Comparison of PCB congener profiles in tissues of PCB-treated rats

Izabela Kania-Korwel[1], Keri C. Hornbuckle[3], Aaron Peck[3], Wiesław W. Sułkowski[2], Gabriele Ludewig[1], Larry W. Robertson[1], Hans-Joachim Lehmler[1]

[1]Department of Occupational and Environmental Health, University of Iowa, Iowa City, U.S.A.
[2]Department of Environmental Chemistry and Technology, University of Silesia, Katowice, Poland
[3]Department of Civil and Environmental Engineering, University of Iowa, Iowa City, U.S.A.

Introduction

Information on the congener–specific distribution of PCBs in tissues of laboratory animals after exposure to technical or environmental PCB mixtures is limited. Typically PCB levels and profiles from adipose tissue, liver, blood and occasionally brain are reported. In the work presented here 120 PCB congeners were extracted from 9 tissues of rats exposed to two different PCB mixtures. One mixture was Aroclor 1254, a well studied technical mixture. The second mixture was an environmental mixture obtained after extraction of soil contaminated with Chlorofen, a highly chlorinated Polish PCB mixture. The study was designed to investigate how different chlorination levels and the structure of PCB congeners affect their distribution and total concentration in selected tissues. Principal Component Analysis and Hierarchical Cluster Analysis were employed to compare tissues of Aroclor and soil extract-treated animals.

Materials and methods

The soil PCB mixture was a hexane-acetone extract from soil collected at the Chlorofen manufacturing site[1]. One-month-old male rats (Sprague Dawley, Harlan) were divided into three groups and injected i.p. with a single dose of the environmental PCB mixture (20 mg/kg b.w., 0.05 mmol/kg b.w.; n = 3) or Aroclor 1254 (16 mg/kg b.w., 0.05 mmol/kg b.w.; n = 4). Control animals received the vehicle alone (corn oil, n = 4). Rats were euthanized on day 7. Blood was collected by cardiac puncture and serum was prepared by centrifugation. Lung, liver, spleen, kidney, brain, skin and adipose tissue were excised *en bloc*.

The extraction of PCBs from tissues was performed as described previously by our laboratory[2]. The PCB extraction from blood was carried out as described by Gill[3]. The PCB analysis was performed using a HP gas chromatograph with a ^{63}Ni μ-ECD detector. 90 chromatographic peaks representing 120 PCB congeners were analyzed. Internal (PCBs # 30 and 204) and recovery (PCBs # 14, 65 and 166) standards were used[4]. The lipid content in tissues was determined gravimetrically from the hexane–acetone extract, while the lipid content in serum was determined using a commercial test kit for total cholesterol and triglycerides (Chol and Trig/GB for Roche/Hitachi 917 system, Roche Diagnostics). The formula presented in[5] was used for the calculation of the total lipid content in serum.
For further analysis the PCB data set was preprocessed with autoscaling[6] and used as input to other methods. Principal Component Analysis and Hierarchical Cluster Analysis (Ward's method with Euclidean distance as a similarity measure) was carried out under Matlab (Mathworks, Natick, MA, USA, version 6.5.1).

Results

The PCB levels in method blanks were 44±15 ng for tissues and 4.1±1.2 ng for blood and serum. Recoveries of the recovery standard were 89–91 % for tissues and 75–83 % for blood and serum. Corrections of total concentration were made for recoveries < 100%. Total PCB concentrations and lipid adjusted values are presented in Table 1. The highest concentration in both treatment groups was found in the adipose tissue. High concentrations were also observed in the skin. PCB levels were in general higher in tissues from soil extract-treated animals compared to Aroclor 1254-treated animals. Lipid adjusted PCB levels were similar in all tissues with the exception of the spleen where the lipid adjusted PCB levels were much higher compared to the other tissues investigated. In addition on a molar as well as a weight adjusted basis, the total PCB content was significantly higher in the spleen ($P < 0.005$) but significantly lower in the skin ($P < 0.05$,) in the soil extract treatment group compared to the Aroclor group (ANOVA with Tukey post-hoc test).

Table 1. Total PCB concentration in rat tissues and lipid adjusted values (means ± S.D.).

	PCB concentration in Aroclor 1254 treated rats		PCB concentration in soil extract treated rats	
tissue	total [ng/g]	lipid adjusted [ng/g]	total [ng/g]	lipid adjusted [ng/g]
adipose	31544 ± 20944	808 ± 162	42172 ± 20152	979 ± 152
brain	471 ± 78	n. d.	681 ± 134	n. d.
blood	94 ± 14	n. d.	184 ± 61	n. d.
heart	821 ± 58	421 ± 95	1068 ± 70	575 ± 19
kidney	1493 ± 533	556 ± 122	2058 ± 846	788 ± 261
liver	2451 ± 482	768 ± 156	4727 ± 1097	1441 ± 239
lung	1415 ± 614	553 ± 25	2841 ± 1410	794 ± 94
serum	153 ± 4	530 ± 46	197 ± 20	411 ± 28
skin	8336 ± 1007	551 ± 16	6983 ± 1063	595 ± 147
spleen	2080 ± 574	2040 ± 1732	7005 ± 540*	4512 ± 609

* n = 2; n.d. = not determined

The **hierarchical cluster analysis (HCA)** shows high grouping tendency in the data (Fig. 1). HCA reveals two main groups: Aroclor 1254, tissues from Aroclor 1254-treated animals and tissues from the control animals are in one group. Chlorofen, soil extract and tissues from soil extract-treated animals are in a separate group. In the Aroclor 1254 treatment cluster three sub-groups can be observed. The first sub-group consists of brain, heart, lung and kidney, the second sub-group consists of "storage" tissues, such as adipose, skin, liver and blood or serum, and the third sub-group is the spleen. With exception of the spleen, these sub-groups are not further grouped into individual tissues. In the soil extract cluster three sub-groups of tissues can be distinguished. The first sub-group contains adipose tissue, skin, blood and serum. The liver is found in the second sub-group with kidney, heart and brain and not in the "storage group" as with the Aroclor 1254 treatment cluster. The last group contains Chlorofen, soil extract and spleen. The lung samples are distributed among different clusters. In contrast to our observations with Aroclor 1254, tissues group closely together within these three sub-groups. Analysis of the clustering behavior of individual congeners by HCA does not reveal any additional information. They do not group according to chlorination level, metabolic or structure groups or number of ortho-chlorine substituents. The only distinguishing features are the abundance and frequency with which each congener is found in the respective tissues.

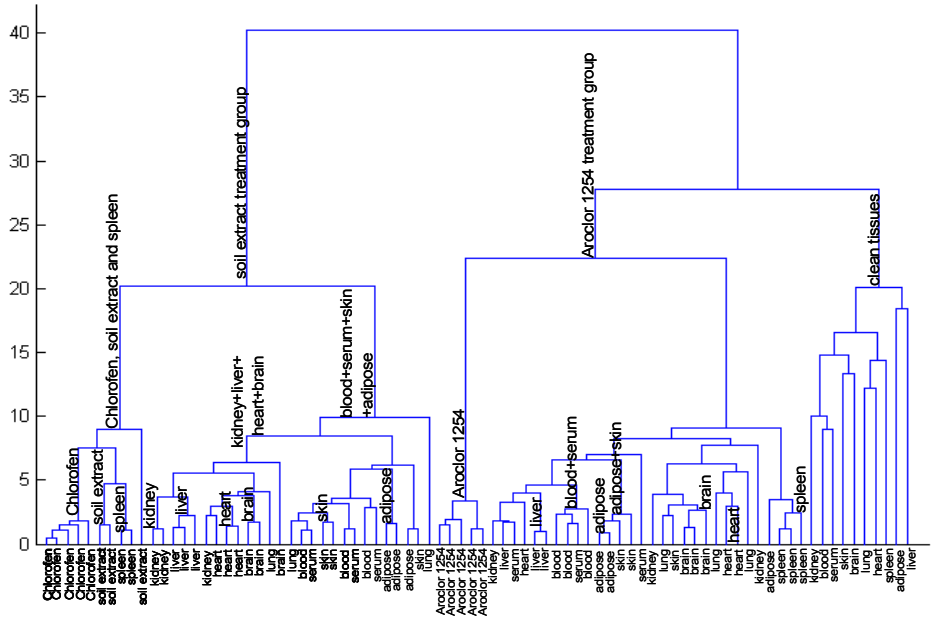

Fig. 1. Dendrogram obtained by Hierarchical Cluster Analysis of all tissues.

Principal Component Analysis (PCA) was used to examine the PCB congener distributions in the tissues. PCA confirmed the results from the HCA and provided additional information on the role of substitution pattern in the distribution of congeners in tissues. The PCA was performed on the same normalized data as described above. More than 35 Principal Components (PCs) are required to describe over 99% of the data variance. We analyzed 3 PCs which explain 51.98% of the data variance. The first two PCs account for 27.59 and 14.73% of the data variance. The projection of objects defined by PC1 and PC2 shows 4 natural groups of objects that are separated from each other (Fig. 2), i.e. (1) Aroclor 1254, (2) tissues from Aroclor 1254-treated animals, (3) tissues from control animals, and (4) a group containing Chlorofen, soil extract and tissues from soil extract-treated animals. The better separation of the Chlorofen group is observed in the PC1 and PC3 projection (Fig. 3). The sub-groups of Chlorofen, soil extract and tissues can be distinguished in this projection. PC3 explains 9.66% of total variance.

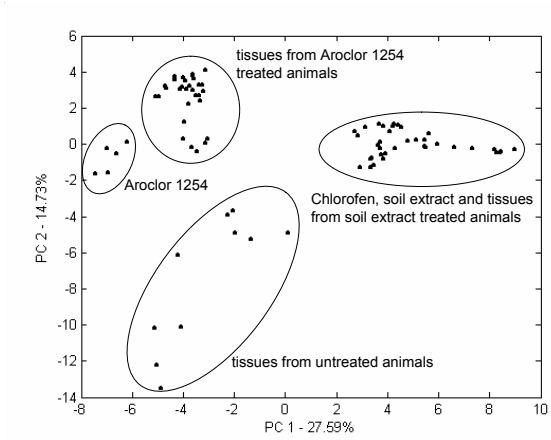

Fig. 2. Projection of PC1 and PC2.

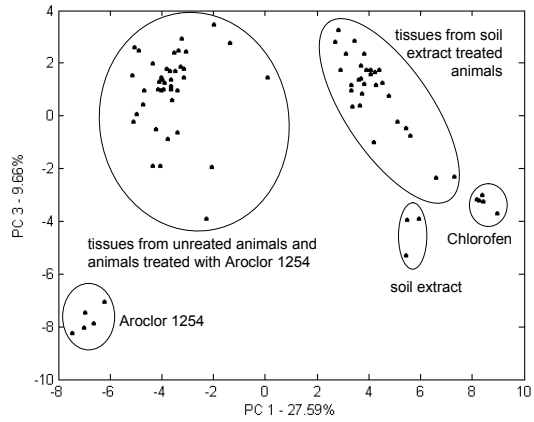

Fig. 3. Projection of PC1 and PC3.

As shown in Figs. 2 and 3, PC1 distinguishes the Chlorofen from the Aroclor group, PC2 separates the control animals from all other samples, and PC3 separates each parent mixture from tissues treated with the respective parent mixture. Some additional information can be obtained from the analysis of the loadings (Table 2), i.e. the contribution of each congener into the construction of PC1 and PC3.

Most congeners with high loadings on PC1 (except of PCB # 183, 129 and 195) are persistent, highly-chlorinated (i.e., 7-9 chlorine substituents) congeners with no vicinal H atoms. These congeners are characteristic for the Chlorofen group (i.e., Chlorofen, soil extract and tissues of soil extract-treated rats). Congeners with a medium degree of chlorination have the lowest loading on PC1. These congeners are characteristic for the Aroclor group (i.e., Aroclor 1254, tissues of Aroclor 1254-treated and control animals). One common feature of congeners with high loadings on PC3 are a 2,4,4' substitution

pattern. These congeners also have o,m-vicinal H atoms or are persistent. No structural relationships can be found for congeners with the lowest loadings in this group or the congeners contributing to the construction of PC 2.

Table 2. Variable loadings in PCA analysis.

Principal component	Loading value	PCB congener	Structure and degradation characteristic*
PC1	**>0.15**	**178+129, 187+182+175, 183, 180, 201, 194, 203+196, 205, 206, 207, 208+195**	**highly chlorinated and persistent**
PC1	**< -0.15**	**81+87, 97, 101, 110+77, 118, 128, 163+138**	**2,4 substitution pattern predominant**
PC2	**>0.15**	**85, 107, 137+176+130, 146, 170+190, 163+138, 153+105+132, 202+171+156**	**o,p substitution pattern and/or persistent**
PC2	**< -0.15**	**18, 44, 45, 46, 56+60, 84+92, 91, (193)**	**easily degradable, (persistent)**
PC3	**>0.15**	**28, 47, 66, 74, 118, 153+105+132, 191, (193)**	**2,4,4'-structure (persistent)**
PC3	**< -0.15**	**41+71, 70+76, 82, 84+92, 134, 52, 95, 97, 110+77, 141, 151, 174**	**Easily degradable, 2,2',3 substitution pattern predominant**

* **In case of coeluting congeners, not all coelutent may show the structural feature listed.**

Discussion

The highest total PCB concentration was in adipose tissue, which is in agreement with previous reports. In the soil extract-treated animals the total PCB levels were in general higher compared to the respective organs in the Aroclor 1254-treated animals, which is in part due to the higher molar weight of the soil extract mixture. Lipid-adjusted total PCB concentrations were similar in all tissues with the exception of the spleen. To our knowledge this is the first report about the unusually high retention of PCB in the spleen compared to other organs on a lipid adjusted basis. In addition, on a molar and a molar weight adjusted basis (data not

shown), the PCB concentration was significantly higher in the spleen and significantly lower in the skin in the soil extract treatment group relative to the Aroclor group. Considering that the time course of the experiment was only seven days, these differences most likely reflect differences in the redistribution of PCBs between the two treatment groups. PCBs are initially distributed into highly perfused organs such as the liver, lung, kidney and spleen[2,7]. Subsequently, PCBs are redistributed into poorly perfused storage organs such as the adipose tissue and the fat depots under the skin. We hypothesize that, relative to the Aroclor treatment group, the significantly higher PCB levels in the spleen of soil extract-treated animals are due to a slower redistribution of the highly chlorinated soil extract from highly perfused organs such as the spleen and into storage tissues such as the skin. In comparison the redistribution apparently happens faster in the tissues of animals treated with medium chlorinated Aroclor 1254.

Hierarchical cluster analysis of PCB profiles in tissues shows two main clusters, i.e. a Chlorofen and an Aroclor cluster. Control animals group with the Aroclor samples which is consistent with the fact that the "background" contamination results from Aroclors (Fig. 2). In Aroclor and soil extract treatment groups the association of tissues with each other was different. Unlike the spleen PCB profile from Aroclor-treated animals, which resembled the profile from other tissues, the PCB profile of the spleen from soil extract-treated animals was most similar to the soil extract profile itself. This resembles a recently reported observation with mice exposed to PCB 84, where the enantiomeric fraction of PCB 84 in the spleen was very similar to the parent racemic compound, while all other organs showed a significant change of the enantiomeric fractions[2]. These observations, similarly to the significantly higher lipid adjusted PCB levels in the spleen of both treatment groups, suggest that the spleen, especially of soil extract-treated animals, still serves as an initial PCB depot at day seven. A possible reason for this phenomenon could be that mechanisms and/or degree of uptake and/or clearance of PCBs in the spleen is different compared to other organs.

Principal Component Analysis confirms the groupings observed in the HCA but also gives a few additional details. Highly chlorinated, persistent congeners (Chlorofen) and medium chlorinated congeners (Aroclor 1254) contribute to construction of PC1, thus differentiating the soil extract and the Aroclor 1254 treatment group from each other (Table 2). Such congeners therefore need to be taken into consideration for the risk assessment of Chlorofen or soil contaminated with this mixture. PC2 distinguished mainly tissues from Aroclor 1254 and untreated animals, while, within a treatment group, PC 3 separated the parent mixture from tissues of animals treated with the respective treatment group (Fig.

3). This is another indication that the composition of PCB mixtures is altered in biological matrixes.

The conclusion from this study is that, during the initial redistribution phase, the chlorination level of PCB mixtures influences to some degree the total PCB concentration in tissues such as the spleen. In addition, the PCB profiles in selected tissues may (soil extract) or may not (Aroclor 1254) be different to each other in animals receiving the same treatment. This suggests that the distribution, redistribution and/or metabolic processes of PCBs differ depending on the degree of chlorination and, possibly, the chlorine substitution pattern of individual congeners. An understanding of such processes however is necessary to understand the toxicities of different PCB mixtures.

Acknowledgements

This work was supported by the Environmental Health Sciences Research Center of the University of Iowa (ES05605) and the University of Kentucky Superfund Basic Research Program (ES07380). I. Kania-Korwel gratefully acknowledges the support of a Fulbright Junior Research Grant and a Kosciuszko Foundation Grant.

References

1 Sułkowski W. W. Kania-Korwel I., Robertson L., Szafran B., Lulek J. (2003) Fres. Environ. Bull. 12(2), 158
2 Lehmler H.-J. Price D.J., Garrison A.W., Birge W.J., Robertson L.W. (2003) Fres. Environ. Bull. 12, 254
3 Gill U. S. Schwartz H. M., Whearley B. (1996) Chemosphere. 32(6), 1055
4 Hornbuckle K.C. Smith G.L., Miller S.M., Eadie B.J., Lansing M.L. (2004) J Geophysical Research-Oceans
5 Voorspoels S. Covaci A., Maervoet J., Schepens P. (2002) Bull. Environ. Contam. Toxicol. 69, 22
6 Vandeginste B.G.M. Massart D.L., Buydens L.M.C., de Jong S., Lewi P.J., Smeyers-Verbeke J., *Handbook of Chemometrics and Qualimetrics*. Vol. B. 1998, Amsterdam: Elsevier
7 Brandt I., Mohammed A. and Slanina P. (1981) Toxicology. 21(4), 317