ACCUMULATION AND METABOLISM OF COPLANAR PCB CONGENERS AND INDUCTION OF CYTOCHROME P450 IN BLACK-TAILED GULL AND BLACK-FOOTED ALBATROSS

Hisato Iwata[1], Nobuhiro Fujii[1], Tatsuya Kunisue[1], Mafumi Watanabe[1], Shinsuke Tanabe[1], Hiroyuki Tanaka[2], Haruo Ogi[3] & Yasuyuki Shibata[4]

Center for Marine Environmental Studies, Ehime University, Tarumi 3-5-7, Matsuyama, 790-8566, Japan[1]
National Research Institute of Fisheries and Environment of Inland Sea, Maruishi 2-17-5, Ohno-cho, Saeki-gun, Hiroshima, 739-0452, Japan[2]
Faculty of Fisheries, Hokkaido University, Minato-cho 3-1-1, Hakodate, 041-3611, Japan[3]
National Institute of Environmental Studies, Onogawa 16-2, Tsukuba, 305-0053, Japan[4]

Introduction

Persistent organic pollutants (POPs) including planar halogenated aromatic hydrocarbons (PHAHs) are widely distributed,[1] and biomagnified in higher trophic birds, particularly inhabiting the local source through the food web.[2] These exposures have resulted in a number of adverse effects on their reproductive potential, such as deformities and lethality of embryos.[3]

PHAHs bind to AhR in the cytoplasm of cell and then this ligand-activated AhR is translocated into nucleus and dimerized with ARNT protein. This heterodimer complex participates in regulating the transcription of cytochrome P450 1A (CYP 1A) and the other genes. Eventually, changes in these translated products are speculated to associate with disruption of cell cycle control, apoptosis, oxidative stress and endocrine signalling.[4] These integrated knowledges indicate that accurate measurements of the AhR-mediated responses including CYP1A may lead to the detection of PHAH exposure and their subtle effects.[5]

Coplanar PCB congeners are structural analogues with TCDD, which share a broad spectrum of biological and toxicological responses including CYP1A induction. It has been reported that the contribution of coplanar PCBs to total TEQs in wildlife is generally much greater than chlorinated dioxins and furans.[6] Therefore, we have attempted to measure CYP as a biomarker in wildlife, and to relate the CYP expression level to the PHAH exposure. Particularly in species from relatively less polluted areas, little information is available on induction of hepatic CYP1A. Here, we report on the current residue levels of coplanar PCB congeners in seabirds, and further evidences of the induction of CYP1A by coplanar PCBs.

Materials and Methods

Liver samples of 13 black-tailed gulls (BTG; *Larus crassirostris*) and 13 black-footed albatross (BFA; *Diomedea nigripes*) were collected from Rishiri Island, Hokkaido in 1999 and from the

North Pacific in 1998, respectively. All the liver tissues were immediately collected after the collection. Liver slices were frozen in liquid nitrogen and stored at −80℃ until microsome preparation. Liver samples for coplanar PCB analyses were stored at −20℃ until chemical analysis. Microsomal fractions from liver samples were prepared according to the method of Guengerich with slight modification.[7] Protein concentrations were determined by the BCA assay. Ethoxyresorufin *O*-deethylase (EROD) activity was measured using a multiwell plate reader according to the method of Kennedy *et al.*[8] Total CYP content was determined spectrally by the method of Omura and Sato.[9] The analysis of coplanar PCB congeners was performed following the procedures described elsewhere.[10] Coplanar PCB congeners were determined using a GC-MSD. TEQs derived from non- (IUPAC 77, 126, and 169) and mono-*ortho* coplanar PCB congeners (IUPAC 105, 118, and 156) were calculated using toxic equivalency factors proposed for avian species.[11]

Correlations among the hepatic level of CYP activity, and hepatic coplanar PCB concentrations of the seabirds were examined by Spearman rank correlation. The Mann-Whitney U-test was used for the detection of statistical differences by species.

Results and Discussion

Total TEQs ranged from 14-160 pgTEQ/g wet wt in BTG and from 72-500 pgTEQ/g wet wt in BFA. PCB126 made a greater contribution to total TEQs, and followed by PCB77. The contribution of PCB126 TEQ and PCB77 TEQ accounted for almost 60-90% and 5-20% to the total TEQs, respectively. The mono-*ortho* coplanar congeners less contributed. Literature surveys on the effects based on TEQs accumulated in bird species showed that the LOAEL and ED_{50} values determined for several endpoints including liver weight decrease, brain asymmetry, and CYP1A induction were mostly in the range of 10-100 pgTEQ/g in avian eggs and embryos.[12,13] Most of the TEQs calculated in the liver of both species exceeded the effective levels.

In order to clarify whether CYP1A is induced by coplanar PCBs, the relationship between EROD and coplanar PCB congener levels was examined (Fig. 1). In BTG, TEQs from PCB126 (p=0.050) and PCB169 (p=0.011) exhibited significant positive correlations with EROD, while a significant correlation between PCB77 and EROD was not observed. In BFA, PCB169 (p=0.015) was only correlated with EROD, and PCB77 and PCB126 were not. No correlation was observed between EROD activity and individual mono-*ortho* PCB congeners in both species, probably due to the low potential of mono-*ortho* PCBs to activate AhR. These results indicate that AhR is specifically activated by non-*ortho* coplanar PCB congeners, and CYP isozyme that is responsible for EROD activity, probably CYP1A, is induced in both species.

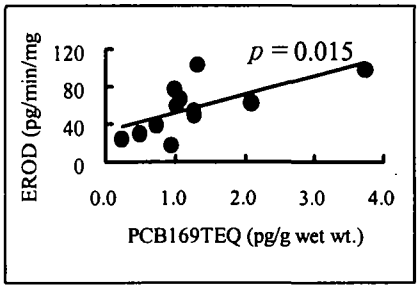

Fig. 1. Relationship between TEQs and EROD activities in the liver of

Regarding the lack of correlations with PCB77 in BTG, and with PCB77 and PCB126 in BFA, preferential metabolism of PCB77 and PCB126 by CYP1A induced was speculated. To ensure this hypothesis, the concentrations of the metabolizable congeners were normalized to relatively recalcitrant congeners, and the relationship between the PCB congener ratio and EROD activity was further evaluated. The statistical analyses showed that EROD activity was negatively correlated with PCB77/PCB126 (p=0.001) and PCB77/PCB169 (p=0.003) ratios in BTG, and

with PCB77/PCB169 (p=0.013) and PCB126/PCB169 (p=0.020) in BFA (Fig. 2). These results suggest that PCB77 is preferentially metabolized by CYP induced by non-*ortho* coplanar PCBs in BTG, and PCB77 and PCB126 in BFA.

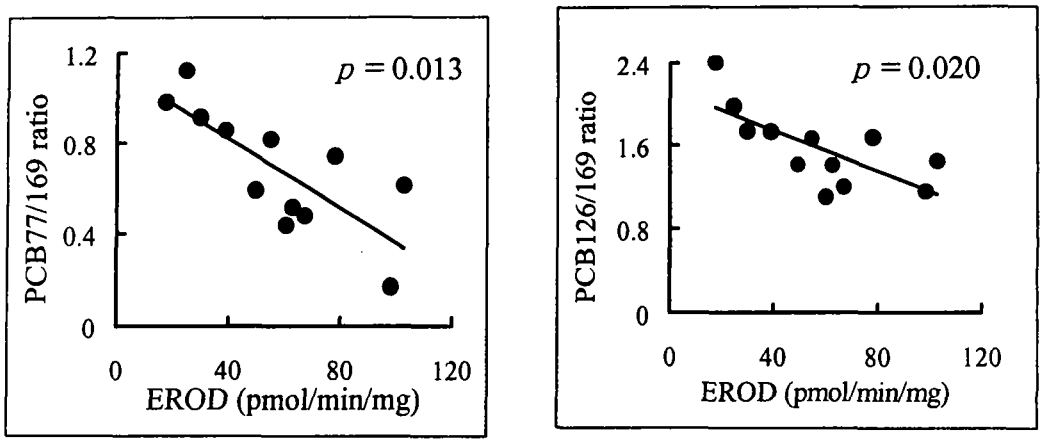

Fig. 2. Relationships between coplanar PCB congener ratios and EROD activities in the liver of BFA.

In order to confirm whether CYP1A induction is coplanar PCB congener-specific action, non-planar POPs including DDT and its metabolites, chlordane compounds, hexachloro-cyclohexane isomers, and hexachlorobenzene were also analyzed in livers of both species, and rank correlations were examined between the residue levels and EROD activity. The results showed that no systematic correlations were found for these non-planar POPs (data not shown).

Our results showed specific activation of AhR by non-*ortho* coplanar PCB congeners, and the following CYP1A induction in seabirds. This study also indicates the species-specific metabolic capacity of CYP1A, which may be one of factors responsible for differences in AhR mediated responses among species.

Acknowledgement

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