Community Reference Laboratory
for Dioxins and PCBs in Feed and Food

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Correlation between pentachlorophenol (PCP) and dioxins in contaminated guar gum from India

based on data as available on 27 August 2007

On 24 July 2007, the Rapid Alert System for Food and Feed (RASFF) of the European Commission received a notification from the competent authorities of Switzerland concerning a finding of a **serious contamination by dioxins and pentachlorophenol in guar gum originating from India**. This contamination incident was reported through the RASSF on 25 July 2007 to all Member States by alert notification 2007.0499 (and additions).

The contamination levels of dioxins and pentachlorophenol found in numerous batches of guar gum vary considerably. The **initially found high levels of up to 480 pg WHO-PCDD/F-TEQ/g product and 4 mg PCP/kg** gave reason for serious concern. Analyses of samples collected to follow up these findings confirmed these high levels in certain batches; even higher levels were detected in few cases. However, also low contaminated or uncontaminated guar gum was found. The dioxin pattern confirms that the **presence of dioxins is related to the presence of pentachlorophenol**.

As regards the **reference point of action for unacceptable levels of dioxins and pentachlorophenol in guar gum**, the Commission services have sent the following information to the competent authorities of the Member States in the interest of an uniform approach within the EU:

- **Pentachlorophenol** should be absent in guar gum (and also other food products). Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC (OJ L 70, 16.3.2005, p.1) does not establish a MRL for pentachlorophenol. However it is foreseen in a draft Commission Regulation amending Regulation 396/2005 currently notified to WTO for comments that for pentachlorophenol **the default MRL of 0.01 mg/kg (limit of quantification) would apply for all foods and feeds**. Currently national MRLs exist of 0.01 mg/kg and 0.05 mg/kg. **Therefore any level of pentachlorophenol in guar gum exceeding 0.01 mg/kg taking into account the measurement uncertainty is to be considered as unacceptable**.
- As regards **dioxins**: No maximum levels have been established for dioxins in guar gum by 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). However to determine what is to be considered as an unacceptable level, reference can be made to the **maximum level existing for vegetable oils and fats which is 0.75 pg WHO-PCDD/F-TEQ /g fat (which in the case is of pure vegetable oils and fats also 0.75 pg WHO-PCDD/F-TEQ /g product)** or reference can also be made to the **action level** set by 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) for **fruits vegetables and cereals which is 0.4 ng/kg product (= 0.4 pg/g product)**. Following the requirement that contaminant levels shall be kept as low as can reasonably be achieved by following good practices at all the stages of production, processing and distribution (Article 2 (2) of Council Regulation (EEC) 315/93 of 8 February 1993 laying down Community procedures for contaminants in food**, 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 PCDD/F-WHO-TEQ/kg product). Levels higher than 0.75 pg WHO-PCDD/F-TEQ/g product are to be considered as unacceptably contaminated with dioxins.**

The question was raised whether PCP analyses would be sufficient as screening method to make sure that guar gum samples do not exceed the level of 0.75 pg WHO-PCDD/F-TEQ /g product. For evaluation of the **correlation between PCP- and dioxin levels in guar gum**, the Commission provided all data of analyses of guar gum batches, as available on 23 August 2007. According to the information available here it is assumed that all contaminated guar gum samples came from one Indian company. From altogether 151 batches, 84 were analysed for dioxins and 57 for PCP. From these, 51 samples were analysed for both PCP and dioxins. Based on these data, factors can be derived for calculation of an individual result from “mg PCP/kg guar gum” to “pg WHO-PCDD/F-TEQ/g guar gum”. Table 1 summarizes the results:

	mg PCP/kg	pg WHO-PCDD/F-TEQ/g	Factor for conversion "mg/kg PCP" to "pg TEQ/g"
No of samples	57	84	51
Min	0,02	0,05	0,86
10 % Percentile	0,05	0,25	5,47
25 % Percentile	0,28	2,28	8,49
Median	1,10	13,00	16,43
Mean	4,31	49,33	24,81
75 % Percentile	2,18	27,45	41,33
90 % Percentile	5,42	92,04	52,00
95 % Percentile	15,08	156,00	76,55
99 % Percentile	55,81	539,13	100,75
Max	80,00	738,00	101,50

Table 1: Frequency distribution of levels of PCP and dioxins and resulting conversion factors for guar gum samples from India (all samples as available on 23 August 2007)

These data show an **extremely wide range of levels of PCP (0.02 to 80 mg/kg;** factor of 4000 between minimum and maximum**) and dioxins (0.05 to 738 pg WHO-PCDD/F-TEQ/g;** factor of about 15000 between minimum and maximum**).** The **conversion factors** for calculation of dioxin levels (in pg WHO-PCDD/F-TEQ/g) from PCP levels (in mg/kg) **range between about 1 and 100**, with an average (mean) of about 25 and a median of 16.4. 90 % of all samples have a factor below 52 and 95 % below 77.

These extremely wide ranges can have different reasons:

1. Guar gum batches can have been contaminated by **different batches of PCP**. As an example, Hermann Brunner reported in his thesis (1990) results of 2.04 and 2.67 µg BGA-TEQ/g for two PCP products (Witophen P and Rhone Poulenc) respectively 0.51 and 0.09 pg BGA-TEQ/g for two Na-Pentachlorphenol products (Dowicide and Preventol).
2. During processing of guar gum, dioxin levels could possibly change, e.g. by **formation** from precursors at heating processes.
3. Analytical results might be unreliable in some cases. As an example, batch number 11560 was analysed by the Dioxin-CRL (Freiburg, Germany) and the CRL for pesticides with single residue methods (Stuttgart, Germany) with a result of 457 pg

WHO-PCDD/F-TEQ/g and 33.4 mg/kg PCP, whereas another lab found 406 pg WHO-PCDD/F-TEQ/g and 4 mg/kg in the same batch. This reported low level of 4 mg/kg PCP seems to be a considerable underestimation of the "true value" (factor of 8 too low). **These considerable underestimations of the PCP levels can be the result of insufficient extraction due to wrong pH values:** According to reports of CVUA Münster, Germany, a sample highly contaminated with dioxins (485 pg WHO-PCDD/F-TEQ/g) had a relatively low level of about 5 mg/kg PCP when directly extracted with an organic solvent (acetonitrile). However, **extraction with acidified acetonitrile** (mixed in a proportion of 9:1 with 2.5 n HCl) **resulted in a 10fold increase** to about 59 mg/kg PCP. CRL Stuttgart uses the QuEChERS-method (extraction with acetonitrile, in the beginning acidified with 0.4 % acetic acid and later modified to acidification with citrate salts; pH 5; see http://www.crl-pesticides.eu/library/docs/cvuas/QuechersForGuarGum.pdf). As a result, the control of the correct pH value is important to make sure that both pentachlorphenol and Na-Pentachlorphenol can be extracted from a sample.

4. The reported analytical results might be **unreliable at the lower concentration ranges** due to the fact that in the beginning of the crisis, high levels of PCP and extremely high levels of dioxins were found, and industry / laboratories might have tried to make sure that guar gum samples with these high levels are quickly identified. However, the sensitivity of the analytical methods might have been insufficient in some cases to determine reliably lower levels of contamination as requested by the reference points of action for unacceptable levels set by the Commission. Reliable analyses in such a wide range of contamination (factors between minimum and maximum levels found to be in the range between 4000 and 15000) would require two different analyses: one screening method to detect samples in the range of high contamination (e.g. above 1 mg/kg PCP and above 10 pg WHO-PCDD/F-TEQ/g), then performance of another analysis to analyse reliably in the range of the reference points of action for unacceptable levels (range of 0.01 mg/kg PCP and 0.75 pg WHO-PCDD/F-TEQ/g). From practical experience it is known that it is difficult to get such duplicate analyses paid for and therefore these are not performed in all cases. However, without a screening for high contamination levels, samples with high levels of dioxins and PCP can cause a cross-contamination within the laboratory causing elevated levels in otherwise uncontaminated samples, and performance of analyses aiming to detect high levels would not meet the analytical reliability required to detect low levels.

5. For PCP, some results are reported as "< 1 mg/kg" which would indicate that the method is a factor of 100 less sensitive than required. Some results are reported as one-digit result, e.g. "3 mg/kg" indicating that it might have been a rough analysis estimating the presence of PCP rather than a method confirming a certain level exactly. Also for dioxin results, it is unclear whether the sensitivity was always low

enough to get reliable data also for the range of low contamination. It has to be assumed that all results are reported as upper bound results, as required by Commission Regulation (EC) No 1881/2006: Upper bound concentrations are calculated on the assumption that all the values of the different congeners below the limit of quantification (LOQ) are equal to the limit of quantification. However, it is unclear whether these results cause an overestimation of dioxin levels in lower concentration ranges due to relatively high LOQs. This could be seen from analytical reports showing the lower and upper bound concentrations. Unfortunately, these data are not available here.

As a result, **a constant ratio between PCP and dioxin levels cannot be expected**. In particular the use of the available data for the **low concentration range** for calculation of a correlation between PCP and dioxin levels **seems to be extremely critical.**

For comparison: At the Belgium dioxin crisis, one batch of polychlorinated biphenyls (PCB) contaminated considerable parts of the food chain: It is assumed that about 25 - 40 kg f a PCB product (waste) was discarded into about 107 t of fat which then contaminated thousands of tons of feeding stuff. In this particular case, it was possible to derive a constant ratio between PCB- and dioxin levels and to calculate action levels for PCBs which allowed to make sure that samples not exceeding these action levels for PCBs would also not exceed tolerances for dioxins.

The Indian guar gum case is different: It is unknown how many batches of PCP might have caused this contamination, and in addition how the ratio might have been influenced by formation processes and analytical problems. Therefore, a more complex statistical approach was used.

All data from guar gum samples and guar gum containing samples made available to CRL Freiburg by the Commission were previously cleaned from any redundantly occurring results. All of the remaining data sets (51) for which both PCP (mg/kg) and Total-TEQ (pg/g) are available, were then included in an evaluation of a possible correlation between the PCP and TEQ sample concentrations.

CRL Freiburg's own data (with determination of PCP at CRL Stuttgart) had shown a very high correlation (see figure 1 "Correlation TEQ-PCP CRL Freiburg"; R2 = 0,9999).

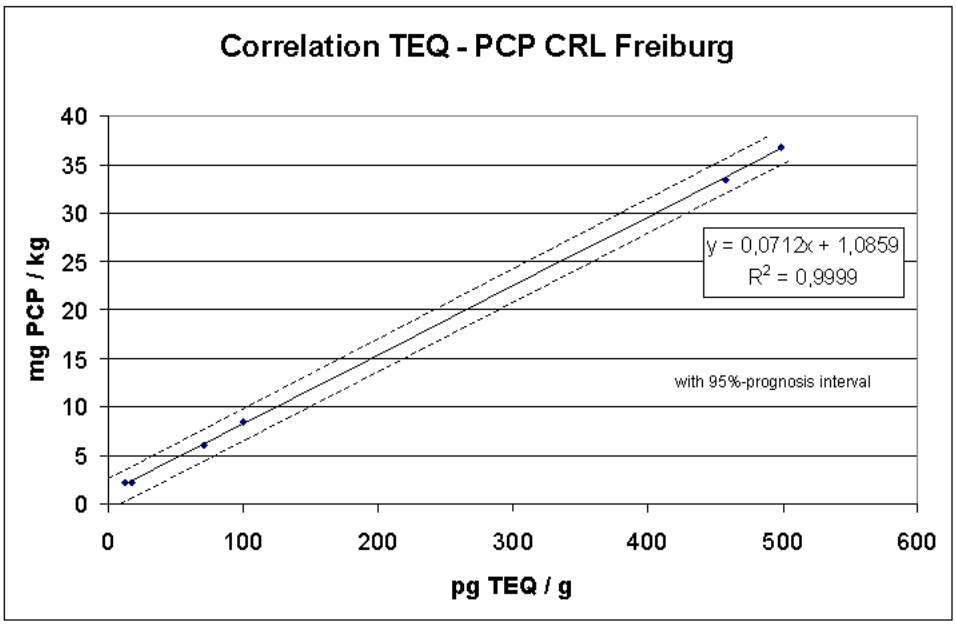

Figure 1: Correlation TEQ-PCP according to results of CRL Freiburg (determination of dioxins at CRL for dioxins and PCBs Freiburg, Germany, and of PCP at CRL for pesticides/single residue methods Stuttgart, Germany)

This correlation is not so good for all available data put together in one plot (see figure 2 “Correlation TEQ-PCP (1)”; R2 = 0,8139).

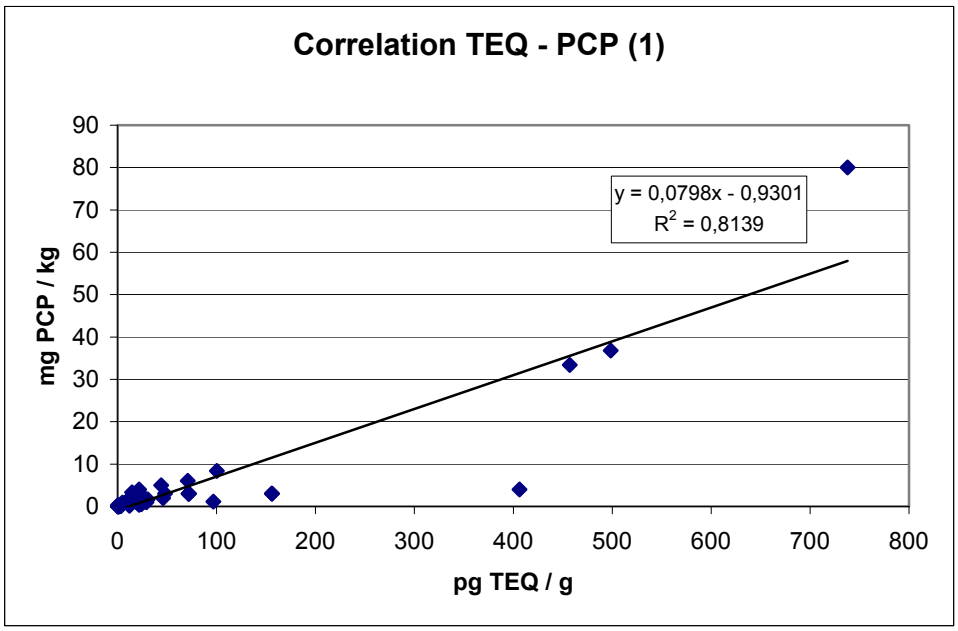

Figure 2: Correlation TEQ-PCP (1) for all data (no of samples: 51)

Next, results at higher concentrations (above 200 pg TEQ/g) were excluded for a possibly better approach of the concentration range of interest, namely PCP at a 0.01 to 0.02

mg/kg range and Total-TEQ around the 0.75 pg TEQ/g level. Results however show a dramatic drop in the square correlation coefficient (see figure 3 “Correlation TEQ-PCP (2)”; R2 = 0,3933).

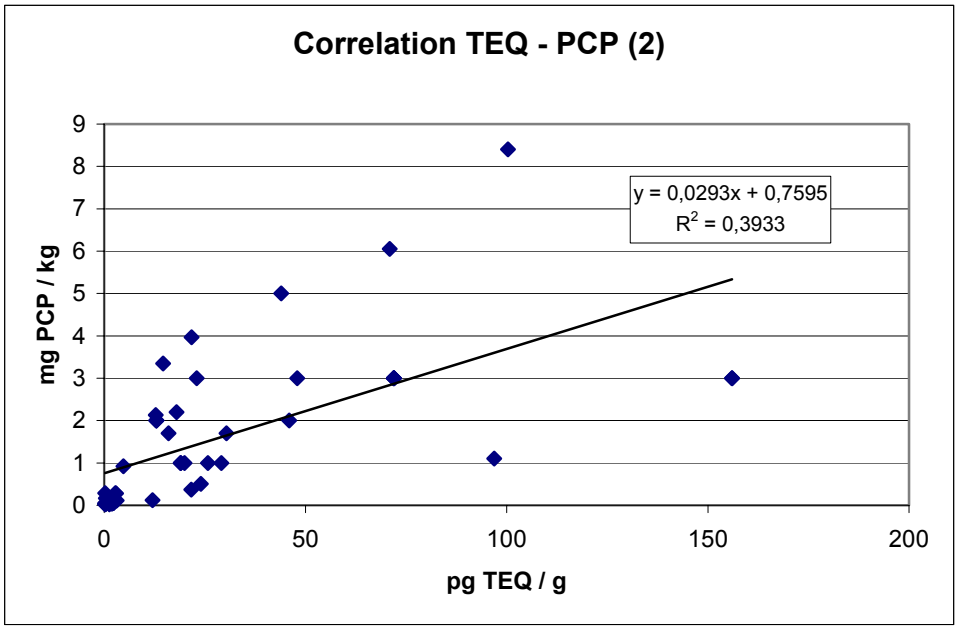

Figure 3: Correlation TEQ-PCP (2) excluding concentration levels above 200 pg TEQ/g

Further, including only samples below 14 pg TEQ/g, the R2 value went back up again to 0,7526 (see figure 4 “Correlation TEQ-PCP (3)”), and for samples below 5 pg TEQ/g, R2 again dropped down to just 0,3977 (see figure 5 “Correlation TEQ-PCP (4)”), which means that there is no linear correlation at all.

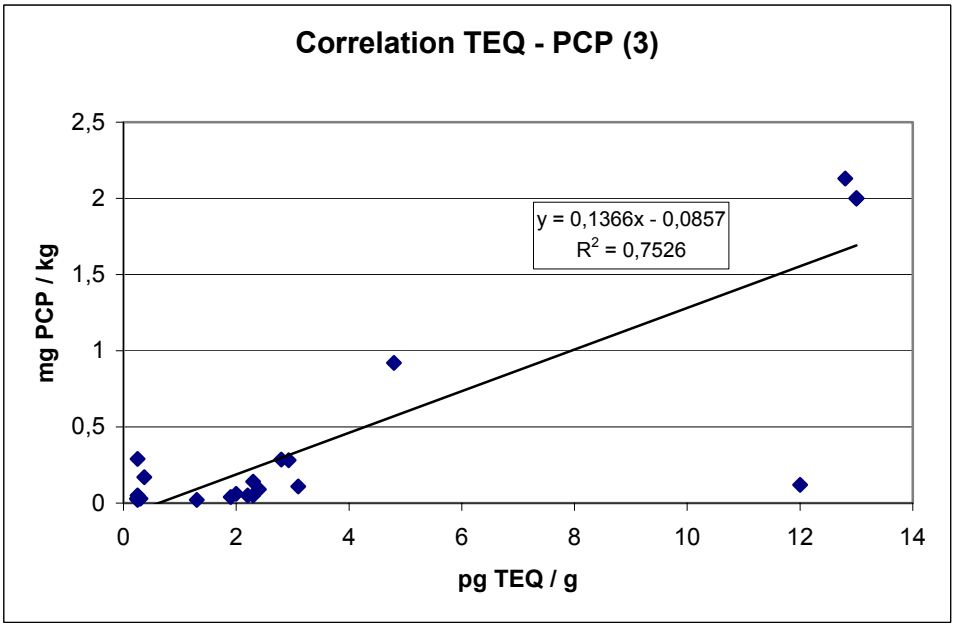

Figure 4: Correlation TEQ-PCP (3) excluding concentration levels above 14 pg TEQ/g

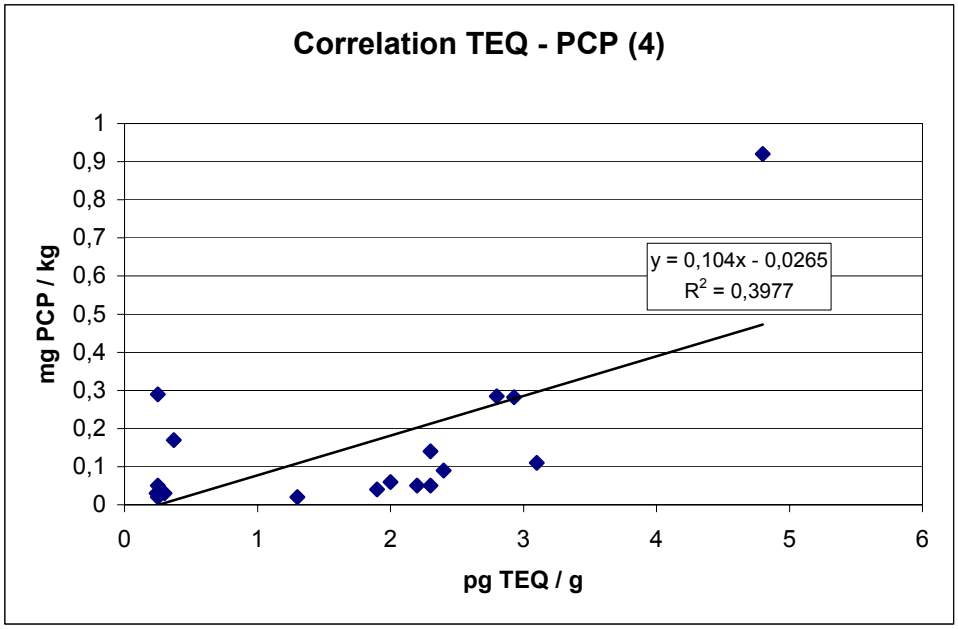

Figure 5: Correlation TEQ-PCP (4) excluding concentration levels above 5 pg TEQ/g

These evaluations confirm the **concern regarding the reliability of the available data in the lower concentration range**, as outlined earlier. Therefore, only samples with levels above 1 mg PCP/kg were used for calculation of the conversion factor. Table 2 summarizes the results. Only the sample with reported levels of 406 pg WHO-PCDD/F-TEQ/g and a PCP-level of 4 mg/kg was eliminated as obvious outlier due to a considerable underestimation of the true PCP level, as the CRL Stuttgart found 33.4 mg/kg PCP in the same batch (see earlier discussion).

Statistical parameters	PCP (mg/kg)	dioxins (pg/g)	Conversion factor from mg PCP/kg to pg TEQ/g
	1	19	19,0
	1	19	19,0
	1	20	20,0
	1	25,8	25,8
	1	29,1	29,1
	1,1	96,9	88,1
	1,7	16,0	9,4
	1,7	30,4	17,9
	2	13	6,5
	2	13	6,5
	2	13	6,5
	2	46	23,0
	2,13	12,8	6,0
	2,2	18,0	8,2
	3	23	7,7
	3	48	16,0
	3	72	24,0
	3	72	24,0
	3	72	24,0
	3	72	24,0
	3	156	52,0
	3	156	52,0
	3,35	14,6	4,4
	3,97	21,7	5,5
	5	44	8,8
	6,05	71,0	11,7
	8,4	100,3	11,9
	33,4	457,0	13,7
	36,8	498,4	13,5
	80	738	9,2
no of samples	30	30	30
Min	1,0	12,8	4,4
10 % Percentile	1,0	13,0	6,5
25 % Percentile	1,8	19,0	8,3
Median	3,0	37,2	14,8
mean	7,4	99,6	19,6
75 % Percentile	3,3	72,0	24,0
90 % Percentile	10,9	186,1	31,4
95 % Percentile	35,3	479,8	52,0
99 % Percentile	67,5	668,5	77,6
max	80,0	738,0	88,1

Table 2: Frequency distribution of levels of PCP and dioxins and resulting conversion factors for guar gum samples with PCP levels above 1 mg/kg

Based on these data, the following conclusions can be drawn:

- The median conversion factor of 15 would result in a dioxin level of 0.15 pg WHO-PCDD/F-TEQ/g product for a PCP level of 0.01 mg/kg.
- The 95th percentile of the conversion factor is 52 resulting in a dioxin level of 0.5 pg WHO-PCDD/F-TEQ/g product.
- The maximum conversion factor of 88 would result in a dioxin level of 0.88 pg WHO-PCDD/F-TEQ/g product exceeding the maximum acceptable level of 0.75 pg WHO-PCDD/F-TEQ/g guar gum as recommended by the Commission.
- Whether this maximum conversion level is another outlier, cannot be clarified because of lack of data for evaluation of the reliability of this analytical result.

These findings are in agreement to the following observations:

- The CRLs (determination of PCP at CVUA Stuttgart and of dioxins at CVUA Freiburg) found batches with conversion factors from about 6 to 13, depending on the concentration ranges with a tendency to higher factors with higher ranges.
- This is confirmed from the regression lines in figures 2 and 4: For all data (including highly contaminated samples up to 738 pg TEQ/g), a factor (calculated as 1/slope) of 12.5 was derived for conversion “mg PCP/kg” to “pg TEQ/g”. For a range up to 14 pg TEQ/g, the conversion factor calculated from the slope is 7.3. Both are in very good agreement with the CRL findings.
- If no acidified solvent (e.g. acetonitrile) is used or the pH value is not correct at liquid/liquid distribution steps, PCP is extracted only to about 10 %. Therefore, samples with conversion factors much higher than 20 should be checked for the extraction and clean up procedures. If PCP was not extracted with an acidified solvent or the pH value is not correct at liquid/liquid distribution steps, there is reason to assume that the reported PCP level seems can be unreliable.

These calculations do not include measurement uncertainty.

Conclusions

- **More reliable data in the low contamination range** are necessary to answer the question finally whether PCP screening is a fully sufficient substitute for determination of dioxins with confirmatory methods.
- It is extremely important to make sure that **PCP is extracted from guar gum with acidified acetonitrile** and to make sure that the pH value is correct at liquid/liquid distribution steps in order to avoid considerable losses of PCP and/or Na-PCP (of 90 %, as shown with one sample).
- The **extraction and clean up procedure for PCP should be provided together with the analytical result** in order to make sure that a low level of PCP is not the result of an insufficient extraction or clean up procedure. It **must be clear that an acidified solvent was / is used.** For existing data, this question should be clarified in order to allow a re-evaluation of the ratio between TEQ- and PCP-levels. In particular samples with a conversion factor about 20 might be suspicious for an extraction of PCP not with an acidified solvent. Future reports of PCP results in guar gum should include information about the kind of extraction. Questions regarding the analysis of pentachlorophenol in guar gum are addressed by the CRL Stuttgart.
- **The analytical methods applied have to be able to determine the analytes at the reference points for action for unacceptable levels of PCP of 0.01 mg/kg and dioxins of 0.75 pg WHO-PCDD/F-TEQ/g.**
- This might require use of a **separate analytical screening method for high contamination levels and of a routine method for determination of residues in the range of the reference points** in order to meet the ranges of linearity and to avoid a cross-contamination within the laboratory (causing elevated levels in otherwise uncontaminated samples).
- The derived **conversion factors for calculation of a dioxin level based on PCP determinations are applicable only to this particular contamination case**. Other cases of PCP contamination can be caused by different PCP products or other production processes with an influence on the ratios between these analytes.