BIOCHEMICAL EFFECTS OF AN ENVIRONMENTAL CHLOROFEN MIXTURE IN COMPARISON WITH AROCLOR 1254 IN RATS

Izabela Kania-Korwel[2], Gabriele Ludewig[2], Larry W. Robertson[2], Keri C. Hornbuckle[3], Aaron Peck[3], Parvaneh Espandiari[4], C. Gary Gairola[4], Wiesław W. Sułkowski[1], Hans-Joachim Lehmler[2]

[1]Department of Environmental Chemistry and Technology, University of Silesia, Katowice, Poland
[2]Department of Occupational and Environmental Health, University of Iowa, Iowa City, U.S.A.
[3]Department of Civil and Environmental Engineering, University of Iowa, Iowa City, U.S.A.
[4]Graduate Center for Toxicology, University of Kentucky, Lexington, U.S.A.

Introduction

Polychlorinated biphenyls (PCBs) are environmental contaminants of great environmental concern, although their manufacture was banned in most countries in the 70's. The total world production was estimated to be 1 million tons[1]. This number does not include many mixtures manufactured in Eastern and Central European countries. Only limited information about these mixtures is currently available. The total production[2] and the congeners profile[3] have been reported recently for the Polish PCB mixture Chlorofen. However, no extensive toxicological studies were performed with Chlorofen, which is in contrast to the well studied Aroclors, especially Aroclor 1254. This is surprising because Chlorofen has a unique congener profile when compared to all other technical PCB mixtures such as Aroclors, Clophens or Kanechlors[3].

Early experiments studied the effect of Chlorofen on the mortality of fish, daphnia and snail in a concentration range from 0.1 to 100 mg/L[4]. Daphnia was the most sensitive of these three species with an LD_{50} of 1 mg/dm^3 after 24 h. In fish Chlorofen caused a decrease in body weight, while it had no effect on snail. In this study Chlorofen added to the water was more toxic compared to a layer of the mixture on the aquarium walls. In an *in vitro* study the Ah receptor response to Chlorofen along with other technical mixtures was reported[5]. The relative potencies compared to TCDD were in the range of 3.8×10^{-8} to 7.6×10^{-7}, almost an order of magnitude less compared to all Aroclors studied.

To our knowledge no *in vivo* mammalian toxicity studies with Chlorofen or environmental mixtures from Chlorofen contaminated sites have been performed. The present study investigates the biological effects in male rats of a soil-extract from a highly contaminated soil from the Chlorofen manufacturing site. The congener profile of this soil extract is very similar to Chlorofen although some lower chlorinated congeners are present, probably because of biodegradation or atmospheric deposition. The biological effect was compared with Aroclor 1254. The total PCB levels as well as the PCB congener profile in the liver were also determined to allow a better understanding of the effects of the two different mixtures on relevant liver enzymes.

Materials and methods

The soil PCB mixture was a hexane-acetone extract from soil collected at the Chlorofen manufacturing site[6]. One month old male rats (Sprague Dawley, Harlan) were randomly divided into three groups and injected i.p. with a single dose of the environmental PCB mixture (0.05 mmol/kg b.w.; n = 3) or Aroclor 1254 (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. The liver was excised *en bloc*.

A series of blood assays was performed using a commercial test kit (VET 16 veterinary test rotor for Analyst benchtop chemistry system, Hemagen, Waltham, Massachusetts, US). These tests did not show any differences between control and PCB treated animals suggesting that no acute liver or kidney damage occurred as a result of the treatment. Liver microsomes were obtained as described previously[7]. Microsomal protein concentration was determined by the method of Lowry[8]. Total cytochrome P-450 content was quantified by the method of Omura and Sato[9]. The resorufin assay was performed as described by Burke, Mayer and Lubet[10-12].

The extraction of PCBs from tissues was based on EPA methods[13-15]. PCB extraction from blood was described by Gill[16]. The PCB analysis was performed using a HP gas chromatograph with a ^{63}Ni μ-ECD detector. Ninety chromatographic peaks representing 120 PCB congeners were determined.

Results

Body- and tissue weight, total cytochrome P-450, as well as EROD and PROD activity for each treatment group are summarized in Table 1. Mean liver weight was significantly elevated in the soil extract-treated animals compared to controls and Aroclor 1254-treated rats. No differences were observed for body and thymus weights. No increased total cytochrome P-450 enzyme content was observed, although both mixtures increased EROD and PROD activities in the livers as

compared to controls. In the soil extract-treated animals PROD (pentoxyresorufin-*O*-dealkylase, CYP2B) activity in rat liver microsomes was 4.5 times higher than in Aroclor 1254-treated rats. In the Aroclor 1254-treated rats EROD (ethoxyresorufin-*O*-dealkylase, CYP1A) activity was 28.7 times higher compared to soil extract treated animals.

Table 1. Biological effects of Aroclor 1254 and soil extract in treated rats (means ± S.D.).

Biological effect	**Control (n=4)**	**Aroclor 1254 (n=4)**	**soil extract (n=3)**
Body weight change [%]	33.30 ± 1.47	32.68 ± 1.14	33.67 ± 1.76
Liver weight change [%]	4.98 ± 0.10	4.70 ± 0.47	5.35 ± 0.10*
Thymus weight change [%]	0.37 ± 0.06	0.38 ± 0.04	0.31 ± 0.04
Total CYP450 [nmol/mL]	0.57 ± 0.16	0.60 ± 0.03	0.68 ± 0.13
PROD [nmol/mg protein min]	n.d.	0.62 ± 0.55	2.83 ± 0.72$^{**\,\#\#}$
EROD [nmol/mg protein min]	n.d.	4.03 ± 1.23$^{\#\#}$	0.14 ± 0.10**

* Statistically different from Aroclor 1254, P = 0.02; ** Statistically different from Aroclor 1254, P = 0.001; $^{\#\#}$ Statistically different from control, P = 0.001; n.d. = not detectable.

The average congener profile in the liver of soil extract and Aroclor 1254-treated animals is shown in Figure 1 and 2, respectively. The profile of the corresponding parent mixture is shown for comparison. The environmental mixture, which was extracted from soil contaminated with Chlorofen, has a profile with hepta- and octachlorobiphenyls as main homologue groups (Figures 1 and 3). Aroclor 1254 consists of a wide range of congeners with pentachlorobiphenyls being the main homologue group (Figures 2 and 3).

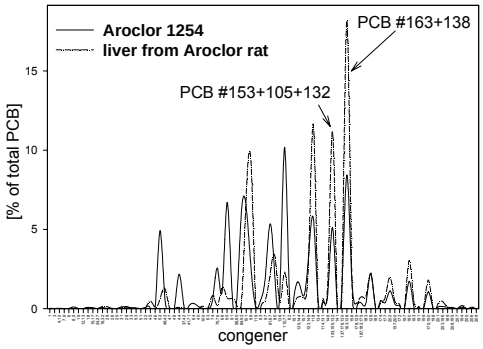

Fig. 1. Congener profile (content as % of total PCB) in soil extract and liver of soil extract treated animals.

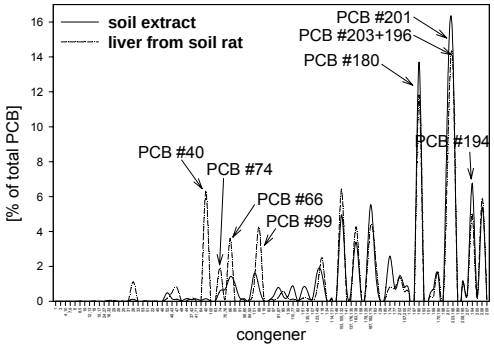

Fig. 2. Congener profile (content as % of total PCB) in Aroclor 1254 and liver of Aroclor 1254 treated animals.

In the liver of Aroclor 1254-treated rats the accumulation of persistent congeners, e.g. PCB #153+105+132 and #163+132, can be observed (Figure 2). The same congeners have a higher content in the liver of soil extract-treated animals (Figure 1). However, in this group a decrease of some of the highest chlorinated PCB congeners can be noticed (e.g., PCB #180, 201, 203+196 and 194). In the Aroclor-treated animals, the levels of lower chlorinated PCBs are reduced while in the soil extract-treated animals some lower chlorinated congeners (e.g., PCB #40, 74, 66, 99) begin to appear. These changes can also be observed in Fig. 3 which shows a relative increase in lower chlorinated homologues (≤ 3 Cl atoms) and decrease in higher chlorinated homologues (≥ 7 Cl atoms) for the soil extract treatment group. The opposite trend can be observed for the Aroclor 1254 treatment group where the relative composition shows an increase in higher chlorinated homologues (≥ 6 Cl atoms) and a decrease in lower chlorinated homologues (≤ 5 Cl atoms).

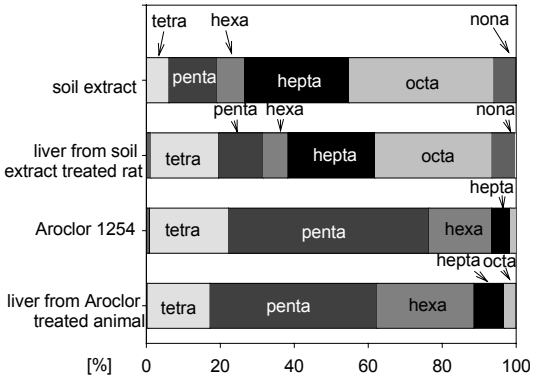

Fig. 3. Approximated percentage of homolog groups in parent mixtures and rat livers.

The distribution of congeners with different number of ortho chlorines in the two mixtures is presented in Fig. 4. In soil extract and liver from soil extract-treated rats tri- and di-ortho substituted congeners are the main group. In Aroclor 1254 and the liver from Aroclor-treated rats, di-ortho substituted congeners are predominant and more of one-ortho and even non-ortho congeners are present. In both treatment groups there is a relative decrease in congeners with 3 or 4 ortho chlorines, while congeners with 1 and/or 2 ortho chlorines increase.

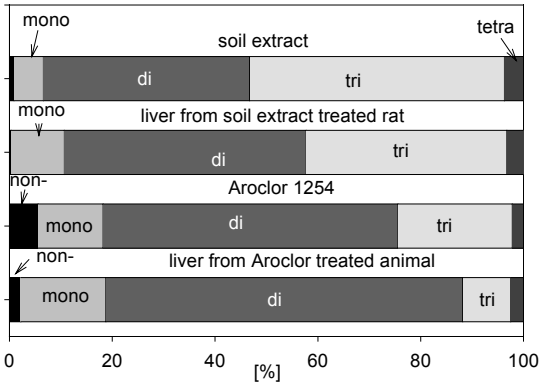

Fig. 4. Approximated percentage of congener with ortho-chlorines in parent mixtures and rat livers.

Metabolic groups were assigned according to congener structure[17]. Their relative content is shown in Fig. 5. In soil extract and the liver from soil extract-treated rats the major metabolic group is 1, i.e. the group consisting of basically non-metabolizable congeners (congeners with no vicinal H atoms). The soil extract treatment group shows a decrease of congeners belonging to group 1 while groups 3 and 4 are increasing compared to the parent mixture. In Aroclor 1254 and the liver from Aroclor-treated rats the main group is 3, i.e. the group which contains congeners with o,m-vicinal H atoms, i.e. CYP1A metabolizable compounds. The

Aroclor liver extract shows an increased contribution of groups 1 and 3 to the overall composition of the mixture relative to Aroclor 1254. On the other hand, congeners belonging to groups 2 (only m,p vicinal H atoms, CYP2B metabolizable) and 4 (congeners with o,m & m,p vicinal H-atoms) show a decrease in comparison to Aroclor 1254.

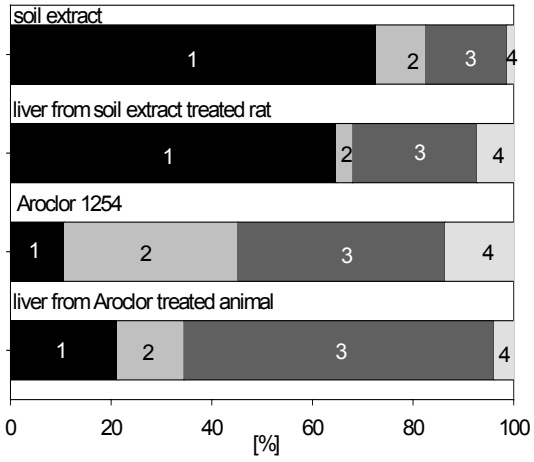

Fig. 5. Approximated percentage congeners belonging to metabolic groups 1 through 4 in parent mixtures and rat livers.

Discussion

Two very different PCB mixtures were used in this study. The dose of PCB mixtures injected into the animals (50 µmol/kg body weight, respectively) was low. This dose was selected to be low enough to mimic typical environmental exposures, but also high enough to ensure positive response in an enzymatic assay. It is well established that as little as 2 mg PCB per kg body weight are sufficient to increase enzymatic activity *in vivo*[18, 19].

Major effects of PCB treatment are loss of body weight, decrease in thymus weight and increase of liver weight. The only effect observed in this study however was an elevated liver mass in the soil extract-treated rats. This increase is due to an enhanced production of PCB metabolizing enzymes in the endoplasmic reticulum of liver cells. The lack of other effects is most likely the result of the low dose or because the statistical power of the experiment was not sufficient to detect subtle effects like thymus mass changes.

The two mixtures have a very different homologue composition. Aroclor samples are high in dioxin-like congeners (CYP1A inducing compounds), i.e. penta chlorinated congeners with mostly di-ortho as well as some non- or mono-ortho substitution patterns. On the other hand the soil extract samples are high in ortho-substituted PCBs (i.e. CYP2B inducing compounds) with highly chlorinated congeners with mostly three ortho Cl-substituents. These differences in the

homologue composition are also reflected in the biological effects. EROD activity (CYP1A), which is characteristic for dioxin like congeners, was elevated in Aroclor 1254 animals. PROD activity (CYP2B), which is characteristic for ortho-substituted PCBs, was strongly induced in soil extract treated animals.

The congener profiles found in the liver of treatment animals reveal distinct changes in the relative congener composition compared to the respective parent mixture. In the Aroclor treatment group, relative levels of persistent, higher chlorinated congeners (metabolic group 1) show an increase in the liver while lower chlorinated congeners decrease or even disappear (metabolic group 2). The opposite is true for the soil extract treatment group where higher chlorinated congeners (metabolic group 1) show a decrease and lower chlorinated congeners increase or begin to appear (metabolic groups 3 and 4). These observations are indicative for metabolic processes. Lower chlorinated congeners present in Aroclor 1254 are readily metabolized, thus resulting in an increased contribution of persistent, higher chlorinated congeners to the overall profile. The changes in the soil extract group are indicative of metabolism of higher chlorinated congeners or, more likely, movement of these congeners into adipose storage tissue. Such processes also explain the relative increase of lower chlorinated congeners compared to higher chlorinated congeners.

Our results show that the high degree of chlorination of the soil extract mixture results in (1) a tissue distribution, (2) metabolism, and (3) biological effects that are drastically different from the medium chlorinated Aroclor 1254. These differences need to be taken into consideration when assessing the risk of exposure to soil contaminated with the Polish PCB mixture Chlorofen.

Acknowledgements

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