Evaluation Manual for the Authorisation of plant protection products and biocides

EU part

Biocides

Chapter 3 Analytical Methods

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Chapter 3 Analytical methods
Category: biocides

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GENERAL INTRODUCTION

This chapter describes the data requirements for the aspect analytical methods and how these are evaluated for the EU framework (§1 - §1.5).

1. EU FRAMEWORK

The procedure for inclusion of active substances in Annex I to Directive 98/8/EC [1] is described under EU framework (§1 - § 1.5) where only the procedure laid down in the EU is described. The NL procedure for evaluation of a substance, described in §2 - §2.5 of the NL part, is reverted to where no EU procedure has been laid down.

1.1. Introduction

The analytical methods are evaluated to establish whether the analytical methods are suitable for pre- and/or post-registration of biocides.

The evaluated analytical methods can be used to support the physical-chemical properties (such as solubility in water and organic solvents and log Kow) see Chapter 2 Physical and chemical properties). The analytical methods are evaluated for the 5-batch analysis of the active substance as manufactured and for the determination of impurities.

The residue analytical methods are evaluated for the determination of residues in vegetable and animal products (see Chapter 5 Residues, residue dossier) and for the determination of residues in water, air and soil (see Chapter 6 Behaviour and fate in the environment). Furthermore, for the determination of residues in body tissues and blood when an active is classified as T/T+, residue analytical methods for are evaluated (See Chapter 4 Human Toxicology).

The residue analytical methods must meet the residue definition and LOQ/MRL set for the appropriate matrices.

The most important Technical Notes for Guidance (TNsG) for this chapter are:

- TNsG on Annex I inclusion [2]
- TNsG on Data Requirements (Chapters 2 and 3) [3]
- TNsG on Product Evaluation [4]

Regarding the analytical methods, the TNsG on Product Evaluation [4] reads as follows:

> These Technical Notes for Guidance do not cover:
>
> evaluation of methods for chemical analysis. Guidance on analytical methods is given in the Technical Notes for Guidance on data requirements.

The guidance documents for plant protection products referred to above are:

- Sanco/3030/99 “Technical Material and Preparations: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A section 5) of directive 91/414” [5].
  http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc13_en.pdf
- Sanco/3029/99 “Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, section 4) and Annex III (part A, Section 5) of directive 91/414” [6].
  http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc12_en.pdf

- Sanco/825/00 ”Guidance document on residue analytical methods” [7].
  http://ec.europa.eu/food/plant/protection/resources/guide_doc_825-00_rev7_en.pdf

In the Technical meetings there is European-wide agreement about the need for guidance regard the analytical methods. The guidance documents mentioned above covering plant protection products can be used for the evaluation of biocidal products.
At the moment there is no European consensus about when these Guidance documents can be used. For this we refer to §2.1 of the NL part.
Additional guidance for the evaluation of analytical methods for biocides has been recently developed. This has been agreed by the 33rd CA meeting (may 2009) [8].

1.2. Data requirements

In order to qualify for inclusion in Annex I to 98/8/EC a dossier, which meets the provisions laid down in Annex IIA, IIB, IIIA and IIIB to 98/8/EC, must be submitted for the active substance (and where appropriate for relevant degradation products, isomers and impurities of active substances and their additives) as well as for the product. The data requirements have been elaborated in the TNsG on data requirements [3].
The data requirements in EU framework are first subdivided into data on the active substance and data on the product. These are then subdivided into ‘common core data’, i.e., data required for each product type and ‘additional data’, data that must be submitted in certain situations (e.g. if the biocidal product may get into contact with food, feedstuffs, agricultural or horticultural soil).

The requirements for the validated analytical methods are summarised in Appendix 2 to this chapter.

The (validation) requirements for the analytical methods of the active substance as manufactured and the product are summarised in Appendix 3 to this chapter.
The residue-analytical methods are summarised in Appendix 4 to this chapter.

Good Laboratory Practice (GLP)
No GLP is required for validation of the analytical methods.
The GLP requirement for studies (where validated analytical methods are used), is elaborated in Chapter 2 Physical and chemical properties (biocides).

Justification for the non-submission of data
Where according to the applicant a certain study is not necessary, a relevant scientific justification should be provided for the non-submission of the particular study.

Pre- and post-registration methods
The Biocides Directive 98/8/EC and the TNsG on data requirements make no distinction between pre- and post-registration methods. According to Chapter 2, section 4 of the TNsG [8] on data requirements the mentioned requirements are well described for both the analytical methods as well as for the residue analytical methods.
Different wording is used for the analytical methods used for monitoring: monitoring methods, enforcement methods or post-registration methods. The wording post-registration methods has been chosen for this chapter
Pre-registration methods are the analytical methods that (possibly once) have been used for (residue) studies required for registration.

The validated analytical method must be suitable for determination of the relevant substances (like active substance and/or relevant degradation products, isomers and

impurities) for the appropriate matrices.
A summary is given in the following table:

Matrix	Relevant substance
In active substance as manufactured [1]	pure active substance [2]
	Significant degradation products, isomers, impurities and additives ≥ 0.1 % w/w
	relevant degradation products, isomers, impurities and additives
In product	pure active substance [2]
In plant and animal material [5]	the compounds as included in the residue definition for the particular matrix (crops type/ kidney/ milk/etc.)
	eco)toxicologically relevant components of the product[4]
In environment (soil, water, air)	the compounds as included in the residue definition for the particular matrix (soil/water/ air)
	(eco)toxicologically relevant components of the product [4]
In animal and human body fluids and tissues [3]	pure active substance [2]
	(eco)toxicologically relevant components of the product [4]

1) This concerns the active substance as manufactured as traded. Where the active substance is not isolated separately during the manufacturing process, but is subjected to further treatment (e.g. dilution or addition of a stabiliser), the result of the treatment is considered as the 'technical substance as manufactured'.
2) Inactive isomers are considered as impurities
3) If the active substance is classified with T or T+
4) The meaning of "(eco)toxicologically relevant components of the product has not been elaborated in European framework.
5) (additional data) may be required if the biocide can get into contact with food, feedstuffs, agricultural or horticultural soil.

1.2.1 Data requirements active substance

Common core data
The text below in grey frames has been taken from Biocides Directive 98/8/EC.
The numbers in these grey frames correspond with the section numbering in Biocides Directive 98/8/EC.

IV. ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION

4.1 Analytical methods for the determination of pure active substances and, where appropriate, for relevant degradation products, isomers and impurities of active substances and their additives (e.g. stabilisers)
4.2. Analytical methods in all relevant environmental media including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following:
(a) Soil

(b) Air
(c) Water: the applicant should confirm that the substance itself and any of its degradation products which fall within the definition of pesticides given for parameter 55 in Annex I to Council Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption(**) can be estimated with adequate reliability at the MAC specified in that Directive for individual pesticides
(d) Animal and human body fluids and tissues
(**) OJ L 229, 30.8.1980, p. 11. Directive as last amended by Directive 91/692/EEC (OJ L 377, 31.12.1991, p. 48).
(d) Animal and human body fluids and tissues
Where an active substance is classified as toxic or highly toxic, analytical methods must be submitted which allow determination of the active substance at the no adverse effect level (NOAEL).

The method should meet standards for certain validation parameters. Typical validation characteristics for residue analytical methods that should be considered are: accuracy, recovery, selectivity (specificity), calibration, precision (repeatability, reproducibility) and limit of quantitation (LOQ). Full descriptions of validated methods must be provided.

The above has been elaborated in the TNsG on data requirements. For a more detailed version see additional guidance on TNsG on Data Requirements [8].

The text below in grey frames originates from the TNsG on data requirements [3].
The numbering in these grey frames follows the section numbering of the TNsG on data requirements [3] to Chapter 2.

4 ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION

- Information on analytical methods is required for assessing compliance with conditions for issuing authorisation for a biocidal product according to Article 5(1c) of the Directive. This information is also required for the post-authorisation control and monitoring purposes, and for the assessment of justifications which should be provided for the methods used for the generation of data as required in accordance with this Directive.
- For substances which are difficult to analyse a description of the problems should be given.
-

The objective of validation of analytical methods is to demonstrate that they are suitable for its intended use. The methods should have the ability to determine all of the analytes included in the residues definitions for enforcement established by the competent authorities (CA). The methods should use commonly available techniques/equipment and avoid hazardous substances (e.g. carcinogenic substances like diazomethane, benzene or chloroform). Enforcement methods require an appropriate limit of quantitation (LOQ), sufficient selectivity, interferences may not exceed 30% of the LOQ, and acceptable recovery and repeatability must be demonstrated.

The method should meet standards for certain validation parameters. Typical validation characteristics for residue analytical methods that should be considered are: accuracy, recovery, selectivity (specificity), calibration, precision (repeatability, reproducibility) and limit of quantitation (LOQ).

4.1 Analytical methods for the determination of pure active substances and, where appropriate, for relevant degradation products, isomers and impurities of active substances and their additives (e.g. stabilisers) [Ann. IIA, IV.4.1.]

- Information on analytical methods is required concerning degradation products, isomers and impurities of the active substance and additives (e.g. stabilisers) which are of toxicological or ecotoxicological concern (i.e. which are relevant for risk assessment) or which are present in quantities ~ 1 g/kg in the active substance as manufactured.
- The description of the method used should include necessary preliminary treatments, details of the equipment and materials used as well as of other conditions, and a recovery rate, interference by other substances and other information on the specificity of the method, and linearity, a limit of determination, intra-laboratory repeatability and where possible inter-laboratory reproducibility in order to allow an assessment of the accuracy and precision of the analysis of the natural active substance used in the different study reports submitted.
- Recovery rates should be determined at the level of the measurement(s). This means that for the determination of the active ingredient in a formulation or an impurity at a constant level, one recovery rate (measured at the stated composition) is sufficient. In case of the determination of residues or impurities of varying levels the recovery rates should be determined at least at two concentration levels: one near the limit of determination and one at a higher level (usually 2-3 orders of magnitude higher, within the range of the calibration curve).
- An explanation must be provided for any interference occurring which contributes more than ± 3 % to the total quantity determined.
- For the determination of a pure active substance, the calibration range must extend (by at least 20 %) the highest and lowest nominal content of the analyte in relevant analytical solutions. Duplicate calibration determinations must be made at three or more concentrations. Alternatively, five concentrations, each as single measurements, are acceptable. Reports submitted must include the equation of the calibration line and the correlation coefficient and representative and properly labelled documentation from the analysis, e.g. chromatograms.
- For the repeatability in the determination of the pure active substance, in principle a minimum of five determinations must be made. The relative standard deviation (% RSD) must be reported. Outliers identified through an appropriate method (e.g. Dixon's or Grubb's test) may be discarded. Where outliers have been discarded, that fact must be clearly indicated. An explanation as to the reason for the occurrence of individual outliers must be attempted.

4.2 Analytical methods in all relevant environmental media including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following [Ann. IIA, IV.4.2.]:

(a) Soil

- The proposed limit of determination must not exceed a concentration which is of concern with regard to the exposure of non-target organisms. Normally the proposed limit of determination should not exceed 0.05 mg/kg.

(b) Air

- This needs to be submitted e.g. if the substance is volatile (i.e. if the vapour pressure >0.01 Pa) or sprayed, or occurrence in air is otherwise probable.
- The proposed limit of determination must take into account relevant health based limit values or relevant exposure levels.

(c) Water
- The applicant should confirm that the substance itself and any of its degradation products which fall within the definition of pesticides given for parameter 55 in Annex I to Council Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption (OJ No L 229, 30.8.1980, p. 11). This Directive as last amended by Directive 91/692/EEC, OJ No L 377, 31.12.1991, p. 48; and amended by directive 98/83/EC) can be estimated with adequate reliability at the MAC specified in that Directive for individual pesticides.
- Directive 75/440/EC (concerning the quality required of surface water intended for the abstraction of drinking water in the Member States).
- Detection and analytical method(s) for natural water and natural sediment (the natural environment).

Description of an analytical method
Full descriptions of validated methods must be provided. The submitted method description must include the following points:
• Definition of the analyte;
• Apparatus;
• Reagents (including purity as well as full details of standard compounds purity and associated
method of determination or clear reference of origin, if commercially available);
• Analytical procedure including sample processing, extraction, clean up, derivatisation, determination (if appropriate);
• Description of calibration including the use of matrix matched standards (if appropriate);
• Procedure for the calculation of results from raw data;
• Result tables (if results are not presented in separate studies).
The following information should be offered if appropriate:
• Schematic diagram of the analytical procedure;
• Stages where an interruption of the procedure is possible;
• Hazards or precautions required;
• A statement about extraction efficiency of solvents used .

The MAC value is the Maximum Acceptable Concentration.
According to Annex 1, part B to Directive 98/83/EC [9] (to which reference is made in § 4.2 (c)) pesticides should, as regards the quality of water intended for human consumption, be defined as:

– organic insecticides,
– organic herbicides,
– organic fungicides,
– organic nematocides,
– organic acaricides,
– organic algicides,
– organic rodenticides
– organic slimicides,
– related products (such as growth regulators) and their relevant metabolites, degradation and reaction products.

For surface water used for the production of drinking water (see § 4.2 above under (c), second bullet) the criteria apply as stated in 75/440/EEC “concerning the required quality of surface water intended for the production of drinking water in the Member States” (currently included in 2000/60/EC). Also according to Directive 2000/60/EC, establishing a

framework for Community action in the field of water policy, monitoring methods are required for monitoring the quality of surface water and groundwater. Monitoring methods that must be submitted in view of 98/8/EC must meet the criteria as stipulated in 2000/60/EC or derived legislation.
According to 79/869/EEC (also included in 2000/60/EC) pesticides must be determined with “Gas or liquid chromatography after extraction with solvents and purification”.

(d) Animal and human body fluids and tissues
- Where an active substance is classified as toxic or highly toxic, analytical methods must be submitted which allow determination of the active substance at the no adverse effect concentration.

This means that the limit of quantification of the active substance in animal and human body fluids and tissues may not be higher than the concentration of which the effect on mammals is considered unacceptable.
The way in which this should be assessed has not yet been elaborated in EU framework. For this we refer to §2.2.1 of this chapter.

The following guidance applies to the data requirements a to d:
- Methods for the analysis for parent compounds and/or metabolites of concern must be submitted.

For each method and for each relevant representative matrix, the specificity, precision, recovery, and limit of determination must be experimentally determined and reported. In principle, residue methods proposed should be multi-residue methods; a standard multi-residue method must be assessed and reported as to its suitability for residue determination. Where residue methods proposed are not multi-residue methods, or are not compatible with such methods, an alternative method must be proposed. Where this requirement results in an excessive number of methods for individual compounds, a "common moiety method" may be acceptable. For demonstrating the suitability of the method for its purpose, information on performance characteristics should be provided.
Basic validation data are:
• a typical calibration curve for each representative matrix (if studies are necessary);
• the concentration of analyte found in blank samples;
• the concentration level(s) of fortification experiments;
• the number of fortification experiments for each commodity/level combination;
• the mean recovery for each commodity/level combination;
• the relative standard deviation (RSD) of recovery for each commodity/level combination;
• representative, clearly labelled chromatograms (standard, blank, sample at least at the LOQ).

Additional data
The grey-framed text below has been taken from the Biocides Directive 98/8/EC. The numbering in these grey frames follows the section numbering in the Biocides Directive 98/8/EC.

IV. ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION

1. Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant

The above has been elaborated in the TNsG on data requirements.

The text below in grey frames has been taken from the TNsG on data requirements [3]. The numbering in these grey frames follows the numbering of the TNsG on data requirements [3] Chapter 3.

ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION

4.3 Analytical methods including recovery rates and the limits of determination for residues in/on food or feedstuffs and other products where relevant [Ann. IIIA, IV.1.]

- Required if the active substance or the material treated with it is to be used in a manner which may cause contact with food or feedstuffs (e.g. when used for disinfection in food production or transportation, in the food processing industry or catering services), or intended to be placed on, in or near soils in agricultural or horticultural use. This may be the case for product types 1, 2, 3, 6, 8, 14 and 18. In addition, always required for product types 4, 5 and 20.
- Required if the active substance for product type 12 is to be used for the treatment of paper pulp, paper, paperboard or any other product intended for contact with feedstuffs. Food packaging may be covered by Directive 89/109/EC which is under revision to include food-contact paper. It seems that feedstuffs are not covered by other directives.
- Analytical methods for residues in fish and shellfish must be submitted for product type 21.
- Reference can be made to analytical methods covered in paragraph A4.2 above where relevant.

1.2.2 Data requirements product

Common core data
The grey-framed text below has been taken from the Biocides Directive 98/8/EC. The numbering in these grey frames follows the section numbering in the Biocides Directive 98/8/EC.

IV. METHODS OF IDENTIFICATION AND ANALYSIS

4.1. Analytical method for determining the concentration of the active substance(s) in the biocidal product

4.2. In so far as not covered by Annex IIA, paragraph 4.2, analytical methods including recovery rates and the limits of determination for toxicologically and ecotoxicologically relevant components of the biocidal product and/or residues thereof, where relevant in or on the following:
(a) Soil
(b) Air
(c) Water (including drinking water)
(d) Animal and human body fluids and tissues
(e) Treated food or feedingstuffs

The above has been elaborated in the TNsG on data requirements.
The text below in grey frames has been taken from the TNsG on data requirements [3]. The numbering in these grey frames follows the numbering of the TNsG on data

requirements [3] Chapter 2.

4 METHODS OF IDENTIFICATION AND ANALYSIS

- Information on analytical methods is required for assessing compliance with conditions for issuing authorisation for a biocidal product according to Article 5(1c) of the Directive. This information is also required for post-authorisation control and monitoring purposes, and for the assessment of justifications which should be provided for the methods used for generation of data as required for this Directive.
- For products which are difficult to analyse a description of the problems should be given.

4.1 Analytical method for determining the concentrations of the active substance(s) in the biocidal product [Ann. IIB, IV.4.1.]

- A quantitative and, if possible, also a qualitative method for defining the active substance in the product must always be stated.
- In the case of a preparation containing more than one active substance, a method capable of determining each, in the presence of the other, should be provided. If a combined method is not submitted, the technical reasons must be stated.

4.2 In so far as not covered by paragraph A4.2 (data set for the active substance), analytical methods including recovery rates and the limits of determination for toxicologically and ecotoxicologically relevant components of the biocidal product and/or residues thereof, where relevant in or on the following [Ann. IIB, IV.4.2].

Product-type-specific guidance is given here:

a) Soil

- May be required, for example, for product types 2, 3, 8, 10, 11 (preservatives used in cooling towers), 12 (not required for paper mill preservatives) and 21.

b) Air

- Required, for instance when the substance is volatile, or sprayed or occurrence in air is otherwise probable.
- May be required, for example, for product types 8, 11 (preservatives used in cooling towers), 12, 13, 18 and 21.

c) Water (including drinking water)

- Required for all product types if contamination of water cannot be excluded.

d) Animal and human body fluids and tissues

- May be required, for example, for product types 3, 4, 5, 14, 19 and 20.

e) Treated food or feeding stuffs

- Required for product types 3, 4 and 20.

The following is stated in the additional guidance on data requirements regarding methods of identification and analysis [8] regarding the need of additional analytical methods for toxicologically and ecotoxicologically relevant by-products (non-actives) of the biocidal product:

Residue definition

Generally, the CA has to decide, where relevant, which by-products should be monitored in addition to TNsG Part A, 4.2 based on its evaluation on fate and behaviour of the components and the toxicological and ecotoxicological potential.
• By-products of the biocidal product classified as toxic or highly toxic are considered to be the toxicologically relevant components. They must be analysed for monitoring purposes if human exposure cannot be excluded. Validation of the analytical methods employed must be performed.

Limit of quantitation: the LOQ should be set at 0.05 mg/L for body fluids and 0.1 mg/kg for tissues.

• By-products of the biocidal product classified as dangerous for the environment are considered to be the ecotoxicologically relevant components. They must be analysed for monitoring purposes if environmental exposure can not be excluded. Validation of the analytical methods employed must be performed.
Limit of quantitation: the LOQ should correspond to the limits of TNsG Part A, 4.2 (soil, water).

Additional data
The Biocides Directive 98/8/EC requests no additional data for the product regarding the validation of analytical methods.

1.3. Risk assessment

Each analytical method is evaluated separately. A short description of the method with the limit of quantification (LOQ) for the post-registration methods is given in a list of endpoints (see Appendix 1 to this chapter).
The submitted analytical methods are assessesd against the requirements laid down in 98/8/EC and the TNsG on data requirements.

A summary of the (validation) requirements for the analytical methods of the active substance as manufactured and the product are presented in Appendix 3 to this chapter.
A summary of the (validation) requirements for residue-analytical methods is presented in Appendix 4 to this chapter.

Appendix 3 and 4 show that on many points the Biocides Directive contains no specification of the validation requirements. The TNsG on data requirements also lacks a clarification of the validation requirements or these are not clear. For the European evaluation NL therefore – wherever possible - uses the guidance documents for plant protection products as described in §2 of this chapter. Where necessary, expert judgement is used in the evaluation.

MAC values (parameter values) for pesticides in drinking water are specified in Directive 98/83/EC:

The parametric value (0.1 µg/l) applies to each individual pesticide. In the case of aldrin, dieldrin, heptachlor and heptachlor epoxide the parametric value is 0.030 µg/l.

„Pesticiden — total” means the sum of all individual pesticides detected and quantified in the monitoring procedure (parameter value 0.5 µg/l).

1.4. Approval

According to the Directive of the European Parliament and the Council of 16 February 1998 concerning the placing of biocidal products on the market (98/8/EC) it should be investigated whether biocides have, when approved, no unacceptable effect on the environment and in particular the health humans and animals (consideration 8) if used properly for the envisaged purpose, in the light of the current scientific and technical knowledge.
Article 5, 1, c stipulates that Member States only authorise a biocide if the product

Article 5.1
(c) the nature and quantity of its active substances and, where appropriate, any toxicologically or ecotoxicologically significant impurities and co-formulants, and its residues of toxicological or environmental significance, which result from authorised uses, can be determined according to the relevant requirements in Annex IIA, IIB, IIIA, IIIB, IVA or IVB;

1.4.1 Evaluation
The principles for the evaluation as regards the effects on humans, animals and the environment are presented in the Common Principles (Annex VI to 98/8/EC). None of these principles concerns analytical methods.

1.4.2 Decision making
The principles for decision making as regards the effects on humans, animals and the environment are presented in the Common Principles (Annex VI to 98/8/EC). None of these principles concerns analytical methods.

Chapter 5.1 of the TNsG on annex I inclusion [2] describes the General Criteria for inclusion in Annexes I, IA and/or IB.
The text below in the grey frames, including section numbering, has been taken from the TNsG on Annex I inclusion.

Methods of Analysis
- The method of analysis of the active substance as manufactured and for the determination of impurities and co-formulants of toxicological, ecotoxicological or environmental concern or which are present in quantities > 1g/kg in the active substance as manufactured has been validated and shown to be sufficiently specific, linear, accurate and precise.
- The method of analysis of the active substance's residues of toxicological or environmental significance, which result from authorised uses, has been validated and shown to be sufficiently specific, linear, accurate and precise.
- The method for analysis in environmental matrices, as appropriate, must have been validated and shown to be sufficiently sensitive with respect to the levels of concern.

1.5. Developments
Biocidal products are now being evaluated in EU framework. The activities in this context will result in amendments of the existing TNsGs and the first amendments are laid down in additional guidance [8].

3. APPENDICES

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Appendix 1 List of Endpoints

Methods of Analysis

Analytical methods for the active substance

Technical active substance (principle of method)	
Impurities in technical active substance (principle of method)	

Analytical methods for residues

Soil (principle of method and LOQ)	
Air (principle of method and LOQ)	
Water (principle of method and LOQ)	
Body fluids and tissues (principle of method and LOQ)	
Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	

Appendix 2 Data requirements analytical methods active substance and product

Doc. III-A Section No.	BPD Annex Point	Document III-A: Study Summaries - Active Substance
4.	IV.	ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION
4.1	IV.4.1	Analytical methods for the determination of pure active substance and, where appropriate, for relevant degradation products, isomers and impurities of the active substance and additives (e.g. stabilisers)
4.2	IV.4.2	Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following: (a) Soil (b) Air (c) Water: the applicant should confirm that the substance itself and any of its degradation products which fall within the definition of pesticides given for parameter 55 in Annex I to Council Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption (8**) can be estimated with adequate reliability at the MAC specified in that Directive for individual pesticides (d) Animal and human body fluids and tissues
4.3	*IV.1*	*Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant*
Doc. III-B Section No.	BPD Annex point	Document III-B: Study Summaries - Biocidal Product
4.	IV.	METHODS OF IDENTIFICATION AND ANALYSIS
4.1	IV.4.1	Analytical method for determining the concentrations of the active substance(s) in the biocidal product
4.2	IV.4.2	In so far as not covered by Annex IIA, paragraph 4.2, analytical methods including recovery rates and the limits of determination for toxicologically and ecotoxicologically relevant components of the biocidal product and/or residues thereof, where relevant in or on the following: (a) Soil (b) Air (c) Water (including drinking water) (d) Animal and human body fluids and tissues (e) Treated food or feedingstuffs

Appendix 3 Summary of the validation requirements for the analytical methods for biocides (98/8/EC and the TNsG on data requirements): Common core data and Additional data (EU framework)

Validation requirement	In the active substance as manufactured	Product
Yield (= recovery, ≈ accuracy)	For impurities in the active substance as manufactured: -In case of a constant concentration at 1 concentration level -In case of varying concentration at LOQ level and at a higher concentration (usually 2-3 orders higher but still within the calibration curve)	For the active substance in the product: - 1 concentration level (specification level)
Limit of quantification (= LOQ = limit of determination)	yes	
Interference	Statement required if the interference > about 3 % of the total amount determined.	
Specificity	yes	
Linearity	For the determination of the calibration curve of the pure active substance it applies that this must be 20 % wider than the highest and lowest nominal concentration in relevant solutions - duplicate measurements at at least 3 concentrations or - single measurements at 5 concentrations Report comparison of calibration line and coefficient of correlation.	
(intra laboratory) repeatability	At least 5 analyses must be carried out to determine the repeatability of the pure active substance. Report RSD.	
(inter-laboratory) reproducibility	Where possible	

Appendix 4 Summary of the validation requirements for the residue-analytical methods for biocides (98/8/EC and the TNsG on data requirements): Common core data and Additional data (EU framework)

Requirement	Residue-analytical methods (for the compounds included in the residue definition)	Residue-analytical methods (of (eco)toxicologically relevant components of the product)
Analytical method in the following matrix:	Only where relevant <u>Soil</u> <u>Air</u>: only required if the substance is volatile (vapour pressure > 0.01 Pa) or if the substance is sprayed or occurrence in air is otherwise possible. <u>Water</u> Drinking water, surface water used for drinking water, 'natural' water and 'natural' sediment. <u>Animal and human body fluids and tissues</u> Only if the a.s. is (very) toxic. *<u>Food or feedstuffs and other products where applicable</u>* *Required if the a.s. or the treated product can get into contact with food, feedstuffs, agricultural or horticultural soil.* *This may be the case for the a.s. used in product types 1, 2, 3, 6, 8, 14 and 18. Always required for the a.s. used in product types 4, 5 and 20.* *Also required for the a.s. used in product type 12 if the treated material (such as paper pulp) is used for packing food.* *Analytical methods for fish and shellfish are required for the a.s. used in product type 21 (anti-fouling products)*	Only where relevant <u>Soil:</u> may be required for e.g. product types: 2, 3, 8, 10, 11 (conservation products used in cooling towers), 12 (not required for conservation products used in paper factories) and 21. <u>Air</u>: required, e.g. if the substance is volatile or if the substance is sprayed or may in a different way get into the air. May be required for e.g. product types 8, 11 (conservation products used in cooling towers), 12, 13, 18 and 21 <u>Water</u> Required for all product types where contamination of water cannot be ruled out. <u>Animal and human body fluids and tissues</u> May be required for e.g. product types 3, 4, 5, 14, 19 and 20 <u>Food or feedstuffs and other products, where applicable</u> Required for product types 3, 4 and 20

Yield (= recovery ≈ accuracy)	AT LOQ level and at a higher concentration (usually 2-3 orders higher but still within the calibration curve)	yes

Limit of quantification (= LOQ = limit of determination)	Yes Soil: LOQ ≤ 0.05 mg/kg, but not above the concentration that gives cause for concern as regards exposure of non-target organisms. Air: take relevant health based reference values or relevant exposure levels into account Water ≤ MAC = “parameter value” Animal and human body fluids and tissues ≤ no adverse effect concentration *Food and feedstuffs and other products, where applicable*	Yes
Interference		
Specificity	Yes	
Linearity		
(intra laboratory) repeatability	Yes	
(inter-laboratory) reproducibility		
Multiresidue method	Yes	
‘Common moiety method’	May be acceptable	

Appendix 5 Summary of the most important requirements for methods in technical material and formulations based on guidance documents for plant protection products (NL framework)

Required	Technical active substance (a.s.)	Formulations (biocidal products)
Description of the method	Complete description required	Complete description required
Analytical method based on generally available laboratory equipment and laboratory facilities	Not required	Not required, however strongly requested.
Avoid dangerous chemicals	Not required	Not required, however if used the necessity must be explained
Derivatisation	Permitted, but the necessity must be explained when used; supplementary validation is required	Permitted, but the necessity must be explained when used; supplementary validation is required
Multi Residue Method	Not required	Not required
Validation report in each matrix	Only for the technical material	For each formulation type
Validation report for compounds	- Active substance - Significant impurities - Relevant impurities	- Active substance - Relevant impurities
Confirmation method	Required when proposed method is not specific	Required for relevant impurities when the proposed method is not specific
Independent laboratory validation (ILV)	Not required	Not required
Limit Of Quantification (LOQ)	a.s.: not required impurities: required, 0.1% w/w for significant and specification level for relevant impurities	a.s.: not required impurities: required for relevant impurities
Range of the method	a.s.: from lowest to highest concentration (+/- 20%) in technical material impurities: from 0.1% w/w (or specification for relevant impurities) to highest concentration (+/- 20%) in technical material.	a.s.: from lowest to highest concentration (+/- 20%) in technical material. impurities: for relevant impurities from specification to highest concentration (+/- 20%) in technical material
Calibration model (linearity or other)	Required Preferably expressed in mg/kg technical a.s. Based on 5 concentration levels or based on 3 duplicate concentration levels Correlation coefficient ≥ 0.99	Required Preferably expressed in mg/kg formulation Based on 5 concentration levels or based on 3 duplicate concentration levels Correlation coefficient ≥ 0.99

Required	Technical active substance (a.s.)	Formulations (biocidal products)
Interference of matrix	maximum 3% at LOQ	maximum 3% at LOQ
Specificity and identity	Required, it must be possible to determine isomers separately, identity can be determined once	Required, it must be possible to determine isomers separately, in case more active substances are present, it must be possible to analyse these separately
Accuracy / average recovery	a.s.: not required impurities: required (n ≥ 2) at level in relation to specification 70-110 %	a.s.: required (n ≥ 2) at level of formulations impurities: required for relevant impurities (n ≥ 2) See § 2.3.1 for requirements
Repeatability (relative standard deviation)	Required, (n ≥ 5), should meet Horwitz, see § 2.3.1	Required, (n ≥ 5), should meet Horwitz, see § 2.3.1

Appendix 6 Summary of the most important requirements for pre- and post-registration methods for residue-analytical methods based on guidance documents for plant protection products(NL framework)

Required	Pre-registration	Post-registration
Description of the method	Complete description required	Complete description required
Analytical method based on generally available laboratory equipment and laboratory facilities	Not required	Required
Avoidance dangerous chemicals	Not required	Required, the use of Diazomethane (or its salts) for derivatisation is not permitted, unless it is demonstrated that there is no other possibility; the use of an LCMS should also be considered.
Derivatisation	Permitted, but the necessity must be explained when used; supplementary validation is required	Permitted, but the necessity must be explained when used; supplementary validation is required
Multi-Residue Method (MRM)	Not required	Required, unless it can be demonstrated that the analyte cannot be included in an (existing) multi-residue method. A specific method is required in that case.
Validation in each matrix	Required, but for the residue-analytical methods for plant products limited validation is sufficient within the same crop group (additional validation: average recovery / accuracy based on n ≥ 2 concentration levels and repeatability / precision based on n ≥ 3 replicates per level)	Required, but for the residue-analytical methods for plant products one sample matrix per crop group is sufficient, see RIVM [10].
Validation report for compounds	all components included in the residue definition	all components included in the residue definition
Confirmation method	Recommended where method is not specific	Required, unless the first method is sufficiently specific to determine identity
Independent laboratory validation (ILV)	Not required	Required for methods of plant and/or animal origin for the residue-analytical methods for plant products validation of 2 crop groups is sufficient; for the residue-analytical methods for animal products validation of 2 animal products is sufficient
Limit Of Quantification (LOQ)	Required Plant/animal: LOQ at 'relevant level' Soil: LOQ ≤ 0.05 mg/kg or ≤ NOEL or LC_{50} Drinking water: LOQ ≤ 0.1 µg/l	Required Plant/animal: LOQ <= 0.1 mg/kg or LOQ = 0.5-1x MRL where MRL is lower than 0.1 mg/kg. Soil: LOQ ≤ 0.05 mg/kg Drinking water: LOQ ≤ 0.1 µg/l

Required	Pre-registration	Post-registration
	Surface water: LOQ ≤ $NOEC_{daphnia}$ or $EC_{50\ algae}$ µg/l Air: not applicable	Surface water: LOQ ≤ 0.1 µg/l and < $NOEC_{daphnia}$ of $EC_{50\ algae}$ µg/l Air: see Sanco/825/00 for calculation of the required LOQ Body fluids and tissues: 0.05 mg/l (blood); 0.1 mg/kg (meat or liver)
Range of the method	Plant/animal: LOQ-10xLOQ or LOQ-expected residue levels/MRL (whichever is widest) Other: LOQ-10xLOQ	Plant/animal: LOQ-10xLOQ or LOQ/MRL (whichever is widest) Other: LOQ-10xLOQ
Calibration model (linearity or other)	Required Preferably expressed in mg/kg matrix Based on 5 concentration levels or based on 3 duplicate concentration levels Correlation coefficient ≥ 0.99	Required Preferably expressed in mg/kg matrix Based on 5 concentration levels or based on 3 duplicate concentration levels Correlation coefficient ≥ 0.99
Interference of matrix	Required, < 0.3*LOQ (n ≥ 2)	Required, < 0.3*LOQ (n ≥ 2)
Specificity and identity	Required (identification) Interference of metabolites, isomers etc. if necessary for risk assessment	Required (identification)
Accuracy / average recovery	Required n ≥ 5 at 2 concentration levels (LOQ and 10*LOQ) 70-110 % Plant/animal: read *expected residue levels/MRL* instead of *10xLOQ* (whichever is highest)	Required n ≥ 5 at 2 concentration levels (LOQ and 10*LOQ) 70-110 % Plant/animal: read *MRL* (if any) instead of *10xLOQ* (whichever is highest)
Repeatability (relative standard deviation)	Required n ≥ 5 at 2 concentration levels (LOQ and 10*LOQ) Plant/animal: read *expected residue levels/MRL* instead of *10xLOQ* (whichever is highest) RSD < 20 %	Required n ≥ 5 at 2 concentration levels (LOQ and 10*LOQ) Plant/animal: read *expected MRL* instead of *10xLOQ* (whichever is highest) RSD < 20 %
Internal standard	No specific requirements	Where used to calculate concentration, it should be demonstrated that the recovery and repeatability of the internal standard are comparable to the analytes

4. REFERENCES

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“Methods of Identification and Analysis”. This document can be downloaded via the ECB website: http://ecb.jrc.ec.europa.eu/biocides/

10 Classification of crops grown in or imported into the European Union for pesticide residue assessment, RIVM report 613340006/2003