

## DEVELOPMENT OF DIOXIN/FURAN ANALYSIS METHOD FOR CONGENERS OF DIOXIN-LIKE PCB

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### Introduction:

Among the 209 congeners of polychlorinated biphenyls (PCBs), 12 congeners have toxicity similar to 2,3,7,8-TCDD and therefore, are included into the scheme of toxicity equivalency factors as established by WHO expert groups. These so-called dioxin-like PCBs (dl-PCBs) include the following: PCB #77, #81, #105, #114, #118, #123, #126, #156, #157, #167, #169, and #189<sup>1</sup> Most laboratories analyze PCBs by HRGC/HRMS according to US-EPA method 1668<sup>2</sup>. We developed and validated an in-house method for low-resolution mass spectrometer (HRGC/LRMS)<sup>3</sup>, based on the dioxin/furan US-EPA method 8280A<sup>4</sup> for dl-PCBs. Extraction, clean-up of dl-PCBs was performed according to in-house method for dioxin/furan analysis. The dl-PCBs and 2,3,7,8-substituted PCDD/PCDF congeners were well removed from the sample matrix and two fractions were received from the alumina column. With applying this new method, we participated successfully in the international intercalibration QA/QC studies of INTERCAL and UNEP<sup>5,6</sup>. The analytical results are good and in comparison with international laboratories.

### Materials and methods:

#### *Standards and reagents:*

Native and <sup>13</sup>C-labelled standard solutions for PCDD/PCDF and dl-PCB were obtained from Wellington Laboratories (Guelph, ON, Canada) included calibration solutions, sampling spike, extraction spike and recovery spike solutions for HRGC/HRMS as well as standard solutions for HRGC/LRMS. Solvents were from Merck in pesticide grade. Silica (Merck, 100-200 mesh) was activated at 140 °C for 24 h. Activated silica was impregnated with 40 % (w/w) concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>-Silica). Silica impregnated with 20 % (w/w) potassium hydroxide (KOH-Silica), activated at 140 °C during 24 h. Sodium sulfate in granular, was purified by extracting with dichloromethane and activated at 400 °C for 4 h. Neutral alumina (50-160 µm) was activated at 600 °C for 24 h.

#### *Spiking sample and control sample:*

Spiking sample: Twelve native dl-PCBs at concentrations from 1 ng/sample to 10 ng/sample was diluted in 30 ml hexane.

Control sample: Add the internal standards, which contain 10 ng for each of twelve <sup>13</sup>C<sub>12</sub>-labeled dl-PCBs, into the spiking sample.

#### *Multilayer column:*

Glass column (11 mm id) is filled with several layers in the following order (from top down to bottom): Na<sub>2</sub>SO<sub>4</sub> (1 cm)/ 3 layers H<sub>2</sub>SO<sub>4</sub>-Silica (5-7 cm)/ Na<sub>2</sub>SO<sub>4</sub> (1 cm)/ KOH-Silica (2-3 cm)/ Na<sub>2</sub>SO<sub>4</sub> (1 cm)/ glass wool. Apply the spiking sample to the top of column, then rinse the column with 100 ml hexane. Collect whole solution, then add the internal standards, which include 10 ng each <sup>13</sup>C<sub>12</sub>-labeled dl-PCBs.

#### *Alumina column:*

Glass column (10 mm id) is filled with several layers in the following order (from top down to bottom): Na<sub>2</sub>SO<sub>4</sub> (1 cm)/ 4 g Al<sub>2</sub>O<sub>3</sub>/ Na<sub>2</sub>SO<sub>4</sub> (1 cm)/ glass wool. Apply the spiking sample to the top of column, collect the solution (Fraction F0). Elute the column with the following sequence of solvents:

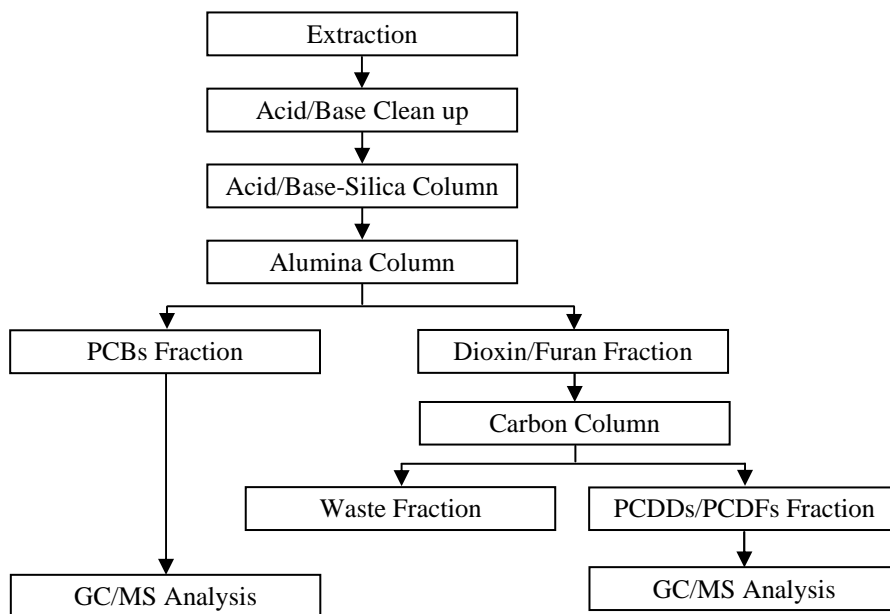
+ 20 ml hexane (Fraction F1),

+ 30 ml of hexane - dichloromethane, 95 : 5 v/v (Fraction F2),

+ 55 ml of hexan - dichloromethane, 50 : 50 v/v (Fraction F3).  
Add the internal standards to the each fraction.

*Sample preparation:*

Add the internal standards into sample. Extract the solid sample by soxhlet with toluen. Extract the liquid sample by homogeneous stirring with dichloromethane. Concentrate the received extract, then dissolve in hexane. Remove interferences by shaking in turn with concentrated H<sub>2</sub>SO<sub>4</sub>, NaCl and KOH solutions. Clean-up the extract on the multilayer column. Separate congeners of PCBs and PCDD/PCDF on the alumina column. Collect the fraction F2 to analyse PCBs and the fraction F3 for PCDD/PCDF. The diagram of sample preparation is presented in Figure 1.



**Figure 1.** Diagram of sample preparation for analysis of PCBs, PCDD/PCDF

*GC/MS analysis:*

Instrument: HRGC/LRMS Agilent 6890/5972A.

Chromatographic conditions:

- BPX-DXN column (60 m, 0.25 mm id, 0.25 µm film thickness), SGE P/N: 054235
- Helium flow velocity: 1 mL/min, constant pressure mode
- Initial temperature: 150 °C, initial time: 2 min
- Temperature program: increase to 220 °C at 20 °C/min, hold at 220 °C in 16 min, increase to 300 °C at 5 °C/min, hold at 300 °C in 2.5 min.
- Injector: 290 °C, Splitless. Interface: 290 °C

Selected Ion Monitoring: M and M+2 with TeCBs, M+2 and M+4 with the other PCBs.

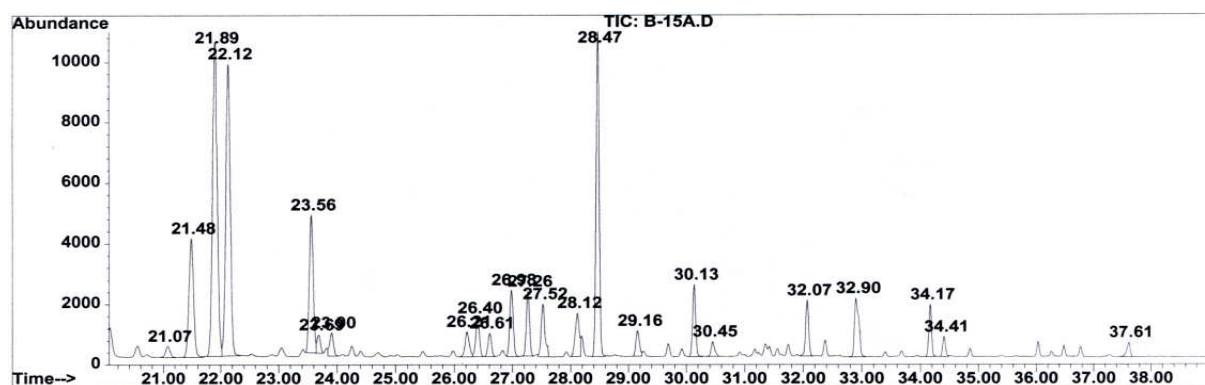
**Results and discussion:**

The amount of dl-PCBs found in the control sample and their percent ratio in the fractions of the multilayer column, alumina column in comparison with the control sample are presented in Table 1. The results shown that the recovery of dl-PCBs is between 86.5% and 98.1% with the multilayer cleanup column. With the alumina column, at least 95.9% of dl-PCBs is eluted in the fraction F2. Except for PCB#77: 88.3% is eluted in the fraction F2, only 9.2% in the fraction F3 for PCDD/PCDF analysis. The fractions F0 and F1 are only sample matrix and interferences, therefore discard that in the GC/MS analysis. The limit of detection (LOD) for each dl-PCB on HRGC/LRMS instrument is 1 pg with signal/ noise > 3. At that LOD, we can analyse all of the environmental and biological samples.

**Table 1.** The amount of dl-PCBs in control sample (ng) and their percentage ratio in fractions in comparison with the control sample (%)

PCB #	Control sample (ng)	Multilayer column (%), n=3	Fractions of alumina column (%), n=3			
			F0	F1	F2	F3
81	0.961	86.5	< 0.7	< 0.8	97.1	< 2.2
77	4.823	87.1	< 0.4	< 0.1	88.3	9.2
123	0.967	96.5	< 1.7	< 0.9	98.1	< 0.6
118	9.736	92.6	< 0.3	< 0.1	97.9	< 0.2
114	0.962	89.8	< 0.9	< 0.8	96.7	< 2.0
105	9.642	90.4	< 0.3	< 0.1	97.2	< 0.2
126	0.983	90.7	< 1.3	< 0.4	95.9	< 1.8
167	1.069	98.1	< 0.7	< 0.7	97.4	< 1.6
156	5.300	96.3	< 0.4	< 0.3	97.9	< 0.2
157	1.065	94.6	< 2.1	< 0.6	98.1	< 1.1
169	1.060	96.0	< 1.5	< 1.1	97.3	< 1.1
189	0.990	97.0	< 1.1	< 0.5	96.1	< 1.6

We analysed PCBs in the following samples: Standard solution S of 12<sup>th</sup> Round and Soil/Sediment B of 10<sup>th</sup> Round of the International intercalibration QA/QC study, which was organized by INTERCAL (Sweden) and UNEP in the UNEP/GEF project on assessment of existing capacity and capacity building needs to analyse persistent organic pollutants (POPs) in developing countries<sup>7</sup>. The total ion chromatogram of sample Soil/Sediment B is presented in Figure 2. The extract ion chromatographs show that excellent separation not only for PCDD/PCDF<sup>8</sup> but also for dl-PCB was obtained with the BPX-DXN capillary column (60 m, 0.25 mm id, 0.25 µm film thickness).



**Figure 2.** Chromatograph of PCBs from Soil/Sediment B of 10<sup>th</sup> Round

Figures 3 and 4 compare the results of dl-PCBs analysis from Standard solution S and Soil/Sediment B of Vietnam-Russian Tropical Centre (VRTC) with international laboratories. We can see that the results of VRTC are very close to average value, median value of 83 laboratories for Standard S<sup>9</sup> and 63 laboratories for Soil/Sediment B<sup>10</sup>. These laboratories have z-score < 2 and are classified as excellent and satisfactory<sup>6</sup>.

Together with our results of PCDD/PCDF analysis from 38 samples of soil, sediment, sludge, clay, ash, fish and standard solution in the international intercalibration QA/QC studies from 10<sup>th</sup> to 15<sup>th</sup> Round of INTERCAL 2005-2010 and 1<sup>st</sup> Round of UNEP 2010, which have z-score for PCDD/PCDF always below 2 and often below 1<sup>11,12</sup>, we find that the development of dioxin/furan analysis method by HRGC/LRMS for congeners of dl-PCBs is reliable and suitable with infrastructure of laboratories in developing countries. Congeners of dl-PCBs are separated from sample matrix, interferences and also PCDD/PCDF fraction. The analytical results of dl-PCBs, PCDD/PCDF from samples of international intercalibration QA/QC studies are very good and in comparison with international laboratories.

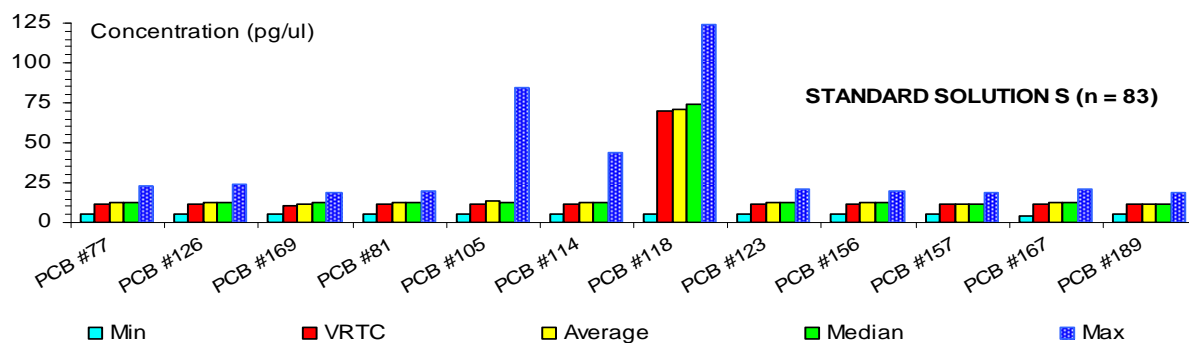


Figure 3. Result of dl-PCBs analysis from Standard solution S of VRTC vs. laboratories

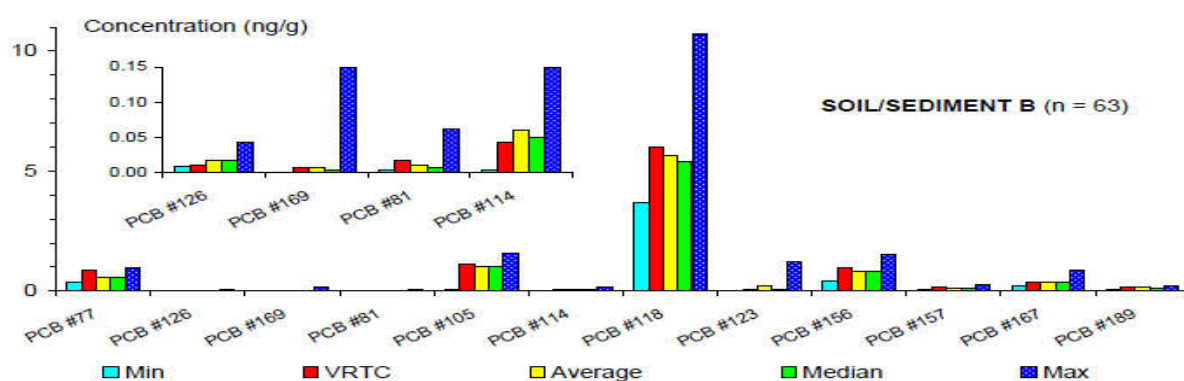


Figure 4. Result of dl-PCBs analysis from Soil/Sediment B of VRTC vs. laboratories

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#### References:

1. Martin Van den Berg, Linda Birnbaum, Albetus T.C. Bosveld, Björn Brunström, Philip Cook... (1998); *Environmental Health Perspectives* 106(12): 775-792.
2. US.EPA Method 1668 (1999), Revision A: *Chlorinated biphenyl congeners in water, soil, sediment, biosolids and tissue by HRGC/HRMS*.
3. VRTC in-house method 45 TQSB 01:2007: *The analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans by HRGC/LRMS*, Hanoi, Vietnam.
4. US.EPA Method 8280A (1996): *The analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans by high resolution gas chromatography/ low resolution mass spectrometry (HRGC/LRMS)*.
5. UNEP (2007), *Guidance for analysis of Persistent Organic Pollutants (POPs)*, Geneva, Switzerland.
6. UNEP (2005), *International Inter-calibration Studies: A Global QA/QC tool for the analysis of POPs under the Stockholm Convention*, UNEP Chemicals, Geneva, Switzerland.
7. UNEP (2008), *Assessment of existing capacity and capacity building needs to analyse persistent organic pollutants (POPs) in developing countries*, UNEP Chemicals, Geneva, Switzerland, .
8. SGE (2004), *Technical report: Development of new capillary columns for dioxin analysis*, TA-0124-C.
9. Bert Van Bavel (2007), *Final report 12<sup>th</sup> round of the international intercalibration study*, Intercal, Sweden.
10. Bert Van Bavel (2005), *Final report 10<sup>th</sup> round of the international intercalibration study*, Intercal, Sweden.
11. Van Bavel B, Lindström G, De Boer J and Fiedler F (2007); *Organohalogen Compounds* 69: 1285-1288.
12. VRTC (2010), *Assessment of the results of analytical method for PCDDs/PCDFs*, Hanoi, Vietnam.