EROD activity as a biomarker of exposure in field and laboratory birds exposed to environmentally relevant PCB mixtures

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Introduction
Expression of the cytochrome P450 CYP1A4 is induced via the aryl hydrocarbon receptor (AhR). Expression of this gene has been used successfully as a biomarker of exposure in avian species exposed to a wide range of environmental contaminants including polycyclic aromatic hydrocarbons (PAHs) and dioxin-like compounds. The CYP1A4 AhR enzyme has preferential specificity for ethoxyresorufin-O-deethylase (EROD) activity, which increases during detoxification and depuration of xenobiotics which has ligand-binding activity with many xenobiotic compounds including PAHs, dioxins, and dioxin-like compounds, such as poly-chlorinated biphenyls (PCBs). Individual PCB congeners can vary in their ability to induce EROD. This variation is an important consideration in establishing EROD as a reliable biomarker for PCB exposure in wild fauna. It has been shown that species exhibit varying levels of EROD induction, largely based on their AhR sequence (Head et al., 2008). Therefore, it is important to characterize EROD activity in species of interest and compare EROD induction to that observed in a laboratory study with known exposure. In this study, we exposed Japanese quail (Coturnix coturnix japonica) to known concentrations of toxicants and compared induction of EROD activity with that observed in tree swallows (Tachycineta bicolor) and eastern bluebirds (Sialia sialis) nesting at a contaminated site.

Purpose: Characterize EROD activity in response to exposure to PCBs in Japanese quail hatchlings. Single congener tested were 126 and 77, mixtures included are PCB mixtures found in tree swallows and spatted sandpiper eggs (respectively) at the Upper Hudson River. EROD activity was measured and dose-response curves were analyzed using the GENMOD procedure to determine differences in EROD activity with compound(s) or gender. Results are compared to those from a previous study using two wildbird species of concern – tree swallows (Tachycineta bicolor) and eastern bluebirds (Sialia sialis).

Methods and Materials – Quail Study
- Site Collection and Preparation: Eggs were collected from the University of Maryland colony, randomly assigned to one of the following groups: control, TRES, SPSA, or PCB 126. Eggs were incubated at 99ºF and 65% humidity. All hatchlings were sacrificed within 24 hours of hatching.
- Hatching and Dissections: Hatchlings were sacrificed by cervical dislocation, and liver was collected for EROD activity analysis.

Methods and Materials – Wild Bird Study
- Field Study: Wild birds were studied in three reference sites: Patuxent Wildlife Refuge, Greenbelt, MD, and the Departments of Animal and Avian Sciences and Veterinary Medicine, University of Maryland, College Park, MD, and Cobleskill Reservoir, Cobleskill, NY for the 2007 and 2008 field seasons. The contaminated field sites were Greenbelt, MD, and Cobleskill Reservoir, Cobleskill, NY for the 2007 and 2008 field seasons. The contaminated sites were Greenbelt, MD, and Cobleskill Reservoir, Cobleskill, NY. Hatchlings were injected in the lab. Eggs were injected at the first sign of development, incubated at 99ºF and 65% humidity. All hatchlings were sacrificed within 24 hours of hatching.
- Assay Procedure: EROD activity was assayed in triplicate on a fluorometer. All experiments were conducted at the University of Maryland, College Park, MD.

Data Analysis: A one-way ANOVA was performed on the PCB 126 data, followed by a Tukey adjustment. A two-way ANOVA was performed on the PCB 126 data using the dose and time as factors, followed by a Tukey adjustment. A one-way ANOVA was performed on the tree swallow and eastern bluebird PCB 77 data separately, followed by a Tukey adjustment (2008 data; Figures 4 and 5).

Results – Quail Study
2006 Data
- EROD Activity in Japanese Quail Treated with PCB 126
- Treatment (µg/egg)
- EROD Activity (nmol/µg protein)
- PRR
- CR
- Combined

2007 Data
- EROD Activity in Japanese Quail Treated with PCB 126
- Treatment (µg/egg)
- EROD Activity (nmol/µg protein)
- PRR
- CR
- Combined

Discussion
These studies offer side-by-side comparisons of the response of EROD activity in a laboratory model and in two species of interest, thereby assessing the use of EROD as a biomarker in the field bird study is a valuable comparison to the Japanese quail to ascertain potential effects and mechanisms of action of single and complex mixtures of PCBs. These data provide a valuable comparison to the GENMOD procedure revealed that the three treatment groups (separated by gender) had a significant cubic component, displaying a quickly mounted response at lower doses, a plateauing effect at medium doses, followed by a second increase in response at higher doses. This might indicate that the response is initiated at low doses, a new set point is established at the medium doses, but the high doses were toxic enough to elicit further increase in response before the compound becomes lethal. However, a more likely explanation is that there is a survivor effect at higher doses. Finally, while there were no statistical differences between males and females across any treatment groups, females seem to be slightly more sensitive to toxicity by these compounds. Our response to PCB 126 was not as high as males; the female response to PCB 77 experienced a decrease at high doses, indicating toxicity; fewer females survived to higher dose of the PCB 126 mixture than the males. Taken together, these studies demonstrate the importance of using a known laboratory model such as the Japanese quail to ascertain potential effects and mechanisms of action of single and complex mixtures of PCBs. These data provide a valuable comparison to effects observed in wild bird species and provide an indication of potential effects that can serve as one basis for assessing potential risk for exposures that are toxicologically relevant.

References:

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