

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for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs (Text with EEA relevance) (repealed)

COMMISSION REGULATION (EC) No 1883/2006

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laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs

(Text with EEA relevance) (repealed)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

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<sup>(1)</sup>, and 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<sup>(2)</sup> provides for maximum levels for dioxins and furans and for the sum of dioxins, furans and dioxin-like PCBs in certain foodstuffs.
- (2) Commission Directive 2002/69/EC of 26 July 2002 laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs<sup>(3)</sup> establishes specific provisions concerning the sampling procedure and the methods of analysis to be applied for the official control.
- (3) The application of new maximum levels for the sum of dioxins, furans and dioxin-like PCBs requires amendments to Directive 2002/69/EC. For reasons of clarity, it is appropriate to replace Directive 2002/69/EC by this Regulation.
- (4) The provisions laid down in this Regulation relate only to the sampling and analysis of dioxins and dioxin-like PCBs for the implementation of Regulation (EC) No 1881/2006 and do not affect the sampling strategy, sampling levels and frequency as specified in Annexes III and IV of 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 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC<sup>(4)</sup>. 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<sup>(5)</sup>.
- (5) A screening method of analysis with proven, widely acceptable validation and high throughput should be used to select the samples with significant levels of dioxins and

dioxin-like PCBs. The levels of dioxins and dioxin-like PCBs in these samples need to be determined by a confirmatory method of analysis. It is therefore appropriate to establish strict requirements for the confirmatory methods of analysis and minimum requirements for the screening method.

- (6) For the sampling of very large fishes, it is necessary that the sampling is specified in order to ensure a harmonised approach throughout the Community.
- (7) In fishes of the same species and originating from the same region, the level of dioxins and dioxin-like PCBs in the fish can be different dependent on the size and or age of the fish. Moreover the level of dioxins and dioxin-like PCBs is not necessarily the same in all parts of the fish. Therefore in case of sampling of fishes, it is necessary that the sampling and sample preparation is specified in order to ensure a harmonised approach throughout the Community.
- (8) It is of major importance that analytical results are reported and interpreted in a uniform way in order to ensure a harmonised enforcement approach throughout the Community.
- (9) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food chain and Animal Health,

HAS ADOPTED THIS REGULATION:

*Article 1*

Sampling 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 I to this Regulation.

*Article 2*

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 II to this Regulation.

*Article 3*

Directive 2002/69/EC is hereby repealed. References to the repealed Directive shall be construed as references to this Regulation.

*Article 4*

This Regulation shall enter into force on the 20th day following its publication in the *Official Journal of the European Union*.

It shall apply from 1 March 2007.

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

Done at Brussels, 19 December 2006.

*For the Commission*

Markos KYPRIANOU

*Member of the Commission*

## ANNEX I

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

#### 1. SCOPE

Samples intended for the official control of the levels of dioxins (PCDD/PCDF) and dioxin like 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.

#### 2. DEFINITIONS

Lot: 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.

- Sublot: 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.
- Incremental sample: a quantity of material taken from a single place in the lot or sublot.
- Aggregate sample: the combined total of all the incremental samples taken from the lot or sublot.
- Laboratory sample: a representative part/quantity of the aggregate sample intended for the laboratory.

#### 3. GENERAL PROVISIONS

##### 3.1. Personnel

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

##### 3.2. Material to be sampled

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

##### 3.3. 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 dioxin-like PCBs, adversely affect the analytical determination or make the aggregate samples unrepresentative.

##### 3.4. 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 part 3.8 of this Annex.

##### 3.5. 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.

##### 3.6. 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.

### 3.7. 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.

### 3.8. 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.

## 4. SAMPLING PLANS

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

### 4.1. Division of lots into sublots

Large lots shall be divided into sublots on condition that the subplot 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 subplot may exceed the mentioned weight by a maximum of 20 %.

TABLE 1

Subdivision of lots into sublots for products traded in bulk consignments

<b>Lot weight (ton)</b>	<b>Weight or number of sublots</b>
≥ 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

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

### 4.2. Number of incremental samples

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The aggregate sample uniting all incremental samples shall be at least 1 kg (see part 3.5 of this Annex).

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

In the case of bulk liquid products the lot or subplot 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 subplot. It is therefore sufficient to take three incremental samples from a lot or subplot 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 part 3.8 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<sup>(6)</sup>, 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).

TABLE 3

Minimum number of incremental samples to be taken from the lot or subplot

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

If the lot or subplot 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 subplot consists of individual packages or units

<b>Number of packages or units in the lot/sublot</b>	<b>Number of packages or units to be taken</b>
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

4.3. 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 3.5).

- 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 to 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.

#### 4.4. Sampling of lots of fish containing whole fishes of different size and/or weight

- The provisions of point 4.3 as regards sample constitution are applicable.
- In case a size or weight class/category is predominant (about 80 % or more of the batch), the sample is taken from fishes with the predominant size or weight. This sample is to be considered as being representative for the whole batch.
- 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 consignment. Specific guidance for such cases is provided in 'Guidance document for the sampling of lots of fish containing whole fishes of different size and/or weight<sup>(7)</sup>'.

#### 4.5. Sampling at retail stage

Sampling of foodstuffs at retail stage shall be done where possible in accordance with the sampling provisions set out in part 4.2 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 subplot.

### 5. COMPLIANCE OF THE LOT OR SUBLOT WITH THE SPECIFICATION

The lot is accepted if the analytical result of a single analysis does not exceed the respective maximum level of dioxins and the sum of dioxins and 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<sup>(8)</sup> analytical result, confirmed by duplicate analysis<sup>(9)</sup>, exceeds the maximum level beyond reasonable doubt taking into account the measurement uncertainty.

The taking into account of the measurement uncertainty may be done 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 subplot is non-compliant if the measured value minus U is above the established permitted level. In case of a separate determination of dioxins and dioxin-like-PCBs the sum of the estimated expanded uncertainty of the separate analytical results of dioxins and dioxin-like PCBs has to be used for the sum of dioxins and dioxin-like PCBs,
- by establishing the decision limit ( $CC\alpha$ ) according to the provisions of Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results<sup>(10)</sup> (point 3.1.2.5 of the Annex — the case of substances with established permitted level) a lot or subplot is non-compliant if the measured value is equal to or above the  $CC\alpha$ .

The present interpretation rules apply for the analytical result obtained on the sample for official control. In case of analysis for defence or referee purposes, the national rules apply.

## ANNEX II

### 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 dioxins (polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF)) and dioxin-like PCBs.

Monitoring for the presence of dioxins in foodstuffs may be performed by a strategy involving a screening method in order to select those samples with levels of dioxins and dioxin-like PCBs that are less than 25 % below or exceed the maximum level. The concentration of dioxins and sum of dioxins and dioxin-like PCBs in those samples with significant levels needs to be determined/confirmed by a confirmatory method.

Screening methods are methods that are used to detect the presence of dioxins and dioxin-like PCBs at the level of interest. These methods shall have a capacity for a high sample throughput and are used to sift large numbers of samples for potential positives. They shall be specifically designed to avoid false negatives.

Confirmatory methods are methods that provide full or complementary information enabling the dioxins and dioxin-like PCBs to be identified and quantified unequivocally at the level of interest.

#### 2. BACKGROUND

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 Organisation and listed in the Appendix to this Annex, and subsequently summed to give the total concentration of dioxin-like compounds expressed as Toxic Equivalents (TEQs).

For the purposes of this Regulation, the accepted specific limit of quantification of an individual congener shall be the concentration of an analyte in the extract of a sample which produces an



instrumental response at two different ions to be monitored with an S/N (signal/noise) ratio of 3:1 for the less sensitive signal and fulfilment of the basic requirements such as e.g. retention time, isotope ratio according to the determination procedure as described in EPA method 1613 revision B.

### 3. QUALITY ASSURANCE REQUIREMENTS TO BE COMPLIED WITH FOR SAMPLE PREPARATION

- 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, aluminium, polypropylene or polyethylene containers. Traces of paper dust must be removed from the sample container. Glassware shall be rinsed with solvents, certified to be free from dioxins or previously controlled for the presence of dioxins.
- 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.
- Perform a blank analysis by carrying out the entire analytical procedure omitting only the sample.
- Sample weight used for the extraction must be sufficient to fulfil the requirements with respect to sensitivity.
- The specific sample preparation procedures used for the products under consideration shall be validated according to 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 rests of muscle meat and fat tissue at the inner side of the skin are carefully and completely scraped off from the skin and that these rests of muscle meat and fat tissue are added to the sample to be analysed.

### 4. REQUIREMENTS FOR LABORATORIES

- 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. For details of acceptance criteria, see part 5.
- Limit of quantification for a confirmatory method shall be in the range of about one fifth of the level of interest.
- 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.
- Laboratory proficiency shall be proved by the continuous successful participation in interlaboratory studies for the determination of dioxins and dioxin-like PCBs in the relevant feed/food matrices.
- 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.

### 5. REQUIREMENTS TO BE MET BY ANALYTICAL PROCEDURE FOR DIOXINS AND DIOXIN-LIKE PCBs

#### **Basic requirements for acceptance of analytical procedures:**

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- *High sensitivity and low limits of detection.* For PCDDs and PCDFs, detectable quantities have to be in the picogram TEQ ( $10^{-12}$ g) range because of extreme toxicity of some of these compounds. PCBs are known to occur at higher levels than the PCDDs and PCDFs. For most PCB congeners sensitivity 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 same sensitivity must be reached as for the PCDDs and PCDFs.
- *High selectivity (specificity).* A distinction is required for PCDDs, PCDFs and dioxin-like PCBs from 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 PCDDs and PCDFs and dioxin-like PCBs) and other congeners. Bioassays shall be able to determine TEQ values selectively as the sum of PCDDs, PCDFs and dioxin-like PCBs.
- *High accuracy (trueness and precision).* 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 estimate of TEQ. 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).

Screening methods may comprise bioassays and GC/MS methods; confirmatory methods are high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) methods. Following criteria have to be complied with on total TEQ value:

	Screening methods	Confirmatory methods
<b>False negative rate</b>	< 1 %	
<b>Trueness</b>		– 20 % to + 20 %
<b>Precision (<math>RSD_R</math>)</b>	< 30 %	< 15 %

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

- Addition of  $^{13}\text{C}$ -labelled 2,3,7,8-chlorine substituted internal PCDD/F standards and of  $^{13}\text{C}$ -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/F 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/F and dioxin-like PCBs). There shall be clear preference, certainly in case of confirmatory methods, of using all 17  $^{13}\text{C}$ -labelled 2,3,7,8-substituted internal PCDD/F standards and all 12  $^{13}\text{C}$ -labelled internal dioxin-like PCB standard.

Relative response factors shall also be determined for those congeners for which no  $^{13}\text{C}$ -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 extraction 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 dibenzodioxins 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 screening methods, the recoveries shall be in the range of 30 to 140 %.
- Separation of dioxins 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).
- Gaschromatographic 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).
- Determination shall be performed according to EPA Method 1613 revision B: Tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS or another with equivalent performance criteria.
- The difference between upperbound level and lower bound level shall not exceed 20 % for foodstuffs with a dioxin contamination of about 1 pg WHO-TEQ/g fat (based on the sum of PCDD/PCDF 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 0,50 pg WHO-TEQ/g product, the difference between upperbound and lowerbound level may be in the range of 25 % to 40 %.

## 7. SCREENING METHODS OF ANALYSIS

### 7.1. Introduction

Different analytical approaches may be performed using a screening method: a pure screening approach and a quantitative approach.

#### Screening approach

The response of samples is compared to that of a reference sample at the level of interest. Samples with a response less than the reference are declared negative, those with a higher response are suspected positives. Requirements:

- A blank and a reference sample(s) have to be included in each test series, which is extracted and tested at the same time under identical conditions. The reference sample must show a clearly elevated response in comparison to a blank.
- Extra reference samples 0,5x and 2x the level of interest shall be included to demonstrate the proper performance of the test in the range of interest for the control of the level of interest.
- When testing other matrices, the suitability of the reference sample(s) has to be demonstrated, preferentially by including samples shown by HRGC/HRMS to contain a TEQ level around that of the reference sample or else a blank spiked at this level.

- Since no internal standards can be used in bioassays, tests on repeatability shall be carried out to obtain information on the standard deviation within one test series. The coefficient of variation shall be below 30 %.
- For bioassays, the target compounds, possible interferences and maximum tolerable blank levels shall be defined.

#### Quantitative approach

The quantitative approach requires standard dilution series, duplicate or triplicate clean up and measuring as well as blank and recovery controls. The result may be expressed as TEQ, thereby assuming that the compounds responsible for the signal correspond to the TEQ principle. This can be performed by using TCDD (or a dioxin/furan/dioxin-like PCB standard mixture) to produce a calibration curve to calculate the TEQ level in the extract and thus in the sample. The result is subsequently corrected for the TEQ level calculated for a blank sample (to account for impurities from solvents and chemicals used), and a recovery (calculated from the TEQ level in a quality control sample around the level of interest). It is essential to note that part of the apparent recovery loss may be due to matrix effects and/or differences between the TEF values in the bioassays and the official TEF values set by the WHO.

#### 7.2. Requirements for methods of analysis used for screening

- GC/MS methods of analysis and bioassays may be used for screening. For GC/MS methods the requirements as laid down in point 6 are to be used. For cell based bioassays specific requirements are laid down in part 7.3 of this Annex and for kit-based bioassays in part 7.4 of this Annex.
- Information on the number of false-positive and false-negative results of a large set of samples below and above the maximum level or action level is necessary, in comparison to the TEQ content as determined by a confirmatory method of analysis. Actual false negative rates shall be under 1 %. The rate of false positive samples shall be low enough to make the use of a screening tool advantageous.
- Positive results have always to be confirmed by a confirmatory method of analysis (HRGC/HRMS). In addition, samples from a wide TEQ-range shall be confirmed by HRGC/HRMS (approximately 2 % to 10 % of the negative samples). Information on correspondence between bioassay and HRGC/HRMS results shall be made available.

#### 7.3. Specific requirements for cell based bioassays

- When performing a bioassay, every test run requires a series of reference concentrations of TCDD or a dioxin/furan/dioxin-like PCB mixture (full dose-response curve with a  $R^2 > 0,95$ ). However, for screening purposes an expanded low level curve for analysing low level samples may be used.
- A TCDD reference concentration (about 3x limit of quantification) on a quality control sheet shall be used for the outcome of the bioassay over a constant time period. An alternative may be the relative response of a reference sample in comparison to the TCDD calibration line since the response of the cells may depend on many factors.
- Quality control (QC) charts for each type of reference material shall be recorded and checked to make sure the outcome is in accordance with the stated guidelines.
- In particular for quantitative calculations, the induction of the sample dilution used must be within the linear portion of the response curve. Samples above the linear portion of the response curve must be diluted and re-tested. Therefore, at least 3 or more dilutions at one time shall be tested.
- The percent standard deviation shall not be above 15 % in a triplicate determination for each sample dilution and not above 30 % between three independent experiments.
- The limit of detection may be set as 3x the standard deviation of the solvent blank or of the background response. Another approach is to apply a response that is above

the background (induction factor 5x the solvent blank) calculated from the calibration curve of the day. The limit of quantification may be set as 5 to 6x the standard deviation of the solvent blank or of the background response or to apply a response that is above the background (induction factor 10x the solvent blank) calculated from the calibration curve of the day.

#### 7.4. Specific requirements for kit based bioassays

- It shall be ensured that the kit-based bioassays have sufficient sensitivity and reliability to be applied for food.
- Manufacturer's instructions for sample preparation and analyses have to be followed.
- Test kits shall not be used after the expiration date.
- Materials or components designed for use with other kits shall not be used.
- Test kits shall be kept within the specified range of storage temperature and used at the specified operating temperature.
- The limit of detection for immunoassays is determined as 3 x the standard deviation, based on 10 replicate analysis of the blank, to be divided by the slope value of the linear regression equation.
- Reference standards shall be used for tests at the laboratory to make sure that the responsiveness to the standard is within an acceptable range.

#### 8. REPORTING OF THE RESULT

Insofar as the used analytical procedure makes it possible, the analytical results shall contain the levels of the individual PCDD/F and PCB congeners and be reported as lowerbound, upperbound 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 lipid content of the sample as well the method used for lipid extraction.

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  $x \pm U$  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 dioxins and dioxin-like-PCBs the sum of the estimated expanded uncertainty of the separate analytical results of dioxins and dioxin-like PCBs has to be used for the sum of dioxins and dioxin-like PCBs.

If the uncertainty of measurement is taken into account by applying  $CC\alpha$  (as described in Annex I, part 5), 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.

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*Status: This is the original version (as it was originally adopted).*

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## Appendix to Annex II

TABLE WHO TEFS FOR HUMAN RISK ASSESSMENT BASED ON THE CONCLUSIONS OF THE WORLD HEALTH ORGANISATION MEETING IN STOCKHOLM, SWEDEN, 15-18 JUNE 1997 (VAN DEN BERG ET AL., (1998) TOXIC EQUIVALENCY FACTORS (TEFS) FOR PCBS, PCDDS, PCDFS FOR HUMANS AND FOR WILDLIFE. *ENVIRONMENTAL HEALTH PERSPECTIVES*, 106(12), 775)

<b>Congener</b>	<b>TEF value</b>
<b>Dibenzo-p-dioxins (PCDDs)</b>	
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	1
1,2,3,4,7,8-HxCDD	0,1
1,2,3,6,7,8-HxCDD	0,1
1,2,3,7,8,9-HxCDD	0,1
1,2,3,4,6,7,8-HpCDD	0,01
OCDD	0,0001
<b>Dibenzofurans (PCDFs)</b>	
2,3,7,8-TCDF	0,1
1,2,3,7,8-PeCDF	0,05
2,3,4,7,8-PeCDF	0,5
1,2,3,4,7,8-HxCDF	0,1
1,2,3,6,7,8-HxCDF	0,1
1,2,3,7,8,9-HxCDF	0,1
2,3,4,6,7,8-HxCDF	0,1
1,2,3,4,6,7,8-HpCDF	0,01
1,2,3,4,7,8,9-HpCDF	0,01
OCDF	0,0001
<b>'Dioxin-like' PCBs Non-ortho PCBs + Mono-ortho PCBs</b>	
<b>Non-ortho PCBs</b>	
PCB 77	0,0001
PCB 81	0,0001
PCB 126	0,1
PCB 169	0,01
<b>Mono-ortho PCBs</b>	
PCB 105	0,0001
PCB 114	0,0005

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

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PCB 118	0,0001
PCB 123	0,0001
PCB 156	0,0005
PCB 157	0,0005
PCB 167	0,00001
PCB 189	0,0001

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

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- (1) [OJ L 165, 30.4.2004, p. 1](#), as corrected by [OJ L 191, 28.5.2004, p. 1](#). Regulation amended by Commission Regulation (EC) No 776/2006 ([OJ L 136, 24.5.2006, p. 3](#)).
- (2) See page 5 of this Official Journal.
- (3) [OJ L 209, 6.8.2002, p. 5](#). Directive as last amended by Directive 2004/44/EC ([OJ L 113, 20.4.2004, p. 17](#)).
- (4) [OJ L 125, 23.5.1996, p. 10](#). Directive as last amended by Regulation (EC) No 882/2004 of the European Parliament and of the Council ([OJ L 165, 30.4.2004, p. 1](#), as corrected by [OJ L 191, 28.5.2004, p. 1](#)).
- (5) [OJ L 65, 5.3.1998, p. 31](#). Decision as amended by the 2003 Act of Accession.
- (6) [OJ L 303, 6.11.1997, p. 12](#).
- (7) [http://ec.europa.eu/food/food/chemicalsafety/contaminants/dioxins\\_en.htm](http://ec.europa.eu/food/food/chemicalsafety/contaminants/dioxins_en.htm)
- (8) The concept of 'upperbound' requires using the limit of quantification for the contribution of each non-quantified congener to the Toxic Equivalent (TEQ).  
The concept of 'lowerbound' requires using zero for the contribution of each non-quantified congener to the TEQ.  
The concept of 'mediumbound' requires using half of the limit of quantification calculating the contribution of each non-quantified congener to the TEQ.
- (9) 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 dioxin contamination incident, confirmation by duplicate analysis might be omitted in case the samples selected for analysis are through traceability linked to the dioxin contamination incident.
- (10) [OJ L 221, 17.8.2002, p. 8](#). Decision as amended by Decision 2004/25/EC ([OJ L 6, 10.1.2004, p. 38](#)).