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Agenda Item 8

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Twenty-sixth Session

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METHODS OF ANALYSIS FOR THE DETERMINATION OF DIOXINS AND PCBS¹

Methods for the determination of dioxins and related compounds

On the 25th session of the Codex Committee on methods of Analysis and Sampling (CCMAS) in Budapest, Hungary, 8 – 12 March 2004, Governments and international organisations were invited to provide proposals and information on the current methods used for the determination of dioxins and related compounds to the delegation of Germany before 15 September 2004.

This initiative has its origin in a request of CCFAC concerning information on methods of analysis for dioxins. 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.

At present a European research project is in progress to improve technologies to determine dioxins (www.dioxins.nl). Within the project several methods will be validated through a collaborative study. However the final report including the validation data of these methods are not yet available.

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.

Dioxins

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are tricyclic aromatic compounds with similar physical, chemical and structural properties. In simplified terms congeners or derivatives of both classes are called "dioxins", although they are differentiated chemically as dioxins or furans. PCDDs include 75 individual compounds and PCDFs 135 different compounds. Figure 3.1a shows the chemical structures. From these 210 theoretically possible congeners, a subset of 17 congeners has chlorine substitutions in the 2, 3, 7, and 8 positions.

The toxicologically most potent dioxin is 2,3,7,8-tetrachlorodibenzodioxin (TCDD).

Because of their chemical stability and high lipophilicity, PCDDs and PCDFs are primarily associated with particulate and organic matter in environmental samples and accumulate in the food chain.

Polychlorinated biphenyls (PCBs) are manufactured as technical products and are liquids with different viscosity. These liquids are mixtures consisting of dozens of different compounds („congeners“) depending on the degree of chlorination.

¹ Prepared by Germany

From a chemical and toxicological point of view, PCBs can be divided into three groups: without chlorine substitution in ortho-position (non-ortho PCBs, „coplanar PCBs“), with one chlorine in ortho-position (mono-ortho PCBs) and di-ortho substituted congeners. The WHO consultation group (WHO 2000) included in the set of dioxin-like compounds 12 of the 209 PCBs. These 12 PCBs considered to share dioxin-like properties are those that have either one or no chlorine substitutions in the ortho positions.

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 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. The 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 (Official Journal of the European Communities L 209, pages 5-14, 6.8.2002).

Due to the lack of feedback from member states, neither a comprehensive register containing information on methods for the determination of dioxins and related compounds in foods can be set up) nor is it possible to assess the usefulness and potential merit of such a register at present.

The list 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 described in columns 5.

Methods reported by governments and organisations

<u>Member state</u>	<u>scope</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 stuffsVDLUFA - 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

References

1. R. Malisch. Evaluation of dioxins and dioxin-like PCBs in the food chain - Part I: Overview of actual assessments. *Deutsche Lebensmittel-Rundschau* 97 (4):129-136, 2001.
2. R. Malisch, E. Bruns-Weller, A. Knoll, P. Furst, R. Mayer, and T. Wiesmuller. Results of an "emergency quality control study" as confirmation of a PCDD/PCDF-contamination of milk and butter samples. *Chemosphere* 40 (9-11):1033-1040, 2000.
3. R. Malisch. Increase of the PCDD/F-contamination of milk, butter and meat samples by use of contaminated citrus pulp. *Chemosphere* 40 (9-11):1041-1053, 2000.