Additional guidance on:

TNsG on Data Requirements, Part A, Chapter 2, Point 4

"Analytical Methods for Detection and Identification"

and

Part B, Chapter 2, Point 4

"Methods of Identification and Analysis"

These Technical Notes for Guidance were adopted during the 33rd meeting of representatives of Members States Competent Authorities for the implementation of Directive 98/8/EC concerning the placing of biocidal products on the market (13-15 May 2009)

TNsG on Data Requirements, Part A, Chapter 2, Point 4 "Analytical Methods for Detection and Identification" with regard to the procedure for evaluation of analytical methods[1]

According to the Directive 98/08/EC for placing of biocidal products on the market the applicant has to supply validated analytical methods required for the determination of the active substances (and where appropriate, for relevant degradation products, isomers and impurities of active substances and their additives), and for relevant residues thereof in/on soil, in air, in drinking and surface water, in body fluids and tissues, and in treated food or feeding stuffs.

The objective of validation of analytical methods is to demonstrate that they are suitable for its intended uses. The methods should have the ability to determine all of the analytes included in the residues definitions for enforcement established by the competent authorities (CA). The methods should use commonly available techniques/equipment and avoid hazardous substances (e.g. carcinogenic substances like diazomethane, benzene or chloroform). Enforcement methods are required to demonstrate appropriate limits of quantitation (LOQ), to be sufficiently selective, so that interfering substances never exceed 30% of the LOQ, and demonstrate acceptable recovery and repeatability.

The method should meet standards for certain validation parameters. Typical validation characteristics for residue analytical methods that should be considered are: accuracy, recovery, selectivity (specificity), calibration, precision (repeatability, reproducibility) and limit of quantitation (LOQ). These parameters are defined below[2]:

Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Recovery

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

[1] Reference documents: Guidance document on residue analytical methods, SANCO/825/00 rev 7; Guidance Document on Pesticide Residue Analytical Methods (OECD), ENV/JM/MONO (2007)17.
[2] ENV/JM/MONO(2007)17

Precision

The precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Selectivity (Specificity)

Selectivity refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences like impurities, degradants, matrix etc. Some regulatory authorities use the term specificity to refer to selectivity.

Calibration

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

Repeatability

Repeatability refers to the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

Reproducibility

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

Limit of quantitation (LOQ)

Limit of quantitation[3], defined from a regulatory perspective as the lowest concentration tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation (RSD) is obtained. The LOQ should be low enough to achieve the intended purpose of the method.

Description of an analytical method

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

- Definition of the analyte;
- Apparatus;
- Reagents (including purity as well as full details of standard compounds purity and associated method of determination or clear reference of origin, if commercially available);
- Analytical procedure including sample processing, extraction, clean up, derivatisation, determination (if appropriate);
- Description of calibration including the use of matrix matched standards (if appropriate);
- Procedure for the calculation of results from raw data;
- Result tables (if results are not presented in separate studies).

The following information should be offered if appropriate:

- Schematic diagram of the analytical procedure;
- Stages where an interruption of the procedure is possible;
- Hazards or precautions required;
- A statement about extraction efficiency of solvents used .

Minimum data requirements

For demonstrating the suitability of the method for its purpose, information on performance characteristics should be provided.

Basic validation data are:

- a typical calibration curve for each representative matrix (if studies are necessary);
- the concentration of analyte found in blank samples;

[3] In Directive 98/08/EC the term "limit of determination" is used, here replaced by "limit of quantitation" (LOQ).

- the concentration level(s) of fortification experiments;
- the number of fortification experiments for each commodity/level combination;
- the mean recovery for each commodity/level combination;
- the relative standard deviation (RSD) of recovery for each commodity/level combination;
- representative, clearly labelled chromatograms (standard, blank, sample at least at the LOQ).

Analytical techniques considered commonly available

- GC detectors: NPD, FPD, ECD, FID, MS, MS^n;
- HPLC detectors: UV, DAD, MS, MS^n, Fluorescence detector, Electrochemical detector;
- HPLC columns: normal phase, reversed phase, ion-exchange, ion-pair; column switching;
- AAS: FAAS, GFAAS, hydride generation AAS;
- ICP: ICP/OES, ICP/MS;
- further analytical techniques in certain cases.

Calibration

Analytical calibration should extend over a range appropriate for the lowest and highest (± 20%) nominal concentration of the analyte in relevant analytical solutions. Duplicate determinations at three or more concentrations or single determinations at five or more concentrations should be performed. Raw data of calibration have to be provided with the studies. The equation of the calibration function and the correlation coefficient (r) should be reported and a typical calibration plot submitted.

Selectivity (Matrix Interference)

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

Number of Fortification Experiments

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

Range of Acceptable Recoveries

In general, the mean recovery at each fortification level and for each commodity should be in the range of 70-110%.

Precision - Repeatability (expressed as relative standard deviation)

The precision of the method in a validation study should be reported as the relative standard deviation (RSD) of repeatability at each fortification level. As specified above, five determinations should be made at each fortification level. In general the RSD should be ≤ 20% per commodity and level. Where outliers have been discarded, this fact, the data of the outlier and the statistical significance must be clearly indicated. A maximum of one outlier may be disregarded at each fortification level.

Confirmatory Techniques

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

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

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

Derivatization

For analysis of some compounds, such as those with high polarity or with poor chromatographic properties, derivatization may be called for. Derivatives may be prepared prior to chromatographic analysis or as part of the chromatographic procedure (pre- or post-column). The use of derivatization methods should be fully reported and justified. The derivative should be stable and its formation reproducible.

When quantification is based on the determination of a derivative, the calibration is preferably conducted using standard solutions of that derivative, unless the derivatization step is an integral part of the detection system. If the derivative is not available as a reference standard, it should be generated within the analytical set by using the same derivatization procedure as that applied for the samples. Under these circumstances, a full justification should be given. The method is considered to be specific to the analyte of interest if the derivatized species is specific to that analyte.

Stability

In case reference is sought with regard to the stability of the samples, the OECD guidance document on pesticide on residue analytical methods (ENV/JM/MONO(2007)17) should be consulted.

Independent Laboratory Validation Studies

Independent laboratory validation (ILV) studies are performed in order to demonstrate the reproducibility of the analytical method. Independent laboratory validation (ILV) studies generally are needed for the determination of residues in plant materials and additionally for methods for the determination of residues in food of animal origin, if such methods are required. An ILV is not required for confirmatory methods.

Usually, an independent laboratory validation should be conducted with samples of the representative commodities and tissues. The sample set (number of samples and fortification levels) of the primary validation has to be applied for the ILV also.

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

An ILV may not be necessary if available published multi-residue methods have been validated for the representative commodities.

Residue analytical methods

Residue analysis from soil

Residue definition

Generally, the CA has to decide, where relevant, which compounds (parent and/or metabolites) should be monitored based on its evaluation on fate and behaviour of the active substance in the environment.

Limit of quantitation

The LOQ must be equal or lower than the relevant NO(A)EC, without exceeding the general limit of 0.05 mg/kg

If the active substance degrades very fast, i.e. DT_{90} values of the active substance and/or the relevant metabolites are lower than 3 days analytical methods for residues in soil are not required except in case of continuous exposure.

Residue analysis from air

Residue definition

Analytical methods must be submitted if the substance is volatile or sprayed or occurrence in air is otherwise probable. Generally, the active substance is considered to be the relevant residue in air for monitoring purposes.

Limit of quantitation

In the case of analytical methods for air regarding **general population**, the limit of quantitation (LOQ) must be equal or lower than the concentration C which is defined as:

$$C = \frac{AEL \cdot 0.1 \cdot 60}{20} \ [mg/m^3 \text{ air}]$$

0.1	safety factor
60	body weight in kg
20	air intake [volume per day in m^3]
AEL_r	overall systemic limit value for the human population as a whole – resembling the AOEL. The lowest AEL value available should be used.

In the case of analytical methods for air concerning professional users, the LOQ should be based on the occupational exposure limit (OEL), when available,which is an air concentration

value expressed in mg/m^3. The OEL defines the health-based airborne concentration limit at the workplace for a certain substance. The defined concentration C should then according to the requirement of EN 482 (Workplace atmospheres-General requirements for the performance of procedures for the measurements of chemical agents) be 1/10 of the OEL:

C = OEL x 0.1

Additionally, the other requirements of the European standard EN 482, e. g. storage stability, reproducibility and expanded uncertainty, have to be fulfilled. These parameters have to be submitted together with the limit of quantitation.

If from international or national sources an OEL is not yet available, the limit of quantitation should be based on the same AEL value as for the general population described above.

The methods must be suitable for detecting both particle associated and gaseous residues.

Residue analysis from water

Residue definition

Generally, the CA has to decide, where relevant, which compounds (parent and/or relevant metabolites) should be monitored based on its evaluation on fate and behaviour of the active substance in the environment and the toxicological and ecotoxicological potential.

Limit of quantitation for drinking water

According to TNsG Chapter 2 Part A 4.2 (c) the LOQ in drinking water must be equal or lower than 0.1 µg/L (EU drinking water limit).

Limit of quantitation for surface water

The LOQ must be equal or lower than the relevant NO(A)EC.

In the case of products of product type 2, which are used in water, the analytical method should be able to analyse 1% of the typical applied concentration.

Residue analysis from body fluids and tissues

Residue definition

Active substances classified as toxic or highly toxic are considered to be the relevant residues in body fluids and tissues. They must be analysed for monitoring purposes. The inclusion of metabolites may be decided by the CA.

Limit of quantitation

The LOQ should be set at 0.05 mg/L for body fluids and 0.1 mg/kg for tissues.

Residue analysis from food and feeding stuffs (relevant for human risk assessment)[4]

Analytical methods are required only, if the intended use of a biocidal product may cause contact with food or feeding stuffs. Therefore, the CA has to decide which food and which feeding stuffs may be contaminated during use of the biocidal product. Generally, the CA has to decide where relevant, which compounds (parent and/or relevant metabolites) should be monitored based on its evaluation on fate and behaviour of the active substance in the relevant food and feeding stuffs and the toxicological potential.

If a maximum residue level (MRL) is available for food and feeding stuff that may be exposed to biocidal products the guidance on analytical methods available for pesticides and/or veterinary medicinal products must be followed.

If the active substance of a biocidal product is not used in plant protection products or in veterinary medicinal products MRLs are not available. In that case the maximum acceptable levels in food and feeding stuffs must be calculated by the CA based on (i) toxicological data and (ii) residue data of the active substance in food and feeding stuffs and (iii) an exposure assessment. This exposure assessment must include all relevant routes of exposure to the consumer (air, water, dust, food etc.). Under consideration of this calculation tolerable residue levels in food and feeding stuffs can be derived and should be used as LOQ.

Validated analytical methods must be available for samples of the representative commodities and tissues.

[4] It must be noted that a framework for dietary risk assessment is currently being developed for food of animal and plant origin. This may have consequences for the present paragraph on "residue analysis from food and feeding stuffs".

TNsG on Data Requirements, Part B, Chapter 2, Point 4 "Methods of Identification and Analysis" regarding the need of additional analytical methods for toxicologically and ecotoxicologically relevant by-products (non-actives) of the biocidal product

Residue definition

Generally, the CA has to decide, where relevant, which by-products should be monitored in addition to TNsG Part A, 4.2 based on its evaluation on fate and behaviour of the components and the toxicological and ecotoxicological potential.

- By-products of the biocidal product classified as toxic or highly toxic are considered to be the toxicologically relevant components. They must be analysed for monitoring purposes if human exposure cannot be excluded. Validation of the analytical methods employed must be performed.

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

- By-products of the biocidal product classified as dangerous for the environment are considered to be the ecotoxicologically relevant components. They must be analysed for monitoring purposes if environmental exposure can not be excluded. Validation of the analytical methods employed must be performed.

Limit of quantitation: the LOQ should correspond to the limits of TNsG Part A, 4.2 (soil, water).