HARMONISED QUALITY CRITERIA FOR CHEMICAL AND BIOASSAYS ANALYSES OF PCDDs/PCDFs IN FEED AND FOOD

PART 1: GENERAL CONSIDERATIONS, GC/MS METHODS

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1 Introduction

Harmonisation of general acceptance criteria for dioxin analyses in feed and food samples is needed. In contrast to methods for determination of food additives, residues (pesticides, drugs used in veterinary medicine) or contaminants, there are no standardized or harmonized methods ("official methods") for determination of dioxins or dioxin-like PCBs in food. There is a different approach for these contaminants: Every lab can use its own method, with the understanding that the lab has to demonstrate that the applied method is fit for the purpose. There is a wide variation in methodology: determination by GC/MS with high resolution, low resolution or tandem mass spectrometry, or by different bioassays. Every determination method is combined with different extraction and clean up procedures. Therefore, there is an uncertainty regarding quality assurance and control across laboratories, and harmonization of acceptance criteria for dioxin analyses is needed to allow free trade if tolerances are developed. These principles should be valid for GC/MS methods as well as for bioassays. Therefore, in two parts quality criteria (QCs) will be presented: in part 1 for methods applying GC/MS determination, in part 2 for bioassays.

2 General considerations

2.1 Upperbound concentrations, lower bound concentrations

For comparison of analytical results to regulatory limits and in general to results from other laboratories, the limit of detection (lowest limit for qualitative identification) and/or limit of determination (lowest limit for quantification) have to be taken into account. For PCDDs/PCDFs analysis, all 17 congeners with 2,3,7,8-substitution have to be determined. For calculation of the TEQ value, the results of each of these congeners is multiplied by the specific TEF factor and then added up. In most cases, a few of the 17 congeners are below the limit of detection and/or limit of determination. This can become critical if many congeners are not determinable or if the toxicologically relevant congeners are not found.

There are different imputation approaches to handle non-detects (1):

1) calculate the contribution of each non-detected congener to the TEQ as zero (lower bound concentrations)
2) calculate the contribution of each non-detected congener to the TEQ as the limit of detection / limit of determination (upper bound concentrations)
3) calculate the contribution of each non-detected congener to the TEQ as half of the limit of detection / limit of determination
4) replacement of a non-detect in a data set by the minimum of usual contribution to the TEQ and LOD
5) multiple imputation with censoring of data

If the contribution of non-detected congeners to the TEQ is calculated as "0", low dioxin contents can be the result of really low levels of the sample or of insufficient detection/determination limits, without considering these detection/ determination limits in the final TEQ calculation. To make sure that low dioxin levels are really the result of low levels in the sample, the concept of tolerances "as upperbound concentrations" was developed. This concept demands the inclusion of the full limit of detection or determination instead of "zero" for not detectable substances: Upperbound concentrations are calculated assuming that all values of the different congeners less than the limit of detection/determination are equal to the limit of detection/determination.

When the limits of determination are high for the decisive congeners and the concept of "upperbound limit of determination" is applied, it results in high numbers of TEQ. This has to be considered for the definition of background contamination, control of tolerances or intake estimates. Especially the use of low resolution mass spectrometers in food analyses or a low weight-in quantity of a sample (for a quick and easy analyses) can cause relatively high values of dioxin contents as upper bound limits of determination. This cannot be seen from a reported TEQ level without knowledge about the results of the individual congeners. For methods with insufficient sensitivity the factor for differences between lower bound and upper bound concentrations can be in the range of 10 to 100, in extreme cases even higher. Thus, for definition of a background contamination or evaluation of exposure, published data must be reviewed critically to avoid that relatively high values are included which are only the result of insufficient detection limits.

For setting and control of tolerances on TEQ basis, the proximity of the level of determination to the appropriate tolerance must be evaluated as part of the decision to accept or reject a food or feedingstuff. High levels of determination relative to the tolerance (see section 3) should lead to the

rejection of a sample analysis result on the basis of poor quality assurance and consequent poor reliability of the estimate of TEQ. As an alternative, some governments may choose to apply upperbound estimates of TEQ, with a preference of the upperbound limit of determination rather than upperbound limit of detection, as a screening approach to remove questionable samples from the marketplace. In the absence of these steps, there is a risk that foods exceeding a maximum level would reach consumers due to insufficient sensitivity. It is the responsibility of laboratories to achieve the required sensitivity to avoid unnecessary rejection of analysis results of foods.

For risk assessment, the application of the upperbound concentrations may lead to an overestimation of the intake, the application of the lowerbound concentrations to an underestimation of the intake. For these purposes, the imputation of half the detection limit yields an acceptable estimate of both the TEQ and its associated standard deviation of uncertainty (see lit. 1).

As a result, it is recommendable for the future that labs report their results as lower bound, upper bound and half detection limit. Then, every information is available to interpret the results according to specific requirements. As minimum requirement, it must be clear from a report which concept was applied.

2.2 Required sensitivity and analytical approaches (HRMS, TMS, bioassays)

Whereas many environmental samples (as soil or sewage sludge) can be analyzed with low-resolution mass spectrometry, measurements of feed, food and human milk or tissue samples have to be performed at ultra-trace levels (usual range 0.1 to 1 pg I-TEQ/g fat in milk, meat and eggs from caged chicken; mean concentrations of 10 pg I-TEQ/g fat in wild fish and farmed freshwater fish, up to 100 pg I-TEQ/g fat and more only in cases of (highly) elevated levels; for food of vegetable origin range 0.1 to 0.5 pg WHO-TEQ/g d.m.). Due to a wide range of fat content of various foods of animal origin, a wide range of PCDD/PCDF contents calculated on fresh weight basis is observed. Therefore, lipid adjusted TEQs are used for reporting dioxin TEQs in animal foods. This helps provide a uniform basis for setting tolerances. For fish it is recommendable to report dioxin results based on the fat content and on fresh weight basis, due to an extremely wide range of the fat content of different sorts of fish.

For reliable analyses of food samples in the range of the normal background contamination, the application of high-resolution mass spectrometry (HRMS) has proved to provide the required sensitivity and specificity. In numerous collaborative studies for determination of PCDDs/PCDFs in different sorts of food, laboratories using HRMS could participate successfully.

The required specificity could be provided by tandem mass spectrometry (TMS; also called MS/MS or MS^2), as well. While MS/MS with sector or quadrupole instruments needs a series of mass analyzers in space, ion traps use one mass analyzer to perform MS/MS in time. As the techniques to achieve MS/MS depend on the type of the mass analyzer employed, a variety of instrument designs has been developed. The advantage of Ion Trap-TMS systems is the much lower price which could reduce analyses costs. However, sensitivity of Ion Trap-TMS instruments is considerably lower than of HRMS instruments: The limit of determination for Ion Trap-TMS systems for TCDD (signal/noise 3/1) can be assumed to be in the range of about 100 to 300 fg, whereas modern HRMS instruments have a LOD of about 3 fg. The lack of sensitivity can be compensated up to a certain degree by much higher sample amounts for extraction and clean up. However, the need to use about 10-fold (or more) higher sample amounts causes many problems

for the availability of sample material and the analytical procedure. Therefore, Ion Trap-TMS could be used as screening method to select elevated levels (like bioassays). However, (unlike bioassays) this screening method gives the possibility to look at congener patterns.

3 Conclusions and recommendations

Generally, the purpose of a method has to be defined clearly (matrix to be analysed, clean-up method, content to be determined reliably, possible limitations). Then, it has to be shown that the method is fit for the purpose by validation and demonstration of basic minimum statistical requirements. Last but not least the methods should prove its successful applicability in collaborative studies.

General statistical parameters were established in other fields of residue analyses and could give an orientation. For example, to allow certification (in national or international commerce) relative to tolerances, laboratories should be able to meet basic requirements as:

- Demonstration of the performance of a method in the range of the tolerance, e.g. 0.5x, 1x and 2x the tolerance with an acceptable coefficient of variation for repeated analyses in the range of interest.
- Limit of detection should be in the range of at least one fifth of the tolerance, to make sure that acceptable coefficients of variations are met in the range of the tolerance.
- Continuous blank controls and spiking experiments or analyses of control samples (preferably, if available, certified reference material) should be performed as internal quality assurance measures.
- Successful participation in interlaboratory studies or proficiency tests are the best way to prove the competence in specific analyses.
- Whenever possible, laboratories that supply analytical data should be accredited by a recognized body to ensure that they are applying analytical quality assurance. As an example, laboratories could be accredited following ISO 17025, supplemented by standard operating procedures and controlled by quality control managers following the principles of OECD for Good Laboratory Practice.

Legislative measures for dioxins in food can comprise "maximum limits", "action levels" and "target levels". Maximum limits can be set at a strict but feasible level in order to discard unacceptably highly contaminated products. Action levels can be set on a lower level to detect increased levels for monitoring. Target levels could be set at a level which would result in an ultimate dietary human weekly exposure below the lower range as recommended by WHO. Some governments have started a discussion of such levels with a factor between maximum limits and action levels being in the range of about 1.5 and between maximum levels and target levels in the range of 5.

If legislative measures are based on these three different levels, analysis for certificates must meet the requirements to check the dioxin content reliably in the range of maximum levels and action levels. For evaluation of exposure and time trends, analysis must be orientated in the target levels.

Specific requirements for dioxin analyses would be:

- The accuracy of the TEQ-based result (closeness of the mean of repeated analyses to the "true" value, determined by reference methods) should be in the range of about +/- 20 % for maximum limits or action levels and of +/- 30 % for target values.
- So far, laboratories applying high resolution GC/high resolution MS methods have shown that a reliable determination of all 17 PCDDs/PCDFs congeners with 2,3,7,8-substitution is possible even in ultra trace levels of below 1 pg WHO-TEQ/g fat (only PCDD/PCDF included). However, the successful participation in intercalibration studies for e.g. soil or sewage samples does not necessarily prove the competence also in the field of food samples with its lower contamination range. Therefore, the continuous participation in interlaboratory studies for determination of dioxins and dioxin-like PCBs in the relevant food matrices is mandatory.

As long as no target values are fixed, for reliable determination in the range of the usual background contamination, the following requirements should be met:

- Sensitivity: For food of vegetable origin in general, a limit of determination of approximately 0.1 pg WHO-TEQ/g dry matter seems to be appropriate to differentiate reliably between samples with elevated dioxin levels and background contamination. However, to follow time trends of background contamination in those matrices, the limit of determination should be at least a factor of 10 lower. For products of land origin and for fish and fish products, a limit of determination of about 0.2 pg WHO-TEQ/g fat (as upperbound limit of determination) seems to be a "marginally acceptable level" of determination and appropriate to differentiate between samples with elevated dioxin levels and background contamination. As the actual background contamination e.g. for milk products or pork meat in some countries is in the range of 0.1 to 0.3 pg WHO-TEQ/g fat, for exact determination and time trends a fully acceptable limit of determination should be below 0.1 pg WHO-TEQ/g fat. These TEQ values should result from congener patterns as found usually in food.
- The difference between upperbound limit of determination and lower bound limit of determination should not exceed the range of 10 to 20 % for food of animal origin with a dioxin contamination of about 1 pg WHO-TEQ/g fat (only PCDD/PCDF included). This requirement should be met for products as butter, beef, cheese or not defatted milk products, whereas for products as skinned fish fillets with a low fat content similar requirements on fresh weight basis can be derived.

Special requirements for GC/MS methods are:

- Recovery control is necessary by addition of ^{13}C-labelled 2,3,7,8-chlorine substituted internal PCDD/F standards. At least one of these congeners for each of the tetra to octa chlorinated homologues groups must be added, with a clear preference of using all 17 ^{13}C-labelled 2,3,7,8-

substituted internal standards. Relative response factors should be determined for those congeners for which no ^{13}C-labelled analogue is added.

- For vegetable food, the addition of the internal standards is mandatory prior to extraction. For food of animal origin, the internal standards can be added either before extraction or after fat extraction, if complete extraction of fat can be demonstrated.
- Prior to GC/MS analysis, a recovery standard must be added.
- The recoveries of the internal standards should be in the range between 50 and 120 %.
- Separation of PCDD/F from interfering chlorinated compounds such as chlorinated diphenyl ethers should be carried out.
- Gaschromatographic baseline 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).
- Identification should be performed according to the principles of EPA Method 1613 revision B: Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS.

References

1 Hoogerbrugge, R. and Liem, A.K.D. (2000) Organohalogen Compounds 45:13 - 16