

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

SECTION 5:

OTHER TEST GUIDELINES

This section encompasses all Test Guidelines which do not fall within the four sections: Section 1: Physical-Chemical Properties (series 100); Section 2: Effects on Biotic Systems (series 200); Section 3: Degradation and Accumulation (series 300); and Section 4: Health Effects (series 400).

**INTRODUCTION TO OECD TEST GUIDELINES ON PESTICIDE RESIDUES CHEMISTRY
SECTION 5 – PART A****Introduction**

1. The objective of OECD Test Guidelines for the pesticide residue chemistry is to assess pesticide exposure by identifying these residues in food or animal feedstuffs for purposes of dietary risk assessment and setting Maximum Residue Levels (MRLs). They have been developed and are based on test methods in use for many years in OECD countries and by the Food and Agriculture Organisation (FAO).

2. Because of the unique nature of each study, the pesticide expected use, and the particular methods needed to elucidate the metabolic pathway for each chemical, the description of the test method cannot be as prescriptive as usually required for other OECD Test Guidelines. Pesticide residue studies are complex; guidelines cannot specify all parameters in advance, but each study must be designed individually. Given these characteristics, the guidelines in Part A of Section 5 include elements that differ from those in the other sections (1-4) of the OECD Guidelines for the Testing of Chemicals.

3. Section 5 – Part A comprises nine Test Guidelines:

TG 501: Metabolism in crops

TG 502: Metabolism in rotational crops

TG 503: Metabolism in livestock

TG 504: Residues in rotational crops (limited field studies)

TG 505: Residues in livestock

TG 506: Stability of pesticide residues in stored commodities

TG 507: Nature of the pesticide residue in processed commodities – High temperature hydrolysis

TG 508: Magnitude of the pesticide residues in processed commodities

TG 509: Crop field trial

Metabolism in crops (TG 501)

4. The purpose of conducting metabolism in crops studies is to elucidate the degradation pathway of the active ingredient, *i.e.* identify the metabolites/degradation products and determine their relative amounts in extractable and non-extractable material. The desired goal of a metabolism in crops study is the identification and characterisation of at least 90% of the total radioactive residue (TRR) in each raw agricultural commodity (RAC) of the treated crop.

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5. Plant metabolism testing is performed for three crops drawn from five crop groups/categories (root vegetables, leafy crops, fruits, pulses and oilseeds, and cereals). If registration is sought for one crop group only, metabolism studies in one crop from that crop group would be sufficient as long as the crop is truly representative of the crop category and the metabolic pathway is elucidated. Where intended use is unique, a separate metabolism study is requested; paddy rice is an example of such a special case. Since the primary purpose of a metabolism in crops study is to identify the chemical components of the residue, the maximum application rate – the proposed Good Agricultural Practices (GAP) application rate – or possibly exaggerated rates should be utilised to provide sufficient material to elucidate metabolic pathways. Dosing takes into consideration: the number, type and timing of pesticide applications (including label directions for timing and minimum intervals between applications), application rate, pre-harvest interval (PHI), and potential for phytotoxicity. The guideline also describes special considerations for metabolism of genetically modified crops.

Metabolism in rotational crops (TG 502)

6. The purpose of conducting metabolism in rotational crop studies is to determine the nature and amount of pesticide residue resulting from the application to bare soil or the primary crop which may accumulate in the rotational crops from soil uptake. The study uses the radiolabelled pesticide active ingredient applied to the soil in a confined test system such as a pot so that the uptake of the pesticide by the rotational crop can be characterised. Usually three different rotational intervals are used being representative of replanting after crop failure, a typical rotation after harvest and a typical rotation in the following year. Studies are not normally required for permanent and semi-permanent crops.

Metabolism in livestock (TG 503)

7. Metabolism in livestock studies are used to determine the qualitative and quantitative metabolism and/or degradation of the active ingredient resulting from pesticide use in/on feed stuffs, direct application to livestock, or premise treatment. Livestock metabolism studies are generally carried out in ruminants (cows or goats) and poultry (chickens). These studies are conducted using radiolabelled test compound. Pig metabolism studies may be necessary if the rat metabolism is significantly different from the ruminant or poultry metabolism.

8. Metabolism in livestock studies provide results which may be considered in determining if livestock feeding studies are required. Dosing is at 1X, where 1X is the reasonable worst case worldwide dietary burden, or at 10 ppm in the diet as received, whichever is greater. Dosing recommendations are for five consecutive daily doses for ruminants and seven for poultry, possibly including trigger values for identification/ characterization of the components of the total radioactive residue (TRR); trigger values are based on total ¹⁴C residues found in extracts and the extracted matrices and provide a regulatory minimum for characterisation/ identification of the residue.

Residues in rotational crops, limited field studies (TG 504)

9. If the results of the metabolism in rotational crops study (TG 502) indicate the potential for uptake of the pesticide or its metabolites at a desired re-cropping interval, then a study on residues in limited field rotational crops is conducted to determine if and at what levels residues occur under field conditions. Limited field rotational studies are conducted with non-radiolabelled pesticide applied to bare soil or the primary crop under the proposed agronomic use practices at the highest seasonal rate in at least two appropriate agricultural regions.

10. Testing is for the purpose of determining uptake of pesticide by rotational crops, but not for environmental fate in the soil. Normally samples are collected at three different plant-back intervals:

30 days after last application, 365 days after last application, and at an intermediate time. Timing would take into consideration agricultural practice and the potential for uptake of soil degradation products. The study results can be used to set plant-back intervals, to provide information used in dietary risk assessment, and to determine whether MRLs will be needed in rotational crops.

Residues in livestock (TG 505)

11. The purpose of the residues in livestock study is to determine levels of pesticide residues transferred to meat, fat, liver, kidney, milk and eggs, following consumption of treated feedstuff (including fodder crops), or direct animal or premise treatment. The study results are used by regulatory authorities to establish the MRLs in food of animal origin and to assess the dietary exposure of consumers following consumption of food commodities of animal origin. Residues in livestock studies are conducted as feeding studies; they are normally carried out in ruminants (dairy cattle) and poultry (chicken). Results of studies in cattle may be extrapolated to other domestic animals (ruminants, horses, pigs, rabbits and others) and results in studies of laying hens may be used for other poultry species. Where metabolic pathways are qualitatively different in the pig as compared with those in ruminants, a pig feeding study is conducted, unless the expected intake by pigs is not significant. Livestock feed levels are at 1X, 3X (or 5X), and 10X where 1X is a reasonable worst case dietary burden. If the registrant anticipates the need for an additional feeding level for risk assessment purposes, another dose level below 1X may be included.

12. In order to conduct residues in livestock studies, detailed knowledge is needed regarding the type of feedstuffs available for livestock, quantities being fed, and which feedstuff components might be used as alternatives and are interchangeable. Each country would use its own feed tables and feed commodities for national/ regional risk assessments. The use of internationally harmonised feed tables will ensure that residues in livestock studies are conducted in such a way as to address the various feeding practices of all OECD countries. The OECD Feedstuffs Derived from Field Crops Table can be found in the Guidance Document entitled Overview of Residue Chemistry Studies (EHS publication, series on Testing and Assessment, No. 64; series on Pesticides, No. 32, 2009). This OECD Table will need to be regularly updated as warranted by the availability of more up-to-date and accurate information and evolving animal production practices.

13. In case of direct animal treatment, the study design should reflect as closely as possible the conditions under which the pesticide is used commercially. All factors that might contribute to the variability of residue levels in animal commodities should be considered and taken into account in the planning and conduct of trials.

Stability of pesticide residues in stored commodities (TG 506)

14. When compiling magnitude of residue (MOR) data from crops, crop products and products of animal origin it is essential to ensure that the residues of all components of the residue definitions (for both risk assessment and enforcement) of a MOR sample remain accurately quantifiable from the time of sampling/ harvest to analysis. If MOR samples are not analysed soon after collection, chemical changes to the components of the residue may take place which may lead to inaccurate results. In cases where it is not possible to analyse MOR samples immediately after they have been taken, the MOR sample should be stored under suitable sub zero °C conditions until analysis. In these cases the stability of residues during storage should be investigated. In other words, applicants need to demonstrate that pesticide residues are stable during frozen storage of the MOR sample to be analysed or show the degree to which residues decline in that period of time.

15. The aim of these studies is to demonstrate the time period during which residues remain stable in representative crops, in processed fractions derived from these crops, and in products of animal origin.

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consequently it is necessary for applicants to ensure that MOR samples are analysed within the period for which stability has been demonstrated in representative commodities. The MOR studies include, but are not limited to, the following: Crop field trials (TG 509); Residues in limited field rotational crops (TG 504); Livestock feeding studies (TG 505); and Processing studies (TG 508).

Nature of the pesticide residue in processed commodities (TG 507)

16. Most food or raw agricultural commodities (RACs) are processed before the consumption. 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.

17. 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. It should 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 Test Guideline provides a way in which such investigations can be conducted.

18. Studies on the nature of the residue in processed commodities are conducted as simulation studies to predict the degradation pathway of the active ingredient under different hydrolysis conditions which represent typical/ generic processing procedures (in most cases hydrolysis will be the predominant effect during processing). These studies 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.

19. When residues are present in raw agricultural commodities that are generally consumed only after processing in either industrial or domestic settings, 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 can be conducted.

20. While recognising that nature of the residue studies in processed commodities are simulation studies, these investigations fulfil several major purposes: (i) provide an estimate of the relative composition of the total residues in the processed commodities; (ii) 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.* residue definition(s) for both risk assessment and enforcement); and (iii) elucidate the degradation pathway of the active ingredient in processed commodities under hydrolytic conditions.

Magnitude of the pesticide residues in processed commodities (TG 508)

21. A wide range of raw agricultural commodities (RAC) are processed before they are consumed. The procedures that are used to produce processed commodities are diverse and varied. Further guidance is available from the Guidance Document on Magnitude of Pesticide Residues in Processed Commodities, (EHS publication, series on Testing and Assessment No. 96, 2008). Processing studies are normally used to determine pesticide residue levels in processed commodities following pesticide application according to label directions, likely leading to measurable residues. Such treatments include pre- or post-harvest pesticide use and direct animal treatment or veterinary use. The processing procedures addressed in this

guideline do not include simple peeling or washing practices or fodder production, as these practices are generally addressed in the supervised residue field trials guideline (TG 509).

22. Information about dilution and concentration of residues and the estimation of processing factors (the ratio of residue levels in the processed commodities to those in the raw agricultural commodity) is used to: (i) conduct refined dietary exposure assessments; (ii) provide residues levels in processed commodities that may be used as animal feedstuffs and thus to allow a more realistic calculation of the dietary burden of livestock; (iii) establish processing factors; and (iv) where a MRL is not established for the processed commodity, monitor compliance with the RAC MRL.

23. This guideline describes how to plan and carry out processing studies. It applies to RACs of plant origin. It also applies to RACs of animal origin. Whether studies on the magnitude of residues in processed commodities are required or not, depends upon the importance of a processed product in the human and/or animal diet; the possibility of residue levels in processed foods/ feeds exceeding the level in a RAC; the level of residue in the plant or plant product to be processed; the physical-chemical properties of the active ingredient or relevant metabolites; and the possibility that degradation products of toxicological significance may be found in animal or plant products after processing.

Crop field trial (TG 509)

24. Crop field trials (also referred to as supervised field trials) studies are conducted to determine the magnitude of the pesticide residue in or on raw agricultural commodities, including feed items, and should be designed to reflect pesticide use patterns that lead to the highest possible residues. The objectives of crop field trials are to: (i) quantify the expected range of residue(s) in crop commodities following treatment according to the proposed or established good agricultural practice (GAP); (ii) determine, when appropriate, the rate of decline of the pesticide residue(s) on commodities of interest; and (iii) determine residue values such as the Supervised Trial Median Residue (STMR) and Highest Residue (HR) for conducting dietary risk assessment; and iv) derive MRLs.

25. Crop field trials may also be useful for selecting residue definitions by providing information on the relative and absolute amounts of parent pesticide and metabolites.

26. This crop field trial Test Guideline provides a harmonized approach to conducting and reporting crop field trials in OECD countries. This guideline, along with the Guidance Document on Crop Field Trials (EHS publication, series on Testing and Assessment, No. 164; series on Pesticides, No. 66, 2011), provides guidance for generation of complete field trial data sets for pesticide uses on crops in comprehensive submissions to all OECD countries. This document also provides guidance on MRL estimations, including the use of the OECD calculator (see below).

MRL Calculator

27. With the aim of harmonizing the calculations of MRLs across OECD countries a statistical calculator for determination of MRLs from valid field residue data has been developed; the calculation procedure is based on "mean + 4sd" methodology. An OECD MRL Calculator User Guide was published (EHS publication, series on Pesticides, No. 56, 2011).

Validation of pesticide residue analytical methods

28. Analytical methods are used to generate the data for estimating dietary exposure assessments, to establish MRLs, and to determine processing factors. Analytical methods are also used in enforcement of any statutory MRLs that may be established. Methods apply to all pesticides used on edible crops/

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livestock and subsequent produce and processed food products, and for products (*e.g.* meat, milk, eggs) from animals that may consume treated crops. Additionally, analytical methods are needed for conducting storage stability studies.

29. The main purpose of the Pesticide Residue Analytical Methods Guidance Document – EHS publication, series on Testing and Assessment, No. 72; series on Pesticides, No. 39, 2007 – is to provide guidance on the residue analytical methods; it also addresses the quality criteria and method validation criteria including independent laboratory validation requirements.

30. The objective of analytical method validation is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. The residue analytical methods Guidance Document describes the procedures to be carried out to validate the analytical methods included as part of an application for approval of an active substance and registration. To be fit for the intended purpose, the method should meet standards for certain validation parameters. Typical validation characteristics for residue analytical methods that should be considered are: recovery, selectivity (specificity), calibration, precision (repeatability, reproducibility), limit of detection (LOD), and limit of quantitation (LOQ). The guidance also addresses the following topics: (i) extraction efficiency and radiochemical procedures; (ii) techniques for confirmatory analysis; (iii) derivatisation methods for detection of an analyte; (iv) common moiety/ 'functional group' and non-specific methods; and (v) method validation criteria and the corresponding information to be reported.

31. The Pesticide Residue Analytical Methods Guidance Document is by its scope analogous to the Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (EHS publication, series on Testing and Assessment, No. 34, 2005) in that it describes qualitative and quantitative validation criteria. Guidance Document 34 is mainly focused on validation and acceptance of test methods in biological systems for hazard assessment. Nonetheless, the principles of validation it describes can be broadly interpreted for tests which use analytical and environmental chemistry to determine dietary exposure to chemicals.

Animal welfare

32. There are several provisions for animal welfare in the pesticide residue chemistry Test Guidelines: (i) controls have been eliminated in the metabolism in livestock study; (ii) if the metabolic profile observed in the ruminant is substantially similar to that in the rat, metabolism studies in swine are not needed; (iii) the residues in livestock study may be waived, under certain circumstances, as long as the metabolism in livestock study is appropriately adjusted; and (iv) for residues in livestock, the results of cattle feeding studies may be extrapolated to other domestic animals (ruminants, horses, pigs, rabbits and others) and the results of laying hen feeding studies to other types of poultry (turkey, goose, duck and others).

Regulatory applicability and use

33. Test Guidelines for residue chemistry have been harmonised at OECD based on test methods in use for many years in OECD countries and by FAO. The harmonised Test Guidelines provide uniform approaches to achieving objectives already established in existing guidelines. These residue chemistry methods have been successfully used for many years to meet specific regulatory needs in determining exposure to pesticides from food or animal feed for purposes of risk assessment and MRL setting. Likewise, they have been used by FAO (Codex) in setting international trade standards. The present OECD residue Test Guidelines have benefited from the long history of use by scientists and regulators of precedent equivalent methods, as well as the use of standards, safeguards and calibration methods inherent in the discipline of analytical chemistry.

34. Each metabolism study calls for a complex approach for characterization of metabolic pathways in plants or livestock and for deriving the residue definition for enforcement purposes. Metabolites are identified and confirmed as a basis for the residues in limited field rotational crops, crop field trials and the residues in livestock studies, which are used to determine levels of pesticides in food or feed. Because of the unique nature of each study and the particular methods needed to elucidate the metabolic pathway for each chemical, guidance cannot be prescriptive. However, each metabolism Test Guideline (Metabolism in Crops, Metabolism in Livestock and Metabolism in Rotational Crops) calls for use of radiolabelled material, with at least 90% recovery to ensure that all significant metabolites are recovered and characterized/ identified. Standard analytical chemistry techniques are used, which call for validation of the identity and quantity of each metabolite detected in the studies. Although each metabolism study for each chemical is unique in character, it is internally validated within the discipline of analytical chemistry. Methods used for enforcement of MRLs are independently confirmed or validated.

35. The Residues in Limited Field Rotational Crops study is used to determine pesticide uptake under field conditions (two small field plots). Study design calls for use of worst case usage assumptions, such as pesticide application at maximum seasonal rate to bare ground prior to planting the rotated crop. Standardized analytical procedures are used to detect the parent pesticides and metabolites identified and confirmed in the Metabolism in Rotational Crops study. The test design has served its purpose well for two decades. Limited field studies are only a step in determining the need for full field studies which would be used to quantitate levels of pesticide residues in rotated crops.

36. The Residues in Livestock guideline calls for use of reasonable worst case diets based on typical international marketing of animal feeds and regional livestock production practices. Ancillary requirements for the analytical phase of a livestock feeding study include method validation for quantitation of residues, as well as demonstrated stability of residues in meat, milk and eggs under controlled conditions of storage. Such requirements provide appropriate analytical rigour to the data. Elements of the Residue in Livestock study design such as study duration, selection of test species, selection of dose levels, sampling methods have been standardized with experience over many years to increase the likelihood that the results of a well conducted study provide appropriate data for the regulator.

37. The Crop Field Trials guideline calls for application of the pesticide under reasonable worst case, outdoor or protected conditions. Although residues in supervised residues trials are known to be variable, various elements of the study design such as number of trials, locations, duration and sampling methods have been standardized with experience over many years to increase the likelihood that the results of a well conducted study provide appropriate data for the regulator.