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 (Text with EEA relevance)

ANNEX III

SAMPLE PREPARATION AND REQUIREMENTS FOR METHODS OF ANALYSIS USED IN CONTROL OF THE LEVELS OF DIOXINS (PCDD/FS) AND DIOXIN-LIKE PCBs IN CERTAIN FOODSTUFFS

1. FIELD OF APPLICATION

The requirements set out in this Annex shall be applied where foodstuffs are analysed for the official control of the levels of 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dioxin-like PCBs) and as regards sample preparation and analytical requirements for other regulatory purposes, including the controls performed by the food business operator to ensure compliance with provisions in Article 4 of Regulation (EC) No 852/2004.

Monitoring for the presence of PCDD/Fs and dioxin-like PCBs in foodstuffs may be performed with two different types of analytical methods:

(a) *Screening methods*

The goal of screening methods is to select those samples with levels of PCDD/Fs and dioxin-like PCBs that exceed the maximum levels or the action levels. Screening methods shall ensure cost-effective high sample-throughput, thus increasing the chance to discover new incidents where high exposure may lead to health risks for consumers. Their application shall aim to avoid false-compliant results. They may comprise bioanalytical and GC/MS methods.

Screening methods compare the analytical result with a cut-off value, providing a yes/no-decision over the possible exceedance of the maximum or action level. The concentration of PCDD/Fs and the sum of PCDD/Fs and dioxin-like PCBs in samples suspected to be non-compliant with the maximum level must be determined or confirmed by a confirmatory method.

In addition, screening methods may give an indication of the levels of PCDD/Fs and dioxin-like-PCBs present in the sample. In case of application of bioanalytical screening methods the result is expressed as Bioanalytical Equivalents (BEQ), whereas in case of application of physico-chemical GC-MS methods it is expressed as Toxic Equivalents (TEQ). The numerically indicated results of screening methods are suitable for demonstrating compliance or suspected non-compliance or exceedance of action levels and give an indication of the range of levels in case of follow-up by confirmatory methods. They are not suitable for purposes such as evaluation of background levels, estimation of intake, following of time trends in levels or re-evaluation of action and maximum levels.

(b) *Confirmatory methods*

Confirmatory methods allow the unequivocal identification and quantification of PCDD/Fs and dioxin-like PCBs present in a sample and provide full information on congener basis. Therefore, those methods allow the control of maximum and action levels, including the confirmation of results obtained by screening methods. Furthermore, results may be used for other purposes such as determination of low background levels in food monitoring, following of time trends, exposure assessment of the population and building of a database for possible re-evaluation of action and maximum levels. They are also important for establishing congener patterns in order to identify the source of a possible contamination. Such methods utilise GC-HRMS. For confirming compliance or non-compliance with the maximum level, also GC-MS/MS can be used.

2. BACKGROUND

For calculation of TEQ concentrations, the concentrations of the individual substances in a given sample shall be multiplied by their respective TEF, as established by the World Health Organisation and listed in the Appendix to this Annex, and subsequently summed to give the total concentration of dioxin-like compounds expressed as TEQs.

Screening and confirmatory methods may only be applied for control of a certain matrix if the methods are sensitive enough to detect levels reliably at the maximum or action level.

3. QUALITY ASSURANCE REQUIREMENTS

— Measures must be taken to avoid cross-contamination at each stage of the sampling and analysis procedure.

— The samples must be stored and transported in glass, aluminum, polypropylene or polyethylene containers suitable for storage without any influence on the levels of PCDD/Fs and dioxin-like PCBs in the samples. Traces of paper dust must be removed from the sample container.

— The sample storage and transportation has to be performed in a way that maintains the integrity of the foodstuff sample.

— Insofar as relevant, finely grind and mix thoroughly each laboratory sample using a process that has been demonstrated to achieve complete homogenisation (e.g. ground to pass a 1 mm sieve); samples have to be dried before grinding if moisture content is too high.

— Control of reagents, glassware and equipment for possible influence of TEQ- or BEQ-based results is of general importance.

— A blank analysis shall be performed by carrying out the entire analytical procedure omitting only the sample.

— For bioanalytical methods, it is of great importance that all glassware and solvents used in analysis shall be tested to be free of compounds that interfere with the detection of target compounds in the working range. Glassware shall be rinsed with solvents or/and heated at temperatures suitable to remove traces of PCDD/Fs, dioxin-like compounds and interfering compounds from its surface.

— Sample quantity used for the extraction must be sufficient to fulfill the requirements with respect to a sufficiently low working range including the concentrations of maximum or action levels.

— The specific sample preparation procedures used for the products under consideration shall follow internationally accepted guidelines.

— In the case of fish, the skin has to be removed as the maximum level applies to muscle meat without skin. However it is necessary that all remaining muscle meat and fat tissue on the inner side of the skin are carefully and completely scraped off from the skin and added to the sample to be analysed.

4. REQUIREMENTS FOR LABORATORIES

— In accordance with the provisions of Regulation (EC) No 882/2004, laboratories shall be accredited by a recognised body operating in accordance with ISO Guide 58 to ensure that they are applying analytical quality assurance. Laboratories shall be accredited following the EN ISO/IEC 17025 standard. The principles as described in the Technical Guidelines for the estimation of measurement uncertainty and limits of quantification for PCDD/F and PCB analysis shall be followed when applicable[(1)].

— Laboratory proficiency shall be proven by the continuous successful participation in interlaboratory studies for the determination of PCDD/Fs and dioxin-like PCBs in relevant food matrices and concentration ranges.

— Laboratories applying screening methods for routine control of samples shall establish a close cooperation with laboratories applying the confirmatory method, both for quality control and confirmation of the analytical result of suspected samples.

5. BASIC REQUIREMENTS TO BE MET BY ANALYTICAL PROCEDURE FOR DIOXINS (PCDD/FS) AND DIOXIN-LIKE PCBS

5.1. **Low working range and limits of quantification**

— For PCDD/Fs, detectable quantities have to be in the upper femtogram (10^{-15} g) range because of extreme toxicity of some of these compounds. For most PCB congeners limit of quantification in the nanogram (10^{-9} g) range is already sufficient. However, for the measurement of the more toxic dioxin-like PCB congeners (in particular non-ortho-substituted congeners) the lower end of the working range must reach the low picogram (10^{-12} g) levels.

5.2. **High selectivity (specificity)**

— A distinction is required between PCDD/Fs and dioxin-like PCBs and a multitude of other, coextracted and possibly interfering compounds present at concentrations up to several orders of magnitude higher than those of the analytes of interest. For gas chromatography/mass spectrometry (GC-MS) methods, a differentiation among various congeners is necessary, such as between toxic (e.g. the seventeen 2,3,7,8-substituted PCDD/Fs, and twelve dioxin-like PCBs) and other congeners.

— Bioanalytical methods shall be able to detect the target compounds as the sum of PCDD/Fs, and/or dioxin-like PCBs. Sample clean-up shall aim at removing compounds causing false non-compliant results or compounds that may decrease the response, causing false-compliant results.

5.3. **High accuracy (trueness and precision, bioassay apparent recovery)**

— For GC-MS methods, the determination shall provide a valid estimate of the true concentration in a sample. High accuracy (accuracy of the measurement: the closeness of the agreement between the result of a measurement with the true or assigned value of the measurand) is necessary to avoid the rejection of a sample analysis result on the basis of poor reliability of the determined TEQ level. Accuracy is expressed as trueness (difference between the mean value measured for an analyte in a certified material and its certified value, expressed as percentage of this value) and precision (RSD_R relative standard deviation calculated from results generated under reproducibility conditions).

— For bioanalytical methods, the bioassay apparent recovery shall be determined.

5.4. **Validation in the range of maximum level and general quality control measures**

— Laboratories shall demonstrate the performance of a method in the range of the maximum level, e.g. 0,5×, 1× and 2× the maximum level with an acceptable coefficient of variation for repeated analysis, during the validation procedure and/or during routine analysis.

— Regular blank controls and spiking experiments or analysis of control samples (preferably, if available, certified reference material) shall be performed as internal quality control measures. Quality control (QC) charts for blank controls, spiking experiments or analysis of control samples shall be recorded and checked to make sure the analytical performance is in accordance with the requirements.

5.5. **Limit of quantification**

— For a bioanalytical screening method, establishment of the LOQ is not an indispensable requirement but the method shall prove that it can differentiate between the blank and the cut-off value. When providing a BEQ-level, a reporting level shall

be established to deal with samples showing a response below this level. The reporting level shall be demonstrated to be different from procedure blank samples at least by a factor of three, with a response below the working range. It shall therefore be calculated from samples containing the target compounds around the required minimum level, and not from a S/N ratio or an assay blank.

— Limit of quantification (LOQ) for a confirmatory method shall be about one fifth of the maximum level.

5.6. **Analytical criteria**

— For reliable results from confirmatory or screening methods, the following criteria must be met in the range of the maximum level for the TEQ value respectively the BEQ value, whether determined as total TEQ or total BEQ (as sum of PCDD/F and dioxin-like PCBs) or separately for PCDD/Fs and dioxin-like PCBs.

	Screening with bioanalytical or physico-chemical methods	**Confirmatory methods**
False-compliant rate[a]	< 5 %	
Trueness		– 20 % to + 20 %
Repeatability (RSD_r)	< 20 %	
Intermediate precision (RSD_R)	< 25 %	< 15 %

a With respect to the maximum levels

5.7. **Specific requirements for screening methods**

— Both GC-MS and bioanalytical methods may be used for screening. For GC-MS methods the requirements as laid down in point 6 are to be used. For cell-based bioanalytical methods specific requirements are laid down in point 7.

— Laboratories applying screening methods for routine control of samples shall establish a close cooperation with laboratories applying the confirmatory method.

— Performance verification of the screening method is required during routine analysis, by analytical quality control and ongoing method validation. There must be a continuous programme for control of compliant results.

— Check on possible suppression of the cell response and cytotoxicity.

20 % of the sample extracts shall be measured in routine screening without and with TCDD added corresponding to the maximum or action level, to check if the response is possibly suppressed by interfering substances present in the sample extract. The measured concentration of the spiked sample is compared to the sum of the concentration of the unspiked extract plus the spiking concentration. If this measured concentration is more than 25 % lower than the calculated (sum) concentration, this is an indication of a potential signal suppression and the respective sample must be submitted to confirmatory analysis. Results shall be monitored in quality control charts.

— Quality control on compliant samples

Approximately 2 % to 10 % of the compliant samples, depending on sample matrix and laboratory experience, shall be confirmed.

— Determination of false-compliant rates from QC data

The rate of false-compliant results from screening of samples below and above the maximum level or the action level shall be determined. Actual false-compliant rates shall be below 5 %.

After a minimum of 20 confirmed results per matrix/matrix group is available from the quality control of compliant samples, conclusions on the false-compliant rate shall be drawn from this database. The results from samples analysed in ring trials or during contamination incidents, covering a concentration range up to, e.g. 2× the maximum level (ML), may also be included in the minimum of 20 results for evaluation of the false-compliant rate. The samples shall cover most frequent congener patterns, representing various sources.

Although screening assays shall preferentially aim to detect samples exceeding the action level, the criterion for determining false-compliant rates is the maximum level, taking into account the expanded measurement uncertainty of the confirmatory method.

— Potential non-compliant results from screening shall always be verified by a full re-analysis of the original sample by a confirmatory method. These samples may also be used to evaluate the rate of false non-compliant results. For screening methods, the rate of false non-compliant results is the fraction of results confirmed to be compliant from confirmatory analysis, while in previous screening the sample had been declared to be suspected to be non-compliant. However, evaluation of the advantageousness of the screening method shall be based on comparison of false non-compliant samples with the total number of samples checked. This rate shall be low enough to make the use of a screening tool advantageous.

— At least under validation conditions, bioanalytical methods shall provide a valid indication of the TEQ level, calculated and expressed as BEQ.

— Also for bioanalytical methods carried out under repeatability conditions, the intra-laboratory RSD_r would typically be smaller than the reproducibility RSD_R.

6. SPECIFIC REQUIREMENTS FOR GC-MS METHODS TO BE COMPLIED WITH FOR SCREENING OR CONFIRMATORY PURPOSES

6.1. **Acceptable differences between upperbound and lowerbound WHO-TEQ levels**

— The difference between upperbound level and lowerbound level shall not exceed 20 % for confirmation of the exceedance of maximum or in case of need of action levels.

6.2. **Control of recoveries**

— Addition of ^{13}C-labelled 2,3,7,8-chlorine-substituted internal PCDD/F standards and of ^{13}C-labelled internal dioxin-like PCB standards must be carried out at the very beginning of the analytical method, e.g. prior to extraction, in order to validate the analytical procedure. At least one congener for each of the tetra- to octa-chlorinated homologous groups for PCDD/Fs and at least one congener for each of the homologous groups for dioxin-like PCBs must be added (alternatively, at least one congener for each mass spectrometric selected ion recording function used for monitoring PCDD/Fs and dioxin-like PCBs). In case of confirmatory methods, all seventeen ^{13}C-labelled 2,3,7,8-substituted internal PCDD/F standards and all twelve ^{13}C-labelled internal dioxin-like PCB standards shall be used.

— Relative response factors shall also be determined for those congeners for which no ^{13}C-labelled analogue is added by using appropriate calibration solutions.

— For foodstuffs of plant origin and foodstuffs of animal origin containing less than 10 % fat, the addition of the internal standards is mandatory prior to extraction. For foodstuffs of animal origin containing more than 10 % fat, the internal standards may be added either before or after fat extraction. An appropriate validation of the extraction efficiency shall be carried out, depending on the stage at which internal standards are introduced and on whether results are reported on product or fat basis.

— Prior to GC-MS analysis, one or two recovery (surrogate) standard(s) must be added.

— Control of recovery is necessary. For confirmatory methods, the recoveries of the individual internal standards shall be in the range of 60 to 120 %. Lower or higher recoveries for individual congeners, in particular for some hepta- and octa- chlorinated dibenzo-p-dioxins and dibenzofurans, are acceptable on the condition that their contribution to the TEQ value does not exceed 10 % of the total TEQ value (based on sum of PCDD/F and dioxin-like PCBs). For GC-MS screening methods, the recoveries shall be in the range of 30 to 140 %.

6.3. **Removal of interfering substances**

— Separation of PCDD/Fs from interfering chlorinated compounds such as non-dioxin-like PCBs and chlorinated diphenyl ethers shall be carried out by suitable chromatographic techniques (preferably with a florisil, alumina and/or carbon column).

— Gas-chromatographic separation of isomers shall be sufficient (< 25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF).

6.4. **Calibration with standard curve**

— The range of the calibration curve shall cover the relevant range of maximum or action levels.

6.5. **Specific criteria for confirmatory methods**

— For GC-HRMS:

In HRMS, the resolution shall typically be greater than or equal to 10 000 for the entire mass range at 10 % valley.

Fulfilment of further identification and confirmation criteria as described in internationally recognised standards, for example, in standard EN 16215:2012 (Animal feed — Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS) and/or in EPA methods 1613 and 1668 as revised.

— For GC-MS/MS:

Monitoring of at least two specific precursor ions, each with one specific corresponding transition product ion for all labelled and unlabelled analytes in the scope of analysis.

Maximum permitted tolerance of relative ion intensities of ± 15 % for selected transition product ions in comparison to calculated or measured values (average from calibration standards), applying identical MS/MS conditions, in particular collision energy and collision gas pressure, for each transition of an analyte.

Resolution for each quadrupole to be set equal to or better than unit mass resolution (unit mass resolution: sufficient resolution to separate two peaks one mass unit apart) in order to minimise possible interferences on the analytes of interest.

Fulfilment of the further criteria as described in internationally recognised standards, for example, in standard EN 16215:2012 (Animal feed — Determination of dioxins and dioxin-like PCBs by GC/HRMS and of

indicator PCBs by GC/HRMS) and/or in EPA methods 1613 and 1668 as revised, except the obligation to use GC-HRMS.

7. SPECIFIC REQUIREMENTS FOR BIOANALYTICAL METHODS

Bioanalytical methods are methods based on the use of biological principles like cell-based assays, receptor-assays or immunoassays. This point establishes requirements for bioanalytical methods in general.

A screening method in principle classifies a sample as compliant or suspected to be non-compliant. For this, the calculated BEQ level is compared to the cut-off value (see point 7.3). Samples below the cut-off value are declared compliant, samples equal or above the cut-off value as suspected to be non-compliant, requiring analysis by a confirmatory method. In practice, a BEQ level corresponding to two-thirds of the maximum level may serve as cut-off value provided that a false-compliant rate below 5 % and an acceptable rate for false non-compliant results are ensured. With separate maximum levels for PCDD/Fs and for the sum of PCDD/Fs and dioxin-like PCBs, checking compliance of samples without fractionation requires appropriate bioassay cut-off values for PCDD/Fs. For checking of samples exceeding the action levels, an appropriate percentage of the respective action level would suit as cut-off value.

If an indicative level is expressed in BEQs, the results from the the sample must be given in the working range and exceeding the reporting limit (see points 7.1.1 and 7.1.6).

7.1. **Evaluation of the test response**

7.1.1. *General requirements*

— When calculating the concentrations from a TCDD calibration curve, values at the higher end of the curve will show a high variation (high coefficient of variation (CV)). The working range is the area where this CV is smaller than 15 %. The lower end of the working range (reporting limit) must further be set significantly (at least by a factor of three) above the procedure blanks. The upper end of the working range is usually represented by the EC_{70} value (70 % of maximal effective concentration), but lower if the CV is higher than 15 % in this range. The working range shall be established during validation. Cut-off values (see point 7.3) must be within the working range.

— Standard solutions and sample extracts shall be tested in triplicate or at least in duplicate. When using duplicates, a standard solution or a control extract tested in four to six wells divided over the plate shall produce a response or concentration (only possible in the working range) based on a CV < 15 %.

7.1.2. *Calibration*

7.1.2.1. Calibration with standard curve

— Levels in samples may be estimated by comparison of the test response with a calibration curve of TCDD (or PCB 126 or a PCDD/F/dioxin-like PCB standard mixture) to calculate the BEQ level in the extract and subsequently in the sample.

— Calibration curves shall contain 8 to 12 concentrations (at least in duplicates), with enough concentrations in the lower part of the curve (working range). Special attention shall be paid to the quality of the curve-fit in the working range. As such, the R^2 value is of little or no value in estimating the goodness of fit in nonlinear regression. A better fit will be achieved by minimising the difference between calculated and observed levels in the working range of the curve (e.g. by minimising the sum of squared residuals).

— The estimated level in the sample extract is subsequently corrected for the BEQ level calculated for a matrix or solvent blank sample (to account for impurities from solvents and chemicals used), and the apparent recovery (calculated from the BEQ level of

suitable reference samples with representative congener patterns around the maximum or action level). For performing a recovery correction, the apparent recovery must always be within the required range (see point 7.1.4). Reference samples used for recovery correction must comply with requirements as given in point 7.2.

7.1.2.2. Calibration with reference samples

Alternatively, a calibration curve prepared from at least four reference samples (see point 7.2: one matrix blank, plus three reference samples at 0,5×, 1,0× and 2,0× the maximum or action level may be used, eliminating the need to correct for blank and recovery if matrix properties of the reference samples match those of the unknown samples. In this case, the test response corresponding to two-thirds of the maximum level (see point 7.3) may be calculated directly from these samples and used as cut-off value. For checking of samples exceeding the action levels, an appropriate percentage of these action levels would suit as cut-off value.

7.1.3. *Separate determination of PCDD/Fs and dioxin-like PCBs*

Extracts may be split into fractions containing PCDD/Fs and dioxin-like PCBs, allowing a separate indication of PCDD/Fs and dioxin-like PCB TEQ levels (in BEQs). A PCB 126 standard calibration curve shall preferentially be used to evaluate results for the fraction containing dioxin-like PCBs.

7.1.4. *Bioassay apparent recoveries*

The 'bioassay apparent recovery' shall be calculated from suitable reference samples with representative congener patterns around the maximum or action level and expressed as percentage of the BEQ level in comparison to the TEQ level. Depending on the type of assay and TEFs[(2)] used, the differences between TEF and REP factors for dioxin-like PCBs may cause low apparent recoveries for dioxin-like PCBs in comparison to PCDD/Fs. Therefore, if a separate determination of PCDD/Fs and dioxin-like PCBs is performed, bioassay apparent recoveries shall be: for dioxin-like PCBs 20 % to 60 %, for PCDD/Fs 50 % to 130 % (ranges apply for TCDD calibration curve). As the contribution of dioxin-like PCBs to the sum of PCDD/Fs and dioxin-like PCBs may vary between different matrices and samples, bioassay apparent recoveries for the sum parameter reflect these ranges and shall be between 30 % to 130 %.

7.1.5. *Control of recoveries for clean-up*

The loss of compounds during the clean-up shall be checked during validation. A blank sample spiked with a mixture of the different congeners shall be submitted to clean-up (at least n = 3) and the recovery and variability checked by a confirmatory method. The recovery shall be within 60 to 120 % especially for congeners contributing more than 10 % to the TEQ-level in various mixtures.

7.1.6. *Reporting Limit*

When reporting BEQ levels, a reporting limit shall be determined from relevant matrix samples involving typical congener patterns, but not from the calibration curve of the standards due to low precision in the lower range of the curve. Effects from extraction and clean-up must be taken into account. The reporting limit must be set significantly (at least by a factor of three) above the procedure blanks.

7.2. **Use of reference samples**

— Reference samples shall represent sample matrix, congener patterns and concentration ranges for PCDD/Fs and dioxin-like PCBs around the maximum or action level.

— A procedure blank, or preferably a matrix blank, and a reference sample at the maximum or action level have to be included in each test series. These samples must

be extracted and tested at the same time under identical conditions. The reference sample must show a clearly elevated response in comparison to the blank sample, thus ensuring the suitability of the test. Those samples may be used for blank and recovery corrections.

— Reference samples chosen for performing a recovery correction shall be representative for the test samples, meaning that congener patterns shall not lead to an underestimation of levels.

— Extra reference samples at, e.g. 0,5× and 2× the maximum or action level may be included to demonstrate the proper performance of the test in the range of interest for the control of the maximum or action level. Combined, these samples may be used for calculating the BEQ-levels in test samples (see point 7.1.2.2).

7.3. **Determination of cut-off values**

The relationship between bioanalytical results in BEQ and results from confirmatory methods in TEQ shall be established (e.g. by matrix-matched calibration experiments, involving reference samples spiked at 0, 0,5×, 1× and 2× the maximum level (ML), with six repetitions on each level (n = 24)). Correction factors (blank and recovery) may be estimated from this relationship but shall be checked in each test series by including procedure/matrix blanks and recovery samples (see point 7.2).

Cut-off values shall be established for decision over sample compliance with maximum levels or for control of action levels, if of interest, with the respective maximum or action levels set for either PCDD/Fs and dioxin-like PCBs alone, or for the sum of PCDD/Fs and dioxin-like PCBs. They are represented by the *lower* endpoint of the distribution of bioanalytical results (corrected for blank and recovery) corresponding to the decision limit of the confirmatory method based on a 95 % level of confidence, implying a false-compliant rate < 5 %, and on a RSD_R < 25 %. The decision limit of the confirmatory method is the maximum level, taking into account the expanded measurement uncertainty.

In practice, the cut-off value (in BEQ) may be calculated from the following approaches (see Figure 1):

7.3.1. *Use of the lower band of the 95 % prediction interval at the decision limit of the confirmatory method*

$$\text{Cut-off value} = \text{BEQ}_{\text{DL}} - s_{y,x} \times t_{\alpha,f=m-2}\sqrt{1/n + 1/m + (x_i - \bar{x})^2 / Q_{xx}}$$

with:

BEQ_{DL}	BEQ corresponding to the decision limit of the confirmatory method, being the ML taking into account the expanded measurement uncertainty
$s_{y,x}$	residual standard deviation
$t_{\alpha,f=m-2}$	student factor (α = 5 %, f = degrees of freedom, single-sided)
m	total number of calibration points (index j)
n	number of repetitions on each level
x_i	sample concentration (in TEQ) of calibration point I determined by a confirmatory method
$\bar{x}$	mean of the concentrations (in TEQ) of all calibration samples

$Q_{xx} = \sum_{j=1}^{m} (x_i - \bar{x})^2$

square sum parameter

i	=	index for calibration point i

7.3.2. Calculation from bioanalytical results (corrected for blank and recovery) of multiple analyses of samples ($n \geq 6$) contaminated at the decision limit of the confirmatory method, as the *lower* endpoint of the data distribution at the corresponding mean BEQ value:

Cut-off value = $BEQ_{DL} - 1{,}64 \times SD_R$

with

SD_R standard deviation of bioassay results at BEQ_{DL}, measured under within-laboratory reproducibility conditions

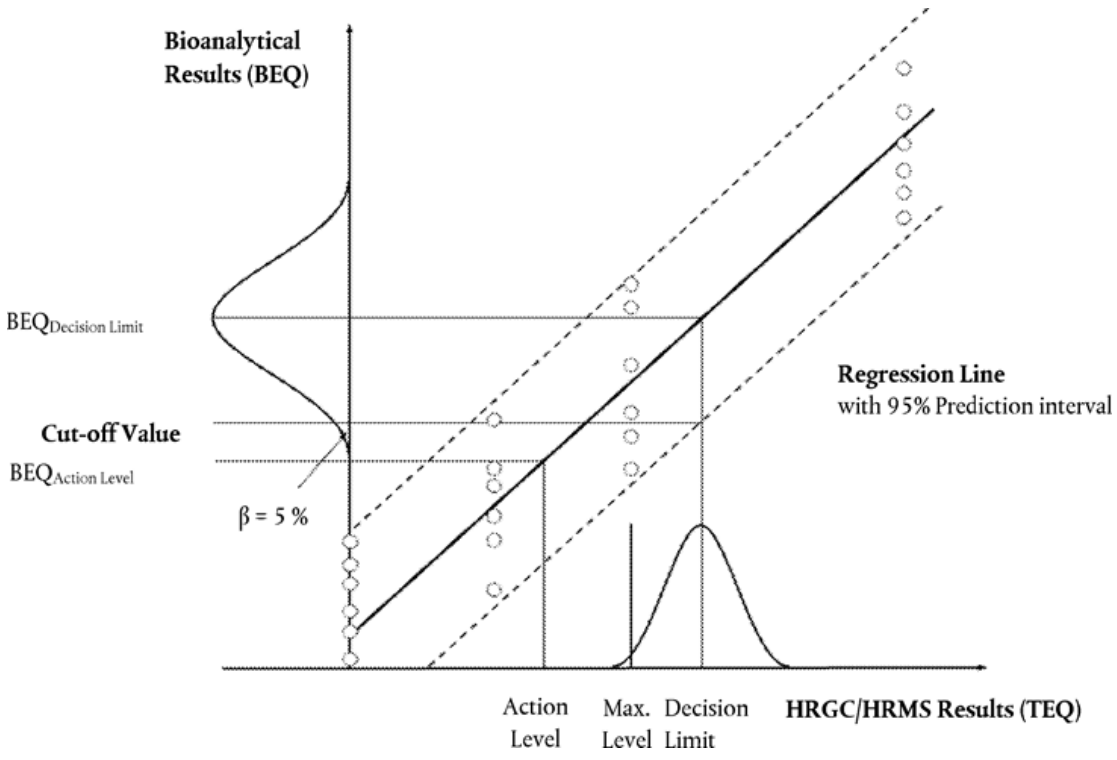
7.3.3. Calculation as mean value of bioanalytical results (in BEQ, corrected for blank and recovery) from multiple analysis of samples ($n \geq 6$) contaminated at two-thirds of the maximum or action level. This is based on the observation that this level will be around the cut-off determined under point 7.3.1 or 7.3.2.

Calculation of cut-off values based on a 95 % level of confidence implying a false-compliant rate < 5 %, and a $RSD_R < 25$ %:

1. from the *lower* band of the 95 % prediction interval at the decision limit of the confirmatory method,

2. from multiple analysis of samples ($n \geq 6$) contaminated at the decision limit of the confirmatory method as the *lower* endpoint of the data distribution (represented in the figure by a bell-shaped curve) at the corresponding mean BEQ value.

Figure 1



7.3.4. *Restrictions to cut-off values*

BEQ-based cut-off values calculated from the RSD_R achieved during validation using a limited number of samples with different matrix/congener patterns may be higher than the TEQ-based maximum or action levels due to a better precision than attainable in routine when an unknown spectrum of possible congener patterns has to be controlled. In such cases, cut-off values shall be calculated from an RSD_R = 25 %, or two-thirds of the maximum or action level shall be preferred.

7.4. **Performance characteristics**

— Since no internal standards can be used in bioanalytical methods, tests on repeatability shall be carried out to obtain information on the standard deviation within and between test series. Repeatability shall be below 20 % and intra-laboratory reproducibility shall be below 25 %. This shall be based on the calculated levels in BEQs after blank and recovery correction.

— As part of the validation process, the test must be shown to discriminate between a blank sample and a level at the cut-off value, allowing the identification of samples above the corresponding cut-off value (see point 7.1.2).

— Target compounds, possible interferences and maximum tolerable blank levels shall be defined.

— The per cent standard deviation in the response or concentration calculated from the response (only possible in working range) of a triplicate determination of a sample extract shall not be above 15 %.

— The uncorrected results of the reference sample(s) expressed in BEQs (blank and at the maximum or action level) shall be used for evaluation of the performance of the bioanalytical method over a constant time period.

— QCcharts for procedure blanks and each type of reference sample shall be recorded and checked to make sure the analytical performance is in accordance with the requirements, in particular for the procedure blanks with regard to the requested minimum difference to the lower end of the working range and for the reference samples with regard to within-laboratory reproducibility. Procedure blanks must be well controlled in order to avoid false-compliant results when subtracted.

— The results from the confirmatory methods of suspected samples and 2 to 10 % of the compliant samples (minimum of 20 samples per matrix) shall be collected and used to evaluate the performance of the screening method and the relationship between BEQs and TEQs. This database might be used for re-evaluation of cut-off values applicable to routine samples for the validated matrices.

— Successful method performance may also be demonstrated by participation in ring trials. The results from samples analysed in ring trials, covering a concentration range up to, e.g. 2× ML, may also be included in the evaluation of the false-compliant rate, if a laboratory is able to demonstrate its successful performance. The samples shall cover most frequent congener patterns, representing various sources.

— During incidents, the cut-off values may be re-evaluated, reflecting the specific matrix and congener patterns of this single incident.

8. REPORTING OF THE RESULT

Confirmatory methods

— The analytical results shall contain the levels of the individual PCDD/F and dioxin-like PCB congeners and TEQ-values shall be reported as lower-bound, upper-bound and medium-bound in order to include a maximum of information in the reporting of the results and thereby enabling the interpretation of the results according to specific requirements.

— The report shall also include the method used for extraction of PCDD/Fs, dioxin-like PCBs and lipids. The lipid content of the sample shall be determined and reported for food matrices with maximum levels expressed on fat basis and with an expected fat concentration in the range of 0-2 % (in correspondence to existing legislation). For other samples, the determination of the lipid content is optional.

— The recoveries of the individual internal standards must be made available in case the recoveries are outside the range mentioned in point 6.2 where the maximum level is exceeded (in this case, the recoveries for one of the two duplicate analysis) and in other cases upon request.

— As the expanded measurement uncertainty is to be taken into account when deciding about the compliance of a sample, this parameter shall also be made available. Thus, analytical results shall be reported as x +/– U whereby x is the analytical result and U is the expanded measurement uncertainty using a coverage factor of 2 which gives a level of confidence of approximately 95 %. In case of a separate determination of PCDD/Fs and dioxin-like-PCBs the sum of the estimated expanded uncertainty of the separate analytical results of PCDD/Fs and dioxin-like PCBs has to be used for the sum of PCDD/Fs and dioxin-like PCBs.

— The results shall be expressed in the same units and with the same number of significant figures as the maximum levels laid down in Regulation (EC) No 1881/2006.

Bioanalytical screening methods

— The result of the screening shall be expressed as compliant or suspected to be non-compliant (‘suspected’).

— In addition, an indicative result for PCDD/F and/or dioxin-like PCBs expressed in BEQ (not TEQ) may be given (see point 1). Samples with a response below the reporting limit shall be expressed as lower than the reporting limit. Samples with a response above the working range shall be reported as exceeding the working range and the level corresponding to the upper end of the working range shall be given in BEQ.

— For each type of sample matrix, the report shall mention the maximum or action level on which the evaluation is based.

— The report shall mention the type of test applied, the basic test principle and kind of calibration.

— The report shall also include the method used for extraction of PCDD/Fs, dioxin-like PCBs and lipids. The lipid content of the sample shall be determined and reported for food matrices with maximum levels expressed on fat basis and with an expected fat concentration in the range of 0-2 % (in correspondence to existing legislation). For other samples, the determination of the lipid content is optional.

— In the case of samples suspected to be non-compliant, the report needs to include a note on the action to be taken. The concentration of PCDD/Fs and the sum of PCDD/Fs and dioxin-like PCBs in those samples with elevated levels has to be determined/confirmed by a confirmatory method.

— Non-compliant results shall only be reported from confirmatory analysis.

Physico-chemical screening methods

— The result of the screening shall be expressed as compliant or suspected to be non-compliant (‘suspected’).

— For each type of sample matrix, the report shall mention the maximum or action level on which the evaluation is based.

— In addition, levels for individual PCDD/F and/or dioxin-like PCB congeners and TEQ-values reported as lower-bound, upper-bound and medium-bound may be given. The results shall be expressed in the same units and with (at least) the same number

of significant figures as the maximum levels laid down in Regulation (EC) No 1881/2006.

— The recoveries of the individual internal standards must be made available in case the recoveries are outside the range mentioned in point 6.2 and in other cases upon request.

— The report shall mention the GC-MS method applied.

— The report shall also include the method used for extraction of PCDD/Fs, dioxin-like PCBs and lipids. The lipid content of the sample shall be determined and reported for food matrices with maximum levels expressed on fat basis and with an expected fat concentration in the range of 0-2 % (in correspondence to existing legislation). For other samples, the determination of the lipid content is optional.

— In case of samples suspected to be non-compliant, the report needs to include a note on the action to be taken. The concentration of PCDD/Fs and the sum of PCDD/Fs and dioxin-like PCBs in those samples with elevated levels has to be determined/ confirmed by a confirmatory method.

— Non-compliance can only be decided after confirmatory analysis.

Appendix

WHO-TEFs for human risk assessment based on the conclusions of the World Health Organisation (WHO) 0151 International Programme on Chemical Safety (IPCS) expert meeting which was held in Geneva in June 2005[(3)]

Congener	TEF value	Congener	TEF value
Dibenzo-p-dioxins ('PCDDs')		*'Dioxin-like' PCBs* ***Non-ortho PCBs + Mono-ortho PCBs***	
2,3,7,8-TCDD	1		
1,2,3,7,8-PeCDD	1	***Non-ortho PCBs***	
1,2,3,4,7,8-HxCDD	0,1	PCB 77	0,0001
1,2,3,6,7,8-HxCDD	0,1	PCB 81	0,0003
1,2,3,7,8,9-HxCDD	0,1	PCB 126	0,1
1,2,3,4,6,7,8-HpCDD	0,01	PCB 169	0,03
OCDD	0,0003		
Dibenzofurans ('PCDFs')		***Mono-ortho PCBs***	
2,3,7,8-TCDF	0,1	PCB 105	0,00003
1,2,3,7,8-PeCDF	0,03	PCB 114	0,00003
2,3,4,7,8-PeCDF	0,3	PCB 118	0,00003
1,2,3,4,7,8-HxCDF	0,1	PCB 123	0,00003
1,2,3,6,7,8-HxCDF	0,1	PCB 156	0,00003
1,2,3,7,8,9-HxCDF	0,1	PCB 157	0,00003
2,3,4,6,7,8-HxCDF	0,1	PCB 167	0,00003
1,2,3,4,6,7,8-HpCDF	0,01	PCB 189	0,00003
1,2,3,4,7,8,9-HpCDF	0,01		
OCDF	0,0003		

Abbreviations used: 'T' = tetra; 'Pe' = penta; 'Hx' = hexa; 'Hp' = hepta; 'O' = octa; 'CDD' = chlorodibenzodioxin; 'CDF' = chlorodibenzofuran; 'CB' = chlorobiphenyl.

(**1**) Guidance Document on Measurement Uncertainty for Laboratories performing PCDD/F and PCB Analysis using Isotope Dilution Mass Spectrometry [link to website], Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food [link to website].

(**2**) Current requirements are based on the TEFs published in: M. Van den Berg et al, Toxicol Sci 93 (2), 223-241 (2006).

(**3**) Martin van den Berg et al., The 2005 World Health Organisation Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicological Sciences 93(2), 223–241 (2006).

Changes to legislation:
There are outstanding changes not yet made to Commission Regulation (EU) 2017/644. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.
View outstanding changes

Changes and effects yet to be applied to the whole legislation item and associated provisions

- Signature words omitted by S.I. 2019/639 reg. 37
- Annex 1 para. 1.1 words substituted by S.I. 2019/639 reg. 38
- Annex 2 s. 2para. 1 words omitted by S.I. 2019/639 reg. 39(a) (This amendment not applied to legislation.gov.uk. Reg. 39 substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 8(12))
- Annex 2 s. 2para. 1 words omitted by virtue of S.I. 2019/639, reg. 39(a) (as substituted) by S.I. 2020/1504 reg. 8(12)
- Annex 2 s. 2para. 6 words substituted by S.I. 2019/639 reg. 39(b) (This amendment not applied to legislation.gov.uk. Reg. 39 substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 8(12))
- Annex 2 s. 2para. 8 words substituted by S.I. 2019/639 reg. 39(c) (This amendment not applied to legislation.gov.uk. Reg. 39 substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 8(12))
- Annex 2 s. 2para. 6 words substituted by S.I. 2019/639, reg. 39(b) (as substituted) by S.I. 2020/1504 reg. 8(12)
- Annex 2 s. 2para. 8 words substituted by S.I. 2019/639, reg. 39(c) (as substituted) by S.I. 2020/1504 reg. 8(12)
- Annex 5 inserted by S.I. 2019/639 reg. 40Sch.