

comisión del codex alimentarius



ORGANIZACIÓN DE LAS NACIONES
UNIDAS PARA LA AGRICULTURA
Y LA ALIMENTACIÓN

ORGANIZACIÓN
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Tema 3 del programa

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PROGRAMA CONJUNTO FAO/OMS SOBRE NORMAS ALIMENTARIAS COMITÉ DEL CODEX SOBRE CONTAMINANTES DE LOS ALIMENTOS 1ª reunión

Beijing (China), 16 - 20 de abril de 2007

CUESTIONES REMITIDAS AL COMITÉ POR LA COMISIÓN DEL CODEX ALIMENTARIUS Y OTROS COMITÉS Y GRUPOS DE ACCIÓN DEL CODEX

A. CUESTIONES FORMULADAS EN LA 29ª REUNIÓN DE LA COMISIÓN DEL CODEX ALIMENTARIUS (Ginebra. Suiza, 2006)

Cuestiones prácticas

Mandato del Comité del Codex sobre Contaminantes de los Alimentos

1. La Comisión adoptó el mandato para el Comité del Codex sobre Contaminantes de los Alimentos. La Comisión acordó que el Comité deberá examinar su mandato en su primera reunión.¹
2. El Comité, por lo tanto, queda invitado a examinar su mandato (expuesto a continuación) e informar a la Comisión si está satisfecho con el mismo, así como las observaciones que considere pertinentes.
 - (a) establecer o ratificar límites máximos permitidos o límites de referencia para los contaminantes y las sustancias tóxicas presentes de forma natural en alimentos y piensos;
 - (b) preparar listas de prioridades de contaminantes y sustancias tóxicas naturales para que el Comité Mixto FAO/OMS de Expertos en Aditivos Alimentarios evalúe sus riesgos;
 - (c) examinar métodos de análisis y muestreo para determinar la presencia de contaminantes y sustancias tóxicas naturales en los alimentos y piensos;
 - (d) examinar y elaborar normas o códigos de prácticas para cuestiones afines; y
 - (e) examinar toda cuestión relativa a los contaminantes y sustancias tóxicas naturales en los alimentos y piensos que le encargue la Comisión.

¹ ALINORM 06/29/41, párr. 26 y Apéndice III

Cuestiones informativas

Aprobación de nuevos trabajos para la elaboración de nuevas normas y textos afines

3. La Comisión aprobó los siguientes temas como nuevos trabajos para el Comité:²
- Revisión del preámbulo de la Norma general del Codex para los contaminantes y las toxinas presentes en los alimentos (Código de trabajo N04-2006);
 - Anteproyecto de Código de prácticas para la prevención y reducción de la contaminación por ocratoxina A en el vino (Código de trabajo N05-2006);
 - Anteproyecto de Código de prácticas para la reducción de la acrilamida en los alimentos (Código de trabajo N06-2006); y
 - Anteproyecto de Código de prácticas para la reducción de la contaminación de los alimentos con HAP de los procesos de ahumado y secado directo (Código de trabajo N07-2006).

Aprobación de proyectos de normas y textos afines en el trámite 8 o en el trámite 5/8

4. La Comisión aprobó los siguientes límites máximos y otros textos en el trámite 8 o n el trámite 5/8:³
- Límites máximos para el plomo en el pescado;
 - Límites máximos para el cadmio en los moluscos marinos bivalvos (excluidas las ostras y vieiras), los cefalópodos (sin vísceras) y el arroz pulido;
 - Apéndice al Código de prácticas del Codex para la prevención y la reducción de la contaminación de nueces de árbol por aflatoxinas. Medidas suplementarias para prevenir y reducir la presencia de aflatoxinas en las nueces del Brasil;
 - Código de prácticas para prevenir y reducir la contaminación en alimentos y piensos por dioxinas y bifenilos policlorados (BPC) análogos a las dioxinas;
 - Revisión de los límites de referencia del Codex para radionucleidos en alimentos objeto de comercio internacional contaminados después de una emergencia nuclear o radiológica; y
 - Norma general del Codex para los contaminantes y las toxinas presentes en los alimentos, incluida la lista I.

Aprobación de proyectos de normas y textos afines en el trámite 5

5. La Comisión aprobó los proyectos de límites máximos que figuran a continuación en el trámite 5 y los adelantó al trámite 6:⁴
- Proyecto de límites máximos para las aflatoxinas totales en las almendras, las avellanas y los pistachos "listos para el consumo"; y
 - Proyecto de límites máximos para el estaño en alimentos enlatados (distintos de las bebidas) y en bebidas enlatadas.

Revocación de normas y textos afines

6. La Comisión aceptó la revocación del siguiente texto:⁵
- Lista de niveles máximos individuales y niveles de orientación del Codex para contaminantes y toxinas.

² ALINORM 06/29/41 párrs. 124-127 y Apéndice VIII

³ ALINORM 06/29/41 párr. 36 y Apéndice IV

⁴ ALINORM 06/29/41 párr. 97 y Apéndice V

⁵ ALINORM 06/29/41 párr. 120 y Apéndice VII

Consulta de expertos FAO/OMS sobre los riesgos para la salud asociados con el metilmercurio, las dioxinas y los BPC análogos a las dioxinas en el pescado, y beneficios para la salud del consumo de pescado

7. La Comisión acordó pedir a la FAO y la OMS que se considerara la posibilidad de convocar una consulta de expertos sobre los riesgos para la salud asociados al consumo de pescado y otros productos marinos y sobre los beneficios para la salud asociados al consumo de pescado y otros productos marinos, con el mandato detallado propuesto por el CCFAC.⁶

B. CUESTIONES FORMULADAS POR OTROS COMITÉS Y GRUPOS DE ACCIÓN DEL CODEX**Cuestiones prácticas****Métodos de análisis para la determinación de las dioxinas y los BCP⁷**

8. El Comité del Codex sobre Métodos de Análisis y Toma de Muestras (CCMAS), en su 27ª reunión (mayo de 2006) acordó pedir al Comité del Codex sobre Contaminantes en los Alimentos (CCCF) que proporcionara información precisa sobre el intervalo de los límites que debían examinarse, así como las matrices a las cuales se aplican esos límites. Y el CCMAS acordó remitir el documento sobre los Métodos de análisis para la determinación de las dioxinas y los BPC a fin de proporcionar información, y que el nuevo trabajo sobre este asunto sólo se reanudaría de acuerdo a la respuesta del CCCF.

9. Por lo tanto, se pide al Comité que examine el documento de los Métodos de análisis para la determinación de las dioxinas y los BCP,⁸ que figura en el anexo de este documento, en respuesta a la petición arriba formulada.

Asuntos informativos

10. No presentaron otra información los comités ni los grupos de acción del Codex.

⁶ ALINORM 06/29/41 párr. 195, ALINORM 06/29/12 párr. 191

⁷ ALINORM 06/29/23 párrs. 10-11 y 92-97

⁸ CX/MAS 06/27/8 (sólo en inglés)

METHODS OF ANALYSIS FOR THE DETERMINATION OF DIOXINS AND PCBs

Background

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 next session (ALINORM 05/28/23 para 123).

This initiative has its origin in a request of the Codex Committee on Food Additives and Contaminants (CCFAC) concerning information on methods of analysis for dioxins. CCFAC is currently drafting a Code of Practice for the Prevention and Reduction of Dioxin and Dioxin-like PCB Contamination in Foods and Feed which was considered (at Step 3) at the 38th session in The Hague, the Netherlands, 24-28 April 2006.

Some delegations in CCFAC expressed the view that although there were no limits in Codex for dioxins, it would be useful to consider the selection of appropriate methods in the Committee, taking into account the work underway in different international organisations.

A lot of work has been done by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (www.who.int).

Due to the heterogeneity in analytical approach JECFA and the European Community do not consider a standardisation of those methods.

Up-to-date there exists no official internationally agreed method for the determination of dioxins and related compound.

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 marker 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 1997 and have to be analysed to determine the PCB-TEQ content. Data for these dioxin-like PCB congeners are still scarce. Due to their chemical and physical properties mono-ortho PCBs and non-ortho PCBs have to be determined separately in most cases. Reliable determinations of non-ortho PCBs in food have been performed by high-resolution MS, as collaborative studies demonstrate.

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. 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 $^{13}\text{C}_{12}$ analogues of the individual dioxins which are added to the sample before analysis and detected separately by the HRMS.

GCxGC

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.

Methods reported by Member Countries

Only two countries have 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 have been summarised in the annexed list of "Methods Reported by Member Countries".

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 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.

The list in Annex 2 is organised as follows:

Each method is referred to the country reporting it.

For each method a general scope is mentioned if indicated by the notifier (column 2).

The principle of the method used is indicated in column 3.

If available a reference is given in column 4.

Information on the validation status is given in column 5.

Criteria approach

The criteria defined (Annex 1) below are based on the validation results of only two methods for the identification of dioxins and related compounds. Due to the lack of sufficient validation data for such methods it is proposed to take into consideration the criteria laid down in 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 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 25 Codex member states and for which some experience exists.

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

1. Requirements for Confirmatory Methods:

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

1.1. Applicability:

All foods and feeding stuffs

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 dioxin-like PCBs) and other 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).

1.3. 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.

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 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 B⁹.

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 %.

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. 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

⁹ Validation data for EPA 1613: Telliard, William A., McCarty, Harry B., and Riddick, Lynn S. "Results of the Interlaboratory Validation Study of US EPA Method 1613 for the Analysis of Tetra- through Octachlorinated Dioxins and Furans by Isotope Dilution GC/MS," *Chemosphere*, 27, 41-46 (1993).

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 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. 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/MS analysis, 1 or 2 recovery (surrogate) standard(s) must be added.

1.6 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 (RSD_R, relative standard deviation calculated from results generated under reproducibility conditions). Following criteria have to be complied with on total TEQ value:

	Confirmatory methods
Trueness	- 20 % to + 20 %
Precision RSD _R	< 15 %

2. Requirements for Screening-Techniques:

Note: GC/MS methods of analysis and bioassays may be used for screening. For cell based bioassays specific requirements are laid down in point 2.5 and for kit-based bioassays in point 2.6. Positive results have always to be confirmed by a confirmatory method of analysis (HRGC/HRMS).

2.1 Applicability:

All foods and feeding stuffs

2.2. Selectivity:

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 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 specific 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.

2.3 Recovery (GC/MS methods):

For screening methods, the recoveries of the internal standards should be in the range of 30% to 140%. For proper use of internal standards refer to 1.5.

2.4. Accuracy in case of a quantitative screening:

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.

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 %. Actual false negative rates should be under 1%.

	Screening methods
False negative rate	< 1%
Precision RSD _R	< 30 %

2.5 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.

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

2.6 Specific requirements for kit-based bioassays¹⁰

- 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 response to the standard is within an acceptable range.

¹⁰ No evidence has 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 feeding stuffs.

ANNEX2:Methods reported by governments and organisations

<u>Member state</u>	<u>Applicability</u>	<u>Principle</u>	<u>Reference</u>	<u>Comment</u>
USA	Food	Ion trap	D. G. Hayward, K. Hooper, and D. Andrzejewski. Tandem-in-time mass spectrometry method for the sub-parts-per-trillion determination of 2,3,7,8-chlorine-substituted dibenro-p-dioxins and -furans in high-fat foods. <i>Analytical Chemistry</i> 71 (1):212-220, 1999.	Not validated
USA	Food	Ion trap HRMS	D. G. Hayward, J. Holcomb, R. Glidden, P. Wilson, M. Harris, and V. Spencer. 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. <i>Chemosphere</i> 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