

Evaluation of enzyme induction in relation to tissue concentrations of PCDDs, PCDF, and PCBs in four bird species from the Tittabawassee River, MI, USA

Timothy B. Fredricks[‡], John L. Newsted^{†,‡}, Rita M. Seston[†], Patrick W. Bradley[†], Steve B. Wiseman[†], Steve J. Bursian[†], John P. Giesy^{†,‡}, Matthew J. Zwiernik[§]

MICHIGAN STATE UNIVERSITY

[†]Department of Zoology, Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824

[‡]Department of Animal Science, Michigan State University, East Lansing, MI, 48824

[§]ENTRIX, Inc. East Lansing, MI

[¶]Department of Biomedical Veterinary Sciences & Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan



ABSTRACT

Polychlorinated dibenzofurans (PCDFs), dibenzo-p-dioxins (PCDDs) and polychlorinated biphenyls (PCBs), ethoxyresorufin- (EROD), methoxyresorufin O-dealkylase (MROD) activity and CYP1A4 gene expression were quantified in nestlings prior to fledging for tree swallows (TS), eastern bluebirds (EB), house wrens (HW), and belted kingfishers (BK) in the Tittabawassee and Chippewa River floodplains near Midland, Michigan. While previous studies have shown that concentrations of PCDF/PCDDs in biota are approximately 10- to 20-fold greater downstream (study areas; SA) of Midland when compared to upstream (reference areas; RA), the toxicological significance of these differences is still being investigated. Seventeen 2,3,7,8-substituted PCDF and PCDD congeners and 12 dioxin-like PCBs were measured liver and whole body nestling homogenates and converted to TEQs using WHOAvian TEF values. Mean EROD activities in TS, EB, HW, and BK from the RA were approximately 170, 1600, 200, 190 while at the SA activities were approximately 630, 4400, 290, and 210 pmol product/mg microsomal protein/min, respectively. Mean MROD activity in TS, EB, HW, and BK from the RAs were <LOQ, 330, 54, and 7.2 while in the SA average activities were 6.3, 820, 200 and 15 pmol product/mg microsomal protein/min, respectively. Liver and whole body concentrations of WHOAvian TEQs at in birds from the SA were about 10-fold greater than RA. In birds from the SA, reported enzyme induction levels were primarily associated with furan exposure, principally 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF. In birds collected from the RA, enzyme induction was associated with both furan and dioxin exposure. In general, PCB concentrations were not significantly associated with enzyme activity levels. With the exception of the BK, CYP1A4 levels were poorly correlated with enzyme activity levels. While EROD/MROD activities and TEQtotal in bird from the SA were greater than RA, the productivity measurements at co-located study sites are comparable between sites.

INTRODUCTION

- Polychlorinated dibenzo-p-dioxins (PCDDs), Dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) in soils and sediments downriver of Midland, MI have been reported to be 10- to 20-fold greater than those collected from an upstream reference area (Figure 1).
- WHOAvian TEQ concentrations in passerine and piscivorous avian species from downriver locations are at least an order of magnitude greater than those observed in birds from reference locations.
- In field studies, it can be difficult to establish definitive cause and effect relationships due to high exposure variability and to interspecies differences in susceptibility (Bishop et al. 1999). Biomarkers based on specific mode-of-action can be used to address uncertainties related to these two factors.
- The toxicity of dioxin-like compounds are mediated through the aryl hydrocarbon receptor (AHR), a ligand-activated nuclear transcription factor (Okey, 2007). Differences in species sensitivity appear to be related, in part, to alterations in amino acid sequences of the AHR ligand binding domain (LBD) (Karchner et al. 2006).
- The field research presented here is part of a large collaborative congener-specific project involving a multi-species egg injection study, primary hepatocyte research, AHR ligand binding domain sequencing, and mRNA response quantification.
- Study species include 3 passerine species (TREE SWALLOW, TS; HOUSE WREN, HW; EASTERN BLUEBIRD, EB) and the BELTED KINGFISHER (BK)
- Field studies were conducted on nest box trails established on both study and reference sites in the Tittabawassee River floodplain in 2004.



OBJECTIVES

- Quantify CYP1A gene expression, CYP1A related enzyme activity, and PCDD/PCDF residue concentrations in field collected samples from the Tittabawassee floodplain for several avian species
- Compare EROD/MROD activities and CYP1A induction levels between these primarily furan exposed birds and existing literature values

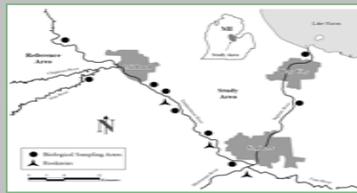


Figure 1. Map of research areas including reference and study areas in the Tittabawassee River floodplain, Midland, MI, USA.

METHODS

- Field studies were conducted on nest box trails established on both study and reference sites in the Tittabawassee River floodplain in 2004.
- Near fledging age nestlings were collected at four study areas (SAs) and 2 reference areas (RAs) (Figure 1), from May through August 2007
- Liver was removed in the field and snap frozen (subsequently stored at -80°C)
- Remaining nestling tissues were stored in tact at -20°C
- Liver tissue was split with ~0.1 g for enzyme activity, ~0.025 g for CYP1A mRNA quantification, and the remaining tissue for residues analyses

Residue Analyses

- Nestlings were homogenized without feathers, lower legs, bill, and liver tissue
- Nestling liver tissue was homogenized and analyzed individually
- Chemical extraction followed EPA method 3540C & 3541
- Chemical analyses followed EPA method 8290

- Results were corrected based on recoveries and non-detected congeners equal to detection limit
- TEQ concentrations are based on avian-specific World Health Organization (WHOAvian) TCDD equivalency factors (Van den Berg et al. 1998)

- Reconstituted whole body nestling concentrations were determined by combining values for liver and nestling homogenates by individual

Enzyme Activity

- Liver microsome preparation and EROD/MROD analyses (Kennedy and Jones 1994; Shipp et al. 1998; Trudeau and Maisonneuve 2001)
- ~0.1000 g liver homogenized (range 0.0934-0.1288 g)
- Induction measured on a "machine" in 96-well plates with a total well volume of 201 ul
- EROD assay wells averaged 28.5 ug/ml microsomal proteins (9.09-46.4 ug/ml)
- MROD assay wells averaged 53.6 ug/ml microsomal proteins (18.4-83.9 ug/ml)
- Results expressed as maximum pmol product/mg microsomal protein/min

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RESULTS

- Total TEQ concentrations in livers of nestlings from the study areas were at least an order of magnitude greater than reference areas.
- ΣPCDD and ΣPCDF TEQ concentrations in all species from the study areas were significantly (p<0.05) greater than that observed in reference areas
- ΣPCB TEQ concentrations in Kingfisher from the study areas were statistically greater (p<0.05) than reference areas
- Species differences in liver TEQ concentrations were observed in both reference and study areas. The rank order (least to greatest) in the reference area for ΣPCDD, ΣPCDF, ΣPCB was:
 - Kingfisher<Tree swallow<Eastern bluebird<House wren
- In the study areas rank order for ΣPCDD was:
 - Kingfisher<Tree swallow<Eastern bluebird<House wren
- For ΣPCDF rank order was:
 - Kingfisher<Tree swallow<House Wren<Eastern Bluebird

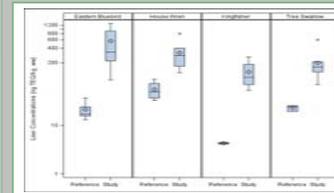


Figure 2. Liver total TEQ (Dioxin concentrations associated with nestling samples collected in the Tittabawassee River floodplain, Midland MI, USA in 2007.

Results

- In study areas, EROD activities in kingfishers, tree swallows and bluebirds were approximately 3 to 5-fold greater than reference areas
- In study areas MROD activities in kingfisher, bluebird and house wrens were 3 to 10-fold greater than reference areas
- EROD and MROD activities in bluebirds, were statistically greater than tree swallows, house wrens, and kingfishers.

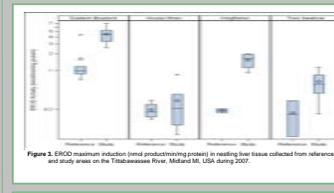


Figure 3. EROD maximum induction (pmol product/mg protein) in nestling liver tissue collected from reference and study areas on the Tittabawassee River, Midland MI, USA during 2007.

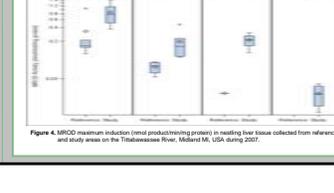


Figure 4. MROD maximum induction (pmol product/mg protein) in nestling liver tissue collected from reference and study areas on the Tittabawassee River, Midland MI, USA during 2007.

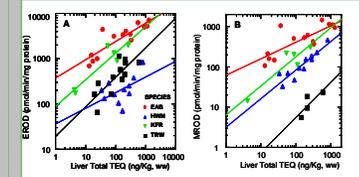


Figure 5. Relationship between total liver WHOAvian TEQ concentrations (ng in nestling livers and EROD (A) and MROD (B) activities.

Results

- Significant (p<0.05) linear relationships were observed for EROD/MROD activities and liver TEQ concentrations in bluebirds (r²=0.008) and house wrens (r²=0.700).
- Slopes for EROD ranged from 0.833 to 3.64 while for MROD, slopes ranged from 0.022 to 0.656 for house wren and bluebirds, respectively.
- A visual comparison of the regression lines for EROD/MROD to liver TEQ concentrations with species being dominate factor in the observed differences in the dose-response relationships.

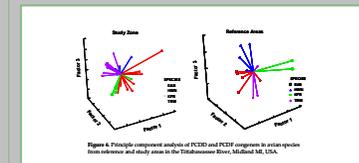


Figure 6. Principal component analysis of PCDF and PCDD congeners in liver species from reference and study areas in the Tittabawassee River, Midland MI, USA.

Results

- Congener profiles at downstream study areas are composed of >80% furan compounds, principally 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF.
- In study area, species were separated on Factor 1 (Hepta, Hexa PCDD(Fs) and Factor 3 (TCDF and PeCDF). These patterns are indicative of a specific source of furan contamination.
- In reference areas, Factor 1 contained all PCDF/F congeners with the exception of TCDF (Factor 2) indicating a more diffuse exposure.

Discussion

- Bishop et al. 1998 (2119), Bishop et al. 1999 (394), and Gentes et al. 2007 (2113) reported primarily background (10.7-34.9 pmol/min/mg; ~4-22 pmol/min/mg, 20.84-38.03 pmol/min/mg) induction for field exposure TS to a variety of chemicals
- Custer et al. 1998 (388) monitored TS exposed to PCBs in Green Bay, WI for EROD/BROD (which were correlated) induction (ranged from 32.07-109.23 and 15.95-45.10 pmol/min/mg, respectively) but was not correlated with TEQs or congener data (results are for NS collected as similar sites not same NS)
- Head and Kennedy 2007 (2114) reported EROD induction and CYP1A mRNA quantification from primary hepatocytes exposed to TCDD from the same samples

Literature Cited

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