

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 (CYP450) isoform 1A4 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 CYP450 1A4 enzyme has preferential specificity for ethoxyresorufin-o-deethylase (EROD) activity, which increases during detoxification of exogenous chemicals. The AhR has ligand-binding activity with many xenobiotic compounds including PAHs, dioxins, and dioxin-like compounds, such as polychlorinated 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 exposures. 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 congeners tested were 126 and 77, mixtures included two PCB mixtures found in tree swallow and spotted 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 were 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

Egg Collection and Injections - Eggs were collected from the University of Maryland colony, randomly assigned treatment groups, and were injected on embryonic day (ED) 0.
Hatching and Dissections - Hatchlings were sacrificed within 24 hours of hatch, livers were harvested and snap frozen in liquid nitrogen. Assay procedure described below.
Data Analysis - Data were analyzed using the GENMOD procedure. Dose, compound, and sex were considered treatment groups. Dose was nested within compound.

Methods and Materials – Wild Bird Study

Field Sites - Wild birds were studied in three reference sites: Patuxent Wildlife Refuge, Greenbelt, MD, and Great Sacandaga Lake, Adirondack Park, New York, for the 2006 field season; Patuxent Wildlife Refuge, Greenbelt, MD, and Cobleskill Reservoir, Cobleskill, NY for the 2007 and 2008 field seasons. The contaminated study site was Remnant Area 3 in the Upper Hudson River near Ft. Edward, NY.
Injection Compounds - PCB 126 was used during the 2006 field season, a PCB mixture based on a profile taken from tree swallow tissue from the Upper Hudson river was used in 2007, and PCB 77 was used in 2008. All eggs from the Upper Hudson River remained untreated.

Egg Injections and Collections - During the 2006 field season, three eggs were collected from each nest, randomly assigned to a treatment group, and injected in the lab. Eggs were injected at the first sign of development, incubated at 99% and 65% humidity and rotated 180° twice a day until three days before hatch. During the 2007 and 2008 field seasons, three eggs from each nest were randomly assigned to treatment groups, injected in the field, and remained in the nest to be incubated naturally.
Hatching and Dissections - Eggs that remained in nests following treatment were transferred to the lab incubator at Day 10; maintained at 99% and 65% humidity. All hatchlings were sacrificed within 24 hours of hatch; livers were collected and immediately snap frozen in liquid nitrogen.
Assay Procedure - Ethoxyresorufin-O-dealkylase (EROD) was assayed in triplicate on a fluorescence 96 microwell Wallace 1420 plate scanner. The assay utilized 200 µL of 1.25 µM ethoxyresorufin (Sigma-Aldrich Chemical) substrate, 10 µL of 0.125 mM NADPH (Sigma-Aldrich Chemical), and 50 µL of microsomal protein. The plate was read for a total of 12 readings over 18 minutes after incubation at 37°C. Reference Japanese quail microsomes that had not been induced were included with each plate. Change in fluorescence units over time were converted to rate of product formation using a 9-point standard curve (0.001-0.1µM). Protein was determined by BCA Protein Assay kit (Pierce Chemical Company, Rockford, IL, USA). Ethoxyresorufin-O-dealkylase activity was calculated as picomoles of product formed/min/mg microsomal protein.

Data Analyses - A one-way ANOVA was performed on the PCB 126 data, followed by a Tukey adjustment (2006 data; Figure 2). A two-way ANOVA was performed on the PCB mixture using site and dose as factors, followed by a Tukey adjustment (2007 data; Figure 3). One-way ANOVAs were performed on the tree swallow and bluebird PCB 77 data separately, followed by a Tukey adjustment (2008 data; Figures 4 and 5).

Results – Quail Study

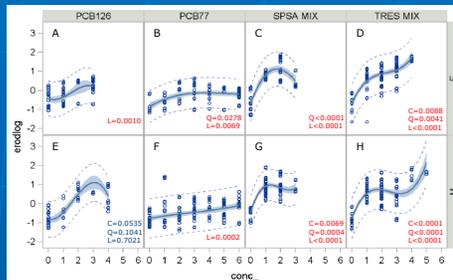


Figure 1: The GENMOD procedure was used to analyze EROD induction in Japanese quail. The cubic component was significant for panels D, G, and H, while the quadratic component was significant for B, and the linear component was significant for panel F ($p < 0.05$).

Results – Wild Bird Study

2006 Data

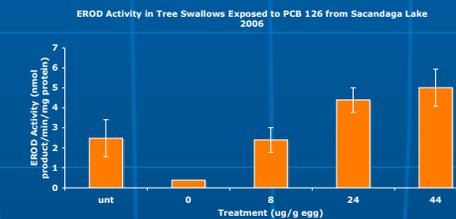


Figure 2: A one-way ANOVA was performed for treatment groups in tree swallows from GSL exposed to PCB 126 in 2006. Eggs from Great Sacandaga Lake were treated with PCB 126; embryos differed in their response to PCB 126 dose ($p = 0.0416$).

2007 Data

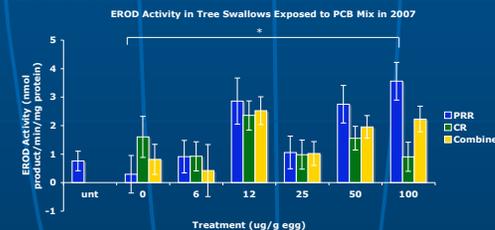


Figure 3: A two-way ANOVA was performed for treatment and site in tree swallows exposed to a PCB mixture at PRR and CR in 2007. There was no significant interaction ($p = 0.0635$) between site and treatment. There was no significant difference for the main effect of site ($p = 0.1055$), however there was a significant difference between 12 ug/g egg treatment and the untreated egg treatment for the main effect of treatment ($p < 0.05$). There was a significant difference ($p < 0.05$) between 100 ug/g egg and 0 ug/g egg treatments at the PRR field site. There was a significant difference between treatment groups in eggs from the sites when combined ($p = 0.026$).

2008 Tree Swallow Data

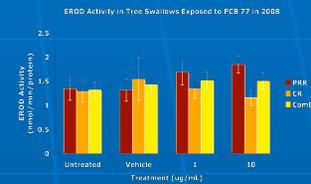


Figure 4: A two-way ANOVA was performed for treatment and site in tree swallows exposed to PCB 77 at PRR and CR in 2008. There was no significant difference across treatments or sites in the 2008 dosed eggs.

2008 Eastern Bluebird Data

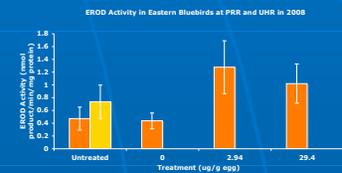


Figure 5: A one-way ANOVA was performed for treatment groups in bluebirds exposed to PCB 77 at PRR in 2008. There was no significant difference across treatment groups in eastern bluebirds from PRR. Student's t-test was performed on untreated bluebirds from PRR and UHR. There was no significant difference between untreated birds from PRR and UHR.

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 allowing an assessment of EROD for utility as a biomarker of exposure in environmentally exposed birds. PCB 126 was very toxic in both quail (Figure 1A and 1E) and tree swallows (Figure 2). The response to PCB 77 was quantitatively lower in both quail (Figure 1B and 1F) and tree swallow (Figure 3) compared to PCB 126, indicating that PCB 77 did not activate the AhR as strongly. This is in contrast to the dioxin-like character of PCB 77, since it is non-ortho-substituted.

The quail study highlights important differences between the two PCB mixtures. First, there was a stronger EROD response to both mixtures compared to PCB 126 (Figure 1), due to high mortality. Our conclusion is that the body was able to mount a greater response to both mixtures, thereby raising the lethal limit of the mixtures. This finding points to the issue of assessing risk from exposure to complex mixtures. Second, the EROD response to the TRES mixture is slightly higher than response to the SPSA mixture, in both males and females, which correlates with the higher mortality of the SPSA mixture; the lethality of the SPSA mixture does not allow the birds to mount the same level of response that the TRES mixture does. Third, statistical analysis using 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 a hormetic response is elicited 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; their level of 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 doses of the TRES mixture than the males.

Taken together, our 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 of toxicological concern.

Reference:

Head, J.A., Hahn, M.E., and Kennedy, S.W. 2008. Key amino acids in the aryl hydrocarbon receptor predict dioxin sensitivity in avian species. *Environmental Science and Technology* 42: 7535-7541.

Acknowledgements:

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