**504**Adopted: 8 January 2007

# **OECD GUIDELINES FOR THE TESTING OF CHEMICALS**

## **Residues in Rotational Crops (Limited Field Studies)**

## **INTRODUCTION**

1. Rotational crops are any field or aquatic crops, which may be produced after the harvest of a pesticide treated primary crop (or in some cases replanting of crops after failure of the pesticide treated primary crop). Limited Field Studies for Residues in Rotational Crops are generally conducted if the results of the Metabolism in Rotational Crop study (see OECD Test Guideline 502 Metabolism in Rotational Crops) (1) indicate that significant accumulation of residues occur through soil uptake into food or feed commodities. Guidance on when to conduct the study and what components of the residue, if any, require analysis may be found in OECD Guidance Document on Overview of Residue Chemistry Studies (2) and the OECD Guidance Document on Definition of the Residue (3).

## **PURPOSE**

2. Studies of Residues in Rotational Crops (Limited Field Studies) are performed to determine the amount of pesticide residues which may be accumulated into rotational crops via soil uptake following realistic agricultural practices. These data may be used to establish crop rotation restrictions, i.e., the time from application to a time when rotational crops can be planted based on residue accumulation, to provide information for assessing the overall significance of the residues for dietary risk assessment and to determine whether maximum residue limits (MRLs) will be needed in rotational crops.

## STUDY CONSIDERATIONS AND TESTING PARAMETERS

## **Study design considerations**

3. The study uses a typical end-use product applied to a field plot, after which suitable rotational crops are grown to assess the amount of pesticide residue taken up into rotational crops under actual field conditions. The study design, in terms of selection of trial sites, pesticide rates and timings and formulation type, primary crop if planted, rotational crops and timings of replanting, and typical agricultural practice, should seek to address situations for which the potential uptake of pesticide soil residues in rotational crops is the highest, either due to mode of application, soil type and soil temperatures, pesticide persistence or other environmental or cultural practices.

## **Crops**

4. Residues in Rotational Crops (Limited Field Studies) are generally not required for uses of pesticides on permanent or semi-permanent crops including, but not limited to, the following commodities or crop groups: asparagus, avocado, banana, berries crop group, citrus fruit crop group, coconut, cranberry, dates, fig, ginseng, globe artichoke, grapes, guava, kiwi fruit, mango, mushrooms, olives, papaya, passion fruit, pineapple, plantain, the pome fruits crop group, rhubarb, the stone fruits crop group, and the tree nuts crop group.

# 504

## OECD/OCDE

5. If the pesticide is to be applied to paddy rice an alternative study design, such as aging the pesticide under paddy conditions prior to rotation to field crops, may be required (4).

#### **Test sites**

- 6. The limited field trials should be conducted at two diverse geographical regions, which are major areas of cultivation or production where the pesticide may be used. If the primary crop is grown in only a limited geographical region, the trials should be conducted over two different test sites within that region. One of the test sites should be a sandy loam soil. However, if the pesticide label instructions of the product limit its use to one soil type other than sandy loam, then the study should be conducted with the soil type specified on the pesticide label. In the case where a crop rotation study after paddy rice cultivation is needed, then the appropriate soil type (e.g., clay or clay loam) and test sites should be selected.
- 7. Untreated control plots should also be included for each test site. It is desirable to use sites on which the test substance has not previously been applied.

## Application of pesticide

- 8. At each of the test sites, the trials should be designed to address residues that may occur in representative rotational crops. If more in keeping with agricultural practice for the rotational crop, it may be suitable to grow a primary crop that is treated prior to harvesting and replanting the rotational crops. If a primary crop is not grown in the trials, the application of pesticide will be to bare soil prior to planting of the rotational crops. Following treatment, the pesticide should be aged under appropriate conditions in the soil for a time approximating the anticipated agricultural practice; this may need to include flooded conditions if paddy rice is the major primary crop.
- 9. The pesticide should be applied (either to primary crop or bare soil according to justification) by the method stated in the directions for use specified on the pesticide label or proposed label at the maximum label rate and the maximum number of applications. If the pesticide is applied to a primary crop, the crop should be maintained and harvested following typical agronomical practices.
- 10. The test substance should be a typical formulation. If more than one formulation type is registered, several factors need to be considered as to which type(s) should be used in order to provide a reasonable worst case. If one formulation has a significantly higher application rate than the others, it should be applied to the plots in which the rotational crops will be grown. If all formulations have similar application rates, but one has been specifically designed to have a longer half-life in the environment, e.g., a controlled release product, then that formulation should be the test substance.
- 11. For practical purposes it may be preferable to apply the maximum seasonal rate in one application rather than applying a number of applications in accordance with the pesticide label. This will be acceptable provided the expected exposure from soil residues that could affect the outcome of residues in the rotational crops are not expected to be different. In either case, the maximum seasonal application rate should be used.

## **Rotational crops tested**

12. At each of the trial sites, three representative crops applicable to the rotations and agricultural practice should be tested to determine the levels of uptake of residues. The representative crops chosen should be from each of the three following crop groupings: root and tuber vegetables; leafy vegetables; and small grains e.g., wheat, barley. An additional representative crop group may also need to be included if a crop important to the rotation is not covered by these crop groupings. For example, in the United States, soybeans are an important rotational crop and represent oilseeds/pulses plant groups. Additional trial sites

may be used if it is not possible to grow all three representative crop groups at the same site due to climatic or other agricultural reasons.

## Timing for replanting of rotational crops

- 13. The limited field study calls for use of three rotational intervals. The rotated crops should be planted after the minimum rotational interval that could be expected as part of agricultural practice, e.g., 7 to 30 days for assessing circumstances of crop failure or closely rotated crops and at 270 to 365 days for crops rotated the following year. An additional rotational interval should be conducted to reflect the anticipated agricultural use of the pesticide, reflecting a typical harvest interval (e.g., 60 to 270 days). Rotational crops must be considered, particularly in commercial vegetable growing with its close crop rotations. The applicant must provide justification if fewer than three rotational intervals are studied.
- 14. In cases where the pesticide applied (e.g., certain herbicides) results in excessive phytotoxicity to rotational crops at 7 to 30 days, an alternative timing for the first rotational interval should be studied. Information regarding planting restrictions due to phytotoxicity should be provided.

#### **Trial maintenance**

15. Trials should be maintained according to usual agricultural practice. Use of pesticide treatments that could interfere with the analysis of the components of the residue definition for the rotational crop study should be avoided. Any unusual features regarding tillage, taking of samples, etc. should be justified according to agricultural practice.

## **Sampling**

- 16. All of the plant parts defined as raw agricultural commodities (RACs), including the leaves of the root and tuber vegetables, should be analyzed. (Annex 3 of the OECD Guidance Document on Overview of Residue Chemistry Studies (2) provides a list of RACs to be analyzed for specific crops.) The RACs should be analyzed for the residues that are relevant to rotational crops, as proposed following critical analysis of the nature and levels of residues in the metabolism in rotational crop study, and considering the appropriate residue definition for rotational crops.
- 17. Recognised sampling methods for supervised field trials should be used (Refer to the FAO Manual 2002, Appendices V and VI for sampling methods and portion of commodities which must apply to the samples taken in the rotational field trial samples) (5).
- 18. Residues should be analyzed in RACs whether they are items that are consumed directly by humans or consumed as feed items by livestock animals. If it could be expected that some crops could be harvested when immature for consumption (e.g., such as young leaf spinach and salad) then both immature and mature samples should be taken in the trials.
- 19. Sampling of the soil for pesticide residue analysis is not required, but may be performed at the discretion of applicant.

## Sample analysis

20. The methods employed to analyze the rotational crops should be specific for all pesticide residues in the subject commodities and should follow acceptable criteria for analytical methods for residue trials. Fortified samples should be run concurrently with those from the rotational crops study to validate the method, but the residues measured should not be corrected for recovery. The required limits of quantitation (LOQs) for rotational crops should be comparable to those for primary crops but should

# 504

## OECD/OCDE

generally be on the order of 0.01-0.05 mg/kg or less. References should be provided to the analytical method used, if standardized and validated. Alternative methods should be submitted as separate studies.

## Storage stability

21. Residues should be analyzed within 30 days of harvesting (and should be stored frozen at less than -18 °C prior to analysis). If samples are to be stored for longer periods, then suitable freezer storage stability data should be available to support the length of storage of residue samples in order to demonstrate that no significant degradation of the residues of concern between sampling and analysis has occurred. Particular care should be taken to ensure all the analytes of interest to the rotational crop field study are included in the storage stability data. Storage stability studies should include not only total residues, but also separate analyses of all components in the residue definition, as far as possible.

## CONSIDERATIONS FOR DATA REPORTING

#### **Data**

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

## **Summary/Introduction**

- (i) The chemical name and formulation of the pesticide and the method of application to the primary, i.e., treated, crop or bare soil. Structures of the pesticide and metabolites may be included in this section.
- (ii) Maintenance of the treated plots.
- (iii) A narrative or a table with an appropriate title that provides the following information:
  - 1. Days between treatment and planting of rotational, crops.
  - 2. Age of crop in days at each sampling point, e.g., at forage, hay and grain stages.
  - 3. Total residues (mg/kg). Parent and all metabolites of concern should also be reported separately, if so determined by the method.
- (iv) If there is uptake of soil residues into rotational crops, a discussion should be provided as to the significance of the residues taken up, at what rotational intervals residues are taken up by rotational crops, i.e., in which crop fractions and at what levels; and at what intervals where no quantifiable residues can be expected to be taken up by rotational crops, if this can be determined.
- (v) Indications of any problems, such as technical difficulties or unusual weather, resulting in necessary deviations from the intended test protocol, and a description of the effects of these deviations on the results of the study.

## Materials/Methods

This section should be in narrative form in the following order and should contain all details with regard to the materials, equipment, experimental design, field plots, and procedures used in conducting the study.

The applicant is encouraged to include drawings and photographs of the plot, equipment and different phases of the study.

#### a) Test Substance

- (i) Chemical name, common name (ANSI, BSI, IS0) (if available), company developmental/experimental name or number; and Chemical Abstracts Service (CAS) name and number and IUPAC chemical name, and chemical structure. The source and purity of the compound should be specified.
- (ii) Active ingredient and type of formulation including the percent by weight of the active ingredient and, for liquid formulations, the weight of the active ingredient per unit of liquid measure.

#### b) Test Sites

- (i) Include a map of the test plots, indicating their location, topography and size, and location and size of the control plots in relation to the test plots, as well as the soil characteristics, i.e., percent sand, silt, clay, and organic matter, pH, and moisture capacity.
- (ii) Include a complete record of daily temperature and daily rainfall throughout the study and how they compare to average temperature and rainfall at the test site. Discuss whether actual temperature recordings and actual rainfall are within average historical values for the residue study period. Describe any meteorological abnormalities that occurred during the conduct of the study.

## c) Crops

- (i) Crop and pesticide use history on the plot for the three year period preceding the study.
- (ii) The date and technique of plot preparation prior to pesticide application.
- (iii) The identity of the primary, i.e., treated, crop; a description of how and when the primary crop was planted; how and when the subject pesticide was applied; the weather, i.e., temperature, rainfall, windspeed and direction, and the condition of the field at time of application; the formulation of the pesticide applied and adjuvants or other compounds added to the spray/application mixture; and the application rate and the application technique. Also, provide a similar description for each of any additional applications made of the subject pesticide. Indicate how much pesticide was applied in comparison to actual use rates and if application technique differed from label recommendations.
- (iv) Dates of planting and harvesting of primary and rotational, crops. A description of any post-treatment crop maintenance, such as use of fertilizers and other pesticides; irrigation, i.e., when applied, how much, and source; and tilling, weeding, etc. The amount of rainfall and irrigation water, accumulated from application to harvest should be reported.
- (v) Sampling times, stages of crop development at times of sampling and sampling techniques for primary, if applicable, and secondary, i.e., rotational, crop RACs.
- (vi) Identify all plant fractions analyzed in the study, such as grain, forage, hay, and straw in the case of small grains, and root and aerial (leafy) portions in the case of root crops, and provide weights of each sample taken for analysis.

## **OECD/OCDE**

#### d) Test methods

#### (i) General

- 1. The date of harvest of the treated crop. Describe what was done to the plot after harvest in preparation for planting of the rotational crops.
- 2. The identity of the rotational crops planted in the study, a description of the procedure used in planting the rotational crops, and days elapsed between planting of crops and treatment with pesticide. A description of all procedures used in the maintenance of the rotational crops as done for the treated crop, the sampling/harvest method and number of samples/replicates should be included.
- 3. Describe handling from the time of taking of the samples until analysis. Provide information on: sample preparation (e.g., chopping) prior to storage, containers, how quickly the samples are put into storage, storage temperature, length of storage (dates of collection, shipping, analysis, etc.); mode of shipping, if applicable and thawing procedures.

#### (ii) Analytical method

- 1. Describe methods fully or reference them if previously submitted, including method validation data, recovery and method sensitivity data. Preparation and handling of the sample throughout the method should be described in detail. Note that methods for metabolites may also be needed. Recovery data should be obtained concurrently with the residue analyses to validate the method and establish its sensitivity (lowest reliable quantification limit). The experimental design of these validation studies should be described including: (a) Identity of the test compounds and crop substrates, (b) Magnitudes of fortification levels, (c) Number of replicates per test compound per level.
- 2. Dates of sample fortification, extraction, and analysis of extracts should be listed. If extracts are not analyzed on the day of preparation, storage conditions should be described.
- 3. Raw data such as sample weights, final volumes of extracts, and peak heights/areas should be furnished for control, fortified (including those for storage stability data) and treated samples to support reported residue values and recoveries.
- 4. Instrumentation should be identified, including equipment and reagents used and the operating conditions of the instrumentation. If the extraction/clean-up procedure is complex, a flow diagram should be submitted.
- 5. Copies of representative chromatograms should be supplied for control, fortified, and treated samples of each crop matrix along with a few sample calculations of residue levels and percent recoveries using the raw data. Examples of calibration curves of analytical standards should also be provided.

#### **Results and Discussion**

This section should contain the scientific results of the study and the relevance of results should be discussed in relation to the proposed uses of the plant protection product. For instance,

- 1. Narrative and tables describing the steps taken in determining the pesticide residues in crop samples. All graphical presentations of the data should be accompanied by the tables of the actual values from which the graphs were constructed.
- 2. A table of structures and chemical names/designations for the parent compound and metabolites.
- 3. Levels of the residues (uncorrected for recovery) should be reported for each crop component for each rotational interval (including control (untreated) samples). The individual values should be listed for all samples (not merely averages or ranges). If the parent pesticide and its metabolites are measured separately, the residues of each should be reported. Recovery percentages (all values, not just averages or ranges) for the pesticide and/or its metabolites should be reported for all crop matrices studied.
- 4. Where samples are not analysed within 30 days, data should be presented showing that the storage did not affect the results of the study. Dates when the samples were collected, frozen, thawed, and analyzed should be provided. Storage duration and temperature of these samples should be specified. Storage stability data showing the behaviour of residues as a function of time in the relevant crop components can be referenced if available from other studies.
- 5. Discussion should be presented as to whether the data indicate consistent results with the metabolism in rotational crops study.
- 6. Deviations from the intended test protocol and the effects on the results should be described.

#### Conclusion

A conclusion must be reached as to whether quantifiable residues are expected following use of the pesticide at maximum seasonal application rates and timings. If residues above limits of quantification are found in the rotational crop matrices, the results can be summarized, preferably in a table, showing either the ranges or maximum residues for each crop sample. A discussion should be provided as to the significance of the residues taken up, at what rotational intervals residues are taken up by rotational crops, i.e., in which crop fractions and at what levels; and at what interval no quantifiable residues can be expected to be taken up by rotational crops.

## Study report

- 23. The study report should contain the following information:
  - 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.
  - Justification for the selection of the formulation(s) used in the study.
  - Rationale for the selection of the test sites, primary crop (if used), and crops that were rotated.
  - Description and soil characteristics (i.e., % sand, % silt, % clay, % organic matter, pH, cation exchange capacity, and moisture capacity) for each test site.

# 504

## OECD/OCDE

- Temperature monitoring data and a description of the general climatic conditions at each test site for the duration of the study.
- Include a map of the test plots, indicating their location, topography and size, and location and size of the control plots in relation to the test plots.
- Application rate, method of application to the soil or primary crop, number and timing of applications.
- The rotational intervals and a rationale for their selection.
- A description of all the procedures used in planting, maintenance, and harvesting of the primary crop (if applicable), and the rotational crops, including irrigation, application of fertilizers and other maintenance chemicals.
- The sampling times (age of crop in days) for rotational crop RACs; stages of crop development at each sampling point, e.g., at forage, hay and grain stages and number of samples/replicates.
- A rationale for the analytes determined in the study in consideration with the results of the metabolism in rotational crop study.
- Full details pertaining to the analytical methods, including instrumentation, equipment and reagents used and the operating conditions of the instrumentation.
- A description of the preparation and handling of the samples throughout the method. Flow diagrams of extraction/clean-up procedures should be provided for complex methods.
- Analytical data for residues of concern in each crop matrix. Raw data such as sample weights, final volumes of extracts, and peak heights/areas should be furnished for control, fortified (including those for storage stability data) and treated samples to support reported residue values and recoveries.
- Analytical responses of standards (calibration curves).
- Method validation data, recovery and method sensitivity data.
- Copies of representative chromatograms should be supplied for control, fortified, and treated samples of each crop matrix.
- Dates of sample fortification, extraction, and analysis of extracts, if extracts are not analyzed on the day of preparation, include extract storage conditions.
- Freezer storage stability data (if required).
- Summary of residue data (all analytes) in rotational crops at the various plantback intervals.
- Discussion of the significance of the residues taken up, at what plant back intervals residues are taken up by rotational crops, i.e., in which crop fractions and at what levels; and at what interval no quantifiable residues can be expected to be taken up by rotational crops.

Conclusion as to whether quantifiable residues are expected following use of the pesticide at
maximum seasonal application rates and timing. If residues above limits of quantification are
found in the rotated crop matrices, the results may be summarized, preferably in a table, showing
either the ranges or maximum residues for each crop sample.

## **LITERATURE**

- (1) OECD Guidelines for the Testing of Chemicals. OECD Test Guideline 502: Metabolism in Rotational Crops.
- (2) OECD Guidance Document on Overview of Residue Chemistry Studies (2006)
- (3) OECD Guidance Document on the Definition of Residue (2006)
- (4) Japan Ministry of Agriculture, Forestry, and Fishing (MAFF) (2000), Data Requirements for Supporting Registration of Pesticides, 3-2-2, Studies of residues in succeeding crops. Notification No. 12-Nouan-8147, 24 November, 2000.
- (5) Food and Agricultural Organization of the United Nations (FAO) (2002). Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed. Rome.
- (6) U.S. Environmental Protection Agency (1996). OPPTS Harmonized Test Guideline 860.1850. Confined Accumulation in Rotational Crops. EPA Report No. 712-C-96-188, August 1996.
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- (10) European Commission. (1997). Appendix C Testing of plant protection products in rotational crops. Document 7524/VI/95 rev. 2, 22/7/97, Directorate General for Agriculture VI B II-1. <a href="http://ec.europa.eu/food/plant/protection/resources/publications\_en.htm">http://ec.europa.eu/food/plant/protection/resources/publications\_en.htm</a>
- (11) Food and Agricultural Organization of the United Nations (FAO). (1986). Guidelines on Pesticide Residue Trials to Provide Data for the Registration of Pesticides and the Establishment of Maximum Residue Limits, Section 2.1 Radiolabelled Studies (Metabolism Studies), Rome.