PCB AND PCB METABOLITES IN SERUM FROM YUSHO PATIENTS 37 YEARS AFTER THE ACCIDENT

Linderholm L[1], Masuda Y[2], Athanasiadou M[1], Bergman Å[1]
[1]Department of Environmental Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden. [2]Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan.

Abstract
Over 1800 people became ill after ingestion of rice oil contaminated with polychlorinated biphenyls (PCBs) in Japan 1968. In the present study, serum samples from 9 Yusho patients collected in 2005, 37 years after the Yusho accident, have been analysed for PCBs and for their hydroxylated (OH-PCB) and methyl sulfone ($MeSO_2$-PCB) metabolites. Liquid-liquid extraction was used for extraction and separation into the three substance groups. Sulfuric acid treatment and silica gel columns were applied before analysis by GC-ECD or GC-MS were performed. CB153 was the major PCB congener followed by CB138, CB180, CB156 and CB187. Mean Σ(10)PCBs ranged from 1.5 to 3.7 ng/g serum with a mean concentration of 2.6 ng/g serum (390 ng/g fat). The characteristic PCB pattern for Yusho patients, high levels of CB156 and low levels of CB118 and CB105 were observed. The Σ(6)OH-PCBs ranged between 0.39-1.3 ng/g serum with a mean value of 0.78 ng/g serum. Relatively high levels of the OH-PCB congener 4'-OH-CB120 was found which might explain the low levels of CB118 due to an enhanced metabolism in Yusho patients caused by an induction of cytochrome P450 enzymes. A $MeSO_2$-PCB pattern similar to other human cohorts was observed but at very low concentrations.

Introduction
In 1968 rice bran oil was contaminated with polychlorinated biphenyls (PCBs) in Japan. Over 1800 people developed a strange skin disease (Yusho disease) that were shown to be caused by ingestion of a certain type of rice oil[1]. The rice oil was found to be contaminated by PCBs. Later on it was also shown that the rice oil was contaminated with polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/PCDFs)[2]. Some typical symptoms in Yusho patients were fatigue, weight loss, acne and dark pigmentation of the skin and nails[3]. PCBs are metabolized to hydroxylated PCBs (OH-PCBs) and methyl sulfone PCBs ($MeSO_2$-PCBs)[4]. An arene oxide is formed as a first step in the metabolism, an oxidation mediated by cytochrome P450. The arene oxide can form OH-PCBs spontaneously or via the enzyme epoxide hydrolase. Alternatively, it can react with glutathione and form $MeSO_2$-PCBs via the mercapturic acid pathway, thiol formation, S-methylation by the intestinal micro flora and oxidation to form PCB methyl sulfones[5]. Some OH-PCBs are retained in blood due to their high affinity to transthyretin, one of the transport proteins for the hormone thyroxine in blood[6]. OH-PCBs have been analysed in blood in relatively high concentration compared to PCBs[7-9]. $MeSO_2$-PCBs are lipophilic and accumulate in adipose tissue but they can also bind to specific proteins, leading to a strong retention certain tissues. For example, some para-substituted $MeSO_2$-PCBs bind to uteroglobine, a secretory protein found in mouse and human lungs. High levels of $MeSO_2$-PCBs were found in the lung of a Yusho patient compared to a control patient[10,11]. The aim of this project was to analyse serum samples from Yusho patients for PCBs, OH-PCBs and $MeSO_2$-PCBs 37 years after the accident.

Materials and methods
Samples: Serum samples from 9 Yusho patients were collected in 2005. The samples came from females (n=5) and males (n=4). The subjects were between 46 and 79 years of age (mean 68).

Clean-up and analysis: The methods used for the extraction, clean-up and instrumental analyses of PCB and PCB metabolites are described elsewhere[8,12]. Shortly, hydrochloric acid and 2-propanol were added to 2-3g of serum before the analytes were extracted with a mixture of n-hexane:MTBE. After re-extraction of the serum, the combined organic phases were washed with a 1% potassium chloride solution. A neutral and a phenolic fraction were received through partitioning with potassium hydroxide. The neutral fraction was partitioned with dried DMSO to isolate methylsulfonyl metabolites of PCBs and DDE from their parent compounds since aryl methyl sulfones partition into DMSO as previously described[13,14]. The methylsulfonyl substituted analytes were extracted from the DMSO by addition of water and n-hexane and the methyl sulfone phase was further cleaned

up on a multilayered silica gel column. The phenolic fraction was derivatized with ethereal diazomethane and further cleaned up with sulfuric acid and a sulfuric acid:silica gel column. The PCBs were cleaned up by a sulfuric acid treatment and a sulfuric acid:silica gel column. The OH-PCBs and the PCBs were analysed with GC-ECD and the $MeSO_2$-PCBs were analysed with GC-MS (ECNI).

Results and discussion

The median levels of the major PCBs and OH-PCBs are presented in Figure 1. CB153 was the major PCB congener followed by CB138, CB180, CB156 and CB187. The rice oil was contaminated with the PCB mixture Kanechlor 400, a mixture that consisted of mostly tri- to penta-chlorinated biphenyls[15]. Relatively high levels of CB156 and low levels of CB118 and CB105 is characteristic for Yusho patients[16]. This pattern was seen in these samples as well. The Σ(10)PCBs ranged from 1.5 to 3.7 ng/g serum (270-580 ng/g fat) with a mean concentration of 2.6 ng/g serum (390 ng/g fat). The major OH-PCB metabolite was 4-OH-CB187 followed by 4-OH-CB146, 4-OH-CB107 and 4'-OH-CB120. Interestingly, the levels of 4'-OH-CB120 in the Yusho patients are relatively high compared to most other studies on OH-PCBs in human serum[7,9,17]. 4'-OH-CB120 is a metabolite of CB118 (after a 1,2-shift). CB118 and CB105 are metabolized to 4'-OH-CB107, a metabolite that is found in the serum as well. The low values of CB118 and CB105 seen in Yusho patients compared to control groups might be due to an induction of cytochrome P450 and, therefore, an enhanced metabolism to the hydroxylated metabolites. The Σ(6)OH-PCBs ranged between 0.39-1.3 ng/g serum with a mean value of 0.78 ng/g serum. The mean ratio of the Σ(6)OH-PCBs to the Σ(10)PCBs was 0.31, ranging between 0.17 and 0.52. This ratio is quite high compared to other studies that reports ratios between 0,08-0,16[8,9,17-19]. Similar high ratios were seen in Latvian men where 4-OH-CB107 by far was the dominating OH-PCB congener[20]. The reason for these high ratios might be due to an old exposure to PCB or induction of cytochrome P450 enzymes. The mean of Σ(10)PCBs in men and women were the same (2.5 ng/g serum). The mean of Σ(6)OH-PCBs were 0.91 ng/g serum in women and 0.62 ng/g serum in men, but the difference was not significant. A comparison of the OH-PCB pattern in men and women is shown in Figure 2. All Yusho patients had a low concentration of $MeSO_2$-PCBs in their blood. The dominating sulfone metabolites were 4-$MeSO_2$-CB87 and 4-$MeSO_2$-CB101 at approximately two orders of magnitude lower levels than the most abundant OH-PCBs.

Similar concentrations of PCB and OH-PCB were found in 36 Yusho patients sampled between 1999 and 2003[16]. The ratio between Yusho patients and controls from that study ranged from 0.47 for CB105 to 8.35 for CB156. $MeSO_2$-PCBs were not detected in the blood in that study. However, Haraguchi and co-workers identified 40 $MeSO_2$-PCBs in lung liver and adipose tissue from one Yusho patient in 1986[10,11].

The present study shows PCB and OH-PCB congener patterns that can be related to exposure to PCB almost four decades earlier.

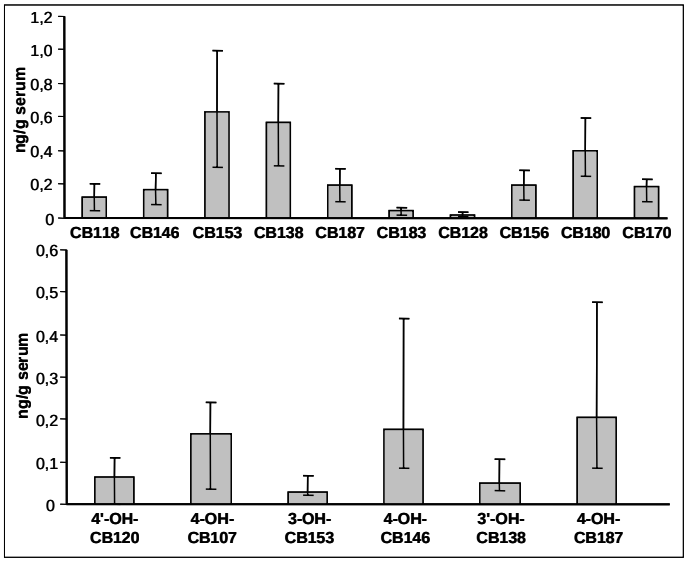

Figure 1. Median concentration (ng/g serum) and range of PCBs and OH-PCBs in serum from Yusho patients.

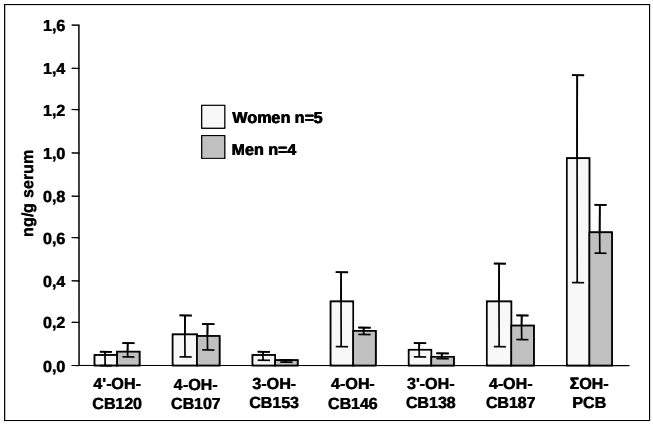

Figure 2. A comparison of OH-PCB pattern in women and men.

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