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Guidance document on pesticide residue analytical methods

[Revision 8 is the version of this guidance document that is currently valid. It is, however, under continuous review and will be updated when necessary. The document is aimed at manufacturers seeking pesticides authorisations and parties applying for setting or modification of an MRL. It gives requirements for methods that would be used in post-registration monitoring and control by the competent authorities in Member States in the event that authorisations are granted. For authorities involved in post-registration control and monitoring, the document may be considered as being complementary to the documents: Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed (for the valid revision visit http://ec.europa.eu/food/plant/protection/resources/publications_en.htm) and the OECD document "Guidance Document on pesticide residue analytical methods", 2007. (ENV/JM/ ENV/JM/MONO(2007)17).

1 Preamble

This document provides guidance to applicants, Member States and EFSA on the data requirements and assessment for residue analytical methods for post-registration control and monitoring purposes. It is not intended for biological agents such as bacteria or viruses. It recommends possible interpretations of the provisions of section 3.5.2 of Annex II of Regulation (EC) No 1107/2009 [1] and of the provisions of section 4, part A of Annex II and section 5, part A of Annex III of Council Directive 91/414/EEC [2]. It also applies to applications for setting or modification of an MRL within the scope of Regulation (EC) No 396/2005 [3]. It has been elaborated in consideration of the 'Guidance Document on pesticide residue analytical methods' of the OECD [4] and SANCO/10684/2009 "Method validation and quality control procedures for pesticide residue analysis in food and feed" [5].

This document has been conceived as an opinion of the Commission Services and elaborated in co-operation with the Member States. It does not, however, intend to produce legally binding effects and by its nature does not prejudice any measure taken by a Member State nor any case law developed with regard to this provision. This document also does not preclude the possibility that the European Court of Justice may give one or another provision direct effect in Member States.

This guidance document must be amended at the latest if new data requirements as referred to in Article 8 (1)(b) and 8 (1)(c) of Regulation (EC) No 1107/2009 will have been established in accordance with the regulatory procedure with scrutiny referred to in Article 79 (4).

2 General

2.1 Good Laboratory Practice

According to Guidance Document 7109/VI/94-Rev. 6.c1 (Applicability of Good Laboratory Practice to Data Requirements according to Annexes II, Part A, and III, Part A, of Council Directive 91/414/EEC) [6] the development and validation of an analytical method for monitoring purposes and post-registration control is not subject to GLP. However, where the method is used to generate data for registration purposes, for example residue data, these studies must be conducted to GLP.

2.2 Selection of analytes for which methods are required

The definition of the residues relevant for monitoring in feed and food as well as in environmental matrices and air is not the subject matter of this document. Criteria for the selection of analytes in case that no legally binding definition is available are given in the respective sections 3 - 8. In addition, sections 5.2, 6.2, 7.2 and 8.2 clarify under which circumstances analytical methods for residues may not be necessary.

2.3 Description of an analytical method and its validation results

Full descriptions of validated methods shall be provided. The submitted studies must include the following points:

- Itemisation of the fortified compounds and the analytes, which are quantified
- Description of the analytical method
- Validation data as described in more detail below
- Description of calibration including calibration data
- Recovery and Repeatability
- Data proving the selectivity of the method
- Confirmatory data, if not presented in a separate study
- References (if needed)

The following information should be offered in the description of the analytical method:

- An introduction, including the scope of the method
- Outline/summary of method, including validated matrices, limit of quantification (LOQ), range of recoveries, fortification levels and number of fortifications per level

- Apparatus and reagents
- instrument parameters used as example if appropriate
- Description of the analytical method, including extraction, clean-up, derivatisation (if appropriate), chromatographic conditions (if appropriate) and quantification technique
- Hazards or precautions required
- Time required for one sample set
- Schematic diagram of the analytical method
- Stages where an interruption of the method is possible
- Result tables (if results are not presented in separate studies)
- Procedure for the calculation of results from raw data
- Extraction efficiency of solvents used
- Important points and special remarks (e.g. volatility of analyte or its stability with regard to pH)
- Information on stability of fortified/incurred samples, extracts and standard solutions (If the recoveries in the fortified samples are within the acceptable range of 70-120 %, stability is sufficiently proven.)

Sometimes it may be necessary for other information to be presented, particularly where special methods are considered.

2.4 Hazardous reagents

Hazardous reagents (carcinogens category I and II [7]) shall not be used. Among these compounds are diazomethane, chromium (VI) salts, chloroform and benzene.

2.5 Acceptable analytical techniques considered commonly available

Analytical methods shall use instrumentation regarded as "commonly available":

- GC detectors: FPD, NPD, ECD, FID, MS, MS^n (incl. Ion Traps and MS/MS), HRMS
- GC columns: capillary columns
- HPLC detectors: MS, MS/MS, HRMS, FLD, UV, DAD
- HPLC columns: reversed phase, ion-exchange, normal phase
- AAS, ICP-MS, ICP-OES

Other techniques can be powerful tools in residue analysis, therefore the acceptance of additional techniques as part of enforcement methods should be discussed at appropriate intervals. Whilst it is recognised that analytical methodology is constantly developing, some time elapses before new techniques become generally accepted and available.

2.6 Multi-residue methods

Multi-residue methods that cover a large number of analytes and that are based on GC-MS and/or HPLC-MS/MS are routinely used in enforcement laboratories for the analysis of plant matrices. Therefore, validated residue methods submitted for food of plants, plant products and foodstuff of plant origin (Section 3) should be multi-residue methods published by an international official standardisation body such as the European Committee for Standardisation (CEN) (e.g. [8 - 12]) or the AOAC International (e.g. [13]). Single residue methods should only be provided if data show and are reported that multi-residue methods involving GC as well as HPLC techniques cannot be used.

If validation data for the residue analytical method of an analyte in at least one of the commodities of the respective matrix group have been provided by an international official standardisation body and if these data have been generated in more than one laboratory with the required LOQ and acceptable recovery and RSD data (see Section 2.9.2), no additional validation by an independent laboratory is required.

2.7 Single methods and common moiety methods

Where a pesticide residue cannot be determined using a multi-residue method, one or where appropriate more alternative method(s) must be proposed. The method(s) should be suitable for the determination of all compounds included in the residue definition. If this is not possible and an excessive number of methods for individual compounds would be needed, a common moiety method may be acceptable, provided that it is in compliance with the residue definition. However, common moiety methods shall be avoided whenever possible.

2.8 Single methods using derivatisation

For the analysis of some compounds by GC, such as those of high polarity or with poor chromatographic properties, or for the detection of some compounds in HPLC, derivatisation may be required. These derivatives may be prepared prior to chromatographic analysis or as part of the chromatographic procedure, either pre- or post-column. Where a derivatisation method is used, this must be justified.

If the derivatisation is not part of the chromatographic procedure, the derivative must be sufficiently stable and should be formed with high reproducibility and without influence of matrix components on yield. The efficiency and precision of the derivatisation step should be demonstrated with analyte in sample matrix against pure derivative. The storage stability of

the derivative should be checked and reported. For details concerning calibration refer to Section 2.9.1.

The analytical method is considered to remain specific to the analyte of interest if the derivatised species is specific to that analyte. However, where – in case of pre-column derivatisation – the derivative formed is a common derivative of two or more active substances or their metabolites or is classed as another active substance, the method should be considered non-specific and may be deemed unacceptable.

2.9 Method validation

Validation data must be submitted for all analytes included in the residue definition for all representative sample matrices to be analysed at adequate concentration levels.

Basic validation data are:

- Calibration data
- Concentration of analyte(s) found in blank samples
- Concentration level(s) of fortification experiments
- Concentration and recovery of analyte(s) found in fortified samples
- Number of fortification experiments for each matrix/level combination
- Mean recovery for each matrix/level combination
- Relative standard deviation (RSD) of recovery, separate for each matrix/level combination
- Limit of quantification (LOQ), corresponding to the lowest validated level
- Representative clearly labelled chromatograms
- Data on matrix effects, e.g. on the response of the analyte in matrix as compared to pure standards

.Further data may be required in certain cases, depending on the analytical method used, and the residue definition to be covered.

2.9.1 Calibration

The calibration of the detection system shall be adequately demonstrated at a minimum of 3 concentration levels in duplicate or (preferably) 5 concentration levels with single determination. Calibration should be generated using standards prepared in blank matrix extracts (matrix matched standards) for all sample materials included in the corresponding validation study (Sections 3 - 8). Only, if experiments clearly demonstrate that matrix effects are not significant (i.e. < 20 %), calibration with standards in solvent may be used. Calibration with standards in solvent is also acceptable for methods to detect residues in air (Section 7).

In case that aqueous samples are analysed by direct injection HPLC-MS/MS calibration shall be performed with standards in aqueous solution.

The analytical calibration must extend to at least the range which is suitable for the determination of recoveries and for assessment of the level of interferences in control samples. For that purpose a concentration range shall be covered from 30 % of the LOQ to 20 % above the highest level (Section 2.9.2).

All individual calibration data shall be presented together with the equation of the calibration. Concentration data should refer to both, the mass fraction in the original sample (e.g. mg/kg) and to the concentration in the extract (e.g. µg/L). A calibration plot should be submitted, in which the calibration points are clearly visible. A plot showing the response factor[1] versus the concentration for all calibration points is preferred over a plot of the signal versus the concentration.

Linear calibrations are preferred if shown to be acceptable over an appropriate concentration range. Other continuous, monotonically increasing functions (e.g. exponential/power, logarithmic) may be applied where this can be fully justified based on the detection system used.

When quantification is based on the determination of a derivative, the calibration shall be conducted using standard solutions of the pure derivative generated by weighing, unless the derivatisation step is an integral part of the detection system. If the derivative is not available as a reference standard, it should be generated within the analytical set by using the same derivatisation procedure as that applied for the samples. Under these circumstances, a full justification should be given.

2.9.2 Recovery and Repeatability

Recovery and precision data must be reported for the following fortification levels, except for body fluids and body tissues (Section 8):

- LOQ 5 samples
- 10 times LOQ, or MRL (set or proposed) or other relevant level (≥ 5 x LOQ) 5 samples

Additionally, for unfortified samples residue levels must be reported:

- blank matrix 2 samples

According to the residue definition the LOQ of chiral analytes usually applies to the sum of the two enantiomers. In this case it is not necessary to determine the enantiomers separately.

[1] The response factor is calculated by dividing the signal area by the respective analyte concentration.

Enantioselective methods would only be required if a single enantiomer is included in the residue definition.

In cases of complex residue definitions (e.g. a residue definition which contains more than one compound) the validation results shall be reported for the single parts of the full residue definition, unless the single elements cannot be analysed separately.

The mean recovery at each fortification level and for each sample matrix should be in the range of 70 % - 120 %. In certain justified cases mean recoveries outside of this range will be accepted.

For plants, plant products, foodstuff (of plant and animal origin) and in feeding stuff recovery may deviate from this rule as specified in Table 1.[2]

Table 1: Mean recovery and precision criteria for plant matrices and animal matrices [4]

Concentration level	Range of mean recovery (%)	Precision, RSD (%)
> 1 µg/kg ≤ 0.01 mg/kg	60 - 120	30
> 0.01 mg/kg ≤ 0.1 mg/kg	70 - 120	20
> 0.1 mg/kg ≤ 1.0 mg/kg	70 - 110	15
> 1 mg/kg	70 - 110	10

If blank values are unavoidable, recoveries shall be corrected and reported together with the uncorrected recoveries.

The precision of a method shall be reported as the relative standard deviation (RSD) of recovery at each fortification level. For plants, plant products, foodstuff (of plant and animal origin) and feeding stuff the RSD should comply with the values specified in Table 1. In other cases the RSD should be ≤ 20 % per level. In certain justified cases, e.g. determination of residues in soil lower than 0.01 mg/kg, higher variability may be accepted.

When outliers have been identified using appropriate statistical methods (e.g. Grubbs or Dixons test), they may be excluded. Their number must not exceed 1/5 of the results at each fortification level. The exclusion should be justified and the statistical significance must be

[2] According to Annex IIA 4.2 of Directive 91/414/EEC the mean recovery should normally be 70 % - 110 % and the RSD should preferably be ≤ 20 %.

clearly indicated. In that case all individual recovery data (including those excluded) shall be reported.

2.9.3 Selectivity

Representative clearly labelled chromatograms of standard(s) at the lowest calibrated level, matrix blanks and samples fortified at the lowest fortification level for each analyte/matrix combination must be provided to prove selectivity of the method. Labelling should include sample description, chromatographic scale and identification of all relevant components in the chromatogram.

When mass spectrometry is used for detection, a mass spectrum (in case of MS/MS: product ion spectrum) should be provided to justify the selection of ions used for determination.

Blank values (non-fortified samples) must be determined from the matrices used in fortification experiments and should not be higher than 30 % of the LOQ. If this is exceeded, detailed justification should be provided.

2.10 Confirmation

Confirmatory methods are required to demonstrate the selectivity of the primary method for all representative sample matrices (Sections 3 – 8). It has to be confirmed that the primary method detects the right analyte (analyte identity) and that the analyte signal of the primary method is quantitatively correct and not affected by any other compound.

2.10.1 Confirmation simultaneous to primary detection

A confirmation simultaneous to the primary detection using one fragment ion in GC-MS and HPLC-MS or one transition in HPLC-MS/MS may be accomplished by one of the following approaches:

- In GC-MS, HPLC-MS, by monitoring at least 2 additional fragment ions (preferably $m/z > 100$) for low resolution system and at least 1 additional fragment ion for high resolution/accurate mass system
- In GC-MS^n (incl. Ion Traps and MS/MS), HPLC-MS/MS, by monitoring at least 1 additional SRM transition

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 (Section 2.9.1), recovery and precision data according to Section 2.9.2 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.

2.10.2 Confirmation by an independent analytical technique

Confirmation can also be achieved by an independent analytical method. The following are considered sufficiently independent confirmatory techniques:

- chromatographic principle different from the original method
  - e.g. HPLC instead of GC
- different stationary phase and/or mobile phase with significantly different selectivity
  - the following are not considered significantly different:
    - in GC: stationary phases of 100 % dimethylsiloxane and of 95 % dimethylsiloxane + 5 % phenylpolysiloxane
    - in HPLC: C18- and C8-phases
- alternative detector
  - e.g. GC-MS vs. GC-ECD, HPLC-MS vs. HPLC-UV/DAD
- derivatisation, if it was not the first choice method
- high resolution/accurate mass MS
- in mass spectrometry an ionisation technique that leads to primary ions with different *m/z* ratio than the primary method (e.g. ESI negative ions vs. positive ions)

It is preferred that confirmation data are generated with the same samples and extracts used for validation of the primary method.

The following validation data are required: calibration data (Section 2.9.1), recovery and precision data (Section 2.9.2) for samples fortified at the respective LOQ ($n \geq 3$) and of a blank sample and proof of selectivity (Section 2.9.3).

2.11 Independent laboratory validation (ILV)

A validation of the primary method in an independent laboratory (ILV) must be submitted for methods used for the determination of residues in plants, plant products, foodstuff (of plant and animal origin) and in feeding stuff. The ILV shall confirm the LOQ of the primary method, but at least the lowest action level (MRL).

The extent of independent validation required is given in detail in sections 3 and 4.

In order to ensure independence, the laboratory chosen to conduct the ILV trials must not have been involved in the method development and in its subsequent use. In case of multi-

residue methods it would be accepted if the ILV is performed in a laboratory that has already experience with the respective method.

The laboratory may be in the applicant's organisation, but should not be in the same location. In the exceptional case that the lab chosen to conduct the ILV is in the same location, evidence must be provided that different personnel, as well as different instrumentation and stocks of chemicals etc have been used.

Any additions or modifications to the original method must be reported and justified. If the chosen laboratory requires communication with the developers of the method to carry out the analysis, this should be reported.

2.12 Availability of standards

All analytical standard materials used in an analytical method must be commonly available. This applies to metabolites, derivatives (if preparation of derivatives is not a part of the method description), stable isotope labelled compounds or other internal standards.

If a standard is not commercially available the standard should be made generally available by the applicant and contact details be provided.

2.13 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.

Data or suitable samples may be available from pre-registration metabolism studies or rotational crop studies or from feeding studies. In cases where such samples are no longer available to validate an extraction procedure, it is possible to "bridge" between two solvent systems (details in [4]). The same applies if new matrices are to be included.

3 Analytical methods for residues in plants, plant products, foodstuff (of plant origin), feedingstuff (of plant origin) (Annex IIA Point 4.2.1 of Directive 91/414/EEC; Annex Point IIA, Point 4.3 of OECD)

3.1 Purpose

- Analysis of plants and plant products, and of foodstuff and feeding stuff of plant origin for compliance with MRL [3].

3.2 Selection of analytes

The selection of analytes for which methods for food and feed are required depends upon the definition of the residue for which a maximum residue level (MRL) is set or is applied for according to Regulation (EC) No 396/2005.

3.3 Commodities and Matrix Groups

Methods validated according to Section 2.9 and 2.10 must be submitted for representative commodities (also called "matrices" by analytical chemists) of all four matrix groups in Table 2.

Table 2: Matrix groups and typical commodities

Matrix group	Examples for commodities
dry commodities (high protein/high starch content)	barley, rice, rye, wheat, dry legume vegetables
commodities with high water content	apples, bananas, cabbage, cherries, lettuce, peaches, peppers, tomatoes
commodities with high oil content	avocados, linseed, nuts, olives, rape seed
commodities with high acid content	grapefruits, grapes, lemons, oranges

Important Note: This list of commodities is not a comprehensive list of commodities/matrices. Applicants may consult regulatory authorities for advice on the use of other commodities.

If samples with high water content are extracted at a controlled pH a particular method or validation for commodities with high acid content is not required.

Where a previously validated method has been adopted to a new matrix group, validation data must be submitted for representative matrices of this group.

If a method is required for a commodity which is difficult to analyse (see Table 3 for examples), full validation data for that specific commodity shall be presented to prove the suitability of the method.

Table 3: Examples of matrices which are difficult to analyse

Matrix group	Examples for Commodities
no group	coffee beans, cocoa beans, herbal infusions, hops, spices, tea, tobacco

The decision on whether a commodity must be considered "difficult to analyse" may depend upon the sample preparation and the analytical method selected for analysis. For example matrices like brassica or onion may be considered "difficult to analyse" if detection techniques like ECD, NPD or UV are used.

3.4 Limit of quantification

Generally, an LOQ of 0.01 mg/kg should be met. Only in justified cases it may be sufficient that the LOQ complies with the lowest MRL in the respective matrix group [3]. For commodities which are difficult to analyse the LOQ must meet 50 % of the MRL of that commodity unless the MRL is set at the LOQ.

3.5 Independent laboratory validation (ILV)

An ILV must be conducted with samples of representative commodities of all matrix groups for which a primary method is required, with the same number of samples and fortification levels. If the primary method is identical for all matrix groups, it is sufficient to perform the ILV for commodities of two of these groups, one of them with high water content.

If a validated primary method is required for commodities difficult to analyse (Section 3.3) an ILV must be performed.

No ILV may be required if a multi-residue method published by an international official standardisation body is sufficiently validated in more than one laboratory (see Section 2.6 for details).

4 Analytical methods for residues in foodstuff (of animal origin) (Annex IIA Point 4.2.1 of Directive 91/414/EEC; Annex Point IIA, Point 4.3 of OECD)

4.1 Purpose

- Analysis of foodstuff and feeding stuffs of animal origin for compliance with MRL [3].

4.2 Selection of analytes

The selection of analytes for which methods for foodstuff of animal origin are required depends upon the definition of the residue for which a maximum residue level (MRL) is set or is applied for according to Regulation (EC) No 396/2005.

4.3 Commodities

A residue analytical method for foodstuff of animal origin shall be provided for the following animal matrices, if an MRL is established or is likely to be proposed:

- Milk
- Eggs
- Meat (e.g. bovine or poultry)
- Fat
- Liver/kidney

Methods must be validated according to Section 2.9 and 2.10.

4.4 Limit of quantification

Generally, an LOQ of 0.01 mg/kg should be met. Only in justified cases it may be sufficient that the LOQ complies with the lowest MRL in the respective matrix [3].

4.5 Independent laboratory validation (ILV)

An ILV must be conducted with samples of representative commodities of all matrices for which a primary method is required, with the same number of samples and fortification levels. If a primary method is identical for all matrices listed under Section 4.3, it may be sufficient to perform the ILV with at least two of these matrices.

5 Analytical methods for residues in soil (Annex IIA, Point 4.2.2 of Directive 91/414/EEC; Annex Point IIA, Point 4.4 of OECD)

5.1 Purpose

Monitoring, enforcement of restrictions, post-registration control, emergency measures in the case of an accident, surveillance of buffer zones to surface waters.

5.2 Selection of analytes

The residue definition for monitoring purposes in soil is based on the assessment of fate and ecotoxicology and may include the active substance and/or relevant metabolites.

EFSA Conclusions provide recommendations for the analytes "relevant for monitoring" in soil for active substances which were already peer reviewed. However such a definition may be subject to national legal provisions.

- Analytical methods for residues in soil may not be necessary, if more than 90 % of the start concentration of the active substance and its relevant metabolites are degraded within 3 days (DT_{90} < 3 d).
- Methods for naturally occurring non-toxic substances are usually not required.

5.3 Samples

Methods must be validated according to Section 2.9 and 2.10 with representative soil of crop growing. Characteristics of the soil sample (e.g. soil type, pH and organic matter/carbon content) should be provided in the method description to support its selection.

5.4 Limit of quantification

Usually, the limit of quantification for residues in soil should be 0.05 mg/kg.

If the toxic concentration (LC_{50}) for the most sensitive non-target organism is lower than 0.05 mg/kg (= 75 g/ha) [3] the LOQ must comply with this LC_{50} value. For phytotoxic herbicides the LOQ should also comply with the EC_{10}-value of the most sensitive crop.

[3] Expected concentrations in soil can be calculated from the application rate of an active substance (in [g/ha]) using the following equation:

$$c = \frac{\text{application rate}}{\text{soil depth} \cdot \text{soil density}}$$

$$\text{with soil depth}: 10\,[\text{cm}]; \text{soil density}: 1.5\,[\text{g/cm}^3]:$$

$$c = \text{application rate} \cdot \frac{1}{1500} \left[\frac{mg}{kg}\right]$$

478 Methods for highly phytotoxic compounds possibly demand highly sophisticated equipment
479 to meet the required LOQ. Therefore techniques that are not considered as commonly
480 available can be accepted, if justified.

6 Analytical methods for residues in water (Annex IIA, Point 4.2.3 of Directive 91/414/EEC; Annex Point IIA; Point 4.5 of OECD)

6.1 Purpose

Enforcement of the drinking water limit [14] or the groundwater limit [15] of 0.1 µg/L, post-registration control, emergency measures in the case of an accident.

6.2 Selection of analytes

The residue definition for monitoring purposes in drinking water and surface water is based on the assessment of fate and ecotoxicology and may include the active substance and/or relevant metabolites.

EFSA Conclusions provide recommendations for the analytes relevant for monitoring in drinking water/groundwater and surface water for active substances which were already peer reviewed. However such a definition may be subject to national legal provisions.

- Analytical methods for residues in water may not be necessary, if more than 90 % of the start concentration of the active substance and its relevant metabolites are degraded within 3 days (DT_{90} < 3 d).
- Methods for naturally occurring non-toxic substances are usually not required.

6.3 Samples

Methods must be validated according to Section 2.9 and 2.10 for the following matrices:

- Drinking water or groundwater
- Surface water (freshwater, e.g. from rivers or ponds)

In the method description the sampling site should be provided. For the surface water used in method validation quality data shall be provided to demonstrate that the sample is a typical surface water in terms of its inorganic load (e.g. conductivity, hardness, pH) and its organic load (e.g. dissolved organic carbon content (DOC)).

Provided that a method has been successfully validated for surface water at the LOQ required for drinking water, no further validation in drinking water is required.

6.4 Limit of quantification

For drinking water or groundwater the limit of quantification must meet 0.1 µg/L [14]. For surface water the LOQ must comply with the lowest effect concentration [16] mentioned in Table 4.

512 **Table 4: Effect concentrations relevant for setting of LOQs in surface water**

	Acute test	Long-term test
Fish	LC_{50}	NOEC
Daphnia	EC_{50}	NOEC
Chironomus sp	EC_{50}	NOEC
Algae	EC_{50}	
Higher aquatic plants	EC_{50}	

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514 ## 6.5 Direct injection

515 In case that HPLC-MS/MS is used the direct injection of water samples is desirable, provided
516 that it complies with the LOQ. In that case recovery data cannot be calculated. Thus,
517 calibration and precision data have to be presented, only.

7 Analytical methods for residues in air (Annex IIA, Point 4.2.4 of Directive 91/414/EEC; Annex Point IIA; Point 4.7 of OECD)

7.1 Purpose

Monitoring of the exposure of operators, workers or bystanders and working place.

7.2 Selection of analytes

For air analyte selection is governed by the safety of operators, workers and/or bystanders as the primary criterion, and comprises the active substance in most cases.

EFSA Conclusions provide recommendations for the analytes relevant for monitoring for active substances which were already peer reviewed.

- Methods may not be necessary if the application technique makes an exposure unlikely. However, consideration should be given to spray drift and particle associated substances which can cause relevant exposures. Therefore, in such cases an analytical method is also required for substances with a low vapour pressure (< 10^{-5} Pa).
- Methods are usually not required for naturally occurring non-toxic substances and substances which are not classified as T+, T, Xi, Xn and are not labelled with the following symbols according to GHS:

7.3 Samples

Methods shall be validated according to Section 2.9 with air at 35 °C and at least 80 % relative humidity (RH). In justified cases (e.g. heat sensitive analyte) and if it is shown that a method does not work at 35 °C and 80 % RH, other conditions are applicable (e.g. ambient temperature and normal humidity).

7.4 Limit of quantification

If a limit was established according to Council Directive 98/24/EC [17], the LOQ should comply with this value. If no limit is set the LOQ should comply with the concentration c calculated from the $AOEL_{inhalative}$ (in [mg/kg bw d]) according to the following equation:

$$c = AOEL_{inhalative} \cdot \frac{\text{safety factor} \cdot \text{body weight}}{\text{air intake}}$$

548 $\text{with safety factor} : 0.1; \text{body weight} : 60\,[\text{kg}]; \text{air intake} : 20\,[\text{m}^3/\text{day}]$

$$c = AOEL_{inhalative} \cdot 300 \left[\frac{\mu g}{m^3}\right]$$

549 Instead of the $AOEL_{inhalative}$ the $AOEL_{systemic}$ can be used for calculation. In case that no
550 AOEL is available, the ADI-value can be employed instead.

551 In case that inhalation toxicity studies show that an active substance induces local effects on
552 the respiratory tract rather than systemic effects, the LOQ should comply with the $AEC_{inhalation}$
553 [18].

554 7.5 Sorbent characteristics

555 The sorbent must be suitable for enrichment of particle associated and gaseous residues. It is
556 sufficient to quote literature proving that the sorbent also adsorbs particle associated residues.
557 For polymer based sorbents such a proof is not required.

558 7.6 Further validation data

559 The retention capacity of the sorbent material must be proven. This may be carried out by
560 determining the recovery of the analyte, added onto the sorbent in a small volume of solvent,
561 at defined air temperature and relative humidity, after passage of a defined volume of air
562 (> 100 L) for at least 6 hours. The capacity is considered sufficient if no significant
563 breakthrough occurs.

564 It is desirable to submit data on the extractability of the analyte from the sorbent and on the
565 storage stability of the analyte loaded onto the sorbent.

566 7.7 Confirmatory methods

567 No confirmatory methods are required for the determination of residues in air if sufficient
568 confirmatory methods are available for the determination in soil or water.

8 Analytical methods for residues in body fluids and tissues (Annex IIA, Point 4.2.5 of Directive 91/414/EEC; Annex Point IIA Point 4.8 of OECD)

8.1 Purpose

Detection of intoxications in humans and animals.

8.2 Selection of analytes

EFSA Conclusions provide recommendations for the analytes relevant for monitoring in body fluids and tissues for active substances which were already peer reviewed. This may include analytes defined as “relevant for monitoring” and classified as toxic or very toxic (T, T+) or are classified according to GHS as follows: Acute toxicity (cat. 1 - 3), CMR (cat. 1) or STOT (cat. 1). This may also include analytes that exhibit high acute toxicity in humans or animals equivalent to those classifications.

8.3 Samples

Methods must be validated according to Section 2.9 and 2.10 with the following matrix groups:

- Body fluids (either blood, serum, plasma or urine)
- Body tissues (either meat, liver or kidney)

Methods for body fluids and tissues should be validated with the matrix which is most suitable to prove intoxication. If a primary method for food of animal origin (Section 4) with sufficient sensitivity covers the respective tissue no additional method or validation study for body tissue is required.

8.4 Sample set

- LOQ 5 samples
- control 2 samples

No validation data for an elevated concentration level (10 x LOQ) are required for body fluids and tissues.

8.5 Limit of quantification

The LOQ shall meet 0.05 mg/L for body fluids and 0.1 mg/kg for body tissues.

9 Summary - List of methods required

Table 5 gives an overview on the methods that may be required. It can be used by the applicant to check prior to submission of the application whether all required studies are addressed.

Table 5: Completeness check of analytical methods for monitoring purposes and post-registration control

Matrix group / crop group	Residue definition for monitoring	LOQ	Methods		
			Primary method	Confirmatory method	Independent lab validation
Dry commodities (high protein/high starch content)					
Commodities with high water content					
Commodities with high oil content					
Commodities with high acid content					
Commodities which are difficult to analyse					
Milk					
Eggs					
Meat					
Fat					
Kidney/liver					
Soil					Not necessary
Drinking water					Not necessary
Surface water					Not necessary
Air					Not necessary
Body fluids					Not necessary
Body tissues					Not necessary

10 Abbreviations

AAS	atomic absorption spectroscopy
ADI	acceptable daily intake
$AOEL_{inhalative}$	acceptable operator exposure level for exposure by inhalation; according to [19]
$AEC_{inhalation}$	adverse effect concentration for exposure by inhalation [18]
$AOEL_{systemic}$	acceptable operator exposure level concerning systemic effects [19]
CEN	European Committee for Standardisation
DAD	diode array detector
DT_{90}	time required for 90 % degradation
EC_{10}	concentration showing 10 % effect
EC_{50}	concentration showing 50 % effect
ECD	electron capture detector
EFSA	European Food Safety Authority
ESI	electrospray ionisation
FID	flame ionisation detector
FLD	fluorescence detector
FPD	flame photometric detector
GC	gas chromatography
HPLC	high-performance liquid-chromatography
HRMS	high resolution mass spectrometry
ICP	inductively coupled plasma
ILV	independent laboratory validation
LC_{50}	concentration showing 50 % lethal effect
LOQ	limit of quantification (here: lowest successfully validated level)
MRL	maximum residue level
MS	mass spectrometry
MS^n	multiple-stage mass spectrometry (with $n \geq 2$)
NOEC	no observed effect concentration
NPD	nitrogen phosphorus detector
OECD	Organisation of Economic Cooperation and Development
OES	optical emission spectroscopy

RSD	relative standard deviation (coefficient of variation) (here: within laboratory)
SRM	selected reaction monitoring
UV	ultraviolet (detector)

11 References

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[4] OECD (2007) Guidance Document on Pesticide Residue Analytical Methods. OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 72 and Series on Pesticides No. 39. ENV/JM/MONO (2007) 17.

[5] European Commission, Directorate General Health and Consumer Protection (2010) Guidance Document on Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, SANCO/10684/2009

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[11] European Committee for Standardisation (CEN) EN 12393-2:2008. Non-fatty foods. Multiresidue methods for the gas chromatographic determination of pesticide residues. Methods for extraction and clean-up.

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