QUALITY ASSURANCE IN PLANAR PCBs ANALYSIS – VALIDATION OF THE METHOD AND RESULTS FROM FRESHWATER FISH ANALYSIS

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Introduction

Nowadays there are a lot of well-established methods for routine analysis of non-ortho PCB congeners (No. 77, 81, 126 and 169) in fish. Although these coplanar congeners are much less toxic than usually monitored polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs), their occurrence in more than three order of magnitude was made these analysis greatly worthwhile for the survey of toxic equivalent concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD (TEQ). The aims of this study were: (i) optimization of extraction step for lipid portion isolation (together with studied compounds), which may be considered as one of the most critical step in analysis, (ii) validation of ultra-trace analytical method for non-ortho PCBs determination and interlaboratory comparison, and (iii) survey for planar PCBs levels in freshwater fish from Czech Republic to assess health risk due to dietary intake of these compounds, in terms of TEQ.

Methods and Materials

For the extraction step optimization the homogenized fish muscle material was prepared: chub (Leuciscus cephalus) and barbel (Barbus barbus), 2:1 (w/w). Different extraction techniques and solvent mixtures (or pure solvents) were tested: ultrasonic extraction (hexane:acetone, 2:1, v/v) after and without 3 hours dessication after Nasulphate mixing, Soxhlet extraction (pentane:dichloromethane, 1:1, v/v, and hexane:dichloromethane, 1:1, v/v), extraction with Sxtce apparatus (hexane EN 1528, diethyl ether EN 1528, hexane ČSN 570146 – as dried material, and hexane ISO 1443 – after hydrolysis). Extracted lipids were determined gravimetrically.

The validation of ultra-trace method for planar PCBs determination was carried out as a part of homogeneity testing of candidate reference material chub (Leuciscus cephalus) muscle (from the bottom reaches of Elbe river) in CHRONO project: "Chub and herring as reference materials for ortho and non-ortho chlorobiphenyls", and consisted of following parts:
(a) "between-batch variance" homogeneity testing: 20 analysis from 20 tins (each selected from 150 pieces batch)
(b) "within-batch variance" homogeneity testing: 5 analysis from pooled content of 3 tins
(c) "clean-up error" testing: 5 analysis from pooled extract of 3 tins
(d) "GC error" testing: 10 analysis of non-ortho PCB standard solution

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(e) interlaboratory comparison of planar PCB determination in candidate reference material chub and other RIVO-DLO intralaboratory materials (cod liver, eel muscle).

Fish tissue samples (20 g fish muscle dessicated with 50 g of anhydrous sodium sulphate) were extracted with Soxhlet extractor (170 ml of mixture pentane: dichloromethane, 1:1, v/v, for 7 hours). Before extraction samples were spiked with $^{13}$C isotopes (each 3.2 ng), incubation time was 16 hours. Lipid removal was carried out with disposable silica columns (30 g of SiO$_2$, 10% H$_2$O), analytes were eluted with 100 ml of hexane. Separation of non-ortho PCB group was done with the HPLC-PYE system, on Cosmosil PYE column (2/-1-pyrenal/ethylysilica, 250×4.6 mm, 5 μm particles, operated at 0°C with hexane as eluent, 0.5 ml.min$^{-1}$). Fraction of monitored analytes (9-18 ml) was collected, and after addition of internal standard (16 ng of $^{13}$C PCB 101) evaporated and transferred to 200μl of isoctane. Final identification and quantification was carried out by HRGC-LRMS-NCI (HP 6890 coupled to HP 5973, ionization gas methane) on DB-5ms column (60m×0.25mm×0.25μm phase).

Fish samples for planar PCB survey in Czech freshwater ecosystem were collected in summer 1999 from four localities on major rivers: Dříteč–Němčice (Elbe river), Všenory–Jiřišťen (berounka river), Štekná–Radomyšl (Ota river) and Uherské Hradiště–Jarov (Morava river). The whole set consisted of 17 samples and included these different species: chub (Leuciscus cephalus), barbel (Barbus barbus), perch (Perca fluviatilis) and bream (Abramis brama).

Results and Discussion

The comparison of studied extraction techniques is summarized in Figure 1. As can be seen from picture, the highest amounts of lipids were obtained when extraction was performed with Soxtex apparatus, but unfortunately due to the limited sample size this technique is not suitable for planar PCB isolation. The best choice is using Soxhlet extraction with mixture pentane:dichloromethane (1:1, v/v) for the almost identical boiling points of these solvents.

From the planar PCB method validation there were obtained satisfactory low values of uncertainty associated with clean-up or GC error in determination of these compounds, as same as regarding between or within batch variance, see Table 1. Recoveries of $^{13}$C labelled PCB analogues were in the acceptable range of 77-89%, with rsd 3.0-3.7%.

Table 1: Comparison of relative standard errors in planar PCBs analysis

<table>
<thead>
<tr>
<th>RSD [%]</th>
<th>PCB 77</th>
<th>PCB 81</th>
<th>PCB 126</th>
<th>PCB 169</th>
</tr>
</thead>
<tbody>
<tr>
<td>rsd between batch</td>
<td>4.2</td>
<td>8.3</td>
<td>4.8</td>
<td>9.4</td>
</tr>
<tr>
<td>rsd within batch</td>
<td>2.2</td>
<td>5.3</td>
<td>6.9</td>
<td>7.0</td>
</tr>
<tr>
<td>rsd inhomogeneity</td>
<td>3.6</td>
<td>6.4</td>
<td>0.0</td>
<td>6.3</td>
</tr>
<tr>
<td>rsd clean-up error</td>
<td>4.8</td>
<td>8.4</td>
<td>3.0</td>
<td>6.9</td>
</tr>
<tr>
<td>rsd GC error</td>
<td>4.0</td>
<td>4.0</td>
<td>3.4</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* $\text{rsd}_{\text{inhomogeneity}} = \text{rsd}_{\text{between batch}} - \text{rsd}_{\text{within batch}}$

Results of interlaboratory comparison of planar PCB determination in chub are summarized in Table 2. (observed values from RIVO-DLO were taken from initially stability testing of candidate reference material at t=0). Excellent agreement in determined non-ortho PCB levels as well as

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very low values of standard deviations were found except for PCB 169 (probably due to greatly
difficult determination at this concentration level).

Table 2: Interlaboratory comparison of planar PCB determination in chub material (in ng/kg
tissue)

<table>
<thead>
<tr>
<th></th>
<th>PCB 77</th>
<th>PCB 81</th>
<th>PCB 126</th>
<th>PCB 169</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICT, CZ (n=20)</td>
<td>160.9</td>
<td>5.4</td>
<td>21.4</td>
<td>2.5</td>
</tr>
<tr>
<td>stdv</td>
<td>6.8</td>
<td>0.4</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>RIVO-DLO, NL (n=5)</td>
<td>177</td>
<td>5.9</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>stdv</td>
<td>1.2</td>
<td>0.2</td>
<td>1.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Results from analyzed Czech freshwater fish samples (including non- and mono-ortho PCB) were
expressed as TEQ equivalents (using currently valid TEFs for human exposure 5) with values in
range of 1-12 ng/kg. Contributions from non-ortho PCB to TEQ value were higher than from
mono-ortho CBs, the major ones resulted from CB 126, CB156 and CB 118. The highest levels of
planar PCBs were detected in barbel samples, less in chub and bream and lowest values were
obtained in perch samples (due to very low amount of lipids, i.e. typically < 1%). Obtained TEQ
values are comparable with reported data from fish caught from heavily polluted rivers in Western
Europe 6-8.

Acknowledgments

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References

   1823.
   417.
5. van der Berg M., Birnbaum L., Bosveld A.T.C., Brunström B., Cook P., Feeley M., Giesy
   J.P., Hanberg A., Hasegava R., Kennedy S.W., Kubiak T., Larsen J.Ch., van Leeuwen
   F.X.R., Liem A.K.D., Noit C., Peterson R.E., Poellinger L., Safe S., Schrenk D., Tillitt D.,
   Compounds 32,344.
Figure 1: Comparison of different extraction methods for the isolation of lipids from fish muscle (n=6)