Evaluation Manual for the Authorisation of Plant protection products and Biocides according to Regulation (EC) No 1107/2009

EU part

Plant protection products

Chapter 3 Analytical Methods

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Board for the authorisation of Plant protection products and Biocides

Chapter 3 Analytical methods

Category: Plant protection products

General introduction

This chapter describes the data requirements for the aspect analytical methods and how these are evaluated for the EU framework Regulation (EC) No 1107/2009 [1] (§1 - §1.5).

Substances that are approved under Regulation (EC) No 1107/2009 and were approved under Directive 91/414/EEC [2] are included in Commission Implementing Regulation (EU) No 540/2011 [3].

The chapter describes the procedures following the data requirements as laid down in Commission Regulation (EU) No 283/2013 for active substances and in Commission Regulation (EU) No 284/2013 for plant protection products. These data requirements apply for active substances submitted after 31 December 2013 and for plant protection products submitted after 31 December 2015.

A concept guidance is available on the interpretation of the transitional measures for the data requirements for chemical active substances according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 (SANCO/11509/2013 – rev. 0.1).

For further information on the former data requirement as laid down in Commission Regulation (EU) No 544/2011 for active substances and in Commission Regulation (EU) No 545/2011 we refer to the Evaluation Manual for Authorisation of plant protection products according to Regulation (EC) No 1107/2009 version 1.0

1. EU framework

In this document, the procedures for the evaluation and re-evaluation of active substances as laid down in the EU are described; the NL procedure for evaluation of a substance is reverted to when no EU procedure has been laid down. The NL-procedure for the evaluation of an active substance is described in §2 - §2.5 of part 2 of the Evaluation Manual (plant protection products). This document aims to give procedures for the approval of active substances and inclusion in Commission Implementing Regulation (EU) No 540/2011 [3]

1.1. Introduction

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

The evaluated analytical methods are –*inter alia*- used for the 5-batch analysis of the technical substance as manufactured (see Chapter 2 Physical and chemical properties, (plant protection)), for analysis of residues in animal and plant products (see Chapter 5 residues, residue dossier (plant protection)) and for analysis of residues in water, air and soil (see Chapter 6 Behaviour and fate in the environment (plant protection)).
Analytical methods that can be used for monitoring and control of the use of plant protection products are evaluated as well.

The main guidance documents for this chapter are:

- SANCO/3030/99 rev. 4, 11/07/2000 "*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*" [4]. Currently, august 2012 no updated version of this SANCO document is available. Therefore, under Regulation (EC) No 1107/2009 this version of the document is used until an adapted version is available.
  SANCO/3029/99 rev.4, 11/07/2000 "*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" [5]. Currently (august 2012), no updated version of this SANCO document is available. Therefore, under Regulation (EC) No 1107/2009 this version of the document is used until an adapted version is available.

- SANCO/825/00 rev.8.1, 16/11/2010 "*Guidance document on residue analytical methods*" [6]. This SANCO document has been recently updated (2010) so this current version will be used under Regulation (EC) No 1107/2009.

The requirements from the guidance documents are applicable as soon as they have been laid down and studies started after that date should fully comply.

For studies that were started before this date all subjects that should be validated according to the mentioned documents must be dealt with.
An acceptable explanation should be given for any item that does not meet the requirements of the documents.

Because there is much confusion about terminology such as LOQ or limit of detection the definition of these terms is given in Appendix 6 to this chapter.

In this chapter no distinction is made between the terms 'accuracy' and 'trueness';
the term is in all cases defined as trueness, whereas the term 'accuracy' is used to keep in line with the text of the law. This has been done to avoid confusion. This may, e.g.,
be the result of the literal translation of the English terms into Dutch whereas this is in fact impossible.

1.2. Data requirements

In order to qualify for inclusion in Commission Implementing Regulation (EU) No 540/2011 [3] a dossier that meets the provisions laid down in Commission Regulation (EU) No 283/2013 [7] and Commission Regulation (EU) No 284/2013 of Regulation (EC) No 1107/2009 [8] 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 plant protection product.

Generally, EU and OECD guidelines for the protocol of experiments are mentioned in Commission Communications 2013/C 95/01 [9]

If the applicant holds the view that a certain study is not necessary, a relevant scientific justification may be provided for the non-submission of the particular study.
Deviations from the standard validation of analytical methods should always be justified.

Individual readings of the validations should be submitted; summaries and average values are not acceptable.

There should be no doubt about the identity and the purity of the tested substance for each study.

No GLP is required for validation of the analytical methods. Studies carried out after 25 July 1993 using these analytical methods, however, should be carried out under GLP.

It is important to distinguish between the analytical methods required for monitoring and the methods used for residue studies. (Validation) requirements differ because the purpose of the analytical methods is different.

The validated analytical method must be suitable for the 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*	pure active substance#
	impurities ≥ 0.1% w/w
	relevant impurities
	additives where present
In plant protection product	pure active substance#
	relevant impurities where these can (theoretically) be formed during production or storage of the plant protection product
	relevant co-formulants
In environment (soil, water, air)	the compounds as included in the residue definition for the particular matrix (soil/water/ air)
In plant and animal material	the compounds as included in the residue definition for the particular matrix (type crops/kidneys/milk/etc)

*) 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 active substance as manufactured.
#) inactive isomers are considered as impurities

Different terms are used for the analytical methods used for monitoring: monitoring methods, enforcement methods or post-registration methods. The term post-registration methods has been chosen for this chapter because this is also used in the guidance documents.
This includes only the methods used for enforcement.

Validation of these analytical methods should demonstrate that the method is suitable for determination of residues by enforcement bodies. The post-registration methods are included as a separate requirement in Commission Regulation (EU) No 283/2013 under section 4 (active substance); they are described in Commission Regulation (EU) No 284/2013, under section 5. The requirements for post-registration methods are elaborated in guidance document SANCO/825/00 rev 8.1 [6].

Pre-registration methods are the analytical methods that have (possibly only once) been used for (residue) studies required for registration. Validation of these analytical methods should demonstrate that the results produced in the experiments are reliable.

The data requirements, and the fact whether or not they are required for certain fields of use and the corresponding guidelines, have been summarised in the overview tables in the appendices to this chapter.

1.2.1 Data requirements active substance

The data requirements for the active substance, and the fact whether or not they are required for certain fields of use and the corresponding guidelines, are given in the summarising table; see Appendix 1 to this chapter.

Data requirements analytical methods for the active substance and impurities in the active substance as manufactured

Methods for determination of the pure active substance concentration in the active substance as manufactured are given in Guidance document Sanco/3030/99 [4] for pre- as well as for post-registration methods. The requirements are, however, the same for both purposes.

The grey-framed text below has been taken from Commission Regulation (EU) No 283/2013. The numbering in these grey frames follows the section numbering of this Commission Regulation. Any necessary additions to the text have been added below the grey frames. Question numbers (NL as well as EU) are given below the headings.

Introduction

The provisions of this Section cover analytical methods used for the generation of pre-approval data and required for post-approval control and monitoring purposes.

Descriptions of methods shall be provided and include details of equipment, materials and conditions used.

On request, the following shall be provided:
(a) analytical standards of the purified active substance;
(b) samples of the active substance as manufactured;
(c) analytical standards of relevant metabolites and all other components included in all monitoring residue definitions;
(d) samples of reference substances for the relevant impurities.

Where possible, the standards referred to in points (a) and (c) shall be made commercially available and, on request, the distributing company shall be named.

Regulation (EC) No 1107/2009 provide the following descriptions for impurities, (relevant) metabolites, as follows:

- ‘impurity’ means any component other than the pure active substance and/or variant which is present in the technical material (including components originating from the manufacturing process or from degradation during storage).

- ‘metabolite’ means any metabolite or a degradation product of an active substance, safener or synergist, formed either in organisms or in the environment.

A metabolite is deemed relevant if there is a reason to assume that it has intrinsic properties comparable to the parent substance in terms of its biological target activity, or that it poses a higher or comparable risk to organisms than the parent substance or that it has certain toxicological properties that are considered unacceptable. Such a metabolite is relevant for the overall approval decision or for the definition of risk mitigation measures.

Methods used for the generation of pre-approval data
(283/2013; 4.1)

Methods for the analysis of the active substance as manufactured (283/2013; 4.1.1)

The analytical methods section only covers analytical methods required for post-registration control and monitoring purposes.
For analytical methods used for generation of data as required in Regulation (EC) No 1107/2009 or for other purposes the applicant has to provide a justification for the method used; where necessary separate guidance will be developed for such methods on the basis of the same requirements as defined for methods for post-registration control and monitoring purposes.

4.1.1. Methods for the analysis of the active substance as manufactured

Methods shall be provided, with a full description, for the determination of:
(a) pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009;
(b) significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured.

The applicability of existing CIPAC methods shall be assessed and reported. In case of use of a CIPAC method, further validation data shall not be required, but example chromatograms shall be submitted, where available.

The specificity of the methods shall be determined and reported. In addition, the extent of interference by other substances present in the active substance as manufactured (such as impurities or additives), shall be determined.

The linearity of methods shall be determined and reported. The calibration range shall extend (by at least 20 %) beyond the highest and lowest nominal content of the analyte in relevant analytical solutions. Either duplicate determinations at three or more concentrations or single determinations at five or more concentrations shall be made. The equation of the calibration line and the correlation coefficient shall be reported and a typical calibration plot shall be submitted. In cases where a non-linear response is used, this shall be justified by the applicant.

The precision (repeatability) of the methods shall be determined and reported. A minimum of five replicate sample determinations shall be made and the mean, the relative standard deviation and the number of determinations shall be reported.

For the determination of the active substance content, an assessment of accuracy of the method shall be made by an assessment of the interference and precision.

As regards additives and significant and relevant impurities:

— the accuracy of the methods shall be determined on at least two representative samples at levels appropriate to the batch data and material specification. The mean and the relative standard deviation of the recoveries shall be reported,
— the experimental determination of the limit of quantification (LOQ) shall not be required. However, it shall be demonstrated that the methods are sufficiently precise to analyse

significant impurities at levels appropriate to the material specification and relevant impurities at a concentration equivalent to at least 20 % less than the specification limit.

CIPAC and AOAC methods can be used without validation. CIPAC methods can be obtained via http://www.cipac.org/.

Description of analytical methods for the determination of impurities (non-active components arising from the manufacturing process or from the degradation during storage), which are of toxicological, ecotoxicological or environmental concern or which are present in quantities ≥ 1 g/kg in the active substance as manufactured

For analytical methods used for generation of data as required in Regulation (EC) No 1107/2009 or for other purposes the applicant has to provide a justification for the method used; where necessary separate guidance will be developed for such methods on the basis of the same requirements as defined for methods for post-registration control and monitoring purposes.

Methods for risk assessment
(283/2013; 4.1.2)

4.1.2 Methods for risk assessment

Methods shall be submitted, with a full description, for the determination of non-isotope-labelled residues in all areas of the dossier, as set out in detail in the following points:

(a) in soil, water, sediment, air and any additional matrices used in support of environmental fate studies;
(b) in soil, water and any additional matrices used in support of efficacy studies;
(c) in feed, body fluids and tissues, air and any additional matrices used in support of toxicology studies;
(d) in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies;
(e) in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies;
(f) in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies;
(g) in water, buffer solutions, organic solvents and any additional matrices used in the physical and chemical properties tests.

The specificity of the methods shall be determined and reported. Validated confirmatory methods shall be submitted if appropriate.

The linearity, recovery and precision (repeatability) of methods shall be determined and reported.

Data shall be generated at the LOQ and either the likely residue levels or ten times the LOQ. Where relevant, the LOQ shall be determined and reported for each analyte.

Linearity must be determined for the active substance and the significant and relevant impurities in the active substance as manufactured, in the relevant concentration range. Although the Regulation only mentions linearity, other calibration functions are permitted as well. Not all detection systems or analytical methods will have a linear relationship.
Use of a non-linear relationship should be justified. Otherwise, the same requirements apply as for a linear relationship.
According to SANCO/3030/99 [4] determination of the accuracy is not required for analysis of the active substance in the active substance as manufactured. This accuracy therefore only needs to be determined for the significant and relevant impurities.
Where the method for the impurities in the active substance as manufactured includes no separation of the impurities (from the active substance as manufactured) before the analysis, a statement, e.g. an estimation of the precision based on the analytical technique used, is sufficient for accuracy.

Methods for post-approval control and monitoring purposes
(283/2013; 4.2)

4.2. Methods for post-approval control and monitoring purposes

Methods, with a full description, shall be submitted for:
(a) the determination of all components included in the monitoring residue definition as submitted in accordance with the provisions of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin;
(b) the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2;
(c) the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible;
(d) the analysis in body fluids and tissues for active substances and relevant metabolites.

As far as practicable these methods shall employ the simplest approach, involve the minimum cost, and require commonly available equipment.

The specificity of the methods shall be determined and reported. It shall enable all components included in the monitoring residue definition to be determined. Validated confirmatory methods shall be submitted if appropriate.

The linearity, recovery and precision (repeatability) of methods shall be determined and reported.

Data shall be generated at the LOQ and either the likely residue levels or ten times the LOQ. The LOQ shall be determined and reported for each component included in the monitoring residue definition.

For residues in or on food and feed of plant and animal origin and residues in drinking water, the reproducibility of the method shall be determined by means of an independent laboratory validation (ILV) and reported.

<u>Derivatisation</u>
Derivatisation is permitted but additional validation should demonstrate that the derivatisation yield is high enough.

Determination identity
It must be possible to determine the identity of the analytes (generally, a non-specific method is not acceptable). This can be achieved by using a sufficiently selective analytical method (see e.g. SANCO/825/00 rev. 8.1 [6]) or by submitting a confirmatory method. Such a method must then deviate sufficiently from the original method. A confirmatory method is not required where the method as such is already sufficiently selective, such as, e.g., an LC/MS/MS method, or GC/MS where at least 3 mass fragments with m/z > 100 are included in the analysis.

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).

Determination of the 'limit of quantification', LOQ
Although the Regulation says nothing about determination of the LOQ for relevant and/or significant impurities, this should according to SANCO/3030/99 [4] be determined for all relevant and/or significant impurities. The LOQ may not be determined by a calculation based on the signal/noise ratio (noise) 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.

Validation requirements
A review of the validation requirements for the analytical methods of the technical substance as manufactured is given in Appendix 3 to SANCO/3030/99 [4], and Appendix 1 to this chapter.

Data requirements analytical methods residues

Pre-registration analytical methods residues
The requirements are described in SANCO/3029/99 [5]; Regulation (EC) No 1107/2009 does not contains any requirements. See §1.3.2 for further information about the evaluation methodology of pre-registration methods.

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 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 [5] and Appendix 4 to this chapter.

Linearity
Regulation (EC) No 1107/2009 does not specifically mention linearity but this aspect should be determined according to SANCO/825/00 rev. 8.1 [6], SANCO/3029/99 [5] and SANCO/3030/99 [4].

Other calibration functions are permitted as well. Not all detection systems or analytical methods will have a linear relationship. Use of a non-linear relationship should be justified; otherwise the same requirements apply as for a linear relationship.
The minimum range to be studied is 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 determined fall within the studied range. Extrapolation can only be accepted with a sound justification.

Determination identity
It must be possible to determine the identity of the analytes by using a sufficiently selective analytical method (see e.g. SANCO/825/00 [6]) or by submitting a confirmatory method. Such a method must then be sufficiently different from the original method.
A confirmatory method is not required where the method as such is already sufficiently selective, such as, e.g., an LC/MS/MS method, or GC/MS where at least 3 mass fragments are included in the analysis.

Determination of the 'limit of quantification' LOQ)
The LOQ may not be determined by a calculation based on the signal/noise ratio (noise) 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.

An overview of the validation requirements for the residue-analytical methods is given in SANCO/825/00 [6] and Appendix 1 to this chapter.

Independent laboratory validation, ILV
The proposed analytical method(s) must also 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 multi residue analytical method validated in the relevant matrices; this was decided in the expert meeting during EPCO 11 2004.

Plant products to be validated
Crops are divided into 4 representative groups. The requested crops are classified into one of the groups. A validation should be carried out per representative crop group.

- cereals and dry crops (e.g. barley, wheat, rye, oats)
- crops with a high water content (e.g. lettuce, tomato, cherry, strawberry)
- crops with a high fat content (e.g. nuts, oilseed rape, linseed)
- fruit with a high acid content (e.g. lemon, orange, grapefruit)

Deviation from this classification is possible for specific crops that are very difficult to analyse such as hops and tea. A separate validation should then be carried out for these crops.

Animal products to be validated
An analytical method in animal material is only required for material for which an MRL and

residue definition has been laid down.
Where a residue definition for animal products has been laid down, the following parts must be validated:
- milk
- eggs
- meat
- fat (only where the log P_{ow} (other name: log K_{ow}) is > 3, and the metabolism studies indicate that residues clearly exceed 0.01 mg/kg in fat
- kidneys and liver, only where a specific MRL has been laid down.

An agriculturally representative soil type should be used for validation of the method.
Soil type and origin of the soil should be given to be able to assess whether this is the case.
Where appropriate, soil composition may also be used to demonstrate that the soil is agriculturally representative, e.g., by determining pH, clay content and organic carbon content.

See for Commission Regulation (EU) No 546/2011 [10] the following website: http://eur-lex.europa.eu/Notice.do?checktexts=checkbox&val=574598%3Acs&pos=2&page=1&lang=en&pgs=10&nbl=2&list=607713%3Acs%2C574598%3Acs%2C&hwords=&action=GO&visu=%23texte

The analytical method must be validated separately in drinking water as well as surface water. Where the method for surface water has been validated, a statement can be submitted that validation in drinking water is not required. This is judged on a case-by-case basis.

For surface water, the origin of the water sample and the characteristics (pH, DOC, hardness, salt content…) must be reported.

The concentration required for determination of the limit of determination for surface water depends on the target species and can be derived from toxicity tests (LC_{50}, NOEC or EC_{50}); see SANCO/825/00 [6].

Limit values can be taken from:
- Commission Directive 2000/39/EC [11] establishing a first list of indicative occupational exposure limit values
- Commission Directive 2006/15/EC [12] establishing a second list of indicative occupational exposure limit values
- Commission Directive 2009/161/EU [13] establishing a third list of indicative occupational exposure limit values

The relevant exposure level can also be calculated from the AOEL; see SANCO/825/00 [6].

Air of room temperature and normal humidity as well as air of 35 °C and 80% humidity should be used for method validation. Where the results at 35 °C and 80% humidity are acceptable, measurement at room temperature is no longer required.

Where the submitted method for determination in air is as such insufficiently selective (not suitable to determine the identity of the compound), the question for a confirmation can be dropped if the method for analysis in water can be used for this purpose.
The methods should, however, be sufficiently different; see SANCO/825/00.

1.2.2 Data requirements plant protection product

The data requirements, and the fact whether or not they are required for certain fields of use and the corresponding guidelines, are summarised in the overview table, see Appendix 2 to this chapter.

Data requirements analytical methods for the active substance and impurities in the plant protection product

Methods for determination of the active substance concentration in the plant protection product are given in Guidance document SANCO/3030/99 [4] for pre- as well as for post-registration methods. The requirements are, however, the same for both purposes.

Generally, EU and OECD guidelines for the protocol of experiments are mentioned in Commission Communications 2013/C 95/02 [14]

The grey-framed text below has been taken from Commission Regulation (EU) No 284/2013. The numbering in these grey frames follows the section numbering in this Commission Regulation. Any necessary additions to the text have been added below the grey frames. Question numbers (NL as well as EU) are given below the headings.

Analytical methods

Introduction

The provisions of this Section cover analytical methods used for the generation of pre-authorisation data and required for post-authorisation control and monitoring purposes.

Descriptions of methods shall be provided and include details of equipment, materials and conditions used.

On request, the following shall be provided:
(a) analytical standards of the purified active substance and of the plant protection product;
(b) samples of the active substance as manufactured;
(c) analytical standards of relevant metabolites and all other components included in all monitoring residue definitions;
(d) samples of reference substances for the relevant impurities.

In addition, the standards referred to in points (a) and (c) shall, where possible, be made commercially available and, on request, the distributing company shall be named.

Article 3 of Regulation (EC) No 1107/2009 provide the following descriptions for impurities, relevant metabolites as follows:

- ‘impurity’ means any component other than the pure active substance and/or variant which is present in the technical material (including components originating from the manufacturing process or from degradation during storage).
- ‘metabolite’ means any metabolite or a degradation product of an active substance, safener or synergist, formed either in organisms or in the environment.

A metabolite is deemed relevant if there is a reason to assume that it has intrinsic properties comparable to the parent substance in terms of its biological target activity, or that it poses a higher or comparable risk to organisms than the parent substance or that it has certain toxicological properties that are considered unacceptable. Such a metabolite is relevant for

the overall approval decision or for the definition of risk mitigation measures.

Methods used for the generation of pre-authorisation data
(284/2013; 5.1)

Methods for the analysis of the plant protection product
(284/2013; 5.1.1)

5.1.1. Methods for the analysis of the plant protection product

Methods shall be provided, with a full description, for the determination of:
(a) active substance and/or variant in the plant protection product;
(b) relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage;
(c) relevant co-formulants or components of co-formulants, where required by the national competent authorities.

In the case of a plant protection product containing more than one active substance and/or variant a method capable of determining each, in the presence of the other, shall be provided. If a combined method is not submitted, the technical reasons shall be stated.

The applicability of CIPAC methods shall be assessed and reported. In case of use of a CIPAC method, further validation data shall not be required, but example chromatograms shall be submitted, where available.

The specificity of the methods shall be determined and reported. In addition, the extent of interference by other substances present in the plant protection product (such as impurities or co-formulants), shall be determined.

The linearity of methods shall be determined and reported. The calibration range shall extend (by at least 20 %) beyond the highest and lowest nominal content of the analyte in relevant analytical solutions. Either duplicate determinations at three or more concentrations or single determinations at five or more concentrations shall be made. The equation of the calibration line and the correlation coefficient shall be reported and a typical calibration plot shall be submitted. In cases where a non-linear response is used, this shall be justified by the applicant.

The precision (repeatability) of the methods shall be determined and reported. A minimum of five replicate sample determinations shall be made, and the mean, the relative standard deviation and the number of determinations shall be reported. The accuracy of the methods shall be determined on at least two representative samples at levels appropriate to the material specification. The mean and the relative standard deviation of the recoveries shall be reported.

For relevant impurities and, where necessary, for relevant co-formulants the limit of quantification (LOQ) shall be determined and reported and shall be at a concentration of analyte, which is of toxicological or environmental significance, or at the concentration which is formed during storage of the product, where relevant.

CIPAC and AOAC methods can be used without validation in case validation in the requested formulation type has already been carried out by these organisations. CIPAC methods can be obtained via http://www.cipac.org/.

Linearity must be determined for the active substance and the relevant impurities in the plant protection product, in the relevant concentration range.

This accuracy must be determined for the active substance and relevant impurities.
These relevant impurities in the plant protection product are defined as “impurities that are (eco)toxicologically or environmentally dangerous and which may, on the basis of theoretical considerations, be formed during production or during storage of the plant protection product”.

An analytical method for determination of the active substance in the plant protection product must in principle be validated for each type of formulation (for details see §2.2.3 of the NL part of the Evaluation Manual (PPP).

The exact requirements for the methods under question 5.1 are only described in the guidance document SANCO/3030/99 [4]; no requirements are given in Regulation (EC) No 1107/2009.

Some important points in this guidance document are:
- a ‘common moiety’ method, where a specific group of a molecule is determined instead of the molecule itself, is generally not acceptable
- derivatisation is permitted but requires supplemental validation
- determination of active substance and relevant impurities must be possible
- determination of the identity of relevant impurities in the plant protection product must be possible

Validation requirements
The validation requirements for the analytical methods for the plant protection product are given in Appendix 3 to SANCO/3030/99 [4] and Appendix 2 to this chapter.

Methods for the determination of residues
(284/2013; 5.1.2)

5.1.2. Methods for the determination of residues

Methods shall be submitted, with a full description, for the determination of non-isotope-labelled residues in all areas of the dossier, as set out in detail in the following points:
(a) in soil, water, sediment, air and any additional matrices used in support of environmental fate studies;
(b) in soil, water and any additional matrices used in support of efficacy studies;
(c) in feed, body fluids and tissues, air and any additional matrices used in support of toxicology studies;
(d) in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies;
(e) in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies;
(f) in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies;
(g) in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests.

The specificity of the methods shall be determined and reported. Validated confirmatory methods shall be submitted if appropriate.

The linearity, recovery and precision (repeatability) of methods shall be determined and reported.

Data shall be generated at the LOQ and either the likely residue levels or ten times the LOQ. The LOQ shall be determined and reported for each component in the residue definition.

Methods for post-authorisation control and monitoring purposes
(284/2013; 5.2)

5.2. Methods for post-authorisation control and monitoring purposes

As far as practicable these methods shall employ the simplest approach, involve the minimum cost, and require commonly available equipment.

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.1.1 can be applied.

The provisions set out in point 5.1.1 shall apply.

Methods, with a full description, shall be submitted for the determination of residues:
— in or on plants, plant products, processed food commodities, food and feed of plant and animal origin,
— in body fluids and tissues,
— in soil,
— in water,
— in air, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible.

The applicant may deviate from such requirement by showing that the methods submitted in accordance with the requirements set out in point 4.2 of Part A of the Annex to Regulation (EU) No 283/2013 can be applied.

The specificity of the methods shall enable all components included in the monitoring residue definition to be determined. Validated confirmatory methods shall be submitted if appropriate.

The linearity, recovery and precision (repeatability) of methods shall be determined and reported.

Data shall be generated at the LOQ and either the likely residue levels or ten times the LOQ. The LOQ shall be determined and reported for each component included in the monitoring residue definition.

For residues in or on food and feed of plant and animal origin and residues in drinking water, the reproducibility of the method shall be determined by means of an independent laboratory validation (ILV) and reported.

1.3. Assessment

Each analytical method is assessed separately. Method and LOQ are presented in a list of endpoints (see Appendix 5 to this chapter).

Where as result of an outlier test (Dixons or Grubbs test) the required number of measuring points cannot be met, acceptability will be judged on a case-by-case basis. Only one outlier may be present per series of the same data (e.g. repeatability at 1 concentration level). Judgement is based on aspects such as the cause of the outlier and the extent to which the outlier affects the results.

1.3.1 Analytical methods for active substance and plant protection product

The submitted analytical methods are assessed against the guidance document 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*" [4].
Currently (January 2014), no updated version of this SANCO-document is available. Therefore, under Regulation (EC) No 1107/2009 this version of the document is used until an adapted version is available.
This guidance document concerns analytical methods for 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 plant protection product.
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.

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 plant protection 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 [4] 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 – technical substance as manufactured
The guidance document contains no clear description of the requirements to be met as regards accuracy. The NL evaluation is used for the EU evaluation (see Appendix 3).

Accuracy – plant protection 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

Linearity
The guidance document contains a clear description of the requirements to be met as regards linearity; these are included in Appendix 1 and 2 to this chapter.

1.3.2 Pre-registration analytical method residues
The submitted analytical methods are assessed against guidance document 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*" [5].
Currently (January 2014), no updated version of this SANCO-document is available. Therefore, under Regulation (EC) No 1107/2009 this version of the document is used until an adapted version is available. Regulation (EC) No 1107/2009 does not contain data requirements.

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 feedingstuffs
- 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 explanation is given.

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 of the residue definition.

Validation requirements
An overview of the validation requirements for the residue-analytical methods is given in Appendix 2 to Sanco/3029/99 [5] and Appendix 4 to this chapter.

1.3.3 Post-registration analytical method residues
The submitted analytical methods are assessed against guidance document SANCO/825/00 "*Guidance document on residue-analytical methods*" [6].

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 feedingstuffs
- 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 [6].
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, with samples of representative commodities of all matrices, and animal material to demonstrate that the method is also effective in a different laboratory.
- Confirmatory methods are required to demonstrate the selectivity of the primary method for all representative sample matrices.
  The following validation data are required for the additional fragment ions (MS and HRMS) or the additional SRM transition (MS_n and MS/MS): calibration data ,recovery and precision data for samples fortified at the respective LOQ (n = 5) and for 2 blank samples. For all mass spectrometric techniques a mass spectrum (in case of single MS) or a product ion spectrum (in case of MS_n) should be provided to justify the selection of the additional ions.
- Extraction efficiency: The extraction procedures used in residue analytical methods for the determination of residues in plants, plant products, foodstuff (of plant and animal

origin) and in feeding stuff should be verified for all matrix groups for which residues ≥ LOQ are expected, using samples with incurred residues from radio-labelled analytes.

See also SANCO/10232/2006 “Quality control procedures for pesticide residues analysis” [15] 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.

1.4. Approval

This section describes the approval criteria for active substances (section 1.4.1) and plant protection products (section 1.4.2 and 1.4.3). For the EU approval procedure of active substances a representative formulation has to be included in the dossier. Therefore section 1.4.1 to 1.4.3 apply. For the zonal applications of plant protection products only section 1.4.2 and 1.4.3 apply.

1.4.1 Approval of the active substance

Regulation (EC) No 1107/2009 *Annex II* provides the procedure and criteria for the approval of an active substances, substances and synergists. Point 3 of *Annex II* of Regulation (EC) No 1107/2009 gives the criteria for the approval of an active substance. The text specifically applicable to the aspects identity, physical-chemical properties and analytical methods is presented below.

3.3. Relevance of metabolites
Where applicable the documentation submitted shall be sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.

3.4. Composition of the active substance, safener or synergist

3.4.1. The specification shall define the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.

3.4.2. The specification shall be in compliance with the relevant Food and Agriculture Organisation specification as appropriate, where such specification exists. However, where necessary for reasons of protection of human or animal health or the environment, stricter specifications may be adopted.

Methods of analysis

3.5.1. The methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, shall have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.

3.5.2. The methods of residue analysis for the active substance and relevant metabolites in

plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.

3.5.3. The evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6).

Point 4 of Annex II of Regulation (EC) No 1107/2009 gives criteria for substitution. The texts specifically applicable to the aspect physical-chemical properties and analytical methods are presented below. In the chapter “Generic aspects” of the Evaluation Manual 2.0, more information is provided on criteria for substitution

4. Candidate for substitution
An active substance shall be approved as a candidate for substitution pursuant to Article 24 where any of the following conditions are met:
- it contains a significant proportion of non-active isomers,

Point 5 of Annex II of Regulation (EC) No 1107/2009 gives information on low risk substances. The texts specifically applicable to the aspect physical-chemical properties and analytical methods are presented below. In the chapter “Generic aspects” of the Evaluation Manual 2.0, more information is provided on low risk substances.

5. Low-risk active substances
An active substance shall not be considered of low risk where it is or has to be classified in accordance with Regulation (EC) No 1272/2008 as at least one of the following:
- explosive,
- corrosive.

1.4.2 Evaluation of plant protection products

The principles for the evaluation (the Uniform Principles) regarding analytical methods are presented in Commission Regulation (EU) No 546/2011 [10]. These concern the relevant sections of the introductory principles, the general principles and the specific principles Analytical methods.
The specific principles Analytical methods are in the text below printed in a grey frame. This text, including numbering, is the literal text from Commission Regulation (EU) No 546/2011.

2.6. Analytical methods

Member States shall evaluate the analytical methods proposed for post-registration control and monitoring purposes, to determine:

2.6.1. for formulation analysis:
the nature and quantity of the active substance(s) in the plant protection product and, where appropriate, any toxicologically, ecotoxicologically or environmentally significant impurities and co-formulants.

This evaluation will take into consideration the following information:
(i) the data on analytical methods as provided for in the Annex to Regulation (EU) No

544/2011 and the results of the evaluation thereof;
(ii) the data on analytical methods as provided for in the Annex to Regulation (EU) No 545/2011 and in particular:
- the specificity and linearity of the proposed methods,
- the importance of interferences,
- the precision of the proposed methods (intra-laboratory repeatability and inter-laboratory reproducibility);
(iii) the limit of detection and determination of the proposed methods for impurities.

2.6.2. for residue analysis:

the residues of the active substance, metabolites, breakdown or reaction products resulting from authorized uses of the plant protection product and which are of toxicological, ecotoxicological or environmental significance.

This evaluation will take into consideration the following information:
(i) the data on analytical methods as provided for in the Annex to Regulation (EU) No 544/2011 and the results of the evaluation thereof;
(ii) the data on analytical methods as provided for in the Annex to Regulation (EU) No 545/2011 and in particular:
- the specificity of the proposed methods,
- the precision of the proposed methods (intra-laboratory repeatability and inter-laboratory reproducibility),
- the recovery rate of the proposed methods at appropriate concentrations;
(iii) the limit of detection of the proposed methods;
(iv) the limit of determination of the proposed methods.

The requirement for determination of the reproducibility in a different laboratory (ILV) became defunct in the elaboration of the requirements; see 5.1 in Appendix III of this document.

1.4.3 Decision making for plant protection products

The principles for decision making as regards analytical methods are presented in Commission Regulation (EU) No 546/2011 [10]. These concern the relevant sections of the introductory principles, the general principles and the specific principles Analytical methods. The specific principles Analytical methods are in the text below printed in a grey frame. This text, including numbering, is the literal text from Commission Regulation (EU) No 546/2011.

2.6. Analytical methods

The methods proposed must reflect the state of the art. The following criteria must be met in order to permit validation of the analytical methods proposed for post-registration control and monitoring purposes:

2.6.1. for formulation analysis:

the method must be able to determine and to identify the active substance(s) and where appropriate any toxicologically, ecotoxicologically or environmentally significant impurities and co-formulants;

2.6.2. for residue analysis:
(i) the method must be able to determine and confirm residues of toxicological,

ecotoxicological or environmental significance;

(ii) the mean recovery rates should be between 70% and 110% with a relative standard deviation of ≤ 20%;

(iii) the repeatability must be less than the following values for residues in foodstuffs:

Residue level mg/kg	Difference mg/kg	Difference in %
0,01	0,005	50
0,1	0,025	25
1	0,125	12,5
>1		12,5

Intermediate values are determined by interpolation from a log-log graph;
(iv) the reproducibility must be less than the following values for residues in foodstuffs:

Residue level mg/kg	Difference mg/kg	Difference in %
0,01	0,01	100
0,1	0,05	50
1	0,25	25
>1		25

Intermediate values are determined by interpolation from a log-log graph;
(v) in the case of residue analysis in treated plants, plant products, foodstuffs, feedingstuffs or products of animal origin, except where the MRL or the proposed MRL is at the limit of determination, the sensitivity of the methods proposed must satisfy the following criteria:
Limit of determination in relation to the proposed provisional or Community MRL:

MRL mg/kg	Limit of determination mg/kg
> 0,5	0,1
0,5-0,05	0,1-0,02
<0,05	MRL x 0,5

The left-hand column of the table above shows the established MRL and the corresponding required LOQ is given in the right-hand column.

1.5. Developments

Clarity is required whether for establishment of the required LOQ for determination of the residues in surface water, which is derived from the toxicity tests the safety factors as described in EU guidance document 8075/VI/97 should be used.

2. Appendices

Appendix 1 Requirements regarding the active substance

Unless indicated otherwise, the question must always be answered.

EU question	NL question	description	Explanation / requirements	Method / guideline
4.1.1	A4.1.1a	Description of analytical methods for the analysis of the active substance as manufactured	• Description of the method • The applicability of existing CIPAC methods must be reported	SANCO/3030/99
4.1.2	A4.1.2a	Description of analytical methods for the determination of impurities (non-active components arising from the manufacturing process or from the degradation during storage), which are of toxicological, ecotoxicological or environmental concern or which are present in quantities ≥ 1 g/kg in the active substance as manufactured	• Description of the method	SANCO/3030/99
	A4.1.2a	Analytical methods for determination of additives in the technical substance as manufactured.	• E.g., a stabiliser • Description of the method	
4.1.3.1	A4.1.3.1a	Specificity of the methods submitted for question 4.1.1 and 4.1.2	• Demonstrate specificity • Determination interference other substances present • Explanation of interference of other substances present if they represent more than ± 3% of the total measured concentration • The identity of the impurities must (if applicable only once) be determined during method validation, either by using detectors that provide information about the identity, or with a confirmatory method. Remark: retention time alone is not sufficient to demonstrate identity. • Representatively labelled documents (e.g., chromatogrammes)	SANCO/3030/99
4.1.3.2	A4.1.3.2a	Linearity of the methods submitted for question 4.1.1 and 4.1.2	• Linearity over an appropriate range • The mathematical equation of the calibration line + graphic representation • The correlation coefficient should be at least 0.99	SANCO/3030/99
4.1.3.3	A4.1.3.3a	Accuracy of the methods submitted for question 4.1.1 and 4.1.2	• SANCO/3030/99 only indicates that an average recovery of 2 determinations is required at specification level. A requirement for the average recovery is not given in this guidance document. See Appendix 3 for the requirement regarding average recovery in NL framework	SANCO/3030/99
4.1.3.4	A4.1.3.4g	Repeatability of the methods submitted for question 4.1.1 and 4.1.2	- of at least 5 determinations • Relative Standard Deviation (RSD) • Indication whether outliers have been discarded from evaluation • Acceptable explanation for the existence of the outliers (discarding outliers without acceptable explanation is not permitted)	SANCO/3030/99
4.2.1 - 4.2.5	*A4.2.1a - A4.2.5a*	*Only concerns the residue-analytical methods intended for post-registration (enforcement/monitoring).Pre-registration methods must be submitted together with the corresponding studies*		

4.2.1	A4.2.1a	Analytical methods for determination of residues on plants, plant products, foodstuffs (of plant or animal origin) and feedingstuffs	• Description of the methods for determination of all components that are included in the residue definition to be able to investigate whether or not the established MRLs are exceeded For each method and representative matrix: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Independent Laboratory Validation (ILV) • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00
4.2.2	A4.2.2a	Analytical method for determination of residues in soil	• Description of the method for analysis of soil for parent compound and relevant metabolites For each method: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00
4.2.3	A4.2.3a A4.2.3b	Analytical method for determination of residues in water	• Description of the method for analysis of water (drinking water, groundwater and surface water) for parent compound and relevant metabolites For each method: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00
4.2.4	A4.2.4a	Analytical method for determination of residues in air	• Description of the method for analysis of air for the active substance and toxicologically relevant metabolites that are formed during or immediately after application Method is required unless it can be demonstrated that operators, workers or bystanders will most probably not be exposed. For each method: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00

4.2.5	A4.2.5a	Analytical method for determination of residues in body fluids and tissues	Analytical method for determination of residues of parent compound and relevant metabolites in body fluids and tissues. Only required for substances classified as toxic or very toxic. For each method: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00

-relevant impurities: impurities that are toxicologically and/or ecotoxicologically or environmentally relevant;
-significant impurities: impurities of which the concentration in the active substance as manufactured ≥ 1 g/kg;
-impurities: other components than the pure active substance formed in the active substance as manufactured during manufacturing or degradation during storage (including non-active isomers);

Appendix 2 Requirements regarding the plant protection product

Unless indicated otherwise, the question must always be answered.

EU question	NLquestion	description	explanatory notes	Method / guideline
5.1.1	P05.1.1a	Description of analytical methods for the determination of the active substance in plant protection products	• Description of the method • Where the plant protection product contains more than 1 active substance, a method must be described which enables determination of each active substance in the presence of the other. Technical reasons must be given where no combined method is submitted. • The applicability of existing CIPAC methods must be reported.	SANCO/3030/99
5.1.2	P05.1.2a	Description of analytical methods for the determination of impurities (non- active components arising from the manufacturing process or from degradation during storage) which are of toxicological, ecotoxicological or environmental concern, in the preparation	• Description of the method • Expert judgement is required to decide whether an analytical method is required	SANCO/3030/99
	-	Description of analytical methods for the determination of formulants or constituents of formulants in the plant protection product	• Description of the method • Required where these substances are relevant.	
5.1.3.1	P05.1.3.1a	Specificity of the methods submitted for question 5.1.1 and 5.1.2	• Demonstrate specificity • Determine interference of other substances in the plant protection product • Clarification of interference of other substances where these constitute more than ± 3% of the total concentration as determined	SANCO/3030/99
5.1.3.2	P05.1.3.2a	Linearity of the methods submitted for question 5.1.1 and 5.1.2	• Linearity over an appropriate range • The mathematical equation of the calibration line + graphic representation • The correlation coefficient should be at least 0.99 • Representatively labelled documents (e.g. chromatogrammes)	SANCO/3030/99
5.1.3.3	P05.1.3.3a	Accuracy of the methods submitted for question 5.1.1 and 5.1.2	• SANCO/3030/99 indicates that an average recovery of 2 determinations is required at specification level. See §1.3.1 for the requirements regarding average recovery.	SANCO/3030/99
5.1.3.4	P05.1.3.4a	Repeatability of the methods submitted for question 5.1.1 and 5.1.2	• of at least 5 determinations • Relative Standard Deviation (RSD) • Indication whether outliers have been excluded from evaluation • Acceptable explanation for the existence of the outliers	SANCO/3030/99

EU question	NLquestion	description	explanatory notes	Method / guideline
5.2	*P05.2.1a t/m P05.2.5a*	*Only concerns the residue-analytical methods intended for post-registration (enforcement/monitoring).Pre-registration methods must be submitted together with the corresponding studies These are the same methods as described in Appendix 1, under questions 4.2 but 91/414/EEC contains the questions in the substance part (Annex II) as well as in the plant protection product part (Annex III). The question are therefore for reasons of completeness also included here.*		
5.2	P05.2.1a	Description of analytical methods for the determination of residues (all components included in the residue definition proposed (see point 8) to enable compliance with MRLs to be determined or to determine dislodgeable residues	• Description of the methods for determination of all components that are included in the residue definition to be able to investigate whether or not the established MRLs are exceeded For each method and representative matrix: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Independent Laboratory Validation (ILV) • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00
	P05.2.2a	Description of methods for analysis of soil for parent compound and metabolites of toxicological, ecotoxicological or environmental concern	• Description of the method for analysis of soil for parent compound and relevant metabolites For each method: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00
	P05.2.3a	Description of methods for analysis of water for parent compound and metabolites of toxicological, ecotoxicological or environmental concern	• Description of the method for analysis of water (drinking water, groundwater and surface water) for parent compound and relevant metabolites For each method: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00
	P05.2.4a	Description of methods for analysis of air for active substance and metabolites, formed during or shortly after application, of toxicological, ecotoxicological or environmental concern	• Description of the method for analysis of air for the active substance and toxicologically relevant metabolites that are formed during or immediately after application Method is required unless it can be demonstrated that operators, workers or bystanders will most probably not be exposed. For each method: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00

EU question	NLquestion	description	explanatory notes	Method / guideline
	P05.2.5a	Analytical methods for parent compound and toxicologically, ecotoxicologically or environmentally significant metabolites in body fluids and tissues	Analytical method for determination of residues of parent compound and relevant metabolites in body fluids and tissues. Only required for substances classified as toxic or very toxic. For each method: • Specificity (if necessary with an extra confirmatory method) • Repeatability • Limit of quantification (LOQ) • Individual and average recovery, total relative standard deviation and relative standard deviation for each fortification level	SANCO/825/00

- relevant impurities: impurities that are toxicologically and/or ecotoxicologically or environmentally relevant;
- impurities: other components than the pure active substance formed in the active substance as manufactured during manufacturing or degradation during storage (including non-active isomers);

Appendix 3 Summary of the most important requirements for methods in technical material and formulations (NL framework)

Required	Technical a.s.	Formulations
Description of the method	Complete description required	Complete description required
Analytical method based on generally available laboratory equipment and laboratory facilities	Not required, but the necessity must be explained when used	Not required, but the necessity must be explained when used
Avoid dangerous chemicals	Not required, but the necessity must be explained when used	Not required, but the necessity must be explained when used
Derivatisation	Permitted, but the necessity must be explained when used; supplementary validation	Permitted, but the necessity must be explained when used; supplementary validation
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 technical a.s. Based on 5 concentration levels or based on 3 duplicate concentration levels Correlation coefficient ≥ 0.99

Interference of matrix	maximum 3%	maximum 3%
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 §1.3.1 for requirements
Repeatability (relative standard deviation)	Required, (n ≥ 5), should meet Horwitz, see §1.3.1	Required, (n ≥ 5), should meet Horwitz, see §1.3.1

Appendix 4 Summary of the most important requirements for pre- and post-registration methods for residue-analytical methods (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	Permitted, but the necessity must be explained when used; supplementary validation
Multi Residue Method	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 of the residue definition	all components of 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, but 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 Surface water: LOQ ≤ $NOEC_{daphnia}$ or $EC_{50\ algae}$ µg/l Air: not applicable	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 Surface water: LOQ ≤ 0.1 µg/l and < $NOEC_{daphnia}$ of $EC_{50\ algae}$ µg/l Air: see SANCO/825/00 for calculation LOQ
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

Extraction efficiency	No specific requirements	The extraction procedures used in residue analytical methods for the determination of residues in plants, plant products, foodstuff (of plant and animal origin) and in feeding stuff should be verified for all matrix groups for which residues ≥ LOQ are expected.

Appendix 5 List of Endpoints (LOEP)

Methods of Analysis

Analytical methods for the active substance (283/2013, point 4.1)

Technical as (principle of method)	
Impurities in technical as (principle of method)	
Preparation (principle of method)	

Analytical methods for residues (283/2013, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin	
Food of animal origin	
Soil	
Water surface	
drinking/ground	
Air	

Monitoring/Enforcement methods

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)	
Soil (principle of method and LOQ)	
Water (principle of method and LOQ)	
Air (principle of method and LOQ)	
Body fluids and tissues (principle of method and LOQ)	

Appendix 6 Definition terms

	Linearity (Lineariteit)	Precision (Precisie)	Trueness (Juistheid)	Selectivity (Selectiviteit)	Limit of Quantification /Quantification (Bepalingsgrens)
Definition	Linear relationship between response and amount (concentration) of the component to be determined	The closeness of agreement in the analytical results of the same sample	Extent of the agreement between the average of a series of measured values and the actual value	The property of a method to distinguish between the component to be determined and other substances (such as exclusion of Interference/interfering effects)	Lowest concentration of the component in the sample of which the measured value can still be determined with a certain (un)certainty
Other frequently used terms		ruggedness	Accuracy is often used, although not fully correct	The term specificity is often used. An analytical method is specific where it only reacts to the component to be determined. Specificity can be considered as the ultimate selectivity	Limit of determination (not to be confused with limit of detection)
How determined		Repeatability RSD Reproducibility	Trueness can be determined by means of recovery after addition of a standard (standard addition)		

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