OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Nature of the Pesticide Residues in Processed Commodities - High Temperature Hydrolysis

INTRODUCTION

1. Most food or raw agricultural commodities (RACs) are processed before the general public consumes them. In fact, most RACs are consumed in multiple processed forms, for example cooked potatoes, fried potatoes, chips. The processes that are used (industrial or domestic) to produce these processed foods are diverse and varied.

2. Metabolism studies in crops traditionally submitted as part of guideline requirements establish the residue definition in the harvested commodity. These studies however do not necessarily elucidate the nature of the residue in/on processed commodities. As processed foods are major commodities in the diet and in commerce, the nature of the potential transformation products in these commodities should be determined.

3. Studies on the nature of the residue in processed commodities are conducted as model studies to predict the degradation pathway of the active ingredient. They permit the identification of the degradation products that result from residues in a RAC when subjected to certain generic processing procedures, and include the determination of the relative amount of degradation products.

PURPOSE

4. When residues are present in raw agricultural commodities that are generally consumed only after processing in either industrial or domestic 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 agricultural commodity, it should first be determined 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 investigations may be conducted.

5. While recognising that Nature of the Residue studies are model studies, investigations into the nature of residues in processed commodities fulfil several major purposes:

- to provide an estimate of the relative composition of the total residues in the processed commodities.

- to identify the major components of the terminal residue in processed commodities, thus indicating the components to be included in the magnitude of residue studies (i.e. to determine the residue definition(s) for both risk assessment and enforcement).

- to elucidate the degradation pathway of the active ingredient in processed commodities under hydrolytic conditions.

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CONDUCT OF STUDIES

General Considerations

6. Several processing operations have been identified as being representative of the most widely used industrial and domestic food processing technologies. These include:

   - cooking vegetables, pulses and cereals in water
   - preparation of fruit preserves
   - preparation of fruit juices
   - preparation of edible oil
   - preparation of alcoholic beverages, *inter alia* beer and wine
   - preparation of bread
   - preparation of instant noodles
   - frying of vegetables, meat and fish
   - fermentation of milk and vegetables

   It is assumed that simple physical procedures, for example milling and pressing, will not influence the nature of residues, where there is no change in temperature during processing.

7. The nature of processing procedures is such that it would be impossible to conduct a processing study using radiolabelled chemicals in a manner that accurately reflects industrial or domestic practices. Likewise, it would not be readily practicable and yet expensive to process samples with incurred radiolabelled residues from nature of the residue studies in plants or livestock.

8. The parameter or factor which is most likely to affect the nature of the residue during many processing operations is hydrolysis. Processes such as heating would generally inactivate enzymes present in the substrate, leaving primarily simple hydrolysis as the degradation mechanism. Therefore, within a core procedure it is hydrolysis—characterised by temperature, time and pH—that influences the nature of residues. For example, pasteurisation of juice from pressed fruit is the core procedure in preparation of fruit juice.

9. On the basis of the water solubility of the active ingredient (as reported in the physical chemical properties section), no model hydrolysis studies are necessary for substances with a water solubility of < 0.01 mg/L. Substances with a solubility $\geq$ 0.5 mg/L are considered to be of high water solubility in the context of these studies, while the solubility in between these two values (>0.01 but <0.5 mg/L) are defined as low water solubility in this context.

10. Studies may not be necessary where only simple physical operations, not involving a change in temperature during processing of the plant or the plant product, are involved—such as washing, trimming, pressing, or milling—or where the distribution of residues between peel and pulp is the only process.

11. For the purposes of determining the nature of the residue in processed fractions, model studies are considered as being the best approach. This guideline therefore describes a range of hydrolytic conditions that may be employed to simulate normal processing practices.

12. Since the substrate itself is not likely to have a major effect upon the processing procedure (apart from governing the pH level in some situations), the guideline does not require the presence of the commodity during these model investigations.
13. The determination of whether the residue has been sufficiently characterised and identified will depend on the level of radioactivity characterised/identified, on the chemical structure of the active ingredient and identified degradation products, and on the toxicity of chemicals that are structurally similar to potential degradation products. Where the structure of a degradation product is identical to another registered pesticide chemical and the information is in the public domain, the applicant should provide this information.

14. Due to the fact that these studies are conducted in a closed system where loss can only occur by precipitation, by adherence to the surface of vessels, and by degradation to gaseous products the accountability/recovery of the TRR after hydrolysis should be 90%. If this is not achieved, a justification must be given. The desired goal of such a study is the identification and characterisation of at least 90% of the remaining TRR. In some rare cases it may not be possible to identify significant proportions of the TRR especially when the pesticide is extensively degraded to numerous low level and/or unstable components. In this case it is important for the applicant to demonstrate clearly the presence and levels of the components, and if possible, attempt to characterise them.

**Hydrolytic Conditions**

15. Hydrolysis data (required as part of the physical-chemical properties of an active ingredient) are normally generated at temperatures between 0°C and 40°C for a time chosen to allow observance of degradation up to at least 70% at pH 4, 7 and 9. The objective of these studies is primarily related to environmental conditions. Therefore, they are not interchangeable with the required data outlined in the types of model studies described here. As such, physico-chemical data will not substitute for the data obtained from studies carried out in accordance with this guideline as the processing operations described herein typically involve higher temperatures but normally for much shorter periods and, in some cases, at more extreme pH values. Reactions are therefore faster and may lead to the formation of different degradation products.

16. Table 1 summarises typical conditions (temperature, time and pH) which prevail for each of the processing operations.

<table>
<thead>
<tr>
<th>Type of process</th>
<th>Critical operation</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking vegetables, cereals</td>
<td>Boiling</td>
<td>100 (1)</td>
<td>15 - 50 (2)</td>
<td>4.5 - 7</td>
</tr>
<tr>
<td>Fruit preserves</td>
<td>Pasteurisation</td>
<td>90 - 95 (3)</td>
<td>1 - 20 (4)</td>
<td>3 - 4.5</td>
</tr>
<tr>
<td>Vegetable preserves</td>
<td>Sterilisation</td>
<td>118 - 125 (5)</td>
<td>5 - 20 (6)</td>
<td>4.5 - 7</td>
</tr>
<tr>
<td>Fruit Juice</td>
<td>Pasteurisation</td>
<td>82 - 90 (7)</td>
<td>1 - 2 (8)</td>
<td>3 - 4.5</td>
</tr>
<tr>
<td>Oil</td>
<td>Raffination</td>
<td>190 - 270 (9)</td>
<td>20 - 360 (10)</td>
<td>6 - 7</td>
</tr>
<tr>
<td>Beer</td>
<td>Brewing</td>
<td>100</td>
<td>60 - 120</td>
<td>4.1 - 4.7</td>
</tr>
<tr>
<td>Red wine (11)</td>
<td>Heating of grape mash</td>
<td>60</td>
<td>2 (12)</td>
<td>2.8 - 3.8</td>
</tr>
<tr>
<td>Bread</td>
<td>Baking</td>
<td>100 - 120 (13)</td>
<td>20 - 40 (14)</td>
<td>4 - 6</td>
</tr>
<tr>
<td>Instant noodle</td>
<td>Steam and dehydration</td>
<td>100</td>
<td>1-2</td>
<td>9 (15)</td>
</tr>
<tr>
<td></td>
<td>(by frying or hot air)</td>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 (frying)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;80 (air)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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(1) Temperature of the vegetables during cooking
(2) Time the vegetables or cereals are kept at 100°C
(3) Temperature within the fruit preserves during pasteurisation
(4) Time the fruit preserves are kept at 90 - 95°C
(5) Temperature within the vegetable preserves during sterilisation
(6) Time the preserves are kept at 118 - 125°C
(7) Temperature of the fruit juice during pasteurisation
(8) Time the fruit juice is kept at 82 - 90°C
(9) Temperature of the deodorization during raffination
(10) Time of the deodorization
(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 is kept at 100 - 120 °C
(15) Wheat flour is kneaded with 0.1-0.6% Kansui (alkaline water containing 20% K₂CO₃ and 3.3% Na₂CO₃)

17. In essence, most of the processing operations given in paragraph 6 fall within the range of the parameters given in table 1. Only a few operations are not covered, for example fermentation or frying.

18. From the details given in table 1 a further reduction in the parameters can be made and three representative sets of hydrolytic conditions can be defined. A compilation is given in table 2. These should be used as appropriate to investigate the effects of hydrolysis for the relevant processing operations.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>pH</th>
<th>Processes represented</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>20</td>
<td>4</td>
<td>Pasteurisation</td>
</tr>
<tr>
<td>100</td>
<td>60</td>
<td>5</td>
<td>Baking, Brewing, Boiling</td>
</tr>
<tr>
<td>120*</td>
<td>20</td>
<td>6</td>
<td>Sterilisation</td>
</tr>
</tbody>
</table>

* closed system under pressure (e.g. autoclave or similar)

19. The extreme conditions that would be required to mimic the temperature and time of the deodorization during raffination and to mimic the pH of instant noodles (table 1), have been omitted from this set of representative conditions. The necessity for these studies should be discussed on a case-by-case basis with regulatory authorities.

20. In contrast, hydrolysis of parent compounds during the preparation of wine is already addressed in the study conducted as part of the physical chemical properties section (i.e., the routine hydrolysis studies following OECD Guideline 111) as conditions are likely to be comparable to the lower temperatures used in the wine making process. Nevertheless, the potential for changes in the parent compound or its predominant metabolite during the fermentation process is not covered by the hydrolytic model conditions and should be discussed with regulatory authorities.

21. Conditions of temperature and time that might be found in the preparation of meat and fish are not represented. The necessity of such studies should be discussed on a case-by-case basis with regulatory authorities.
TEST METHOD

Test material

22. Usually, hydrolysis studies simulating processing are conducted with the radiolabelled active ingredient. However, studies on the nature of residues may be conducted with representative component(s) of the residue as defined in food (derived from crop metabolism, confined rotational crop, or livestock metabolism studies). The residue definition of a pesticide might consist of the active ingredient plus one or more metabolites, a single or several metabolites (or degradation products). If the active ingredient is the major residue in food, it is appropriate to use the active ingredient only. If a unique plant metabolite is the predominant residue in the food, then it may be appropriate to use the metabolite only. Generally the use of mixtures is not recommended and in such situations, separate studies are preferred. However, this should be considered on a case-by-case basis as well as the question of major metabolites coming from more than one active ingredient. It may be that, for example, comparison of the structures of distinct metabolites with the active ingredient and hydrolysis products may suggest that additional studies are unnecessary.

23. Radiolabelled active ingredients are used to elucidate the possible degradation pathway and for quantitation of the extent of degradation. As these studies are conducted without a crop/animal matrix, bound residues are not relevant. However, hydrolysis products may be insoluble and therefore may precipitate out of solution or adhere to the sides of the reaction vessel. The active ingredient should be labelled so that the degradation pathway can be traced as far as possible. The radiolabel should be placed in a suitable position of the molecule so that all significant moieties or degradation products can be adequately tracked. If multiple ring structures or significant side chains are present in the molecule, separate studies reflecting labelling of each ring or side chain will normally be required if it is anticipated that cleavage between these moieties may occur. The same labelling positions as in the available metabolism studies are preferable and should be used. A scientifically based rationale may be submitted in lieu of conducting studies with multiple radiolabels, if no cleavage is anticipated. Any cleavage of linkages between rings or chains which can be predicted, e.g. from crop metabolism and hydrolysis studies, should be taken into account.

24. In choosing the position to be labelled, assurance should be provided that a stable position is selected. The preferred isotope is $^{14}$C, although $^{32}$P, $^{35}$S, or other radioisotopes may be more appropriate if no carbons or only labile carbon side chains exist in the molecule. If a potentially labile side chain is chosen, a study will be considered adequate if all significant radioactivity is identified and found to be associated with the active ingredient, and not related to loss of the label from the basic structure of the active ingredient molecule.

25. The use of tritium ($^3$H) as a label is not permitted due to the possibility of hydrogen exchange with the water.

26. The specific activity of the radiolabelled active ingredient should be adequate to meet the data requirements of the nature of residue study (quantitation of about 0.01 mg/kg total TRR). In cases where the radiochemical purity at the time of application is below 95%, appropriate justification should be given.

27. The use of stable isotopes such as $^{13}$C, $^{15}$N or deuterium $^2$D (non exchangeable) together with the radiolabel isotope is encouraged, to aid in the identification of degradation products by various spectroscopic methods [mass spectrometry (MS), or nuclear magnetic resonance (NMR)].
Experimental conditions

28. Regardless of the potential range of uses of the plant protection product, all three representative hydrolysis conditions should be investigated.

29. In these model studies the active ingredient should be dissolved in sterilised buffer medium. The pH of the test solution must be checked with a calibrated pH meter at the beginning and at the end of the experiment to a precision of at least ± 0.1. Buffer solutions can be prepared according to the tables given in OECD Guideline 111 (see paragraph 48 (c).

30. A proposed value for the concentration of a water soluble active ingredient in the studies required here is 1.0 mg/L. If the proposed value is not achieved a justification should be given. The use of miscible solvents is recommended only in the case of low water soluble substances (of water solubility, >0.01 but <0.5 mg/L, in this case). The amount of solvent should not exceed 1 percent, and the solvent should not interfere with the hydrolysis process. Nevertheless, in certain circumstances, two concentrations may be used in order to facilitate identification.

31. During the study it is important to maintain the temperature of the test solution to at least within ± 5°C of each of the required temperatures. In case of studies where an autoclave or similar instrumentation is used, a higher deviation may be acceptable. Normally natural light plays no role in processing especially in industrial situations and processes. In the case of photo-labile active ingredient the studies should be carried out using any method that is suitable to avoid any photolytic effects.

Analysis

32. In the initial stage of the hydrolysis study, the radioactivity of an aliquot is analysed to determine the TRR by liquid scintillation counting (LSC) analysis and to assure that no significant loss occurs during the study, e.g. by adherence to the vessels. All necessary attempts should be made to solubilise solid residues.

33. Samples may be analysed directly by chromatography or may be extracted with a series of solvents or solvent mixtures with various polarities and other characteristics depending on the nature of the expected residues. The resultant extracts are defined as the ‘extractable residues’ analogous to extractable residues from metabolism studies. It is anticipated that a large proportion of the radiolabel will be extractable; however, insoluble residues may sometimes form. Attention should be given to residues adhering to the vessels used. All necessary attempts should be made to dissolve solid residues. The characterisation and identification of extractable residues is summarised in table 3.

34. Identification refers to the exact structural determination of components of the total radioactive residue (TRR). Characterisation refers to the elucidation of the general nature/characteristics of the radioactive residue. Terms used to characterise residues include organo-soluble, water or aqueous soluble, neutral, acidic or alkaline, polar, non-polar, etc. Characterisation may also involve descriptions of chemical moieties known to be present in the molecule based on conversion to a common structure or due to reactivity with particular reagents. The degree of characterisation refers to how close the assignment comes to complete structural identification.
<table>
<thead>
<tr>
<th>Relative amount (% TRR)</th>
<th>Concentration (mg/L)</th>
<th>Required Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Substances with high water solubility (&gt; 0.5 mg/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10</td>
<td>&lt; 0.01</td>
<td>No action if no toxicological concern</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>0.01 – 0.05</td>
<td>Characterize. Only attempt to confirm identity if straightforward, e.g., a reference compound is available or the identification is known from a previous study.</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>&gt; 0.05</td>
<td>Characterisation/identification needs to be decided on a case-by-case basis taking into account how much has been identified.</td>
</tr>
<tr>
<td>≥ 10</td>
<td>-</td>
<td>Identify using all possible means.</td>
</tr>
</tbody>
</table>

| ≥ 10 | > 0.05 | Significant attempts to identify should be made especially if needed to establish a degradation pathway, ultimately characterisation might be accepted. |

| **Substances with low water solubility (0.01-0.5 mg/L)** | | |
| < 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 | Significant attempts to identify should be made especially if needed to establish a degradation pathway, ultimately characterisation might be accepted. |
| ≥ 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 degradation pathway, ultimately characterisation might be accepted. |
| ≥ 10 | > 0.05 | Identify using all possible means. |

35. When identification of radioactive residues is not accomplished, the degree of characterisation required for a portion of the total radioactivity will depend on several factors including the amount of residue present, the amount of the TRR already identified, toxicological concern over the class of compounds and/or degradation products, the suspected significance of the residue as determined by characterisation already performed and the capability of analytical methods to detect characterised but unidentified residues (i.e., by conversion to a common moiety). Conversion to a common moiety is acceptable for the characterisation of multiple low concentration components. However, conversion to the common moiety to alleviate identification of a significant portion of the residues is not an acceptable approach.

36. Typically, identification is achieved either by co-chromatography of the degradation product with known standards using two dissimilar systems or by techniques capable of positive structural identification such as mass spectrometry (MS), nuclear magnetic resonance (NMR), etc. In the case of co-chromatography, chromatographic techniques utilising the same stationary phase with two different solvent systems are not adequate for the verification of the degradation product identity, since the methods are not independent. Identification by co-chromatography should be obtained using two dissimilar, analytically independent systems, such as reverse and normal phase thin layer chromatography (TLC) or TLC and high...
performance liquid chromatography (HPLC). Provided that the chromatographic separation is of suitable quality, then additional confirmation by spectroscopy is not required. Unambiguous identification can also be obtained using methods providing structural information such as gas chromatography/mass spectrometry (GC-MS), liquid chromatography/mass spectrometry (LC-MS), liquid chromatography/tandem mass spectrometry (LC-MS/MS), and NMR. If the degradation product is determined to be of minimal importance due to its low absolute level, identification by co-elution with putative synthetic degradation products as reference standards using one chromatographic technique e.g. reverse phase HPLC, will be acceptable. This is meant as rough guidance and may not apply to situations where a degradation product is suspected to be of particular toxicological concern.

37. The stereochemistry of degradation products generally does not need to be determined. If identified degradation products with stereochemical centres are to be included in the residue definition and have toxicological concerns, the ratio of the stereoisomers may need to be addressed in the magnitude of residue studies.

38. New extraction and analysis techniques may be appropriate to utilise as a substitute for the techniques mentioned above. State of the art technology should be used, as appropriate, to fully elucidate the degradation pathway.

**Characterisation/Identification of Extractable Residues**

39. The strategy for identification and characterization of degradation products in the nature of the residue study is similar to that used for crop metabolism studies as described in table 3. The radioactivity threshold values shown in table 3 reflect the characterisation or identification needed following application of the radiolabelled test compound. It takes into account the range of possible different water solubilities of the active ingredients tested. If TRR is 0.01 mg/L or less in a solvent fraction, no differentiation of the radioactivity would be needed, unless there are toxicological concerns over residues occurring at lower levels.

40. If the combined concentration of the new metabolites or breakdown products is >0.01 mg/L refer to table 3 for trigger values relating to the identification/characterisation of extractable residues within the specific solvent. The exception to this would be toxicological concerns over potential residues which might occur at lower levels; this includes polar fractions. However, low-level individual residues (in terms of both mg/L and percent of total residues) do not typically need to be identified if the major components of the residue have been identified.

41. It is noted that trigger values (on a concentration basis) are not absolute standards, but approximate guides as to how much characterisation is adequate. The amount of effort required for characterization of the radioactivity depends on the water solubility of the component(s), i.e. in the case of low water solubility, greater effort would be required compared to situations of high water solubility. However, in many cases, a potentially important degradation product may partition into multiple fractions because of solubility characteristics. In order for trigger values to apply, particularly in cases where the TRRs are distributed among numerous fractions, it should be demonstrated by chromatographic analysis of each fraction, that no single component is distributed among the various fractions in such amounts that the combined level (or sum) of the component significantly exceeds the trigger value.

**Storage stability**

42. Ideally samples should be stored at/or below –18°C. Storage under any other conditions needs to be recorded and justified.
43. Determinations as to whether sample integrity was maintained during collection, sample preparation, and storage should be made. Such analyses should show that the basic profile of radiolabelled residues has not changed throughout the duration of the study. It is impossible to spike samples before the identity of the residue and the length of time needed for hydrolysis studies are known. Storage stability data are not normally necessary for samples analysed within 6 months of collection, provided evidence is given that attempts were made to limit degradation of residues by appropriate storage of samples and extracts during the analytical phase of the study.

44. If instability of the active ingredient is suspected or observed, based on information from metabolism studies in crops or other information, steps should be taken to safeguard the integrity of the study. In those cases where a Nature of the Residues in Processed Commodities study cannot be completed within 6 months of sample collection, evidence should be provided that the identity of residues did not change during the period between collection and final analysis. This can be done by analyses of representative samples early in the study and at its completion. Such analyses should show that the basic profile of radiolabelled residues has not changed throughout the duration of the study.

45. If changes are observed (e.g., disappearance of a particular HPLC peak or TLC spot), additional analyses or another Nature of the Residues in Processed Commodities study with a shorter collection to analysis interval may be necessary.

DATA REPORTING

Considerations for Data Reporting

46. The following elements should be considered during the design, conduct and reporting of the study.

**Summary/Introduction**

a. Testing strategies employed and the rationale for the selection of these hydrolysis conditions should be given.

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

c. The routes of degradation observed should include a complete description of the identity and quantity of all major components of the total radioactive residues. It is preferable that the previously mentioned information be summarised in a narrative form including with tables and/or figures.

d. A conclusion concerning the qualitative nature of the TRRs.

**Materials/Methods**

e. Test substance

   (i) Identification of the test pesticide active ingredient (a.i.), including chemical name; common name American National Standards Institute (ANSI), British Standards Institution (BSI), or International Standards Organisation (ISO); company
developmental/experimental name; and Chemical Abstracts Service (CAS) number and IUPAC chemical name.

(ii) Chemical structure(s) for the active ingredient and degradation products constituting the residue should be provided and a cross reference of all different developmental codes or experimental names should be provided in either an overview document or as an appendix to the study. Certificates of analysis describing the purity and the identity of standards used in the identification process should be provided if available.

(iii) For radiolabelled test material, report the purity, nature of the radiolabel and its source and the specific activity of the test substance in MBq/mg. If radioactive impurities occur at significant levels (i.e. > 5%) then the identity of radiolabelled impurities, if any, derived from the test material should also be reported. The site(s) of labelling in the molecule for radiolabelled test material should be provided. A rationale should be provided for selection of radiolabellers other than \(^{14}\)C and for site(s) of labelling in the molecule (where possible, emphasis is placed on labelling the ring position).

(iv) With regard to the specific activity of the test substance, it should be reported as MBq/mg, with a sample calculation to show how the analyst arrived at radioactivity concentrations (mg/L) from the experimental data. Sufficient information on counts should be provided so that the relevant Regulatory Authority can verify the concentration, expressed as mg active ingredient/L in the various chromatographic fractions.

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

f. **Test facility**

A description of the overall testing environment utilised for the study including, as appropriate, a record of environmental conditions experienced during the course of the study at the testing facility.

g. **Hydrolytic conditions**

(i) A rationale or statement provided by the applicant for selection of a certain hydrolytic condition when deviating from the proposals made in this guideline should be given.

(ii) The sampling procedure used or a rationale for the direct analysis of a sample should be given.

h. **Application of the pesticide**

(i) The actual concentrations used in the study including solvent used and its amount, where appropriate, should be given.

(ii) An explanation or rationale for any significant deviation in the concentration.

i. **Sample handling and storage stability**

(i) A description of the handling for samples.
A description of the conditions and length of storage of collected samples.

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

**j. Analytical methods used for the analyses of radioactive residues**

(i) The analytical methods in the nature of residue study to determine the components of the residue should be described.

(ii) Method for quantitation of total recovered radioactivity provided in narrative, tabular format, or figure.

(iii) A description of sample preparation prior to liquid scintillation analyses where appropriate.

(iv) A quantitative accountability of the total radioactivity recovered from the test vessel at the beginning and at the end of the study. Significant losses should be discussed.

(v) Details of analytical method parameters including descriptions of equipment used for determining total radioactivity in each sample. Radioassay methods using quench correction (automated or not) should describe quench correction methodology and report methods applied to decrease quench, as applicable.

(vi) Details of radioactive counting data for selected representative samples should be provided to calculate concentrations (mg/L, as active ingredient equivalents) found and limit of detection.

**k. Extraction and fractionation of radioactivity**

(i) A complete description, accompanied by a flow sheet or diagram depicting the overall extraction and fractionation strategies (schema) employed in the hydrolysis study.

(ii) A discussion of and rationale for the selection and extraction sequence for the extracting solvent (polar vs. non-polar) used.

(iii) Radiochemical extraction efficiencies calculated and reported.

(iv) The efficiency of separation and purification for all fractionation and isolation techniques employed in the study (i.e. solvent partitioning, ion exchange, or exclusion column chromatography, HPLC using gradient elution, 2-dimensional thin layer radio autography employing multiple solvent systems) should be reported for a representative sample.

(v) Data to account for or track the loss of radioactivity in each subsequent step of the fractionation and isolation procedure should be provided and attempts made by the applicants to minimise these losses should be discussed.

(vi) The amount of radioactivity in each sample fraction should be quantified and reported in terms of total radioactivity (MBq), and as mg/L (as active ingredient equivalents) of total radioactivity recovered in the original sample matrix analysed.
1. Characterisation and identification of radioactivity

(i) A tabular listing and description of all known metabolites and degradation products of the active ingredient (model compounds, including their structure and purity) used to facilitate the characterisation and/or identification of unknown sample degradation products.

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

(iii) Photographs (or radio-analytical imaging detection) of thin-layer chromatographic (TLC) plates, radioautograms, or output from other appropriate imaging systems that were critical to the identification should be provided. Samples or reproductions of HPLC/GC chromatograms including mass spectral scans, etc., should also be submitted. Regardless of the chromatographic technique used, chromatograms showing the behaviour of the analytical standards should also be included in the report.

(iv) Details of additional confirmatory analytical procedures used to separate and characterise/identify degradation products (i.e. ion exchange, or exclusion chromatography, derivatisation, etc.) and determinative methods [i.e. mass spectroscopy in electron ionisation (EI) and chemical ionisation (CI) modes] used for ultimate identification of degradation products.

(v) A description of all instrumentation, equipment, and reagents used, including operating conditions of the instrumentation utilised for the separation, characterisation, and identification of radioactive residues should be submitted.

(vi) Explanation for all lost radioactivity in each fraction. The amount reported should be expressed as mg/L (as active ingredient equivalents) of total radioactivity recovered from the particular fraction analysed.

(vii) A report of each of the major degradation products/components and, if possible, provide information on the chemical nature of discrete (minor) degradation products/components.

(viii) Any and all additional information the applicant considers appropriate and relevant to provide a complete and thorough description of the conduct of the hydrolysis study and the determination of the TRRs.

Results and Discussion

m. Test strategies

A discussion should be included describing deviations from the intended testing protocols or from strategies as a result of unusual experimental problems (e.g. difficulties in extraction, fractionation, and characterisation of residues). It should include a discussion of the impact or effects, if any, of those deviations on the results of the study.

n. Hydrolytic degradation
Discussion, preferably accompanied by a flow chart format, of the routes of degradation observed should be provided. For discussion purposes, the observed route of degradation should be compared and contrasted to known and previously reported metabolic pathways in crop metabolism studies and observed in animal metabolism studies conducted with the particular chemical. Based on the results of the characterisation and/or identification studies, the degradation pathway should be proposed, including a table with associated chemical structures and names (CAS and IUPAC as available). Any postulated (but not identified) intermediates should also be clearly indicated in the pathway.

**o. Characterisation and/or identification and distribution of TRRs**

(i) Use a tabular or graphic format. Identify all major components of TRR, including name, structure, and quantity (expressed both as mg/L active ingredient equivalents) and %TRR.

(ii) The applicant should provide as much information as possible on all significant unidentified and/or uncharacterised components of the terminal residue, and their quantities.

(iii) Statistical treatment(s). Include representative examples of any statistical tests applied to the raw data obtained during sampling/analyses in the course of the hydrolysis study. Provide the limit of quantification (LOQ) for radioactivity determination and chromatographic separation.

(iv) Any and all additional information the applicant considers appropriate and relevant to provide a complete and thorough description of the hydrolysis study including quality control measures/precautions taken to ensure validity of all aspects of the study.

**p. Conclusion**

(i) The routes or pathways, mechanisms involved and extent or degree of degradation observed should be included.

(ii) The results of validation studies conducted on radiolabelled samples, if conducted, should also be discussed.

**q. Tables**

(i) Name, structure, purity, for all reference standards, metabolites and degradation products utilised in study.

(iii) HPLC/GC retention times and TLC Rf values for active ingredient, metabolites, degradation products, related compounds and model compounds under different column, solvent (elution) conditions.

**r. Figures**

(i) Overall extraction and fractionation strategies or schema employed.

(ii) Distribution of radioactivity in various ion exchange (exclusion) or preparative HPLC/GLC fractions.
Degradation flow diagrams or charts.

Appendices

(i) Representative chromatograms, spectra, etc. (as applicable).

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

(iii) Other: Any relevant material not fitting in any of the other sections of this report should be appended.

Test report

47. The study report should contain the following information:

a. Identification of the test pesticide active ingredient (a.i.), including chemical name; common name [American National Standards Institute (ANSI), British Standards Institution (BSI), or International Standards Organization (ISO)]; company developmental/experimental name; and Chemical Abstracts Service (CAS) name and number and IUPAC chemical name.

b. A description of the radiolabelled test substance(s) and a justification for the positions of radiolabelling, the radiopurity, nature of the radiolabel, specific activity (reported as MBq/mg), source, identity of significant radiolabelled impurities, if any.

c. Name, structure, and purity of reference standards, metabolites and degradation products utilised in the study.

d. A description of the overall testing environment utilised for the study.

e. A careful and full description of the extraction and fractionation of radioactivity, including reports on the amount of radioactivity in each sample fraction, quantified in terms of total radioactivity (MBq) and as concentration (mg/L, as active ingredient equivalents) compared to the amount used.

f. A complete description of all instrumentation, equipment, and reagents used, including operating conditions of the instrumentation utilised for the separation, characterisation, and identification of radioactive residues.

g. Characterisation and/or identification of radioactive residues, to include data for all major components.

h. A description of the chromatographic behaviour [e.g., HPLC and/or GC retention times, TLC reference (Rf) values] of active ingredient, degradates, and related reference standards. Representative radiochromatograms of sample extracts and chromatograms of the analytical standards, as well as any spectral data supporting the identity of degradation products, should also be included.

i. Information of the storage stability for all major components of the total radioactive residues.
j. Quantitative information on the recovery of the radioactive residue via the extraction methods used.

k. A detailed discussion, accompanied by a degradation pathway, of the routes of degradation observed.

l. Conclusions on pathways or routes of degradation and extent of degradation observed.

LITERATURE


