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ESTIMATION OF LOQ FOR THE ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS, IN PARTICULAR PCDD/FS AND PCBS

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Introduction

A "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" was prepared in cooperation with the European Union Reference Laboratory (EURL) for Polycyclic Aromatic Hydrocarbons (EURL PAH), the EURL for Heavy Metals in Feed and Food (EURL HM), the EURL for Mycotoxins (EURL Mycotoxins), and the EURL for Dioxins and PCBs in Feed and Food. The part of the document concerning analysis of PCDD/Fs and PCBs was prepared by the EURL for Dioxins and PCBs in close cooperation with the core working group for measurement uncertainty in PCDD/Fs and PCBs analysis of the EURL/NRL network.

The guidance document presents two different concepts (see table 1) for estimation of LOD (limit of detection) and/or LOQ (limit of quantification) in official feed and food analysis corresponding to the requirements of the different fields of application:

- In the fields of heavy metals, PAHs and mycotoxins, preference is given to the estimation procedure based on blank (matrix) samples, if available. As an alternative, LOD/LOQ may be estimated based on a calibration model using spiked blank (matrix) samples[1,2,3].
- In the analysis of persistent organic pollutants, in particular polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs), using isotope-dilution mass spectrometry, LOQs are assessed either by signal-to-noise ratios or calibration experiments, taking into account procedural blank samples. Results are calculated as sum parameters taking into account congener concentrations or estimated LOQs. Therefore, the focus is primarily set on the estimation of LOQs. The calibration approach as described for heavy metals, PAHs and mycotoxins may also be applied.

Concepts for estimation of LOQs

The two different approaches as generally defined in the respective EU regulations[4,5] for PCDD/Fs and PCBs, are further described and specified in the framework of the guidance document. The flow chart (see figure 1) gives an overview of the procedures.

Derivation of LOQs from signal-to-noise estimate

The signal-to-noise (S/N) ratio approach identifies a specific LOQ for each congener within each sample reflecting day-to-day method performance, related e.g. to the efficiency of extraction and clean-up, changes in sensitivity of the detection system over time and background noise levels. A congener-based LOQ is defined as the concentration of an analyte in the extract of a sample, which produces an instrumental response on two different diagnostic ion mass traces with a given S/N ratio for both diagnostic ions. The respective higher LOQ value is used for further calculations.

EU regulations[4,5] require a minimum S/N ratio of 3:1 for characteristic ion intensities versus the respective background noise. Therefore laboratories shall establish the S/N ratio preferably based on a noise height N of $2 \times \sigma_{noise}$ (σ_{noise} = standard deviation of the noise).
The LOQ is then calculated as the concentration corresponding to a signal (S), which is 3 times the noise height N:
$LOQ_{S/N=3} = 3 \times N = 3 \times \sigma_{noise}$
σ_{noise}: Standard deviation of the baseline noise
N: Noise height measured from the baseline: $N = 2 \times \sigma_{noise}$
Before a congener-LOQ is calculated, various procedures, including e.g. visual check of chromatogram, selection of noise range and peak baseline and check of procedural blanks, must be followed. In addition, specific identification criteria, in particular for relative ion intensities must be met.

Estimation of LOQs from calibration standards
If the noise level is too small to perform a reliable signal-to-noise ratio calculation or no noise level is measurable[6], LOQs can be estimated from low-level calibration standards, provided that matrix effects and interferences caused by the test sample do not contribute to variability and bias of the analytical results.
Congener LOQs equal the lowest standard concentration meeting criteria for retention time, relative ion intensities and acceptable and consistent deviation of relative response factors (≤ 30 %, measured at least at the start and at the end of a sample series) as described in respective EU Regulations[4,5]. Sample-specific congener-LOQs are then calculated by taking into account sample intake, final extract volume and the recovery of the internal standard.
Laboratories may use alternative approaches provided that identification and quantification criteria specified in EU regulations[4,5] are fulfilled.

Use of procedural blanks
Procedural blanks representative of every batch of test samples providing information on method performance, such as effects/interferences from the chemical measurement process shall be monitored in QC charts and checked for acceptance of a batch of samples. If acceptance criteria are met, estimated LOQs according to the above mentioned procedures are applied. In case these criteria are not met, the analyst must check, if the batch of samples has to be repeated.
Alternatively, if calculated LOQs or measured analyte contents of procedural blanks are higher than the analyte contents in test samples of the same batch, the values estimated/measured in the procedural blanks are applied as LOQs for these test samples.

Estimation of LOQs for sum parameters
In PCDD/F and DL-PCB analysis, legal limits are only given for the TEQ-based sum-parameters WHO-PCDD/F-TEQ, WHO-PCB-TEQ, and WHO-PCDD/F-PCB-TEQ[7,8]. Therefore, according to EU legislation[4,5], the LOQ associated with a WHO-TEQ sum parameter must not exceed the respective target limit of quantification (target-LOQ), being approximately one fifth of the maximum level to be checked. Following a practical approach, WHO-TEQ values are calculated for the procedural blank representative for the respective series of samples. In case an individual congener cannot be quantified, the LOQ for that congener is used (upper bound concept) for TEQ calculation. These WHO-TEQ values of the procedural blank are then used as LOQs, representing the laboratory's contribution to blank signals.

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6. Kotz A et al. (2012); Organohalogen Compd 74:156-159
7. Commission Regulation (EC) No 1881/2006 of 19 December 2006
8. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002

Table 1: Approaches for the estimation of LOD/LOQ described for the different fields of application:

	Signal-to-noise ratio	Blank (matrix) samples	Procedural blanks	Calibration (spiked blank samples)
Heavy metals		x		x
Mycotoxins		x		x
PAHs		x		x
PCDD/Fs and PCBs (LOQ)	x		x	x

Figure 1: Flow chart for estimation of LOQ in the field of PCDD/Fs and PCBs using isotope dilution mass spectrometry