

Proficiency test on the determination of PCDD/Fs and PCP in guar gum and follow-up analyses

Kotz A¹, Malisch R¹, Wahl K¹, Hädrich J¹, Adamovic K¹, Gerteisen I¹, Leswal S¹, Podestat U¹, Schächtele J¹, Stumpf C¹, Tritschler R¹, Winterhalter H¹

¹ EU Community Reference Laboratory (CRL) for Dioxins and PCBs in Feed and Food, State Laboratory for Chemical and Veterinary Analysis (CVUA), Freiburg, Germany

Abstract

A proficiency test on the determination of PCDD/Fs, PCBs and PCPs in guar gum was organized by the CRL for Residues of Pesticides - Single Residue Methods, Stuttgart, Germany and the CRL for Dioxins and PCBs in Feed and Food, Freiburg, Germany, in 2008. The concentration range and congener pattern in the PT samples reflected the range of contamination of guar gum originating from India in 2007. More than 50 laboratories participated in this PT analyzing PCDD/Fs in both samples, with more than 2/3 of the laboratories reporting results within the range of +/- 2 z-scores of the assigned value. Additionally further tests due to some elevated levels reported in the PT were performed at the CRL for Dioxins and PCBs focusing on different extraction techniques and solvents. Overestimation by more than a factor of two in results from application of CALUX bioassays was found for both samples, mainly due to differences between REP-datasets and TEF-values for higher chlorinated PCDD/Fs, here especially for 1,2,3,4,6,7,8-HpCDD.

Introduction

Guar gum is an edible thickening agent extracted from guar beans. Food grade guar gum powder is authorized as food additive and used as a thickening, emulsifying, binding and gelling additive in a very wide range of foodstuffs. Industrial grade guar gum powder is used in various non-food sectors. India produces approximately 80 % of the world's total production of guar beans.

In July 2007, a contamination by PCDD/Fs and pentachlorophenol (PCP) in guar gum originating from India was found. Contamination levels of PCDD/Fs and PCP in certain batches of guar gum were very high (up to a range of 100 mg/kg PCP and nearly 1000 ng WHO-PCDD/F-TEQ /kg product). To ensure a uniform approach within the EU, the Commission's services derived the following reference points of action for unacceptably high levels of dioxins and pentachlorophenol in guar gum:

- Pentachlorophenol: Any level of pentachlorophenol in guar gum exceeding 0.01 mg/kg taking into account the measurement uncertainty is considered as un-acceptable.
- Dioxins: Levels of dioxins (PCDD/F) in guar gum should be lower than 0.75 pg WHO-PCDD/F-TEQ /g product (or 0.75 ng WHO-PCDD/F-TEQ /kg product). Levels higher than 0.75 pg WHO-PCDD/F-TEQ /g product are considered as unacceptably contaminated with dioxins.^{1,2,3,4}

It was decided to organize a study on determination of dioxins (PCDD/F), PCBs (dioxin-like PCBs and indicator PCBs) and pentachlorophenol (PCP) in guar gum samples.

Two samples of guar gum were sent out for analysis with one sample covering very roughly the range of the above mentioned reference points and one sample clearly exceeding these reference points, however, not reflecting the range of extremely high contaminated samples. After performance of the study, these two samples were kept available for the participants as proficiency test-checked reference material with consensus values derived from the participants' results.

Due to some elevated levels found while performing a test for sufficient homogeneity and during the proficiency test itself, further tests on the extraction of guar gum and determination of PCDD/F were performed. Some elevated results for PCDD/F congeners and the Total-TEQ raised questions with regards to the effectiveness of the extraction of PCDD/Fs from guar gum samples and the "trueness" of the derived consensus values.

Materials and Methods

Proficiency test:

77 laboratories registered for this PT. 54 laboratories reported results for PCDD/F and PCB for samples A and 53 for sample B using GC/MS methods. 9 laboratories reported results for sample A and B using bioassays. Two different batches of guar gum contaminated with PCP and PCDD/F were selected. These two batches very roughly covered the PCDD/F concentration range between the reference point of action at 0.75 ng WHO-PCDD/F-TEQ/kg product and clearly elevated levels with more than a factor of 50 above the reference point of action. There was no fortification with the analytes of interest or drying of the samples prior to bottling or distribution.

The test for sufficient homogeneity was performed according to “The international harmonized protocol for the proficiency testing of analytical chemistry laboratories”⁵. The test portions of sample A and B were analyzed using Twisselmann hot extraction with ethanol/toluene (70/30, v/v). There was no pre-treatment, e.g. drying, of the samples before extraction.

The following extraction and clean-up steps and GC/MS measurement were applied:

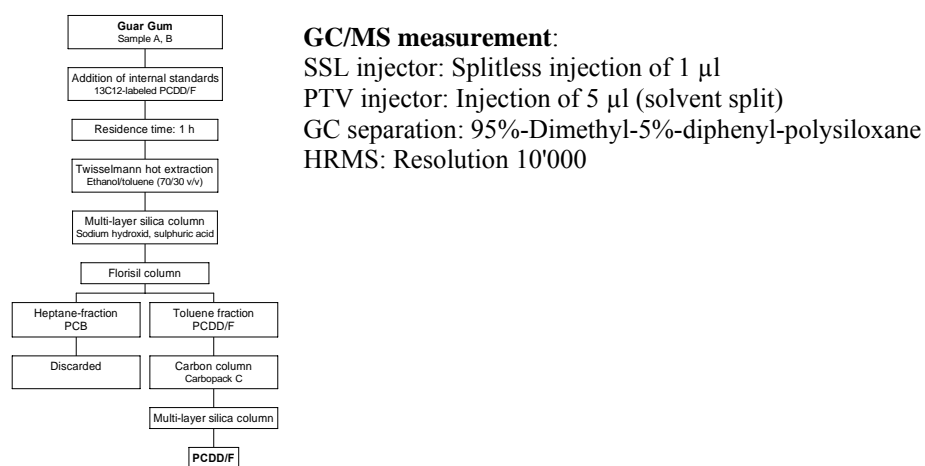


Figure 1: Extraction, clean-up and GC/MS measurement of PCDD/F in guar gum

Results and Discussion

The determination of the assigned values for WHO-PCDD/F-TEQ and individual PCDD/F congeners was performed according to “The international harmonized protocol for the proficiency testing of analytical chemistry laboratories” (IUPAC Technical Report, Pure Appl. Chem, Vol. 78, No. 1, pp-145-196, 2006) by estimation of the assigned values as the consensus of participants’ results (using only GC/MS results). The Huber robust mean was taken as assigned value after exclusion of extreme outliers (outside the range of ± 50 % of the median of all reported results) and examination of the distribution of the remaining results using histogram and kernel density estimation, if necessary. The Huber robust mean was additionally compared with the robust mean of all values calculated using Algorithm A (ISO 13528:2005) and the median of all values.

For sample A, 87 % of reported results were used for calculation of the assigned value for WHO-PCDD/F-TEQ, for PCDD/F congeners between 77 – 92 % (mean of 85 %) of all values were used. For sample B, 85 % were used for the WHO-PCDD/F-TEQ and between 65 and 90 % (mean of 83 %) for PCDD/F congeners.

The assigned value was not calculated for several PCDD/F congeners, WHO-PCB-TEQ, dl-PCBs and indicator PCBs if no unimodal distribution of results was found after elimination of outliers or if mainly LOQs were reported for congeners.

Commission Regulation (EC) No 1883/2006 sets performance criteria for confirmatory methods, among them for trueness - 20 % to + 20 %. In order to check this criterion for WHO-PCDD/F-TEQ results, the target deviation σ_p is set as 10 %. For individual congeners, 20 % was chosen.

Participants' z-scores were calculated as:

$$z = (x - x_a) / \sigma_p$$

x_a : assigned value

x : participants result

σ_p : target deviation (fitness-for-purpose-based "standard deviation for proficiency assessment")

Acceptable z-scores for WHO-PCDD/F-TEQ results lie between - 2 and + 2. Not acceptable z-scores lie outside the range of - 3 to + 3 for individual congeners and sum parameters.

Congener pattern and WHO-TEQ

The two samples showed the typical PCDD/F congener pattern found in technical PCP products. The most abundant congeners in both samples were OCDD, 1,2,3,4,6,7,8-HpCDD and OCDF with several orders of magnitude higher concentrations compared to the lower chlorinated congeners. 1,2,3,4,6,7,8-HpCDD showed the highest contribution to the WHO-PCDD/F-TEQ with 60 to 70 %.

The consensus value for WHO-PCDD/F-TEQ for sample B was nearly two orders of magnitude higher than in sample A. Additionally, the WHO-PCDD/F-TEQ of sample A was about two orders of magnitude higher than the estimated WHO-PCB-TEQ.

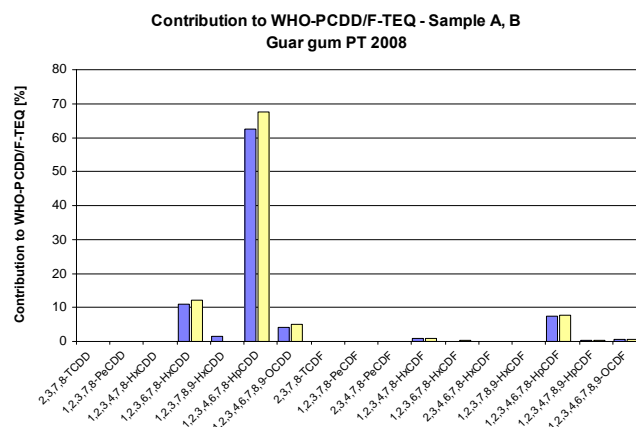


Figure 2: Contribution of PCDD/F congeners to WHO-PCDD/F-TEQ in guar gum samples A and B

Z-scores and distribution of results

The number and percentage of z-scores of WHO-PCDD/F-TEQ in the satisfactory range $-2 < z\text{-score} < 2$ are shown in table 1, the distribution in table 2. The distribution of the z-scores was comparable for samples A and B. Considerably more z-scores were above the satisfactory range, which reflects the tendency to an overestimation of the actual concentrations. An effect of the different concentration levels (factor of 50 between sample A and B) on the distribution of the results was observed. The results of both samples showed homogeneity of variance.

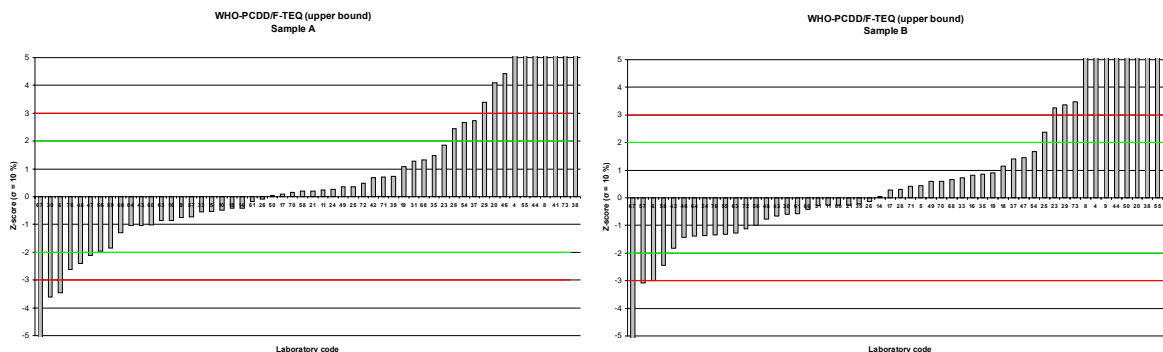


Figure 3: Z-score distribution for guar gum samples A and B

Table 1: Distribution of z-scores of WHO-PCDD/F-TEQ for sample A and B:

Z-score	Sample A (n = 54)	Sample B (n = 53)
No. of WHO-PCDD/F-TEQ values (Percentage)		
$-2 < z < 2$	36 (67 %)	37 (70 %)
$z > 2$	13 (24 %)	13 (25 %)
$z > 3$	10 (19 %)	12 (23 %)
$z < -2$	5 (9 %)	3 (9 %)
$z < -3$	2 (4 %)	2 (4 %)

Further tests at the CRL Freiburg

The follow-up analyses focused on different solvents and solvent mixtures (including different proportions of acetone) using different extraction techniques (Twisselmann, Soxhlet and ASE). The results showed an increase of higher chlorinated PCDD congeners and no significant changes for higher chlorinated PCDFs using increasing percentages of acetone, or pure acetone in the extraction solvents for ASE, Twisselmann and Soxhlet extraction. Also the elevated temperature normally applied in these three extraction methods seems to have an influence on the increase of the levels. Additionally the extraction of different amounts of guar gum (0.1 - 20 g of guar gum PT sample A) using ASE and Twisselmann extraction with ethanol/toluene were compared. Considerably higher levels for WHO-PCDD/F-TEQ and HpCDD were found for 0.1 g of guar gum, no differences were found for OCDD between different sample intake. This could be due to the higher variability for the lowest sample intake of 100 mg. Additionally considerably lower values were found for TEQ and individual congeners for the extraction of 20 g of sample using ASE. The addition of unlabeled and ^{13}C -labeled PCP standards to contaminated and not contaminated guar gum before extraction with acetone using Twisselmann extraction showed no significant differences.

In the framework of the test for sufficient homogeneity additional parameters influencing the PCDD/F results were tested, especially concerning the extremely high levels reported for sample B in some cases. The focus was set on the influence of different GC injection techniques on the PCDD/F results. Therefore these two different techniques were applied:

- Split/splitless injector: splitless injection of 1 μl
- PTV injector: injection of 5 μl in solvent split mode

Especially for the higher contaminated sample B the PTV injection of the same extracts showed considerably higher WHO-PCDD/F-TEQ results (median was about 50 % higher for PTV compared to SSL) and a different congener pattern with higher contributions of lower chlorinated congeners, especially penta- and hexachlorinated dibenzo-p-dioxins, to the WHO-PCDD/F-TEQ. As a reason for this elevated levels and considerably different congener patterns for PTV injection, particles of graphite, possible from ferrules used to fix and tighten the GC column in the injector, were identified.

Results from laboratories performing bioassays

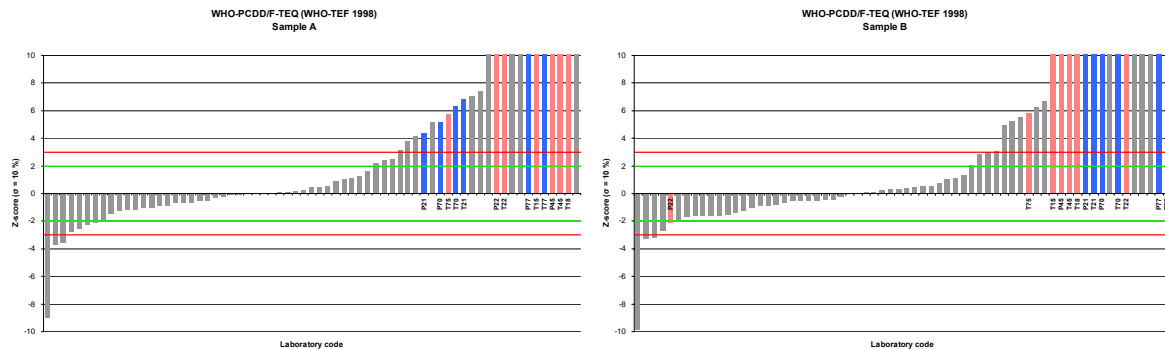


Figure 4: Z-scores for Bioassay-TEQ results for sample A and B in comparison with GC/MS results (blue: XDS-CALUX results, red: DR-CALUX: Total-TEQ)

An overestimation by more than a factor of two with CALUX bioassay was found for both concentration levels. When using the specific **REP-values** published for the two different cell lines^{6,7}, the results of the GC/MS and bioassay analysis were better comparable, due to the significant differences of REP- and TEF-values for the most abundant congener 1,2,3,4,6,7,8-HpCDD.

	WHO-TEF ₁₉₉₈	DR-CALUX-REP ₂₀₀₄ ⁶	REP ₂₀₀₄ / TEF ₁₉₉₈
1,2,3,4,6,7,8-HpCDD	0,01	0,02	2

	WHO-TEF ₁₉₉₈	XDS-CALUX-REP ₂₀₀₁ ⁷	REP ₂₀₀₁ / TEF ₁₉₉₈
1,2,3,4,6,7,8-HpCDD	0,01	0,031	3,1

Thus Z-scores were calculated for total-TEQs results of all labs using GC/HRMS not only with TEF-factors, but also with DR-CALUX-REPs (2004), and with XDS-CALUX-REPs (2001), and compared to bioassay results.

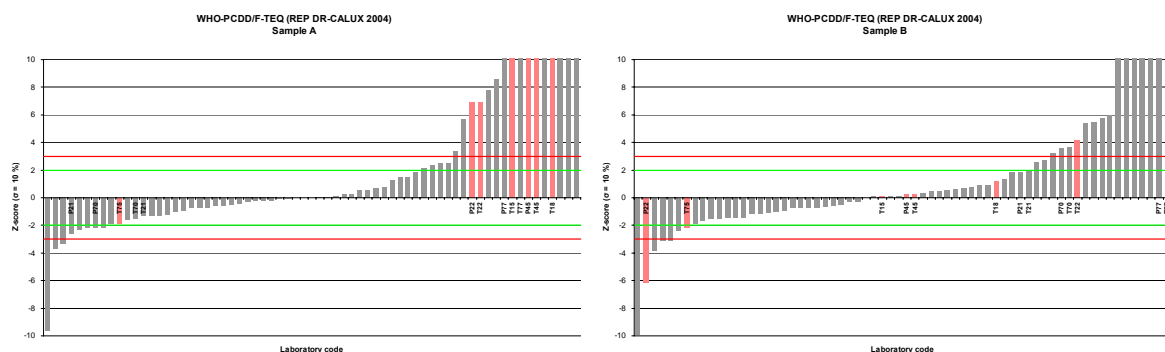


Figure 5: Z-scores for Bioassay-TEQ results for sample A and B in comparison with GC/MS results calculated from a BDS-REP data set (2004) (red: DR-CALUX: Total-TEQ)

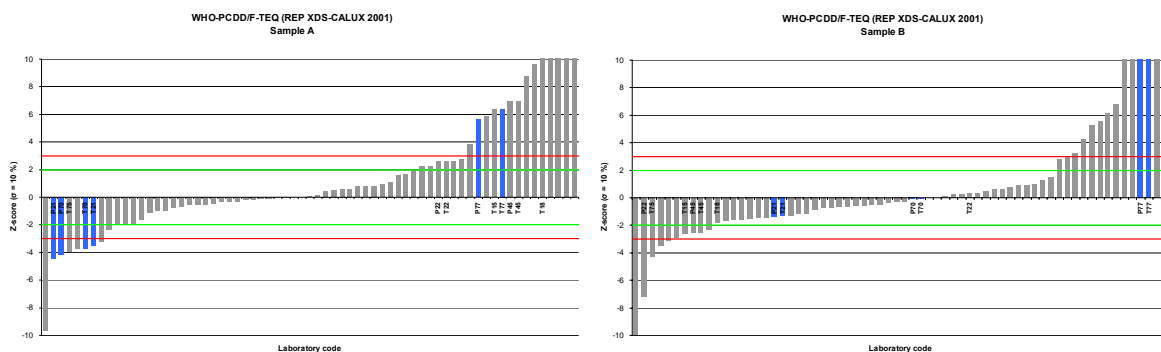


Figure 6: Z-scores for Bioassay-TEQ results for sample A and B in comparison with GC/MS results calculated from a XDS-REP data set (2001) (blue: XDS-CALUX: Total-TEQ)

For sample A, especially the XDS-CALUX-REP data set leads to GC/HRMS results better comparable to those from bioassays. For sample B, both the DR-CALUX-REP and the XDS-CALUX-REP sets lead to GC/HRMS results better comparable to those from bioassays. The differences between *REP-datasets* and *TEF-values* for higher chlorinated PCDD/Fs, here especially for 1,2,3,4,6,7,8-HpCDD may be one, but certainly not the only contributing factor to overestimation.

Acknowledgements

We would like to thank the European Commission for the financial support of the work of the Community Reference Laboratory for Dioxins and PCBs in Feed and Food Freiburg.

References

1. Publication of CRL for Dioxins and PCBs in Feed and Food, 23 August 2007, Contamination of Guar Gum from India with Pentachlorophenol (PCP) and Dioxins, www.crl-dioxin-freiburg.eu
2. Wahl, K, Kotz, A, Malisch, R, Haedrich, J, Anastassiades, M, Sigalova, I, 2008. *Organohalogen Compd* 70
3. Commission Regulation (EC) 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in food (OJ L 364, 20.12.2006, p. 5)
4. Commission Recommendation 2006/88/EC of 6 February 2006 on the reduction of the presence of dioxins, furans and PCBs in feedingstuffs and foodstuffs (OJ L42, 14.2.2006, p. 26)
5. IUPAC Technical Report, *Pure Appl. Chem*, Vol. 78, No. 1, pp-145-196, 2006
6. Scippo, M-L, Eppe, G, De Pauw, E, Maghuin-Rogister, G, 2004. *Talanta* 63, 1193-1202
7. Brown, DJ, Chu, M, Van Overmeire, I, Chu, A, Clark, GC, 2001. *Organohalogen Compd* 53, 211-214