MEASUREMENT OF HYDROXYLATED PCB METABOLITES (OH-PCBS) IN ARCHIVED SERUM FROM 1950-60'S CALIFORNIA MOTHERS

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Abstract

Since the persistent hydroxylated PCB metabolites (OH-PCBs) are toxicologically active, we conducted a pilot study to characterize and quantify OH-PCBs to elucidate the association between prenatal OH-PCB metabolites and adverse effects on adult offsprings' development. We used GC-ECD and GC-NCI/MS to analyze PCBs and OH-PCBs in 30 archived third trimester or postpartum serum specimens collected in the 1950-60's California mothers from a large cohort study (Child Health and Development Studies, CHDS). The blood levels of OH-PCB metabolites were dependent on PCB levels (R^2=0.34, $p<0.05$). The average ratio of Σ_8OH-PCBs to Σ_{11}PCBs was 0.16±0.09. These ratios seem to be inversely related, albeit rather weakly (R^2=0.18, $p<0.05$), to PCB concentrations, possibly due to the faster elimination of OH-PCBs as a result of the enhanced induction of phase II enzymes and conjugation reactions. Σ_8OH-PCB metabolites showed significant difference between the higher and lower exposed mothers ($p<0.05$). 4-OH-CB187 was the primary metabolite followed by 4-OH-CB107. We found that 1950-60's California mothers were exposed to OH-PCBs, particularly more to para-positioned ones. Therefore, we will focus the exposure to those OH-PCB metabolites on future analyses and try to explore epidemiologically the relationship between prenatal exposure of OH-PCB metabolites and adult offsprings' status of thyroid function, development, and reproduction.

Introduction

Many adverse human health and developmental effects of PCBs have been reported. In addition, their hydroxylated metabolites (OH-PCBs) are also retained in the body [1] and may contribute to the effects, at least in part. OH-PCBs are transferred to the fetus via the placenta [2,3,4] and may contribute to hypothyroidism [5,6], and possibly chronic adverse health effects in adult offspring. Maternal OH-PCBs may result in some endocrine related problems in offspring after birth and possibly in adulthood, such as impaired reproductive system [7] and others (e.g., impaired development of neurosensory and immune systems). In the body, PCBs are biotransformed to more water soluble forms (e.g., OH-PCBs), via cytochrome P450 enzyme-mediated oxidation and OH-PCB metabolites of para- and meta-substituted OH with adjacent chlorine atom have relatively high affinity to transthyretin (TTR) [8]. In addition, humans can be exposed to OH-PCBs by other environmental inputs such as precipitation and surface water [9], and consumption of fish [10]. This pilot study was proposed as part of California Child Health and Development Studies (CHDS) funded by the National Institute of Health (NIH) that is a longitudinal cohort study with more than 20,000 pregnant women enrolled. The original study was designed to find associations between prenatal organochlorine exposures to the adult offsprings' health (e.g., thyroid and male reproduction, and thyroid and development). Since, to our knowledge, there have been no human epidemiological studies on the prenatal exposures to OH-PCBs and adverse health effects on the adult offspring, we attempted to characterize and quantify the levels of specific OH-PCB metabolites from 1950-60's Californian maternal serum exposed to environmental PCBs.

Materials and Methods

From 1959 to 1967, more than 20,000 pregnant women were enrolled in a large cohort study, CHDS. Aliquots of 700 maternal serum specimens were transferred to the Department of Toxic Substances Control, Berkeley, CA for PCB and OH-PCB analyses. Among the first 100 specimens analyzed for PCBs and organochlorine pesticides, a subset (N=30) selected from both higher (N=15) and lower ends (N=15) of PCB concentrations was analyzed for eight OH-PCB metabolites. Each batch for the OH-PCB analysis consisted of one reagent blank (water), 1 control sample, and 10 serum samples. Before extraction, 4'-OH-CB159 was added to all samples as a surrogate internal standard. The blood extraction method was

adopted to separate the PCBs and OH-PCBs from maternal serum by using MTBE:hexane (1:1,v/v), denaturation (6M HCl), KCl (1%) wash, and KOH phase separation [1]. The extracts in neutral fractions (e.g., PCBs) were cleaned up by using deactivated Florisil column chromatography. After the acidification and derivatization, the extracts in phenolic fractions (e.g., OH-PCBs) were cleaned up by using concentrated H_2SO_4 (98%) and then Pasteur pipette column chromatography packed with acidic silica gel (1:2, w/w) and activated silica gel. Eleven PCB congeners were analyzed on a Varian 3800 GC-ECD (Varian Inc., Walnut Creek, CA) equipped with RTX-5MS capillary column (60m × 0.25 mm i.d., 0.25 μm thickness, Restek, Bellefonte, PA) and DB-XLB (60 m × 0.25 mm i.d., 0.25 μm thickness, J&W Scientific, Folsom, CA). Nine OH-PCB metabolites, including an internal standard, were determined as methyl derivatives (MeO-PCBs) by using a Varian 1200 GC-NCI/MS (Varian Inc., Walnut Creek, CA) equipped with DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, USA). The most intense ions, either molecular ion or fragmentation ion, were monitored. GC temperature program completely resolved the possible co-elutions (e.g., 4-OH-CB153/4-OH-CB146, 3'-OH-CB138/4'-OH-CB130, and 3'-OH-CB180/4'-OH-CB172). The samples with low surrogate recoveries were excluded from the data summary.

Results and Discussion

Eight OH-PCBs (penta, hexa, and hepta-OH-PCBs) were observed; 4-OH-CB107, 3-OH-CB153, 4-OH-CB146, 3'-OH-CB138, 4'-OH-CB130, 4-OH-CB187, 3'-OH-CB180 and 4'-OH-CB172. Average recovery of internal standard (4'-OH-CB159) was 89±24%. 4-OH-CB187, 4-OH-CB107, and 4-OH-CB146 were detected in quantifiable level in almost all serum samples, while 4'-OH-CB130 and 3'-OH-CB180 were not detected or detected below the detection limits. The concentrations of OH-PCBs here are presented as ng/mL wet wt. since they are preferentially bound to blood protein rather then lipid. Σ_8OH-PCB metabolites varied from 0.21 to 1.01 ng/g wet wt. with an average of 0.58±0.29 in the higher exposed mothers, and from 0.13 to 0.71 ng/g wet wt. with an average of 0.37±0.18 in lower exposed mothers, and showed significant difference between the two groups ($p<0.05$) (Figure 1).

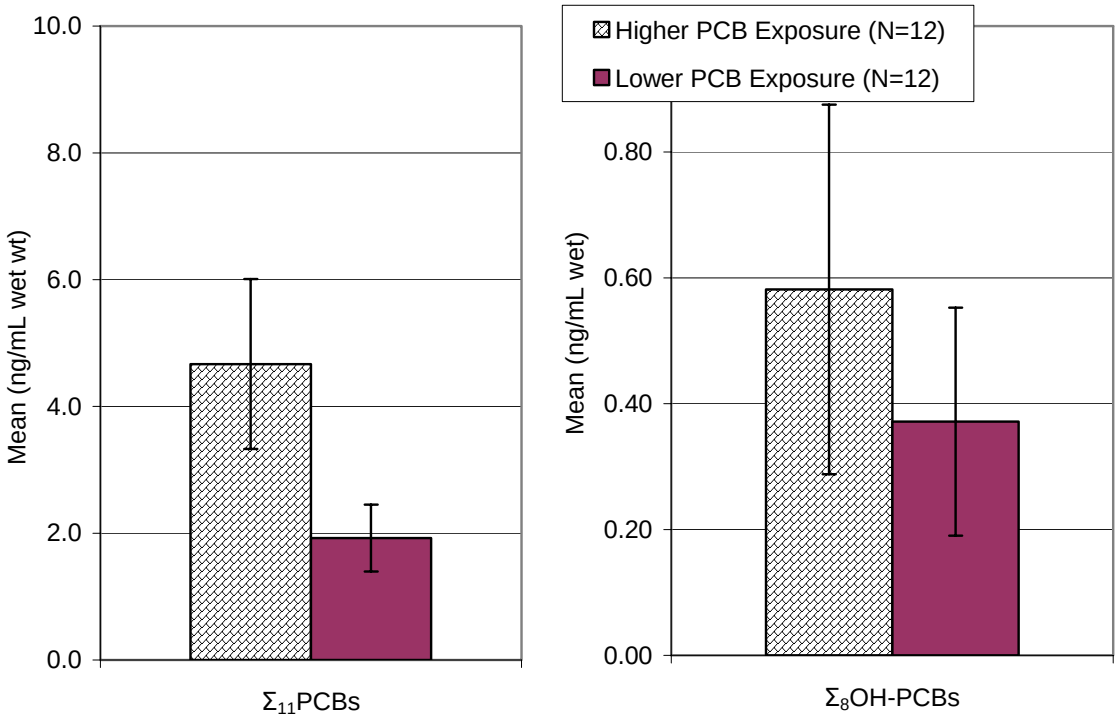

Figure 1. Concentrations of PCBs and OH-PCBs in high and low exposed California mothers. Error bars indicate standard deviation.

The concentrations of OH-PCB metabolites observed in this study were comparable to a Slovakia cohort study and our median concentrations fell between the high and the relatively lower exposure areas [1]. Σ_8OH-PCBs correlated with Σ_{11}PCBs (R^2=0.34, $p<0.05$), indicating the blood levels of OH-PCB metabolites were dependent on PCB levels (Figure 2a). Ratios of Σ_8OH-PCBs to Σ_{11}PCBs ranged from 0.05 to 0.37 with an average of 0.16±0.09. These ratios seem to be inversely related, albeit rather weakly (R^2=0.18, $p<0.05$), to PCB concentrations (Figure 2b), possibly due to the faster elimination of OH-PCBs as a result of the enhanced induction of phase II enzymes and conjugation reactions. 4-OH-CB187 was a primary metabolite followed by 4-OH-CB107. We observed about four times higher concentrations of 4-OH-CB107 than in the Slovakia cohort study where the prenatal exposure to this metabolite congener was significantly related to the deficit of offsprings' neurodevelopment in the 16 months' follow up [11]. We found that 1950-60's California mothers were exposed to OH-PCBs, particularly more to para-positioned OH-PCB metabolites (4-OH-CB107 and 4-OH-CB187) than meta-positioned OH-PCBs (e.g., 3-OH-CB153, 3'-OH-CB138). Therefore, we will focus the exposure to those OH-PCB metabolites on future analyses. We plan to conduct additional serum analysis of OH-PCBs to explore epidemiologically the relationship between prenatal exposure of OH-PCB metabolites and adult offsprings' status of thyroid function, development, and reproduction.

Acknowledgements and Disclaimer
This study was funded by the U.S. National Institutes of Health (grant #: R01ES012460, R01ES012231, and N01-HD-4-3367). The authors would like to thank the many people who assisted with transfer of the specimens and analysis, especially Piera Cirillo and Dr. Olga Kalantzi. The ideas and opinions expressed herein are those of the authors and do not necessarily reflect the official position of the California Department of Toxic Substances Control.

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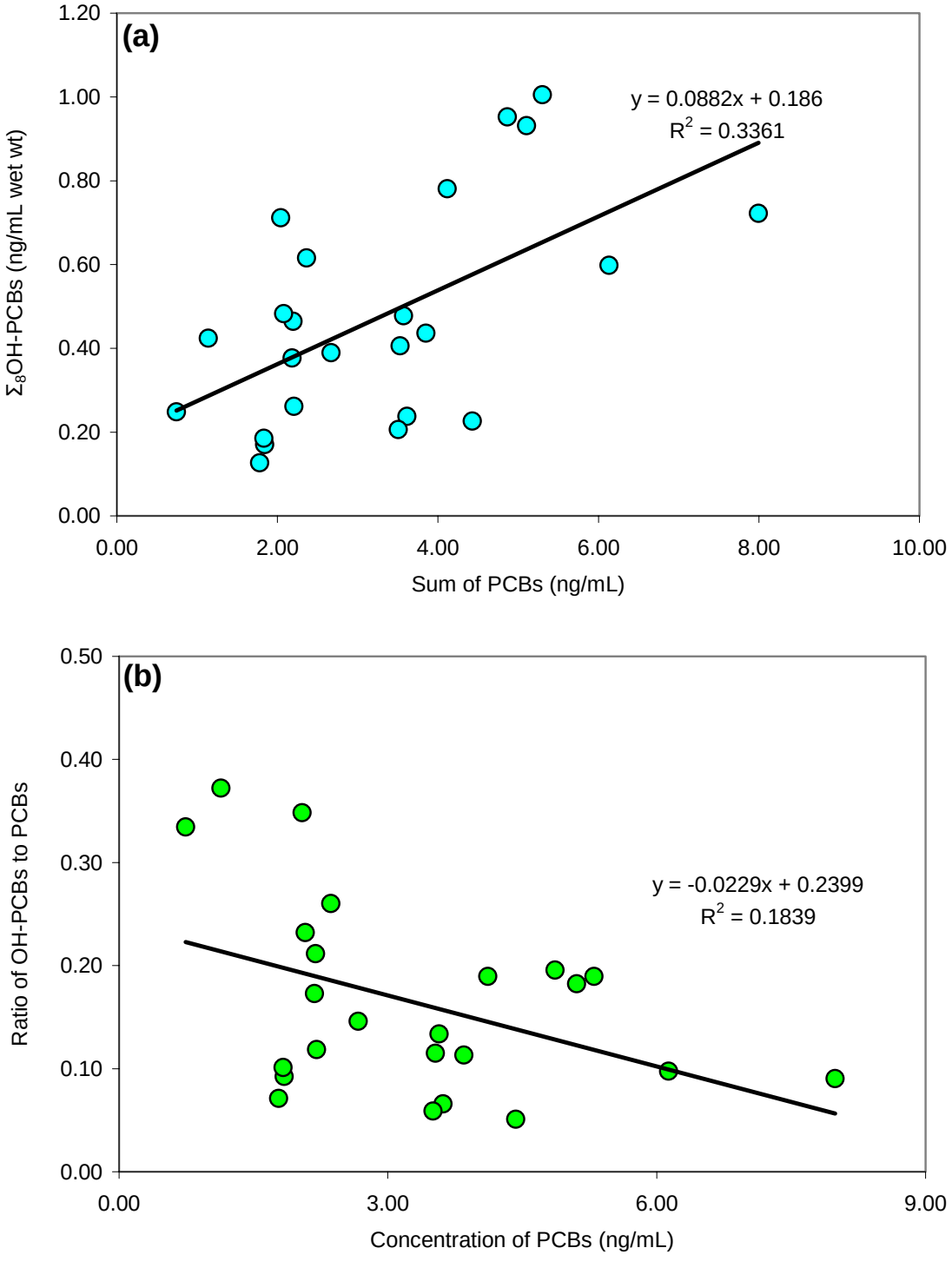

Figure 2. Correlation between PCBs and OH-PCB metabolites in 1950-60's California mothers' serum (a) and inverse relationship between PCB concentrations and the ratios of OH-PCBs to PCBs (b).