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COMMISSION REGULATION (EU) No …/..

of XXX

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) 252/2012

(Text with EEA relevance)

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COMMISSION REGULATION (EU) No …/..

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(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules[1], in particular Article 11 (4) thereof,

Whereas:

(1) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs[2] provides for maximum levels for non-dioxin-like PCBs, dioxins and furans and for the sum of dioxins, furans and dioxin-like PCBs in certain foodstuffs.

(2) Commission Recommendation 2011/516/EU of 23 August 2011 on the reduction of the presence of dioxins, furans and PCBs in feed and food[3] sets out action levels in order to stimulate a pro-active approach to reduce the presence of polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like PCBs in food. Those action levels are a tool for competent authorities and operators to highlight those cases where it is appropriate to identify a source of contamination and to take measures for its reduction or elimination.

(3) Commission Regulation (EC) No 1883/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs[4] establishes specific provisions concerning the sampling procedure and the methods of analysis to be applied for the official control.

(4) The application of new maximum levels for non-dioxin like PCBs, established following the availability of a scientific opinion from the European Food Safety

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[1]   OJ L 165, 30.4.2004, p. 1.
[2]   OJ L 364, 20.12.2006, p. 5.
[3]   OJ L 218, 24.8.2011, p. 23.
[4]   OJ L 364, 20.12.2006, p. 32.

Authority (EFSA) on non dioxin-like PCBs and also to provide a harmonisation at Union level and the update of the criteria for screening methods require significant amendments. Therefore, for reasons of clarity, it is appropriate to replace Regulation (EC) 1883/2006 by this Regulation.

(5)   The provisions laid down in this Regulation relate only to the sampling and analysis of dioxins, dioxin-like PCBs and non dioxin-like PCBs for the implementation of Regulation (EC) 1881/2006. They do not affect the sampling strategy, sampling levels and frequency as specified in Annexes III and IV to Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 83/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC[5]. They do not affect the targeting criteria for sampling as laid down in Commission Decision 98/179/EC of 23 February 1998 laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products[6].

(6)   A screening method of analysis with widely acceptable validation and high throughput can be used to identify the samples with significant levels of PCDD/Fs and dioxin-like PCBs (preferably selecting samples exceeding action levels and ensuring the selection of samples exceeding maximum levels). The levels of PCDD/Fs and dioxin-like PCBs in these samples need to be determined by a confirmatory method of analysis. It is therefore appropriate to establish appropriate requirements for the screening method making sure that the false-compliant rate with respect to maximum levels is below 5% and strict requirements for the confirmatory methods of analysis. Furthermore, confirmatory methods with sufficient sensitivity allow the determination of levels also in the low background range. That is important for to follow time trends, exposure assessment and for the re-evaluation of maximum and action levels.

(7)   For the sampling of very large fish, it is necessary that the sampling is specified in order to ensure a harmonised approach throughout the Union.

(8)   In fish of the same species originating from the same region, the level of dioxins, dioxin-like PCBs and non-dioxin-like PCBs can be different depending on the size and/or the age of the fish. Moreover, the level of dioxins, dioxin-like PCBs and non-dioxin-like PCBs is not necessarily the same in all parts of the fish. Therefore, it is necessary that the sampling and sample preparation is specified in order to ensure a harmonised approach throughout the Union.

(9)   It is important that analytical results are reported and interpreted in a uniform way in order to ensure a harmonised enforcement approach throughout the Union.

(10)   The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health and neither the European Parliament nor the Council have opposed them,

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[5]   OJ L 125, 23.5.1996, p.10.
[6]   OJ L 65, 5.3.1998, p. 31.

HAS ADOPTED THIS REGULATION:

Article 1

For the purposes of this Regulation, the definitions and abbreviations set out in Annex I shall apply.

Article 2

Sampling for the official control of the levels of dioxins, furans, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs listed in Section 5 of the Annex to Regulation (EC) No 1881/2006 shall be carried out in accordance with the methods set out in Annex II to this Regulation.

Article 3

Sample preparation and analyses for the official control of the levels of dioxins, furans and dioxin-like PCBs in foodstuffs listed in Section 5 of the Annex to Regulation (EC) No 1881/2006 shall be carried out in accordance with the methods set out in Annex III to this Regulation.

Article 4

Analyses for the official control of the levels of non-dioxin-like PCBs in foodstuffs listed in Section 5 of the Annex to Regulation (EC) No 1881/2006 shall be carried out in accordance with the requirements for analytical procedures set out in Annex IV to this Regulation.

Article 5

Regulation (EC) 1883/2006 is hereby repealed.

References to the repealed Regulation shall be construed as references to this Regulation.

Article 6

This Regulation shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

It shall apply from the date of entry into force.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels,

For the Commission
José Manuel BARROSO
The President

DEFINITIONS AND ABBREVIATIONS

I.   **DEFINITIONS**

For the purposes of this Regulation the definitions laid down in Annex I to Commission Decision 2002/657/EC of 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results[7] shall apply.

Further to these definitions, the following definitions shall apply for the purposes of this Regulation:

1.1.   *Action level* means the level of a given substance, as laid down in Annex to Recommendation 2011/516/EU, which triggers investigations to identify the source of that substance in cases where increased levels of the substance are detected.

1.2   *Screening methods* can be used for selection of those samples with levels of PCDD/Fs and dioxin-like PCBs that exceed the maximum levels, or the action levels. They should allow a cost-effective high sample-throughput, thus increasing the chance to discover new incidents with high exposure and health risks of consumers. Screening methods are based on bioanalytical or GC-MS methods. Results from samples exceeding the cut-off value need to be verified by a full re-analysis from the original sample by a confirmatory method.

1.3   *Confirmatory methods* are methods that provide full or complementary information enabling the PCDD/Fs and dioxin-like PCBs to be identified and quantified unequivocally at the level of interest. At present such methods utilize gas chromatography/high resolution mass spectrometry (GC-HRMS) or gas chromatography/tandem mass spectrometry (GC-MS/MS).

1.4.   *Bioanalytical methods* means methods based on the use of biological principles like cell-based assays, receptor-assays or immunoassays. They do not give results at the congener level but merely an indication[8] of the TEQ level, expressed in Bioanalytical Equivalents (BEQ) to acknowledge the fact that not all compounds present in a sample extract that produce a response in the test may obey all requirements of the TEQ-principle.

1.5.   *Bioassay apparent recovery* means the BEQ level calculated from the TCDD or PCB 126 calibration curve corrected for the blank and then divided by the TEQ level determined by the confirmatory method. It attempts to correct factors like the loss of PCDD/PCDFs and dioxin-like compounds during the extraction and clean-up steps, co-extracted compounds increasing or decreasing the response (agonistic and antagonistic effects), the quality of the curve fit, or differences between the TEF and

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[7]   OJ L 221, 17.8.2002, p. 8.
[8]   Bioanalytical methods are not specific to those congeners included in the TEF-scheme. Other structurally related AhR-active compounds may be present in the sample extract which contribute to the overall response. Therefore, bioanalytical results cannot be an estimate but rather an indication of the TEQ level in the sample.

the REP values. The bioassay apparent recovery is calculated from suitable reference samples with representative congener patterns around the level of interest.

1.6. *Semi-quantitative methods* means methods which give an approximate indication of the concentration of the putative analyte, while the numerical result does not meet the requirements for quantitative methods.

1.7. *The accepted specific limit of quantification of an individual congener in* a sample is the lowest content of the analyte that can be measured with reasonable statistical certainty, fulfilling the identification criteria as described, 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.

The limit of quantification of an individual congener can be defined as

- the concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions to be monitored with a S/N (signal/noise) ratio of 3:1 for the less intensive raw data signal.

Alternative approach, if for technical reasons the signal-to-noise calculation does not provide reliable results:

- The lowest concentration point on a calibration curve that gives an acceptable ($\leq$ 30 %) and consistent (measured at least at the start and at the end of an analytical series of samples) deviation to the average relative response factor calculated for all points on the calibration curve in each series of samples[9]

1.8. *Upper-bound* means the concept which requires using the limit of quantification for the contribution of each non-quantified congener.

1.9. *Lower-bound* means the concept which requires using zero for the contribution of each non-quantified congener.

1.10. *Medium-bound* means the concept which requires using half of the limit of quantification calculating the contribution of each non-quantified congener.

1.11. *Lot* means an identifiable quantity of food delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor or markings. In the case of fish and fishery products, also the size of fish shall be comparable. In case the size and/or weight of the fish is not comparable within a consignment, the consignment may still be considered as a lot but a specific sampling procedure has to be applied.

1.12. *Sublot* means designated part of a large lot in order to apply the sampling method on that designated part. Each sublot must be physically separated and identifiable.

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[9] The LOQ is calculated from the lowest concentration point taking into account the recovery of internal standards and sample intake.

1.13.   *Incremental sample* means a quantity of material taken from a single place in the lot or sublot.

1.14.   *Aggregate sample* means the combined total of all the incremental samples taken from the lot or sublot.

1.15.   *Laboratory sample*: a representative part/quantity of the aggregate sample intended for the laboratory.

II.   ABBREVIATIONS USED

BEQ	Bioanalytical Equivalents
GC	Gas chromatography
HRMS	High resolution mass spectrometry
LRMS	Low resolution mass spectrometry
MS/MS	Tandem mass spectrometry
PCB	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-p-dioxins
PCDF	Polychlorinated dibenzofurans
QC	Quality control
REP	Relative potency
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
TCDD	Tetrachlorodibenzodioxin
U	Expanded measurement uncertainty

METHODS OF SAMPLING FOR OFFICAL CONTROL OF LEVELS OF DIOXINS (PCDD/PCDF), DIOXIN-LIKE PCBs AND NON-DIOXIN-LIKE PCBs IN CERTAIN FOODSTUFFS

I.   **SCOPE**

Samples intended for the official control of the levels of dioxins (PCDD/PCDF), dioxin-like PCBs and non-dioxin-like PCBs, hereafter referred to as dioxins and PCBs, in foodstuffs shall be taken according to the methods described in this Annex. Aggregate samples thus obtained shall be considered as representative of the lots or sublots from which they are taken. Compliance with maximum levels laid down in Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs shall be established on the basis of the levels determined in the laboratory samples.

II.   **GENERAL PROVISIONS**

1.   **Personnel**

Sampling shall be performed by an authorised person as designated by the Member State.

2.   **Material to be sampled**

Each lot or sublot, which is to be examined, shall be sampled separately.

3.   **Precautions to be taken**

In the course of sampling and preparation of the samples, precautions shall be taken to avoid any changes, which would affect the content of dioxins and PCBs, adversely affect the analytical determination or make the aggregate samples unrepresentative.

4.   **Incremental samples**

As far as possible incremental samples shall be taken at various places distributed throughout the lot or sublot. Departure from such procedure shall be recorded in the record provided for under point II.8 of this Annex.

5.   **Preparation of the aggregate sample**

The aggregate sample shall be made up by combining the incremental samples. It shall be at least 1 kg unless not practical, e.g. when a single package has been sampled or when the product has a very high commercial value.

6. **Replicate samples**

The replicate samples for enforcement, defence and reference purposes shall be taken from the homogenised aggregate sample, unless such procedure conflicts with Member States' rules as regard the rights of the food business operator. The size of the laboratory samples for enforcement shall be sufficient to allow at least for duplicate analyses.

7. **Packaging and transmission of samples**

Each sample shall be placed in a clean, inert container offering adequate protection from contamination, from loss of analytes by adsorption to the internal wall of the container and against damage in transit. All necessary precautions shall be taken to avoid any change in composition of the sample, which might arise during transportation or storage.

8. **Sealing and labelling of samples**

Each sample taken for official use shall be sealed at the place of sampling and identified following the rules of the Member States.

A record shall be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

III. **SAMPLING PLAN**

The sampling method applied shall ensure that the aggregate sample is representative for the (sub)lot that is to be controlled.

1. **Division of lots into sublots**

Large lots shall be divided into sublots on condition that the sublot can be separated physically. For products traded in large bulk consignments (e.g. vegetable oils) Table 1 shall apply. For other products Table 2 shall apply. Taking into account that the weight of the lot is not always an exact multiple of the weight of the sublots, the weight of the sublot may exceed the mentioned weight by a maximum of 20%.

Table 1: Subdivision of lots into sublots for products traded in bulk consignments

Lot weight (ton)	Weight or number of sublots
≥1 500	500 tonnes
> 300 and < 1 500	3 sublots
≥ 50 and ≤ 300	100 tonnes
< 50	--

Table 2: Subdivision of lots into sublots for other products

Lot weight (ton)	Weight or number of sublots
≥ 15	15-30 tonnes
<15	-

2.   **Number of incremental samples**

The aggregate sample uniting all incremental samples shall be at least 1 kg (see point II.5 of this Annex).

The minimum number of incremental samples to be taken from the lot or sublot shall be as given in Tables 3 and 4

In the case of bulk liquid products the lot or sublot shall be thoroughly mixed insofar as possible and insofar it does not affect the quality of the product, by either manual or mechanical means immediately prior to sampling. In this case, a homogeneous distribution of contaminants is assumed within a given lot or sublot. It is therefore sufficient to take three incremental samples from a lot or sublot to form the aggregate sample.

The incremental samples shall be of similar weight. The weight of an incremental sample shall be at least 100 grams.

Departure from this procedure must be recorded in the record provided for under point II.8 of this Annex. In accordance with the provisions of Commission Decision 97/747/EC of 27 October 1997 fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products[10], the aggregate sample size for hen eggs is at least 12 eggs (for bulk lots as well for lots consisting of individual packages, tables 3 and 4 shall apply).

Table 3: Minimum number of incremental samples to be taken from the lot or sublot

Weight or volume of lot/sublot (in kg or litre)	Minimum number of incremental samples to be taken
< 50	3
50 to 500	5
> 500	10

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[10]   OJ L 303, 6.11.1997, p. 12.

If the lot or sublot consists of individual packages or units, then the number of packages or units which shall be taken to form the aggregate sample is given in Table 4.

Table 4: Number of packages or units (incremental samples) which shall be taken to form the aggregate sample if the lot or sublot consists of individual packages or units

Number of packages or units in the lot/sublot	Number of packages or units to be taken
1 to 25	at least 1 package or unit
26 to 100	about 5 %, at least 2 packages or units
> 100	about 5 %, at maximum 10 packages or units

3.   **Specific provisions for the sampling of lots containing whole fishes of comparable size and weight**

Fishes are considered as being of comparable size and weight in case the difference in size and weight does not exceed about 50 %.

The number of incremental samples to be taken from the lot are defined in Table 3. The aggregate sample uniting all incremental samples shall be at least 1 kg (see point II.5.).

−   In case the lot to be sampled contains small fishes (individual fishes weighing < about 1 kg), the whole fish is taken as incremental sample to form the aggregate sample. In case the resulting aggregate sample weighs more than 3 kg, the incremental samples may consist of the middle part, weighing each at least 100 grams, of the fishes forming the aggregate sample. The whole part to which the maximum level is applicable is used for homogenisation of the sample.

The middle part of the fish is where the centre of gravity is. This is located in most cases at the dorsal fin (in case the fish has a dorsal fin) or halfway between the gill opening and the anus.

−   In case the lot to be sampled contains larger fishes (individual fishes weighing more than about 1 kg), the incremental sample consists of the middle part of the fish. Each incremental sample weighs at least 100 grams.

For fishes of intermediate size (about 1-6 kg) the incremental sample is taken as a slice of the fish from backbone to belly in the middle part of the fish.

For very large fishes (e.g. > about 6 kg), the incremental part is taken from the right side (frontal view) dorso-lateral muscle meat in the middle part of the fish In case the taking of such a piece of the middle part of the fish would result in a

significant economic damage, taking of three incremental samples of at least 350 grams each may be considered as being sufficient, independently of the size of the lot or alternatively an equal part of the muscled meat close to the tail part and the muscle meat close to the head part of one fish may be taken to form the incremental sample being representative for the level of dioxins in the whole fish.

4.   **Sampling of lots of fish containing whole fishes of different size and/or weight**

–   The provisions of point III.3 as regards sample constitution shall apply.

–   In case a size or weight class/category is predominant (about 80 % or more of the lot), the sample is taken from fishes with the predominant size or weight. This sample is to be considered as being representative for the whole lot.

–   In case no particular size or weight class/category predominates, then it must be ensured that the fishes selected for the sample are representative for the lot. Specific guidance for such cases is provided in "Guidance document on sampling of whole fishes of different size and/or weight[11]."

5.   **Sampling at retail stage**

Sampling of foodstuffs at retail stage shall be done where possible in accordance with the sampling provisions set out in point III.2 of this Annex.

Where this is not possible, an alternative method of sampling at retail stage may be used provided that it ensures sufficient representativeness for the sampled lot or sublot.

IV.   **COMPLIANCE OF THE LOT OR SUBLOT WITH THE SPECIFICATION**

1.   AS REGARDS NON-DIOXIN-LIKE PCBS

The lot is accepted, if the analytical result does not exceed the maximum level of non-dioxin-like PCBs as laid down in Regulation (EC) No 1881/2006 taking into account the measurement uncertainty.

The lot is non-compliant with the maximum level as laid down in Regulation (EC) No 1881/2006, if the upperbound analytical result confirmed by duplicate analysis[12], exceeds the maximum level beyond reasonable doubt taking into account the measurement uncertainty.

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[11]   http://ec.europa.eu/food/food/chemicalsafety/contaminants/dioxins_en.htm
[12]   The duplicate analysis is necessary to exclude the possibility of internal cross-contamination or an accidental mix-up of samples. The first analysis, taking into account the measurement uncertainty is used for verification of compliance. In case the analysis is performed in the frame of a contamination incident, confirmation by duplicate analysis might be omitted in case the samples selected for analysis are through traceability linked to the contamination incident

The measurement uncertainty may be taken into account according to one of the following approaches:

–   by calculating the expanded uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95%. A lot or sublot is noncompliant if the measured value minus U is above the established permitted level,

–   by establishing the decision limit (CCα) according to the provisions of Commission Decision 2002/657/EC of 14 August 2002 (point 3.1.2.5 of the Annex I to that Decision — the case of substances with an established permitted level). A lot or sublot is noncompliant if the measured value is equal to or above the CCα.

The abovementioned rules shall apply for the analytical result obtained on the sample for official control. In case of analysis for defence or reference purposes, the national rules apply.

2.   **AS REGARDS DIOXINS (PCDD/PCDF) AND DIOXIN-LIKE PCBs**

The lot is accepted, if the result of a single analysis

–   performed by a screening method with a false-compliant rate below 5% indicates that the level does not exceed the respective maximum level of PCDD/Fs and the sum of PCDD/Fs and dioxin-like PCBs as laid down in Regulation (EC) No 1881/2006;

–   performed by a confirmatory method does not exceed the respective maximum level of PCDD/Fs and the sum of PCDD/Fs and dioxin-like PCBs as laid down in Regulation (EC) No 1881/2006 taking into account the measurement uncertainty.

For screening assays a cut-off value shall be established for the decision on the compliance with the respective levels of interest set for either PCDD/Fs, or for the sum of PCDD/Fs and dioxin-like PCBs.

The lot is noncompliant with the maximum level as laid down in Regulation (EC) No 1881/2006, if the upperbound analytical result obtained with a confirmatory method and confirmed by duplicate analysis[13], exceeds the maximum level beyond reasonable doubt taking into account the measurement uncertainty.

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[13]   The duplicate analysis is necessary to exclude the possibility of internal cross-contamination or an accidental mix-up of samples. The first analysis, taking into account the measurement uncertainty is used for verification of compliance. In case the analysis is performed in the frame of a contamination incident, confirmation by duplicate analysis might be omitted in case the samples selected for analysis are through traceability linked to the contamination incident

The measurement uncertainty may be taken into account according to one of the following approaches:

–   by calculating the expanded uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95%. A lot or sublot is noncompliant if the measured value minus U is above the established permitted level. 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 estimated expanded uncertainty of the sum of PCDD/Fs and dioxin-like PCBs,

–   by establishing the decision limit (CCα) according to the provisions of Commission Decision 2002/657/EC of 14 August 2002 (point 3.1.2.5 of the Annex I to that Decision — the case of substances with established permitted level) a lot or sublot is noncompliant if the measured value is equal to or above the CCα.

The abovementioned rules shall apply for the analytical result obtained on the sample for official control. In case of analysis for defence or reference purposes, the national rules apply.

V.   EXCEEDANCE OF ACTION LEVELS

Action levels serve as tool for selection of samples in those cases where it is appropriate to identify a source of contamination and to take measures for its reduction or elimination. Screening methods shall establish appropriate cut-off values for selection of these samples. The efforts necessary to identify a source and to reduce or eliminate the contamination shall be deployed only if exceedance of the action level is confirmed by duplicate analysis using a confirmatory method and taking into account the measurement uncertainty[14].

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[14]   Identical explanation and requirements for duplicate analysis for control of action levels as in footnote 12 for maximum levels.

ANNEX III

SAMPLE PREPARATION AND REQUIREMENTS FOR METHODS OF ANALYSIS USED IN OFFICAL CONTROL OF THE LEVELS OF DIOXINS (PCDD/PCDF) 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 for other regulatory purposes.

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

(a)   selection of those samples with levels of PCDD/Fs and dioxin-like PCBs that exceed the maximum levels, or the action levels. For this, screening methods may be used. Their application should aim at avoiding false-compliant results. The reported concentration of PCDD/Fs and the sum of PCDD/Fs and dioxin-like PCBs in those samples with significant levels needs to be determined/confirmed by a confirmatory method.

(b)   determination of the levels of PCDD/Fs and dioxin-like PCBs in food samples including in the range of low background levels. This is important in order to follow time trends, exposure assessment of the population and to build a database for possible re-evaluation of action and maximum levels. This goal is achieved by confirmatory methods with sufficient sensitivity. They are also important for establishing congener patterns in order to identify the source of a possible contamination.

2.   **CLASSIFICATION OF METHODS BY THEIR DEGREE OF QUANTIFICATION**[15]

Qualitative methods give a yes / no response on the presence of analytes of interest, with no quantified indication of the concentration of the putative analyte. These methods may have the potential for providing semi-quantitative results but are used solely for report of a yes / no decision as indication of levels above or below certain ranges, e.g. limit of detection, limit of quantification or cut-off values.

For control of maximum and action levels for PCDD/Fs and dioxin-like compounds in food, screening methods may be applied which are based on comparison of the analytical result with a cut-off value and give a yes/no-decision for indication for the possible exceedance of the level of interest. For this purpose, bioanalytical methods were introduced. Generally, also physico-chemical methods could be developed; however, with regard to the TEQ-based maximum and action levels and the complex

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[15]   Adapted to PCDD/Fs and dioxin-like compounds from "Guidelines for the validation of screening methods for residues of veterinary medicines", EU Reference Laboratories (EURLs) for residues of veterinary medicines and contaminants in food of animal origin in Fougeres, Berlin and Bilthoven, 20/1/2010, http://ec.europa.eu/food/food/chemicalsafety/residues/lab_analysis_en.htm

analysis with required determination of the relevant individual congeners, there are no practical examples.

Semi-quantitative methods give an approximate indication of the concentration which may be useful as information on the range of the analyte concentration and helpful for the analyst in deciding the calibration range for the confirmatory test subsequently to be performed and for quality control purposes. Examples include:

- bioanalytical methods which are able to detect the analytes of interest, include a calibration curve, give a yes/no-decision for indication for the possible exceedance of the level of interest and allow to report the result as Bioanalytical Equivalents (BEQ), being an indication of the TEQ value in the sample,

- physico-chemical test (e.g. GC-MS/MS or GC-LRMS) where the measured method precision characteristics do not meet the requirements for quantitative tests.

Quantitative methods meet the same requirements for accuracy, dynamic range, and precision as confirmatory tests. When the quantification is required, these methods shall be validated as confirmatory methods, as detailed in this document for PCDD/Fs and dioxin-like PCBs. Such methods are based on GC-HRMS and GC-MS/MS.

3. **BACKGROUND**

For calculation of Toxic Equivalents (TEQ) concentrations, the concentrations of the individual substances in a given sample shall be multiplied by their respective Toxic Equivalency Factor (TEF), as established by the World Health Organization 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 level of interest (action or maximum level).

4. **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

homogenization (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 interest.

–   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.

5.   **REQUIREMENTS FOR LABORATORIES**

–   In accordance with the provisions of Regulation (EC) No 882/2004, laboratories shall be accredited by a recognized 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.

–   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.

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

6.1.   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.

6.2. 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.

6.3. 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.

6.4. Validation in the range of level of interest and general quality control measures

– Laboratories shall demonstrate the performance of a method in the range of the level of interest, e.g. 0.5x, 1x and 2x the level of interest 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.

6.5. 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 level of interest.

6.6. Analytical criteria

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

	Screening with bioanalytical or physico-chemical methods	Confirmatory methods
False-compliant rate*	< 5%	
Trueness		- 20% to + 20%
Repeatability (RSD$_r$)	< 20%	
Within-laboratory reproducibility (RSD$_R$)	< 25%	< 15%

* with respect to the maximum levels

6.7. 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 7 of this Annex are to be used. For cell based bioanalytical methods specific requirements are laid down in point 8 of this Annex.

– 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 on-going 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 2,3,7,8-TCDD added corresponding to the level of interest, 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 analyzed in ring trials or during contamination incidents, covering a concentration range up to e.g. 2x 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 at detecting samples exceeding the action level, the criterion for determining false-compliant rates is the maximum level, taking into account the measurement uncertainty of the confirmatory method.

– Potential noncompliant 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-noncompliant results. For screening methods, the rate of "false-noncompliant 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 noncompliant. 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.

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

7.1. General requirements

– The difference between upper-bound level and lower bound level shall not exceed 20% for foodstuffs with a contamination of about 1 pg WHO-TEQ/g fat (based on the sum of PCDD/Fs and dioxin-like PCBs). For foodstuffs with a low fat content, the same requirements for contamination levels of about 1 pg WHO-TEQ/g product have to be applied. For lower contamination levels, for example 0.5 pg WHO-TEQ/g product, the difference between upper-bound and lowerbound level may be in the range of 25% to 40%.

7.2. 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 17 ^{13}C-labelled 2,3,7,8-substituted internal PCDD/F standards and all 12 ^{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, 1 or 2 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%.

7.3. 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).

7.4. Calibration with standard curve

– The range of the calibration curve shall cover the relevant range of levels of interest.

7.5. 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, 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 2 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 minimize possible interferences on the analytes of interest.

• Fulfilment of the further criteria as described, 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

8. **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 8 establishes requirements for bioanalytical methods in general.

A screening method in principle classifies a sample as compliant or suspected to be noncompliant. For this, the calculated BEQ level is compared to the cut-off value (see 8.3.). Samples below the cut-off value are declared compliant, samples equal or above the cut-off value as suspected to be noncompliant, requiring analysis by a confirmatory method. In practice, a BEQ level corresponding to 2/3 of the maximum level may serve as the most suitable cut-off value ensuring a false-compliant rate below 5% and an acceptable rate for false-non compliant results. 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 level of interest would suit as cut-off value.

Furthermore, in the case of certain bioanalytical methods, an indicative level expressed in BEQs may be given for samples in the working range and exceeding the reporting limit (see 8.1.1. and 8.1.6.).

8.1.   **Evaluation of the test response**

8.1.1.   General requirements

–   When calculating the concentrations from a TCDD calibration curve, values at the lower and 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 (8.3) must be well within the working range.

–   Standard solutions and sample extracts shall be tested at least in duplicate. When using duplicates, a standard solution or a control extract tested in 4 to 6 wells divided over the plate shall produce a response or concentration (only possible in the working range) based on a CV<15%.

8.1.2.   Calibration

8.1.2.1. 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 minimizing the difference between calculated and observed levels in the working range of the curve (e.g. by minimizing the sum of squared residuals).

– The estimated level in the sample extract is subsequently corrected for the BEQ level calculated for a matrix/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 level of interest). For performing a recovery correction, the apparent recovery must always be within the required range (see point 8.1.4.). Reference samples used for recovery correction must comply with requirements as given in point 8.2.

8.1.2.2. Calibration with reference samples

Alternatively, a calibration curve prepared from at least 4 reference samples (see point 8.2: one matrix blank, plus three reference samples at 0.5x, 1.0x and 2.0x the level of interest) around the level of interest may be used, eliminating the need to correct for blank and recovery. In this case, the test response corresponding to 2/3 of the maximum level (see 8.3) 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.

8.1.3. 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.

8.1.4. Bioassay apparent recoveries

The "bioassay apparent recovery" shall be calculated from suitable reference samples with representative congener patterns around the level of interest and expressed as percentage of the BEQ level in comparison to the TEQ level. Depending on the type of assay and TEFs[16] 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 25% 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%.

8.1.5. 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

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

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.

8.1.6.   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.

8.2.   **Use of reference samples**

–   Reference samples shall represent sample matrix, congener patterns and concentration ranges for PCDD/Fs and dioxin-like PCBs around the level of interest (maximum or action levels).

–   A procedure blank, or preferably a matrix blank, and a reference sample at the level of interest 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. These 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. 0.5x and 2x the level of interest may be included to demonstrate the proper performance of the test in the range of interest for the control of the level of interest. Combined, these samples may be used for calculating the BEQ-levels in test samples (8.1.2.2).

8.3.   **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, 0.5x, 1x and 2x the maximum level (ML) , with 6 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 (8.2).

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 levels of interest 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 measurement uncertainty.

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

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

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

with:

BEQ$_{DL}$ BEQ corresponding to the decision limit of the confirmatory method, being the ML including 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 determind by a confirmatory method

$\overline{x}$ mean of the concentrations (in TEQ) of all calibration samples

$Q_{xx} = \sum_{j=1}^{m}(x_i - \overline{x})^2$ square sum parameter, i = index for calibration point i

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

$$\text{Cut-off value} = \text{BEQ}_{DL} - 1.64 \times \text{SD}_R$$

with

SD$_R$ standard deviation of bioassay results at BEQ$_{DL}$, measured under within-laboratory reproducibility conditions

8.3.3. Calculation as mean value of bioanalytical results (in BEQ, corrected for blank and recovery) from multiple analysis of samples (n≥6) contaminated at 2/3 the level of interest. This is based on the observation that this level will be around the cut-off determined under 8.3.1 or 8.3.2.
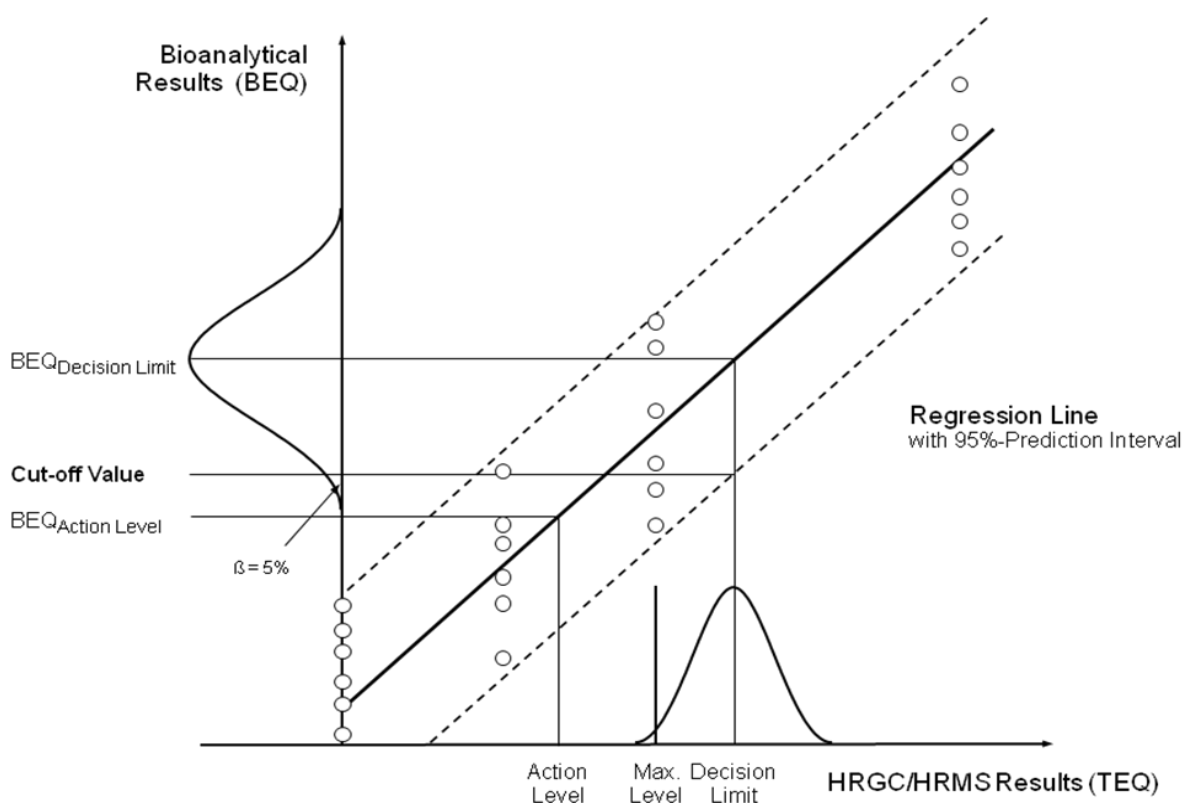
Figure 1. Calculation of cut-off values based on a 95% level of confidence implying a false-compliant rate < 5%, and a RSD_R < 25%: 1. from the *lower* band of the 95% prediction interval at the decision limit of the confirmatory method, 2. from multiple analysis of samples ($n \geq 6$) contaminated 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.

8.3.4.   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 levels of interest 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 level of interest shall be preferred.

8.4.   **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%, intra-laboratory reproducibility 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 8.1.2).

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

–   The percent 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 level of interest) shall be used for evaluation of the performance of the bioanalytical method over a constant time period.

–   Quality control (QC) charts 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 analyzed in ring trials, covering a concentration range up to e.g. 2x 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.

9.   REPORTING OF THE RESULT

Confirmatory methods

–   Insofar as the used analytical procedure makes it possible, the analytical results shall contain the levels of the individual PCDD/F and dioxin-like PCB congeners and 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 samples with maximum or action levels expressed on fat basis and an expected fat concentration in the range of 0 - 2 %

(in correspondence to existing legislation), for other samples is the determination of the lipid content optional.

– The recoveries of the individual internal standards must be made available in case the recoveries are outside the range mentioned in point 7.2, in case the maximum level is exceeded and in other cases upon request.

– As the uncertainty of measurement 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.

– If the uncertainty of measurement is taken into account by applying CCα (as described in Annex II, point IV. 2), this parameter shall be reported.

– 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.

Bioanalytical screening methods

– The result of the screening shall be expressed as compliant or suspected to be noncompliant ("suspected").

– In addition, a result for PCDD/F and/or dioxin-like PCBs expressed in Bioanalytical Equivalents (BEQ) (not TEQ) may be given (see Annex III, point 2).

– If measurement uncertainty on the calculated BEQ-level is given e.g. as standard deviation, it must be based on at least a triplicate analysis (including extraction, clean up and determination of the test response) of the sample.

– Samples with a response below the reporting limit shall be expressed as lower than the reporting limit.

– For each type of sample matrix, the report shall mention the level of interest (maximum level, 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 samples with maximum or action levels expressed on fat basis and an expected fat concentration in the range of 0 - 2 % (in correspondence to existing legislation), for other samples is the determination of the lipid content optional.

Appendix to ANNEX III

WHO-TEFs for human risk assessment based on the conclusions of the World Health Organization (WHO) – International Programme on Chemical Safety (IPCS) expert meeting which was held in Geneva in June 2005 (Martin van den Berg et al., The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicological Sciences 93(2), 223–241 (2006))

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.

ANNEX IV

SAMPLE PREPARATION AND REQUIREMENTS FOR METHODS OF ANALYSIS USED IN OFFICAL CONTROL OF THE LEVELS OF NON DIOXIN-LIKE PCBS (PCB # 28, 52, 101, 138, 153, 180) IN CERTAIN FOODSTUFFS

1.   Applicable detection methods:

Gas Chromatography / Electron Capture Detection (GC-ECD), GC-LRMS, GC-MS/MS, GC-HRMS or equivalent methods.

2.   Identification and confirmation of analytes of interest:

- Relative retention time in relation to internal standards or reference standards (acceptable deviation of +/- 0.25 %).

- Gas chromatographic separation of all six indicator PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180) from interfering substances, especially co-eluting PCBs, in particular if levels of samples are in the range of legal limits and non-compliance is to be confirmed.

  [Congeners often found to co-elute are e.g. PCB 28/31, PCB 52/69 and PCB 138/163/164. For GC-MS also possible interferences from fragments of higher chlorinated congeners have to be considered.]

- For GC-MS techniques:

  - Monitoring of at least:

    - two specific ions for HRMS,

    - two specific ions of m/z > 200 or three specific ions of m/z >100 for LRMS,

    - 1 precursor and 2 product ions for MS-MS.

  - Maximum permitted tolerances for abundance ratios for selected mass fragments:

    Relative deviation of abundance ratio of selected mass fragments from theoretical abundance or calibration standard for target ion (most abundant ion monitored) and qualifier ion(s):

Relative intensity of qualifier ion(s) compared to target ion	GC-EI-MS (relative deviation)	GC-CI-MS, GC-MS^n (relative deviation)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %

> 10 % to 20 %	± 20 %	± 30 %
≤ 10%	± 50 % (*)	± 50 % (*)

(*)   Sufficient number of mass fragments with relative intensity > 10 % available, therefore not recommendable to use qualifier ion(s) with a relative intensity of less than 10 % compared to the target ion.

- For GC-ECD:

  Confirmation of results exceeding the tolerance with two GC columns with stationary phases of different polarity.

3.   Demonstration of performance of method:

Validation in the range of the level of interest (0.5 to 2 times the level of interest) with an acceptable coefficient of variation for repeated analysis (see requirements for intermediate precision in point 8).

4.   Limit of quantification:

The blank values shall not be higher than 30% of the level of contamination corresponding to the maximum level[17].

5.   Quality control:

Regular blank controls, analysis of spiked samples, quality control samples, participation in interlaboratory studies on relevant matrices.

6.   Control of recoveries:

- Use of suitable internal standards with physico-chemical properties comparable to analytes of interest.

- Addition of internal standards:

  - Addition to products (before extraction and clean-up process);

  - Addition also possible to extracted fat (before clean-up process), if maximum level is expressed on fat basis.

- Requirements for methods using all six isotope-labelled indicator PCB congeners:

  - Correction of results for recoveries of internal standards,

  - Generally acceptable recoveries of isotope-labelled internal standards are between 50 and 120 %;

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[17]   It is highly recommendable to have a lower contribution of the reagent blank level to the level of a contaminant in a sample. It is in the responsibility of the laboratory to control the variation of blank levels, in particular, if the blank levels are subtracted.

- Lower or higher recoveries for individual congeners with a contribution to the sum of the six indicator PCBs below 10 % are acceptable.

- Requirements for methods using not all six isotope-labelled internal standards or other internal standards:

  - Control of recovery of internal standard(s) for every sample,

  - Acceptable recoveries of internal standard(s) between 60 and 120 %,

  - Correction of results for recoveries of internal standards.

- The recoveries of unlabelled congeners shall be checked by spiked samples or quality control samples with concentrations in the range of the level of interest. Acceptable recoveries for these congeners are between 70 and 120 %.

7. **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.

8. **Performance characteristics: Criteria for the sum of the six indicator PCBs at the level of interest:**

Trueness	- 30 to + 30 %
Intermediate precision (RSD%)	≤ 20 %
Difference between upper and lower bound calculation	≤ 20 %

9. **Reporting of results**

– Insofar as the used analytical procedure makes it possible, the analytical results shall contain the levels of the individual PCB congeners and 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 PCBs and lipids. The lipid content of the sample shall be determined and reported for food samples with maximum levels expressed on fat basis and an expected fat concentration in the range of 0 - 2 % (in correspondence to existing legislation), for other samples is the determination of the lipid content optional.

– The recoveries of the individual internal standards must be made available in case the recoveries are outside the range mentioned in point 6, in case the maximum level is exceeded and in other cases upon request.

–   As the uncertainty of measurement 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%.

–   If the uncertainty of measurement is taken into account by applying CCα (as described in Annex II, point IV.1), this parameter shall be reported.

–   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.