THIRD ROUND OF WHO-COORDINATED EXPOSURE STUDY: ANALYSIS OF PCDDs, PCDFs AND PCBs IN HUMAN MILK

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Introduction

To ensure the reliability of exposure data and to improve comparability of analytical results from different laboratories, WHO Regional Office for Europe and the WHO European Centre for Environment and Health, Bilthoven Division, have coordinated a number of inter-laboratory quality assessment studies. The fourth round on levels of PCBs, PCDDs and PCDFs in human milk was conducted between February 1996 and April 1997. The objective was to identify laboratories, whose results could be accepted by WHO for exposure assessment studies. The final report presents the results of the study and a list of accepted laboratories for each of the studied compounds (1). As only the State Institute for Chemical and Veterinary Analysis of Food met all criteria for analyses of PCDDs, PCDFs, dioxin-like PCBs, marker PCBs and fat in human milk, this laboratory was selected as reference laboratory for the 3rd round of the WHO exposure study. This paper describes the method applied in this study and the validation of the results, presented elsewhere in this volume (2).

Methods

Solvent extraction, clean-up and determination by GC-MS of three groups of compounds (PCDDs/ PCDFs, dioxin-like PCBs and marker PCBs) was carried out following the same procedure as used for the WHO interlaboratory quality assessment study (1): After freeze-drying of the whole sample, fat and contaminants of interest are extracted in a hot extraction device (”Twisselmann extractor”) with ethanol / toluene (70/30) for 8 hrs. After evaporation of the solvent, the crude extract contains polar co-extractives which are removed by solving the residue in butyl methyl ether (gives purified fat after evaporation). An aliquot of 3 g is spiked with ^{13}C-labeled internal standards (17 PCDD/Fs, 5 non-ortho PCBs [37, 77, 81, 126, 169], 6 mono-ortho PCBs [28, 60, 105, 118, 156, 189] and 7 di-ortho PCBs [52, 101, 153, 138, 180, 194 and 209]). Gel permeation chromatography on Bio Beads S-X3 removes fat (in four runs with 0.75 g fat each). A silica column impregnated with sulfuric acid removes remaining oxidizable substances. A florisil column separates PCDD/F from PCBs.

The PCDD/F-fraction is purified on a Carbopack B-column (automated version) or on a Carbopack C-column (manual version). After addition of 1234-$^{13}C_{12}$-TCDD, this fraction is evaporated to a final volume of 20 µl. Determination is performed by HRGC/HRMS (Fisons Autospec; resolution 10,000; injection of 5 µl, DB5-MS; 5-point calibration curve).

On a Carbopack B-column, PCBs are separated into three fractions of first di-ortho PCBs (elution with hexane), then mono-ortho PCBs (elution with hexane / toluene; 92.5/7.5) and finally non-ortho PCBs (reversed elution with toluene). After addition of $^{13}C_{12}$-PCB 80 as recovery standard, the fractions are evaporated to a final volume of 60 µl (non-ortho PCBs) or 500 µl (mono-ortho and di-

ortho PCBs). These different groups are determined by HRGC/HRMS (Fisons Autospec; resolution 10,000; injection of 1 µl split/splitless, DB5-MS; 5-point-calibration curve) in three separate runs.

In addition to the 4th round of the WHO interlaboratory quality assessment study in human milk, the same steps of the method were also successfully applied in interlaboratory studies for the determination of PCDD/F in milk powder (3) or with modification of the fat extraction in eggs (4), milk and butter (5) and for PCDD/F and dioxin-like PCBs in different matrices (6 , 7).

Results and Discussion

The accuracy of results depends on systematic errors and random components. Systematic errors can be checked by analysis of reference material or in interlaboratory studies. The most important random component in dioxin analysis according to our experience is the possibility of cross contamination from highly contaminated samples.

A rigid quality control programme was carried out to guarantee a maximum of accuracy, including blank samples, spiked vegetable oil samples on different levels, and five different kinds of quality control samples (two butter samples and two egg samples of different levels of contamination and WHO pooled breast milk samples which remained from the WHO interlaboratory quality assessment study (1)). Except the availability of the particular reference material, this validation is part of the general quality control programme and applied in the daily routine of analysis of all kinds of samples. Therefore, comprehensive validation data are available showing the accuracy for WHO breast milk samples in context with the accuracy in the general daily routine. As a result, the validation of all results of the study is a complex picture. The individual steps reflect “worst case validation” (data are collected in separate runs and not simultaneously in one sequence) and can be summarized as follows:

Reagent blank samples

For PCDD/F, the median of 363 blank samples analysed since 1995 is 0.1 pg I-TEQ/g fat (as upperbound limit of determination). In most cases, tetra- through hexa-substituted congeners are not detectable. Therefore, the upperbound calculated blank is more an indication of the limit of determination than a reagent blank which could be considered for possible substraction. For PCB, the limit of determination could be lowered when the final volume was reduced to 500 µl. After this modification the median of 17 blank samples is 0.03 pg mono-ortho PCB-TEQ/g fat, in comparison to 0.10 pg mono-ortho PCB-TEQ/g fat for a final volume of 5 ml as used before. For non-ortho PCBs, the final volume was reduced from 300 µl to 60 µl resulting in a median of 0.05 pg non-ortho PCB-TEQ/g fat for 17 blank samples, in comparison to 0.08 pg non-ortho PCB-TEQ/g fat for a final volume of 300 µl. Therefore, the influence of reagent blank samples is negligible for human milk samples. As a result of this low limit of determination, the mean of the difference between upper and lower-bound concentrations is 0.2% for samples with a range of PCDD/F and PCB contamination as usual for human milk and therefore negligible in this range.

Spiked vegetable oil samples

As part of the routine quality control, refined vegetable oil (sunflower oil) is spiked on different levels with PCDD/Fs (range 0.25 to 20 pg I-TEQ/g fat) and PCBs (range 1 to 40 pg WHO-TEQ/g fat). Most data are available for PCDD/F control in the range of 1 pg I-TEQ/g fat which has been the usual background contamination in many sorts of food of animal origin for years. Without elimination of any data, all 79 spiking experiments at a level of 1.05 pg I-TEQ/g fat had a recovery of 102 % with a CV of 7.5 % (for result on TEQ basis), with a CV for the individual congeners 2378-TCDD, 12378-PeCDD and 23478-PeCDF between 9 and 12 %. Due to changes in the calibration standards, spiking

experiments with PCBs were performed on numerous different levels, with a limited number of experiments available on each level, so far. Most data are available for the level 40 pg WHO-PCB-TEQ/g fat (resulting from 29 pg WHO-mono-ortho-PCB-TEQ/g fat and 11 pg WHO-non-ortho-PCB-TEQ/g fat). At this level, for results calculated as TEQs recovery was 95 % for mono- and non-ortho-PCBs, with a CV of 1.8 % for mono-ortho PCB and 5.2 % for non-ortho PCB. For the individual congeners, CV for marker PCBs (138, 153, 180) was between 1.1 and 3.4 %, for mono-ortho PCBs (118, 156, 157, 189) between 1.4 and 7.8 % and for PCB 126 10.3 %.

Butter fat and egg fat samples as quality control samples

Since 1994, butter fat has been used as quality control. Two levels are available: sample "butter A" with a mean of 0.53 pg I-TEQ/g fat, sample "butter B" with 1.18 pg I-TEQ/g fat. 47 replicates of sample "butter A" had a CV for I-TEQ of 9.4 % and 32 replicates of sample "butter B" of 5.3 %. Since 1996, also two levels of egg fat are available. 28 replicates from sample "egg A" had a CV for I-TEQ (mean: 0.70 pg I-TEQ/g fat) of 9.8 %, 20 replicates from sample "egg B" (mean: 4.46 pg I-TEQ/g fat) a CV of 6.1 %. In the samples above 1 pg I-TEQ/g fat, the CV for 2378-TCDD, 12378-PeCDD and 23478-PeCDF is in the range between 7 and 12 %.

These samples have been used since 2001 also as quality control samples for PCB analysis. 7 replicates from "butter B" had a CV of 8.3 % for a level of 1.65 pg WHO-PCB-TEQ/g fat, 8 replicates from "egg B" had a CV of 6.5 % for a level of 3.1 pg WHO-PCB-TEQ/g fat. The decisive PCB-congeners had CVs between 3 and 10 % (138, 153, 180: range 4 – 10%; 105, 118, 156, 167: range 3 – 10 %; 126: range 8 – 10 %).

WHO pooled breast milk samples remaining from WHO interlaboratory assessment study

Five pools of WHO breast milk samples remaining from the WHO interlaboratory assessment study (1) were included as quality control samples. In comparison to the consensus value, the following recoveries were found for sum results (in brackets: CV in %): PCDD/F (as WHO-TEQ) 82.2 (10.5), non-ortho-PCB (as WHO-TEQ) 83.5 (16.5), mono-ortho-PCB (as WHO-TEQ) 81.7 (11.0), sum of marker PCBs (138, 153, 180) 106.5 % (9.7), fat 99.8 % (3.8). In comparison to the results as submitted for the interlaboratory assessment study by CVUA Freiburg, the recoveries were as follows (in brackets: CV in %): PCDD/F 96.9 (7.4), non-ortho-PCB 83.5 (14.7), mono-ortho-PCB 90.7 (10.8), sum of marker PCBs (138, 153, 180) 100.8 % (10.9), fat 92.8 % (7.2). The CV for the decisive individual congeners were for PCDD/F (2378-TCDD, 12378-PCDD, 123678-HxCDD, 23478-PeCDF) in the range 7 to 12 %, for PCB 126 15 %, for mono-ortho PCBs (118, 156, 157) in the range 8 – 13 %, for marker PCBs (138, 153, 180) 10 – 12 % and for fat 4 %. These measurements again meet the criteria for acceptance.

Participation in Food 2001

As additional external quality control, CVUA Freiburg participated in Food 2001 which included the determination of PCDD/F and dioxin-like PCBs in breast milk. Samples of the WHO field study and of Food 2001 were analysed together. The results of the congeners which were decisive for the WHO interlaboratory study (2378-TCDD, 12378-PCDD, 123678-HxCDD, 23478-PeCDF, PCB 126, PCB 118, PCB 156, and PCB 157) were in good correspondence with the consensus value (recoveries in the range 78 to 103 %). Calculated on WHO-TEQ basis, recovery was 87 % of the consensus value. For fat, recovery was 98 % of the consensus value.

Duplicate analysis as "overlapping sandwich method"

Samples were analysed in a way which can best be described as "overlapping sandwich method": a large portion of the samples was analysed as duplicate analyses, with the duplicate analyses being

performed in sequences with samples from other countries and with different quality control samples. This makes sure that the results of all samples from different countries have the same reliability, even in the situation where receipt of the various samples by the reference laboratory spanned a period of more than one year. In addition, it is possible to calculate the repeatability standard deviation, if at least 15 samples of a specific matrix are analysed in duplicate. From 69 samples analysed, 38 samples were analysed as duplicates and 5 as triplicates. The duplicate analysis gave a CV of 2.3 % for WHO-PCDD/F-TEQ and of 4 – 6 % for the congeners 2378-TCDD, 12378-PeCDD, 123678-HxCDD and 23478-PeCDF. For the sum PCB-TEQ, the CV was 4.0 %, and for individual PCBs (marker PCBs 138, 153 and 189; mono-ortho 118, 156, 157, 189 and non-ortho 126) between 3 and 9 %.

Conclusion

The comprehensive quality control proves that the validation of results of all samples from the third round of the WHO exposure study meets the requirements for acceptance of results as requested for the fourth round of WHO interlaboratory quality assessment study on levels of PCBs, PCDDs and PCDFs in human milk, and of the recommendations for harmonised quality criteria for analyses of PCDD/Fs in feed and food (8).

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