# HYDROXYLATED METABOLITES OF POLYCHLORINATED BIPHENYLS (PCBS) IN CALIFORNIA WILD BIRDS

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**Abstract**

Hydroxylated PCB metabolites (OH-PCBs) are persistent and toxicologically active compounds. We characterized and quantified OH-PCBs from kestrel plasma, fail-to-hatch peregrine falcon eggs, and a dead chick. The kestrels nested in the San Francisco Bay region, and the peregrines throughout California. PCBs and OH-PCB metabolites were measured using HRGC-HRMS and GC-NCI/MS, respectively. Kestrel plasma samples carried high levels of $\Sigma_{16}$PCBs (6.53-45.4 ng/g wet wt). OH-PCB metabolites were detected at lower levels (<5% of parent compounds for most samples). However, $\Sigma_8$OH-PCB levels were believed high enough (0.15-2.11 ng/g wet wt) to disrupt thyroid homeostasis in animals and humans. Compared to human blood, the ratios of OH-PCBs to PCBs were lower and the OH-PCB profiles were different, suggesting different metabolic pathways and capacities. OH-PCB levels in the peregrine falcon eggs (5.72 and 8.65 ng/g dry wt) and chick tissue (20.9 ng/g dry wt), were very high, and levels of their parent PCBs were among the highest in the world. These high levels of PCBs and OH-PCB metabolites are of concern, as they may contribute to developmental deficits or behavioral abnormalities in the chick (known to occur in PCB and PBDE-dosed rats and mice at similar levels) or to risks of fail-to-hatch eggs and chick mortality.

**Introduction**

Polychlorinated biphenyls (PCBs) were introduced as highly effective dielectrics, and the production and use of PCBs were banned in most worlds during the 1970s-80s. Nevertheless, due to their environmental persistence and high lipophilicity, they accumulate in the food chain, and concerns remain regarding potentially important consequences for public and environmental health. Hydroxylated metabolites in the body are formed via biotransformation of PCBs, catalyzed by the P450 monooxygenase enzyme system. PCB metabolites (OH-PCBs) are retained in the body of humans[1] and wildlife[2,3], as well as in the abiotic environment[4]. These levels are of toxicological concern because OH-PCBs are associated with perturbed thyroid homeostasis[5, 6], neurodevelopmental deficits[7,8], and possibly with impaired growth in animals. The OH-PCB metabolites may be more potent endocrine disruptors (especially thyroid) than their parent compounds[9]. Hydroxylation pathways involve either the direct insertion of a hydroxyl group to the parent compound or the formation of an arene oxide with a 1,2, shift. The direct addition of the hydroxyl group leads to a sterochemical resemblance to thyroxine (T4), resulting in binding to transthyretin (TTR), a major T4 transport protein. Significantly, in human cord blood the levels of OH-PCB metabolites may approach those of the parent PCB compounds[10]. Such findings prompt continued investigation of the associations between exposure to these endocrine disrupting compounds (EDCs) and reproductive, developmental and neurological health. We investigated the metabolic fates of PCBs in California wildlife samples by measuring levels of PCBs and some of their specific OH-PCB metabolites retained in the blood and/or the eggs.

**Materials and Methods**

Plasma samples (0.11 g-0.39 g) were collected during 2007 from nine kestrels brought to the Lindsay Wildlife Hospital in Walnut Creek, CA for rehabilitation. A set of California peregrine falcon fail-to-hatch eggs and a dead chick were selected from a larger study[11]. The kestrels nested in the San Francisco Bay region, and the peregrines throughout California. Plasma samples were extracted for PCBs and OH-PCB metabolites using standard extraction and phase-separation techniques[1]. Eggs and chick (about 0.5 g) were extracted for PCBs using lyopilization and ASE extraction[11]. Eggs and chick were extracted for OH-PCBs

using standard plasma extraction technique. Due to the small volume of plasma sample, the final extracts were concentrated to 10 μL. PCB and OH-PCB levels were measured using isotope dilution/HRGC-HRMS (ThermoFinngan MAT95, Bremen, Germany) equipped with DB-5MS (60 m × 0.25 mm i.d., 0.25 μm thickness, J & W Scientific, Folsom, CA) and a Varian 1200 gas chromatography /mass spectrometry (Varian Inc., Walnut Creek, CA) equipped with DB-5MS (30 m × 0.25 mm i.d., 0.25 μm thickness, J & W Scientific, Folsom, CA), respectively. OH-PCBs were methylated by using diazomethane. The GC temperature program completely resolved the possible co-elutions (e.g., 3-OH-CB153/4-OH-CB146, 3'-OH-CB138/4'-OH-CB130, and 3'-OH-CB180/4'-OH-CB172). The most intense ions were monitored; $[(M-CH_3)^-]$ and $[(M+2-CH_3)^-]$ for 4-OH-CB187, 4'-OH-CB159, and 4'-OH-CB172, and $[(M-HCl)^-]$ or $[(M+2-HCl)^-]$ for the rest of congeners. These chemical analyses were performed in the ultra clean laboratory of the Department of Toxic Substances Control, Berkeley, CA. Eight penta-hepta OH-PCBs were quantified. Recoveries of surrogate compound (4'-OH-CB159) averaged 75±6%. Human serum (SRM1589a, National Institute of Standards and Technology, Gaithersburg, MD) was used as a standard reference material for PCB analysis. The pooled serum samples were used as in-house control samples for OH-PCB analyses as described elsewhere[1]. The concentrations of OH-PCBs here are presented as ng/g wet or dry wt. and, for comparison, PCBs were also reported on a wet/dry weight basis. $^{13}C_{12}$ labeled PCB standards were purchased from Wellington Labs. Inc., Guelph, ON, Canada. The OH-PCB standards were purchased from Wellington Laboratory (TerraChem Inc., Shawnee Mission, KS, USA) or donated by Åke Bergman (Stockholm University, Sweden).

**Results and Discussion**

Kestrel plasma samples carried high levels of $\Sigma_{16}$PCBs (6.53-45.4 ng/g wet wt), and OH-PCB metabolites were detected at lower levels (<5% of parent compounds for most samples). Levels of OH-PCBs and PCBs weakly correlated ($R^2$=0.2, $p<0.05$). OH-PCB levels ranged from 0.15 to 2.11 ng/g wet wt, comparable to those (0.01-2.03) of Bald Eagle plasma from British Columbia and Southern California[12]. These levels are believed high enough to disrupt thyroid homeostasis in animals and humans[5]. 4-OH-CB187 was predominant in all kestrel plasma samples, followed by 4-OH-CB146 (Figure 1). This profile was different from human serum pool (n=3) collected from Sacramento, CA in 2002, showing the dominance of 4-OH-CB187 followed by 4-OH-CB107 and 3-OH-CB153. Although the levels of OH-PCBs and PCBs were comparable to those found in human blood, the ratios of OH-PCBs to PCBs were lower, suggesting different metabolic pathways and capacities.

Peregrine falcon eggs showed high levels of ΣPCBs (16.8-21.0 μg/g dry wt) and ΣOH-PCB (5.72 and 8.65 ng/g dry wt). The OH-PCB level was higher than Norwegian peregrine falcon eggs[13] and Faroe Islands' Fulmar eggs[14]; 2.1 ng/g wet wt. and 4.0 ng/g fresh wt, respectively. The OH-PCB level in chick tissue (20.9 ng/g dry wt) was higher than the highest liver concentrations of great cormorants in Japan[15] and common buzzard from Belgium[3]; 2.00 and 13.7 ng/g wet wt., respectively. These differences are likely to attribute to the very high PCB exposure levels in our chick (119 μg/g dry wt). Peregrine falcon eggs and chick showed 4'-OH-CB130 and 3'-OH-CB180, metabolites rarely detected in blood of wildlife and human. 4-OH-CB187 and 4-OH-CB146 were predominant among these wild bird eggs and chick.

These high levels of PCBs and OH-PCB metabolites in the California wildlife are of concern since they may contribute to developmental deficits or behavioral abnormalities in the chick (known to occur in PCB and PBDE-dosed rats and mice at similar levels) or to the risks of fail-to-hatch eggs or chick mortality. The 4-OH-CB187, the predominant OH-metabolite detected in wildlife, has been shown to be toxicologically active[5,8]. Further investigation of levels and distribution of OH-PCBs is warranted to better understand the relationship between these emerging endocrine disrupters and environmental health, including effects on wildlife.

**Acknowledgements and Disclaimer**

The views expressed herein are those of the authors and do not necessarily reflect those of Department of Toxic Substances Control, California Environmental Protection Agency. We thank the many ornithologists and bird enthusiasts who have enabled the US peregrine populations to survive DDT-induced egg-shell thinning and reproductive failure in the mid-1950s-60s, and to restore the California breeding pair population from a low of 2 known to >200 over the past thirty years.

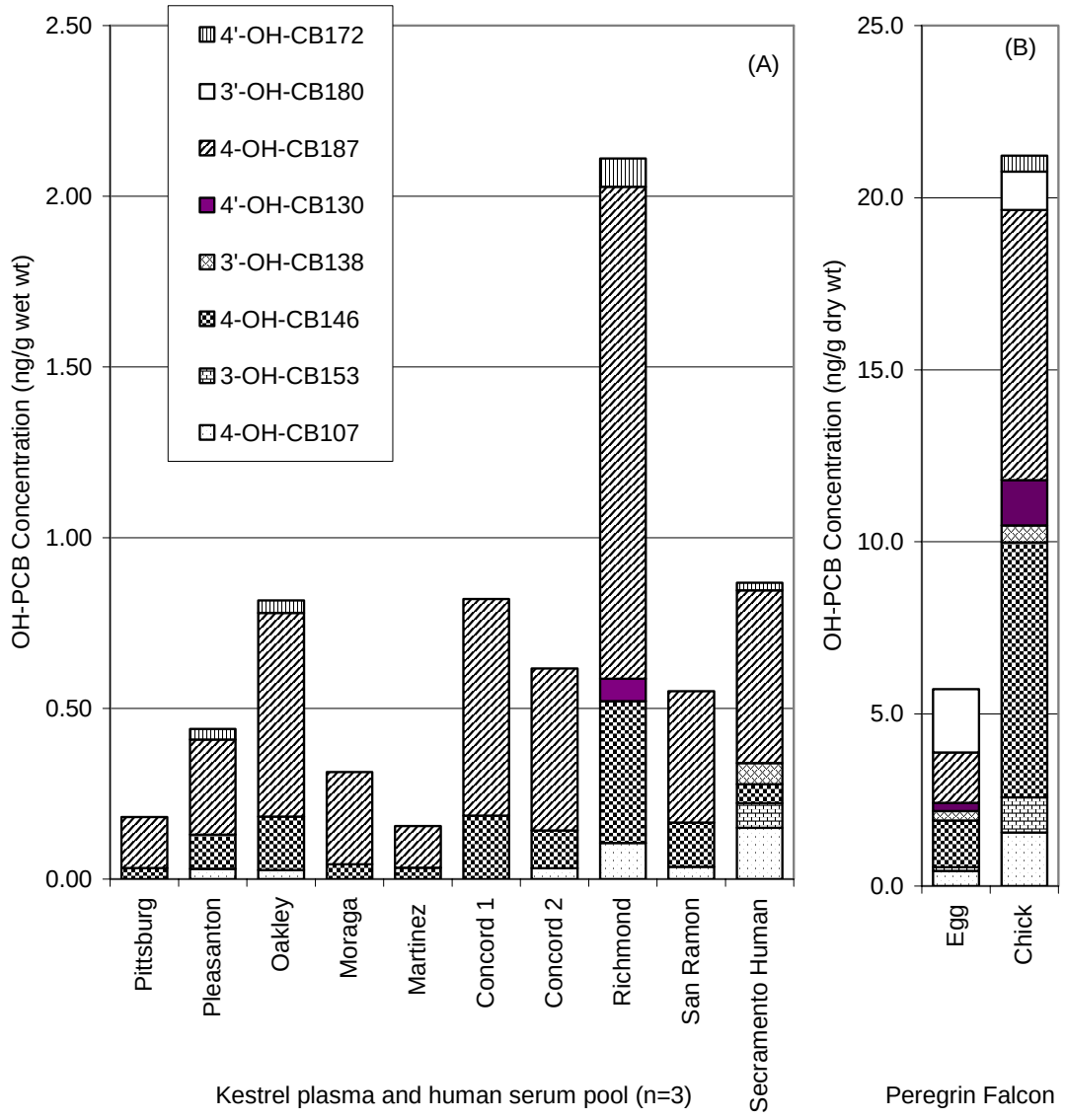


Figure 1. Concentrations of OH-PCBs measured from northern California kestrel plasma and human serum (A) and California peregrine falcon eggs and chick (B).

**References**


1. Park JS, Linderholm L, Charles MJ, Athanasiadou M, Petrik J, Kocan A, Drobna B, Trnovec T, Bergman A, Hertz-Picciotto I. *Environ Health Perspect* 2007; 115(1):20-27.
2. Park JS, Kalantzi O, Kopec D, Petreas M. *Organohalogen Compds* 2007; 69:1733-1736.
3. Jaspers VL, Dirtu AC, Eens M, Neels H, Covaci A. *Eviron Sci Technol* 2008; On-line available. In press.
4. Ueno D, Darling C, Alaee M, Campbell L, Pacepavicius G, Teixeira C, Muir D. *Eviron Sci Technol* 2007; 41(6):1841-1848.
5. Otake T, Yoshinaga J, Enomoto T, Matsuda M, Wakimoto T, Ikegami M, Suzuki E, Naruse H, Yamanaka T, Shibuya N *et al*. *Environ Res* 2007; 105(2):240-246.
6. Meerts I, Assink Y, Cenijn PH, van den Berg JHJ, Weijers BM, Bergman A, Koeman JH, Brouwer A. *Toxicol Sci* 2002; 68(2):361-371.
7. Kenet T, Froemke RC, Schreiner CE, Pessah IN, Merzenich MM. *Proceedings of the National Academy of Sciences of the United States of America* 2007; 104(18):7646-7651.

8. Negishi T, Takahashi M, Tashiro T. *Organohalogen Compds* 2007; 69:424-427.
9. Sandau CD: Analytical Chemistry of Hydroxylated Metabolites of PCBs and Other Halogenated Phenolic Compounds in Blood and Their Relationship to Thyroid Hormone and Retinol Homeostasis in Humans and Polar Bears. Carleton University, Ottawa, Ontario. *PhD Dissertation* 2000.
10. Park JS, Bergman A, Linderholm L, Athanasiadou M, Charles MJ, Kocan A, Petrik J, Drobna B, Trnovec T, Hertz-Picciotto I. *Chemosphere* 2008; 70(9): 1676-1684.
11. Hooper K, Holden A, Chun C, Linthicum J, Walton BJ. *Organohalogen Compds* 2007; 69:2715-2718.
12. McKinney MA, Cesh LS, Elliott JE, Williams TD, Garcelon DK, Letcher RJ. *Environ Sci Technol* 2006; 40(20):6275-6281.
13. Berger U, Herzke D, Sandanger TM. *Anal Chem* 2004; 76: 441-452.
14. Fängström B, Athanasiadou M, Athanassiadis I, Weihe P, Bergman A. *Ambio* 2005; 34(3):184-187.
15. Hasegawa J, Matsuda M, Ohnishi H, Enomoto T, Kwawano M, Wakimoto T. *Organohalogen Compds* 2007; 69:1495-1498.