# SELECTION AND ANALYSIS BY LC/LC/GC-ECD OF INDICATOR CONGENERS OF MONO- AND NON-*ortho* PCBs and PCDD/Fs

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## INTRODUCTION

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) and non- and mono-*ortho* substituted polychlorinated biphenyl's, also called planar PCBs (non-/ mono-*ortho* PCBs, or pPCBs) are ultra trace level contaminants found all over the world. Usually, these compounds are analysed by high-resolution gas chromatography/ high-resolution mass spectrometry (HRGC/HRMS) after an extensive clean-up. The expensive instrumentation in combination with a rather laborious clean-up results in high prices of the analyses. As a consequence, some types of ecological and ecotoxicological investigations, in which numerous samples has to be analysed to give statistically significant results, will become so expensive that they will be almost impossible to undertake.

The aim of the present study was to develop a more cost effective analysis method which can be used for ecological and ecotoxicological investigations similar to the ones described above.

In biota the inter special variation in the relative PCDD/F and pPCB levels (the "congener profile") is low as long as all individuals lives in the same geographical area and no point sources is present within the area. This is nowadays the case in e.g. the Baltic where the main source of PCDD/F and pPCB is supposed to be long range air pollution and release from the sediment pool. In cases then the congener profile is constant it is possible to predict all individual congeners by only analysing a few congeners called indicator congeners. By an appropriate choice of indicator congeners and analysis methods cost effective analysis procedures can be developed.

We will now describe how we selected indicator congeners for PCDD/F, mono- and non-*ortho* PCBs in Baltic Herring. We will also describe a cost effective method to analyse these congeners in Baltic Herring.

## SELECTION OF INDICATOR CONGENERS

The selection of indicator congeners was based on three criteria: 1) abundance; 2) ease of analysis; 3) toxicological relevance. At the time of the selection our laboratory had been analysing 60 Baltic herring samples for PCDD/F and 13 samples for pPCB. The results of these analyses were used in the selection.

PCDD/F: Only 15 PCDD/F (all 2378-substituted) congeners were present in more than 90% of the samples. Of the remaining congeners 23478-pentachlorodibenzofuran (PeCDF) were chosen as the prime indicator candidate. It is present in high levels, is easy to analyse and has an high toxic equivalency factor (TEF). Most of the PCDD/F correlated well with 23478-PeCDF. The hepta and octa substituted congeners showed however lower correlation, probably due to bad chromatographic properties on the polar GC columns used in the analysis. 2378-Tetrachlorodibenzo-p-dioxin and -dibenzofuran (TCDD and TCDF) also show lower correlation. This is probably due to an actual difference in tissue levels originating from variations in the metabolic activities between different individuals. The correlation between TCDD and TCDF was however good and TCDF was therefore included as an additional indicator congener.

pPCB: Results were available for three non-*ortho* PCBs: 3,3',4,4'-tetra- (TeCB), 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl (HxCB) (IUPAC #77, #126 & #169, respectively), and for two mono-*ortho* PCBs: 2,3,3',4,4'- and 2,3',4,4',5-PeCB (IUPAC #105 & #118). PCB #126 and #105 were chosen as indicators for PCB #169 and #118, respectively, with the same motivation as above. PCB #77 also had to be determined due to low correlation to PCB #126.

TABLE 1. Summary of the selected indicator congeners

| Compound class | "Indicator" | Used to obtain/ predict: |
|---|---|---|
| PCDD/F | TCDF | TCDF and TCDD |
| | 23478-PeCDF | 23478- and 12378-PeCDF<br>12378- PeCDD<br>123478-, 123678- and 234678-HxCDF<br>123478-, 123678- and 123789-HxCDD<br>1234678-HeptaCDD/F and OctaCDD |
| non-*ortho* PCB | PCB #77 | PCB #77 |
| | PCB #126 | PCB #126 and #169 |
| mono-*ortho* PCB | PCB #105 | PCB #105 and #118 |

## EVALUATION OF THE INDICATOR CONGENERS

To evaluate the indicator congeners the Σ and NTEQ (Nordic toxic equivalent) levels of PCDD/F and pPCB were calculated from the indicator congener levels in a large number of herring samples, and the results were compared to the actual Σ and NTEQ levels, see Figure 1.

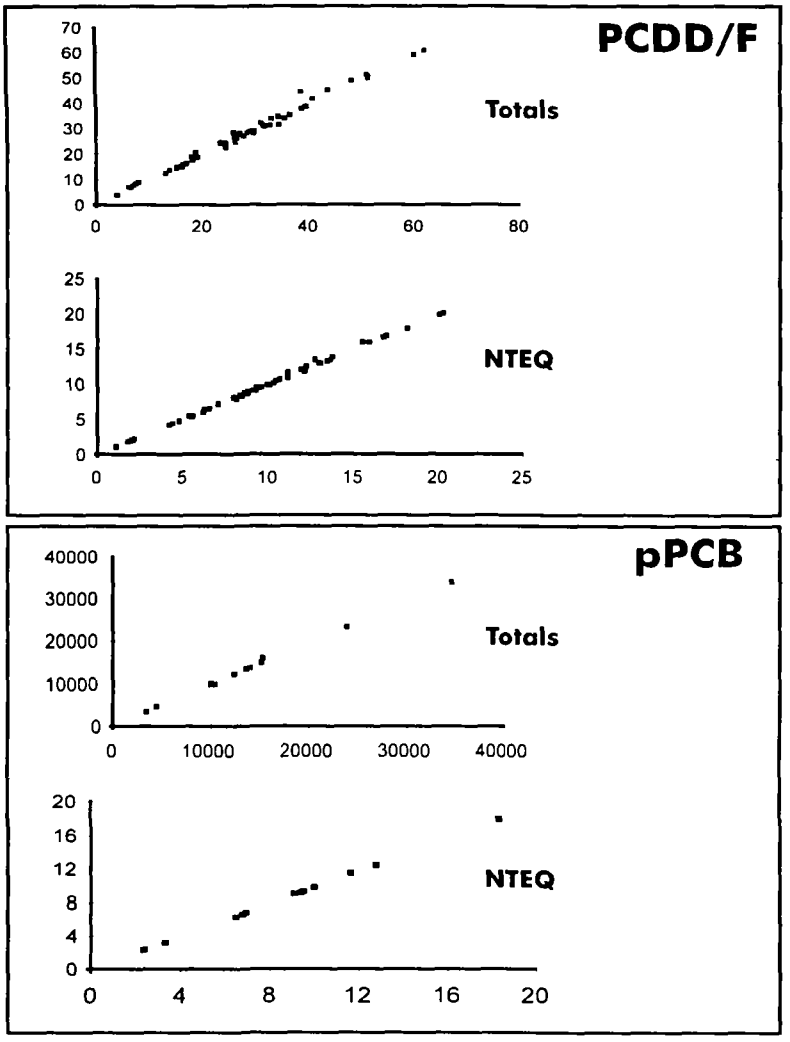


Figure 1: Predicted levels (ppt, fresh weight) of 2378-substituted PCDD/F and pPCB (y-axis) plotted vs. the actual values (x-axis).

## EXPERIMENTAL

Extraction and Clean-up: The herrings were blended with sodium sulphate and were column extracted with acetone/n-hexane 2.5:1 and n-hexane/diethyl ether 9:1[1,2]. The solvent was evaporated and the lipid weights were determined gravimetically. To remove the major part of the lipids the extract were saponified with 1% potassium hydroxide in 99.5% ethanol over night at ambient temperature. The hydrolysates were then diluted with *Milli*-Q water and were passed through solid phase extraction (SPE) cartridges filled with C18-silica. The SPE cartridges were then washed carefully and dried with argon gas. At

this stage one SPE cartridge filled with 40% H2SO4 on silica was coupled to the outlet of each of the C18 cartridges and the tandem-SPE cartridges were eluted with n-hexane. The SPE equipment used allowed for parallel processing of up to 24 samples.

On-line separation and analysis: Separation of the indicator congeners from other persistent lipophilic compounds as well as all other PCDD/F and PCB congeners requires a maximum of selectivity. To obtain enough selectivity two-dimensional high-performance liquid chromatography (LC-LC) was used. The first HPLC column was an silica column and the second an PYE (2-(1-pyrenyl)ethyldimethylsilylated silica) column. n-Pentane was used as the mobile phase. The effluent from the PYE column is passing through an UV-detector followed by a loop interface connected to an gas chromatograph equipped with an electron capture detector (GC-ECD). The UV-detector is used for direct determination of PCB #105 - the indicator for mono-*ortho* PCB. All other indicators had to be determined by the GC-ECD because of low levels.

Following injection an appropriate fraction of the effluent from the silica column is transferred onto the PYE column by an heart-cut technique. The PYE column is eluted in the forward direction until PCB #105 has passed the UV-detector. The PYE column is then back-flushed, and the resulting back-flush peak is transferred on-line into the GC using the loop interface. The GC is operated under CSV (Concurrent Solvent Evaporation) conditions to concentrate the large volume of solvent entering the GC[3]. To shorten the analysis time and to protect the analytical column and the detector the GC is equipped with an early vapour exit[3]. The whole LC/LC/GC-ECD equipment is fully automized and computer controlled.

## RESULTS

The data produced by the LC/LC/GC-ECD procedure is comparable with the classical GC/ high-resolution mass spectrometry analyses. Examples of GC-ECD chromatograms from the analysis of an herring sample is given in Figure 2.

## DISCUSSION

The method developed is sensitive enough to allow the determination of PCDD/F and pPCB in less than 0.5 gram of herring fat. This sensitivity is sufficiently high to allow individual analysis of herrings as long as the individuals has reached one year of age. The method is both faster and more cost effective than e.g. the Stalling method[4]. At the moment the extraction is the bottle-neck of the method at our laboratory. If the extraction could be speeded up one person would be able to process about 20 samples including blanks per working day.

The method presented can be considered as a specialised method designed to solve a specific problem, viz. determining the levels of Σ and NTEQ levels of PCDD/F and pPCB in Baltic herring. However, the method can easily be adopted to other situations as long as the congener profiles remains stable. After such a modification a calibration of the method has to be done by comparative analysis with an existing method to get the ratios between the indicator congeners and the congeners that should be predicted.

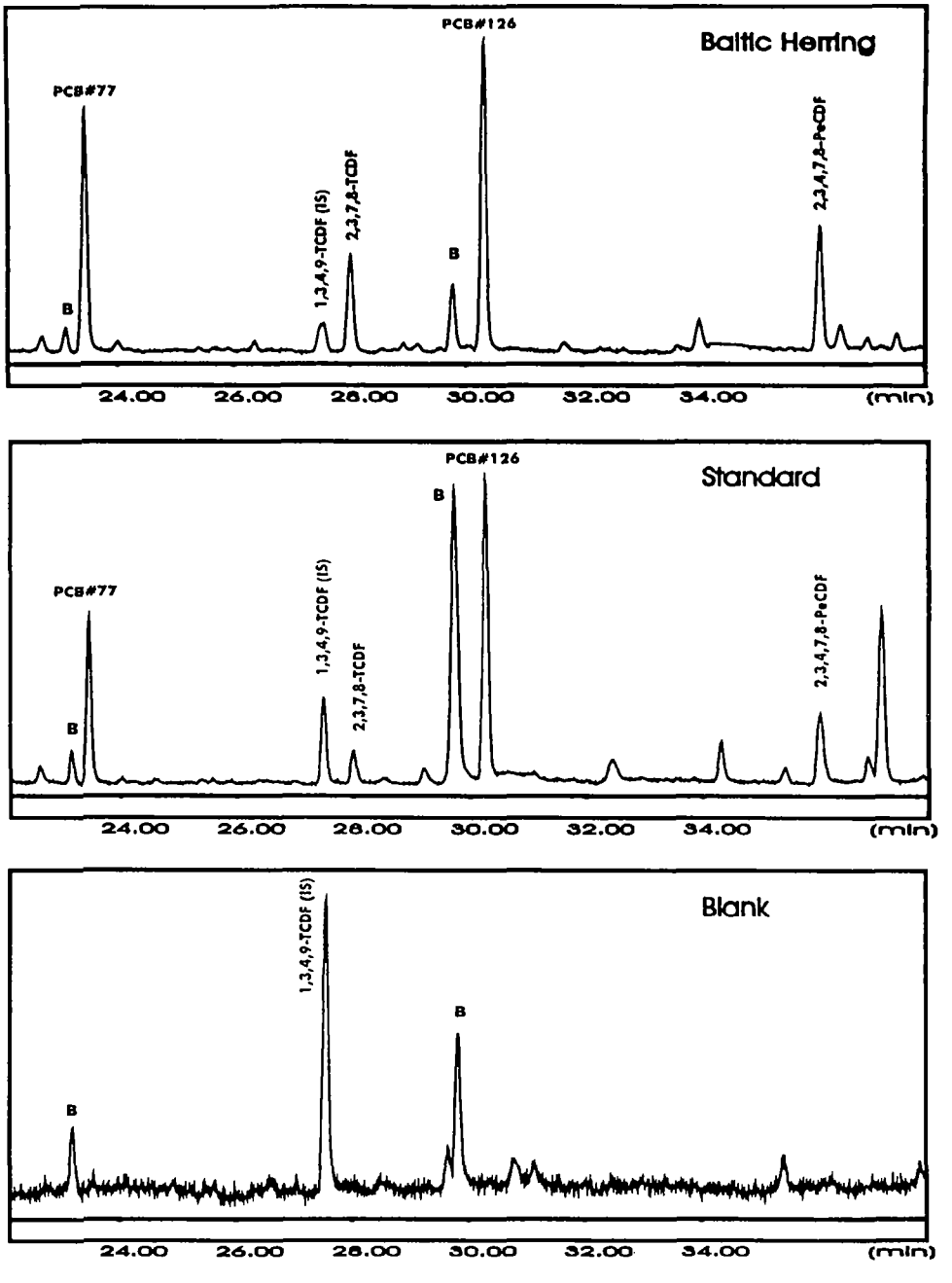


*Figure 2:* Gas chromatograms from the analysis of indikators for PCDD/F (2378-TeCDF and 23478-PeCDF) and pPCB (PCB# 77 and PCB#126) in Baltic herring, a standard and a method blank. IS denotes the internal standard and B denotes column bleed peaks.

## REFERENCES


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