codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 2 **CX/CF 09/3/2-Add.1**
March 2009

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

Third Session
Rotterdam, the Netherlands, 23 – 27 March 2009

MATTERS REFERRED TO THE COMMITTEE BY THE CODEX ALIMENTARIUS COMMISSION AND/OR OTHER CODEX COMMITTEES/TASK FORCES

A. MATTERS ARISING FROM THE 31st SESSION OF THE CODEX ALIMENTARIUS COMMISSION

Matters for action

Committee on Natural Mineral Waters: Amendments to the Standard on Natural Mineral Waters[1]

1. The Commission noted the proposal of Kenya to initiate new work on the completion of the Section on methods of analysis in the Codex Standard on Natural Mineral Waters (CODEX STAN 108-1981) in view of the fact that, in the standard, there was no indication of specific methods of analysis and sampling procedures available for a number of chemical substances mentioned in Sections 3.2.17 (Surface active agents), 3.2.18 (Pesticides and PCBs), 3.2.19 (Mineral oil) and 3.2.20 (Polynuclear aromatic hydrocarbons), and the proposal to revise the Section on Hygiene to make it easier to use and consistent with the Recommended International Code of Hygienic Practice for Collecting, Processing and Marketing of Natural Mineral Waters (CAC/RCP 33-1985). The Chair of the Committee on Natural Mineral Waters also noted that the proposal from Kenya had been presented orally at the last session of the Committee on Natural Mineral Waters, however it had not been examined by the Committee as it fell outside the mandate given to the Committee by the 30th Session of the Commission. The Commission further noted that the project document had been considered at the last session of the Executive Committee and, after some discussion, agreed to refer the issue on the methods of analysis raised in Project Document 22 to the Committees on Contaminants in Foods, on Pesticide Residues and on Methods of Analysis and Sampling for review in their respective areas of competence as a matter of priority, especially whether further work was warranted and desirable. (The Project Document 22 is attached as Annex I).

2. The Commission also requested the Committee concerned, as mentioned above, to inform the Executive Committee and the Commission about their findings in order to allow the Commission to take an informed decision on this matter at its next session (ALINORM 08/31/REP, para. 106-108).

3. The Committee is invited to consider this matter and to report its views to the 62nd CCEXEC and 32nd CAC.

B. MATTERS ARISING FROM OTHER COMMITTEES

Sampling: Methods of Analysis for Dioxins and Dioxin-like PCBs

30th Session Codex Committee on Methods of Analysis

4. The The Committee recalled that at its last session it had been agreed to prepare a discussion paper aimed at answering the question from the Committee on Contaminants in Foods (CCCF) on the applicability of the methods for the indicated ranges and commodities concerned, review the validation data for the methods, and set criteria for dioxin analysis, for consideration at the present session.

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1 ALINORM 08/31/REP, paras 106-108

5. The Delegation of Germany, speaking as Chairperson of the electronic working group, referring to the discussion paper in CX/MAS 09/30/12, highlighted that this document was not to intended to gather a long list of analytical methods for detection of dioxins and dioxin-like PCBs but to provide criteria approach on selection of detection methods for monitoring and recommended that the Committee should consider further the procedure for establishing these criteria in Codex and whether this paper should be forwarded to the CCCF.

6. The Committee noted that the list in Annex II did not intend to identify any status and purposes (confirmative or screening purposes), but simply provided information of methods available submitted by government and organizations and agreed to add a footnote in Annex II to state this point. It was also noted that generally high-resolution gas chromatography/high resolution mass spectrometry methods (GC-HRMS) was used as a confirmatory method and GC-MS methods were used for screening.

7. The reference "MS" was added to the title *GCxGC* for clarity and the number of Codex members in the EC was updated. In Annex 4, information from Japan on methods for confirmation for fish, tea, meat, dairy products, milk and egg submitted was deleted as they were repetition and a scientific reference regarding TEQ – value for determination of animal feed was added for the screening method provided by Belgium.

8. The Committee agreed to forward this discussion paper as amended above with some editorial changes, for consideration by the Committee on Contaminants in Foods.

9. The Committee is invited to consider the Discussion Paper on Methods of Analysis for Dioxins and Dioxin-like PCBs (attached as Annex II) and report its views to the next session of CCMAS.

Aflatoxin Sampling Plan for Almonds, Hazelnuts and Pistachio

61st Session of the Executive Committee of the Codex Alimentarius Commission

10. The Executive Committee discussed the need to refer the Proposed Draft Aflatoxin Sampling Plan for Almonds, Hazelnuts and Pistachios, to the Committee on Methods of Analysis and Sampling in order to ensure consistency with general sampling texts, the criteria approach for methods of analysis and existing methods for the determination of aflatoxins. Some Members pointed out that the Proposed Draft Sampling Plans was directly related to the Draft Maximum Levels for Total Aflatoxin in Almonds, Hazelnuts and Pistachios forwarded to the Commission for adoption and that the maximum levels could not be adopted without the sampling plans. The Committee recognized the importance of the adoption of these maximum levels in order to protect consumers' health and therefore recommended that the Commission adopt the Proposed Draft Sampling Plan as proposed by the CCCF and forward it to CCMAS for further consideration (ALINORM 08/31/3A, para. 49).

11. The 31st CAC adopted the Aflatoxin Sampling Plans for Almonds, Hazelnuts and Pistachios at Step 5/8 (see paragraph 2 of CX/CF 09/3/2).

30th Session Codex Committee on Methods of Analysis and Sampling[2]

12. The Delegation of the European Community, referring to its written comment in CRD 12 regarding paragraph 10 of the Aflatoxin Sampling Plan for Almonds, Hazelnuts and Pistachio as incorporated in Codex General Standard for Contaminants and Toxins in Foods (GSCTF) (CODEX STAN 193-1995), proposed to replace the text "dry grind with vertical cutter mixer type mill and a 50 g test portion" to "such that each laboratory sample shall be finely ground and mixed thoroughly using a process that has been demonstrated to provide the lowest sample preparation variance." and amend the text for Decision Rule to read "If the aflatoxin test result corrected for recovery is less than or equal to 15ng/g total aflatoxin, taking into account the measurement uncertainty, then accept the lot". Those proposals were also applied to amend the similar texts for Aflatoxin for ready-to-eat treenuts. One error was corrected, replacing RSD_r with RSD_R on the last row of recommended values in Table 2.

13. The Delegation of Iran asked for clarification of the above addition on measurement uncertainty and wondered as to whether this might significantly impact on the Aflatoxin Sampling plan and the maximum levels for aflatoxins in Almonds, Hazelnuts and Pistachio in the GSCTF. The Delegation noted that Annex I of the Aflatoxin Sampling Plan addressed analytical variance only.

[2] ALINORM 09/32/23, paras 9-11

14. After some discussion, the Committee agreed to refer back to the Committee on Contaminants in Foods the consideration of the above proposed amendments and to ask whether the Use of Analytical Results: Sampling Plans, Relationship between the Analytical Results, the Measurement uncertainty, Recovery Factors and Provisions in Codex Standard[3] had been duly taken into account in the Aflatoxin Sampling Plan.

15. The Committee is invited to consider the amendments proposed by CCMAS and to clarify whether the measurement uncertainty recovery factors and provisions in Codex standards had been taken into account in the aflatoxin sampling plan.

[3] Procedural Manual of the Codex Alimentarius Commission.

ANNEX I

PROJECT DOCUMENT NO. 22: PROPOSALS FOR AMENDMENTS ON CODEX STANDARD FOR NATURAL MINERAL WATERS (CODEX STAN 109-1981, REV.1-1997) (Prepared by Kenya)

1. Purpose and Scope of the Standard

The purpose of the amendment is to provide essential guidance relating to food safety essential quality, hygiene, and labelling, for the purpose of protecting the health of the consumer and ensuring fair practices in food trade.

This Standard applies to all packaged natural mineral waters offered for sale as food. It does not apply to natural Mineral waters sold or used for other purposes.

2. Relevance and Timeliness

The Consumption and global trade in packaged natural mineral water has been steadily increasing over the years. This raises concerns over its safety as food. There is therefore an urgent need to identify methods of analysis and sampling, and set maximum limits for the substances referred to in Clause 3.2.17 to 3.2.20 and microbiological maximum limits indicated in Clause 4.4

3. Main Aspects to be amended

During the 8th session of Codex Committee on Natural Mineral waters held in Lugano, Switzerland on 11th -15th February, 2008, the Delegation of Kenya, referring to Footnotes 3 and 4 of the current Standard, drew the attention of the Committee to the fact that in the Standard there was no indication of specific methods of analysis and sampling available for surface active agents, pesticides and Polychlorinated Biphenyls (PCBs), mineral oil and polynuclear aromatic hydrocarbons and this created some problems in practical application of the Standard. The Delegation also proposed to review the section on hygiene as in their view it was not easy to interpret and apply the microbiological specifications as they stand in the Standard.

4. An Assessment against the *Criteria for the Establishment of Work Priorities*

a) Consumer protection from the point of view of health and fraudulent trade practices. Quality of the Natural Mineral water meets consumer needs and minimum requirements on food safety.

b) International and regional Market for packaged Natural Mineral waters has tremendously increased in the recent past.

c) The Standard for Natural Mineral water has successfully been drafted. Available ISO methods of analysis and sampling such as,

ISO 2456:1986 for surface active agents, GLC for Pesticides,

HPLC for PCBs and GC-MS for mineral oil can be adopted to make the Standard implementable for substances identified in clauses 3.2.17 to 3.2.20.

5. Relevance to Codex Strategic Objectives

This amendment proposal is consistent with the Codex Commission strategic Plan 2008-2013 goal 1; Promoting sound regulatory frameworks, page 3.

6. Information on the Relation between the Proposal and other Existing Codex Documents.

The need for this amendment was noted, by the Kenya Delegation during the 8th session of Codex meeting on Natural Mineral waters held in Lugano, Switzerland, Feb 11th – 15th 2008. However, the members felt that the scope of the meeting was limited to the health related substances on Clauses 3.2.1-3.2.16 which had been approved by the Commission in 2007 July, CAC meeting. Therefore the Secretariat of the Codex Committee of Natural Mineral water informed the members who would like to prepare a project document following process and format as described in the Codex procedure Manual 17th Edition Page 21 is free to do so before end of March, 2008.

7. Identification of any Requirements for and Availability of Expert Scientific Advice

Codex Committee on Methods of Analysis and sampling (CCMAS) and Joint Experts on Microbiological Risk Assessment (JEMRA)

8. Identification of any Need for Technical Input to the Standard from External Bodies so that this can be planned for

The technical input of Joint Experts on Microbiological Risk Assessment (JEMRA) to simplify the tables under Clause 4.4 to make them easy for the regulatory authorities to enforce and the exploiters to implement will be needed. An exhaustive list of harmful Microorganisms to be tested in the Natural Mineral water should be developed. Generally, Clause 4.4 should be re-structured and the tables properly labelled and titled to make the information contained there-in meaningful.

9. The Proposed Timeline for Completion of the Amendment Including the Start Date, and Date for Adoption by the Commission

Start date	2008 (June-July)
Proposed date for amendment at step 5/8	2009
Proposed date for adoption by the commission	2009 (June-July)

ANNEX II

DISCUSSION PAPER ON METHODS OF ANALYSIS FOR DIOXINS AND DIOXIN-LIKE PCBS

(Prepared by the electronic working group (Australia, Belgium, Brazil, Estonia, European Community, Finland, Ireland, Japan, Norway, Romania, Republic Korea, Turkmenistan, Uganda, Grocery Manufacturers Association (GMA)) led by Germany with contributions of Belgium, Germany, Japan, Norway, Romania and Republic of Korea.

Methods of Analysis for the determination of dioxins and PCBs

Background

This initiative has its origin in a request of the Codex Committee on Food Additives and Contaminants (CCFAC) which while drafting a Code of Practice for the Prevention and Reduction of Dioxin and Dioxin-like PCB Contamination in Foods and Feed (adopted as in ALINORM 06/29/12 Appendix XXVI by the Codex Alimentarius Commission (ALINORM 06/29/41) expressed the view that although there were no limits in Codex for dioxins, it would be useful to consider the selection of appropriate methods of analysis for dioxins in the Committee taking into account the work underway in different international organisations.

At the 26th session of the Codex Committee on Methods of Analysis and Sampling (CCMAS) in Budapest, Hungary, 4 - 8 April 2005, the Committee decided to inform the Codex Committee on Food Additives and Contaminants (CCFAC) about the status of its work on methods of analysis for dioxins (CX/FAC 06/38/2-Add.1).

The Committee requested the delegation of Germany to revise the paper with the view of converting the already reported methods used for the determination of dioxins and related compounds into criteria. Furthermore all governments and international organisations were again invited to provide information on currently used methods for dioxin analysis to the delegation of Germany before the next session (ALINORM 05/28/23 para. 123).

By its 27th session the Committee had forwarded a request for clarification on the purpose of the methods to the Committee on Contaminants in Food (CCCF)(ALINORM 06/29/3 para. 95).

In reply the CCCF had forwarded to the CCMAS the ranges for the determination of dioxin and PCBs as well as the matrices for which these levels were to be applied and requested the Committee to also indicate for the different methods the highest level that can be reliably analysed (ALINORM 07/30/41, para. 24 and Appendix XIV Part 1)(see tables in Annex 3)

In its 28th session the Committee noted the reply from the CCCF and considered whether the Committee should proceed with the development of methods for dioxins and dioxin-like PCBs or to apply the criteria approach for the determination of dioxins and dioxin-like PCBs. The Committee agreed to a proposal of Germany to discontinue consideration of methods for dioxins under Agenda Item 5b) but to consider this issue under a separate agenda item (Other Business and Future Work). The Committee agreed that the Delegation of Germany would lead an electronic working group open to all members and observers in order to update the document CX/MAS 06/27/8 in the light of the remarks made by CCCF; answer the questions on the applicability of the methods for the indicated ranges and commodities concerned; review the validation data for the methods; and set criteria for dioxin analysis. A discussion paper should be prepared that would be considered as a separate Agenda Item at the next session.

Recommendation

The working group recommends that the 30th session of CCMAS considers this paper and the criteria set therein for methods of analysis for the determination of dioxins and PCBs. The members of CCMAS should discuss the further procedure for establishing these criteria in Codex and whether this paper should be forwarded to CCCF.

Methods used to determine dioxins and related compounds

PCDDs/PCDFs are normally found as complex mixtures in varying composition in different matrices. Their identification and quantification requires a highly sophisticated analysis, because it is necessary to separate the toxic (17 congeners with 2,3,7,8 - chlorine substitution) from the less-toxic congeners. Usually, PCDDs/PCDFs are determined by capillary-GC/MS (gas chromatography / mass spectrometry) methods.

In the past, PCB analyses mainly focused on the determination of total PCBs or indicator congeners (PCBs 28, 52, 101, 138, 153 and 180), which are the predominant PCB congeners found in humans and food stuffs of animal origin). However, the toxicity of these PCB congeners appears to be relatively low. Based on the available toxicological information, the non-ortho PCBs 77, 81, 126 and 169 and the mono-ortho congeners 105, 114, 118, 123, 156, 157, 167 and 189 were assigned a toxic equivalency factor (TEF) by a WHO expert group in 1998 with revision in 2005 and have to be analysed to determine the PCB-TEQ content. Due to their chemical and physical properties mono-ortho PCBs and non-ortho PCBs have to be determined separately from dioxins in most cases.

GC-HRMS

Gas Chromatography combined with High Resolution Mass Spectrometry is currently the only technique able to provide the required sensitivity and selectivity for analysis and detection of dioxins and dioxin-like PCBs in low contaminated food and feedingstuffs. Contrary to the biological screening techniques (that measure the sum of the toxic dioxins in the sample), GC-HRMS allows to separate and detect the individual dioxins that contribute to the sum of toxic dioxins in a sample. The main difference of HRMS compared with low resolution MS is the fact that HRMS has significant more separating power (resolution) to allow separation of the dioxin-borne ions from other interfering ions. In that way HRMS is able to detect dioxins at very low levels without interference from other compounds. To assure reliable detection, generally, quantification is performed by addition of isotope-labelled 13C12 analogues of the individual dioxins which are added to the sample at the beginning of the analytical procedure and detected separately by the HRMS.

GCxGC MS

In environmental analysis complex mixtures like dioxins, PCBs and brominated flame retardants require high separating power to enable the detection of all individual compounds. Conventional single column capillary gas chromatography offers much separation but often suffers from co-eluting compounds or (unknown) interferences.

In comprehensive two-dimensional gas chromatography (GCxGC) two independent separations are applied to an entire sample. The sample is first separated on a normal-bore capillary column under programmed-temperature conditions. The effluent of this column then enters a thermal (or cryo) modulator, which traps each subsequent small portion of eluate, focuses these portions and releases the compounds into a second column for further separation. The second separation is made to be fast enough (e.g. 5 - 10 s) to permit the continual introduction of subsequent, equally small fractions from the first column without mutual interference.

Cell-Based Bioassay

Apart from chromatographic techniques, several cell-based bioassays are available. Although individual congeners cannot be quantified, adequate clean-up enables to distinguish between dioxins and dioxin-like PCBs, so that cell-based bioassays are considered an adequate screening tool for assessing compliance with maximal limits for dioxin-like PCBs and/or PCDDs/PCDFs.

Methods reported by Member Countries (in 2006)

Only two countries provided further information on methods for the detection and identification of dioxins and related compounds which have been used in their countries to control the presence of those chemicals.

The reported methods are summarised in the list of "Methods reported in 2006 by governments and organisations" (Annex 2).

In addition three countries commented to the request to provide methods to identify dioxins and related compounds.

Two of these countries expressed their favour in having method criteria which have to be fulfilled by the procedure (“fit-for-purpose”) instead of individual accepted methods. Countries referred to the European Community and its Commission Regulation (EC) No 1883/2006 of 19 December 2006 laying down the methods of sampling and analysis for the official control of dioxins and the determination of dioxin-like PCBs in certain foodstuffs.

Criteria approach

The criteria defined (Annex 1) below are based on the validation results of several methods for the identification of dioxins and related compounds. It is proposed to take into consideration the criteria laid down in Commission Regulation (EC) No 1883/2006 of 19 December 2006 laying down the methods of sampling and analysis for the official control of dioxins and the determination of dioxin-like PCBs in certain foodstuffs and Commission Directive 2002/70/EC of 26 July 2002 establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feeding stuffs (Official Journal of the European Communities L 209, pages 5-14 and 15-21, 6.8.2002), which are already valid in 27 Codex member states and for which some experience exists.

ANNEX 1: Criteria for Methods for the Determination of Dioxins/Furans and dioxin-like PCBs

1. Criteria for Confirmatory Methods and Screening-Techniques:

Note: Confirmatory methods are usually high-resolution gas chromatography/high resolution mass spectrometry methods.

Note: GC-MS methods of analysis and bioassays may be used for screening.

For cell based bioassays specific requirements are laid down in point 2. and for kit-based bioassays in point 3. Positive results (around the level of interest) have to be confirmed by a confirmatory method of analysis (GC-HRMS).

1.1. Applicability (Matrix and Range):

All foods and feeding stuffs (relevant matrices and ranges (received by CCCF) are listed in Annex 3).

1.2. Selectivity:

A distinction is required for PCDDs, PCDFs and dioxin-like PCBs from a multitude of other, co-extracted and possibly interfering compounds present at concentrations up to several orders of magnitude higher than those of the analytes of interest. 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). 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 twelve dioxin-like PCBs) and other non toxic congeners.

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).

For bioassays, the target compounds, possible interferences and maximum tolerable blank levels should be defined. Bioassays should be able to determine TEQ values selectively as the sum of PCDDs, PCDFs and dioxin-like PCBs.

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. The rate of false positive samples should be low enough to make the use of a screening tool advantageous.

A blank sample has to be included in each test series, which is extracted and tested at the same time under identical conditions. Additionally reference samples have to be analysed regularly. The reference sample must show a clearly elevated response in comparison to a blank. In the case of bioassays extra reference samples 0.5 × and 2 × 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 specific matrices, the suitability of the reference sample(s) has to be demonstrated, preferentially by including samples shown by GC-HRMS to contain a TEQ level around that of the reference sample or else a blank spiked at this level.

1.3. Limits of detection:

For PCDDs and PCDFs, detectable quantities have to be in the low femtogram TEQ (10^{-15} 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.

1.4. Limits of quantification, differences between upperbound and lowerbound level:

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.

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 (2005)/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 should not exceed 40 %.

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.

1.5 Recovery:

Control of recovery is necessary. The recoveries of the individual internal standards should be in the range of 60 % to 120 % for confirmatory methods, in the range of 30% to 140% for GC/MS-screening methods.

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).

Use of internal standards:

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, 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 ^{13}C-labelled 2,3,7,8-substituted internal PCDD/F standards and all 12 ^{13}C-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 ^{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 can be added either before extraction or after fat extraction. The same specifications apply for the analysis of feeding stuff of plant as well as animal origin.

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-HRMS analysis, 1 or 2 recovery (surrogate) standard(s) must be added.

1.6 Accuracy (trueness and precision):

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. The determination should provide a valid estimate of the true concentration in a sample. 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 confirmatory methods the compliance with the criteria listed below should be demonstrated for values around (0.5 x; 1 x; 2 x) the level of interest.

The determination of the accuracy in case of a quantitative screening method 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.

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. For screening methods the coefficient of variation actual false negative rates should fulfil the criteria listed below in a particular matrix at the lowest concentration of the relevant ranges The requirements apply to concentrations of 1 pg/g fat or higher.

	Screening methods	Confirmatory methods
False negative rate	< 1%	
Trueness*		– 20 % to + 20 %
Precision RSD_R*	< 30 %	< 15 %

*on total TEQ value

2. 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× 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.

- 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× 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× the solvent blank) calculated from the calibration curve of the day. The limit of quantification may be set as 5× to 6× 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× the solvent blank) calculated from the calibration curve of the day.

- Information on correspondence between bioassay and GC-HRMS results should be made available.

3. Specific requirements for kit-based bioassays

- 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× 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 response to the standard is within an acceptable range.

Annex 2 Methods reported by governments and organisations[4]

Reported by (Member state)	Applicability (Matrix)	Principle of Detection	Reference	Status of validation
USA	Food	Ion trap	D. G. Hayward et al., Tandem-in-time mass spectrometry method for the sub-parts-per-trillion determination of 2,3,7,8 -chlorine-substituted dibenzo-p-dioxins and -furans in high-fat foods. *Analytical Chemistry* 71 (1):212-220, 1999.	Not validated
USA	Food	Ion trap, HRMS	D. G. Hayward et. al., Quadrupole ion storage tandem mass spectrometry and high-resolution mass spectrometry: complementary application in the measurement of 2,3,7,8 -chlorine substituted dibenzo-p-dioxins and dibenzofurans in US foods. *Chemosphere* 43 (4-7): 407-415, 2001.	Not validated
Germany	Feed	HRMS	Determination of PCDDs, PCDFs and selected coplanar (non-ortho-) PCBs in feeding stuffs *VDLUFA - Collection of methods*, VDLUFA-Verlag Darmstadt, Germany, VDLUFA (1996b) Band VII: Umweltanalytik – Dioxine in Futtermitteln 3.3.2.4	**Validated**
Germany	Soil, Sewage Sludge and Compost	HRMS	Determination of PCDDs, PCDFs and selected coplanar (non-ortho-) PCBs in soil, sewage sludge and compost *VDLUFA - Collection of methods* VDLUFA-Verlag Darmstadt, Germany, VDLUFA (1996b) Band VII: Umweltanalytik – Dioxine in Böden, KS und Komposten 3.3.2.3.	**Validated**
Germany	Food	HRMS	Determination of PCDDs and PCDFs in foods of animal origin P. Fürst, CVUA Münster, Germany	**Validated**
Germany	Food	HRMS	Determination of PCDD/F and dl-PCB in food and feed; E. Bruns-Weller, A. Knoll, LAVES, Lebensmittelinstitut Oldenburg, Germany; R. Malisch, E. Bruns-Weller, A. Knoll, P. Fürst, R.Mayer, T. Wiesmüller: Results of an “emergency quality control study" as confirmation of a PCDD/PCDF-contamination of milk and butter samples. *Chemosphere* 40, 1033 -1040, 2000	**Validated**
Japan	Food/Fish	Cell-based bioassay (CALUX)	Tsutsumi et al., Validation of the CALUX bioassay for screening of PCDD/Fs and dioxin-like PCBs in retail fish, *Analyst* 128, 486-492, 2003	**Validated**
Japan	Food/Fish	HRMS	Tsutsumi et al., Evaluation of an aqueous KOH digestion followed by hexane extraction for analysis of PCDD/Fs and dioxin-like PCBs in retailed fish, *Anal Bioanal Chem*. 375, 792-798, 2003	**Validated**
Romania	Food/Milk/ Cheese		Francesca Santelli, Floriana Boscaino, Determination of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzo-p-furans (PCDFs) and polychlorinated biphenyls (PCBs) in buffalo milk and mozzarella cheese, *European Food Research and Technology* 223 (1), 51-56, 2006	**Not validated**

[4] The list is only provided as information of methods submitted by government and organizations

Annex 3 - Ranges and Matrices for the Determination of Dioxin and PCBs

1. Foodstuff

All levels are expressed in World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

Matrix	**Dioxins and furans**	**Dioxin-like PCBs**	**Sum of dioxins, furans and dioxin-like PCBs**
Meat and meat products (incl. poultry)	0.2 – 12.0 pg/g fat	0.2 – 12.0 pg/g fat	0.3-24.0 pg/g fat
Fish and fishery products (incl. shell fish)	0.3 – 8.0 pg/g fresh weight*	1.0 – 12.0 pg/g fresh weight*	1.0 – 18.0 pg/g fresh weight*
Milk and dairy products	0.5 – 6.0 pg/g fat	0.5 – 6.0 pg/g fat	1.0 – 12.0 pg/g fat
Eggs and egg products	0.5 – 6.0 pg/g fat	0.5 – 6.0 pg/g fat	1.0 – 12.0 pg/g fat
Animal fat	0.2 – 12.0 pg/g fat	0.2 – 12.0 pg/g fat	0.3 – 24.0 pg/g fat
Vegetable oils and fats	0.15 – 1.5 pg/g fat	0.15 – 1.5 pg/g fat	0.3 – 3.0 pg/g fat
Marine oils	0.4 – 4.0 pg/g fat	1.5 – 12.0 pg/g fat	2.0 – 15.0 pg/g fat
Fruits, vegetables, nuts and cereals and derived products	0.1 – 1.0 pg/g fresh weight*	0.1 – 0.5 pg/g fresh weight*	0.2 – 1.5 pg/g fresh weight*
Foods for infants and young children			
- infant formulae	0.2 – 1.5 pg/g fat	0.1 – 1.5 pg/g fat	0.2 – 3.0 pg/g fat
- baby food (meat, egg and dairy based)	0.2 – 2.0 pg/g fat	0.2 – 2.0 pg/g fat	0.3 – 4.0 pg/g fat
- baby food (grain, vegetable, fish based)	0.025 – 0.2 pg/g product*	0.025 – 0.2 pg/g product*	0.05 – 0.4 pg/g product*
Food supplements	0.15 – 4.0 pg/g fat	0.15 – 12.0 pg/g fat	0.3 – 15.0 pg/g fat

* Ranges of levels are expressed on a fresh weight or product basis given the very wide range of fat content that can be observed in the concerned foodstuffs or the very low content of fat in the foodstuff. If results are expressed on a fat/lipid basis, the lower end of the range remains valid but much higher levels than the upper end of the range can be observed.

Comments:

Germany:

1.Foods for infants and young children:

For products with a fat content above 20 % the following concentration ranges mark the lower end of the capacity of analytical measurement with acceptable measurement uncertainty:

• Action levels of about 0.3 pg WHO-PCDD/F-TEQ/g fat or 0.3 pg WHO-PCB TEQ/g fat

• For checking of compliance (MRL): levels of about 0.8 pg WHO-PCDD/F-PCB-TEQ/g fat

2. As the above indicated ranges take into account the actual contamination scenario it should be clear that lower analytical levels should be achievable. For example 0.5 pg WHO-TEQ/g fat for milk is approx. the ubiquitous background contamination for milk and dairy products.

2. Feed material / Feedingstuff

All levels are expressed in World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

Matrix	**Dioxins and furans**	**Dioxin-like PCBs**	**Sum of dioxins, furans and dioxin-like PCBs**
Feed materials of plant origin	0.15 -1.5 pg/g product**	0.15 -1.5 pg/g product**	0.25 -2.5 pg/g product**
Feed materials and additives of mineral origin, trace elements	0.2 – 10 pg/g product**	0.2 – 10 pg/g product**	0.3 – 20 pg/g product**
Animal fat	0.2 – 6 pg/g product**	0.2 – 6 pg/g product**	0.3 – 9 pg/g product**
Feed materials of animal origin other than fat	0.15 –1.5 pg/g product**	0.15 –1.5 pg/g product**	0.25 –2.5 pg/g product**
Fish meal	0.25 – 4 pg/g product**	0.5 – 15 pg/g product**	0.75 – 16 pg/g product**
Fish oil	1– 12 pg/g product/fat	3 – 24 pg/g product/fat	4 – 30 pg/g product/fat
Fish feed / pet food	0.5 – 4.5 pg/g product**	1 – 10 pg/g product**	1 – 10 pg/g product**
Premixtures	0.2 – 10 pg/g product**	0.2 – 10 pg/g product**	0.3 – 20 pg/g product**
Compound feed	0.15 –1.5 pg/g product**	0.15 –1.5 pg/g product**	0.3 – 3 pg/g product**

** Levels are relative to a feedingstuff with a moisture content of 12 %. Ranges of levels are expressed on product basis given the very wide range of fat content that can be observed in feed materials / feedingstuffs or the very low content of fat in the feed materials / feedingstuffs. If results are expressed on a fat/lipid basis, the lower end of the range remains valid but much higher levels than the upper end of the range can be observed.

Annex 4 – Information on Methods for the Determination of Dioxin and PCBs given by the members of the eWG

Reporting member: Germany

Purpose	**Principle**	**False negative rate**	**Trueness***	**Precision RSD_R***	**LOQ for 2,3,7,8 TCDD**	**differences between upperbound and lowerbound level****	**Reference**
		[%]	**[%]**	**[%]**	**[pg/g]**	**[%]**	
Screening (S)			----------				
Confirmation (C)	HRGC/ HRMS (various matrices, see applicability)	----------	+/- 20 %	< 10 %	0.01 – 0.05 (depends on matrices)	15 % difference at 1 pg TEQ/ g fat	Determination of PCDD/F and dl-PCB in food and feed, E. Bruns-Weller, A. Knoll, LAVES, Lebensmittelinstitut Oldenburg, Germany R. Malisch, E. Bruns-Weller, A. Knoll, P. Fürst, R.Mayer, T. Wiesmüller: Results of an "emergency quality control study" as confirmation of a PCDD/PCDF-contamination of milk and butter samples. *Chemosphere* 40, 1033 -1040, 2000
	HRGC/ HRMS (various matrices)		+/- 20 %	< 15 %	See comment	< 20 % difference at 1 pg/g fat	Determination of PCDDs and PCDFs in foods of animal origin P. Fürst, CVUA Münster, Germany

* Levels expressed in World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

**** Indicate also corresponding TEQ , e.g.: 20 % difference at 1 pg TEQ / g fat**

Comments:

Upperbound LOQ for all food and feed matrices regulated in the EU is at least a factor of 10 lower than the maximum level and action level.

Applicabitity				
Matrix	**Principle of validation**	**Validated Range * [pg/g]**		
		Dioxins and furans	**Dioxin-like PCBs**	**Sum of dioxins, furans and dioxin-like PCBs**
Feed material	In house validation, continuous participation in proficiency tests	0.03 pg/g product* (to a feedingstuff with a moisture content of 12 %)	0.01 pg/g product* (to a feedingstuff with a moisture content of 12 %)	0.04 pg/g product* (to a feedingstuff with a moisture content of 12 %)
Meat and meat products (incl. poultry)	In house validation, continuous participation in proficiency tests	0.05 pg/g fat	0.05 pg/g fat	0.10 pg/g fat
Fish and fishery products (incl. shell fish)	In house validation, continuous participation in proficiency tests	0.05 pg/g fresh weight*	0.05 pg/g fresh weight*	0.10 pg/g fresh weight*
Milk and dairy products	In house validation, continuous participation in proficiency tests	0.10 pg/g fat*	0.05 pg/g fat*	0.15 pg/g fat*
Eggs and egg products	In house validation, continuous participation in proficiency tests	0.10 pg/g fat*	0.10 pg/g fat*	0.20 pg/g fat*
Vegetable oils and fats	In house validation, continuous participation in proficiency tests	0.05 pg/g fat*	0.05 pg/g fat*	0.10 pg/g fat*
Marine oils	In house validation, continuous participation in proficiency tests	0.10 pg/g fat*	0.10 pg/g fat*	0.20 pg/g fat*
Fruits, vegetables	In house validation, continuous participation in proficiency tests	0.05 pg/g fresh weight*	0.10 pg/g fresh weight*	0.15 pg/g fresh weight*
Foods for infants and young children	In house validation, continuous participation in proficiency tests	0.020 pg/g product*	0.005 pg/g product*	0.025 pg/g product*

* Levels expressed in World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

Comments:

Only the lowest validated level is given

Annex 4 – Information on Methods for the Determination of Dioxin and PCBs given by the members of the eWG

Reporting member: Romania							
Purpose	**Principle**	**False negative rate** [%]	**Trueness*** [%]	**Precision RSD_R*** [%]	**LOQ** [pg/g]**	**differences between upperbound and lowerbound level***** [%]	**Reference**
Screening (S)	***GC-HRMS***		----------		10^{-9}		Francesca Santelli, Floriana Boscaino, Determination of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzo-p-furans (PCDFs) and polychlorinated biphenyls (PCBs) in buffalo milk and mozzarella cheese , *European Food Research and Technology* 223 (1), 51-56, 2006
Confirmation (C)		--------					

* Levels expressed in World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

**** Indicate LOQ for 2,3,7,8 TCDD in pg/g**

***** Indicate also corresponding TEQ , e.g.: 20 % difference at 1 pg TEQ / g fat**

Comments:

Applicabitity

Matrix	**Principle of validation**	**Range *** [pg/g]		
		Dioxins and furans	**Dioxin-like PCBs**	**Sum of dioxins, furans and dioxin-like PCBs**
Milk and dairy products		0.5 – 6.0	0.5 – 6.0	1.0 – 12.0

* Levels expressed in World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

Annex 4 – Information on Methods for the Determination of Dioxin and PCBs given by the members of the eWG

Reporting member: JAPAN							
Purpose	**Principle**	**False negative rate [%]**	**Trueness* [%]**	**Precision RSD_R***	**LOQ [pg/g]****	**differences between upperbound and lowerbound level [%]**	**Reference**
Screening (S)	Cell-based bioassay (CALUX)	No available data	----------	25.0–36.4% (2.3–3.9 pg TEQ/g fresh weight)	0.16	Not defined	Tsutsumi et al., *Analyst*, 2003, 128, 486-492.

Purpose	**Principle**	**False negative rate [%]**	**Trueness* [%]**	**Precision RSD_R***	**LOQ [pg/g]****	**differences between upperbound and lowerbound level [%]**	**Reference**
Confirmation: Fish (analysis of certified reference material)	HRGC/HRMS	----------	-21 – +16% for certified isomers in CRM (CARP-1, National Research Council Canada)	0.8–6.9% for certified isomers in CRM (CARP-1, National Research Council Canada)	Approx. 0.01	Approx. 5% difference in fish samples containing 1.4-1.6 pg TEQ/g fat (based on PCDD/Fs only)	Tsutsumi et al., *Anal Bioanal Chem.*, 2003, 375, 792-798.

* Levels expressed in World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

**** LOQ for 2,3,7,8 TCDD in pg/g**

Comments:

Fish (analysis of certified reference material):

The values in the trueness and precision columns for the HRGC/HRMS row were calculated for individual certified isomer concentrations in the certified reference fish material, CARP-1 (not expressed based on total TEQ concentrations).

Applicability

The concentrations were calculated following lower bound level concept. The detailed information is described in the manuscripts sited below.

Amakura et al., *J. Food Hyg. Soc. Japan*, 2002, 43, 312-321.

Tsutsumi et al., *Analyst*, 2003, 128, 486-492.

Amakura et al., *J. Food Hyg. Soc. Japan*, 2005, 46, 148-152.

Tsutsumi et al., *J. Food Hyg. Soc. Japan*, 2007, 48, 8-12.

Tsutsumi et al., *Organohalogen Compounds*, 2007, 69, 2371-2374.

Matrix	**Principle of validation**	**Principle**	**Range * [pg/g]**		
			Dioxins and furans	Dioxin-like PCBs	Sum of dioxins, furans and dioxin-like PCBs
Fish		Cell-based bioassay (CALUX)	0–1.7 (fresh weight) †	0.011–7.2 (fresh weight) †	0.011–8.9 (fresh weight) †
Fish and fishery products		HRGC/HRMS			0.024-14 (fresh weight) †
Fish, meat, fruits, vegetables, cereals, milk, dairy products, eggs, fats		HRGC/HRMS			0-1.5 (fresh weight) †
Baby food		HRGC/HRMS			<0.0010–0.14 (product) †
Food supplements (Fish oil products)		HRGC/HRMS	<0.10–37(product) †	<0.10–450(product) †	<0.10–480 (product) †

†Levels expressed in the World Health Organization (WHO) toxic equivalent using the 1998 WHO-TEFs

Applicabitity

The concentrations were calculated following lower bound level concept. The detailed information is described in the manuscripts sited below.

Ministry of Agriculture, Forestry and Fisheries: Dioxins and PCBs surveillance of animal products and fishery commodities in FY 2005 (in Japanese).
(http://www.maff.go.jp/j/press/2006/20061027press_2.html, http://www.maff.go.jp/j/press/syouan/tikusui/080125.html)

Ministry of Agriculture, Forestry and Fisheries: Dioxins and PCBs surveillance of animal products and fishery commodities in FY2006 (in Japanese).
(http://www.maff.go.jp/j/press/2006/20061027press_2.html)

Matrix	**Principle of validation**	**Principle**	**Range * [pg/g]**		
			Dioxins and furans	Dioxin-like PCBs	Sum of dioxins, furans and dioxin-like PCBs
Fish		HRGC/HRMS	0-14(fresh weight)	0-16 (fresh weight)	0-19 (fresh weight)
Shellfish		HRGC/HRMS	0-0.43 (fresh weight)	0-0.33 (fresh weight)	0-0.62 (fresh weight)
Prawn,Shrimp, Crab		HRGC/HRMS	0-0.46 (fresh weight)	0-0.69 (fresh weight)	0-1.1 (fresh weight)
Cuttlefish, Others		HRGC/HRMS	0-1.1(fresh weight)	0-0.95 (fresh weight)	0-2.0 (fresh weight)
Cereals		HRGC/HRMS	<0.003-0.0071 (fresh weight)	<0.0004-0.011 (fresh weight)	<0.003-0.013 (fresh weight)
Soya bean		HRGC/HRMS	<0.003-0.0048 (fresh weight)	<0.0004 (fresh weight)	<0.003-0.0048 (fresh weight)
Vegetables		HRGC/HRMS	<0.003-0.087 (fresh weight)	<0.0004-0.015 (fresh weight)	<0.003-0.087 (fresh weight)
Fruits		HRGC/HRMS	<0.003-0.038 (fresh weight)	<0.0004-0.0095 (fresh weight)	<0.003-0.047 (fresh weight)
Milk		HRGC/HRMS	0-0.023 (fresh weight)	0-0.010 (fresh weight)	0-0.023 (fresh weight)
Meat		HRGC/HRMS	0-1.3 (fresh weight)	0-0.30 (fresh weight)	0-1.5 (fresh weight)
Meat (poultry)		HRGC/HRMS	0-0.082 (fresh weight)	0-0.15 (fresh weight)	0-0.23 (fresh weight)
Milk products (cheese)		HRGC/HRMS	0-0.10 (fresh weight)	0-0.098 (fresh weight)	0-0.15 (fresh weight)
Egg (incl. dried egg yolk, dried egg white)		HRGC/HRMS	0-3.9 (fresh weight)	0-0.28 (fresh weight)	0-4.2 (fresh weight)

* Levels expressed in the World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

Annex 4 – Information on Methods for the Determination of Dioxin and PCBs given by the members of the eWG

Reporting member:

BELGIUM

Purpose	**Principle**	**False negative rate** **Dioxins/furanes**	**False negative rate** **Sum dio/fur Co-PCBs** **[%]**	**Trueness*** **[%]**	**Precision RSD_R*** **Dioxins/furans**	**Precision RSD_R*** **Co-PCBs**	**LOQ** **[pg/g]****	**differences between upperbound and lowerbound level***** **[%]**	**Reference**
Screening (S)	CALUX Eggs Milk Fish Meat feed	 0 0 0 0 0	 0 0 1.69 0 0	----------	 0.3 0.3 0.4 0.16 0.1	 0.3 0.3 0.4 0.3 0.07	 0.5 1 0.78 0.5 0.25		 Validated in house Accreditated lab Feed: TEQ–value determination of animal feed; emphasis on CALUX bioassay validation [Vonderperren *et. al*., Talanta 63 (2004) 1227 – 1280]
Confirmation (C)			----------						

* Levels expressed in World Health Organization (WHO) toxic equivalent using the 2005 WHO-TEFs (toxic equivalency factors)

**** Indicate LOQ for 2,3,7,8 TCDD in pg/g**

***** Indicate also corresponding TEQ , e.g.: 20 % difference at 1 pg TEQ / g fat**