RESULTS OF A QUALITY CONTROL STUDY OF DIFFERENT ANALYTICAL METHODS FOR DETERMINATION OF PCDD/PCDF IN KALE SAMPLES

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ABSTRACT

A quality control study was performed to evaluate the results of three laboratories for determination of PCDD/PCDF in kale samples. These laboratories applied different extraction and clean-up methods, used different GC-columns and GC/MS-equipment and different standard solutions. One laboratory compared the application of another extraction and clean up method, as well. Thus, four different methods were tested. Two kinds of homogenized kale samples had to be analyzed: One set of kale sample reflects background contamination (samples from Wackersdorf area), one set of samples reflects the contamination of an highly industrialized area (samples from Duisburg area).

The results are in a good agreement. Slightly different results for most individual congeners were of minor importance. Differences in the 2,3,7,8-TCDF content are caused obviously by the specifity of the used capillary columns and not by differences of the extraction or clean up procedures. Thus, the laboratory's individual methods proved to give correct results. The specifity of the PCDD/PCDF separation of the GC column is an important factor, however.

KEY WORDS: PCDD, PCDF, analysis, GC/MS-determination, collaborative study, quality control, kale samples

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INTRODUCTION

National German authorities (Bund/Länder-Arbeitsgruppe DIOXINE) initiated a dioxin reference program. For this, different samples (e.g. soil and food) from the same origin should be analyzed over years to determine temporal trends of the dioxin contamination. Kale samples reflect the air contamination, espacially in the winter time, very well. Thus, three states („Länder") named laboratories for participation in this part of the dioxin reference program: Chemische Landesuntersuchungsanstalt Freiburg for Baden-Württemberg, Staatliches Lebensmitteluntersuchungsamt Oldenburg for Lower Saxony, and Bayerisches Landesamt für Umweltschutz, branch Wackersdorf, for Bavaria. In order to make sure that the data are comparable, the institutes had to participate in a quality control study, first. The „AK Bioindikationen / Wirkungsermittlung der Landesämter und -anstalten für Umweltschutz" (chairman: Dr. Peichl) gave advice in selection of the samples sites and organized the raising of the plants.

EXPERIMENTAL

Two kinds of kale samples had to be analyzed:

- kale samples from the Wackersdorf area (in the north-eastern part of Bavaria) which shows a background contamination of a mainly rural / forested area
- kale samples from the Duisburg area (in North Rhine-Westphalia) which is a highly industrialized area with elevated PCDD/PCDF-contamination.

The samples in Wackersdorf were raised by the UMEG (Gesellschaft für Umweltmessungen und Umwelterhebungen), Karlsruhe, the samples in Duisburg by the Landesumweltamt NRW (North Rhine-Westphalia), Essen. The kale plants were grown in these areas between August and December 1996 and unwashedly homogenized and frozen. For each location, three replicates had to be analyzed.

Analytical method of E. Bruns-Weller and A. Knoll, Oldenburg

The frozen sample was thawed up, mixed with sodium sulphate, and extracted with n-hexane/acetone in a chromatography column. After evaporation of the solvent, the extract was spiked with $^{13}C_{12}$-labelled congeners. For clean-up, column chromatography was used (sulfuric acid impregnated silica gel; alumina [Alumina B-Super I]). After clean up, $^{13}C_6$-1,2,3,4-TCDD was added as recovery standard. GC/MS parameters: 50 m Ultra 2 fused silica capillary column, Finnigan MAT 95 mass spectrometer with 7,000 - 10,000 resolution power.

Analytical method of H. Thoma, Wackersdorf

The samples were freeze-dried and extracted with toluene in a Soxhlet extractor (24 h). After evaporation of the solvent, the extract was spiked with 17 $^{13}C_{12}$-labelled congeners. For clean-up, column chromatography was used (alumina [Alumina B-Super I], sulfuric acid impregnated silica gel). After clean up, $^{13}C_6$-1,2,3,4-TCDD was added as recovery standard. GC/MS parameters: 60 m Supelco SP 2331 for all PCDD/F besides OCDF; for determination of OCDF: DB 5. MS: VG Autospec.

Analytical method no. 1 of R. Malisch, Freiburg: freeze-drying, Soxhlet-extraction with ethanol/toluene

The samples were thawed up, spiked with all 2,3,7,8-substituted $^{13}C_{12}$-labelled PCDD/PCDF congeners, freeze-dried and extracted with toluene/ethanol (30/70) for 8 h in a Soxhlet extractor. After filtration and evaporation, the residue was purified on a sulfuric acid impregnated silica gel column, gelchromatography, florisil column and carbon column (Carbopack B). The whole procedure does not use any halogenated solvents or benzene. Additionally, the procedure allows to recycle and to reuse about 70 - 80 % of the solvent waste.

As recovery standard, ^{13}C-labelled 1,2,3,4-TCDD was used. GC/MS-detection was performed on a VG AutoSpec at 10,000 resolution using a 60 m DB-Dioxin. For confirmation, a DB 5-MS was used. (Table 1 and 2 show the results of the DB Dioxin column, with the exception of 1,2,3,7,8-PeCDD, where the results of the DB 5-MS column were selected for the calculation of the I-TEQ.) The AS 200 autosampler injected 5 μl into the Multinjector of a Carlo Erba Mega GC.

Analytical method no. 2 of R. Malisch, Freiburg: extraction of wet kale with acetone and n-heptane by means of a high-efficiency disperser

The sample was thawed up, spiked with all 2,3,7,8-substituted $^{13}C_{12}$-labelled PCDD/PCDF congeners and extracted twice with acetone and once with n-heptane by means of a high-efficiency disperser. After each extraction step, the extract was centrifuged and the supernatant liquid decanted. After addition of water to the combined extracted phases, the lower water/acetone-phase was separated. The heptane-phase was evaporated to dryness. The residue was purified on a sulfuric acid impregnated silica gel column, gelchromatography, florisil column and carbon column (Carbopack B). GC/MS-determination was the same as described for method no. 1.

RESULTS AND DISCUSSION

All samples had taken up the PCDD/PCDF burden from the environmental air. 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. The comparability of the standard solutions was not checked for this quality control study. However, in former times the Oldenburg and Freiburg laboratory had checked the comparability of their standards in earlier control test succesfully.

Table 1 shows the results in ng/kg dry weight for the kale sample with background contamination and table 2 for the kale sample with elevated PCDD/PCDF contamination (mean of 3 replicates for each laboratory).

Table 1: Results of the kale sample with background PCDD/F contamination (ng/kg dry weight; mean of 3 replicates; totals including 2,3,7,8-congeners)

congener	Olden-burg	Wackers-dorf	Freiburg - method 1	Freiburg - method 2	mean of all samples	minimum of all samples	maximum of all samples
2,3,7,8-TCDD	0.04	0.07	0.06	0.06	0.06	0.04	0.08
Total TCDD	3.84	4.26	4.92	4.92	4.50	3.37	5.66
1,2,3,7,8-PeCDD	0.08	0.19	0.12 [1)]	0.10 [1)]	0.12	0.07	0.21
1,2,3,7,8-PeCDD			0.33 [2)]	0.30 [2)]			
Total PeCDD	2.26	3.08	3.06	2.87	2.86	2.15	3.38
1,2,3,4,7,8-HxCDD	0.05	0.12	0.08	0.08	0.08	0.05	0.13
1,2,3,6,7,8-HxCDD	0.12	0.16	0.19	0.18	0.16	0.12	0.19
1,2,3,7,8,9-HxCDD	0.14	0.16	0.14	0.13	0.14	0.13	0.17
Total HxCDD	2.19	2.52	2.67	2.66	2.51	2.14	2.74
1,2,3,4,6,7,8-HpCDD	0.95	1.21	1.30	1.24	1.17	0.92	1.34
Total HpCDD	2.08	2.57	2.73	2.70	2.52	2.02	2.82
OCDD	2.36	3.09	3.54	3.32	3.08	2.21	3.76
2,3,7,8-TCDF	1.21	0.84	0.82	0.69	0.89	0.63	1.25
Total TCDF	12.99	14.26	17.61	16.08	15.44	11.03	20.49
1,2,3,7,8-PeCDF	0.22	0.46	0.31	0.27	0.31	0.21	0.49
2,3,4,7,8-PeCDF	0.25	0.40	0.29	0.27	0.30	0.24	0.42
Total PeCDF	3.77	4.65	4.12	3.79	4.30	3.36	5.81
1,2,3,4,7,8-HxCDF	0.26	0.25	0.19	0.17	0.22	0.17	0.27
1,2,3,6,7,8-HxCDF	0.13	0.20	0.16	0.14	0.16	0.12	0.21
2,3,4,6,7,8-HxCDF	0.09	0.18	0.14	0.12	0.13	0.07	0.18
Total HxCDF	1.01	1.82	0.86	0.76	1.11	0.70	1.87
1,2,3,4,6,7,8-HpCDF	0.34	0.27	0.40	0.38	0.35	0.26	0.42
1,2,3,4,7,8,9-HpCDF	0.00	0.00	0.04	0.04	0.02	0.00	0.04
Total HpCDF	0.43	0.11	0.58	0.52	0.48	0.35	0.61
OCDF	0.23	0.29	0.30	0.28	0.28	0.22	0.31
I-TEq (NATO/CCMS)	0.43	0.60	0.47	0.51	0.55	0.42	0.64
I-TEq (NATO/CCMS)	0.38 [3)]						
dry weight (%)	18.8	19.9	20.7	20.7	20.0	18.4	20.8

[1)] data from DB5-MS-confirmation

[2)] data from DB Dioxin

[3)] with the assumption of 0.78 ng/kg for 2,3,7,8-TCDF as average of the other laboratories

Table 3: Results of the kale sample with elevated PCDD/F contamination (ng/kg dry weight; mean of 3 replicates; totals including 2,3,7,8-congeners)

congener	Olden-burg	Wackers-dorf	Freiburg - method 1	Freiburg - method 2	mean of all samples	minimum of all samples	maximum of all samples
2,3,7,8-TCDD	0.50	0.57	0.56	0.55	0.55	0.39	0.61
Total TCDD	71.47	32.68	68.48	65.32	59.63	30.69	75.30
1,2,3,7,8-PeCDD	1.10	1.80	1.26 [1)]	1.11 [1)]	1.32	0.96	1.91
1,2,3,7,8-PeCDD			2.77 [2)]	2.64 [2)]			
Total PeCDD	34.38	39.09	35.16	32.12	35.64	27.73	42.33
1,2,3,4,7,8-HxCDD	0.33	0.66	0.53	0.49	0.50	0.28	0.74
1,2,3,6,7,8-HxCDD	1.00	1.30	1.24	1.12	1.16	0.98	1.36
1,2,3,7,8,9-HxCDD	1.09	0.94	0.86	0.79	0.92	0.72	1.15
Total HxCDD	16.07	15.67	15.64	14.81	15.55	13.49	16.54
1,2,3,4,6,7,8-HpCDD	5.50	6.40	6.08	5.37	5.84	5.11	6.92
Total HpCDD	11.78	12.89	12.23	11.35	12.06	10.34	13.79
OCDD	14.45	20.24	16.34	14.68	16.43	13.66	26.07
2,3,7,8-TCDF	36.60	10.79	11.04	10.35	17.19	9.24	39.46
Total TCDF	347.76	241.60	323.98	284.01	302.03	243.57	370.44
1,2,3,7,8-PeCDF	5.46	8.93	6.18	5.47	6.51	4.80	9.47
2,3,4,7,8-PeCDF	6.09	5.57	4.67	3.92	5.06	3.27	6.42
Total PeCDF	102.36	84.77	87.49	84.05	93.29	70.98	106.75
1,2,3,4,7,8-HxCDF	4.77	2.95	2.57	2.30	3.15	2.07	5.09
1,2,3,6,7,8-HxCDF	2.27	2.68	2.28	2.03	2.32	1.87	2.78
1,2,3,7,8,9-HxCDF	0.00	< 0.01	0.00	0.00			
2,3,4,6,7,8-HxCDF	1.45	1.93	1.59	1.45	1.60	1.34	2.00
Total HxCDF	20.00	25.82	12.10	11.02	17.23	10.05	27.22
1,2,3,4,6,7,8-HpCDF	4.77	3.00	4.18	3.76	3.93	2.68	5.02
1,2,3,4,7,8,9-HpCDF	0.36	0.45	0.47	0.41	0.43	0.36	0.49
Total HpCDF	7.05	0.76	6.35	5.68	5.71	3.58	7.35
OCDF	3.31	3.48	3.44	2.93	3.29	2.74	3.93
I-TEq (NATO/CCMS)	9.23	7.05	6.03	5.31	7.28	5.55	9.40
I-TEq (NATO/CCMS)	6.92 [3)]						
dry weight (%)	21.1	21.5	23.5	23.5	22.4	20.8	23.8

[1)] data from DB5-MS-confirmation

[2)] data from DB Dioxin

[3)] with the assumption of 10.7 ng/kg for 2,3,7,8-TCDF as average of the other laboratories

The basic idea of the study was to find out whether the results of these three laboratories applying four different extraction and clean-up methods, using different GC/MS-equipment and different standard solutions, are comparable.

The results of the four different methods are in a good correspondence. Slightly different results for the most individual congeners were of minor importance: The most congeners were found in very good correspondence. Above all, differences in 2378-TCDF in the Duisburg samples are obvious. As reason for this, the use of different GC columns is asumed: Ultra 2 with its DB5-separation characteristic obviously doesn't separate all TCDF congeners from 2378-TCDF, giving a too high value. If a DB Dioxin or SP 2331 had been used, probably a chromatographic interference could have been avoided. With the assumption of the average 2378-TCDF content of the Freiburg and Wackersdorf laboratory found also in the Oldenburg

laboratory, the I-TEQ-value of the Duisburg sample would be remarkably lower and exactly in the same range as for the Wackersdorf and Freiburg laboratory (6.92 pg I-TEQ/kg d.w. instead of 9.23 pg I-TEQ/kg d.w.).

A similar problem occurs with the determination of 1,2,3,7,8-PeCDD on a DB-Dioxin-column: On DB Dioxin, two other PeCDD isomers (1,2,4,6,7-PeCDD and 1,2,4,8,9-PeCDD) can overlap 1,2,3,7,8-PeCDD (1). Thus, the Freiburg laboratory injected the samples for confirmation purposes on a DB 5-MS. The 1,2,3,7,8-PeCDD content on a DB5-MS-column was about 40 % of the content which was determined on a DB-Dioxin column. This again is a confirmation of the importance of the consideration of the separation power of the chromatographic columns.

As a result, all extraction and clean up methods give the same results. The standard solutions are in good correspondence. It is important, however, to avoid chromatographic interferences in samples of vegetable origin. 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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