CONFIRMATION OF A PCDD/PCDF CONTAMINATION OF MILK AND BUTTER SAMPLES BY AN „EMERGENCY COLLABORATIVE STUDY"

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INTRODUCTION

In March 1998, the Chemische Landesuntersuchungsanstalt Freiburg detected a raising dioxin contamination in milk and dairy products (1). In all samples, the increase of the I-TEQ-values was caused above all by 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD. It was clear from the number of samples analyzed that this increase was not a local problem but concerned samples from probably all parts of Germany and some other European states, as well. A single sample from milk from tankers (collecting milk from farms on tours to the dairy) exceeded the proposed guide line for PCDD/F in milk fat for being marketable (2) causing severe pressure to find the source and confirm this trend. Thus, in the middle of March 1998 four other dioxin laboratories of the official food control in Germany were asked to confirm this trend in four butter samples (2 from Schleswig-Holstein, the northernmost state of Germany, and 2 from Baden-Württemberg, the south-western state of Germany) as well as in milk from a farm where the source could be traced back to the use of citrus pulp from Brazil which was used as component in feed stuff (up to 25 %). All laboratories were asked to analyze the samples as soon as possible under the available routine conditions at that time, without spending time in optimization of methods or GC/MS conditions. Thus, it was a sort of „emergency analysis" when the results arrived roughly two to three weeks after the first announcement of the samples by telephone call (time including shipment).

EXPERIMENTAL

Four butter samples had to be analyzed:

- two butter samples produced in two different dairies in Schleswig-Holstein (which is the northernmost state of Germany) collected randomly on the market in January 1998 in the Freiburg area (no. 574.1 and 633.1),
- two butter samples produced in two different dairies in Baden-Württemberg (which is the south-western state of Germany) collected randomly on the market in January 1998 in the Freiburg area (no. 708.1 and 1496.1).

Additionally, one milk sample from a farm in the Freiburg area with a high dioxin contamination had to be analyzed. For this, the raw milk was centrifuged for 5 min at 3000 rpm, the upper cream layer was separated and shipped for analysis.

Analytical methods

For extraction of fat, different methods were used:

- Butter and cream samples were mixed with sodium sulfate and extracted with with n-hexane/acetone in a chromatography column (Bruns-Weller and Knoll, Oldenburg).
- Butter samples were warmed up for liquefaction, mixed with sodium sulfate and extracted with pentane (Fürst, Münster).
- Cream samples were extracted in a separatory funnel by shaking with ethanol, diethylether and pentane. After phase separation, the aequous layer was separated and extracted with pentane. The combined organic layers were washed with sodium sulfate, dried by sodium sulfate and evaporated (Fürst, Münster).
- Butter was warmed up to 55 °C in a centrifuge tube and centrifuged 5 min at 3000 rpm. The upper butterfat was taken for analysis. The cream sample was freeze dried and extracted in a Soxhlet with hexane (Malisch, Freiburg).
- Butter samples and the cream samples were ground with anhydrous sodium sulfate to bind any water. The pulverized mixture was filled into a chromatography column and fat was extracted with n-hexane/acetone (2 + 1) (Mayer, Oberschleißheim).
- The butter samples were dissolved in hexane. The hexane layer was removed carefully and the $^{13}C_{12}$-labelled PCDD/PCDF-standard containing all 17 2,3,7,8-substituted PCDD/PCDFs was added. The cream sample was freeze dried and extracted in a Soxhlet with hexane after addition of the $^{13}C_{12}$-labelled PCDD/PCDF-standard. The hexane was completely evaporated for gravimetric fat determination with a rotary evaporator (Wiesmüller, Potsdam).

The clean up-procedures used different steps, as well:

- column chromatography with sulfuric acid impregnated silica gel; carbon column (Carbopack/Celite 545) and alumina column (Alumina 90) (Bruns-Weller and Knoll, Oldenburg).
- After gelchromatographic separation of fat, the extract was purified on a florisil column and carbon column (Carbopack C/celite) (Fürst, Münster).

- After gelchromatographic separation of fat, the extract was purified on a sulfuric acid impregnated silica gel column, florisil column and carbon column (Carbopack B) (Malisch, Freiburg).
- Clean-up with three chromatographic steps (mixed acid-base silica, charcoal, florisil) (Mayer, Oberschleißheim)
- Purification was done first by refluxing the sample with hexane / silica-sulfuric acid (44 %) for 15 min, chromatography on a carbon column (Envicarb) and on an alumina B super I for dioxin analysis column (Wiesmüller, Potsdam).

All laboratories used $^{13}C_{12}$-labelled congeners as internal standards. Most laboratories added the internal standards to an aliquot of the extracted fat. As recovery standard, $^{13}C_6$-1,2,3,4-TCDD or $^{13}C_{12}$-1,2,3,4-TCDD were used.

GC/MS-parameters:
- 50 m Ultra 2 fused silica capillary column, Finnigan MAT 95 mass spectrometer with 7,000 - 10,000 resolution power (Bruns-Weller and Knoll, Oldenburg)
- VG AutoSpec at 10,000 resolution using a 60 m DB-5MS (injection of 2 μl) (Fürst, Münster).
- VG AutoSpec at 10,000 resolution using a 60 m DB-5MS. The AS 200 autosampler injected 5 μl into the Multinjector of a Carlo Erba Mega GC (Malisch, Freiburg).
- AutoSpec Ultima mass spectrometer at 10,000 resolution using a DB-5MS capillary column (Mayer, Oberschleißheim)
- Carlo Erba GC 8000 series, coupled to a VG Autospec Ultima, at 10,000 resolution using a DB-XLB (30 m x 0,25 mm x 0.25 μm) (Wiesmüller, Potsdam).

RESULTS AND DISCUSSION

All samples had taken up the PCDD/PCDF burden from contaminated 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.

Table 1 shows the results in pg/g fat of the butter and milk samples and the coefficient of variation for each sample calculated from the results of all participants. In the last row, the mean of all coefficients of variation was calculated for each congener to summarize the variation for samples with roughly the same concentration range.

The basic idea of the study was to confirm the elevated 2,3,7,8-TCDD level (usually in the range of about 0.1 pg/g fat) and 1,2,3,7,8-PeCDD (usually in the range of 0.2 pg/g fat). All laboratories confirmed these findings very closely. In the milk sample obtained from a farm with use of contaminated citrus pulp as component in feedstuff, the increased levels of certain PCDF could be confirmed, as well (2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF and OCDF). The only difference worth mentioning is the obviously too high 2,3,7,8-TCDF-content of one laboratory in the butter samples which was excluded from the calculation of the mean.

The coefficient of variation for the I-TEQ-value for the results of all five participating laboratories was 11 % (range 9.4 to 13.1 % in five samples). The variation of the results of the toxicologically most important congeners 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-

PeCDF was in the range of 15 %. 2,3,7,8-TCDD which was most important with respect to the increased contamination of the milk had a coefficient of variation of 9.1 % (range 7.6 to 12.2). The HxCDD/F and HpCDD/F with higher concentration range had a coefficient of variation in the range of 20 to 30 %, and OCDD/F was in the range of 40 %. This good correspondence was obtained between five laboratories applying different extraction and clean-up methods, using different GC/MS-equipment and different standard solutions under „emergency conditions". As a result, all extraction and clean up methods give the same results.

The results proved that the increased dioxin levels could be detected in butter from northern Germany even in slightly higher amounts than in butter from the south-west of Germany where the first signals had been seen. These low number of samples were not representative for the whole market, however, but were an indication, that other regions were involved, as well, on the national and international level: With use of contaminated citrus pulp from Brazil as ingredient for feed, the PCDD/F contamination of milk was increased which can be seen with increased levels of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD as first indication.

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		butter no. 574.1 mean	cv (%)	butter no. 633.1 mean	cv (%)	butter no. 708.1 mean	cv (%)	butter no. 1496.1 mean	cv (%)	milk mean	cv (%)	mean of all
2,3,7,8-	**TCDD**	**0.62**	**7.8**	**0.55**	**7.6**	**0.48**	**9.5**	**0.36**	**12.2**	**2.03**	**8.4**	**9.1**
1,2,3,7,8-	**PeCDD**	**0.52**	**14.5**	**0.49**	**14.1**	**0.40**	**16.1**	**0.37**	**17.3**	**1.55**	**9.9**	**14.4**
1,2,3,4,7,8-	HxCDD	0.14	21.9	0.13	35.4	0.12	34.4	0.11	38.6	0.31	27.2	31.5
1,2,3,6,7,8-	HxCDD	0.26	16.8	0.31	11.6	0.33	12.6	0.36	10.8	0.44	15.1	13.4
1,2,3,7,8,9-	HxCDD	0.11	28.5	0.13	27.1	0.13	18.0	0.12	11.6	0.24	18.9	20.8
1,2,3,4,6,7,8-	HpCDD	0.41	12.1	0.52	25.1	0.58	16.9	0.79	11.2	0.73	16.2	16.3
	OCDD	0.59	36.0	0.84	61.8	0.64	54.1	0.64	37.6	0.83	35.8	45.1
2,3,7,8-	**TCDF** [1]	**0.11**	**15.9**	**0.14**	**19.1**	**0.12**	**20.8**	**0.11**	**20.7**	**0.16**	**32.0**	**21.7**
2,3,7,8-	TCDF [2]	0.05	277.3	0.06	326.3	0.06	228.8	0.05	275.4	0.16	73.6	236.3
1,2,3,7,8-	PeCDF	0.06	61.3	0.06	47.9	0.05	56.6	0.05	63.8	0.09	49.5	55.8
2,3,4,7,8-	**PeCDF**	**0.65**	**18.6**	**0.70**	**13.4**	**0.55**	**17.2**	**0.54**	**16.9**	**1.50**	**15.7**	**16.4**
1,2,3,4,7,8-	HxCDF	0.55	12.1	0.59	16.0	0.40	17.0	0.43	15.7	1.76	12.6	14.7
1,2,3,6,7,8-	HxCDF	0.33	13.6	0.35	15.5	0.25	13.5	0.25	11.4	0.93	14.5	13.7
2,3,4,6,7,8-	HxCDF	0.49	27.2	0.42	23.5	0.34	40.3	0.35	32.4	1.80	16.7	28.0
1,2,3,7,8,9-	HxCDF	< 0.05	n.d.	< 0.04	n.d.	< 0.03	n.d.	< 0.03	n.d.	< 0.05	n.d.	n.d.
1,2,3,4,6,7,8-	HpCDF	0.42	18.4	0.41	20.1	0.27	24.5	0.28	25.5	1.90	16.5	21.0
1,2,3,4,7,8,9-	HpCDF	0.06	77.4	0.09	57.3	0.06	60.8	0.06	56.0	0.33	55.2	61.3
	OCDF	0.50	21.7	0.46	42.9	0.28	73.6	0.28	43.6	2.71	15.8	39.5
I-TeQ (NATO, 1988)		**1.41**	**10.9**	**1.37**	**9.8**	**1.14**	**11.9**	**1.00**	**13.1**	**4.16**	**9.4**	**11.0**

[1] without lab no. 4

[2] including lab no. 4