THE NORWEGIAN POPs IN FOOD-STUDY: 15 YEARS OF PROFICENCY TESTING

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Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/PCDFs), dioxin-like polychlorinated biphenyls (dl-PCBs) and brominated flame retardants (BFRs) are persistent, organic pollutants (POPs) that are globally distributed in practically all environmental compartments. As they are extremely resistant to decomposition and highly lipophilic, bioaccumulation and biomagnification are properties typical for these substances.
Effects on humans when exposed to POPs are many and adverse, including cancer, endocrine disruption, developments and reproduction disorders, prenatal mortality and immunosuppression[1, 2].
Humans are exposed mainly through the diet, with food of animal origin usually being the predominant source. Maximum and action limits for concentration of several POPs in food and feed, are set in the European Union as well as in other countries.

In order to ensure sufficient quality of chemical analysis, official control laboratories within the EU as well as all analytical test laboratories which are accredited according to ISO/IEC 17025:2005 have to demonstrate their competence by participation in proficiency testing. The Norwegian Institute of Public Health has been organizing Interlaboratory Comparison (ILC) studies on the determination of PCDDs/PCDFs, dl-PCBs, indicator PCBs as well as selected BFRs in three natural food items annually since year 2000[3]. Up to 104 laboratories from 46 countries world-wide have reported results. This has provided a powerful tool for evaluating the quality and uncertainty in the analysis of dioxin-like compounds and other POPs in different food items such as meat, fish, eggs and dairy products[2].

Materials and methods

Target Compounds

From 2000-2003 the participants in the study were asked to determine PCDDs/Fs and dl-PCBs. In 2004 the study was expanded with 8 PBDEs and the HBCDs, and finally, in 2005, six indicator PCBs were included. Today, the analytes to be determined are all seventeen 2,3,7,8-substituted PCDDs/PCDFs, the four non-ortho substituted PCBs #77, 81, 126 and 169 and the eight mono-ortho substituted PCBs #105, 114, 118, 123, 156, 157, 167 and 189. Also and if wished for, the laboratories are offered the opportunity to determine eight PBDEs #28, 47, 99, 100, 153, 154, 183 and 209, six indicator PCBs #28, 52, 101, 138, 153 and 180, total HBCD and its three isomers (α-, β-, γ-HBCD).

Test Material

The test materials consist of three (non-spiked) naturally contaminated food products. When needed, a product known to be highly contaminated is mixed with a background contaminated product so to obtain a reasonable contamination level. Homogenization of solid foodstuffs is performed by repeatedly grinding and mixing the food items. Liquid samples are stirred, if necessary at elevated temperatures. No further treatment of the samples, like for example freeze drying, is performed. Sub samples of the homogenates are placed into carefully cleaned screw-cap bottles and stored at -20 °C until shipment.
For all rounds, standard solutions of all analytes dissolved in n-nonane, were provided by Cambridge Isotope Laboratories, Inc., Andover, MA, and shipped together with the food samples.

Testing of homogeneity

To assure the homogeneity of the solid samples without wasting time and valuable sample material and add to the uncertainty when determining trace levels analytes, we have adopted an indirect approach using electrolytic conductivity measurements after addition of sodium chloride. To measure background conductivity, boiling water is added to 10 g of homogenate followed by shaking and ultrasonification. After centrifugation and filtration, conductivity is measured. Salt is then added to approximately 10% of the total sample homogenate in such amounts as to double the natural conductivity. This sub-sample is then added to the total sample prior to further homogenization. Homogeneity of the test material is demonstrated by comparing the conductivity in

water extracts of 10 times 10 g of sample taken at random from the total batch. Relative standard deviations between the sub-samples are typically between 0.5-2.0 percent.

Statistical analysis
Several approaches have been evaluated in order to find a suitable method for the calculation of the consensus values of the analytes. As high background contaminations or false LOD are obvious challenges in these analysis and results in data sets that are very rarely normal distributed, the following methods has been found most fitted for the treatment of the results:

-For the dioxins and the dl-PCBs:
1: Congener-by-congener medians are calculated from the data reported by all laboratories using the reported detection limit for non-detected congeners.
2: Values two times the calculated medians are then defined as outliers and removed from the data set.
3: The consensus values are defined as the median of the remaining data set which then is close to normally distributed, and thereby allowing calculation of the consensus mean and relative standard deviation (RSD).

-For indicator PCBs, PBDEs and HBCD:
1: Non-detected congeners are removed from the data set prior to consensus calculation.
2: Outliers are defined as those values above two times the median of all values and are removed from the data set.
3: The consensus values are defined as the median of the remaining data for each congener.

The consensus of the lipid content is calculated as the mean after removal of values outside ± 2 SD.

Toxic equivalencies (TEQs) are calculated from the consensus values for PCDD/Fs and dl-PCBs using the toxic equivalency factors (TEFs) derived by WHO in 1998 and 2006. Z-scores of TEQs as well as the sum of indicator PCBs, PBDEs and total HBCDs is calculated for each laboratory according to the following equation

$$Z = (x-X)/\sigma$$

where x = reported value; X = consensus value; σ = target value for standard deviation.
A σ-value of 0.02 was chosen, i.e. Z-scores of +1 and -1 reflect deviations of 20% from the consensus value.

Results and discussion:
Historical participation
The number of participants has varied over the years but has been stable between 85 and 90 participants the last couple of years (figure 1). On the other hand, the number of participating countries has been increasing steadily over the years (Figure 2) and we are now covering all 5 continents.

Figure 1: Number of Participants

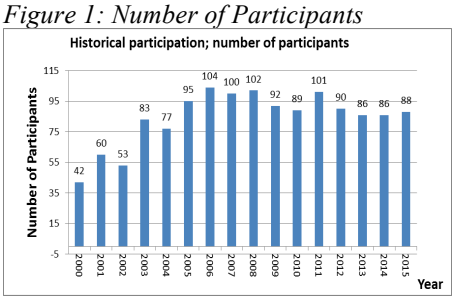
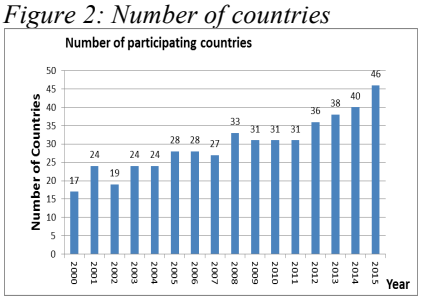


Figure 2: Number of countries



Calculations of Consensus Values

All data received are entered into a database and assessed using the method described above to calculate consensus values of the congeners.

Direct comparison between samples to assess the fitness of the method of calculations is difficult due to the wide range of matrix types and differences contaminations levels. Nevertheless, a clear trend observed is that the consensus median and consensus mean of the congeners usually are in good agreement, indicating a non-skewed distribution of eligible results and hence a good statistical method for the calculations of consensus values. For the dioxins, and the dl-PCBs, however, the difference between the median and mean increases when concentrations decreases, indicating a larger contribution from both high reported detection limits used for the calculation of upper-bound concentrations, and an increasing impact of background contamination. This is illustrated in Figure 3 and 4 below, were the consensus values of the dioxins and dl-PCBs of a medium contaminated sample of beef and a very low contaminated sample of cheese (both samples from the 2015-round of the study) is plotted against the difference in percent between the consensus median and consensus mean. As can be read from the plots, the median decreases in numerical value compared to the mean (negative difference) as the concentrations decreases.

*Figure 3: Illustration of the difference between mean and median as a function of congener concentrations in a sample of beef (*Beef-2015, consensus total TEQ: 0.42 pg TE/g fresh weight

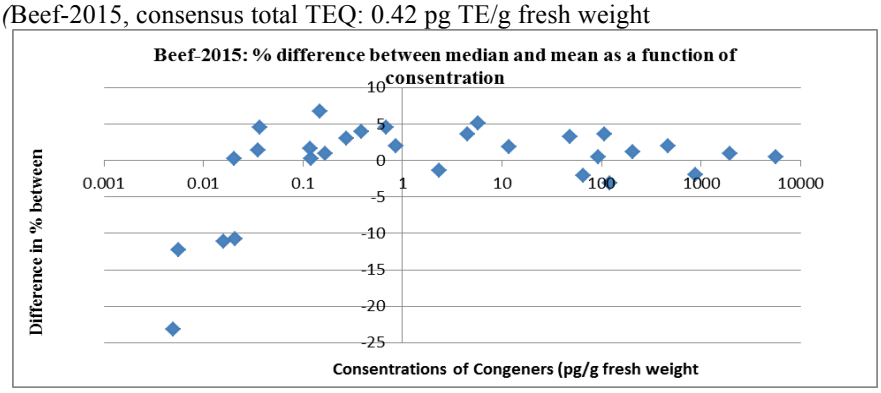
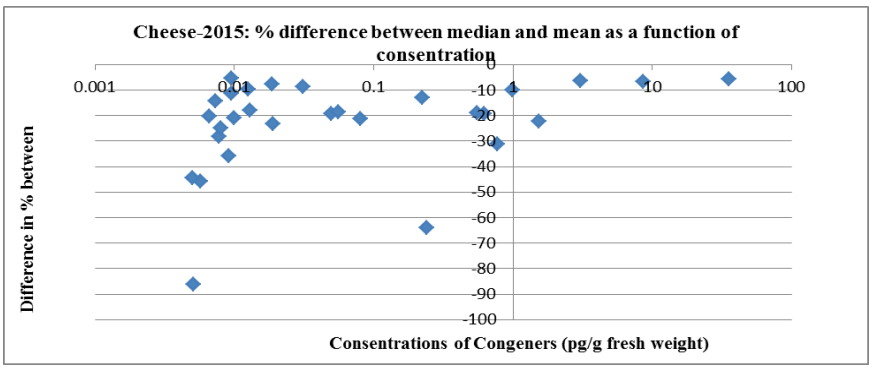


Figure 4: Illustration of the difference between mean and median as a function of congener concentrations in a sample of cheese (Cheese-2015, consensus total TEQ: 0.055 pg TE/g fresh weight)



Performance of the participating laboratories expressed as z-score values

The z-score is a measure of the number of standard deviation units (σ) an observation is deviating from the consensus value. It is used to measure how the participants are performing in the study. A σ-value of 0.2 for the TEQ z-scores has been selected to satisfy the performance criteria of the European Commission for methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs[4]. A z-score value of a measurement of ±1 is then describing a deviation of ±20 % from the consensus value.

In the last 5 rounds of the Norwegian ILC studies on POPs in food, z-scores within ±1for the total TEQ were achieved by an average of 75 % of the participating laboratories. For the sum of indicator PCB, an average of 63

% of the participants had a z-score of ±1 and for the PBDEs (excluding BDE-209 due to few results reported) 64 % of the laboratories achieved the same result. In the figures below the z-score as a function of contamination level is summarized for the 15 different matrixes analyzed during the 5 last years of the study. A clear tendency of decreasing performance with decreasing contamination levels is observed.

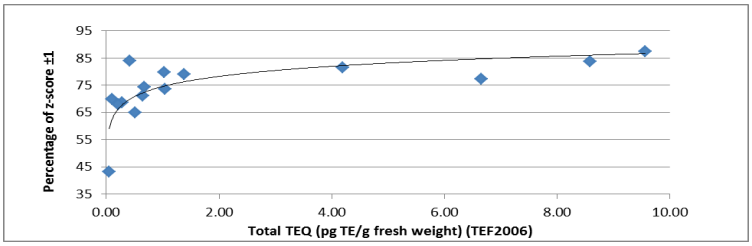


Figure 5: Z-scores ±1 for Total TEQs

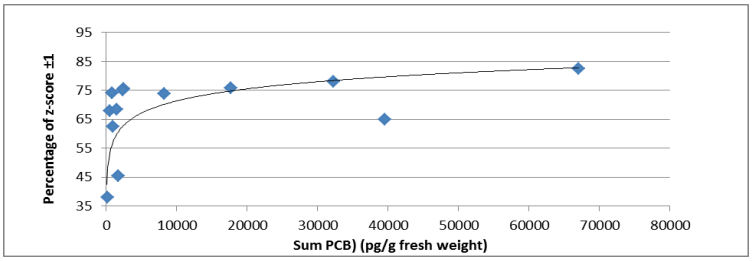


Figure 6: Z-scores ±1 for the sum of i-PCBs

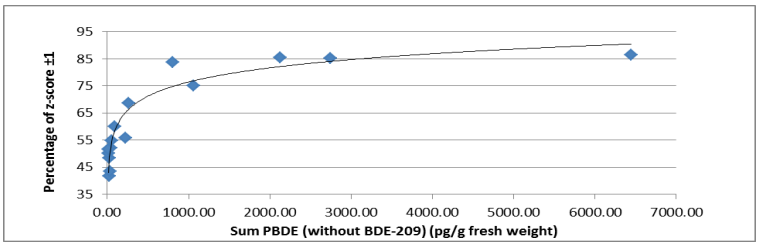


Figure 7: Z-scores ±1 for PBDEs (without BDE-209)

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