Determination of PCDD/PCDF in PCB products and milk samples; correlation between PCB- and PCDD/PCDF-contamination of milk samples

Rainer Malisch
Chemische Landesuntersuchungsanstalt (State Institute for Chemical Analysis of Food)
Bissierstr.5, D-79114 Freiburg, Germany

INTRODUCTION

The contamination of PCB products with polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) is well known: A mass food poisoning in western Japan in 1968 was caused by polychlorinated biphenyls (PCB) which induced a disease called "Yusho". Investigations revealed that PCDF could be detected in Kanechlors and "Yusho Oil" (1-2). PCDF were detected in other PCB products, also (3-5). A review on industrial sources for PCDD/PCDF (5,6) summarizes the results of an isomer specific analysis in PCB dielectric fluids from capacitors and transformers (7).

Regarding these findings of PCDF in PCB, we determined PCDF in milk samples with high amounts of PCB and checked whether there was a relation between PCB- and PCDF-concentration. As "high amount", we defined residues exceeding the tolerances by a factor of two or more. For PCB in milk, the following tolerances were set in Germany (in mg/kg milk fat): PCB 138 0.05, PCB 153 0.05, PCB 180 0.04.

As earlier reported (8), a monitoring program was used in our institute to detect sources for PCB contamination of milk samples. During the monitoring program between 1998 and 1992, 40 raw milk samples from producers exceeded the tolerances. These were analyzed in 1993 for PCDF contamination. Additionally, the PCB-products Clophen A 30 and Clophen A 60 were analyzed to compare the PCDD-PCDF-congener pattern between these PCB products and the milk samples.

EXPERIMENTAL

The basic steps of the analysis were presented earlier (9, 10). For development of the method, we considered some clean up-columns of the method of Fürst et al. (12) and modified them in a way that the whole procedure does not use any halogenated solvents or ben-

zene. 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 we can generally recycle about 70 - 80 % of the solvent waste and reuse it. The method proved to work well in a collaborative study (13). The method for raw milk samples contains the following steps:

- separation of cream by centrifugation of 200 ml raw milk (10 min at 4000 rpm)
- freeze drying of separated cream layer
- Soxhlet extraction with hexane
- spiking of 3 g fat with all 13C-labeled PCDD/PCDF-congeners
- gelchromatographic separation of 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 injection of the lowest concentrated standard with 12.5 fg TCDD/µl gave a signal to noise of at least 10:1 (with +/- 2 standard deviations about the mean noise; theoretically according to specification and achievable in the daily routine: 15:1).
- Selected results were confirmed on a 60 m DB-Dioxin column.

For determination of PCDD/PCDF in PCB products, 200 µg Clophen A 30 or 200 µg Clophen A 60 were spiked with 13C-labeled PCDD/PCDF. The PCB were separated by means of duplicate use of a florisil column (second time to remove small amounts of PCB co-eluted with PCDD/PCDF in separation on the first florisil column). Completeness of separation of PCB was checked by capillary GC/ECD. Afterwards, the PCB-free extract was purified on a Carbopack C/celite-column. After addition of 13C-labeled 1234-TCDD, PCDD/PCDF were determined in the same way as described for the milk samples (first detection on a DB5-MS-column, then confirmation on a DB-Dioxin column).

RESULTS AND DISCUSSION

Table 1 gives the results of the determination of PCDD/PCDF in the PCB products Clophen A 30 and Clophen A 60 before use. Both products show the expected contamination with PCDF, only, and here mainly with TCDF, PCDF and HxCDF. Generally, the higher chlorinated Clophen A 60 (contains 58 % chlorine) is more contaminated than Clophen A 30 (contains 42 % chlorine). Whereas the PCDF-con-

tamination in Clophen A 60 resembles the degree and pattern described in the literature (5, 6), the Clophen A 30 analyzed in our institute exceeded the published concentrations considerably.

	Clophen A 30	Clophen A 60
2378-TCDD	0.0	0.0
12378-PeCDD	0.0	0.1
123478-HxCDD	0.0	0.2
123678-HxCDD	0.8	0.0
123789-HxCDD	0.0	0.0
1234678-HpCDD	5.6	2.5
OCDD	31.1	14.9
2378-TCDF	1032.6	2287.7
12378-PeCDF	135.8	465.2
23478-PeCDF	509.2	1921.9
123478-HxCDF	301.4	1604.2
123678-HxCDF	65.3	157.6
234678-HxCDF	50.6	369.5
123789-HxCDF	0.0	42.8
1234678-HpCDF	43.7	480.6
1234789-HpCDF	22.5	321.7
OCDF	15.7	639.2
Total TCDD	0.7	0.4
Total PeCDD	0.0	2.0
Total HxCDD	1.2	1.8
Total HpCDD	5.6	3.0
Total TCDD-OCDD	38.7	22.1
Total TCDF	6376.6	15785.7
Total PeCDF	2402.4	11654.6
Total HxCDF	804.8	4455.8
Total HpCDF	108.3	1517.0
Total TCDF-OCDF	9707.7	34052.2
Total PCDD/PCDF	9746.4	34074.4
I-TEQ	407.2	1439.2

Table 1. PCDD/PCDF in the PCB products Clophen A 30 and Clophen A 60 (before use; in µg/kg).

In 1993, we analyzed 97 milk samples. From these, 92 were collected from miscellanous milk tanks in a dairy (representing producers in different parts of our supervision district; mainly from the Black Forest) and 5 from the market. Their PCDD/PCDF-contamination was low (in the mean about 0.8 pg I-TEQ/g fat). For comparison: In Germany, the following tolerances (in pg I-TEQ/g fat) are recommended (11): < 0.9 pg as aspired objective; > 3 recommendation for restriction of consumption and examination of possible sources; > 5 prohibition of consumption. Figure 1 shows the frequency distribution.

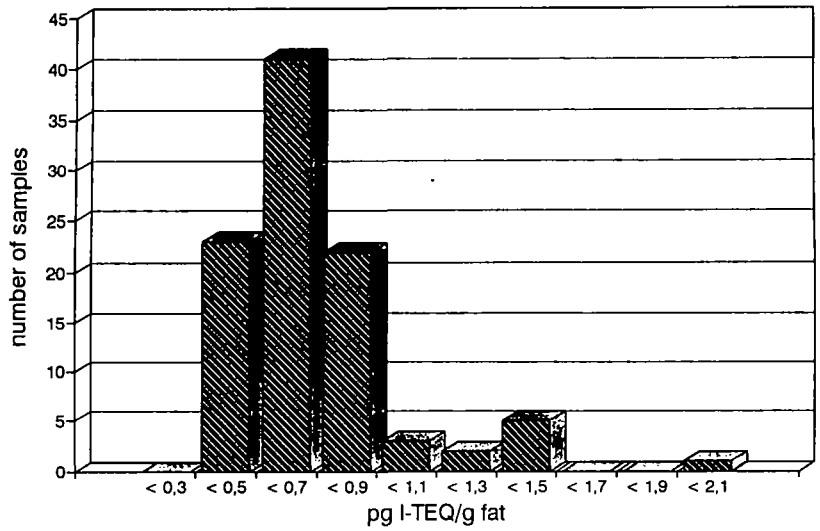

Figure 1. Frequency distribution of PCDD/PCDF-contamination of milk samples (97 samples from 1993)

In comparison to these milk samples, the 40 samples with PCB-contamination above the tolerances were rouhgly three times higher contaminated with PCDD/PCDF: in the mean, about 2.7 pg I-TEQ/g fat; 15 samples exceeded the recommended tolerance of 3 pg I-TEQ/g fat. Figure 2 shows the frequency distribution.

Figure 3 shows the correlation between the amount of PCB 153 and I-TEQ in the PCB-contaminated milk samples. There is a clear tendency of higher I-TEQ-values with rising PCB 153-contamination. However, there is not a strict correlation allowing to conclude from a determined PCB amount to a I-TEQ amount.

In Clophen A60 (before use), the highest concentration of congeners with 2378-substitution were found for 2378-TCDF (2287 µg/kg), 23478-PeCDF (1921 µg/kg) and 123478-HxCDF (1604 µg/kg). On the contrary to this, 2378-TCDF was determined in the milk samples only to about one fifteenth of the 23478-PCDF amount which was the predominant congener here (in the mean, as µg/kg fat: 2378-TCDF 0.22, 23478-PeCDF 3.60, 123478-HxCDF 1.41). In the PCB-contaminated milk samples, 23478-PeCDF contributes to about 65 % of the I-TEQ (all furans as a sum to about 76 % of the I-TEQ). In comparison to the 97 milk samples without elevated PCB-contamination, the contamination with furans is about 3-5 times higher, whereas PCDD amounts are in the same order of magnitude.

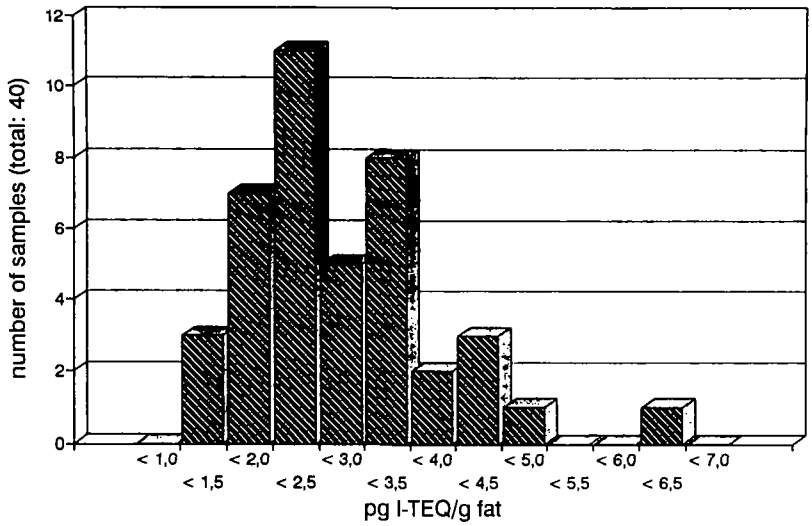

Figure 2. Frequency distribution of PCDD/PCDF-contamination of milk samples contaminated with PCB above the tolerance level (40 samples from 1988-1992)

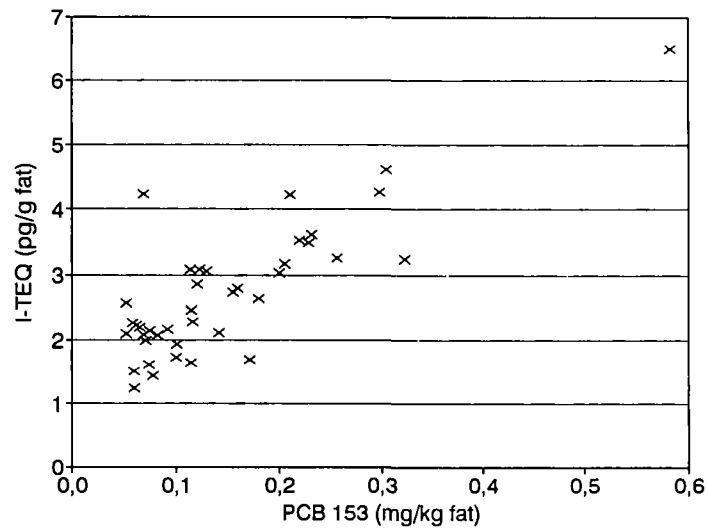

Figure 3. Correlation between PCB 153- and I-TEQ-amounts

CONCLUSION

Milk samples with PCB contamination above the tolerance levels were about three times higher contaminated with PCDD/PCDF (ex-

pressed in pg I-TEQ/g fat) than samples not exceeding the PCB tolerance levels. Specifically, furans were found in significantly higher amounts, although the pattern does not reflect the PCDF-contamination of Clophen A 60 (before use) closely. There is no strict correlation allowing to predict the I-TEQ value exactly depending on the determined PCB 153 (or other) congener. Milk with PCB 153-contamination exceeding about 0.2 to 0.3 mg/kg fat (4 to 6 times the tolerance) exceeded the recommended PCDD/PCDF-tolerances for restrictions of consumption. Milk with PCB 153-contamination in the range between 0.05 (tolerance level) and about 0.2 to 0.3 mg/kg had I-TEQ-values above the aspired PCDD/PCDF-objective, but mainly below the recommended PCDD/PCDF-tolerance for restrictions of consumption. Thus, the strict PCB tolerance levels protect the consumer from elevated PCDD/PCDFlevels, also.

REFERENCES

1) Nagayama J., M. Kuratsune and Y. Masuda (1976) Bull. Environ. Contam. Toxicol. 15:9-13
2) Masuda Y. and H. Kuroki (1982): Polychlorinated Dibenzofurans and Related Compounds in Patients with "Yusho", in: Pergamon Series on Environmental Science Vol.5 "Chlorinated Dioxins and Related Compounds - Impact on the Environment", pp. 561-569
3) Bowes G.W., M.J. Mulvihill, M.R. DeCamp and A.S.Kende (1975) J. Agric. Food Chem. 23:1222-1223
4) Bowes, G.W., M.J. Mulvihill, B.R.T. Simoneit, A.L. Burlingame and R.W. Risebrough (1975) Nature 256:305-307
5) Fiedler, H. (1991): Industrielle Quellen von PCDD/PCDF, Organohalogen Compounds 6:213-238
6) Fiedler, H. (1993): Sources and Emission, pp. 401-417 in "The Toxicology Forum - Current Views on the Impact of Dioxins and Furans on Human Health and the Environment", Berlin, Germany, November 9-11, 1992; The Toxicology Forum, Washington, D.C.
7) Brown, J.F., J.C. Carnahan, S.B. Dorn, J.T. Groves, W.V. Ligon, R.J. May, R.E. Wagner and S.B. Hamilton (1988) Chemosphere 17:1697-1702
8) Kypke-Hutter, K. and R. Malisch (1989) Z. Lebensm. Unters. Forsch. 188:127-134
9) Malisch, R. (1993): Determination of Dioxins and Furans in Coloured Candle Wax, Dioxin '93, Vol. 11, pp. 321-323. Detailed publication submitted to Chemosphere (Chlorinated Dioxins and Related Compounds 1993)
10) Malisch, R. and Metschies, M. (1993): Development of Analytical Methods for Determination of Dioxins: Advantages of Tritiumlabeled TCDD and Carbon 14-labeled OCDD, Dioxin '93, Vol. 11, pp. 135-138. Detailed publication submitted to Chemosphere (Chlorinated Dioxins and Related Compounds 1993)
11) Bundesgesundheitsamt and Umweltbundesamt (1993): Dioxine und Furane - ihr Einfluß auf Umwelt und Gesundheit, Bundesgesundhbl. Sonderheft/93, pp.3-14
12) Fürst, P., C. Fürst, H.A. Meemken and W. Gröbel (1989) Z. Lebensm. Unters. Forsch. 189:33-345
13) Malisch, R., P. Schmid, R. Frommberger and P. Fürst (1994): Collaborative study of different analytical methods for determination of PCDD/PCDF in eggs, Dioxin '94, Kyoto, Japan