the FAO Panel.

The reports of recent JMPR Meetings can provide some additional examples for the content of the section on definition of residues.

The summary hereunder provides an example:

Residue definition

Happyplant (common name of F64) is extensively metabolised and the most important pathways for metabolism are common to wheat, peanut and sugar beet. The nature of the residue found in wheat after foliar spray application, seed treatment and as a rotational crop was similar.

Hydroxylation was the major metabolic process of happyplant in plants. The majority of the metabolites are simply multiple structural isomers of monohydroxylated desthio derivative F64*M1* and their conjugates [glucosides and malonyl-glucosides, and happyplant-dihydroxy-olefin and its conjugates. Oxidative hydroxylation led to isomers of happyplant-dihydroxy-diene and their conjugates. Although the sum of these compounds and their conjugates were as high as 42% of the TRR in an individual crop matrix, these conjugated and/or hydroxylated metabolites represented individually < 10% of the TRR in the plant matrices.

The proportion of parent happyplant in TRR was low (days after last application are given in brackets): in wheat grain 1%, (48 days) wheat forage 5% (6 days), wheat hay 3% (26 days), and straw 6% (48 days), in [list residues in other plant parts from additional metabolism studies if available], rotational crops < 1% if detected at all.
Irrespective of the crop or application mode, the major metabolites found in all crops were happyplant-desthio [35.4% of TRR in wheat forage, 15.9% of TRR in wheat grain, 28.2% of TRR in peanut hay, 28% of TRR in sugar beet tops] and triazolylalanine, triazolyl-hydroxy-propionic acid and triazolylacetic acid. Other minor metabolites resulting from the molecule cleavage were happyplant-benzylpropyl-diol, happyplant-triazolyl-ethanol and its glucoside, and happyplant-triazolyl-sulphonic acid-ethanol-glucoside.
The major plant metabolite, happyplant-desthio was slowly metabolised in wheat. It was the dominating constituent of the residue in forage (77% of TRR) and straw (72% of TRR) at harvest. However, it was only detected in small amounts in grain (0.07 mg/kg), where the residue was mainly made up by triazolylacetic acid and triazolylalanine. No free 1,2,4-triazole was detected in any matrix either in the target plant metabolism studies or in the confined rotational crops study.

The metabolic profiles for milk and the edible tissues and organs of goats showed that parent happyplant was a major compound in all tissues and organs (> 10% of TRR), but only of minor importance in milk (< 1-3% of TRR). Compounds detected in all matrices in the study with phenyl-labelled F64M1 were F64M1 (except for milk), and conjugates of hydroxy F64M1, dihydroxy-F64M1, hydroxy-methoxy-F64M1. Following the administration of triazole

labelled happyplant the only label specific metabolite identified was thiocyanate: 41.1% of TRR in milk, 29.6% in muscle, 12.4% in fat, 9% in kidney and 2% in liver. Triazole derivatives or free 1,2,4-triazole were not found at concentrations above 0.01 mg/kg in any goat matrix under investigation. Free triazole and happyplant-triazolyl-ethanol were detected in all matrices of laying hen. Free triazole did not exceed a residue level of 0.04 mg/kg.

The most abundant metabolite was happyplant-S-glucuronide. F64M1 was also present in all sample materials, but in much lower concentrations than happyplant-S-glucuronide. An exception was fat in hen and goat (16.1%) and in hen eggs, in which F64M1 was predominant. In eggs and all edible tissues of hen metabolite happyplant-S-methyl was additionally identified. Animal feeding studies showed that the residues are not concentrated in fat of meat or milk cream. As the total residue is composed of several hydroxy derivatives and their conjugates, the Meeting concluded that the residues of happyplant are not fat soluble.

There are analytical procedures for the determination of happyplant residues in various combinations. A GC/MS multi residue method has been validated for the determination of F64M1. An LC-MS/MS total residue method converts happyplant, its metabolites and their conjugates to a mixture of happyplant sulphonic acid and F64M1. Another method is suitable for the determination of F64M1, 3-hydroxy-F64M1 and 4-hydroxy-F64M1 and conjugates that can be converted to one of these compounds via acid hydrolysis in/on matrices of animal origin by HPLC-MS/MS. The major part of TRR (58-84%) is recovered with this method.

Supervised trials indicated that residues measured as the sum of happyplant sulphonic acid and happyplant-desthio were higher than the happyplant-desthio alone.

The Meeting noted that 1,2,4-triazole, triazolyl-acetic acid and triazolyl-alanine may derive from several sources. Field trials performed in USA indicated that the sum of conjugates of triazolyl-alanin and triazolyl-acetic acid amounted to a maximum of 0.92 mg/kg and 1.76 mg/kg in barley and wheat grain, 0.66 mg/kg in canola seed and 3.39 in peanut meat. Free 1,2,4 triazole was not detected in any of the samples above LOQ (0.01 or 0.02 mg/kg). These findings agree with the information obtained from metabolism studies. As these compounds may be present in food commodities from different sources they are not suitable for enforcement purposes. The relatively low level of conjugated residues in food commodities and the low toxicity of triazolyl-acetic acid and triazolyl-alanine (max ADI of 1 mg/kg) do not justify their inclusion for dietary risk assessment.

Solution for exercise 6.2 Expression of sum of residues included in residue definition and rounding of residue values

1.
Residues of acephate in rank order are: <0.025, 0.036, 0.04, 0.042, 0.065, 0.09, 0.1 and 0.69 mg/kg
Residues of methamidophos in rank order are: <0.01, 0.021, <0.025, <0.025, 0.046, <0.05, 0.05 and 0.38 mg/kg

As methamidophos is a pesticide on its own the MRLs for acephate are expressed as the parent compound alone.
For methamidophos the MRLs will be evaluated taking into account the residues derived from the use of acephate and residues derived from the use of methamidophos and the larger estimated maximum value will be used as MRL.

2.

Acephate	Methamidophos	Sum 1	Sum 2
0.036	<0.05	0.411	0.536
0.065	<0.01	0.14	0.165
0.69	0.38	3.54	4.49
0.09	0.05	0.465	0.59
0.04	0.021	0.1975	0.25
0.1	0.046	0.445	0.56
0.042	＜0.025	0.2295	0.292
<0.025	<0.025	0.2125	0.275

Note:
Sum1 for long term risk assessment
Sum 2 for short term risk assessment

3. The residues expressed as acephate equivalent

For long term risk assessment the residues are: 0.14, 0.20, 0.21, 0.23, 0.41, 0.45, 0.47 and 3.54 mg/kg
For short term risk assessment the residues are: 0.17, 0.25, 0.28, 0.29, 0.54, 0.56, 0.59 and 4.49 mg/kg

4
Median values for acephate: 0.0535 mg/kg; for methamidophos: 0.035 mg/kg
The median values for long term dietary risk assessment is **0.320 mg/kg acephate equivalent**.

REMEMBER:
(a) report the residue values in the evaluation unrounded (as they were provided in the original report with maximum 3 significant figures),
(b) perform the calculations where needed and report the values in the appraisal with a minimum of 2 significant figures
(c) calculate the median values from the unrounded figures
(d) use the unrounded STMR value for further calculations (e.g. STMR-P in processed commodities)

(e) round the calculated STMR values only as the last step and report the value rounded to 2 significant figures

Note the above principles shall also be used in all cases (e.g. entering the residue values into the OECD calculator, calculation of processing factors and animal burden)

Solutions for Exercise 7.1. Summarising GAP Information

GAP Summary Table – happychloronid foliar sprays.

Country	Crop	Formulation and conc		Max product applic rate or product spray conc	Max applic rate kg ai/ha	Spray conc kg ai/hL	No. of applics	Interval between applics, days	PHI, days
Australia	apples	WG	100 g/kg	35 g/hl	-	0.0035	6	7-10 Note[53] 14-21 Note[54]	28
Belgium	apples	EC	250 g/l	150 ml/ha	0.0375			10	14
Brazil	apples	EC	250 g/l	14 ml/hl		0.0035	8		5
France	apples	EC	250 g/l	0.015 l/hl		0.00375	3	10	30
Italy	apples	EC	250 g/l	15 ml/hl		0.00375	4		14
Poland	apples	EC	250 g/l	0.2 l/ha	0.05		3	7-14	14
Spain	apples	EC	250 g/l	20 ml/hl		0.005	3-5	7-10 12-18 Note[55]	14
France	apricot	EC	250 g/l	0.02 l/hl		0.005	3	12-14	14 GS[56]
Belgium	asparagus	EC	250 g/l	0.5 l/ha	0.125				GS[57]
Spain	asparagus	EC	250 g/l	0.5 l/ha	0.125		3	14-21	30
Brazil	avocado	EC	250 g/l	20 ml/hl		0.005	4	14	14 GS[58]
Australia	bananas	EC	250 g/L	400 mL/ha Note[59]✈[60]	0.10		6		1
Brazil	bananas	EC	250 g/l	0.4 l/ha ✈	0.1		5	14-21	7
Central America	bananas	EC	250 g/l	0.4 l/ha✈	0.1		8	15-20	0
Brazil	beans	EC	250 g/l	0.3 l/ha	0.075		3	14-15	25
Belgium	beets	EC	250 g/l	0.5 l/ha	0.125				21
Belgium	broccoli	EC	250 g/l	0.5 l/ha	0.125		2		14
Germany	broccoli	EC	250 g/l	0.4 l/ha	0.1		3	14-21	21
UK	broccoli	EC	250 g/l	0.3 l/ha	0.075		Note	14	21
Belgium	Brussels sprouts	EC	250 g/l	0.5 l/ha	0.125		2		21

[53] Before petal fall.
[54] After petal fall
[55] Intervals of 7-10 days until diameter of fruit is 1 cm, then intervals of 12-18 days.
[56] Growth stage instruction. Application up to the stage where the stone has hardened.
[57] Growth stage instruction. Spray after harvest.
[58] Growth stage instruction. Apply until fruit are around 5 cm in diameter.
[59] Apply with a water-miscible oil.
[60] Aerial application approved.

Country	Crop	Formulation and conc		Max product applic rate or product spray conc	Max applic rate kg ai/ha	Spray conc kg ai/hL	No. of applics	Interval between applics, days	PHI, days
France	Brussels sprouts	EC	250 g/l	0.5 l/ha	0.125		3		21
Germany	Brussels sprouts	EC	250 g/l	0.4 l/ha	0.1		3	14-21	21
UK	Brussels sprouts	EC	250 g/l	0.3 l/ha	0.075		Note[61]		21
Germany	bulb vegetables	EC	250 g/l	0.4 l/ha	0.1		3	7-14	21
Belgium	cabbage	EC	250 g/l	0.5 l/ha	0.125		2		21
France	cabbage	EC	250 g/l	0.5 l/ha	0.125		3		21
UK	cabbage	EC	250 g/l	0.3 l/ha	0.075		Note		21
Germany	cabbage, drumhead	EC	250 g/l	0.4 l/ha	0.1		3	14-21	21
Germany	cabbage, savoy	EC	250 g/l	0.4 l/ha	0.1		3	14-21	21
Australia	carrots	EC	250 g/L	500 ml/ha	0.125	-	6	10-14	7
Belgium	carrots	EC	250 g/l	0.5 l/ha	0.125		3		14
Brazil	carrots	EC	250 g/l	0.60 l/ha	0.15		8	7	15
France	carrots	EC	250 g/l	0.5 l/ha	0.125		3		14
Germany	carrots	EC	250 g/l	0.4 l/ha	0.1		3	14-21	21
Italy	carrots	EC	250 g/l	0.5 l/ha	0.125		3-4	7-14	7
Belgium	cauliflower	EC	250 g/l	0.5 l/ha	0.125		2		14
Brazil	cauliflower	EC	250 g/l	20 ml/hl		0.005	5	7	14
France	cauliflower	EC	250 g/l	0.5 l/ha	0.125		3		14
Germany	cauliflower	EC	250 g/l	0.4 l/ha	0.1		3	14-21	21
Italy	cauliflower	EC	250 g/l	0.5 l/ha	0.125		3-4	7-14	14
UK	cauliflower	EC	250 g/l	0.3 l/ha	0.075		Note	14	21
Belgium	celery	EC	250 g/l	0.5 l/ha	0.125		3	14	14
France	celery	EC	250 g/l	0.5 l/ha	0.125		3		14
Italy	celery	EC	250 g/l	0.5 l/ha	0.125		3-4	7-14	21
Spain	celery	EC	250 g/l	20 ml/hl		0.005	4	7-14	14
Belgium	Chinese cabbage	EC	250 g/l	0.5 l/ha	0.125		2	14	14
Brazil	cucumber	EC	250 g/l	10 ml/hl		0.0025	5	10	1
Italy	cucumber	EC	250 g/l	0.5 l/ha	0.125		3-4	7-14	7

[61] Total dose for season 0.9 litres product per hectare.

Country	Crop	Formulation and conc		Max product applic rate or product spray conc	Max applic rate kg ai/ha	Spray conc kg ai/hL	No. of applics	Interval between applics, days	PHI, days
Germany	cucumber (green-house)	EC	250 g/l	0.8 l/ha	0.2		3	5-14	3
Germany	cucumber (open land)	EC	250 g/l	0.4 l/ha	0.1		3	5-14	3
USA	cucurbit veg Note[62]	WG	200 g/kg	0.56 kg/ha	0.112		3		3
Brazil	egg plant	EC	250 g/l	30 ml/hl		0.0075	6	7	3
Germany	forage turnip	EC	250 g/l	0.4 l/ha	0.1		2		28
USA	Fruiting veg Note[63] Note[64]	WG	200 g/kg	0.67 kg/ha	0.134		2		3
Brazil	garlic	EC	250 g/l	0.5 l/ha	0.125		6	7	14
Spain	garlic	EC	250 g/l	0.5 l/ha	0.125		3-4	7-14	30
Belgium	grapes	EC	250 g/l	0.12 l/ha	0.03				
Brazil	grapes	EC	250 g/l	12 ml/hl		0.003	6	14	21
USA	grapes	WG	200 g/kg	0.84 kg/ha	0.168		1		14
France	grapes (vines)	EC	250 g/l	0.12 l/ha	0.03		3	14	
USA	hops	WG	200 g/kg	1.68 kg/ha	0.336		1		14
France	Japanese pear	EC	250 g/l	0.015 l/hl		0.00375	3	10	30
Spain	lettuce	EC	250 g/l	0.5 l/ha	0.125		3	10-14	14
Spain	loquat	EC	250 g/l	20 ml/hl		0.005	5	14-21	14
Australia	macadamia	EC	250 g/L	50 ml/hl	-	0.0125	6	21-28	GS[65]
Brazil	mango	EC	250 g/l	50 ml/hl		0.0125	3	14	7 GS[66]
USA	nectarines	WG	200 g/kg	1.12 kg/ha	0.224		3		5
Switzerland	oilseed rape	SC	62.5 g/l	2 l/ha	0.125		1		GS[67]
UK	oilseed rape	EC	250 g/l	0.5 l/ha	0.125		Note[68]		GS[69]

[62] Cucurbit vegetables crop group: chayote, Chinese waxgourd, citron melon, cucumber, edible gourd, gherkin, momordica species, muskmelon (includes cantaloupe and honeydew), pumpkin, summer squash:, winter squash, watermelon.
[63] Fruiting vegetables crop group: egg plant, ground cherry, pepino, peppers (bell, chili, cooking, pimento, sweet), tomatillo, tomato.
[64] Restraint for use on tomatoes. Use only on plants that will produce tomatoes greater than 1 inch in diameter when mature.
[65] Growth stage instruction. Begin at nut set and continue until late December.
[66] Growth stage instruction. Continue applications until small fruits have formed.
[67] Growth stage instruction. 1 application between beginning of flowering and full flowering.

Country	Crop	Formulation and conc		Max product applic rate or product spray conc	Max applic rate kg ai/ha	Spray conc kg ai/hL	No. of applics	Interval between applics, days	PHI, days
Spain	olive	EC	250 g/l	60 ml/hl		0.015	1-2	14-21	14
Belgium	paksoi	EC	250 g/l	0.5 l/ha	0.125		2	14	14
Brazil	papaya	EC	250 g/l	30 ml/hl		0.0075	4	7-10	14
France	peach	EC	250 g/l	0.02 l/hl		0.005	3	12-14	14 GS
Italy	peach	EC	250 g/l	30 ml/hl		0.0075	2-3 1-2 GS[70]		7
USA	peach	WG	200 g/kg	1.12 kg/ha	0.224		3		5
Australia	pears	WG	100 g/kg	35 g/hl	-	0.0035	6	7-10 Note 14-21 Note	28
Belgium	pears	EC	250 g/l	150 ml/ha	0.0375			10	14
France	pears	EC	250 g/l	0.015 l/hl		0.00375	3	10	30
Italy	pears	EC	250 g/l	15 ml/hl		0.00375	4		14
Poland	pears	EC	250 g/l	0.2 l/ha	0.05		3		14
Spain	pears	EC	250 g/l	20 ml/hl		0.005	3-5	7-10 12-18 Note	14
USA	plums	WG	200 g/kg	1.12 kg/ha	0.224		3		5
USA	pome fruits Note[71]	WG	200 g/kg	1.12 kg/ha	0.224		2		7
Australia	potato	EC	250 g/L	500 ml/ha	0.125	-	6	10-14	7
Brazil	potato	EC	250 g/l	0.3 l/ha	0.075		4		7
Italy	potato	EC	250 g/l	0.5 l/ha	0.125		3-4	7-14	14
Spain	potato	EC	250 g/l	0.8 l/ha	0.2		3-4	12	30
USA	prunes	WG	200 g/kg	1.12 kg/ha	0.224		3		5
France	quinces	EC	250 g/l	0.015 l/hl		0.00375	3	10	30
Brazil	rice	EC	250 g/l	0.3 l/ha✈[72]	0.075		1		45
Switzerland	rye	EC	250 g/l	0.5 l/ha	0.125		1		GS[73]
Brazil	strawberry	EC	250 g/l	40 ml/hl		0.010	6	14	7
Germany	sugar beet	EC	250 g/l	0.4 l/ha	0.1		2		28
Italy	sugar beet	EC	250 g/l	0.3 l/ha	0.075		3	14-21	21
Spain	sugar beet	EC	250 g/l	0.5 l/ha	0.125		1-3	21-28	30
Switzerland	sugar beet	EC	250 g/l	0.4 l/ha	0.1		1-2		

[68] Total dose for season 1.0 litre product per hectare
[69] Growth stage instruction. End of flowering.
[70] Growth stage instruction. 2-3 treatments at bud break. 1-2 treatments pre-harvest.
[71] Pome fruit crop group: apples, crabapples, pears, quince, loquat, Mayhaw, oriental pear.
[72] Aerial application approved.
[73] Growth stage instruction. Only one application from BBCH39 to 61.

Country	Crop	Formulation and conc		Max product applic rate or product spray conc	Max applic rate kg ai/ha	Spray conc kg ai/hL	No. of applics	Interval between applics, days	PHI, days
Brazil	summer squash	EC	250 g/l	14 ml/hl		0.0035	4	10	3
Switzerland	sunflower	SC	62.5 g/l	2 l/ha	0.125		1		GS[74]
Australia	tomato	EC	250 g/L	500 ml/ha	0.125	-	6	10	3
Brazil	tomato	EC	250 g/l	50 ml/hl		0.0125	3	7	14
France	tomato	EC	250 g/l	0.5 l/ha	0.125		3		20
Italy	tomato	EC	250 g/l	0.5 l/ha	0.125		3-4	7-14	7
Spain	tomato	EC	250 g/l	64 ml/hl		0.016	2-4	7-10	7
Spain	tomato	EC	250 g/l	0.8 l/ha	0.2		2-4	7-10	7
Switzerland	wheat	EC	250 g/l	0.5 l/ha	0.125		1		GS[75]
UK	wheat	EC	250 g/l	0.3 l/ha	0.075		Note[76]		GS[77]

GAP Summary Table – happychloronid seed treatments.

Crop	Country	Formulation and concentration		Max product application rate per 100 kg seed	Max application rate, g ai per 100 kg seed	Use instructions and notes
Barley	Australia	FS	120 g/l	280 ml	33.6	Apply diluted with water, using slurry treatment equipment
Wheat	Australia	FS	120 g/l	280 ml	33.6	Apply diluted with water, using slurry treatment equipment

[74] Growth stage instruction. 1 application at appearance of first inflorescence.
[75] Growth stage instruction. Only one application from BBCH31 to 61.
[76] Total dose for season 0.3 litres product per hectare
[77] Growth stage instruction. Apply from ear fully emerged stage of the crop to before grain early milk-ripe stage (GS 59-71).

Solution for Exercise 7.2 Evaluation of bridging trials

The results suggest similar residue levels. However, it should be proven with statistical method. Where we have 4 or more data sets then the method of linear regression can be used.

Proceed as follow:

1. Calculate the average of residue values as they give the best estimate of the residue on the treated crop

Average residue SL	Average residue WG
0.355	0.465
0.01	0.02
0.395	0.33
0.03	0.025

2. Select one of the data sets as independent variable (e.g. average residues derived from the trials with SL formulation) and enter the values in '*Input X Range*' of MS Excel regression function available in the 'Data Analysis' tool. Enter the residue values from treatment with WG formulation in the 'Input Y Range'. Select the output range and click OK.
3. The calculation is performed and the gives the following output results:

SUMMARY OUTPUT

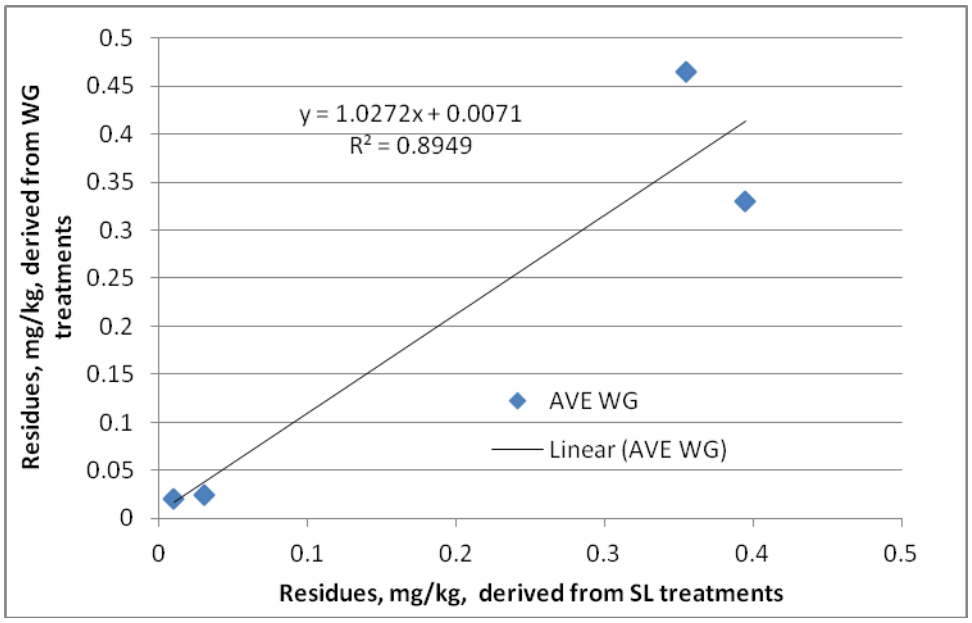
Regression Statistics	
Multiple R	0.946018
R Square	0.89495
Adjusted R Square	0.842424
Standard Error	0.088688
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.134019	0.134019	17.0384	0.053982
Residual	2	0.015731	0.007866		
Total	3	0.14975			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.007	0.066	0.108	0.924	-0.278	0.292	-0.278	0.292
X Variable 1	1.027	0.249	4.128	0.054	-0.044	2.098	-0.044	2.098

4. If the 95% confidence limits of the intercept enclose 0, and the confidence limits for slope (indicated as X variable 1) enclose 1, then the 2 data sets are not different. Note the highlighted values in the output table.
5. The chart of the two data sets looks like:



Conclusion:

As the residue levels derived from SL and WG formulations are not significantly different, only the higher values from each pair of the bridging trials should be included in the dataset for STMR and maximum residue level estimation.

That means, from these 4 pairs of bridging trials, the selected residues for further evaluation are: 0.465, 0.02, 0.395 and 0.03 mg/kg.

Solution for Exercise 7.3 Evaluation of azoxystrobin residue in stone fruits

The Meeting agreed that the data on cherry, peach, and plum obtained according to the US GAP for stone fruit are statistically not different and could be used to support a “stone fruit” commodity group maximum residue level.

Based on the residues obtained on peach (the residue dataset with largest number of values and including the highest residue value), the Meeting estimated a maximum residue level for azoxystrobin in stone fruit of 2 mg/kg and an STMR value of 0.74 mg/kg.

HR value is not necessary as the compound has no ARfD established.

Solution for Exercise 7.4 Checking validity of report on supervised trial with triazophos in rice

The report was submitted by a national Government applying the template prepared by FAO for submitting supervised trial data by government institutions.

Therefore the condensed report format can be accepted.

The report provides most of the essential information required by the template.

Missing and unclear points:

Description of sampling: the sampled portion of the crop and the number of primary samples is missing

Conditions of samples during the 3 days elapsed between sampling and arrival to the laboratory.

Sample processing: how the 20 g husked rice grain (GC0649) portion was prepared from 2 kg sample

Conclusion:

The precise description of the crop part sampled, method of sample processing and the portion of commodity analysed are essential basic information which are required for evaluation of residue data for estimation of maximum residue levels and STMR, HR residues.

Though the condensed format is acceptable, however, because of the missing essential information the report should not be accepted.

Experience shows that typically with condensed format reports vital information is missing. For example, if doubts arise about spray equipment calibration, no details are available.

Solutions for Exercise 7.5. Evaluation of supervised trials conditions in *lychee*

Residues of acar resulting from supervised trials on lychee carried out with 500 WP formulations in USA in 2004

Corp/Location,	Application					PHI	Residue		Reference/
	kg ai/ha	l/ha	kg ai/hl	No.	RTI	days	mg/kg		Field ID
US GAP: 500 WP/ 480 SC, at 0.40-0.56 kg ai/ha (0.09-0.12 kg ai/hl, number of applications 1, PHI 1 day									
Mauritius	0.56	1188	0.05	2	20	1	1.998	2.9[1]	PR 08268
Homestead, FL	0.57						1.946	2.8[1]	04-FL33
Mauritius	0.57	1193	0.05	2	21	1	2.594	3.6[1]	PR 08268
Homestead, FL	0.57						2.553	3.7[1]	04-FL34
Mauritius	0.57	1193	0.05	2	21	1	2.291	3.3[1]	PR 08268
Homestead, FL	0.57						1.545	2.2[1]	04-FL35

RTI: retreatment interval

1./ The residues reported are corrected for the loss during storage.

Evaluation

The US GAP specifies one application at maximum 0.56 kg ai/ha with a PHI of 1 day

3 trials were performed at the same site with two pesticide treatments with maximum GAP dosage rate 20-21 days apart. The plots were treated on different days within a short period of time.

Residues ranged from 1.545 to 2.594 ppm acar equivalents. The highest residues were observed in samples from the 04-FL34 trial (2.553 ppm and 2.594 ppm), representing the highest average residue of 2.574 ppm).

The average residues in/on lychee fruits, corrected for the loss during storage, one day after the 2nd application were 2.85 3.25, and 3.65 mg/kg.

Observation on the trials conditions:

The trials were conducted and reported properly, the sprayers were calibrated and the actually applied amount of pesticide was close to the target rate.

The number of trials meets the minimum US requirements for minor crops.

Two applications were made instead of one specified on the label (and reported in the GAP table). As the first application was made 20 days earlier, taking into account the fast growing of fruits, the application conditions may be considered within GAP.

Sampling, sample preparation and analysis were carried out correctly.

The residues in stored samples decomposed by about 33% within the first week of storage, and they remained constant (relative standard deviation 9.1%) afterwards. It indicates that, though the sample processing was carried out in deep-frozen conditions, the residues most likely decomposed during sample processing. However, based on a single trial a correction factor cannot be estimated. Furthermore, similar decomposition can be expected during regulatory control, adjusting the measured residues for the loss during storage is not acceptable

The trials cannot be considered independent as they were carried out at the same site on the same crop variety with the same sprayer within a short period of time.

Conclusion: the results of these supervised trials cannot be used for estimation of maximum and median residue levels.

Solution for Exercise 7.6: Evaluation of supervised trials conditions in *papaya*

Tasks:

Taking into consideration the basic requirements of sampling, sample processing and analysis evaluate the implementation of supervised trials in papaya.

- Validate the study conditions (study material, test system, compliance with GAP, application conditions,
- Identify independent trials
- Summarise the residues in tabulated form
- Select residue data suitable for estimation of maximum residue levels

Solution

Sampling: See solution for exercise 5.2.

The trials were conducted in compliance with US GAP.

Test system was selected correctly.

The handling of test materials was correct and well described.

The experimental field was well maintained and all maintenance pesticide and fertilizer application had been recorded.

The analyses of samples were carried out within a short period of time with properly validated method. The method performance was verified with concurrent recoveries.

The trials were conducted at different sites and they are independent.

Summary of residues of acar resulting from supervised trials on papaya carried out with 500 WP formulations in USA in 2003.

Crop variety/Location,	Application					PHI days	Residue[1] mg/kg	Reference/ Field ID
	kg ai/ha	l/ha	kg ai/hl	No.	RTI			
US GAP: 500 WP/ 480 SC, at 0.40-0.56 kg ai/ha, number of applications 1, PHI 1 day								
Red Lady Homestead, FL	0.57	1393	0.04	2	21	1	0.11 0.14	PR 08270 03-FL19
Gold Haleiwa, HI	0.57	477	0.12	2	21	1	0.62 1.9	PR 08270 03-HI01
Kapoho Keaau, HI	0.58	963	0.06	2	22	1	0.81 0.76	PR 08270 03-HI02

RTI: retreatment interval 1./ All harvested samples were cut into fractions (1/8 to 1/2) to reduce sample size, put into bags in the field, transferred to frozen storage and kept frozen until analysis. Note, that this practice is not permitted by the Codex Guideline on Good Laboratory Practice (ftp://ftp.fao.org/codex/alinorm03/al03_41e

Conclusion:

Well implemented and documented trials.

However, taking into account the uncertainties derived for sample size reduction in the field the residue data is not suitable for estimation of maximum residue levels.

Furthermore, the number of trials is not sufficient for Codex purposes for estimation of maximum residue levels.

Solutions for Exercise 8.1. Evaluation of Data from Supervised Residue Trials and Processing Studies – Estimation of Maximum Residue Levels

Evaluation of zappacarb residues on pome fruit

Completed Worksheets

Supervised trials, validation checks

Trial	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?
TRIAL C107	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	74-111 (n=27)		238 days ✓	**yes**
TRIAL J107	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	74-111 (n=27)		239 days ✓	**yes**
TRIAL 104	apple	✓	✓	✓	**?**	whole fruit	✓	HPLC7	✓	72-120 (n=46)		165 days ✓	**yes**
TRIAL 5	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	72-120 (n=46)	c = 0.01	152 days ✓	**yes**
TRIAL 12	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	72-120 (n=46)		162 days ✓	**yes**
TRIAL 105	apple	✓	✓	✓	**?**	whole fruit	✓	HPLC7	✓	72-120 (n=46)		197 days ✓	**yes**
TRIAL 97	apple	✓	✓	✓	**?**	whole fruit	✓	HPLC7	✓	72-120 (n=46)	c = 0.019	133 days ✓	**yes**
TRIAL 98	apple	✓	✓	✓	**?**	whole fruit	✓	HPLC7	✓	72-120 (n=46)		133 days ✓	**yes**
TRIAL 101	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	72-120 (n=46)	c = 0.068 **?**	139 days ✓	**? no**
TRIAL S105	apple	✓	✓	✓	**?**	whole fruit	✓	HPLC7	✓	72-120 (n=46)		137 days ✓	**yes**
TRIAL J102	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	72-120 (n=46)		167 days ✓	**yes**
TRIAL J103	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	72-120 (n=46)		135 days ✓	**yes**

Trial	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?
TRIAL 4	apple	✓	✓	✓	?	whole fruit	✓	HPLC7	✓	72-120 (n=46)	c = 0.01	144 days ✓	**yes**
TRIAL 20	apple	✓	✓	✓	?	whole fruit	✓	HPLC7	✓	72-120 (n=46)		137 days ✓	**yes**
TRIAL 103	apple	✓	✓	✓	?	whole fruit	✓	HPLC7	✓	72-120 (n=46)		190 days ✓	**yes**
TRIAL S105	apple	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	72-120 (n=46)		162 days ✓	**yes**
REPORT 6A	apple	✓	✓	✓	✓	whole fruit	✓	?	?	?		?	**no**
REPORT 6NO	apple	✓	✓	✓	✓	whole fruit	✓	?	?	?		?	**no**
REPORT 24IE	apple	✓	✓	✓	✓	whole fruit	✓	✓	✓	✓		✓	**yes**
REPORT 24	apple	✓	✓	✓	✓	whole fruit	✓	✓	✓	✓		✓	**yes**
TRIAL L8	pear	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	102-123		475 days ?	**? yes**
TRIAL L13	pear	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	102-123		500 days ?	**? yes**
TRIAL P106	pear	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	102-123		494 days ?	**? yes**
TRIAL P107	pear	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	102-123		473 days ?	**? yes**
TRIAL J108	pear	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	102-123	c = 0.014	479 days ?	**? yes**
TRIAL J109	pear	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	102-123	c = 0.01	470 days ?	**? yes**

Trial	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?
TRIAL J110	pear	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	102-123	c = 0.01	481 days **?**	**? yes**
TRIAL 121	pear	✓	✓	✓	✓	whole fruit	✓	HPLC7	✓	102-123		474 days **?**	**? yes**
REPORT 7NAGANO	pear	✓	✓	✓	✓	whole fruit	✓	HPLC6	✓	✓		✓	**yes**
REPORT P7	pear	✓	✓	✓	✓	whole fruit	✓	HPLC6	✓	✓		✓	**yes**
REPORT P14N1	pear	✓	✓	✓	✓	whole fruit	✓	HPLC6	✓	✓		✓	**yes**
REPORT P14N2	pear	✓	✓	✓	✓	whole fruit	✓	HPLC6	✓	✓		✓	**yes**
REPORT P19F	pear	✓	✓	✓	✓	whole fruit	✓	HPLC6	✓	✓		**?**	**no**
REPORT P19S	pear	✓	✓	✓	✓	whole fruit	✓	HPLC6	✓	✓		**?**	**no**
REPORT P19I	pear	✓	✓	✓	✓	whole fruit	✓	HPLC6	✓	✓		**?**	**no**
REPORT P19T	pear	✓	✓	✓	✓	whole fruit	✓	HPLC6	✓	✓		**?**	**no**

Residue interpretation table

Residue interpretation table for zappacarb residues on apples. GAP and trial conditions are compared for treatments considered valid for MRL and STMR estimation.

Crop	Country	Form	Use pattern				Trial	Residues mg/kg
			kg ai/ha	kg ai/hl	No of appl	PHI days		
Pome fruit	US GAP	WP	0.56		1	7		
Apple	US trial	WP	0.56	0.12	1	7	Trial 104	0.058
Apple	US trial	WP	0.56	0.12	1	7	Trial 5	0.58
Apple	US trial	WP	0.56	0.12	1	7	Trial 12	0.20 (0.13) 1/
Apple	US trial	WP	0.55	0.12	1	7	Trial 105	0.16
Apple	US trial	WP	0.56	0.12	1	7	Trial 97	0.15
Apple	US trial	WP	0.56	0.12	1	7	Trial 98	0.22
Apple	US trial	WP	0.58	0.12	1	7	Trial 105	0.18
Apple	US trial	WP	0.55	0.12	1	7	Trial J102	0.18
Apple	US trial	WP	0.56	0.12	1	7	Trial J103	0.37
Apple	US trial	WP	0.54	0.12	1	7	Trial 4	0.17
Apple	US trial	WP	0.55	0.12	1	7	Trial 20	0.049
Apple	US trial	WP	0.56	0.12	1	7	Trial 103	0.19
Apple	US trial	WP	0.56	0.12	1	7	Trial 8105	0.38
Apple	Japan GAP	SC	1.4		1	7		
Apple	Japan trial	SC	1.2	0.02	1	7	No 24Ie	0.24
Apple	Japan trial	SC	1.0	0.02	1	7	No 24	0.26

1/ The residue on day 14 (0.20 mg/kg) exceeded the residue on day 7 (0.13 mg/kg).

Residue interpretation table for zappacarb residues on pears. GAP and trial conditions are compared for treatments considered valid for MRL and STMR estimation.

Crop	Country	Form	Use pattern				Trial	Residues mg/kg
			kg ai/ha	kg ai/hl	No of appl	PHI days		
Pome fruit	US GAP	WP	0.56		1	7		
Pear	US trial	WP	0.55	0.12	1	7	Trial L8	0.10
Pear	US trial	WP	0.56	0.12	1	7	Trial L13	0.24
Pear	US trial	WP	0.55	0.13	1	7	Trial P106	0.14
Pear	US trial	WP	0.56	0.12	1	7	Trial P107	0.13 (0.076) 1/
Pear	US trial	WP	0.54	0.12	1	7	Trial J108	0.16
Pear	US trial	WP	0.55	0.12	1	7	Trial J109	0.094
Pear	US trial	WP	0.55	0.12	1	7	Trial J110	0.097
Pear	US trial	WP	0.53	0.12	1	7	Trial 121	0.29
Pear	Japan GAP	SC	1.4		1	1		
Pear	Japan trial	SC	1.2	0.02	1	1	P14N1	0.42
Pear	Japan trial	SC	1.2	0.02	1	1	P14N2	0.90 (0.82) 2/

1/ The residue on day 14 (0.13 mg/kg) exceeded the residue on day 7 (0.076 mg/kg).

2/ The residue on day 3 (0.90 mg/kg) exceeded the residue on day 1 (0.82 mg/kg).

Summary of residue data selected for STMR, HR and maximum residue level estimation (rank order, median underlined):

Apples: 0.049, 0.058, 0.15, 0.16, 0.17, 0.18, <u>0.18</u>, 0.19, 0.20, 0.22, 0.37, 0.38, 0.58 mg/kg.

Pears: 0.094, 0.097, 0.10, <u>0.13</u>, <u>0.14</u>, 0.16, 0.24, 0.29 mg/kg

Pome fruits: 0.049, 0.058, 0.094, 0.097, 0.10, 0.13, 0.14, 0.15, 0.16, 0.16, <u>0.17</u>, 0.18, 0.18, 0.19, 0.20, 0.22, 0.24, 0.29, 0.37, 0.38, 0.58 mg/kg

Recommendations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR, mg/kg	HR, mg/kg
FP 0226	Apple	0.7	0.18	0.58
FP 0230	Pear	0.5	0.135	0.29
FP 0009	**Pome fruits**	**0.7**	**0.17**	**0.58**

GAP on which the estimations are based: US GAP for Pome fruit: 0.56 kg ai/ha, 7days PHI.

Food processing

Raw agricultural commodity (RAC)	Processed commodity	Calculated processing factors = residue in processed ÷ residue in RAC.	Median or best estimate
Apples 0.89 mg/kg 2.1 mg/kg	Washed apples 0.63 1.8	0.71, 0.86	0.8
Apples 0.89 mg/kg 2.1 mg/kg	Wet pomace 1.6 3.6	1.8, 1.7	1.8
Apples 0.89 mg/kg 2.1 mg/kg	Apple Juice 0.20 0.22	0.22, 0.10	0.16

Raw agricultural commodity (RAC)	Processed commodity	Processing factor (PF)	Raw commodity		Processed commodity	
			STMR	HR	STMR-P =STMR×PF	HR-P =HR×PF
Apples	Wet pomace	1.8	0.17		0.31	
	Apple Juice	0.16	0.17		0.027	

The HR-P calculation is relevant only where commodity units maintain their integrity through the process.

Recommendations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR-P, mg/kg	HR-P, mg/kg
JF 0226	Apple juice		0.027	

Solutions for Exercise 8.2. Evaluation of Data from Supervised Residue Trials and Processing Studies – Estimation of Dietary Intake

Evaluation of happyfos residues on tomatoes

Completed Worksheets

Supervised trials, validation checks

Study	Crop treated	Trial design	Calibrated sprayer?	Plot size OK?	Field sample size OK?	Commodity analysed	Commodity in line with Codex analytical portion?	Identity of analyt method	Method OK for commodity?	Concurrent recoveries OK?	Residue in sample from control plot?	Freezer storage OK	Valid trial?
R110991	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	71-99% n=14	<LOQ	112 days ✓	**yes**
R81099I	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	71-99% n=14	<LOQ	193 days ✓	**yes**
4841-T	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	87-102% n=10	<LOQ	163 days ✓	**yes**
10PS4510	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	87-102% n=10	<LOQ	164 days ✓	**yes**
R63099S	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	82-105% n=10	<LOQ	181 days ✓	**yes**
R74099S	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	82-105% n=10	<LOQ	183 days ✓	**yes**
4260-TMN	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	72-102% n=17	<LOQ	103 days ✓	**yes**
5361-CY	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	72-102% n=17	<LOQ	194 days ✓	**yes**
1521-TA37	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	82-105% n=10	<LOQ	118 days ✓	**yes**
2117-T37P	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	72-106% n=12	<LOQ	63 days ✓	**yes**
D2760-NMT	tomato	✓	✓	✓	✓	whole fruit	✓	B5150	✓	78-122% n=23	dry pomace 0.05 mg/kg	73 days ✓	**yes**

Residue interpretation table

Residue interpretation table for happyfos residues on tomatoes. GAP and trial conditions are compared for treatments considered valid for MRL, STMR and HR estimation.

Crop	Country	Form	Use pattern				Study	Residues, mg/kg		
			kg ai/ha	kg ai/hl	No of appl	PHI days		happyfos	happyfos oxon	happyfos + oxon expressed as happyfos
Tomatoes	Spanish GAP	WG		0.11		14				
Tomatoes	Spanish trial	WG	1.1	0.13	3	14	10PS4510	0.05	0.03	0.08
Tomatoes	Spanish trial	WG	1.0	0.1	2	14	R63099S	0.18	0.11	0.30
Tomatoes	Spanish trial	WG	1.0	0.1	2	14	R74099S	0.08	0.05	0.13
Tomatoes	Italian trial	WG	1.0	0.1	2	14	R11099I	0.14	0.05	0.19
Tomatoes	Italian trial	WG	1.0	0.1	2	14	R810991	0.08	0.03	0.11
Tomatoes	French trial	WG	0.84	0.1	4	14	1521-TA37	0.40	0.15	0.56
Tomatoes	Cyprus trial	WP	1.5	0.1	2	15	4260-TMN	0.38	0.06	0.44
Tomatoes	Cyprus trial	WP	1.5	0.1	2	15	5361-CY	0.65	0.12	0.78

Happyfos + happyfos oxon, expressed as happyfos
= happyfos residue + (330/314) x happyfos oxon residue

Summary of residue data selected for STMR, HR and maximum residue level estimation (rank order, median underlined)

Happyfos residues in tomatoes: 0.05, 0.08, 0.08, 0.14, 0.18, 0.38, 0.40, 0.65 mg/kg. Propose a maximum residue level of 0.7 or 1 mg/kg, with a preference for 1 mg/kg.

Residues for estimation of dietary intake: 0.08, 0.11, 0.13, <u>0.19</u>, <u>0.30</u>, 0.44, 0.56, 0.78 mg/kg.

Recommendations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR, mg/kg	HR, mg/kg
VO 0448	Tomato	1 mg/kg	0.245 mg/kg	0.78 mg/kg

GAP on which the estimations are based: Spain, foliar application of WG formulation at a spray concentration of 0.11 kg ai/hl, with harvest 14 days later.

Food processing

Raw agricultural commodity (RAC)	Processed commodity	happyfos + oxon expressed as happyfos mg/kg		Calculated processing factors.	Median or best estimate
		2117-T37P	D2760-NMT		
Tomatoes		1.48	1.41		
	Washed tomatoes	1.91	0.36	1.3, 0.26	1.3
	Tomato paste	6.05	1.59	4.1, 1.1	4.1
	Tomato puree	2.68	1.02	1.8, 0.72	1.8
	Tomato juice	1.38	0.44	0.93, 0.31	0.93
	Canned tomato	0.58		0.39	0.39

Raw agricultural commodity (RAC)	Processed commodity	Processing factor (PF)	Raw commodity		Processed commodity	
			STMR	HR	STMR-P =STMR×PF	HR-P 1/ =HR×PF
Tomatoes	Tomato paste	4.1	0.245		1.0	
	Tomato puree	1.8	0.245		0.44	
	Tomato juice	0.93	0.245		0.228	
	Canned tomato	0.39	0.245	0.78	0.096	0.30

The HR-P calculation is relevant only where commodity units maintain their integrity through the process.

Recommendations

Codex commodity number	Codex commodity	Maximum residue level, mg/kg	STMR-P, mg/kg	HR-P, mg/kg
	Tomato juice		0.228	

	Tomato paste		1.0	
	Tomato puree		0.44	
	Canned tomato		0.096	0.30

Dietary intake calculations

Understanding the IEDI calculation

There are 13 diets. Choose Diet B and Diet F for this exercise.

		Grams per person per day	
Codex Code	Name	Diet B	Diet F
FP 0226	Apple	60.5	39.4
FB 0269	Grapes	128.5	44.0
MM 0095	Meat 20% as fat	23.3	26.3
MM 0095	Meat 80% as muscle	93.2	105.0
VO 0448	Tomato	185.0	40.9
JF 0448	Tomato juice	0.5	15.2
	Tomato paste	1.3	4.5
	Tomato peeled (canned tomato)	0.4	3.2
TN 0085	Tree nuts	21.5	10.2
VC 0432	Watermelon	43.1	6.0
	Wine	76.8	25.6

Body weight for Diet B and Diet F = 60 kg.

Factors are required for calculating the grams of RAC (raw agricultural commodity) that produced a gram of processed product.

For tomatoes, 1.25 g of tomatoes produced 1 g of tomato juice or 1 g of peeled tomatoes, while 4 g of tomatoes produced 1 g of tomato paste.

For grapes, 1.4 g grapes produced 1 gram wine.

IEDI calculation on RACs

Codex Code	Name	STMR or STMR-P mg/kg	B diet	B intake	F diet	F intake
FP 0226	Apple	0.42	60.5	25.4	39.4	16.5
FB 0269	Grapes	0.02	128.5	2.6	44.0	0.9
MM 0095	Meat 20% as fat	0.95	23.3	22.1	26.3	24.9
MM 0095	Meat 80% as muscle	0.04	93.2	3.7	105.0	4.2
VO 0448	Tomato	0.245	185.0	45.3	40.9	10.0

TN 0085	Tree nuts	0.03	21.5	0.6	10.2	0.3
VC 0432	Watermelon	0.02	43.1	0.9	6.0	0.1
TOTAL µg/person per day				100.6		56.9

IEDI calculation on RACs and processed commodities.

Subtract the consumption of processed commodity (adjusted for production factor) from the consumption of RAC (raw agricultural commodity).

Codex Code	Name	STMR or STMR-P mg/kg	B diet	B intake	F diet	F intake
FP 0226	Apple	0.42	60.5	25.4	39.4	16.5
FB 0269	Grapes	0.02	21.0	0.4	8.2	0.2
MM 0095	Meat 20% as fat	0.95	23.3	22.1	26.3	24.9
MM 0095	Meat 80% as muscle	0.04	93.2	3.7	105.0	4.2
VO 0448	Tomato	0.245	178.4	43.7	0	0
JF 0448	Tomato juice	0.228	0.5	0.11	15.2	3.5
	Tomato paste	1.0	1.3	1.3	4.5	4.5
	Tomato peeled	0.096	0.4	0.04	3.2	0.31
TN 0085	Tree nuts	0.03	21.5	0.6	10.2	0.3
VC 0432	Watermelon	0.02	43.1	0.9	6.0	0.1
	Wine	0.005	76.8	0.4	25.6	0.1
TOTAL µg/person per day				98.7		54.6

Calculate intake as % of ADI.

Express ADI as µg per person: ADI (mg/kg bw) × 60 × 1000 = 1800 µg per person

Express estimated intake (µg/person per day) as % of ADI.

Diet B, IEDI as % of ADI = 100×98.7/1800 = 5.5 %

Diet F, IEDI as % of ADI = 100×54.6/1800 = 3.0 %

IESTI Calculation

			Total population		Children	
	STMR or STMR-P	HR or HR-P	Case	IESTI, µg/kg bw per day	Case	IESTI, µg/kg bw per day
Apple		1.3	2a	30.97	2a	76.18
Grapes		0.09	2a	1.00	2a	2.73
Meat 20% as fat		2.2	1	3.42	1	6.03
Meat 80% as muscle		0.1	1	0.62	1	1.10
Tomato		0.78	2a	8.82	2a	17.30
Tree nuts		0.11	1	0.22	1	0.16
Watermelon		0.02	2b	1.79	2b	4.65

			Total population		Children	
	STMR or STMR-P	HR or HR-P	Case	IESTI, µg/kg bw per day	Case	IESTI, µg/kg bw per day
Wine	0.005		3	0.10	3	0.02

The IESTI for children ranged from <1 % to 30 % of ARfD for the total population and <1 % to 80 % of ARfD for children.

IEDI calculation

Spreadsheet: IEDI_calculation14_FAO.xlt

Data to be entered.
Compound: HAPPYFOS
ADI = 0.03 mg/kg bw
STMRs for all the commodities

Tomato STMR: 0.245 mg/kg
Tomato paste STMR: 1.0 mg/kg
Tomato juice STMR: 0.228 mg/kg
Tomato peeled STMR: 0.096 mg/kg (the canned tomato from the processing trials).

Because tomato processing data are available for the 3 processed commodities, we should enter the tomato STMR using “Tomato (excl juice, excl paste, excl peeled)”.

Results: Intakes = 0-5% of ADI.

IESTI calculation

Spreadsheet: IESTI_calculation11_FAO.xlt

Data to be entered
Compound: HAPPYFOS
ARfD = 0.1 mg/kg bw
Tomato HR: 0.78 mg/kg

Results: Intakes = 8-10 % for adults and 20 % for children.

IEDI

HAPPYFOS () International Estimated Daily Intake (IEDI) ADI = 0 - 0.0300 mg/kg bw

		STMR or	Diets: g/person/day		Intake = daily intake: µg/person									
		STMR-P	A		B		C		D		E		F	
Codex Code	Commodity	mg/kg	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake
FP 0226	Apple (incl juice)	0.42	0.3	0.1	60.5	25.4	18.5	7.8	39.9	16.8	50.8	21.3	39.4	16.5
FE 0269	Grape (incl dried, incl juice, excl wine)	0.02	1.9	0.0	21.0	0.4	25.6	0.5	11.5	0.2	11.2	0.2	8.2	0.2
MM 0095	Meat from mammals: 20% as fat	0.95	5.5	5.3	23.3	22.1	7.7	7.3	11.0	10.5	18.0	17.1	26.3	24.9
MM 0095	Meat from mammals: 80% as muscle	0.04	22.2	0.9	93.2	3.7	30.8	1.2	44.1	1.8	72.2	2.9	105.0	4.2
VO 0448	Tomato (excl juice, excl paste, excl peeled)	0.245	1.3	0.3	178.4	43.7	102.8	25.2	53.4	13.1	1.6	0.4	0.0	0.0
JF 0448	Tomato juice	0.228	5.2	1.2	0.5	0.1	0.4	0.1	2.1	0.5	6.9	1.6	15.2	3.5
-d	Tomato paste	1	0.5	0.5	1.3	1.3	3.5	3.5	1.0	1.0	3.8	3.8	4.5	4.5
-d	Tomato, peeled	0.096	0.1	0.0	0.4	0.0	0.5	0.0	0.4	0.0	4.9	0.5	3.2	0.3
TN 0085	Tree nuts	0.03	4.2	0.1	21.5	0.6	3.9	0.1	3.0	0.1	5.5	0.2	10.2	0.3
VC 0432	Watermelon	0.02	6.1	0.1	43.1	0.9	47.1	0.9	25.8	0.5	4.4	0.1	6.0	0.1
-	Wine	0.005	1.3	0.0	76.8	0.4	1.1	0.0	15.4	0.1	68.8	0.3	25.6	0.1
	Total intake (µg/person)=			8.6		98.7		46.7		44.5		48.4		54.7
	Bodyweight per region (kg bw) =			60		60		60		60		60		60
	ADI (µg/person)=			1800		1800		1800		1800		1800		1800
	%ADI=			0.5%		5.5%		2.6%		2.5%		2.7%		3.0%
	Rounded %ADI=			0%		5%		3%		2%		3%		3%

HAPPYFOS () International Estimated Daily Intake (IEDI) ADI = 0 - 0.0300 mg/kg bw

		STMR or	Diets: g/person/day		Intake = daily intake: µg/person									
		STMR-P	G		H		I		J		K		L	M
Codex Code	Commodity	mg/kg	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake
FP 0226	Apple (incl juice)	0.42	14.4	6.0	10.1	4.2	2.2	0.9	0.0	0.0	9.8	4.1	17.9	7.5	36.3	15.2
FE 0269	Grape (incl dried, incl juice, excl wine)	0.02	1.2	0.0	3.5	0.1	2.2	0.0	0.2	0.0	2.0	0.0	5.9	0.1	15.4	0.3
MM 0095	Meat from mammals: 20% as fat	0.95	11.0	10.4	17.9	17.0	6.1	5.8	5.7	5.4	16.4	15.6	12.2	11.6	31.7	30.1
MM 0095	Meat from mammals: 80% as muscle	0.04	43.8	1.8	71.5	2.9	24.5	1.0	22.9	0.9	65.7	2.6	48.9	2.0	126.6	5.1
VO 0448	Tomato (excl juice, excl paste, excl peeled)	0.245	22.8	5.6	4.1	1.0	12.3	3.0	1.8	0.4	32.8	8.0	0.4	0.1	27.3	6.7
JF 0448	Tomato juice	0.228	0.0	0.0	0.8	0.2	0.1	0.0	7.2	1.6	0.0	0.0	2.4	0.5	45.2	10.3
-d	Tomato paste	1	0.1	0.1	2.1	2.1	0.6	0.6	0.4	0.4	0.6	0.6	1.4	1.4	1.2	1.2
-d	Tomato, peeled	0.096	0.2	0.0	14.5	1.4	0.2	0.0	0.0	0.0	0.3	0.0	0.8	0.1	1.2	0.1
TN 0085	Tree nuts	0.03	16.3	0.5	15.7	0.5	9.7	0.3	1.9	0.1	19.1	0.6	29.0	0.9	5.6	0.2
VC 0432	Watermelon	0.02	39.3	0.8	14.0	0.3	2.5	0.1	13.6	0.3	8.4	0.2	14.5	0.3	13.6	0.3
-	Wine	0.005	1.0	0.0	0.9	0.0	6.8	0.0	0.1	0.0	3.4	0.0	3.6	0.0	31.0	0.2
	Total intake (µg/person)=			25.2		29.6		11.8		9.2		31.8		24.5		69.6
	Bodyweight per region (kg bw) =			55		60		60		60		60		55		60
	ADI (µg/person)=			1650		1800		1800		1800		1800		1650		1800
	%ADI=			1.5%		1.6%		0.7%		0.5%		1.8%		1.5%		3.9%
	Rounded %ADI=			2%		2%		1%		1%		2%		1%		4%

IESTI

HAPPYFOS () International estimate of short term intake (IESTI) for Acute RfD= 0.100 mg/kg bw (100 µg/kg bw)

GENERAL POPULATION Maximum %ARfD: 30%

Codex Code	Commodity	STMR or STMR-P mg/kg	HR or HR-P mg/kg	Large portion diet			Unit weight			Varia-bility factor	Case	IESTI µg/kg bw/day	% acute RfD rounded
				Country	Body weight (kg)	Large portion, g/person	Unit weight, g	Country	Unit wt, edible portion, g				
TN 0085	Tree nuts	-	0.11	JPN	52.6	107	-	-	ND	ND	1	0.22	0%
TN 0660	Almonds	-	0.11	JPN	52.6	74	-	-	ND	ND	1	0.15	0%
FP 0226	Apple	-	1.3	USA	65.0	1348	110	FRA	100	3	2a	30.97	30%
FP 0226	Apple	-	1.3	USA	65.0	1348	200	JPN	200	3	2a	34.96	30%
FP 0226	Apple	-	1.3	USA	65.0	1348	138	USA	127	3	2a	32.04	30%
FP 0226	Apple	-	1.3	USA	65.0	1348	162	SWE	149	3	2a	32.92	30%
FP 0226	Apple	-	1.3	USA	65.0	1348	155	BEL	140	3	2a	32.54	30%
TN 0295	Cashew nut	-	0.11	Thai	53.5	200	-	-	ND	ND	1	0.41	0%
TN 0664	Chestnuts	-	0.11	FRA	52.2	373	-	-	ND	ND	1	0.79	1%
FB 0269	Grape (excl wine)	-	0.09	AUS	67.0	513	125	FRA	118	3	2a	1.00	1%
FB 0269	Grape (excl wine)	-	0.09	AUS	67.0	513	150	JPN	150	3	2a	1.09	1%
FB 0269	Grape (excl wine)	-	0.09	AUS	67.0	513	456	SWE	438	3	2a	1.87	2%
TN 0666	Hazelnut	-	0.11	AUS	67.0	70	-	-	ND	ND	1	0.11	0%
MM 0095	Meat from mammals other than marine mammals: 20% as fat	-	2.2	AUS	67.0	104	-	-	ND	ND	1	3.42	3%
MM 0095	Meat from mammals other than marine mammals: 80% as muscle	-	0.1	AUS	67.0	417	-	-	ND	ND	1	0.62	1%
TN 0672	Pecan	-	0.11	AUS	67.0	23	-	-	ND	ND	1	0.04	0%
VO 0448	Tomato	-	0.78	FRA	52.2	387	105	FRA	102	3	2a	8.82	9%
VO 0448	Tomato	-	0.78	FRA	52.2	387	150	JPN	150	3	2a	10.26	10%
VO 0448	Tomato	-	0.78	FRA	52.2	387	85	UNK	85	3	2a	8.32	8%
VO 0448	Tomato	-	0.78	FRA	52.2	387	123	USA	123	3	2a	9.46	9%
VO 0448	Tomato	-	0.78	FRA	52.2	387	150	BEL	143	3	2a	10.04	10%
TN 0678	Walnut	-	0.11	FRA	52.2	145	-	-	ND	ND	1	0.31	0%
VC 0432	Watermelon	-	0.02	USA	65.0	1939	3000	JPN	3000	3	2b	1.79	2%
VC 0432	Watermelon	-	0.02	USA	65.0	1939	4518	USA	2078	3	2b	1.79	2%
-	Wine	0.005	-	FRA	52.2	1006	-	-	ND	ND	3	0.10	0%

HAPPYFOS () International estimate of short term intake (IESTI) for Acute RfD= 0.100 mg/kg bw (100 µg/kg bw)

CHILDREN UP TO 6 YEARS Maximum %ARfD: 90%

Codex Code	Commodity	STMR or STMR-P mg/kg	HR or HR-P mg/kg	Large portion diet Country	Body weight (kg)	Large portion, g/person	Unit weight Unit weight, g	Country	Unit wt, edible portion, g	Varia-bility factor	Case	IESTI µg/kg bw/day	% acute RfD rounded
TN 0085	Tree nuts	-	0.11	AUS	19.0	28	-	-	ND	ND	1	0.16	0%
TN 0660	Almonds	-	0.11	USA	15.0	13	-	-	ND	ND	1	0.10	0%
FP 0226	Apple	-	1.3	USA	15.0	679	110	FRA	100	3	2a	76.18	80%
FP 0226	Apple	-	1.3	USA	15.0	679	200	JPN	200	3	2a	93.49	90%
FP 0226	Apple	-	1.3	USA	15.0	679	138	USA	127	3	2a	80.83	80%
FP 0226	Apple	-	1.3	USA	15.0	679	162	SWE	149	3	2a	84.66	80%
FP 0226	Apple	-	1.3	USA	15.0	679	155	BEL	140	3	2a	83.01	80%
TN 0295	Cashew nut	-	0.11	Thai	17.1	99	-	-	ND	ND	1	0.64	1%
TN 0664	Chestnuts	-	0.11	FRA	18.9	196	-	-	ND	ND	1	1.14	1%
FB 0269	Grape (excl wine)	-	0.09	AUS	19.0	342	125	FRA	118	3	2a	2.73	3%
FB 0269	Grape (excl wine)	-	0.09	AUS	19.0	342	150	JPN	150	3	2a	3.04	3%
FB 0269	Grape (excl wine)	-	0.09	AUS	19.0	342	456	SWE	438	3	2b	4.86	5%
TN 0666	Hazelnut	-	0.11	FRA	18.9	27	-	-	ND	ND	1	0.16	0%
MM 0095	Meat from mammals other than marine mammals: 20% as fat	-	2.2	AUS	19.0	52	-	-	ND	ND	1	6.03	6%
MM 0095	Meat from mammals other than marine mammals: 80% as muscle	-	0.1	AUS	19.0	208	-	-	ND	ND	1	1.10	1%
TN 0672	Pecan	-	0.11	AUS	19.0	22	-	-	ND	ND	1	0.13	0%
VO 0448	Tomato	-	0.78	FRA	18.9	215	105	FRA	102	3	2a	17.30	20%
VO 0448	Tomato	-	0.78	FRA	18.9	215	150	JPN	150	3	2a	21.27	20%
VO 0448	Tomato	-	0.78	FRA	18.9	215	85	UNK	85	3	2a	15.91	20%
VO 0448	Tomato	-	0.78	FRA	18.9	215	123	USA	123	3	2a	19.04	20%
VO 0448	Tomato	-	0.78	FRA	18.9	215	150	BEL	143	3	2a	20.65	20%
TN 0678	Walnut	-	0.11	FRA	18.9	53	-	-	ND	ND	1	0.31	0%
VC 0432	Watermelon	-	0.02	AUS	19.0	1473	3000	JPN	3000	3	2b	4.65	5%
VC 0432	Watermelon	-	0.02	AUS	19.0	1473	4518	USA	2078	3	2b	4.65	5%
-	Wine	0.005	-	FRA	18.9	89	-	-	ND	ND	3	0.02	0%

Solutions for exercise 8.3 Evaluation of residues derived from supervised trials in *passion fruits*

Taking into account that up to 5 times GAP dose rate did not lead to residues above at or above 0.05 mg/kg at shorter than recommended PHI, the Meeting estimated a maximum residue level, an STMR value and HR value of 0.05, 0.01 and 0.04 mg/kg, respectively. (Statistical calculation methods are not applicable for this situation as 5XGAP considered by them as GAP trial data . (NAFTA 0.07, OECD 0.09))

Solutions for Exercise 9.1. Estimation of an EMRL

The task

Estimate the violation rates if an EMRL was established at 0.1, 0.5, 1, 2 or 5 mg/kg for each set of data.

Determine the critical data set. Determine that the critical data set is sufficient and is not selected data from a specific region of DDT usage.

Estimate a suitable EMRL for DDT residues in:

MM 0095 Meat (from mammals other than marine mammals) (fat)

1. Calculate percentage of samples exceeding selected DDT residue concentrations of 0.1, 0.5, 1, 2 or 5 mg/kg.

Percentages exceeding 1.0% are shown in bold.

Country	Commodity	No. of samples	Percentage of samples exceeding DDT residue, mg/kg				
			0.1 mg/kg	0.5 mg/kg	1 mg/kg	2 mg/kg	5 mg/kg
Australia 1989-94	beef (fat)	39854	**3.6**		0.073		0.005
Australia 1989-94	sheep (fat)	29270	**8.0**		0.044		0
Australia 1989-94	pig (fat)	15900	**1.4**		0.050		0.013
Germany, 1993	meat[78] (fat)	777	**7.3**	0.13	0	0	0
Germany, 1993	sheep (fat)	87	**37**	**2.3**	**1.15**	0	0
New Zealand, 1990-94	lambs (fat)	965			**1.9**	0.21	0
New Zealand, 1990-94	adult sheep (fat)	548			**2.2**	0.73	0
New Zealand, 1990-94	Adult bovine (fat)	739			0.68	0	0
New Zealand, 1990-94	Suckling calves (fat)	1211			**2.6**	0.83	0.08
New Zealand, 1990-94	Pigs (fat)	925			**1.1**	0.43	0.11
New Zealand, 1992-93	Lambs (fat) Note[79]	403			**32.5**	**17.6**	**3.2**
Norway, 1990-94	Bovine (fat)	537		0.19	0		
Norway, 1990-94	Pigs (fat)	537		0.19	0		
Norway, 1990-94	Sheep (fat)	149	0	0	0		
Norway, 1990-94	Moose (fat)	169	0	0	0		
Thailand, 1993	Cattle meat (fat)	30	**10**	0			
Thailand, 1994	Cattle meat (fat)	123	**8.9**	0			
Thailand, 1993	Pig meat (fat)	65	**9.2**	0			
Thailand, 1994	Pig meat (fat)	157	**5.1**	0			
USA, 1991	Cattle (fat)	4650	0.86	0.13	0.043		0
USA, 1991	Sheep (fat)	347	**1.15**	0.00	0.000		0
USA, 1991	Hogs (fat)	643	0.78	0.47	0.31		0
USA, 1992	Cattle (fat)	1546	**3.82**	0.45	0.13		0
USA, 1992	Sheep (fat)	342	**7.89**	**1.46**	0.29		0
USA, 1992	Hogs (fat)	3604	**1.78**	0.31	0.14		0.055
USA, 1993	Cattle (fat)	4032	**3.84**	0.40	0.15		0
USA, 1993	Sheep (fat)	1107	**5.87**	0.54	0.18		0
USA, 1993	Hogs (fat)	1488	**2.08**	0.27	0.13		0.067
USA, 1994	Cattle (fat)	3955	**3.72**	0.28	0.10		0.025
USA, 1994	Pigs (fat)	1457	**3.71**	0.34	0.14		0.069
USA, 1994	Sheep and goats (fat)	900	**13.0**	**2.22**	0.22		0

[78] Except sheep.
[79] Lambs from a region with known DDT history.

Interpretation

If violation rates of 0.5 to 1 % are generally unacceptable in trade, estimates of residue levels corresponding to 0.1-0.2 % violations are needed.

Smaller datasets are generally inadequate to distinguish small differences at the 0.1 % level (equivalent to 1 sample in 1000). Effectively, the limit to observations with 1000 samples is 0.1 %.

DDT residues in the New Zealand lambs from the region with a known DDT history should be excluded from the analysis of the remaining monitoring data with no such connection.

Observation of the violation rates at 1 mg/kg where more than 500 samples are available, suggests that the New Zealand data population is different and would be the critical population.

Country	Commodity	No. of samples	% of samples exceeding 1 mg/kg
Australia 1989-94	beef (fat)	39854	0.073
Australia 1989-94	sheep (fat)	29270	0.044
Australia 1989-94	pig (fat)	15900	0.050
Germany, 1993	meat[80] (fat)	777	0
New Zealand, 1990-94	lambs (fat)	965	**1.9**
New Zealand, 1990-94	adult sheep (fat)	548	**2.2**
New Zealand, 1990-94	Adult bovine (fat)	739	0.68
New Zealand, 1990-94	Suckling calves (fat)	1211	**2.6**
New Zealand, 1990-94	Pigs (fat)	925	**1.1**
Norway, 1990-94	Bovine (fat)	537	0
Norway, 1990-94	Pigs (fat)	537	0
USA, 1991	Cattle (fat)	4650	0.043
USA, 1991	Hogs (fat)	643	0.31
USA, 1992	Cattle (fat)	1546	0.13
USA, 1992	Hogs (fat)	3604	0.14
USA, 1993	Cattle (fat)	4032	0.15
USA, 1993	Sheep (fat)	1107	0.18
USA, 1993	Hogs (fat)	1488	0.13
USA, 1994	Cattle (fat)	3955	0.10
USA, 1994	Pigs (fat)	1457	0.14
USA, 1994	Sheep and goats (fat)	900	0.22

Further examination of the New Zealand data shows that violation rates at 2 mg/kg would be 0.21, 0.73, 0, 0.83 and 0.43 % for the commodities studied.

Violation rates at 5 mg/kg would be 0.08 and 0.11 % for meat (fat) from suckling calves and pigs and below this for the other meats. These estimated violation rates are close to the target 0.1-0.2 %, suggesting that 5 mg/kg would be suitable for the EMRL.

On the basis of the residue data received from the government of New Zealand, the Meeting (JMPR, 1996) concluded that the EMRL for DDT in meat (fat) recommended by the 1993 JMPR should be increased to 5 mg/kg, thus confirming the existing temporary CXL.

[80] Except sheep.

Recommendation

Compound:	DDT
Commodity:	MM 0095 Meat (from mammals other than marine mammals)
EMRL	5 (fat) mg/kg

Solutions for Exercise 10.1. Evaluation of food processing data

1. Calculate the processing factor for each processed commodity in each trial.

Pirimicarb residues in apples

APPLES country, year (variety)	Commodity	Residues mg/kg	Processing factors	Ref
Italy, 2000 (Red Chief)	apples	0.06		IT20-00-S391
	wet pomace	0.10	1.67	
	dry pomace	0.33	5.5	
	apple juice	0.03	0.50	
France, 2003 (Golden)	apples	0.08		AF/7359/SY/1
	dry pomace	0.40	5.0	
	apple juice	0.06	0.75	
France, 2003 (Golden)	apples	0.08		AF/7359/SY/2
	dry pomace	0.44	5.5	
	apple juice	0.06	0.75	
France, 2003 (Golden)	apples	0.05		AF/7359/SY/3
	dry pomace	0.38	7.6	
	apple juice	0.05	1.0	

Pirimicarb residues in tomatoes

TOMATOES country, year (variety)	Commodity	Residues mg/kg	Processing factors	Ref
Italy, 1997 (Red River)	tomatoes	0.13		IT33-97-E379
	tomato juice	0.08	0.62	
	tomatoes canned	0.02	0.15	
France, 2003 (Quest)	tomatoes	0.43		AF/7363/SY/1
	tomato juice	0.37	0.86	
	tomatoes canned	0.39	0.91	
France, 2003 (Quest)	tomatoes	0.37		AF/7363/SY/2
	tomato juice	0.57	1.54	
	tomatoes canned	0.51	1.37	
France, 2003 (Quest)	tomatoes	0.47		AF/7363/SY/3
	tomato juice	0.33	0.70	
	tomatoes canned	0.51	1.09	

TOMATOES country, year (variety)	Commodity	Residues mg/kg	Processing factors	Ref
France, 2003 (Quest)	tomatoes tomato juice tomatoes canned	0.56 0.28 0.37	 0.50 0.66	AF/7363/SY/4

Trifloxystrobin residues in grapes.

GRAPES country, year	Commodity	Residues mg/kg	Processing factors	Ref
Germany, 1996	berries wine	1.01 <0.02	 <0.020	gr01396
Germany, 1996	berries wine	0.37 <0.02	 <0.054	gr01496
Germany, 1997	berries wine	0.71 <0.02	 <0.028	gr45597
Germany, 1997	berries wine	0.66 <0.02	 <0.030	gr46597
Germany, 1995	berries wine	0.44 <0.02	 <0.046	CGD03
Switzerland, 1995	berries wine	0.22 0.05	 0.23	2035/95
Switzerland, 1995	berries wine	0.58 0.17	 0.29	2036/95
Germany, 1995	berries wine	1.01 <0.02	 <0.020	951047008
Germany, 1996	berries wine	1.23 <0.02	 <0.016	gr01196
Germany, 1996	berries wine	0.35 <0.02	 <0.057	gr01296
France, 1996	berries wine	0.64 0.03	 0.047	FRA-DE17
France, 1996	berries wine	0.94 0.10	 0.106	FRA-KJ58
Switzerland, 1998	berries wine	0.22 <0.02	 <0.091	SWZ-98-3-211.051
Switzerland, 1998	berries wine	0.15 <0.02	 <0.13	SWZ-98-3-211.052
Switzerland, 1998	berries wine	0.13 <0.02	 <0.15	SWZ-98-3-211.060
Switzerland, 1998	berries wine	0.25 0.04	 0.16	SWZ-98-3-211.061

GRAPES country, year	Commodity	Residues mg/kg	Processing factors	Ref
Italy, 1996	berries	0.16		ITA-2084-96
	wine	<0.02	<0.13	
Italy, 1996	berries	1.36		ITA-2085-96
	wine	0.10	0.074	

Hexythiazox residues in oranges.

ORANGES country, year	Commodity	Residues mg/kg	Processing factors	Ref
USA (CA), 2006 (Valencia)	whole fruits	0.29		TCI-06-142
	juice	<0.02	<0.069	
	pulp, dried	0.78	2.69	
	citrus oil	60	207	
USA (CA), 2006 (Valencia)	whole fruits	0.44		TCI-06-142-01
	juice	<0.02	<0.046	
	pulp, dried	0.76	1.73	
	citrus oil	32	73	
Italy, 2002 (Navel 115)	whole fruit	0.67		A2058 IT2
	marmalade	0.18	0.27	
	juice	0.15	0.22	
Spain, 2002 (New Holl)	whole fruit	0.44		A2058 PA2
	marmalade	0.06	0.14	
	juice	0.13	0.30	
Spain, 2002 (Navel New Gold)	whole fruit	0.85		A2058 ES2
	marmalade	0.09	0.11	
	raw juice	0.33	0.39	
	dry pomace	2.4	2.82	
	final juice	0.22	0.26	

2. Make the best estimate of processing factor from the experimentally derived values.

RAC	Processed commodity	Processing factors	Median or best estimate
PIRIMICARB			
Apples	apple pomace, dry	5.0, 5.5, 5.5, 7.6	**5.5**
	apple juice	0.50, 0.75, 0.75, 1.0	**0.75**
Tomato	tomato juice	0.50, 0.62, 0.70, 0.86, 1.54	**0.70**
	tomato, canned	0.15, 0.66, 0.91, 1.09, 1.37	**0.91**

RAC	Processed commodity	Processing factors	Median or best estimate
TRIFLOXYSTROBIN			
Grapes	wine	<0.016, <0.020, <0.020, <0.028, <0.030, <0.046, 0.047, <0.054, <0.057, 0.074, <0.091, 0.106, <0.13, <0.13, <0.15, 0.16, 0.23, 0.29	**0.065**
HEXYTHIAZOX			
Oranges	juice	<0.046, <0.069, 0.22, 0.30, 0.39	**0.22**
	dried pomace (dried pulp)	1.73, 2.69, 2.82	**2.69**
	citrus oil	73, 207	**140**
	marmalade	0.11, 0.14, 0.27	**0.14**

3. Use the processing factors and the STMR of the RAC to produce an STMR-P value for each processed food or feed commodity.

RAC	STMR	HR	Processed commodity	Processing factor	STMR-P	HR-P
PIRIMICARB						
Apples	0.18[81]		apple pomace, dry	5.5	**0.99**	
Apples	0.18[81]		apple juice	0.75	**0.14**	
Tomatoes	0.105[82]		tomato juice	0.70	**0.074**	
Tomatoes	0.105[82]	0.25[83]	canned tomato	0.91	**0.096**	**0.23**
TRIFLOXYSTROBIN						
Grapes	0.15[84]		wine	0.065	**0.0098**	
HEXYTHIAZOX						
Oranges	0.11[85]		orange juice	0.22	**0.024**	
Oranges	0.11[85]		citrus pulp, dry	2.69	**0.30**	

[81] Pirimicarb FP 0009 Pome fruits STMR 0.18 mg/kg
[82] Pirimicarb VO 0050 Fruiting vegetables other than cucurbits STMR 0.105 mg/kg.
[83] Pirimicarb VO 0050 Fruiting vegetables other than cucurbits HR 0.25 mg/kg.
[84] Trifloxystrobin FB 0269 Grapes STMR 0.15mg/kg.
[85] Hexythiazox FC 0001 Citrus fruits (whole fruit) median 0.11 mg/kg

NOTE: The STMR for citrus fruits applies to the edible portion (citrus flesh), but the processing factor refers to the raw agricultural commodity (RAC). Therefore, the STMR-P for citrus processed commodities is calculated from the median RAC value, not the STMR.

Solutions for Exercise 11.1. Calculation of livestock dietary burden.

Summary of dietary burden calculations

		US-Canada	EU	Australia	Japan
Max	beef	4.19	24.49	**31.43**	2.04
	dairy	7.83	16.73	**21.62**	5.14
	broiler	0.16	0.05	0.35	1.58
	layer	0.16	**1.78**	0.35	0.02
Mean	beef	2.42	8.38	**11.29**	1.19
	dairy	3.10	6.06	**8.34**	2.95
	broiler	0.16	0.05	0.35	0.53
	layer	0.16	**0.58**	0.35	0.02

Select dietary burdens for integration with results of livestock feeding studies.

	Maximum	Mean
Beef, for residues in tissues	31.43 ppm	11.29 ppm
Dairy, for residues in milk	21.62 ppm	8.34 ppm
Poultry, for residues in tissues	1.78 ppm	0.58 ppm
Poultry, for residues in eggs	1.78 ppm	0.58 ppm

Solutions for Exercise 11.2. Evaluation of livestock feeding studies.

Estimation of residue levels in tissues and milk that relate to the dietary burdens - interpolation

Feeding study values		Max dietary burden	Calculated by interpolation	
Dosing, conc in dry wt feed, ppm	**Highest residues**, mg/kg	Conc in dry wt feed, ppm	Residue (mg/kg) corresponding to dietary burden	
Muscle tissue				
5	<0.1 Note[86]	8.26	0.146	HR for muscle
15	0.24			
Kidney tissue				
5	<0.1 Note[87]	8.26	0.129	HR for offal, supports MRL
15	0.19			
Fat tissue				
5	1.7	8.26	1.86	HR for fat, supports MRL for meat (fat)
15	2.2			

Feeding study values		Mean dietary burden	Calculated by interpolation	
Dosing, conc in dry wt feed, ppm	**Mean residues**, mg/kg	Conc in dry wt feed, ppm	Residue (mg/kg) corresponding to dietary burden	
Muscle tissue				
0	0	3.35	0.034	STMR for muscle
5	<0.1 Note[88]			
15	0.15			
Kidney tissue				
0	0	3.35	0.041	STMR for offal
5	<0.1 Note[89]			
15	0.185			
Fat tissue				
0	0	3.35	0.583	STMR for fat
5	0.87			

[86] At 5 ppm dosing, residues in muscle were all <0.1 mg/kg. Accept that 0.1 mg/kg is the highest.
[87] At 5 ppm dosing, residues in kidney were both <0.1 mg/kg. Accept that 0.1 mg/kg is the highest.
[88] At 5 ppm dosing, residues in muscle were all <0.1 mg/kg. The mean is likely to be much below 0.1 mg/kg. It is better to rely on the residue levels from the 15 ppm dosing.
[89] Kidney. Similar situation to muscle.

Feeding study values		Dietary burden	Calculated by interpolation	
Dosing, conc in dry wt feed, ppm	**Mean residues**, mg/kg	Conc in dry wt feed, ppm	Residue (mg/kg) corresponding to dietary burden	
Milk				
5 15	0.083 0.152	7.41 max	0.100	supports MRL for milk
Milk				
0 5	0 0.083	3.21 mean	0.053	STMR for milk
Milk fat				
5 50	0.765 8.813	7.41 max	1.20	supports MRL for milk fat
Milk fat				
0 5	0 0.765	3.21 mean	0.491	STMR for milk fat

Recommendations table.

CCN	Commodity	Recommended MRL, mg/kg	STMR, mg/kg	HR, mg/kg
MM 0095	Meat (from mammals other than marine mammals)	*3* (fat)	*0.58* fat 0.034 muscle	*1.86* fat 0.15 muscle
MO 0105	Edible offal (Mammalian)	*0.2*	*0.041*	*0.13*
ML 0106	Milks	*0.2*	*0.053*	
FM 0183	Milk fats	*2*	*0.49*	

Solutions for Exercise 13.1. IEDI and IESTI calculations for dietary intake

Summary of IEDI calculations

BIFENAZATE

Pesticide: BIFENAZATE (219) ADI = 0-0.01 mg/kg

Residue definition: (for compliance with the MRL and for estimation of dietary intake): Sum of bifenazate and bifenazate diazene (diazenecarboxylic acid, 2-(4-methoxy-[1,1'-biphenyl-3-yl] 1-methylethyl ester), expressed as bifenazate. The residue is fat soluble.

Diet	A	B	C	D	E	F
Total intake (µg/person)=	5.9	101.6	37.7	41.5	53.2	30.6
Bodyweight per region (kg bw) =	60	60	60	60	60	60
ADI (µg/person)=	600	600	600	600	600	600
%ADI=	1.0%	16.9%	6.3%	6.9%	8.9%	5.1%
Rounded %ADI=	1%	20%	6%	7%	9%	5%

Diet	G	H	I	J	K	L	M
Total intake (µg/person)=	22.7	29.0	12.7	10.7	15.4	20.1	52.5
Bodyweight per region (kg bw) =	55	60	60	60	60	55	60
ADI (µg/person)=	550	600	600	600	600	550	600
%ADI=	4.1%	4.8%	2.1%	1.8%	2.6%	3.7%	8.7%
Rounded %ADI=	4%	5%	2%	2%	3%	4%	9%

For bifenazate IEDI details, see JMPR Report (2006)[90].

Note that the values produced by the latest IEDI calculator may not agree exactly with the 2006 version because there have been changes in diets and modifications of processed food calculations.

[90] JMPR. 2006. Bifenazate IEDI. *FAO Plant Production and Protection Paper*, 187:289-292.

MANDIPROPAMID

Pesticide: MANDIPROPAMID (231) ADI = 0-0.2 mg/kg
Residue definition: (for compliance with the MRL and for estimation of dietary intake for plant and animal commodities): mandipropamid.

Diet	A	B	C	D	E	F
Total intake (µg/person)=	35.8	318.0	88.8	176.9	137.7	239.4
Bodyweight per region (kg bw) =	60	60	60	60	60	60
ADI (µg/person)=	12000	12000	12000	12000	12000	12000
%ADI=	0.3%	2.6%	0.7%	1.5%	1.1%	2.0%
Rounded %ADI=	0%	3%	1%	1%	1%	2%

Diet	G	H	I	J	K	L	M
Total intake (µg/person)=	237.3	81.8	76.3	56.6	39.4	291.0	261.9
Bodyweight per region (kg bw) =	55	60	60	60	60	55	60
ADI (µg/person)=	11000	12000	12000	12000	12000	11000	12000
%ADI=	2.2%	0.7%	0.6%	0.5%	0.3%	2.6%	2.2%
Rounded %ADI=	2%	1%	1%	0%	0%	3%	2%

For mandipropamid IEDI details, see JMPR Report (2008)[91].
Note that the values produced by the latest IEDI calculator may not agree exactly with the 2008 version because there have been changes in diets.

SPIRODICLOFEN

Pesticide: SPIRODICLOFEN (237) ADI = 0-0.01 mg/kg
Residue definition: (for compliance with the MRL and for estimation of dietary intake) for plant commodities: spirodiclofen.
(for compliance with the MRL) for animal commodities: spirodiclofen.
(for estimation of dietary intake) for animal commodities: the sum of spirodiclofen and spirodiclofenenol, expressed as spirodiclofen.
The residue is fat-soluble.

[91] JMPR. 2008. Mandipropamid IEDI. *FAO Plant Production and Protection Paper*, 193:426-427.

Diet	A	B	C	D	E	F
Total intake (µg/person)=	2.3	52.8	22.2	25.4	27.6	17.9
Bodyweight per region (kg bw) =	60	60	60	60	60	60
ADI (µg/person)=	600	600	600	600	600	600
%ADI=	0.4%	8.8%	3.7%	4.2%	4.6%	3.0%
Rounded %ADI=	0%	9%	4%	4%	5%	3%

Diet	G	H	I	J	K	L	M
Total intake (µg/person)=	9.6	12.5	3.4	3.2	13.4	10.5	27.2
Bodyweight per region (kg bw) =	55	60	60	60	60	55	60
ADI (µg/person)=	550	600	600	600	600	550	600
%ADI=	1.8%	2.1%	0.6%	0.5%	2.2%	1.9%	4.5%
Rounded %ADI=	2%	2%	1%	1%	2%	2%	5%

For spirodiclofen IEDI details, see JMPR Report (2009)[92]

Summary of IESTI calculations

CYROMAZINE

Pesticide: CYROMAZINE (169) ARfD = 0.1 mg/kg bw
Residue definition: Definition of residues for compliance with MRL and for estimation of dietary intake for plants and animal commodities: cyromazine.

Pesticide	Population	IESTI as %ARfD		
		Range	List where IESTI>100 % ARfD	
			food	IESTI as %ARfD
Cyromazine	general population	0-140 %	Head cabbage Spinach (bunch)	120 % 140 %
Cyromazine	children up to 6 years	0-440 %	Head cabbage Spinach (bunch)	280 % 440 %

For cyromazine IESTI details, see JMPR Report (2007)[93].

[92] JMPR. 2009. Spirodiclofen IEDI. *FAO Plant Production and Protection Paper*, 196:360-362.
[93] JMPR. 2007. Cyromazine IESTI. *FAO Plant Production and Protection Paper*, 191:358-360.

Note that the values produced by the latest IESTI calculator may not agree exactly with the 2007 version because additional dietary information from more countries has been received since 2007.

FLUOPICOLIDE

Pesticide: FLUOPICOLIDE (235) ARfD: 0.6 mg/kg bw (women of childbearing age), ARfD: unnecessary for other groups of the population.
Definition of the residue (for compliance with the MRL) for plant and animal commodities: fluopicolide. Definition of the residue (for estimation of dietary intake) for plant and animal commodities: fluopicolide and 2,6-dichlorobenzamide measured separately.
The residue is fat-soluble.

Pesticide	Population	IESTI as %ARfD		
		Range	List where IESTI>100 % ARfD	
			food	IESTI as %ARfD
Fluopicolide	women of childbearing age	0-70 %		

For fluopicolide IESTI details, see JMPR Report (2009)[94].

BIFENTHRIN

Pesticide: BIFENTHRIN (178) ARfD 0.01 mg/kg bw
Definition of the residue: (for compliance with the MRL and for estimation of dietary intake) for plant and animal commodities: bifenthrin (sum of isomers).
The residue is fat soluble.

Pesticide	Population	IESTI as %ARfD		
		Range	List where IESTI>100 % ARfD	
			food	IESTI as %ARfD
Bifenthrin	general population	0-230 %	Strawberry	230 %
	children up to 6 years	0-430 %	Strawberry	430 %

For bifenthrin IESTI details, see JMPR Report (2010)[95].

[94] JMPR. 2009. Fluopicolide IESTI. *FAO Plant Production and Protection Paper*, 196:374-376.
[95] JMPR. 2010. Bifenthrin IESTI. *FAO Plant Production and Protection Paper*, 200:464-468.

ANNEX

PARTICIPANT'S EVALUATION OF THE COURSE Date ……

	Monday am	Monday pm	Tuesday am	Tuesday pm	Wednesday am	Thursday am	Thursday pm	Friday am
	Introduction Identity Properties	Env fate Sampling and analysis	Metabolism. Residue def	Residue eval, supervised trials	Trials selection.. Monit data	Residue eval, processing, dietary intake	Livestock residues	MRL expression Supervised trials, protocol preparation
I was clear about the purpose of this module								
The content of this module was relevant to my job and/or professional development								
There was adequate coverage of the subject								
The module added to my understanding of the subject								
The facilitator was knowledgeable about the technical issues in the module								
Participants' questions were answered clearly								
The documents and visual presentations were fit for purpose								
The time allocated was adequate								
The rooms, seating arrangements and equipment were fit for purpose								

Assessment using a 1 to 10 grading scale:

1	2	3	4	5	6	7	8	9	10
Definitely NO									Definitely YES

PARTICIPANT'S EVALUATION OF THE COURSE

Module	Comments and suggestions, if any
Introduction Identity. Physical and chemical properties.	
Environmental fate. Sampling and analysis.	
Metabolism. Definition of residues.	
Residue evaluation, supervised trials. Food processing.	
Selection of supervised trials. Monitoring data – spices, EMRLs.	
Residue evaluation, supervised trials. Food processing. Dietary intake.	
Residues in livestock.	
MRL expression Protocol for supervised trials.	
Any other comments	

Thank you for your response and cooperation

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