Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

13-Aug-2007

English - Or. English

ENV/JM/MONO(2007)17
Unclassified

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

SERIES ON TESTING AND ASSESSMENT
Number 72

SERIES ON PESTICIDES
Number 39

GUIDANCE DOCUMENT ON PESTICIDE RESIDUE ANALYTICAL METHODS

JT03230940

English - Or. English

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. 72

and

Series on Pesticides

No. 39

Guidance Document on Pesticide Residue Analytical Methods

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris, 2007

Also published in the Series on Testing and Assessment:

No. 1, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals (1993; reformatted 1995, revised 2006)*

No. 2, *Detailed Review Paper on Biodegradability Testing (1995)*

No. 3, *Guidance Document for Aquatic Effects Assessment (1995)*

No. 4, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment (1995)*

No. 5, *Report of the SETAC/OECD Workshop on Avian Toxicity Testing (1996)*

No. 6, *Report of the Final Ring-test of the Daphnia magna Reproduction Test (1997)*

No. 7, *Guidance Document on Direct Phototransformation of Chemicals in Water (1997)*

No. 8, *Report of the OECD Workshop on Sharing Information about New Industrial Chemicals Assessment (1997)*

No. 9, *Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application (1997)*

No. 10, *Report of the OECD Workshop on Statistical Analysis of Aquatic Toxicity Data (1998)*

No. 11, *Detailed Review Paper on Aquatic Testing Methods for Pesticides and industrial Chemicals (1998)*

No. 12, *Detailed Review Document on Classification Systems for Germ Cell Mutagenicity in OECD Member Countries (1998)*

No. 13, *Detailed Review Document on Classification Systems for Sensitising Substances in OECD Member Countries 1998)*

No. 14, *Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries (1998)*

No. 15, *Detailed Review Document on Classification Systems for Reproductive Toxicity in OECD Member Countries (1998)*

No. 16, *Detailed Review Document on Classification Systems for Skin Irritation/Corrosion in OECD Member Countries (1998)*

No. 17, *Environmental Exposure Assessment Strategies for Existing Industrial Chemicals in OECD Member Countries (1999)*

No. 18, *Report of the OECD Workshop on Improving the Use of Monitoring Data in the Exposure Assessment of Industrial Chemicals (2000)*

No. 19, *Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation (1999)*

No. 20, *Revised Draft Guidance Document for Neurotoxicity Testing (2004)*

No. 21, *Detailed Review Paper: Appraisal of Test Methods for Sex Hormone Disrupting Chemicals (2000)*

No. 22, *Guidance Document for the Performance of Out-door Monolith Lysimeter Studies (2000)*

No. 23, *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (2000)*

No. 24, *Guidance Document on Acute Oral Toxicity Testing (2001)*

No. 25, *Detailed Review Document on Hazard Classification Systems for Specifics Target Organ Systemic Toxicity Repeated Exposure in OECD Member Countries (2001)*

No. 26, *Revised Analysis of Responses Received from Member Countries to the Questionnaire on Regulatory Acute Toxicity Data Needs (2001)*

No 27, *Guidance Document on the Use of the Harmonised System for the Classification of Chemicals Which are Hazardous for the Aquatic Environment (2001)*

No 28, *Guidance Document for the Conduct of Skin Absorption Studies (2004)*

No 29, *Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media (2001)*

No 30, *Detailed Review Document on Hazard Classification Systems for Mixtures (2001)*

No 31, *Detailed Review Paper on Non-Genotoxic Carcinogens Detection: The Performance of In-Vitro Cell Transformation Assays (2007)*

No. 32, *Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies (2000)*

No. 33, *Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures (2001)*

No. 34, *Guidance Document on the Development, Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment (2005)*

No. 35, *Guidance notes for analysis and evaluation of chronic toxicity and carcinogenicity studies (2002)*

No. 36, *Report of the OECD/UNEP Workshop on the use of Multimedia Models for estimating overall Environmental Persistence and long range Transport in the context of PBTS/POPS Assessment (2002)*

No. 37, *Detailed Review Document on Classification Systems for Substances Which Pose an Aspiration Hazard (2002)*

No. 38, *Detailed Background Review of the Uterotrophic Assay Summary of the Available Literature in Support of the Project of the OECD Task Force on Endocrine Disrupters Testing and Assessment (EDTA) to Standardise and Validate the Uterotrophic Assay (2003)*

No. 39, *Guidance Document on Acute Inhalation Toxicity Testing (in preparation)*

No. 40, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures Which Cause Respiratory Tract Irritation and Corrosion (2003)*

No. 41, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures which in Contact with Water Release Toxic Gases (2003)*

No. 42, *Guidance Document on Reporting Summary Information on Environmental, Occupational and Consumer Exposure (2003)*

No. 43, *Draft Guidance Document on Reproductive Toxicity Testing and Assessment (in preparation)*

No. 44, *Description of Selected Key Generic Terms Used in Chemical Hazard/Risk Assessment (2003)*

No. 45, *Guidance Document on the Use of Multimedia Models for Estimating Overall Environmental Persistence and Long-range Transport (2004)*

No. 46, *Detailed Review Paper on Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances (2004)*

No. 47, *Detailed Review Paper on Fish Screening Assays for the Detection of Endocrine Active Substances (2004)*

No. 48, *New Chemical Assessment Comparisons and Implications for Work sharing (2004)*

No. 49, *Report from the Expert Group on (Quantitative) Structure-Activity Relationships [(Q)SARs] on the Principles for the Validation of (Q)SARs (2004)*

No. 50, *Report of the OECD/IPCS Workshop on Toxicogenomics (2005)*

No. 51, *Approaches to Exposure Assessment in OECD Member Countries: Report from the Policy Dialogue on Exposure Assessment in June 2005 (2006)*

No. 52, *Comparison of emission estimation methods used in Pollutant Release and Transfer Registers (PRTRs) and Emission Scenario Documents (ESDs): Case study of pulp and paper and textile sectors (2006)*

No. 53, *Guidance Document on Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms) (2006)*

No. 54, *Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application (2006)*

No. 55, *Detailed Review Paper on Aquatic Arthropods in Life Cycle Toxicity Tests with an Emphasis on Developmental, Reproductive and Endocrine Disruptive Effects (2006)*

No. 56, *Guidance Document on the Breakdown of Organic Matter in Litter Bags (2006)*

No. 57, *Detailed Review Paper on Thyroid Hormone Disruption Assays (2006)*

No. 58, *Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models in the Assessment of New and Existing Chemicals (2006)*

No. 59, *Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-Day Oral Toxicity Study in Laboratory Rats (2006)*

No. 60, *Report of the Initial Work towards the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1A) (2006)*

No. 61, *Report of the Validation of the 21-Day Fish Screening Assay fort he Detection of Endocrine Active Substances (Phase 1B) (2006)*

No. 62, *Final OECD Report of the Initial Work towards the Validation of the Rat Hershberger Assay: Phase-1, Androgenic Response to Testosterone Propionate, and Anti-Androgenic Effects of Flutamide (2006)*

No. 63, *Guidance Document on the Definition of Residue (2006)*

No. 64, *Guidance Document on Overview of Residue Chemistry Studies (2006)*

No. 65, *OECD Report of the Initial Work towards the Validation of the Rodent Uterotrophic Assay – Phase One (2006)*

No. 66, *OECD Report of the Validation of the Rodent Uterotrophic Bioassay - Phase 2: Testing of Potent and Weak Oestrogen Agonists by Multiple Laboratories (2006)*

No. 67, *Report on the Validation of the Uterotrophic Bioassay: Additional Data Supporting the Test Guideline on the Uterotrophic Bioassay in Rodents (2007)*

No. 68, *Summary Report of the Uterotrophic Bioassay Peer Review Panel, including Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2006)*

No 69, *Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models (2007)*

No. 70, *Report on Preparation of GHS Implementation by the OECD Countries (2007)*

No. 71, *Guidance Document on the Uterotrophic Bioassay – Procedure to Test for Antioestrogenicity (2007)*

Also published in the Series on Pesticides:

No.1 *Data Requirements for Pesticide Registration in OECD Member Countries: Survey Results* (1993)

No.2 *Final Report on the OECD Pilot Project to Compare Pesticide Data Reviews* (1995)

No.3 *Data Requirements for Biological Pesticides* (1996)

No.4 *Activities to Reduce Pesticide Risks in OECD and Selected FAO Countries. Part I: Summary Report* (1996)

No.5 *Activities to Reduce Pesticide Risks in OECD and Selected FAO Countries. Part II: Survey Responses* (1996)

No.6 *OECD Governments' Approaches to the Protection of Proprietary Rights and Confidential Business Information in Pesticide Registration* (1998)

No.7 *OECD Survey on the Collection and Use of Agricultural Pesticide Sales Data: Survey Results* (1999)

No.8 *Report of the OECD/FAO Workshop on Integrated Pest Management and Pesticide Risk Reduction* (1999)

No.9 *Report of the Survey of OECD Member Countries' Approaches to the Regulation of Biocides* (1999)

No.10 *Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies* (2000)

No.11 *Survey of Best Practices in the Regulation of Pesticides in Twelve OECD Countries* (2001*)*

No.12 *Guidance for Registration Requirements for Pheromones and Other Semiochemicals Used for Arthropod Pest Control* (2001)

No.13 *Report of the OECD Workshop on Sharing the Work of Agricultural Pesticide Reviews*

No.14 *Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies.*

No. 15 *Persistent, Bioaccumulative and Toxic Pesticides in OECD Member Countries, (2002)*

No. 16 *OECD Guidance for Industry Data Submissions for Pheromones and Other Semiochemicals and their Active Substances (Dossier Guidance for Pheremones and Other Semiochemicals) (2003)*

No. 17 *OECD Guidance for Country Data Review Reports for Pheremones and Other Semiochemicals and their Active Substances (Monograph Guidance for Pheremones and other Semiochemicals), (2003)*

No. 18 *Guidance for Registration Requirements for Microbial Pesticides (2003)*

No. 19 *Registration and Work sharing, Report of the OECD/FAO Zoning Project (2003)*

No. 20 *OECD Workshop on Electronic Tools for data submission, evaluation and exchange for the Regulation of new and existing industrial chemicals, agricultural pesticides and biocides (2003)*

No. 21 *Guidance for Regulation of Invertebrates as Biological Control Agents (IBCAs) (2004)*

No. 22 *OECD Guidance for Country Data Review Reports on Microbial Pest Control Products and their Microbial Pest Control Agents (Monograph Guidance for Microbials) (2004)*

No. 23 *OECD Guidance for Industry Data Submissions for Microbial Pest Control Product and their Microbial Pest Control Agents (Dossier Guidance for Microbials) (2004)*

No. 24 *Report of the OECD Pesticide Risk Reduction Steering Group Seminar on Compliance (2004)*

No. 25 *The Assessment of Persistency and Bioaccumulation in the Pesticide Registration Frameworks within the OECD Region*

No. 26 *Report of the OECD Pesticide Risk Reduction Group Seminar on Minor Uses and Pesticide Risk Reduction*

No. 27 *Summary Report of the OECD Project on Pesticide Terrestrial Risk Indicators (TERI) (2005)*

No. 28 *Report of the OECD Pesticide Risk Reduction Steering Group: Seminar on Pesticide Risk Reduction through Good Container Management (2005)*

No. 29 *Report of the OECD Pesticide Risk Reduction Steering Group Seminar on Risk Reduction through Good Pesticide Labelling (2006)*

No. 30 *Report of the OECD Pesticide Risk Reduction Steering Group: The Second Risk Reduction Survey (2006)*

No. 31 *Guidance Document on the Definition of the Residue (2006)*

No. 32 *Guidance Document on Overview of Residue Chemistry Studies (2006)*

No. 33 *Overview of Country and Regional Review Procedures for Agricultural Pesticides and Relevant Documents (2006)*

No. 34 *Frequently Asked Questions about Work Sharing on Pesticide Registration Reviews (2007)*

No. 35 *Report of the OECD Pesticide Risk Reduction Steering Group Seminar "Pesticide Risk Reduction through Better Application Technology" (2007)*

No. 36 *Analysis and Assessment of Current Protocols to Develop Harmonised Test Methods and Relevant Performance Standards for the Efficacy Testing of Treated Articles/Treated Materials (2007)*

No. 37 *Report on the OECD Pesticide Risk Reduction Steering Group Workshop "Pesticide User Compliance (2007)'*

No. 38 *Survey of the Pesticide Risk reduction Steering Group on Minor Uses of Pesticides (2007)*

Published separately:

OECD Guidance for Country Data Review Reports on Plant Protection Products and their Active Substances-Monograph Guidance (1998, revised 2001, 2005)

OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substances-Dossier Guidance (1998, revised 2001, 2005)

Report of the Pesticide Aquatic Risk Indicators Expert Group (2000)

ABOUT THE OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 30 industrialised countries in North America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides and Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and the Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

FOREWORD

This document provides guidance on the residue analytical methods. Analytical methods are used to generate the data for estimating dietary exposure assessments, to establish Maximum Residue Levels (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/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.

In 2003, the OECD initiated work to develop harmonised Test Guidelines and Guidance Documents on pesticide residue chemistry. Harmonised guidelines are essential to further work sharing goals of the Working Group on Pesticides for pesticide registration and re-registration. The harmonisation is based on guidelines currently used in Australia, Canada, Japan, the United States, the European Union and the Food and Agriculture Organisation (FAO) to provide for determination of pesticide exposure in food or animal feedstuffs. Data derived from such guidelines will not only be used by industry to fulfil pesticide registration requirements in countries/regions, but could also support FAO's development of recommendations on MRLs.

The Test Guidelines and Guidance Documents were drafted by an OECD Expert Group on Pesticide Residue Chemistry (RCEG), chaired by the United States and composed of experts from Australia, Canada, Germany, Italy, Japan, the Netherlands, New Zealand, the United Kingdom, the United States, the European Commission, the European Food Safety Authority, FAO and CropLife International/ BIAC. A small Steering Committee organised the work and identified issues for the Expert Group; it is composed of roughly one Expert Group member per different region (North America, Europe, Asia and Oceania) and organisation (EC, FAO and OECD). The work was carried out by drafting groups drawn from the Expert Group, one for each guideline and guidance document.

The RCEG reported to the Registration Steering Group/Working Group on Pesticides (WGP) which had management oversight of the initial phase of development up to production of draft proposals. The draft documents were submitted to the Working Group of National Co-ordinators of the Test Guidelines Programme (WNT).

The pesticide residue chemistry project consists of several phases.

The first phase of the RCEG's activities, which began in 2004, consisted of the development of five Test Guidelines (501: *Metabolism in Crops*; 502: *Metabolism in Livestock*; 503: *Metabolism in Rotational Crops*; 504: *Residues in Limited Field Rotational Crops*; and 505: *Residues in Livestock*), and two Guidance Documents (*Definition of Residue* and *Overview of Residue Chemistry Studies*).

The second phase began in early 2006. Drafts of two Test Guidelines and one Guidance Document were circulated for WGP comment in November 2006. The RCEG met at US EPA in Arlington, Virginia from 16-18 January, 2007 to finalise these documents. The documents were sent to the WNT on 30 January, 2006 before their finalisation. The two draft Guidelines (*Stability of Pesticide Residues in Stored Commodities* and *Nature of the Pesticide Residues in Processed Commodities – High Temperature Hydrolysis*) and the Guidance Document on *Pesticide Residue Analytical Methods*, were approved by the 19th WNT Meeting in March 2007.

This document is published on the responsibility of the Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals of the OECD.

TABLE OF CONTENTS

INTRODUCTION

1. The main purpose of this document is to provide guidance on the residue analytical methods. Analytical methods are used to generate the data for estimating dietary exposure assessments, to establish Maximum Residue Levels (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/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.

2. If the applicants propose a single residue method for enforcement purposes, this document provides guidance on method validation criteria including independent laboratory validation requirements. In general, the quality criteria for pre-registration and single residue post-registration methods are very similar. Aspects specific to single residue post-registration methods are highlighted in paragraphs 48 and 49. Full validation is warranted for representative commodities whereas fortification experiments, which can serve as reduced validation data sets, are generated within supervised field studies. A full method validation need not be generated every time a certain method is used.

3. It is important to note that the methods include the analytes in accordance with the residue definition for the particular pesticide. The residue definition used for dietary risk assessment purposes may differ from that used for MRL enforcement purposes, as described in the OECD Guidance Document on Overview of Residue Chemistry Studies (see paragraph 77 (a) of this document), thus resulting in different analytical methods. In cases where one method cannot cover all compounds of the residue definition in question, more than one method may be necessary.

4. For post-registration methods for enforcement, the official surveillance laboratories prefer multi-residue methods, which could include a large number of analytes. For MRL enforcement, the methodology applied in multi residue methods is different from country to country and strongly depends on the available equipment and the capability of the individual laboratory. This guidance is not intended to replace or override the regulatory authority multi-residue methods. For such methods, validation criteria are described in separate documents (see paragraphs 77 (b), 77 (c), 77 (d) and 77 (e) of this document). When the analyte is not amenable to the multi-residue method techniques, a single residue method may be provided.

PURPOSE

5. The objective of analytical method validation is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. This guidance 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. In most cases more than one method is needed for meeting the following objectives during method development and validation.

6. The method(s) should: have the ability to determine all of the likely analytes that may be included in the residue definition (both for risk assessment and enforcement); be sufficiently selective so that interfering substances never exceed 30% of the limit of analytical quantitation (LOQ); demonstrate acceptable recovery and repeatability; cover all crops, animals, and feed items being treated. If significant residues occur, cover processing fractions and drinking water; and cover all edible animal commodities if animals are likely to consume treated crops. However, some regulatory authorities will establish maximum residue limits for edible animal commodities for trade purposes, although no residues are expected in those commodities. Enforcement methods are therefore required to demonstrate appropriate limits of quantitation and to establish MRLs at LOQ.

DEFINITION OF VALIDATION PARAMETERS

7. 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). These parameters are defined below.

Recovery

8. Recovery is the amount measured as a percentage of the amount of analyte(s) (active substance and relevant metabolites) originally added to a sample of the appropriate matrix, which contains either no detectable level of the analyte or a known detectable level. Recovery experiments provide information on both precision and trueness (bias), and thereby the accuracy of the method.

Selectivity (Specificity)

9. Selectivity refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour. Some regulatory authorities use the term specificity to refer to selectivity.

Calibration

10. Calibration refers to the ability of a detection system to produce an acceptable, well defined, correlation between the instrumental response and the concentration of the analyte in the sample. The analyte concentration to be measured should be within the defined dynamic range of the instrument.

Repeatability

11. Repeatability refers to the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time. The repeatability (within-run effect) includes contributions from any part of the procedure that varies within a run, including contributions from normal gravimetric and volumetric errors, heterogeneity of the test material, and other procedural errors during the analysis.

Reproducibility

12. Reproducibility refers to the closeness of agreement between independent results obtained with the same method on identical test material obtained but under different conditions. Within-laboratory or intra-laboratory reproducibility or single-laboratory reproducibility (run effect) contributes to day-to-day variations in the analytical system due to changes of analyst, batches of reagents, recalibration of instruments and laboratory environment (e.g. temperature changes). Between-laboratory or inter-laboratory or multiple-laboratory reproducibility (laboratory effect) contributes to additional variations such as variations in calibration standards, differences between local interpretations of a protocol, differences in equipment or reagent source, or environmental factors, such as differences in average climatic conditions.

Limit of Detection (LOD)

13. The limit of detection of an analytical procedure is the lowest amount of an analyte in a sample that can be detected but not necessarily quantitated as an exact value. At the limit of detection, a positive identification can be achieved with reasonable and/or previously determined confidence in a defined matrix using a specific analytical method. The LOD is typically not required. However, if needed for a refined assessment (or some other purpose), an explanation of how the LOD was derived should be provided.

Limit of quantitation (LOQ)

14. Limit of quantitation (LOQ), defined from a regulatory perspective as the lowest concentration tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation (RSD) is obtained, also referred to as the limit of determination (LOD) or Lowest Limit of Method Validation (LLMV) (see paragraph 77 (e) through 77 (l) inclusive). The LOQ should be low enough to achieve the intended purpose of the method. From an analytical perspective, 6-10 times the standard deviation of the noise provides an estimate of the LOQ, which is then verified by the fortification experiments. If not stated otherwise, this document refers to the LOQ from the regulatory perspective.

GENERAL ASPECTS

15. This guidance is divided into sections addressing the needs for residue analytical methods. The following topics are discussed: extraction efficiency/radio-validation, confirmatory techniques; derivatization; common moiety and non-specific methods, method validation criteria and the corresponding information to be reported

Analytes Under Consideration

16. As noted above, methods of analysis for pre-registration purposes generally apply to analytes included in the residue definition used in the dietary risk assessment. The method(s) should be capable of determining the active ingredient and/or relevant metabolites (transformation products) in the presence of the sample matrix. Where the sample contains more than one isomer, analogue, etc., of an active substance or relevant metabolite, the method(s) should distinguish between individual isomers/analogues when necessary for the conduct of dietary risk assessments.

17. Post-registration methods might consider different analytes than pre-registration methods, depending on the definition of the relevant residue for MRL enforcement purposes (see OECD Guidance Document on Overview of Residue Chemistry Studies, as referenced in paragraph 77 (a) of this document).

Extraction Efficiency / Radio-Validation

18. Residue analytical methods should be able to measure all components of the residue definition. If the residue definition includes conjugated or bound residues the method should include appropriate techniques for releasing "bound" residues.

19. Extraction efficiency is regarded as key for the development of methods, and data should be provided for the solvents and conditions (temperature, pH, time) typically used. Poor extraction efficiency can be a major source of bias in a method. Extraction efficiency may significantly influence the accuracy of the analytical results. However, extraction efficiency cannot be verified by traditional recovery studies carried out with samples fortified shortly before analysis. Rigorous validation of the efficient extraction of all residues included in the residue definition can only be performed with samples bearing residues through the route by which they would normally reach the sample. This is generally the case in metabolism studies, where the efficiency of extraction can be determined by means of radio-labelled analytes. Note that an IUPAC report (see paragraph 77(n) of this guideline) on bound xenobiotic residues in food commodities of plant and animal origin has recommended: “The extraction procedures used in residue analytical methods should be validated using samples [with incurred residues] from radio-labelled studies ….”

20. Ideally, the commodities of interest from the metabolism and rotational crop studies should be retained for determining the extraction efficiency of the post-registration methods and methods for collection of magnitude of residue data from supervised field trials and rotational crop studies. Justification for the commodities selected should be included in the study report. The retained commodities should be subjected to the extraction procedures from the analytical methods of interest so the extraction efficiency can be readily determined using radiochemical procedures (combustion analysis, liquid scintillation counting and chromatographic analyses using a radio detector). The efficiency can be compared to the relative amount extracted from the metabolism study, wherein the commodities are subjected to rigorous extraction procedures designed to remove most, if not all, of the potential analytes of interest. This comparison is known as radio-validation and should be conducted for the extraction schemes

from all methods, if possible. Alternatively, comparative extraction efficiency studies including the frequently used extraction solvents, such as acetone + water, ethyl acetate, and acetonitrile, can be conducted on samples from metabolism studies for compounds expected in the residue definition(s).

21. The testing of extraction efficiency can be either part of the metabolism study or the method development study. In any case, the results of the investigations should be cited in the relevant method validation studies since they are essential for the development of both types of methods (pre-registration and post-registration).

22. Full radio-validation experiments including additional clean-up steps and recovery experiments ("accountability studies") are warranted in exceptional cases, e.g. when pre-registration methods use common moiety approaches or when extensive enzymatic cleavage steps are included.

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

Confirmatory Techniques

24. In general, additional confirmatory analysis will not be needed when the primary residue method(s) is shown to be specific for the analyte(s) of interest and the source of the analyte(s) / residue is known. This is typically the case for methods being exclusively developed for pre-registration or data generation purposes. On a case-by-case basis, additional confirmation may be necessary, for example when the first method is an immunoassay or for confirmation of the identity of degradation products formed during sample storage.

25. A confirmatory technique is used for single residue post-registration or MRL enforcement methods to demonstrate their selectivity, unless highly specific techniques are employed (see below). The properties of the analyte should be considered when deciding on an appropriate technique.

26. The development of a separate confirmatory method is not generally needed when the original method is based on mass spectrometry or another highly specific method. For example, GC/MS is considered to be highly specific for the analyte provided at least three fragment ions with an m/z ratio of greater than 100 are used for identification/ quantification. The ions selected should be reported and the reasons for their selection given. In case of HPLC/MS-MS, the method is regarded as highly specific when two ion transitions have been validated. Under these prerequisites, an additional confirmatory method is not necessary.

27. The following techniques are considered acceptable confirmatory techniques: GC/MS or LC/MS, provided that a sufficient number of ions are monitored and the reasons for their selection given; HPLC/DAD, if the UV spectrum is characteristic in samples spiked at the limit of quantitation. In this case, an UV-spectrum obtained under the conditions of the determination should be submitted. Other acceptable confirmatory techniques include an alternative chromatographic principle deviating from the original method (HPLC ⇔ GC); an alternative detection technique; derivatization (if it was not the first choice method) and significantly different chromatographic stationary or mobile phases of different selectivity.In addition, variation of partitioning and clean-up steps can also be useful for confirmation.

Derivatization

28. For analysis of some compounds, such as those with high polarity or with poor chromatographic properties, derivatization may be called for. Derivatives may be prepared prior to chromatographic analysis or as part of the chromatographic procedure (pre- or post-column). The use of derivatization methods should be fully reported and justified. The derivative should be stable and its formation reproducible. When quantification is based on the determination of a derivative, the calibration is preferably conducted using standard solutions of that derivative, unless the derivatization step is an integral part of the detection system. If the derivative is not available as a reference standard, it should be generated within the analytical set by using the same derivatization procedure as that applied for the samples. Under these circumstances, a full justification should be given. The mean yield and precision of the derivatization step should be demonstrated where possible. The method is considered to be specific to the analyte of interest if the derivatized species is specific to that analyte. However, when the derivative formed is a common derivative of two or more active substances or metabolites or is classed as another active substance, the method should be considered non-specific.

Common Moiety Methods and Non-Specific Methods

29. For some analytes, specific residue analytical methods might be unavailable or difficult to perform. In these cases, conversion to a common moiety is valid when all components containing that moiety are considered toxicologically important and when no single component is an adequate marker of residue concentration.

30. For the generation of plant and animal product residue trial data in cases where it is likely that a multi-component residue definition will be needed for risk assessment purposes, a common moiety method may be used.

31. The use of non-specific methods is generally discouraged. The choice of appropriate methods should take into consideration the needs of both risk assessment and MRL compliance. If multiple compounds are included in the definition of the residue for enforcement purposes, this might result in an excessive number of methods. Under these circumstances, a "common moiety method" may be warranted. Disadvantages of using non-specific or common moiety methods are:

a) When a non-specific method has been used, the identity of the source of the analyte is likely to be questionable. For example, the method may also detect breakdown products either containing a moiety common to the intended analyte, or which have been derivatized to a common species, or which cannot be resolved from the target analyte. Such methods may also be subject to interferences from other similarly structured compounds.

b) When analysing active substance content in a product that has undergone storage as part of a storage stability study, stability/degradation may be impossible to determine with a method that is not specific to the active substance.

c) When the method determines a moiety common to two or more distinct active substances with differential toxicities, it is desirable to identify the origin of the residue, enabling the risk assessment to be carried out on the toxicologically significant residue components.

32. In practice, data may have to be generated in such a way as to give the regulatory authority flexibility to establish two separate residue definitions where appropriate, one for risk assessment and a second for MRL compliance monitoring. In such cases, where possible, applicants should either separately analyse for the individual components of the residue definition, rather than carrying out a common moiety

method; or carry out first analyses according to a common moiety approach and a second series of analyses of the field trial samples for a suitable indicator molecule in parallel, if the common moiety methodology is unsuitable for practical routine monitoring and enforcement of the MRL at reasonable cost. The availability of appropriate methods for monitoring purposes should be considered.

33. Non-specific and common moiety methods will be acceptable only in exceptional circumstances where there is no other practical means of determining the target analyte. In these cases, full justification should be provided. This should include an explanation as to why the compound cannot be determined by a specific analytical technique. When common moiety methods are proposed, validation data should be presented separately for all relevant components of the residue definition.

VALIDATION OF PRE-REGISTRATION METHODS

34. In general, residue analytical methods should be validated for all matrices for which the method is used. The extent of validation depends on the information already available and reported. Full validation data (as described in the following sections) should be provided only for new methods or when existing methods are significantly changed (e.g. change of solvent systems or quantitation techniques). Such changes may be required when adapting methods to different commodities. When an existing method, which has been previously validated, is adapted to other "comparable" commodities within a category (as described in Annex I), usually reduced or limited validation sets are sufficient. Reduced validation sets (sometimes referred to quality control data sets) are typically reported within supervised field trial reports, whereas full validation data are included in separate GLP reports.

Number of Matrices for Validation

35. In the case of studies involving plant material, the number of commodities is dependent on the use of the product. Validation data should be submitted for all sample matrices to be analysed and should be carried out for all components of the residue definition for dietary risk assessment.

36. If applicable, full validation experiments should be performed predominantly on one raw agricultural commodity (RAC) from each of the representative commodity categories as listed in Annex I. In case of the commodities with high protein and high starch content, it is not necessary to perform a full validation set for a representative matrix of both commodities. Instead select one dry (low moisture) commodity to represent both groups.

37. The commodity categorization scheme is not intended to imply that if the method is successfully validated using the representative commodity, the method will work for all commodities in the same category. A case for matrix comparability and a reduced validation data set might be considered where two or more very similar commodities are to be analysed (see Annex 1). Reduced validation data for commodities belonging to the same commodity category are acceptable and are still needed for all commodities for which an MRL is sought.

38. Commodities containing high percentages of soluble natural products such as e.g. tobacco, hops, coffee, tea and spices might interfere with the analytes under consideration. Interference can vary depending on the methodology selected and the properties of the compounds. In case of these difficult matrices, full validation data are generally called for to prove the suitability of the method.

39. Methods for the determination of residues in processed commodities should be validated. If the method is substantially the same for both the raw agricultural commodity (RAC) and the processed commodity, then a limited or reduced validation may suffice.

40. If animals are likely to consume treated crops and if feeding studies are required/submitted, methods for determination of residues in products of animal origin should be validated in the following matrices: milk, eggs, and all edible tissues. The tissues normally include cattle muscle, fat, liver, and kidney as well as poultry muscle, fat, and liver. In most cases, the recovery data for cattle commodities are valid for products of goats, hogs, horses, sheep, and poultry.

Validation Levels

41. Data should be generated for two fortification levels appropriate to the proposed LOQ and likely residue levels or 10 x LOQ. Concurrent recoveries should also be conducted during residue trial sample analysis and reported with residue trial results.. The limit of quantitation (LOQ) required will depend upon the sensitivity necessary for the risk assessment or MRL enforcement; in general, it should be in the range of 0.01 to 0.05 mg/kg for each analyte under consideration. On a case-by-case basis, higher levels are acceptable (e.g. for difficult matrices) if there are no toxicological concerns.

42. Samples to be utilised for recovery determinations should be of the untreated commodity, to which a known quantity of analyte is added and the whole sample analysed to reduce sampling error. New technologies require less sample material resulting in the need for increased homogeneity. The results should be compared to the known analyte "content" of the sample. Control (unfortified) samples should be analysed concurrently to determine any contamination by the analyte of interest or interference.

Number of Fortification Experiments

43. For generating full validation data sets, analysis of five replicates at each of the two fortification levels should be performed together with two control samples. Lower numbers of samples should be justified. For a reduced data set, at least three determinations at each validation level plus one control (for proving sample is free of detectable residue) should be used.

Calibration

44. The analytical calibration should extend over a range appropriate to the lowest and highest nominal concentration of the analyte in relevant analytical solutions. Either duplicate determinations at three or more concentrations or single determinations at five or more concentrations should be used. The equation of the calibration curve and a regression parameter, e.g. the correlation coefficient (r), must be reported, and a typical calibration plot submitted. When a non-linear calibration is used, an explanation (including how calibration accuracy is to be maintained) should be provided. Possible matrix enhancement or suppression effects of sample co-extractives, on the chromatography system or detection system response should be addressed. When appropriate, the detection system may be calibrated (matrix matched standards) using standard solutions in a matrix similar to that of the samples to be analysed.

45. If linear calibration has been demonstrated, single point calibration can be used. Note that the calibration model should be a model that covers only two decades of concentration levels (e.g. 0.01-1.0 mg/kg) and the single point level should lie within the validated calibration range. In general, multiple replicates of a calibration standard at one concentration level (comparable to the amount being expected in the sample) are used for evaluation.

Use of Internal and Procedural Standards

46. Procedural standards are considered to be standards that are generated by subjecting the reference standard to some or all of the sample preparation procedures specified in the method. Methods using procedural standards generated from a derivatization step may be acceptable under certain conditions. If the derivatized standard is unstable or cannot be provided, the petitioner must provide data to demonstrate the efficiency and reproducibility of the procedure.

47. An internal standard is a chemical added in known quantity, at a specified stage in analysis, to facilitate determination of the identity and/or quantity of the analyte. The use of an internal standard method is only acceptable under certain circumstances, usually when added to the final extract (prior to quantitation). If used in this manner, the internal standard should show behaviour similar to that of the analyte(s) of interest. It should not degrade and not be prone to matrix effects. However, the use of an internal standard throughout the entire procedure to correct for recoveries is not acceptable unless data are available on numerous samples of each matrix to show that the analyte and internal standard behave very similarly in each step (extraction, cleanup, etc.). An example of an internal standard method that is fully acceptable is the use of stable isotopes (e.g. 2H, 13C) for facilitating quantitation by mass spectrometry.

METHODS FOR MRL ENFORCEMENT (POST REGISTRATION)

48. Generally, post-registration methods are only used for those matrices of plant and animal origin where a MRL is set. Applicants are encouraged to consult regulatory authorities for guidance on establishing MRLs when all residues are reported below the LOQ. If no MRL is set, there is no need for the applicant to provide any further information on post-registration methods.

However, some regulatory authorities will establish MRLs for trade purposes, although no residues are expected in those commodities. Enforcement methods are therefore required to demonstrate appropriate limits of quantitation and to establish MRLs at LOQ.

49. In general, the methods should cover the analytes included in the definition of the residue relevant to MRL setting and enforcement. A discussion on selection of residues for inclusion in MRLs may be found in the OECD Guidance Document on Overview of Residue Chemistry Studies (see paragraph77 (a) of this document). The methods should be fast, easy to perform, use commonly available techniques/equipment and avoid hazardous substances (e.g. chloroform, benzene). The acceptance of techniques as part of enforcement methods should be discussed at appropriate intervals. It is recognised that analytical methodology is constantly developing; however, time may elapse before new techniques become generally accepted and available to enforcement laboratories.

50. For post-registration methods, a multi-residue method approach is clearly preferred compared to a single residue method even if its recovery is not as good as that of a specific individual method as, generally, the enforcement laboratories do not have sufficient capacity to apply individual methods to all compounds that may possibly be present.

51. If applicable, the applicant should first check the suitability of an existing multi-residue method approach. Most of today's state of the art multi-residue methods fulfilling the needs of enforcement laboratories with regard to speed, LOQ and number of analytes covered are based on HPLC/MS-MS or GC/MS quantitation. If one of the multi-residue methods is found to be acceptable as a post-registration method, refer to the documents cited in paragraph 4 of this guidance for validation requirements. If the validated multi-residue method does not include separate chromatograms or spectra for unambiguous

confirmation of positive findings, it may be necessary for the applicant to provide special confirmatory methods.

52. Verification that a new compound can be analyzed by a commonly established multi-residue method approach is preferably done by a modular and tiered approach testing different key steps. A multi-residue method check involves at least the following steps:

a) Mass spectrometry: selection of a suitable ionization technique, suitable ions and transitions (quantitation and confirmatory purposes);

b) Chromatographic behavior: selection of suitable HPLC or GC conditions (for GC: first vaporization behavior);

c) Clean-up: selection of suitable procedures (e.g. solid phase extraction, filtration, liquid/liquid partition);

d) Extraction: selection of suitable solvent systems (e.g. methanol/water, acetonitrile/water, acetone) (see paragraphs 18-23 of this guideline);

e) Calibration: selection of an appropriate calibration function and procedure for standard preparation.

The sequence depends on the selection of the envisaged quantitation technique. For example, vaporization behaviour needs to be checked first in the case of GC methods.

For MRL enforcement, the methodology applied in multi residue methods is different from country to country and strongly depends on the available equipment and the capability of the individual laboratory. This guidance is not intended to replace or override the regulatory authority multi-residue methods. Further validation criteria are described in separate documents (see paragraphs 77(b), 77(c), 77(d) and 77(e) of this document.

Independent Laboratory Validation Studies (Post-Registration)

53. Independent laboratory validation (ILV) studies generally are not needed for pre-registration methods. The requirements on ILVs for multi-residue and single-residue methods are different in different parts of the world. Whereas for US registration, independent laboratory validation studies are only required for single-residue methods proposed by applicants, in Europe independent laboratory validation studies are typically also required for proving the suitability of established multi-residue methods.

54. The post registration method(s) should be suitable for the determination of all compounds included in the residue definition for compliance with the MRL. The suitability of the method(s) should be proven by appropriate experiments. At least one matrix should be independently validated - typically the most difficult target crop / commodity for which an MRL is set. One important purpose of the post-registration method could be to detect any misuse. For some regulatory authorities, the need for an ILV is therefore not necessarily limited to target crops. In some European countries, ILV data are typically required for one representative commodity from each commodity category as defined in Annex I. In case of a dry commodity, one representative commodity can be selected either from the high protein or the high starch category. Further discussion on the number of commodities to be included in the ILV may be found in paragraphs 56 and 57 of this document.

55. The laboratory chosen to conduct the ILV trials must not have been involved in the method development and in its subsequent use. Provided this criterion is met, the laboratory chosen to conduct the ILV trials may be in the applicant's organisation, but must not be at the same location. If the chosen laboratory requires communication with the developers of the method to carry out the analysis, this should be reported. Also any subsequent additions or modifications to the original method should be also reported.

Number of Representative Matrices for Independent Validation

56. ILV data should be submitted for a range of one to four raw agricultural commodities (RAC) selected from each of the commodity categories listed in Annex I. The commodity selected should be representative of the category. In the case of commodities with high protein and high starch content, it is not necessary to perform an ILV for a representative matrix of both categories, but rather include one dry (low moisture) commodity in the validation.

57. For ILV studies of post-registration methods for the determination of residues in products of animal origin, the following animal commodities should be used as appropriate, if an MRL is established or is likely to be proposed: milk, eggs, meat and/or fat, and kidney and/or liver.

Validation Levels for ILVs: Limit of Quantitation - Maximum Residue Levels

58. The ILV should include fortifications at the LOQ and the MRL. Selection of the appropriate LOQ for regulatory purposes is discussed in Paragraph 14 of this guidance. If the residue levels are low, the LOQ should be 0.01-0.05 mg/kg. The selection of an appropriate LOQ depends on the analyte/matrix combination. However, the applicants are encouraged to develop methods which allow the determination of residues at low LOQs by using state-of-the art technology. In any case where a high LOQ is selected (e.g. for difficult matrices) a full justification should be given by the applicant.

Number of Fortification Experiments

59. Recovery data should be generated for the following fortification levels: LOQ (5 samples); 10 x LOQ or MRL, whichever is greater (5 samples); and controls (2 samples).

If matrices are difficult to analyse and the expected residue levels are of minor toxicological importance (e.g. for minor uses), a reduced sample set may be acceptable. However, six samples (three at each fortification level) and one control sample are the minimum.

Calibration

60. Analytical calibration should extend over a range appropriate to the lowest and highest nominal concentration of the analyte in relevant analytical solutions. Duplicate determinations at three or more concentrations or single determinations at five or more concentrations should be performed. Raw data of calibration have to be provided with studies.

MINIMUM PERFORMANCE CHARACTERISTICS FOR METHODS

61. For demonstrating the suitability of the method for its purpose, information on performance characteristics should be provided. The performance characteristics specified below are of importance for both the pre-registration and the single residue post-registration methods.

Range of Acceptable Recoveries

62. In general, the mean recovery at each fortification level and for each commodity should be in the range given in Table 1. In certain justified cases, recoveries outside of this range will be accepted for matrices which are difficult to analyse, e.g. tobacco, hops, coffee, tea and spices, providing that precision data are acceptable, or in cases of very low concentration levels. If matrix effects are noted, recoveries may be corrected by using matrix-matched standards.

Selectivity (Matrix Interference)

63. Uncorrected recoveries and blank (control) values should be reported. Blank values in the area of analytical interest (untreated samples and procedural blanks) have to be determined from the matrices used in fortification experiments and should not be higher than 30% of the LOQ. If this is exceeded, detailed justification should be provided. Matrix effects such as peak suppression and enhancement can also occur with some techniques such as HPLC/MS-MS and GC. Therefore, standard solutions should be added to the final volume of an untreated sample ("quality control samples") to check for these effects.

Precision - Repeatability (expressed as relative standard deviation)

64. The precision of the method in a validation study should be reported as the relative standard deviation (RSD) of repeatability at each fortification level. As specified above, five determinations should be made at each fortification level. In certain justified cases (e.g. in cases of difficult matrices or very low concentration levels) a higher variability may be accepted. The correlation between the concentration level and the repeatability is given in Table 1.

Values for repeatability were calculated from 0.67x Horwitz equation:

$$RSD = 2^{(1-0.5\log C)}$$

Where C is concentration (1 mg/kg = 10^{-6}).

Table 1: Laboratory Repeatability Criteria for Analysis of Pesticide Residues[1]

Concentration level	Repeatability (relative standard deviation)	Range of mean % recovery
≤ 1 µg/kg	35	50 - 120
> 1 µg/kg ≤ 0.01 mg/kg	30	60 - 120
> 0.01 mg/kg ≤ 0.1 mg/kg	20	70 - 120
> 0.1 mg/kg ≤ 1.0 mg/kg	15	70 - 110
> 1 mg/kg	10	70 - 110

[1]Guidelines on Good Laboratory Practice in Residue Analysis, CAC/GL 40-1993, Rev.1-2003 [see paragraph 77 (c)]

65. When outliers have been identified using appropriate statistical methods (e.g. Grubbs or Dixons test), this should be justified. A maximum of one outlier may be disregarded at each fortification level. Where more than one outlier has been identified at one fortification level, additional validation samples might be included and an explanation provided.

66. The precision of the method in an ILV study for single residue post-registration methods should be reported as repeatability. Because of the small number of laboratories involved (2), results cannot be put together to define a between-laboratory reproducibility. Therefore for each individual laboratory the same RSD criteria apply as for the pre-registration methods (see Table 1).

67. If unacceptable variability of results is noted during validation, efforts should be made to identify and control those method parameters with a major influence on method performance (ruggedness testing). The ruggedness of an analytical method is the resistance to change in the results produced when minor changes are made from the conditions described in the procedure.

STABILITY INVESTIGATIONS

Stability of the Analytes in Stored Extracts of the Final Volume

68. Ideally, the validation samples are analysed within 24 hours after initial extraction. Under some circumstances they may be stored longer under ambient conditions, e.g. in the autosampler or in a refrigerator, e.g. if the analyses cannot be completed within one working day. In this case, information on the storage stability of the analytes in extracts and in the final volume should be provided.

69. The relevant information on the stability in the initial extract can sometimes be derived from metabolism studies. Typically in metabolism studies, chromatographic profiles of extracts are investigated over time periods ranging from days to months. If the analytes were stable under comparable conditions in similar solvent systems, any degradation during short-term storage is unlikely.

70. The relevant information on the stability in the final or any intermediate step can be derived from the fortification experiments performed during method validation. If the recoveries in the fortified samples are within the acceptable range of 70 - 120%, stability is sufficiently proven.

71. Only for exceptional cases, further and separate investigations are called for, e.g. in cases where rapid degradation of the analyte is expected. During these separate investigations, the recovery data of stored extracts / final volumes are compared with the data from freshly prepared extracts. For the test, it is sufficient to select representative matrices. If stable, extracts obtained from all commodity categories specified in Annex I do not need to be analyzed. The storage conditions tested should be reported and should reflect typical storage conditions applied during analysis.

72. The requirements for long term stability of extracts under freezer conditions are covered by the OECD Guideline on Stability of Pesticide Residues in Stored Analytical Samples [see paragraph 77(m)].

Stability of Working (Fortification/Calibration) Solutions

73. If stability under controlled storage conditions has been demonstrated, the fortification and calibration solutions can be used over an extended time period. Otherwise, the solutions have to be prepared freshly on a daily basis.

74. The duration of the stability test should reflect typical usage. In general, they are used over a periods of several days or weeks. In general, solutions are used over a periods of several days or weeks. The test conditions, e.g. appropriate solvent systems, ambient temperature or refrigerator, light/dark, should be selected to reflect usual storage conditions applied within the conduct of analyses.

75. For testing, the stability of the stored solutions (typically in peak area or peak height) should be compared with freshly prepared fortification and/or calibration solutions. The concentrations should be chosen so that potential degradation can be observed. If no concentration dependency is observed, it is not necessary to investigate all concentrations applied. In order to obtain reliable data, at least three injections of stored and freshly prepared solutions should be compared.

STUDY REPORT

76. This section describes general information that should be included when describing analytical methods used with residue chemistry studies.

A. Introduction.

(i) Scope or applicability. Describe suitable commodities/matrices and source of method .e.g., the PAM, company reports, etc.

(ii) Principles of the analytical procedure, including identification of the chemical species determined and the limits of detection (if needed) and quantitation.

B. Materials and methods.

(i) Standard Compounds

(*1*) Description e.g. Chemical name, CAS number, Chemical structure, molecular formula and mass, purity, expiration data, storage conditions
(*2*) Preparation of stock solutions.
(*3*) Preparation of calibration solutions.

(ii) Procedure. Describe detailed analytical procedure in a stepwise fashion, with special emphasis on reagents or procedural steps requiring special precautions to avoid safety or health hazards.

(*1*) Preparation of sample.
(*2*) Extraction — demonstrate efficiency, if relevant (e.g. dry crop substrates, bound residues, etc.); radio-validation data may be provided in a separate report at the discretion of the petitioner.
(*3*) Fortification, if applicable—i.e. during method validation runs.
(*4*) Clean-up.

(*5*) Derivatization (if any).
(*6*) Chromatographic conditions/mobile phase composition if chromatographic separation is used
(*7*) Stability of standard solutions and extracts

(iii) Instrumentation.

(*1*) Description (e.g. make/model, type/selectivity of detectors, columns (packing materials, size), carrier gases, etc.).
(*2*) Operating conditions (e.g., flow rates, temperatures, voltage, chromatography conditions, etc.).
(*3*) Calibration procedures.

(iv) Interferences. Describe any interferences, such as:

(*1*) Sample matrices.
(*2*) Other pesticides.
(*3*) Solvents.
(*4*) Laboratory ware.

(v) Confirmatory techniques.

(vi) Describe modifications or potential problems, if any, in the analytical method (detail circumstances and corrective action to be taken).

(vii) Calculations. Describe in a stepwise fashion

(*1*) Calibration factors.
(*2*) Analyte in sample.

(viii) Other. Any and all additional information considered appropriate and relevant to provide a complete and thorough description of residue analytical methodology and the means of calculating the residue results.

C. Performance. Describe expected performance of method.

(i) Recovery (expected mean and range of recoveries). Include the individual recovery values, average recoveries, and relative standard deviation thereof for each component of the residue of concern in each commodity tested during the method validation.

(ii) Precision.

(iii) Limits of detection (if needed) and quantitation (provide definition).

(iv) Ruggedness testing, if performed.

(v) Limitations.

D. Representative Chromatograms. The following representative chromatograms should be included in the study report.

(i) Blank control.

(ii) Analytical/matrix standards

(iii) Lowest fortification levels

(iv) Treated samples

E. Conclusions. Summarize applicability of analytical procedure for measuring specific test compounds in various test substrates, availability of equipment, interferences, stability, etc.

REFERENCES

77. All references cited in the text are listed below.

(a) Organisation for Economic Co-operation and Development (OECD). 2006. Guidance Document on Overview of Residue Chemistry Studies. ENV/JM/MONO (2006)32. 61 pp.

(b) European Union Health & Consumer Protection Directorate General (SANCO). 2006 Doc. No. SANCO/10232/2006 (dated: March 24, 2006): Quality control procedures for pesticide residue analysis (limited to Method validation requirements; p. 11 ff and confirmatory techniques)

(c) Codex Alimentarius Commission (CAC). 1993. CAC/GL 40-1993, Rev.1-2003, Guidelines on Good Laboratory Practice in Residue Analysis

(d) U.S. Environmental Protection Agency 1996. Residue Chemistry Test Guidelines. OPPTS 860.1360 Multiresidue Method

(e) SANCO. 2004. SANCO/825/00 rev 7, 17.03.2004: Guidance document on residue analytical methods (post-registration requirements for annex II and annex III)

(f) European Food Safety Authority (EFSA). 2006. Document (adopted May 17, 2006): Opinion of the Scientific Panel on Analytical methods

(g) European Union (EU)/Germany. 2003., Anforderungen an Analysenmethoden zur Bestimmung fuer Pflanzenschutmittelrueckstaenden im Rahmen des Zulassungsverfahrens, Nachrichtenbl. Deut. Pflanzenschutzd, 55, 275

(h) Australian Pesticides and Veterinary Medicines Authority (APVMA) 2004. Guidelines for the validation of analytical methods for active constituent, agricultural and veterinary chemical products.

(i) Codex Alimentarius Commission (CAC). 2005. CAC/GL 56-2005, Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative Determination of Residues

(j) Food and Agriculture Organization (FAO) . 2002. Plant Production and Protection Paper 170, Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed.

(k) U.S. Environmental Protection Agency. 1996. Residue Chemistry Test Guidelines. OPPTS 860.1340 Residue Analytical Method.

(l) Pest Management Regulatory Agency (PMRA) 1998. Residue Chemistry Guidelines, Regulatory Directive Dir98-02. Section 3 Residue Analytical Method, Section 4 Multiresidue Method. (Canada)

(m) OECD Guideline for the Testing of Chemicals. 2007. Stability of Pesticide Residues in Stored Analytical Samples. Proposal for a New Test Guideline, January 11, 2007.

(n) Skidmore, M W., G.D. Paulson, H.A. Kuiper, B. Ohlin, and S. Reynolds. 1998. Bound xenobiotic residues in food commodities of plant and animal origin. Pure and Applied Chemistry, 70, 1423-1447.

78. Additional references:

(a) SANCO. 2000. SANCO/3029/99 rev 4, 11.07.2000: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for annex II and annex III

(b) Commission of the European Communities. 1997. Working document 7028/VI/95 rev. 3, 22.07.1997: Metabolism and distribution in plants (for extraction efficiency)

(c) Netherlands RIVM. 2002. Factsheets for the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment Part II. Report 601516009/2002. Chapter 3: Pesticide residue analysis in plant and animal products

(d) Part A2: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 6) and Annex III (part A, Section 8) of Directive 91/414 (Draft, 29 June 2003)

(e) Siebers, J. and R. Haenel. 2003. Handbook of residue analytical methods for agrochemicals. Assessment of residue analytical methods for crops, food, feed, and environmental samples: The approach of the EU. John Wiley, New York.

(f) BVL. 2002. Nachrichtenbl. Deutscher Pflanzenschutzdienst, Vol. 54, 157 (Document, in German, on requirements for residue analytical methods)

(g) Alder, L. 2003. Handbook of residue analytical methods for agrochemicals (Validation of analytical methods for post-registration control and monitoring purposes in the EU; includes information on extraction efficiency) .John Wiley, New York.

(h) Veith, B. 2005. Enforcement methods by registrants - revision of guidance document 825/00 rev. 7. Presented at Fresenius Conference, June 2005)

(i) Alder L. et al. 2006. European Pesticide Residue Workshop 2006, “Residue analysis of 500 high priority pesticides – better by GC/MS or LC/MS/MS?”

(j) Australian Pesticides and Veterinary Medicines Authority (APVMA) .Residue Guideline No. 19 – Residue analytical method; http://www.apvma.gov.au/guidelines/guidln19.shtml

ANNEX 1
COMMODITY CATEGORIES FOR PRE- AND POST-REGISTRATION METHOD VALIDATION

When choosing representative commodities for study to extrapolate to other commodities within the same category, it will be necessary to exercise judgement, e.g. it would be inappropriate to select spices or hops alone to study to be representative of a range of oil content commodities.

Commodity Categories	Commodities included in this category	Typical representative commodities
High water content	Pome fruit Stone fruit Bulb vegetables Fruiting vegetables/cucurbits Brassica vegetables Leafy vegetables and fresh herbs Stem and stalk vegetables Forage/fodder crops Fresh legume vegetables Leaves of root and tuber vegetables Sugar cane Fresh green tea Fungi	Apples, pears Apricots, cherries, peaches Bulb onion Tomatoes, peppers, cucumber, melon Cauliflower, Brussels sprout, cabbage Lettuce, spinach Leek, celery, asparagus Wheat and barley forage, alfalfa, Fresh peas with pods, petit pois, mange tout, broad bean, runner bean, dwarf French bean Sugar beet and fodder beet tops
High oil content	Tree nuts Oilseeds Olives Avocados Hops Cacao beans Coffee beans Spices	Walnut, hazelnut, chestnut Oilseed rape, sunflower, cotton, soybean, peanut
High protein content	Dry legume vegetables/Pulses	Field bean, dried broad bean, dried haricot bean (yellow, white/navy, brown, speckled)
High starch content	Cereal grain Roots of root and tuber vegetables Starchy root crops	Wheat, rye, barley and oat grain Sugar beet and fodder beet roots, carrot Potato, sweet potato
High acid content	Citrus fruit Berries Currants Grapes Kiwifruit Pineapple Rhubarb	Lemon, mandarin, tangerine, orange Strawberry, blueberry, raspberry Black currant, red currant, white currant

Important Note:

The above list of commodities is not a comprehensive list of commodities/matrices and other commodities may be used. Applicants should consult regulatory authorities for advice on the use of other commodities. Generally only one dry commodity can be selected to represent the high protein and high starch commodities.