Evaluation Manual for the Authorisation of plant protection products and biocides

NL part

Biocides

Chapter 3 Analytical Methods

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Board
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Chapter 3 Analytical methods
Category: biocides

GENERAL INTRODUCTION

This chapter describes the data requirements for the aspect analytical methods and how these are evaluated for the NL framework (§2 - §2.5).

2. NL FRAMEWORK

The NL framework (§2 - §2.5) describes the authorisation of biocidal products based on existing substances, included in Annex I, and new active substances. A new substance is a substance not authorised in any of the Member States of the EU on 14 May 2000.
The pesticide that contains such substances may be authorised if the criteria laid down in the Wgb (Plant Protection Product and Biocide Act) 2007 [1] are met.
The product is evaluated according to the Rgb (Plant Protection Products and Biocides Regulations) [2]. The evaluation dossiers must meet the conditions of Annex IIA, IIB, IIIA and IIIB to 98/8/EC.

The NL framework describes the data requirements (§2.2), evaluation methodologies (§2.3), criteria and trigger values (§2.4) for which specific rules apply in the national approvals framework or where the national framework has been elaborated in more detail than the EU framework.

The NL procedure described in §2 - §2.5 of this chapter is used for evaluation of a substance for inclusion in Annex I in case no EU procedure has been described

2.1. Introduction

This chapter describes the analytical methods for which specific rules apply in the national framework or where the national framework has been elaborated in more detail than the EU framework.
The specific rules are applicable to the analytical method for water and are related to the assessment according to the the drinking water criteria in the environmental evaluation.
The other data requirements and evaluation methodologies of the aspect analytical methods meet the criteria as laid down in the EU framework.

As already indicated under EU framework, the validation requirements and evaluation methodologies as described in the guidance documents concerning the analytical methods for plant protection products could also apply to biocidal products. For the NL framework the validation requirements and methodologies included in these guidance documents are (where possible) taken as starting point for the analytical methods for biocides.

2.2. Data requirements

The data requirements for analytical methods are as described under EU framework; see §1.2 of B3. Analytical methods of the EU part of the Evaluation Manual (biocides).
A specification of the validation requirements is on many points lacking from the Biocides Directive 98/8/EC. A clarification of the validation requirements is neither given in the guidance document TNsG Data Requirements or these are not clear.

A summary of these (validation) requirements for the analytical methods of the active substance as manufactured and the product are presented in Appendix 5 to this chapter.
A summary of the residue-analytical methods is presented in Appendix 6 to this chapter.

Post-registration confirmatory methods

Sanco/825/00 [http://ec.europa.eu/food/plant/protection/resources/guide_doc_825-00_rev7_en.pdf] does not clearly indicate how a confirmatory method must be evaluated and what the validation requirements are. In the Netherlands the following minimal data requirements have been laid down for the confirmatory method:

Subject	*Requirement*
The confirmatory method should at least have the same LOQ as the original method	Five times a measurement in the matrix concerned at LOQ level
The confirmatory method should have a clearly different selectivity than the original method (example: an HPLC separation with a C8 or a C18 column will hardly ever give sufficient difference in selectivity)	For each matrix* a chromatogram per method from which the difference in selectivity can be read. In case one of the methods is not based on chromatography, the difference in selectivity should be described
No confirmationconfirmatory method is required if the method as such is sufficiently selective as result of the use of mass selective detection	The choice of the mass fragments should be explained, if applicable provided with a mass selective chromatogram in blank as well as in matrix

*) See Sanco/825/00. In case of plant matrices, data on only one crop need to be submitted if several crops in the application belong to 1 representative crop group.

2.2.1 Data requirements active substance

Data requirements on analytical methods for the determination of the concentration of the active substance in the active substance as manufactured and for determination of significant and/or relevant degradation products, isomers, impurities and additives in the active substance as manufactured.
(EU IIA, IV.4.1)

Methods for determination of the concentration pure active substance in the active substance as manufactured are described as pre- and also as post-registration method in the Guidance document Sanco/3030/99 [http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc13_en.pdf]. The requirements are the same for both purposes.

CIPAC and AOAC methods can be used without a full validation, only selectivity should be determined for the applicable formulation. CIPAC methods can be requested via http://www.cipac.org/.

Accuracy

According to Sanco/3030/99 determination of the accuracy is not required for analysis of the active substance in the active substance as manufactured. This means that this accuracy only needs to be determined for the significant and relevant impurities.
Where the method for the impurities in the technical substance as manufactured does not include extraction or other selective work-up (from the technical substance as manufactured) before the analysis, a statement, e.g. an estimation of the range based on the analytical technique used, is sufficient for accuracy.

Isomers

Where the active substance contains isomers, it should be possible to identify each isomer separately (required for risk assessment and identification of the active

substance).

Specificity
The specificity of the proposed methods should be demonstrated and reported.
The extent to which the study results are affected by other substances present in the active substance as manufactured (e.g. isomers, impurities or additives) should be established as well.
Although interferences by other components when determining the accuracy of the methods proposed for analysis of pure active substance in the active substance as manufactured, can be qualified as systematic deviations, a justification should be provided in all cases in which the interfering components constitute more than ± 3 % of the total concentration determined.
For methods for determination of impurities it should also be shown to what extent the results have been affected by interference by other components.

Determination limit of quantification, LOQ
The LOQ for relevant and/or significant impurities should according to Sanco/3030/99 be determined for all relevant and/or significant impurities. The LOQ may not be determined by a calculation based on the signal/noise ratio of a detector. The LOQ is defined as the concentration that can still be determined with sufficient certainty. The compound under test should for this purpose be added (standard addition) and in case recovery and repeatability of these measurements are acceptable, the added concentration can be accepted as the LOQ. The procedure of the standard addition (if applicable) should be included in the description of the method.

Data requirements analytical methods residues
(EU: IIA, IV.4.2., IIIA IV.1)

A Pre-registration analytical methods residues
The requirements are described in Sanco/3029/99
[http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc12_en.pdf].

Some important points in this guidance document are:

- a non-specific method is generally not acceptable
- derivatisation is permitted but requires supplemental validation
- a ‘common moiety’ method, where a specific group of a molecule is determined instead of the molecule itself, is generally not acceptable
- validation data should be submitted for all matrices that are to be analysed, for all components included in the residue definition.

B Post-registration analytical methods residues
The validation requirements for the residue-analytical methods are described in the document Sanco/825/00
[http://ec.europa.eu/food/plant/protection/resources/guide_doc_825-00_rev7_en.pdf].

The following aspects should be taken into consideration for the residue analytical methods in soil, air, water, animal and human body fluids and tissues, and treated food or feedstuffs:

Linearity
The range to be studied is at least LOQ-MRL or LOQ-10 x LOQ (whichever is widest). It is important to check whether the LOQ, the MRL and the concentrations at which repeatability and recovery have been established fall within the studied range. Extrapolation can only be accepted after a sound justification.

Specificity
The specificity of the method must be such that all components included in the residue definition can be determined, were necessary via application of an extra confirmatory method.

Determination limit of quantification (LOQ)
The LOQ may not be determined by a calculation based on the signal/noise ratio of a detector. The LOQ is defined as the concentration that can be determined with sufficient certainty *in a certain matrix,* for which the compound under test should be added (standard addition) to the particular matrix. Where recovery and repeatability of these measurements are acceptable, the added concentration can be accepted as the LOQ. The procedure of the standard addition (if applicable) should be included in the description of the method.

The following does also apply to the residue-analytical methods in animal and human body fluids and tissues, and treated food and feedstuffs:.

Independent lab validation, ILV
The proposed analytical method(s) for animal and plant products must in addition be validated by an independent laboratory. This may be a laboratory of the same applicant where it should be made plausible that both laboratories have had no contact whatsoever about the particular method, e.g., by submission of a statement of the laboratory managers.
At least 2 matrices (where an MRL has been laid down for animal products as well, 2 matrices should be studied there as well) should be studied, including one with a high water content (for plant material). An ILV is not required where reference can be made to a published and accepted multiresidue-analytical method validated in the relevant matrices.

Soil
(EU: IIA, IV.4.2., IIIA IV.1 a)
The proposed LOQ may not be higher than a concentration that constitutes danger after exposure of non-target species or that causes phytotoxic effects. Normally the proposed LOQ may not be higher than 0.05 mg/kg.
Soil type and origin of the soil should be reported, e.g. by determination of pH, clay content, and organic carbon content.

b) Air
(EU: IIA, IV.4.2., IIIA IV.1 b)

> The proposed LOQ should take the relevant health-based reference values or the relevant exposure levels into account. The relevant exposure level can be calculated from the AOEL, see Sanco/825/00 [http://ec.europa.eu/food/plant/protection/resources/guide_doc_825-00_rev7_en.pdf].

Air of room temperature and normal air humidity should be used for method validation as well as air of 35 °C and 80% air humidity. Where the results at 35 °C and 80% air

humidity are satisfactory, measurement at room temperature is not necessary.

If the provided method for determination in air as such is insufficiently selective (not suitable to determine the identity of the compound), the request for a confirmatory method can be discarded if the analytical method in water can be used for this purpose. In that case the methods should be sufficiently different, see Sanco/825/00.

Water
(EU: IIA, IV.4.2., IIIA IV.1 a)

The analytical method must be validated separately for drinking water as well as for surface water. If the method for surface water has been sufficiently validated, a statement would be sufficient that a validated method for drinking water is not required. This will be assessed on a case-by-case basis.

In the case of surface water, the origin of the water sample and the characteristics (pH, DOC, hardness, salt concentration…) should be reported.

For drinking water the LOQ may not be higher than 0.1 µg/l.
For surface water the LOQ may not be higher than the concentration of which the effect on non-target species is considered unacceptable.
The concentration required to determine the LOQ for surface water depends on the target species and can be derived from toxicity tests (LC50, NOEC or EC50) see Sanco/825/00 [http://ec.europa.eu/food/plant/protection/resources/guide_doc_825-00_rev7_en.pdf].

For the Dutch evaluation the Regeling milieukwaliteitseisen gevaarlijke stoffen oppervlaktewateren (Regulation Environmental Quality Requirements Dangerous Substances Surface Waters [3], as laid down in the Netherlands by virtue of Directive 76/464/EEC is taken into account as well. This in particular is applicable to the LOQ required to assess the quality of surface water against the environmental quality criteria as included for some specific pesticides.

Animal and human body fluids and tissues
(EU: IIA, IV.4.2., IIIA IV.1 d)
If the active substance under assessment is known to be toxic or very toxic and also has been classified as such, suitable analytical methods must be provided.
The TNsG on data requirements, however, state that the LOQ for the analytical method of the active substance in animal and human body fluids and tissues may not be higher than the concentration at which no adverse effects on mammals are observed, for which the lowest NOAEL (no observed adverse effect level) from the list of endpoints is taken.

The LOQ for the analytical method of the active substance in animal and human body fluids and tissues is according to Sanco/825/00: 0.05 mg/l for blood and 0.1 mg/kg for tissue (meat or liver) unless the internal NOAEL < 0.1 mg/kg bw/day. The LOQ for these substances will have to be estimated on the basis of expert judgement.

Animal products to be validated
An analytical method is only required for animal material for which an MRL and residue definition have been laid down.
Where a residue definition for animal products has been laid down, the following materials must be validated:
- milk
- eggs

- meat
- fat (but only if the log Pow (other name: log Kow) is > 3 and the metabolism studies indicate that there are clear residues above 0.01 mg/kg in fat
- kidneys and liver, only if a specific MRL had been laid down.

Treated food or feedstuffs
(EU IIIA IV.1.)
This concerns additional data that may be required if the biocide can get into contact with food, feedstuffs, agricultural and horticultural soil.

Plant products to be validated
Crops are classified into 4 representative groups:
- cereals and dry crops (e.g. barley, wheat, rye, oats)
- crops with a high water concentration (e.g. lettuce, tomato, cherry, strawberry)
- crops with a high fat content (e.g. nuts, oilseed rape, linseed)
- fruit with a high acid concentration (e.g. lemon, orange, grapefruit)

It is possible to deviate from this classification for specific crops that are very difficult to analyse such as hops and tea. A separate validation must then be carried out for these crops. Where the EU guidance document provides no clarity, a report prepared by RIVM is used in which it is for all crops indicated to which category they belong [4]. This document is not a new approach but tries to provide clarity about the different group and category classifications.

2.2.2 Data requirements product
Date requirements analytical methods for the active substance in the product.
(EU IIB, IV.4.1)
Methods for determination of the concentration active substance in the product for pre- as well as post-registration methods are described in Guidance document Sanco/3030/99 [http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc13_en.pdf]. The requirements, however, are the same for both purposes.

Some important issues in the guidance document:
- a ‘common moiety’ method, which determines a specific group of a molecule instead of the molecule itself, is generally not acceptable
- derivatisation is permitted but requires additional validation
- it should be possible to determine active substance/relevant impurities
- it should be possible to determine the identity of relevant impurities in the product

CIPAC and AOAC methods can be used without a full validation, only the selectivity should be determined for the formulation in question. if the validation has already been carried out by these organisations in the requested formulation type. CIPAC methods can be requested via http://www.cipac.org/.

An analytical method for determination of the active substance in the product must in principle be validated for each formulation type.

Specificity
The specificity of the proposed methods should be demonstrated and reported. The extent to which the results are affected by other substances present in the active substance that are present in the product should be established as well.
Although interferences by other components when determining the accuracy of the

methods proposed can be qualified as systematic deviations, a justification should be provided in all cases in which the interfering components constitute more than ± 3 % of the total concentration that has been determined.

2.3. Risk assessment

The risk is evaluated in accordance with the European regulations, see § 1.3 of 3. Analytical methods, EU part.

It should be evaluated whether the submitted analytical methods meet the validation requirements as laid down in the guidance documens for plant protection products, see §1.1 of 3. Analytical methods of the EU part of the Evaluation Manual (biocides). Where necessary, expert judgement is used in the evaluation.

Appendix 5 to this chapter contains a summary of the validation requirements for the analytical methods of the active substance as manufactured and the product as given in these guidance documents and Appendix 6 to this chapter contains a summary of the residue-analytical methods.

If the number of points cannot be met as result of an outlier test (Dixons or Grubbs test) it shall be judged on a case-by-case basis whether this is acceptable. Only one outlier may be present per series of the same data (e.g. repeatability at 1 concentration level). The cause of the outlier and the extent to which the outlier affected the results will be taken into account in the evaluation.

2.3.1 Analytical methods for the active substance and the product

It should be investigated whether the submitted analytical methods meet the requirements laid down in the guidance document Sanco/3030/99 [http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc13_en.pdf]. This is applicable to the analytical methods determining the concentration of the active substance and impurities in technical material and of active substance and relevant impurities in preparations for pre- and post-registration purposes.

Validation is not required where CIPAC or AOAC methods are used for determination of the active substance in the technical material or the product, however selectivity should be determined. Supplementary validation may only be requested where interferences exceed 3%.

It is in principle not permitted that the method used for production of the results deviates from the method as it has been validated (e.g. as regards the calibration line, fewer points used than for validation) but this may be acceptable where a sound justification is given.

The following validation requirements apply in addition to guidance document Sanco/3030/99 [http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc13_en.pdf].

Repeatability

The repeatability of the method for analysis of the concentration active substance and the significant and relevant impurities (in technical material as well as in the product) may per compound not be higher than the Horwitz value. This Horwitz value gives an estimation of the acceptable repeatability on the basis of the concentration (the concentration should always be expressed in fractions).

The Horwitz formula is:

RSD(R) = 2^(1-0.5*logC)
RSD(r) = RSD(R) * 0,67

With: RSD(R) = relative standard deviation between laboratories, repeatability
RSD(r) = relative standard deviation, repeatability

See document Sanco/3030/99 for the Horwitz equation and further explanation.
The table below includes a large number of values.

%	conc.	RSD (r)	%	conc.	RSD (r)
100	1	1.34	10	0.1	1.90
95	0.95	1.35	9	0.09	1.93
90	0.9	1.36	8	0.08	1.96
85	0.85	1.37	7	0.07	2.00
80	0.8	1.39	6	0.06	2.05
75	0.75	1.40	5	0.05	2.10
70	0.7	1.41	4	0.04	2.18
65	0.65	1.43	3	0.03	2.27
60	0.6	1.45	2	0.02	2.41
55	0.55	1.47	1	0.01	2.68
50	0.5	1.49	0.9	0.009	2.72
45	0.45	1.51	0.8	0.008	2.77
40	0.4	1.54	0.7	0.007	2.83
35	0.35	1.57	0.6	0.006	2.89
30	0.3	1.61	0.5	0.005	2.97
25	0.25	1.65	0.4	0.004	3.08
20	0.2	1.71	0.3	0.003	3.21
15	0.15	1.78	0.2	0.002	3.41
10	0.1	1.90	0.1	0.001	3.79

Accuracy – impurities in technical substance as manufactured
The guidance document contains no clear description of the requirements to be met as regards accuracy (Sanco/3030/99); 70-110% is therefore taken for average recovery (as in Sanco/3029/99).

Accuracy – product
Accuracy should meet the following requirements:

% active substance	average recovery %	% relevant impurity	average recovery %
> 10	98-102		
1-10	97-103	>1	90-110
0.1-1	95-105	0.1-1	80-120
<0.1	90-110	<0.1	75-125

2.3.2 Pre-registration analytical methods residues
It should be investigated whether the submitted analytical methods meet the requirements as laid down in the guidance document Sanco/3029/99 [http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc12_en.pdf].

This concerns residue-analytical methods for the active substance and (relevant/significant) metabolites in:
- and/or on plants, plant products, foodstuffs (of plant or animal origin) and feedstuffs
- soil
- water
- air
- body fluids and tissues

for pre-registration purposes as regards:
- residue studies on which the risk assessments for public health are based.
- studies into fate and behaviour of the active substance in food, environment, ecotoxicology and toxicology.

It is in principle not permitted that the method used for production of the results deviates from the method as it has been validated (e.g. as regards the calibration line, fewer points used than for validation) but this may be acceptable where a sound justification is given.

Some important points in this guidance document are:
- a non-specific method is generally not acceptable
- derivatisation is permitted but requires additional validation
- a 'common moiety' method, which determines a specific group of a molecule instead of the molecule itself, is generally not acceptable
- validation data should be submitted for all matrices that are to be analysed, for all components of the residue definition.

Validation requirements
A review of the validation requirements for the residue-analytical methods is given in Appendix 2 to Sanco/3029/99 [http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc12_en.pdf] and Appendix 6 to this chapter.

Post-registration analytical method residues
It should be investigated whether the submitted analytical methods meet the requirements as laid down in the guidance document Sanco/825/00.

This concerns residue-analytical methods for the active substance for post-registration purposes (enforcement and monitoring) in:
- and/or on plants, plant products, foodstuffs (of plant or animal origin) and feedstuffs
- soil
- water
- air
- body fluids and tissues

There are additional requirements for the methods for post-registration use, the so-called monitoring or enforcement methods; these are described in Sanco/825/00.
The most important are:
- The methods may only require generally available laboratory equipment and facilities.
- Harmful chemicals should be avoided where possible. The use of chloroform and benzene is not permitted. The use of diazomethane should, whenever possible, be avoided as well.
- An Independent Lab Validation (ILV) must be carried out for the post-registration method for residues in plant and animal material to demonstrate that the method is

also effective in a different laboratory.

See also Sanco/10476/2003 “Quality control procedures for pesticide residues analysis” [5] for guidelines for the validation of post-registration methods.

Where an analytical method uses chromatographic techniques, representative chromatograms must be provided: blank, standard, sample blank and sample added at the LOQ. The chromatograms should be clearly labelled with at least: sample description, identification of all relevant compounds in the chromatogram and scale, where necessary.

2.4. Approval

A pesticide will only be authorised if the pesticide and its metabolite(s), and if used in compliance with the provisions in or by virtue of the According to the Wgb 2006 [1], in compliance with the Statutory Use Instructions and Directions for Use.

Artikel 28. Toelatingsvoorwaarden

1. Een gewasbeschermingsmiddel wordt toegelaten indien het gewasbeschermingsmiddel voldoet aan de voorwaarde dat:
de fysische en chemische eigenschappen van het gewasbeschermingsmiddel zijn vastgesteld en voor juist gebruik en adequate opslag van het middel aanvaardbaar zijn geacht,

Section 2, b. the content of the active substance or substances and the further composition, colour, form, finishing, packaging and specifications and statements on or with the packaging comply with the regulations laid down by the Minister concerned.

The evaluation of products on the basis of existing active substances already included in Annex I, or new substances, has been laid down in the Decision Common Principles Evaluation Biocides (Bgbbbio) in which it is elaborated that these products are evaluated in compliance with the Common Principles.

2.4.1 Criteria and trigger values

The criteria and trigger values are in accordance with the European regulations, see § 1.4 of of 3. Analytical methods of the EU part of the Evaluation Manual (biocides).

2.4.2 Decision on approval

Decisions on approval are taken in accordance with the European regulations, see § 1.4 of 3. Analytical methods of the EU part of the Evaluation Manual (biocides).

The LOQ of the residue-analytical method for post-registration is (if applicable) compared with the MRL as laid down with the authorisation in question for the aspect residues.

2.5 Developments

The Keuringsdienst van Waren (Food and Consumer Product Safety Authority, VWA) is currently developing a multiresidue method with LC/MS. This will be published after validation.

The term ‘relevant impurities’ gives cause for discussion because it has not clearly be defined what would have to be considered as relevant. The FAO is currently further defining this term.

Proposals for amendment of document Sanco/825/00 [http://ec.europa.eu/food/plant/protection/resources/guide_doc_825-00_rev7_en.pdf] may be forthcoming, which would make it also suitable for multiresidue methods using LC/MS.

3. APPENDICES

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 Soil Air: 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. Water Drinking water, surface water used for drinking water, 'natural' water and 'natural' sediment. Animal and human body fluids and tissues Only if the a.s. is (very) toxic. *Food or feedstuffs and other products where applicable* *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 Soil: 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. Air: 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 Water Required for all product types where contamination of water cannot be ruled out. Animal and human body fluids and tissues May be required for e.g. product types 3, 4, 5, 14, 19 and 20 Food or feedstuffs and other products, where applicable 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
Confirmatory 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 [4]
Validation report for compounds	all components included in the residue definition	all components included in the residue definition
Confirmatory 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

1 Wgb (Plant Protection Product and Biocide Act 2007. See www.overheid.nl/wetten.
2 Rgb (Plant Protection Product and biocide Regulations 2007. See www.overheid.nl/wetten.
3 Regulation environmental quality criteria dangerous substances surface waters. NL acts, orders etc can be obtained via http://wetten.overheid.nl/
4 Classification of crops grown in or imported into the European Union for pesticide residue assessment. Report 613340006/2003. RIVM, the Netherlands, 2003.
5 Sanco/10476/2003 “Quality control procedures for pesticide residues analysis”. http://europa.eu.int/comm/food/plant/protection/resources/qualcontrol_en.pdf