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COMMISSION DIRECTIVE 2002/69/EC

of ► C1 30 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

(Text with EEA relevance)

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COMMISSION DIRECTIVE 2002/69/EC

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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

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 85/591/EEC of 20 December 1985 concerning the introduction of Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption ⁽¹⁾, and ►C1 in particular Article 1 and 4 thereof, ◀

Whereas:

- (1) Commission Regulation (EC) No 466/2001 ⁽²⁾, as last amended by Regulation (EC) No 563/2002 ⁽³⁾, and amended by Council Regulation (EC) No 2375/2001 ⁽⁴⁾ establishes maximum limits for dioxins and furans in certain foodstuffs.
- (2) Council Directive 89/397/EEC of 14 June 1989 on the official control of foodstuffs ⁽⁵⁾ lays down the general principles for the performance of control of foodstuffs. Council Directive 93/99/EEC of 29 October 1993 on the subject of additional measures concerning the official control of foodstuffs ⁽⁶⁾ introduces a system of quality standards for laboratories entrusted by the Member States with the official control of foodstuffs.
- (3) Directive 85/591/EEC has fixed general criteria for methods of sampling and analysis. However, in certain cases it is necessary to lay down more specific criteria and or requirements with which the method of analysis should comply in order to ensure that laboratories use methods of analysis with comparable levels of performance.
- (4) The provisions for sampling and methods of analysis have been drawn up on the basis of present knowledge and they may be adapted to take account of advances in scientific and technological knowledge.
- (5) The provisions laid down in this Directive relate only to the sampling and analysis of dioxins and dioxin-like PCBs for the implementation of Regulation (EC) No 466/2001 and 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 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC ⁽⁷⁾. 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 ⁽⁸⁾.
- (6) An active approach should be pursued in order to obtain comprehensive reliable data on the presence of dioxin-like PCBs in foodstuffs. Requirements should therefore be laid down as

⁽¹⁾ OJ L 372, 31.12.1985, p. 50.

⁽²⁾ OJ L 77, 16.3.2001, p. 1.

⁽³⁾ OJ L 86, 3.4.2002, p. 5.

⁽⁴⁾ OJ L 321, 6.12.2001, p. 1.

⁽⁵⁾ OJ L 186, 30.6.1989, p. 23.

⁽⁶⁾ OJ L 290, 24.11.1993, p. 14.

⁽⁷⁾ OJ L 125, 23.5.1996, p. 10.

⁽⁸⁾ OJ L 65, 5.3.1998, p. 31.

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regards the methods of analysis to be used for the determination of dioxin-like PCBs in foodstuffs.

- (7) A screening method of analysis with proven, widely acceptable validation and high throughput could be used to select the samples with significant levels of dioxins. The levels of dioxins 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.
- (8) The measures provided for in this Directive are in accordance with the opinion of the Standing Committee on the Food chain and Animal Health,

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Member States shall ensure that the sampling for the official control of the levels of dioxins and furans and the determination of the levels of dioxin-like PCBs in foodstuffs is carried out in accordance with the methods described in Annex I.

Article 2

The Member States shall ensure that sample preparation and methods of analyses used for the official control of the levels of dioxins and furans and the determination of the levels of dioxin-like PCBs in foodstuffs comply with the criteria described in Annex II.

Article 3

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 28 February 2003 at the latest. They shall forthwith inform the Commission thereof.

When Member States adopt those provisions, they shall contain a reference to this Directive or be accompanied by such a reference on the occasion of their official publication. Member States shall determine how such reference is to be made.

Article 4

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

Article 5

This Directive is addressed to the Member States.



ANNEX I

METHODS OF SAMPLING FOR OFFICIAL CONTROL OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND THE DETERMINATION OF DIOXIN-LIKE PCBs IN CERTAIN FOODSTUFFS**1. Purpose and scope**

Samples intended for the official control of the levels of dioxins (PCDD/PCDF) content, as well for the determination of the content of dioxin-like PCBs⁽¹⁾ in foodstuffs shall be taken according to the methods described below. 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 466/2001 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

(1) 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).

Congener	TEF value
Dibenzo-p-dioxins (PCDDs)	
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
Dibenzofurans (PCDFs)	
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
<i>'Dioxin-like' PCBs Non-ortho PCBs + Mono-ortho PCBs</i>	
Non-ortho PCBs	
PCB 77	0,0001
PCB 81	0,0001
PCB 126	0,1
PCB 169	0,01
Mono-ortho PCBs	
PCB 105	0,0001
PCB 114	0,0005
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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of packing, packer, consignor or markings. In the case of fish and fishery products, also the size of fish shall be comparable.

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 qualified person as specified by the Member States.

3.2. Material to be sampled

Each lot, which is to be examined, must be sampled separately.

3.3. Precautions to be taken

In the course of sampling and preparation of laboratory samples precautions must 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 practical incremental samples shall be taken at various places distributed throughout the lot or sublot. Departure from this procedure must be recorded in the record provided for under 3.8.

3.5. Preparation of the aggregate sample

The aggregate sample is made up by uniting all incremental samples. It shall be at least 1 kg unless not practical, e.g. when a single package has been sampled.

3.6. Subdivision of aggregate sample in laboratory samples for enforcement, defence and referee purposes

The laboratory samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenised aggregate sample unless this conflicts with Member States' regulations on sampling. The size of the laboratory samples for enforcement shall be sufficient to allow at least for duplicate analyses.

3.7. Packaging and transmission of aggregate and laboratory samples

Each aggregate and laboratory 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 change of composition of the aggregate and laboratory samples, which might arise during transportation or storage.

3.8. Sealing and labelling of aggregate and laboratory samples

Each sample taken for official use shall be sealed at the place of sampling and identified following the Member States' regulations. A record must 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 lot that is to be controlled.

▼B*Number of incremental samples*

In the case of milk and oils, for which a homogeneous distribution of the contaminants in question can be assumed within a given lot, it is sufficient to take three incremental samples per lot which forms the aggregate sample. Reference to the lot number shall be given. For other products, the minimum number of incremental samples to be taken from the lot shall be as given in Table 1.

The aggregate sample uniting all incremental samples shall be at least 1 kg (see point 3.5). The incremental samples shall be of similar weight. The weight of an incremental sample should be at least 100 grams. The weight of the incremental sample is dependent on the size of the particles in the lot. Departure from this procedure must be recorded in the record provided for under 3.8. 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⁽¹⁾, the sample size for hen eggs is at least 12 eggs (for bulk lots as well for lots consisting of individual packages, Tables 1 and 2).

TABLE 1

Minimum number of incremental samples to be taken from the lot

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

If the lot consists of individual packages, then the number of packages, which shall be taken to form the aggregate sample, is given in Table 2.

TABLE 2

Number of packages (incremental samples) which shall be taken to form the aggregate sample if the lot consists of individual packages

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

▼M14.1. *Specific provisions for the sampling of lots containing whole fishes*

The number of incremental samples to be taken from the lot is defined in Table 1. 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 fish (individual fish weighing < 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 can consist of the middle part, weighing each at least 100 grams, of the fish forming the aggregate sample. The whole part to which the maximum level is applicable is used for homogenisation of the sample.
- In case the lot to be sampled contains larger fish (individual fish weighing more than 1 kg), the incremental sample consists of the middle part of the fish. Each incremental sample weighs at least 100 grams. In case the lot to be sampled consist of very large fish (e.g.

⁽¹⁾ OJ L 303, 6.11.1997, p. 12.

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> 6 kg) and taking a piece of the middle part of the fish would result in significant economic damage, taking three incremental samples of at least 350 grams each can be considered sufficient, independently of the size of the lot.

5. Compliance of the lot or subplot with the specification

The lot is accepted if the analytical result of a single analysis does not exceed the respective maximum level as laid down in Regulation (EC) No 466/2001, taking into account the measurement uncertainty.

The lot is non-compliant with the maximum level as laid down in Regulation (EC) No 466/2001, if the analytical result confirmed by duplicate analysis and calculated as the mean of at least two separate determinations exceeds the maximum level beyond reasonable doubt, taking into account the measurement uncertainty.

The taking into account of the measurement uncertainty can 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 %,
- 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 ⁽¹⁾ (point 3.1.2.5 of the Annex — the case of substances with established permitted levels).

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.

⁽¹⁾ OJ L 221, 17.8.2002, p. 8. Decision as last amended by Decision 2004/25/EC (OJ L 6, 10.1.2004, p. 38).

▼B*ANNEX II***SAMPLE PREPARATION AND REQUIREMENTS FOR METHODS OF ANALYSIS USED IN OFFICIAL CONTROL OF THE LEVELS OF DIOXINS (PCDD/PCDF) AND THE DETERMINATION OF DIOXIN-LIKE PCBs IN CERTAIN FOODSTUFFS****1. Objective and field of application**

These requirements should 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 the determination of dioxin-like PCBs.

Monitoring for the presence of dioxins in foodstuffs can 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 30-40 % below or exceed the level of interest. The concentration of dioxins 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 have a capacity for a high sample throughput and are used to sift large numbers of samples for potential positives. They are 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

Because environmental and biological samples (including samples of foodstuffs) in general contain complex mixtures of different dioxin congeners, the concept of Toxic Equivalency Factors (TEFs) has been developed to facilitate risk assessment. These TEFs have been established to express concentrations of mixtures of 2,3,7,8-substituted PCDDs and PCDFs, and more recently, some non-ortho and mono-ortho chlorine substituted PCBs which possesses dioxin-like activity in toxic equivalents (TEQs) of 2,3,7,8-TCDD (see Annex I, footnote 1).

The concentrations of the individual substances in a given sample are multiplied by their respective TEF and subsequently summed to give the total concentration of dioxin-like compounds expressed as TEQs.

The concept of 'upperbound' requires using the limit of quantification for the contribution of each non-quantified congener to the 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.

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For the purposes of this Directive only, the accepted specific limit of quantification of an individual congener is 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.

▼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 should be rinsed with solvents 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.

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- 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.
- There are many satisfactory specific sample preparation procedures, which may be used for the products under consideration. The procedures have to be validated according to internationally accepted guidelines.

4. Requirements for laboratories

- Laboratories shall demonstrate the performance of a method in the range of the level of interest, e.g. 0,5 ×, 1 × and 2 × the level of interest with an acceptable coefficient of variation for repeated analysis. For details of acceptance criteria, see point 5.
- Limit of quantification for a confirmatory method should be in the range of about one fifth of the level of interest, to make sure that acceptable coefficients of variations are met in the range of the level of interest.
- Regular blank controls and spiking experiments or analysis of control samples (preferably, if available, certified reference material) should be performed as internal quality control measures.
- Successful participation in interlaboratory studies that assess laboratory proficiency is the best way to prove the competence in specific analyses. However successful participation in interlaboratory studies for, e.g. soil or sewage samples, does not necessarily prove the competence also in the field of food or feedingstuff samples, which present lower contamination levels. Therefore, the continuous participation in interlaboratory studies for the determination of dioxins and dioxin-like PCBs in the relevant feed/food matrices is mandatory.
- In accordance with the provisions of Directive 93/99/EEC, laboratories should be accredited by a recognised body operating in accordance with ISO Guide 58 to ensure that they are applying analytical quality assurance. Laboratories should be accredited following the ISO/IEC/17025:1999 standard.

5. Requirements to be met by analytical procedure for dioxins and dioxin-like PCBs

Basic requirements for acceptance of analytical procedures:

- *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 should be able to determine TEQ values selectively as the sum of PCDDs, PCDFs and dioxin-like PCBs.
- *High accuracy (trueness and precision).* The determination should 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 measurement) 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 (precision is usually calculated as a standard deviation including repeatability and reproducibility, and indicates the closeness of agreement between the results obtained by applying the experimental procedure several times under prescribed conditions).

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Screening methods can 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
False negative rate	< 1 %	
Trueness		- 20 % to + 20 %
CV	< 30 %	< 15 %

6. Specific requirements for GC/MS methods to be complied with for screening or confirmatory purposes

- Addition of ¹³C-labelled 2,3,7,8-chlorine substituted internal PCDD/F standards (and of ¹³C-labelled internal dioxin-like PCB standards, if dioxin-like PCBs have to be determined) must be carried out at the very beginning or start 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, if dioxin-like PCBs have to be determined) 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 is a clear preference, certainly in case of confirmatory methods, of using all 17 ¹³C-labelled 2,3,7,8-substituted internal PCDD/F standards and all 12 ¹³C-labelled internal dioxin-like PCB standard (if dioxin-like PCBs have to be determined).

Relative response factors should also be determined for those congeners for which no ¹³C-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 can be added either before extraction or after fat extraction. An appropriate validation of the extraction efficiency should 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 should 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 PCDD/F only). For screening methods, the recoveries should be in the range of 30 % to 140 %.
- Separation of dioxins from interfering chlorinated compounds such as PCBs and chlorinated diphenyl ethers should be carried out by suitable chromatographic techniques (preferably with a florisil, alumina and/or carbon column).
- Gaschromatographic separation of isomers should be sufficient (< 25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF).
- Determination should 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 should not exceed 20 % for foodstuffs with a dioxin contamination of about 1 pg WHO-TEQ/g fat (based on PCDD/PCDF only). 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 %.

▼B**7. Screening methods of analysis****7.1. Introduction**

Different analytical approaches can 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 $0,5 \times$ and $2 \times$ the level of interest should 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 are very important to obtain information on the standard deviation within one test series. The coefficient of variation should be below 30 %.
- For bioassays, the target compounds, possible interferences and maximum tolerable blank levels should 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 standard mixture) to produce a calibration curve to calculate the TEQ level in the extract and thus in the sample. This 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 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 point 7.3 and for kit-based bioassays in point 7.4.
- 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 should be under 1 %. The rate of false positive samples should 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 should be confirmed by HRGC/HRMS (approximately 2 % to 10 % of the negative samples). Information on correspondence between bioassay and HRGC/HRMS results should 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 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 could be used.
- A TCDD reference concentration (about $3 \times$ limit of quantification) on a quality control sheet should be used for the outcome of the bioassay over a constant time period. An alternative could 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.

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- Quality control (QC) charts for each type of reference material should 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 three or more dilutions at one time are recommended to be tested.
- The percent standard deviation should 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 $3 \times$ 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 $5 \times$ the solvent blank) calculated from the calibration curve of the day. The limit of quantification may be set as $5 \times$ to $6 \times$ the standard deviation of the solvent blank or of the background response or to apply a response that is above the background (induction factor $10 \times$ the solvent blank) calculated from the calibration curve of the day.

7.4. *Specific requirements for kit-based bioassays* ⁽¹⁾

- Manufacturer's instructions for sample preparation and analyses have to be followed.
- Test kits should not be used after the expiration date.
- Materials or components designed for use with other kits should not be used.
- Test kits should 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 $3 \times$ 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 should 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 should contain the levels of the individual PCDD/F and PCB congeners and be reported as lowerbound, upperbound and mediumbound 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 should 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.

⁽¹⁾ No evidence have yet been submitted of commercially available kit-based bioassays having sufficient sensitivity and reliability to be used for screening for the presence of dioxins at the required levels in samples of foodstuffs and feedingstuffs.