Guidance Document on the Estimation of LOQ for Measurements in the Field of Contaminants in Feed and Food

VERSION 1.0

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Editors

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1. Scope and Field of Application

In 2018, the scope of the European Union Reference Laboratories (EURLs) working in the field of contaminants (EURL for metals and nitrogenous compounds (MN), EURL for mycotoxins and plant toxins (MP), EURL for processing contaminants (PC) and EURL for halogenated POPs in feed and food (POPs)) was extended to additional contaminants. As a consequence an even wider variety of analytes with different physico-chemical properties requiring various analytical techniques have to be covered.

In this "Guidance Document on the Estimation of LOQ for Measurements in the Field of Contaminants in Feed and Food (2022)" the four EURLs working in the field of contaminants aim to describe, in a joint document, one recommended and generally applicable approach for estimation of LOQ. Other approaches may then be used only for sound reasons, e.g. if required by EU legislation or in case of elevated background levels.

This document is focusing primarily on official control and compliance testing against legal limits. Fit-for-purpose LOQs in this context are typically in the range of ≤ 0.2 to 0.5 times the respective legal limits. Secondarily, this general approach can be applied to compounds without specific legislation on analytical methods and/or legal limits, and for monitoring and risk assessment purposes. For the latter cases, the LOQs should be aimed as low as possible, which may require an extension of the relevant working range at the lower end compared to the methods used for compliance testing only.

From a general point of view, it is recalled that much of the data submitted to EFSA for risk assessment is from official controls, i.e. has not been analysed under monitoring/risk assessment conditions, meaning that the LOQs are not always as low as possible. It should be kept in mind, that for a reliable risk assessment numerical data are needed as much as possible to gather the present contamination situation and to assess the potential risk to human and animal health.

The authors acknowledge that the new general approach and the possible exceptions still have its limitations and may not be generally applicable in all cases. The described approach for estimation of LOQ differs from the statistical approaches based on estimation of LOD and derivation of LOQ thereof [1]. It is comparable with the approach as established for pesticide residue analysis in food and feed [2].

The concepts of the "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food (2016)" [3] may therefore still be followed if the resulting LOQs fit the criteria specified in this document. In case the estimation of the limit of detection (LOD) is necessary the aforementioned document can be used.

The terminology in this guidance document is adapted to the guidance document published in 2016 [3].

2. Abbreviations

CPs	Chlorinated paraffins
GEs	Glycidyl esters
HBCDDs	Hexabromocyclododecanes
MCPD	Monochloropropane-1,2-diol
MCPDEs	Monochloropropane-1,2-diol esters
MOH	Mineral oil hydrocarbons
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzo-p-dioxins and dibenzofurans
PCNs	Polychlorinated naphthalenes
PFAS	Per- and polyfluoroalkyl substances

3. Definitions

Trueness: Closeness of agreement between the mean value obtained from a large series of test results and an accepted reference value [4, 5]. Trueness is usually expressed as bias. Bias is the (relative) difference between the measured and the accepted reference value.

Recovery: Recovery is the yield of a preconcentration or extraction stage of an analytical process for an analyte divided by amount of analyte in the original sample. The apparent recovery is the observed value derived from an analytical procedure by means of a calibration graph divided by a reference value [6].

Note: *Recovery and apparent recovery are not always clearly distinguished or defined in the different fields of analysis. Please refer to the respective regulations or guidance documents on analytical criteria for exact definition.*

Precision: Closeness of agreement between independent test results obtained under stipulated conditions and is expressed as the standard deviation or coefficient of variation of the test results [4]. More specific terms for precision, depending on which levels of variability are included, are repeatability, intermediate precision (within-laboratory reproducibility), and reproducibility (between-laboratory reproducibility).

Procedural blank: Sample that does not contain the matrix that is brought through the entire measurement procedure and analysed in the same manner as a test sample [7, 8].

4. Estimation of LOQ

4.1. General approach – Lowest validated level

In EU legislation for contaminants (e.g. Commission Regulation (EU) 2017/644 [9] and (EC) No 152/2009 [10], Commission Regulation (EC) 333/2007 [11]) the limit of quantification (LOQ) is defined as the lowest content of the analyte which can be measured with reasonable statistical certainty in the field of contaminants in food and feed.

A general approach for the estimation of LOQ is the lowest successfully validated level of an analyte, for which it has been demonstrated that the respective criteria for identification, precision and trueness are met.

Depending on the required concentration ranges (e.g. checking of compliance with legal limits or also generating monitoring data for risk assessment), the lowest level(s) included in the validation need to be adapted.

The specific criteria for identification, precision and trueness or recovery are described in respective legislation or compound-specific guidance documents.

Note: This approach could result in higher LOQs compared to other approaches, depending on the applied criteria for identification, precision and trueness in the low concentration range.

4.2. Alternative approaches

If the lowest successfully validated level approach is not suitable to a group of analytes for sound reasons, other defined approaches may be applied. Such reasons might be:

- other approaches defined in EU regulations for certain analytes or established for analytes with similar analysis and physico-chemical properties (i.e. PCDD/Fs, PCBs, PCNs, PBDEs; see chapter 8)
- elevated background levels: analytes for which it is impossible to obtain a sufficiently low procedural blank

4.3. Procedural blanks

Especially for ubiquitous contaminants, analyte signals in the procedural blank may arise, e.g. from laboratory background, solvents, reagents, adsorbents, glassware, instruments, impurities in the standards used, and cross-contamination. In general, procedural blank levels should be minimized as far as possible.

In case it is not possible to consistently obtain signal-free procedural blanks, a constant monitoring of the levels of procedural blanks is an essential part of the method quality control. The inevitable background needs to be taken into account in the estimation of the LOQ. The requirement here is that the lowest level tested during validation shall not be less than 10 times the standard deviation and additionally taking into account the average blank level, if necessary, as determined from at least ten independent procedural blank analyses.

4.4. Flow chart

A flow chart was developed (see figure 1) in order to facilitate the choice of the appropriate LOQ estimation.
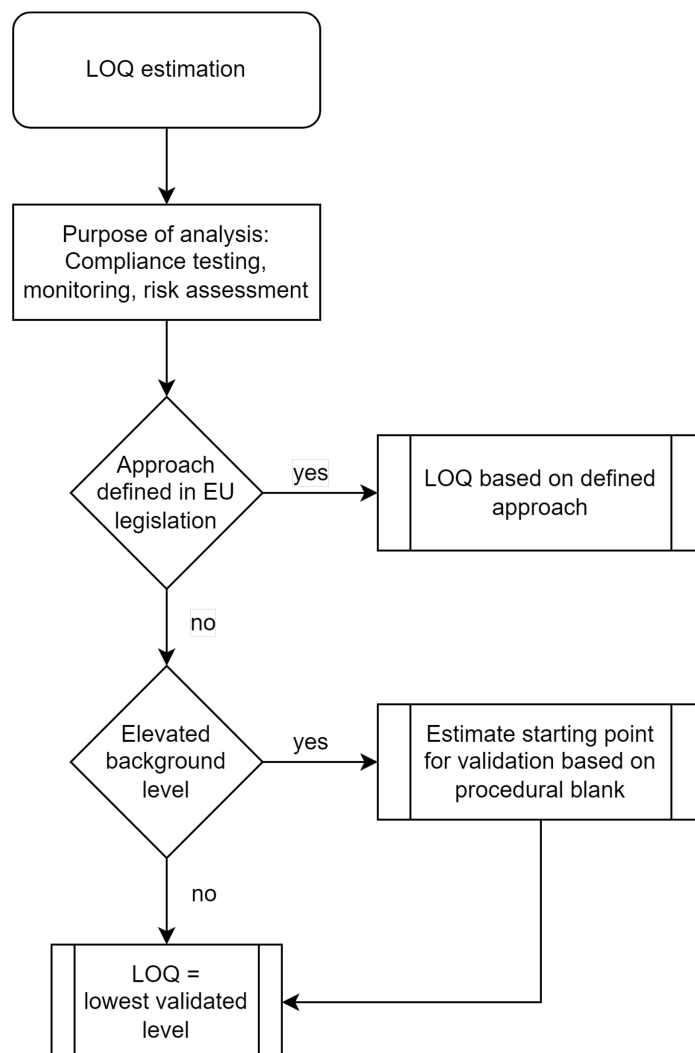
```
┌─────────────────────────┐
│     LOQ estimation       │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│   Purpose of analysis:   │
│   Compliance testing,    │
│ monitoring, risk assessment │
└─────────────────────────┘
            │
            ▼
        ◇ Approach          yes    ┌──────────────────────┐
        defined in EU ──────────►  │ LOQ based on defined │
        legislation ◇              │      approach         │
            │                      └──────────────────────┘
            │ no
            ▼
        ◇ Elevated          yes    ┌──────────────────────┐
        background  ──────────►    │ Estimate starting point │
        level ◇                    │ for validation based on │
            │                      │   procedural blank      │
            │ no                   └──────────────────────┘
            ▼                                │
┌─────────────────────────┐                 │
│        LOQ =             │ ◄───────────────┘
│   lowest validated       │
│        level             │
└─────────────────────────┘
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Figure 1: Flow chart for estimation of LOQ in compliance testing and monitoring/risk assessment

5. Specific Approaches for Metals & Nitrogenous Compounds

For metals and nitrogenous compounds, the general approach of lowest validated level (4.1) applies to most analytes.

For analyte/matrix combinations where relevant samples with sufficient low content (= desired LOQ level) of the analyte are difficult or not possible to find, synthetic blank matrices (a synthetic blank matrix is made from various chemicals to mimic the real food matrix without or very low content of the analyte) spiked with the analyte at the desired LOQ level may be used. A good estimate of the LOQ level can e.g. be found based on procedural blanks and using 10 times the standard deviation as determined from at least ten independent procedural blank analyses [1] as a starting point for the further validation of the LOQ level.

For multi-analyte methods (e.g. multi-element determination by ICP-MS) it is often difficult or not possible to find relevant samples, in which all analytes are present at low (LOQ) level. In such case, a combination of several different samples each with low content of one or several analytes of interest may be used. Alternatively, the approach of using synthetic matrices spiked with the analytes of interest at their respective LOQ levels may be followed.

6. Specific Approaches for Mycotoxins and Plant Toxins

For mycotoxins and plant toxins, the only exception to the general approach mentioned under 4.1, are certain toxin/matrix combinations where the toxins are inherently present at relatively high levels (e.g. gossypol in cotton seeds, hydrocyanic acid in apricot kernels). In these cases the method LOQ can be estimated using a highly similar matrix that is free of the toxin, or contain the toxin at a much lower level than the actual matrix. For example sweet almonds as proxy for bitter apricot kernels for hydrocyanic acid. The LOQ determined this way provides an indication of the LOQ the method can achieve, thereby fulfilling the requirements of ISO 17025.

7. Specific Approaches for Processing Contaminants

The general approach of lowest validated level applies to acrylamide, furan, MCPD, MCPDE, GE and single PAH compounds. For MOH the approach for estimation of LOQ will be described in a separate document. For the processing contaminants, special features apply depending on the group of contaminants. For LOD/LOQ requirements for individual compound groups please refer to Commission Regulation (EU) 333/2007 with amendments. Only PAHs is included in approaches described in the "Guidance Document on the Estimation of LOD and LOQ for Measurements in the field of Contaminants in Feed and Food" [3]. For sample matrixes with very high inherent concentration of the processing contaminants, alternative matrixes with similar properties could be used for the validation (for example for furan in coffee). A list with alternative matrixes will be prepared by the EURL-PC.

8. Specific Approaches for Halogenated POPs

The approaches described in the "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" [3] for the estimation of LOQ are specified for the analysis of PCDD/Fs and PCBs and are closely linked to the EU legal requirements for the analysis of these compounds in feed and food [9, 10]. The approach for PFAS is described in the respective EU regulation [12].

For the other compounds covered by the EURL POPs, currently no legal limits or analytical criteria are defined in EU regulations for official food and feed. However, for brominated flame retardants (BFRs), Commission Recommendation 2014/118/EU [13] describes limits of quantification for monitoring of these compounds in food. In addition guidance documents of the EURL/NRL network for halogenated POPs on the analysis and analytical parameter for the determination of PFAS, CPs and brominated contaminants are available [13, 14, 15].

The extension of the scope of the EURL POPs has increased the number of analyte groups of interest, but also the number of different methods applicable for the new analyses. In addition to the GC-based methods for PCDD/Fs and PCBs, LC-based methods are now included and specific analytical aspects need to be addressed.

In general, the LOD and/or LOQ are estimated for individual compounds. For sum parameters estimation should only be performed if this is required by respective EU regulations.

Some of the approaches described here (see table 1) are very general and applicable to all kind of analytes and methods, whereas other approaches are specific to mass spectrometric detection methods (e.g. low and high mass resolution or tandem mass spectrometry). The procedural blanks approach for chlorinated paraffins (CPs) is considered as a provisional approach and starting point for the estimation of LOQs based on the lowest validated level taking into account procedural blank contributions.

Table 1: Possible approaches for the estimation of LOQ for different groups of contaminants covered by the EURL for halogenated POPs

Approach	Applicable to following groups of halogenated POPs	Justification/ Reference
Lowest validated level (LOQ) (4.1)	CPs, HBCDDs and other halogenated POPs	General approach
Lowest validated level (LOQ) (4.1)	PFAS	[12]
S/N (LOQ) (8.1)	PCDD/Fs, PCBs, PBDEs*, PCNs* (with IS)	[9, 10]
Estimation from Calibration Standards (LOQ) (8.2)	PCDD/Fs, PCBs, PBDEs*, PCNs*, (with IS)	[9, 10]
Sum parameters (8.3)	PCDD/Fs, PCBs	[9, 10]
Procedural blanks (8.4)	CPs	Provisional approach as starting point

*Similar analysis to PCDD/Fs and PCBs

8.1.   Signal-to-noise ratio approach

see "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (2016), chapters 7.1 and 8.1 [3]

8.2.   Lowest calibration standards approach

see "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (2016), chapters 7.2 and 8.2 [3]

8.3.   Sum parameters

see "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (2016), chapter 9 [3]

8.4.   Procedural blanks approach (provisional approach)

As a starting point for the estimation of LOQ according to the general approach (4.1), the lower threshold for validation should first be estimated based on the procedural blank levels using equation 1. For analytes with a negligible matrix effect, but measurable and rather constant procedural blanks, the estimation of this threshold can be based on the standard deviation of multiple procedural blank levels, obtained under intermediate conditions.
At least 10 independent procedural blanks should be analysed.

$$x_{LTV} = x_{blank} + 10 \cdot s_{blank} \quad \text{(equation 1)}$$

x_{LTV}: lower threshold for validation
x_{blank}: Procedural blank contents
s_{blank}: Standard deviation of the procedural blank contents

The lower threshold for validation is the lowest level for which presence of the analyte in samples can be reliably differentiated from the inevitable background. X_{LTV} is the lowest level at which validation can be performed. When the criteria for trueness, precision and identification are met at this level, then the LOQ equals X_{LTV}.

9. References

[1] B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for
Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related
Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org

[2] European Commission, Directorate-General for Health and Food Safety, Analytical
Quality Control and Method Validation Procedures for Pesticide Residues Analysis in
Food and Feed, Document SANTE/11312/2021, implemented by 01.01.2022
Available   from   https://food.ec.europa.eu/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf

[3] Wenzl T; Johannes H; Schaechtele A; Robouch P; Stroka J. Guidance Document on the
Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and
Food. EUR 28099 EN. Luxembourg (Luxembourg): Publications Office of the European
Union; 2016. JRC102946

[4] Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the
performance of analytical methods for residues of pharmacologically active substances
used in food-producing animals and on the interpretation of results as well as on the
methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

[5] International Organization for Standardization (ISO), ISO 5725-1:1994 Accuracy
(trueness and precision) of measurement methods and results — Part 1: General
principles and definitions

[6] D. T. Burns, K. Danzer, A. Townshend, Use of the term "recovery" and "apparent
recovery" in analytical procedures (IUPAC Recommendations 2002), Pure and Applied
Chemistry 74 (2002) 2201–2205

[7] IUPAC Compendium on Analytical Nomenclature, Definitive Rules 1997, 3rd Edition,
IUPAC Orange Book, prepared for publication by J. Inczedy, T. Lengyel, and A.M. Ure,
Blackwell   Science,   Oxford,   1998   [ISBN   0-86542-6155],   Ch.   18.6.1.
https://iupac.org/what-we-do/books/orangebook/

[8] H. Cantwell (ed.) Blanks in Method Validation - Supplement to Eurachem
Guide The Fitness for Purpose of Analytical Methods, (1st ed. 2019). Available from
www.eurachem.org

[9] Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of
sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014

[10] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the
methods of sampling and analysis for the official control of feed

[11] Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down methods
of sampling and analysis for the control of levels of trace elements and processing
contaminants in foodstuffs

[12] Commission Implementing Regulation (EU) 2022/1428 of 24 August 2022 laying down methods of sampling and analysis for the control of perfluoroalkyl substances in certain foodstuffs

[13] Commission Recommendation of 3 March 2014 on the monitoring of traces of brominated flame retardants in food

[14] Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed 2022: https://ec.europa.eu/food/system/files/2022-05/cs_contaminants_sampling_guid-doc-analyt-para_0.pdf

[15] Guidance Document on the Analysis of Chlorinated Paraffins - Sample preparation and quantification in food and feed 2021: https://eurl-pops.eu/news/guidance-document-pfas/guidance-document-pfas

[16] Guidance Document on the Determination of Organobromine Contaminants – Analytical parameters in food and feed 2022: https://eurl-pops.eu/news/guidance-document-bcon-parameters/guidance-document-bcon-parameters-2