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APPENDIX E

PROCESSING STUDIES

- 1 Introduction
- 2 Objectives
- 3 Effect on the nature of the residue
 - 3.1 Objectives
 - 3.2 Study type
 - 3.3 Extent of data required
 - 3.4 Test guidelines
- 4 Effect on the residue levels
 - 4.1 Objectives
 - 4.2 Study types
 - 4.2.1 Balance studies
 - 4.2.2 Follow-up studies
 - 4.3 Planning of processing studies
 - 4.3.1 Importance of the processed product
 - 4.3.2 Consideration of physico-chemical properties
 - 4.3.3 Level of residues in the initial product
 - 4.3.4 Test conditions
 - 4.3.5 Number of studies
 - 4.4 Processing technology
- 5 Evaluation of results
- 6 Reporting
- 7 References

ANNEX 1

Guideline for the conduct of hydrolysis studies to investigate the nature of the potential residue in the products of industrial processing or household preparation

ANNEX 2

Core Processing Procedures

ANNEX 3

Processed commodities per crop group and procedure

1 Introduction

To evaluate the residue behaviour of plant protection products, not only the results of residue tests carried out on plants and plant products are necessary but also the results of residue tests carried out on processed plant products (processing studies) are important.

Even when plant protection products are used correctly, in accordance with Good Agricultural Practice, residues in or on plants and plant products are in many cases unavoidable. Since many of these plants and plant products then are processed before they reach the consumer or before being eaten, processing studies allow a better estimate to be made of consumers' exposure to residues. The results of these studies allow a more realistic calculation of consumers' intake of the active substances in plant protection products and/or their relevant metabolites, and thus a better risk assessment than is possible by calculating the Theoretical Maximum Daily Intake (TMDI). In addition, these studies can produce results relating to residues in commodities that may be used as animal feeding stuffs.

It is not normally necessary to analyse routinely for degradation and reaction products which are not included in the MRL-residue definition in processing studies. Such studies however have to include degradation or reaction products included in the residue definition for the estimation of dietary intake and have to be carried out where there is a reasonable expectation that processing may convert the residues into a species of greater toxicological concern. It should also be noted that a knowledge of the residues in processed plant products is a pre-requisite to estimating transfer factors as referred to in the proposal for an amendment of the Council residue Directive 97/41/EC (OJ No. L184, 12.7.1997, p.33).

The results of processing studies will not only help to give a more realistic estimate of the dietary intake and better comparability of data, but will also help to gain acceptance for maximum residue limits (MRLs) at international level.

In this guideline only general advice is given on how to plan, get up and carry out studies. It may be necessary to produce separate guidelines, if required, for individual crops to work out comparable and repeatable results. The requirements of international trade are taken into account insofar as the guidelines and draft guidelines of international and national organisations and associations are taken into consideration.

2. Objectives of processing studies

Processing studies have four objectives:

- To obtain information about breakdown products or reaction products which may require a separate risk assessment.
- To determine the quantitative distribution of residues in the various intermediate and end products, thus enabling a recognition of reductions and concentrations and to estimate transfer factors (i.e. residue levels in processed commodity/residue levels in raw commodity).
- To enable a more realistic estimate to be made of dietary intake of plant protection products.
- In special cases, to use the test results as a basis for the fixing of Maximum Residue Limits for the various processed foodstuffs of plant origin.

3. Effect on the nature of the residue

3.1 Objectives

The objective of these studies is to establish whether or not breakdown or reaction products arise from residues in the raw commodities during processing which may require a separate risk assessment.

3.2 Study type

The nature of most of the processing procedures is such that it would be impossible to conduct a study using radiolabelled chemicals in a manner representative of the conditions experienced in normal practice. However, the

parameter which is most likely to affect the nature of the residue during most processing operations is hydrolysis, because processes like heating would generally inactivate enzymes present in the substrate, leaving simple hydrolysis as the most important degradation mechanism. The effects of processes other than hydrolysis may also have to be investigated, where the properties of the active substance or metabolites indicate that toxicologically significant degradation products may occur as a result of these processes.

However, in the case of hydrolysis, three different hydrolysis conditions have been chosen to simulate normal processing practice. Since the substrate itself is not likely to have a major effect (apart from governing the pH level in some situations), the presence of the commodity is not necessary during the investigations.

3.3 Extent of data required

The studies are normally conducted with a radiolabelled form of the active substance.

Depending upon the potential uses of the plant protection product one or more studies will be necessary. Under normal conditions a maximum of three hydrolysis studies for the chosen representative conditions will suffice but under certain circumstances (e.g. raffination of oil, where no representative conditions are given) more studies have to be conducted.

3.4 Test guidelines

Specific guidelines to conduct such studies are included in Annex 1.

4. **Effect on the residue levels**

4.1 Objectives

The objectives of these studies are to determine the quantitative distribution of residues in the various intermediate and end products, thus enabling a recognition of reductions and concentrations and to estimate transfer factors. Furthermore they should enable a more realistic estimate to be made of dietary intake of plant protection products. In special cases, the test results could be used as a basis for the fixing of Maximum Residue Limits for the various processed foodstuffs of plant origin.

4.2 Study types

In order to achieve these objectives, two different types of processing studies are suggested: balance studies and follow-up studies.

The basis for the follow-up studies is always dependant on the corresponding balance studies.

These two types of studies differ from one another not only in their objectives but also in their scope.

4.2.1 Balance studies

The term 'processing study' is in principle understood to mean, first and foremost, balance studies (sometimes also known as basic studies). The aim of such studies is where possible to determine the distribution of (or to draw up a 'balance sheet' for) the residues in all intermediate and end products and, where appropriate, the waste products arising from the processing. In this way any concentrations or reductions in residues in individual products can be recognized.

It must be borne in mind, however, that with volatile or heat-sensitive residues, or with residues metabolizing during processing, a 'balance sheet' can be determined only to a limited extent. However, it is intended that all the residues (active substances and relevant metabolites) determined in the original plant product will also be determined in the processed products.

Breakdown or reaction products from hydrolysis studies which require a separate risk assessment must also be determined in the processed products.

4.2.2 Follow-up studies

If as a result of the balance studies the distribution of the residues is known for all intermediate and end products, then more extensive studies can be limited to important end products or intermediate products, i. e. to products which either reach the consumer direct, as an end product, or which are used as the starting product for further processing.

In individual cases, feedingstuffs must also be investigated in this respect (e. g. molasses). Such investigations are specific residue tests, for example to determine concentration factors for specific important products. Since these more extensive studies are based on the balance studies, they are known as follow-up studies.

4.3 Planning of processing studies

The guidelines given here set out the general principles. Recommendations for individual crops and processing studies which deviate from these general guidelines should be obtained from the appropriate individual guidelines that have to be worked out in the future.

The decision as to whether it is necessary to carry out processing studies will depend on:

- the importance of a processed product in the human and animal diet
- the level of residue in the plant or plant product to be processed,
- the physico-chemical properties of the active substance or relevant metabolites, and
- the possibility that degradation products of toxicological significance may be found after processing of the plant or plant product.

The results of the studies for investigation of the nature of the residues have to be taken into account. If no studies are carried out, the detailed reasons must be given.

4.3.1 Importance of the processed product

In considering whether processing studies should be carried out, one has to take into account the importance of the plant or plant product to be processed and the importance of the processed product (see Annex 3). The results of the studies for investigation of the nature of residues have to be taken into account.

4.3.2 Consideration of physico-chemical properties

Important conclusions concerning the behaviour of the active substance and/or its metabolites during processing can be drawn simply by looking at the distribution coefficients for n-octanol/water, hydrolysis stability, heat stability and solubility behaviour. A detailed description of the requirements are given in Annex 1. For example, when the log Pow is greater than three, one can assume that the residue will be concentrated in oil, whereas good water-solubility indicates that residues can be expected in juices.

4.3.3 Level of residues in the initial product

Processing studies are not normally necessary if no significant or no analytically determinable residues occur in the plant or plant product which would be processed, or if the total theoretical maximum daily intake (TMDI) is less than 10% of the ADI. In addition processing studies are not normally required for plants or plant products mostly eaten raw except for those with inedible portions such as citrus, banana or kiwi fruit where data on the distribution of the residue in peel/pulp may be required.

'Significant residues' generally refer to residues above 0.1 mg/kg. If the pesticide concerned has a high acute toxicity and/or a low ADI consideration must be given to conducting processing studies for determinable residues below 0.1 mg/kg.

One must also take into account the fact that in many processes the residues in the original product are diluted during processing. Therefore, processing studies on hops, for example, are only to be carried out if the Maximum Residue Level for dried hops is at or above 5 mg/kg. (.....)

In all other cases, processing studies must, in principle, be contemplated. If no studies are carried out, then detailed reasons must be given.

Preferably only products containing incurred residues should be used for processing. Fortification of the residues, for example, to the maximum permissible levels, either by increasing the application rates, shortening the Pre-harvest Intervals and/or by spiking with the active substance and/or its metabolites in vitro, is not as a rule desirable, though in some cases it may be permissible or even necessary.

4.3.4 Test conditions

In order to estimate transfer factors and to enable a realistic estimate of dietary intake of residues of plant protection products, processing studies representative of the potential uses of the product and of household and industrial preparation of food are usually needed.

When required processing studies (both balance and follow-up) usually only have to be carried out for a core-set of representative processing procedures for representative commodities (see Annex 2).

If it is not possible to derive from the core-set of processing procedures consistent transfer factors applicable to other processed commodities or other crops, then additional processing studies on other crops must be carried out. Additional studies may also be necessary if the estimate of dietary intake exceeds the ADI to allow further refinement of the dietary intake calculation. Additional studies should ideally be carried out on crops which contribute most to the ADI exceedance.

4.3.5 Number of studies

If the processed plant products play an important part in dietary intake or if, in exceptional cases, Maximum Residue Levels are to be set for processed products on the basis of processing studies, at least one reliable balance study (e.g. depending on the recovery of the active substance) and three further follow-up studies are necessary.

It should also be noted that it may be quite sufficient and appropriate to carry out the balance studies on one crop by way of an example for the whole crop group, e. g. to use processing studies on apples for all pome fruit.

Advice on the products to be analysed for the various crops and crop groups is given in Annex 3. Advice on sampling is given in document 7029/VI/95; for products or processed product types not mentioned in Annex 1 of document 7029/VI/95, the applicant, or the person carrying out the trials, must make a justified proposal.

4.4 Processing technology

The technology to be used in processing studies should always correspond as closely as possible to the actual conditions that are normally used in practice. A distinction must therefore be made between preparation in household and industrial processing. Thus, processed products that are prepared in household (e. g. cooked vegetables) should be produced using the equipment and preparation techniques that are normally used in household. On the other hand, industrially produced processed products (e. g. cereal products, preserves, fruit juices, sugar) should be produced using commercially representative technology, i. e. taking into account normal food-technology processes. Important processing practices such as peeling, washing, cooking, baking, frying, canning, freezing and drying have to be studied where appropriate.

If several major commercial processes could be routinely used, then two different processes should be used in the balance studies; i. e. preferably those representative processes that most likely lead to high residues in important processed products. It is possible, for example, that processed products could be obtained from oilseeds by extraction or by pressing, or that apple sauce could be produced using household techniques or industrial processing. In follow-up studies, the process used should be the one that is expected to leave the higher residue levels in the processed product concerned.

5 Evaluation of results

As a result of the studies, it will be possible:

- a) to recognize reductions and concentrations, and
- b) to estimate transfer factors.

If in exceptional cases Maximum Residue Levels for processed products are to be estimated on the basis of the processing studies, then the following procedure is suggested:

From the residue results obtained from the balance studies and from the follow-up studies for the processed product for which a MRL is to be set, an average transfer factor is determined. All available residue results for the plant or plant product which has/have been processed are multiplied by this factor. The figures obtained in this way are evaluated in accordance with the calculation procedure recommended elsewhere. On the basis of the result obtained a MRL is proposed.

6 Reporting

A report on processing studies should include all relevant data in a suitable format. This can, for example, be achieved by using the two-stage method outlined below.

- Tabular report

The trials included in a residue study are reported individually in tabular form. The results of residue analysis of the processed products may also be recorded on these forms.

- Report of the processing study

The presentation of a processing study is, for example, sub-divided into the following sections:

- Objective
- Sample background
- Sample processing
- Preparation, measuring, assessment
- Evaluation, discussion of results.

- Objective

The Objective section of the report again describes the aims of the study in detail and formulates the questions to be dealt with in the study.

- Sample background

This section of the report describes the origin of the raw products used in the processing studies (e. g. from a residue trial, addition trial).

- Sample processing

This section describes the methods used to process the samples. Some commercial processes are quite complex. It may be useful to provide a flow diagram to explain the process.

- Preparation, measuring, assessment

This essentially describes the method used to prepare and analyse the samples.

This section of the report contains the measured results and the methods used to assess the results.

- Evaluation, discussion of results

This section of the report discusses and evaluates the reported analysis results in the light of the questions outlined in the objective section.

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ANNEX 1

Guideline for the conduct of hydrolysis studies to investigate the nature of the potential residue in the products of industrial processing or household preparation

1 Introduction

When residues are present in raw agricultural commodities which are generally consumed only after processing (in either industrial or household situations), it may be necessary to investigate the magnitude of residues in the processed commodities.

Depending upon the type of process involved and upon the chemical nature of the residue in the raw commodity, it may first be necessary to determine whether the nature of the residue in the processed commodities is likely to be different from that in the raw agricultural commodity. This guideline provides a way in which such preliminary investigations may be conducted.

2 Processing types

Six procedures have been identified which are representative of the most widely used processes in industry and the home. These are:

- cooking vegetables in water
- preparation of fruit preserves
- preparation of fruit juices
- preparation of oil
- preparation of beer and wine
- preparation of bread

The nature of most of these procedures is such that it would be impossible to conduct a study using radio labelled chemicals in a manner representative of the conditions experienced in normal practice. However, the parameter which is most likely to affect the nature of the residue during most processing operations is hydrolysis, because processes like heating would generally inactivate enzymes present in the substrate, leaving primarily simple hydrolysis as a degradation mechanism. This guideline therefore describes a range of hydrolytic conditions which may be employed to simulate normal processing practice. Since the substrate itself is not likely to have a major effect (apart from governing the pH level in some situations), the guideline does not require the presence of the commodity during these investigations.

3 Representative hydrolytic conditions

Hydrolysis data are normally generated for a plant protection product at 25°C for one month and at pH 5, 7 and 9. The objective of these studies is primarily related to environmental conditions. By contrast, processing operations typically involve higher temperatures but for much shorter periods and, in some cases, more extreme pH values.

Table 1 summarises the typical conditions which prevail for each type of process, for the significant parameters of temperature, time and pH.

Table 1

Significant parameters during processing operations

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Type of process	Critical operation	Temperature (°C)	Time (min)	pH
Cooking vegetables	Boiling	100 ⁽¹⁾	15 - 50 ⁽²⁾	4.5 - 7
Preserves				
- fruits	Pasteurisation	90 - 95 ⁽³⁾	1 - 20 ⁽⁴⁾	3 - 4.5
- vegetables	Sterilisation	118 - 125 ⁽⁵⁾	5 - 20 ⁽⁶⁾	4.5 - 7
Fruit Juice	Pasteurisation	82 - 90 ⁽⁷⁾	1 - 2 ⁽⁸⁾	3 - 4.5
Oil	Raffination	190 - 270 ⁽⁹⁾	20 - 360 ⁽¹⁰⁾	6 - 7
Beer	Brewing	100	60 - 120	4.1 - 4.7
Red wine ⁽¹¹⁾	Heating of grape mash	60	2 ⁽¹²⁾	2.8 - 3.8
Bread	Baking	100 - 120 ⁽¹³⁾	20 - 40 ⁽¹⁴⁾	4 - 6

(1) Temperature of the vegetables during cooking

(2) Time, the vegetables have 100°C

(3) Temperature within the fruit preserves during pasteurisation

(4) Time, the content of the fruit preserves have 90 - 95°C

(5) Temperature within the vegetable preserves during sterilisation

(6) Time, the content of the preserves have 118 - 125°C

(7) Temperature of the fruit juice during pasteurisation

(8) Time, the fruit juice has 82 - 90°C

(9) Temperature of the desodorisation during raffination

(10) Time of the desodorisation

(11) White wine is not heated

(12) Subsequently either chilled quickly or allowed to cool slowly (overnight)

(13) Temperature within the loaf and on the surface during 20 - 40 minutes

(14) Time, the loaf and the surface have 100 - 120 °C

From the above details, three representative sets of conditions are defined in Table 2. These should be used to investigate the effects of hydrolysis as appropriate for the relevant processing operations.

Table 2

Representative hydrolytic conditions

Error! Bookmark not defined. Temperature (°C)	Time (min)	pH	Processes represented
90	20	4	Pasteurisation
100	60	5	Baking, Brewing, Boiling
120	20	6	Sterilisation

The extreme conditions which would be required to mimic the refining of oil, have been omitted from this set of representative conditions. The major consideration for the processing of oil seeds is the possible concentration or reduction in residues during the pressing or extraction of oil. In most cases, residues will be very low at this stage and only in exceptional circumstances will it be necessary to conduct further studies on the nature of the residue. Because of the extreme conditions, these further studies should be discussed with the regulatory authorities.

By contrast, hydrolytic conditions during the preparation of wine are very mild compared with those in other processes and are therefore not included in Table 2.

4 **Conduct of studies**

Depending upon the potential range of uses of the plant protection product, one or more of the above representative hydrolysis situations should be investigated (an autoclave will be needed for the temperatures above 100°C). The studies should be generally conducted with a radio labelled form of the active substance, in order to maximise the identification of components at the end of the study. The studies must be conducted in compliance with Good Laboratory Practice with reference to Standard Operating Procedures.

In cases where the residue in the raw commodity consists primarily of a metabolite, the need to conduct hydrolysis studies with that metabolite should be considered on a case by case basis. For example, comparison of its structure with those of the parent compound and the hydrolysis products of the parent compound may suggest that additional studies are unnecessary.

5 **Interpretation of results**

An individual hydrolysis product need not be identified if it is clear by calculation that its concentration in the final processed commodities will be less than 0.05 mg/kg. Such calculations should take account of its magnitude in the hydrolysis study (as a proportion of the starting material), dilution (or concentration) factors during processing and the initial residue levels in the raw commodity.

If the hydrolysis products are the same as the transformation products already identified as the residue of toxicological significance in the raw commodity, the same residue methodology principle is appropriate for use in subsequent studies on the magnitude of residues in processed commodities.

ANNEX 2

Core Processing Procedures

Error! Bookmark not defined.processing type	crop ¹⁾
distribution in the edible / non edible portion	citrus bananas
preparation of fruit juice	citrus apples ²⁾
preparation of other fruit products	apples ²⁾ or other fruit
preparation of <u>canned</u> fruit	stone fruit or berries ²⁾
preparation of wine	grapes
preparation of beer	hops barley
cooking vegetables in water	one representative vegetable crop ²⁾ or potatoes
preparation of vegetable juice and other products	one representative vegetable crop ²⁾ potatoes
preparation of oil	rape seed ²⁾
distribution on milling and preparation of bread	wheat
<u>preparation of sugar</u>	<u>sugar beets</u>

¹⁾ Studies with the listed crops only necessary if there is a GAP or expected GAP

²⁾ The selection of crop depends on the use pattern of the pesticide

ANNEX 3

Processed commodities per crop group and procedure

When it comes to the products that are to be tested for residues in a processing study, a distinction should be made according to the objective being pursued. In a balance study, all intermediate products, end products and waste products should if possible be included, in order to guarantee that a "balance sheet" will be obtained (see also 4.2.1). In the follow-up studies, only those intermediate and end products that are still relevant need to be tested. These products, which it is suggested should be tested in a follow-up study, appear in single underlined type in the following list. The residues in the initial material should in all cases be determined and reported as well.

1 Fruits

Beside the already mentioned core processing procedure other procedures may be necessary to conduct like drying.

For individual crop groups, the following crops are suggested:

Crop	Processing type	Products
Citrus fruit	Distribution in the edible/non edible portion	Peel, <u>pulp</u>
Bananas		Peel, <u>pulp</u>
Kiwifruits		Peel, <u>pulp</u>
Pineapples		Peel, <u>pulp</u>
Oranges	Preparation of fruit juice	<u>Industrially produced juice</u> , pomace (wet, dried)
Apples		<u>Industrially produced juice</u> , pomace (wet, <u>dried</u>)
Currants (black)		<u>Juice</u>
Cherries	Preparation of canned fruit	Washed cherries, <u>canned</u>
Strawberry		<u>Canned</u>
Apples	Preparation of other fruit products	Washed apples, <u>apple sauce</u>
Plums		Washed plums, <u>plum puree</u>
Currants		Washed currants, <u>jam/jelly</u>
Strawberry		Washed strawberries, <u>jam</u>
Plums		Prunes
Grapes		Raisins
<u>Oranges</u>		<u>Marmelade</u>
<u>Olives</u>	<u>Preparation of oil</u>	<u>Oil</u>
Wine grapes	Preparation of wine	<u>Juice</u> , <u>wine</u>

2 Vegetables

Beside the already mentioned core processing procedure other procedures may be necessary to conduct like drying, fermentation and concentration.

For individual crop groups, the following crops are suggested:

Crop	Processing type	Products
Carrots	Cooking vegetable in water	Washed, topped and peeled carrots, <u>cooked carrots</u> , cooking liquid
Head cabbage		Inner and outer leaves, <u>cooked head cabbage</u> , cooking liquid
Asparagus		<u>Peeled</u> and <u>cooked asparagus</u> , cooking liquid
Peas without pods		<u>Cooked peas</u> , cooking liquid
Spinach		<u>Cooked spinach</u>
Carrots	Preparation of vegetable juice	<u>Juice</u>
Tomatoes		<u>Juice</u>
Carrots	Preparation of canned vegetable	<u>Canned</u>
Asparagus		<u>Canned</u>
Peas without pods		<u>Canned</u>
Gherkins		<u>Canned</u>
Tomatoes		Washed and peeled tomatoes, <u>canned</u>
Mushrooms		<u>Canned</u>
Melon	Distribution in the edible/non edible portion	Peel, Pulp
Onions	Preparation of other vegetable products	Dried onions, peeled onions
Mushrooms		Dried mushrooms
Head cabbage (white)		<u>Sauerkraut</u> , sauerkraut juice
Tomatoes		<u>Puree</u>
Lettuce		Inner and outer leaves, washing

3 Potatoes

Crop	Processing type	Products
Potatoes	Cooking in water	<u>Peeled potatoes, peel, (...), micro-waved boiled (...), potatoes (unpeeled), cooking liquid.</u>
Potatoes	Preparation of other products	<u>Fried potatoes, (...), crisps, (...)</u>

Fried potatoes should only be investigated in the case of postharvest use of plant protection products or in the case of plant growth regulators shortly before harvest

4 Hops

Crop	Processing type	Products
Hops	Preparation of beer	<u>Beer, brewer's yeast, spent hops and flocs</u>

The report must be state the dilution factor.

5 Oilseeds

Crop	Processing type	Products
Rape seed	Preparation of oil	<u>Crude oil, refined oil, solvent-extracted meal/press cake</u>

6 Cereals

Wheat and rye in particular, and also barley (in the form of mal-ting barley), are included here among the plant products which are of special nutritional importance.

Crop	Processing type	Products
Wheat or rye	Distribution on milling and preparation of bread	<u>Whole-meal flour, whole-grain bread, middlings, total bran (coarse bran, fine bran), flour including toppings (Type 550 or Type 997), wheat germ</u>
Maize	Distribution on milling (and preparation of bread)	Flour, bran, middlings, <u>starch, germ</u> (residues in germ should also be determined in follow-up studies in order to answer questions concerning residues in oil derived from the germ)
Barley	preparation of beer	<u>Brewing malt, malt sprouts, beer, spent grains and flocs, brewer's yeast</u>
Barley		Pot barley
Oats		Oat husk and oat dust, <u>oat flakes</u>

7 Sugar beets

Crop	Processing type	Products
Sugar beets	Preparation of sugar	<u>Pulp, press water, raw juice, thin juice, lime sludge, thick juice, raw sugar, white sugar, molasses</u>