Hyphenated techniques for dioxin analysis: LC-LC-GC-ECD, GCxGC-ECD, and selective PLE with GC-HRMS or bioanalytical detection

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Introduction

The great risks associated with PCDD/F and WHO-PCB exposure through food was obvious in the Belgian dioxin scandal.i It was revealed that feed was contaminated, causing increased levels of PCDD/Fs and PCBs in pork and chicken meat, sometimes exceeding the tolerable level as much as 250 times. The total tolerable weekly intake for PCDD/Fs and dioxin-like PCBs set by the Scientific Committee on Food ii is today 14 pg WHO-TEQ/kg body weight, while the average European citizen is exposed to 8-21 pg WHO-TEQ/kg body weight. More than 90 % of human PCDD/F exposure derives from food. The reduction of human exposure to PCDD/Fs through food consumption is therefore important and necessary to ensure consumer protection. Food of animal origin normally contributes about 80 % of overall exposure. In an attempt to reduce the human PCDD/F exposure Maximum Residue Levels (MRLs) have been set by the European Commission (Council Regulations 466/2001 and 2375/2001). Shortly, the WHO-PCBs will most certainly be included in the legislation, as it shall be reviewed this year, particular with a view to the inclusion of the dioxin-like PCBs.

In order to have a better control of the occurrence and concentrations of these pollutants in various foods, and to trace the sources of exposure it is necessary to use cost-effective monitoring techniques, e.g. screening methods with high sample throughput and low rate of false negatives as suggested by the EU (Commission Directive 2002/69/EC). Such screening approaches are considerably cheaper compared to classical GC-HRMS analysis. But, some of the screening methods used today will not give any information on the relative proportions of the PCDD/Fs and WHO-PCBs, nor of the the congener pattern – only a TEQ value. Further, the precision is usually less than for confirmatory GC-MS methods. Therefore, all potential positives will have to be confirmed by GC-HRMS.

In this paper we will describe a number of hyphenated analytical techniques for screening of food and feed items for WHO-PCB and/or PCDD/F. The techniques range from bioanalytical screening tools (based on Ah-receptor binding; Ah-GFP), via analysis of marker congeners (using LC-LC-GC-ECD) to congener-specific comprehensive 2D GC techniques (GC×GC-ECD), also including efficient sample preparation techniques (based on pressurized liquid extraction; PLE). Their analytical performance will be compared and a cost-benefit analysis will be presented.

Materials and Methods

Samples: Most of the samples used for analytical performance evaluation stem from two EC funded projects “DIAC - Dioxin analysis by using comprehensive gas chromatography” and “DIFFERENCE - PCDD/Fs in food and feed – Reference methods and new certified materials”. Herring for LC-LC-GC-ECD validation was from the National Museum of Natural History.

Dioxin-TEQ analysis by shape-selective PLE and Ah-GFP: Specially made PLE extraction cells were packed with an activated carbon/ Celite mixture followed by the samples. The cells were extracted at 100 bar and 100°C using a Dionex ASE300 with first *n*-heptane (1 cycle), then dichloromethane/*n*-heptane (1:1) (2 cycles) and finally toluene (2 cycles in back-flush). The lipid content was determined gravimetrically using the first two fractions. Fat residues in the toluene fractions were removed by smal columns packed with KOH-silica, silica, and 40% sulphuric acid-silica. Thereafter, the solvent was changed to DMSO, a dilution series of each extract was prepared and the analysis was carried out using a cellbased bioassay iii with genetically modified cells that produce EGFP upon activation of the AhR by ligands such as dioxins.iv All dilutions were tested in triplicates and at two separate occasions (set 1 and 2). The Fluorescence was read using a microplate fluorometer. The TEQ of a sample was obtained using a dilution producing an EGFP induction in the EC_{10} to EC_{50} range of the TCDD calibration curve.

Marker compound analysis by LC-LC-GC-ECD: Samples were column extracted using acetone:hexane (2.5:1) and hexane:diethylether (9:1) and the lipid contents were determined gravimetrically. The lipids were removed using a column packed with KOH-silica, H_2SO_4-silica and silica and four marker congeners, viz. 2,3,4,7,8-PeCDF and PCBs 77, 126, and 157, were analysed using LC-LC-GC-ECD. Two columns were utilised to obtain enough selectivity, viz. silica and PYE (2-(1-pyrenyl)ethyldi-methylsilylated silica) and n-pentane was used as mobile phase. The appropriate eluent fraction from the silica column was heart-cut transferred to the PYE column, which was eluted in the forward direction until 15s before the first marker is expected to elute. The PYE column was then back-flushed via the silica column, and the resulting peak was transferred to the GC using a loop interface. The GC was equipped with an early vapour exit and was operated under concurrent solvent vaporisation conditions. Finally, the four markers were quantified and the remaining sixteen PCDD/Fs, three mono-ortho PCBs, and PCB 169 were estimated using their usual ratios to 2,3,4,7,8-PeCDF, PCB157, and PCB126, respectively.

Isomer-specific analysis by selective PLE and GC-HRMS: Samples were extracted and lipids removed using a Dionex ASE300 with and 40% H_2SO_4-silica loaded extraction cells. Two extraction cycles of n-heptane was used (other conditions as above). The lipid contents were determined separately. The samples were then fractionated on 8% activated carbon: Celite columns into three fractions: 1) bulk PCBs (hexane), 2) mono-ortho PCBs (50% dichloromethane in hexane, and 3) non-ortho PCBs and PCDD/Fs (toluene back-flush). Finally, residual lipids were removed using smal columns packed with KOH-silica, silica, and 40% sulphuric acid-silica and the target compound concentrations were determinged using GC-HRMS.

Isomer-specific analysis by GC×GC-ECD: Sample extraction, lipid determination, and lipid removal were as described for LC-LC-GC-ECD. The samples were then fractionated and purified as described for selective PLE with GC-HRMS detection and analysed by GC×GC-ECD.v

Results and Discussion
All methods tested produce data similar to GC-HRMS, see Figures 1-4.

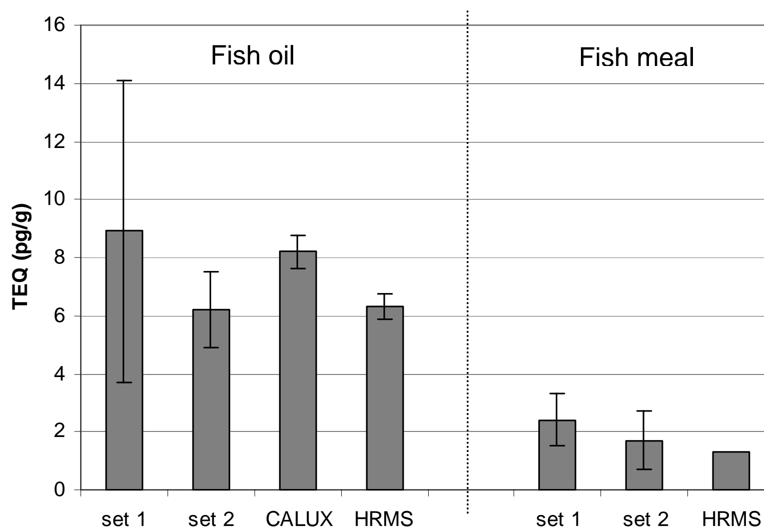

Figure 1. Dioxin content (pg TEQ (lower bound)/g sample) in fish oil and fish meal determined with different methods. Set 1 and 2 refer to the present study, which utilized PLE (n=3). The fish oil (n=6) and fish meal (n=1) CALUX and HRMS values were obtained using traditional extraction procedures. These reference values originate from the EU DIFFERENCE project.

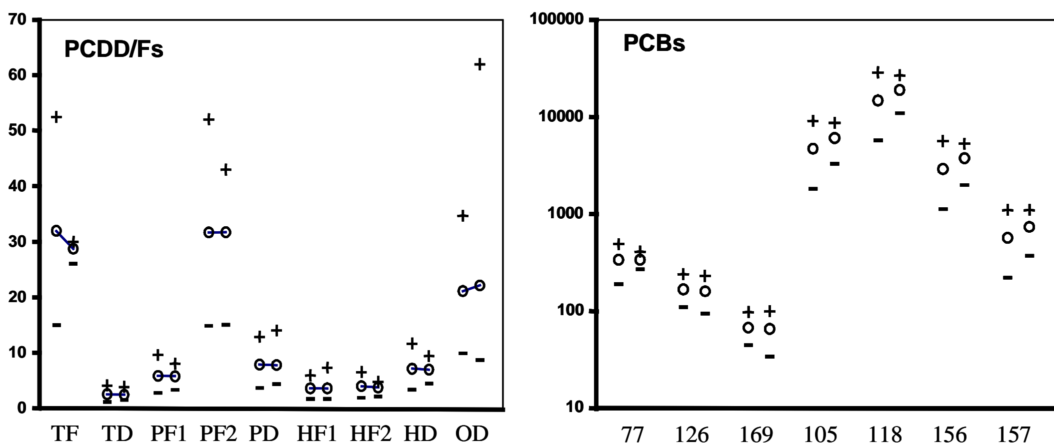

Figure 2: Comparison of average (O), maximum (+) and minimum (–) PCB and PCDD/F levels in herring from Harufjärden, as determined by LC-LC-GC-ECD (left) and GC-HRMS (right). PF1: 12378DF, PF2: 23478DF, PD: 12378DD, HF1: 123478DF, HF2: 123789DF and HD: 123678DD.

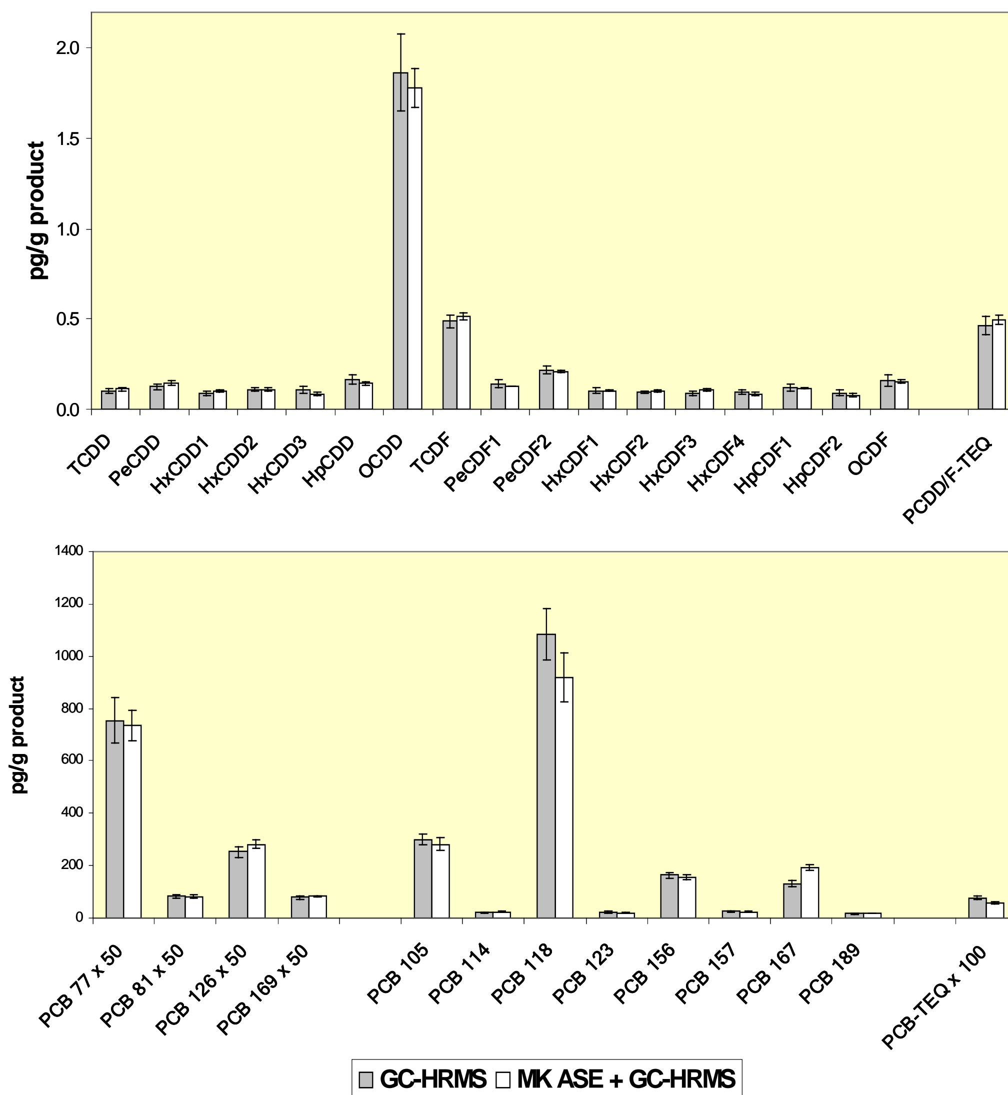

Figure 3. Comparison of the results obtained for PCDD/F and WHO-PCB in a compound feed sample using traditional (column) and PLE extraction, both with GC-HRMS detection. The PCDD/Fs are arranged according to the elution order on 5%-phenyl GC column.

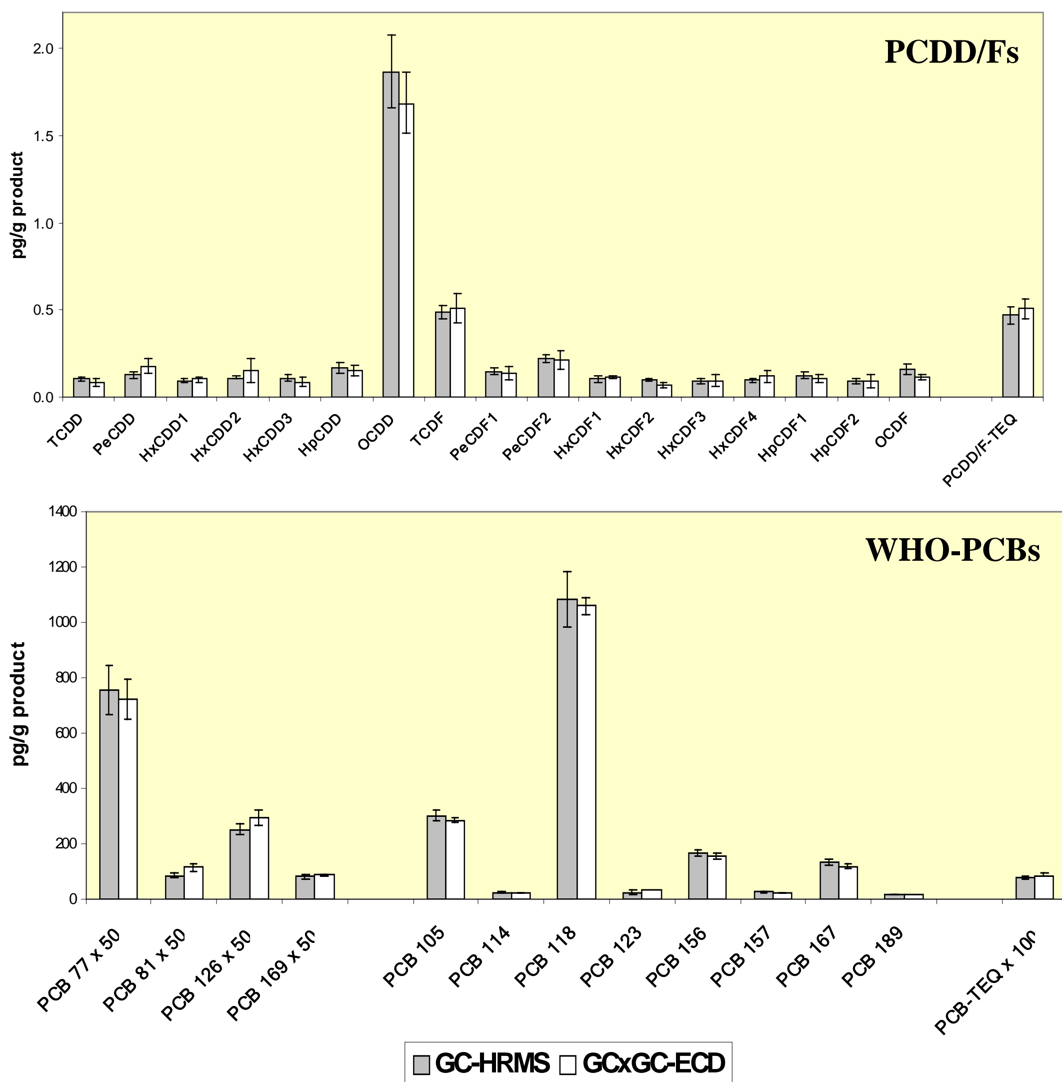

Figure 4. Comparison of the results obtained for PCDD/F and WHO-PCB in a compound feed sample using GC×GC-ECD screening technique and the classic GC-HRMS technique.

However the techniques differ in precision, type of information obtained, degree of automation etc and will therefore have different application areas. Some key aspects are compared in table 1.

Table 1. Comparisson of techniques for food and feed compliance monitoring.

Technique	Type of data	Sensitivity pg TEQ/ g	Precision (CV)	Accuracy	Extraction/ Clean-up	Cost
Shape selective PLE + Ah-GFP	TEQ	≈ 1	≈ 30%	± 100 % (w. reference samples)	Easy	Low
LC-LC-GC-ECD	Isomer-specific through markers	≈ 1 (100 fg/g/ congener)	≈ 20%	± 20-30 %	Moderate	Moderate
Selective PLE + HRMS	Isomer-specific	≈ 1 (10-100 fg/g/ congener)	< 10%	± 10 %	Moderate	High
Trad. sample prep + GC×GC-ECD	Isomer-specific	≈ 1 (20-200 fg/g/ congener)	≈ 10%	± 10 %	Tedious	Moderate

Based on the performance characteristics we would recommend the PLE extraction/clean-up with Ah-GFP detection for monitoring of large sets of samples to determine if the levels are far above, far below or close to a MRL. The LC-LC-GC-ECD technique might find some use in cases where the PCDD/F and PCB congener patterns are constant, but the proportions differ, and it is important to know the TEQ contribution from PCDD/Fs and WHO-PCBs, respectively. The GC×GC-ECD technique might be used for routine congener-specific analysis of PCDD/Fs and WHO-PCBs as soon as the quantification software improves. Finally, the selective PLE procedure might be used to cut the cost of the GC-HRMS verification analysis. However, a combination of shape-selective PLE and GC×GC-ECD would perhaps be the best compromise between cost and analytical performance. This will be investigated in future studies.

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Referenses

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