Collaborative Study of Different Analytical Methods for Determination of PCDD/PCDF in Eggs

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INTRODUCTION

Three laboratories (no. 1 - 3 of the authors) were involved in the analysis of egg samples from a contaminated area in the south western part of Germany. Thus, a collaborative study was performed to test whether the applied methods give comparable results. Additionally, the institute in Münster participated in the ring test.

EXPERIMENTAL

Two kinds of homogenized whole egg samples had to be analyzed:
- egg samples from the market which showed a background contamination according to pretests in the Freiburg laboratory
- egg samples from chicken raised in the contaminated Rheinfelden area with elevated PCDD/PCDF-contamination.

Analytical method of R. Frommberger, Stuttgart:

Method applied is the Smith/Stalling/Johnson-method (1) that was tested succesfully in WHO- (2) and BCR-collaborative study (3).

The sample was freeze dried, homogenized in a blender, mixed with

sodium sulphate (1+1), and the fat was column extracted with cyclohexane/dichlormethane (1+1). The further clean up procedure and GC/MS-analysis are described elsewhere (4, 5).

Analytical method of P. Fürst, Münster:

The basic steps were described for human milk samples (6) and tested succesfully in various WHO collaborative studies.

The method for determination of PCDDs/PCDFs in egg samples contains the following steps:

- extraction of PCDDs/PCDFs along with fat by means of ethanol, diethylether and pentane
- spiking of 3 g fat with eleven 13C-labeled congeners
- gel permeation chromatography with cyclohexane/ethyl acetate (1+1) on Bio Beads S-X3
- separation of PCDDs/PCDFs from PCBs, pesticides and other coextracts on a florisil column
- separation of planar PCDDs/PCDFs from remaining non planar compounds on a Carbopack C/celite column
- addition of recovery standard 13C-1234-TCDD prior to final evaporation step
- analytical determination using HRGC/HRMS on a VG Autospec at a resolution of R = 10,000. Samples are routinely run on a 30m DB-5 column and for quantification partly on a 30 m DB-Dioxin
- quantification by means of a four point calibration curve

Analytical method of R. Malisch, Freiburg:

The basic steps of the analysis were presented earlier (7, 8). For development of the method, some clean up-columns of the method of Fürst et al. (6) were adopted and modified in a way that the whole procedure does not use any halogenated solvents or benzene. This has the advantage of a better protection of the analyst and the environment and of avoiding problems of discharging the waste. The procedure was optimized in a way that generally about 70 - 80 % of the solvent waste can be recycled and reused. Gelchromatographic separation of 3 g fat is performed in four injections with an optimized technical equipment (ABIMED). The method for egg samples contains the following steps:

- freeze drying of the whole egg sample
- Soxhlet extraction with cyclohexane/toluene (1+1)
- spiking of 3.75 g fat with all 13C-labeled PCDD/PCDF-congeners
- gelchromatographic separation of an aliquot of 3.0 g fat
- removal of small amounts of remaining lipophilic and oxidizable substances on a mixed column with layers of silica gel/sulfuric acid, silica gel/NaOH and silica gel
- separation of PCB, chlorinated pesticides, chlorobenzenes,

chlorinated biphenylethers and chlorophenols on a florisil column
- Carbopack C/celite-clean up
- addition of 13C-labeled 1234-TCDD
- concentration to a final volume of 20 µl toluene
- GC/MS on a VG Autospec at 10000 resolution using a 60 m DB5-MS-column. The AS 200 autosampler injected 5 µl into the Mult-injector of a Carlo Erba Mega GC.
- With every acquisition sequence, a 5 point-calibration curve was acquired in duplicate (5 points before and 5 points after the samples). The calibration curve covered the ranges between 0.025 to 2.0 pg/µl for PCDD/F which were expected in a lower concentration range (e.g. 2378-TCDD and 2378-TCDF) and 0.5 to 40 pg/µl for OCDD. Using the above conditions, the duplicate injection of the lowest concentrated standards with 12.5 fg TCDD/µl gave a signal to noise of 21.4 and 24.3 (with +/- 2 standard deviations about the mean noise; theoretically according to specification: 15:1).

Analytical method of P. Schmid, Schwerzenbach:

Extraction of the egg homogenate was based on the procedure for human milk described by Fürst et al. (6):

50 g egg homogenate were mixed with 200 ml water, 10 ml saturated aqueous potassium oxalate solution, and 200 ml ethanol. The mixture was extracted a first time with 100 ml diethyl ether and 150 ml n-pentane and a second time with 100 ml n-pentane. The united extracts were washed twice with each 200 ml 2 % aqueous sodium sulfate solution. After complete evaporation of the solvent the remaining fat was determined and the internal standard (see Table 1) was added.

Table 1. Composition of the 13C-labeled internal standard (pg per sample, dissolved in 5 µl isooctane):

2378-TetraCDD	2.67	2378-TetraCDF	2.67
12378-PentaCDD	2.67	12378-PentaCDF	0.33
		23478-PentaCDF	8.33
123678-HexaCDD	2.67	123478-HexaCDF	2.67
1234678-HeptaCDD	11.67	1234678-HeptaCDF	2.33
OctaCDD	11.00	OctaCDF	2.00

Further treatment of the extracted fat followed the method described by Smith and Stalling (1). The loading and elution of the active carbon column was automatized using a column switching unit. Further treatment of the extract included passing through cesium silicate and silicagel impregnated with sulfuric acid onto an alumina column (9) and elution of PCDDs and PCDFs with n-hexane/dichloromethane (1:1 v/v). After removing the solvent the extract was dissolved in 10 µl isooctane.

A gas chromatograph Fisons HRGC Mega 2 Series equipped with an autosampler A200S was used. Separation was performed on a 20 m x 0.30 mm glass capillary coated with a 0.17 µm film of a polysiloxane containing 12-15 % phenyl (PS 086 from Hüls America, Inc.). The carrier gas was helium at a head pressure of 80 kPa (linear flow rate 50 cm/s). Samples of 1 µl were injected "on column" at 100°C oven temperature. After 1 min the temperature was raised at 20°/min up to 240°C and then at 4°/min up to a final temperature of 280°C. The mass spectrometer (Finnigan MAT 95) was run in electron impact ionization mode at 70 eV electron energy and a mass resolution of 10,000. The two most abundant ions within the molecular ion clusters of the native and 13C-labeled PCDD and PCDF congeners were recorded using multiple ion detection (MID). Quantification was based on signal areas in the mass chromatograms. The response factors used for calculation were determined in a mixture containing known concentrations of all native 2,3,7,8-chlorosubstituted PCDDs and PCDFs.

RESULTS AND DISCUSSION

Table 2 and 3 show the results for the egg sample with background contamination and for the egg sample with elevated PCDD/PCDF-contamination (all results in pg/g fat).

substance	Frommbg. Stuttgt.	Fürst Münster	Malisch Freiburg	Schmid Schwerz.	mean
2378-TCDD	0.28	0.27	0.17	0.44	0.29
12378-PeCDD	0.41	0.39	0.34	0.29	0.36
123478-HxCDD	0.21	0.20	0.22	0.20	0.21
123678-HxCDD	0.93	0.84	0.75	0.78	0.83
123789-HxCDD	0.22	0.18	0.26	0.25	0.23
1234678-HpCDD	4.39	4.23	5.25	4.11	4.50
OCDD	21.70	18.20	37.35	14.16	22.85
2378-TCDF	1.01	0.95	0.88	0.90	0.93
12378-PeCDF	0.41	0.28	0.33	0.60	0.41
23478-PeCDF	0.79	0.53	0.72	0.61	0.66
123478-HxCDF	0.47	0.55	0.48	0.52	0.51
123678-HxCDF	0.56	0.29	0.29	0.36	0.37
123789-HxCDF	0.07	0.04	0.03	0.06	0.05
234678-HxCDF	0.40	0.22	0.25	0.19	0.26
1234678-HpCDF	0.71	0.70	0.63	0.81	0.71
1234789-HpCDF	0.13	0.06	0.07	0.07	0.08
OCDF	0.65	0.80	0.36	1.34	0.79
I-TEQ	1.35	1.15	1.13	1.32	1.24
fat amount (%)	11.40	8.50	9.53	8.93	9.59

Table 2. Results of the egg sample with background contamination

(pg/g fat)

All samples had "incurred" residues from animals which have taken up the PCDD/PCDF burden with the feed; the samples have not been "fortified" or "spiked" by the addition of a known amount of the analyte. Thus, there is no "true" PCDD/PCDF-concentration.

Each laboratory used its own PCDD/PCDF-standard solution for quantification.

substance	Frommbg. Stuttgt.	Fürst Münster	Malisch Freiburg	Schmid Schwerz.	mean
2378-TCDD	0.86	0.87	0.70	0.92	0.84
12378-PeCDD	2.40	1.77	1.83	1.51	1.88
123478-HxCDD	1.96	1.53	1.68	1.22	1.60
123678-HxCDD	9.06	7.10	6.76	5.66	7.15
123789-HxCDD	4.01	1.63	2.30	1.51	2.36
1234678-HpCDD	81.15	75.53	86.11	61.34	76.03
OCDD	234.00	209.33	409.48	124.83	244.41
2378-TCDF	31.00	32.40	26.93	22.68	28.25
12378-PeCDF	13.55	12.80	11.61	8.31	11.57
23478-PeCDF	9.44	6.57	7.16	6.63	7.45
123478-HxCDF	10.30	11.90	11.05	9.01	10.57
123678-HxCDF	4.72	3.13	3.13	2.46	3.36
123789-HxCDF	0.10	0.22	0.27	0.27	0.22
234678-HxCDF	2.20	1.70	1.93	1.35	1.79
1234678-HpCDF	4.98	3.87	5.01	4.11	4.49
1234789-HpCDF	0.84	0.80	0.79	0.67	0.78
OCDF	4.47	5.13	3.43	3.81	4.21
I-TEQ	14.90	12.66	12.51	10.62	12.67
fat amount (%)	11.80	9.80	10.90	10.43	10.73

Table 3. Results for the egg sample with elevated PCDD/PCDF-contamination (pg/g fat)

Results of the four different methods are in a good correspondence. With respect to administrative actions and maximum residue levels for condemnation, the I-TEQ-value is the most important parameter. At both contamination levels, this decisive value was determined in good agreement between all participating laboratories. Thus, the methods give comparable results.

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