SETAC 2008 Annual Meeting

Prof. Giesy and a number of his colleagues, post docs, and students from both Michigan State University and the University of Saskatchewan attended the 29th annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC), which was held November 16-21, Tampa, FL. Prof. Giesy’s group made 22 platform and poster presentations, the most of any group attending the meeting. The titles of the presentations are listed below with links to the presentation.


“Molecular Mechanisms Underlying Differences in Sensitivity of Avian Species to Embryo-toxic Effects of Chlorinated Dioxins and Furans-Recent Advances in the Characterization of Aryl Hydrocarbon receptor 1 (AHR1) in Birds.” With R. Farmahin, S.W. Kennedy, D. Crump, S.P. Jones, L. Mundy, S.J. Bursian, M.J. Zwiernik, M.E. Hahn, and J.A. Head, To: 29th annual meeting, November 16-21, Tampa, FL.

“Application of a Medaka HPG Axis Real Time PCR Array Method to Environmental Chemical Screening.” With X. Zhang, M. Hecker, A. Tompsett, and P.D. Jones.

“Species-specific Accumulation of Polychlorinated Dibenzo-p-dioxins (PCDDs), Dibenzofurans (PCDFs), and Coplanar Polychlorinated Biphenyls (PCBs) in Fishes from the Tittabawassee and Saginaw Rivers (Michigan, USA).” With Y. Wan, P.D. Jones, J. Khim, R.R. Holem, D.P. Kay, S.A. Roark, and J.L. Newsted.


“Multiple Lines of Evidence Risk Assessment of Great Horned Owls (*Bubo virginianus*) Exposed to PCDF/DDs in Midland, MI, USA.” With S.J. Coefield, M.J. Zwiernik, T.B.


“Effects of TCDD, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF Exposure on CYP1A4 and CYP1A5 mRNA Abundance in Japanese Quail (Coturnix japonica), Ring-necked Pheasant (Phasianus colchicus), and Chicken (Gallus gallus) in Ovo.” With S. Wiseman, Y. Yang, P. Jones, Y. Wan, M. Zwiernik, Zoology S. Bursian, J. Herve, S. Kennedy, and J. Newsted.
Introduction

Fish decline in the upper Danube River

"Assessing sediments and fish health using a weight-of-evidence approach and effect-directed analyses – in search for the causes of fish decline in the Danube river"

Henner Hollert
Institute for Environmental Research, RWTH Aachen University
Institute for Zoology of the University of Heidelberg

S. Keiter, M. Böttcher, S. Grund, N. Seitz, J. Otte, K. Bluhm & T. Braunbeck (Department of Zoology, University of Heidelberg, Germany)
K. Wurm (Gewässerökologisches Labor, Starzach, Germany)
E. Higley, J. Giesy & M. Hecker (University of Saskatchewan and ENTRIX, Canada)
H. Olsman, B. van Bavel & M. Engwall (MTM, Örebro University, Sweden)
G. Reifferscheid & W. Manz (Federal Hydrological Institute, Koblenz, Germany)
L. Erdinger (Department of Hygiene, University Heidelberg, Germany)
U. Kammann (Federal Research Centre for Fisheries, Hamburg, Germany)
R. Schönberger & M. Suter (EAWAG, Switzerland)
T. Schulze & W. Brack (UFZ Leipzig, Germany)
J. Otte, C. Andersson, A. Abrahamson & B. Brunström (Uppsala University, Sweden)
L. Yang, C. Zinsmeister & U. Strähle (Institute of Toxicology and Genetic, FZK Karlsruhe)

Introduction

The Freshwater Crisis

Potential impacts?
- Structural changes of habitat
- Change in temperature
- Fish removal (Human & animals)
- Chemical contamination

Effects
- Impairment of health
- Reduction of food supply
- Failing reproduction

Consequence
- Decline of fish population

Reference?
**Introduction**

**Background information**

- Accumulation of contaminants by adsorption to suspended matter in water phase → Sedimentation
- Direct exposure of benthic organism and fish offspring, respectively
- Flood events → Remobilisation of sediment-bound contaminants into water phase

**Purpose of this integrated study**

A pilot study conducted in 2002/03

"Overall, the ecotoxicological hazard potential shown has indeed to be considered as one potential reason for the decline in fish catches at the upper Danube River. However, based on the results of this pilot study, it is not possible to elucidate that chemically induced alterations are responsible for the fish decline."


**Conceptual framework**

- **Line of evidence:**
  - Community structure
  - Bioassays
  - Chemical analyses

**Weight of Evidence – Approaches**

- Triad-Approach according to Chapman (1990)

**Objectives?**

- Assessment of the ecotoxicological contamination of sediments from different sites along the upper Danube River
- Identification of the relevant hazardous substances and their sources
- Verification of the relevance of sediment contamination for the fish decline

Chapman & Hollert (2006): Should the Sediment Quality Triad become a Tetrad, a Pentad or Possibly Even a Hexad? J Soils & Sediments
**Introduction**

- **Bioassays**
  - Acute and mechanism-specific endpoints of the *in vitro* bioassays
    - Cytotoxicity – Cell damage/dead?
    - Embryotoxicity – Teratogenicity of the sediments?
    - Dioxin-like activity – Induction of specific enzymes involved in metabolism of xenobiotics (*via* Ah-receptor)?
    - Endocrine activity – Effects to hormonal balance?
    - Genotoxicity – DNA damage?
    - Alterations in gene expression patterns (*Danio rerio* chip with 20000 genes)
    - Immunotoxicity (hIL8, hIL6 and CD54 in Beas2B and MM39 cells)

**Materials & Methods**

- **Sediment sampling**
  - Bavaria (BfG): Jochenstein
  - Bad Abbach
  - Sediment samples
    - Sampling period: January-February 2006
    - Sampling sites:
      1. Sigmaringen
      2. Lauchert (tributary)
      3. Riedlingen
      4. Schwarzach (tributary)
      5. Rottenacker
      6. Ehingen
      7. Öpfingen

**Results**

- **Genotoxicity of the sediment extracts**
  - Micronucleus assay *in vitro* with RTL-W1 cells

- **Genotoxicity in barbels from the field**
  - Erythrocytes from *Barbus barbus*

- **Dioxin-like activity of the sediment extracts**
  - EROD, GPC:2D:Luc and DR CALUX assays

- **Genotoxicity of whole sediments**
  - Sediment contact Comet-Assay using embryos of *Danio rerio*
### Discussion

**Appraisal of results: dioxin-like activity**

- Tested sediments induced AhR-mediated activities in both dioxin-specific bioassays

- **Danube River 2006:**
  - max. Bio-TEQ 40000 pg/g SEQ (Grund et al. in prep)

- **Danube River 2005:**
  - max. Bio-TEQ 5000 pg/g SEQ (Keiter et al. 2008)

- **Rhine River:**
  - max. Bio-TEQ 1300 pg/g SEQ (Hinger 2003)

- **Bitterfeld:**
  - max. Bio-TEQ 100 000 pg/g SEQ (Brack et al. 2002)

High dioxin-like activities by several sediment extracts

- Effects on health of fish in the Danube River cannot be ruled out

- Identification of the substances by EDA

### Materials & Methods

**Endocrine activity: H295R bioassay**

- **NCI-H295R-cell line:** human adrenocortical carcinoma cell line
- **Ability to produce the steroid hormones of each of the three phenotypically distinct zones found in the adult adrenal cortex**

Screening of effects caused by sediment samples of the Danube River on:
- Synthesis of steroid hormones – ELISA
- Expression of important genes, involved in steroidogenesis – Real time PCR


### Results

**HPLC fractionation of Dioxin-like activities**

EROD assay

- Lauchert
- Grund et al. (in prep)

**Results**

**Multilayer fractionation of Dioxin-like activities**

EROD and DR CALUX assays, chemical analysis

- 25 % by EPA-PAHs, PCBs, PCDD/Fs
- 75 % unknown

**DNA array analyses (Danio rerio)**

In co-operation with the ITG-FZK Karlsruhe, Prof. Dr. Uwe Strähle

**Results**

**Multilayer fractionation of Dioxin-like activities**

EROD and DR CALUX assays, chemical analysis

**Results**

**Multilayer fractionation of Dioxin-like activities**

EROD and DR CALUX assays, chemical analysis

- 25 % by EPA-PAHs, PCBs, PCDD/Fs
- 75 % unknown


Discussion

Appraisal of results: hormone analysis

- Sediment extracts of the sampling sites Riedlingen, Opfingen and Rottenacker caused alterations (>1.5-fold induction) in production of P, T and E2
- No comparable studies
- First investigation of effects of sediment samples to hormone production in H295R cells
- OECD ring test: Validation of a H295R cell line screening test (Hecker et al. 2007)

- Effects on hormonal balance
- Impacts on reproduction/sex ratio/several metabolism pathways in vivo cannot be ruled out

Conclusion & prospects

Conclusion:

- Detection of high genotoxicity in several in vitro bioassays and in the micronucleous assay in situ. High relevance of the in vitro results for the field!
- Toxic effects on state of health of fish population cannot be ruled out
- Detection of high dioxin-like activities of several sediment extracts in both applied test systems
- Imbalance in the complex network of sensitive regulated steps in the synthesis of steroid hormones
- Effects of endocrine disrupting chemicals in sediments of the Danube River to sex ratio/reproduction/metabolism of fish population cannot be ruled out
- Identification of “hot spots” along the Danube River

Conclusion: Determined ecotoxicological contamination of the sediments has to be accounted as an important influencing factor with respect to the decline of fish population in the upper Danube River.

Where do we go?

Exotoxicological potential

Sediment sample

Relevance for in situ situation

In situ investigations

Effect

No

STOP

YES

Chemical Analysis

Identification of relevant contaminants

Identification of fractions

Biomarker

Identification of contaminants

Prospects

Identification of relevant contaminants

STOP

Chemical Analysis

Identification of relevant contaminants
Introduction

Mink (Mustela vison) have been proposed as a model sentinel or surrogate species for assessing the exposure and effects of environmental persistent organic chemicals. Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) that are known to act through the AhR receptor. Livers and metabolites of these compounds are then measured to determine exposure.

AhR-mediated Pathways

Acknowledgements

Jeremy N. Moore
John L. Newsted, PhD.
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Denise P. Kay, PhD.
Paul D. Jones, PhD.
Prof. John P. Giesy, PhD.

Funding

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Methods

• Experiment design
  – Dose: PeCDF: 100, 390, 1600 ng/kg & TCDF: 500, 2000, 9700 ng/kg
  – Time: days 90 and 180

• Biochemical and Molecular methods
  – RACE cDNA cloning: AhR, CYP1A1, CYP1A2, β-actin
  – Gene expression: Real time RT PCR method
  – Western blots: anti-dog CYP1A antibody
  – EROD & MROD activities

• Chemical methods
  – Feed (USEPA methods 8290) and mink tissues (1668)
  – TEFs: PeCDF = 0.3 & for TCDF = 0.1 (van den Berg et al. 2006)
**Relationships among CYP1As endpoints**

### Table 5. Spearman rank correlation coefficients (numbers) and probabilities (*) between expression levels of CYP1A1 mRNA, CYP1A2 mRNA, CYP1A1 protein, EROD, and MROD activity in the liver of mink.

<table>
<thead>
<tr>
<th></th>
<th>CYP1A1 mRNA</th>
<th>CYP1A2 mRNA</th>
<th>CYP1A1 protein</th>
<th>EROD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2 mRNA</td>
<td>0.915 ***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A1 protein</td>
<td>0.732 ***</td>
<td>0.742 ***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EROD</td>
<td>0.751 ***</td>
<td>0.799 ***</td>
<td>0.757 ***</td>
<td></td>
</tr>
<tr>
<td>MROD</td>
<td>0.820 ***</td>
<td>0.859 ***</td>
<td>0.806 ***</td>
<td>0.841 ***</td>
</tr>
</tbody>
</table>

* Sample size, N=49, *** indicates p < 0.001

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**Discussion I**

1. The basic mechanism of CYP1A induction via the AhR mediated pathway is conserved in mink.
2. Predicted protein sequences of CYP1A1 and CYP1A2 indicate that mink have preserved several conserved traits with other mammalian species and are most closely related to marine mammals.
3. TCDF and PeCDF behaved as full AhR agonist and displayed high-intrinsic induction of CYP1A

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**Hypothesis**

Ho: Level of CYP1As expression can be used to indicate the overall exposure to TEQ in mink liver.
### Discussion II

4. Positive correlations between adipose TEQ concentrations and the expression of CYP1A mRNAs and proteins show that adipose concentrations were the best predictors of AhR pathway activation.

5. Plots liver/adipose TEQ concentrations to adipose TEQs along with CYP1A responses indicate that PeCDF may have been sequestered in the liver unlike that observed for TCDF.

### Dietary and Tissue Concentrations

Daily dietary and tissue concentrations of TCDF and/or PeCDF in mink

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Daily dose (ng TEQ/kg bw/d)</th>
<th>Adipose (ng TEQ/kg, ww)</th>
<th>Liver (ng TEQ/kg, ww)</th>
<th>Liver/Adipose ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&lt;LODb &lt;LODd &lt;LODd NA</td>
<td>&lt;LODd &lt;LODd &lt;LODd NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>TCDF</td>
<td>0.98 8.9 ± 2.8 1.2 ± 0.27 0.15 ± 0.06</td>
<td>22 ± 4.4 23 ± 0.22 0.11 ± 0.024</td>
<td>0.62 74 ± 8.2 52 ± 18 0.70 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>PeCDF</td>
<td>3.6 200 ± 21 270 ± 5 1.4 ± 0.24</td>
<td>20 ± 0.22 0.11 0.024</td>
<td>9.5 213 ± 22 360 ± 79 1.8 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Mixture</td>
<td>6.9 274 ± 4.4 350 ± 5 7.1 ± 0.12</td>
<td>2.2 200 ± 21 270 ± 5 1.4 ± 0.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Tissue concentrations are presented as mean ± SD.

b LO = 0.1 ng TEQ/kg, ww

### Discussion III

1. The dose of TEQ required for induction of mink CYP1As, which is AhR-dependent, is lower than that in other well-examined experimental animal, eg. mouse and rat.

1. In B6C3F1 mice liver CYP1As was inducible in a range of 400 – 1000 ng TEQ/g tissue as indicated by EROD activity (DeVito et al., 1997).

2. Wistar (Han) Rat CYP1As was induced in maternal liver at concentration level of 100 ng TEQ/kg (Bell et al., 2007). However,

3. The induction of mink CYP1As occurred at as low as liver 1.5 ng TEQ/kg tissue.

### Sequestration of furans in mink liver

**TEQ ratio (Hepatic/Adipose) vs adipose TEQ**

**Expression of CYP1As vs TEQ**

### Publications

1. Zhang X. et al. (2008) Sequencing and characterization of mixed function monooxygenase genes CYP1A1 and CYP1A2 of Mink (Mustela vison) to facilitate study on dioxin-like compounds. TAAP (In press)


**Background and History**

- Previous research primarily focused on brominated and/or chlorinated halogenated compounds
- Fundamentally different from traditional organic pollutants
- Previously thought to be chemically stable and biologically inert in the environment
- Globally distributed in matrices varying from human blood to polar bear tissue
- Many uncertainties from analytical methods for quantification to toxicity for wildlife and humans

**Physical/Chemical Properties**

- PFOS is a fatty acid analogue
- Log $K_{ow}$ is not useful due to Amphiphilic properties
- Resistant to hydrolysis, photolysis, and biodegradation
- Preferentially retained in liver and blood

**Bioaccumulation and concentration (Laboratory)**

- BAF for trout was calculated to be $0.32 \pm 0.05$, therefore based on lab studies diet does not appear to be the major source for PFOS accumulation in fish
- Enterohepatic recirculation may cause $K_{ow}$ to under predict accumulation

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>$BCF_a$</th>
<th>$K_u$</th>
<th>$K_d$</th>
<th>BCF a</th>
<th>Half-Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muskellunge</td>
<td>Edible</td>
<td>-0.3</td>
<td>0.0041</td>
<td>1050</td>
<td>1452</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Unedible</td>
<td>-0.41</td>
<td>0.0052</td>
<td>4912</td>
<td>1333</td>
<td>2</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Whole</td>
<td>-0.53</td>
<td>0.0045</td>
<td>3614</td>
<td>152</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Carcass</td>
<td>-0.0445</td>
<td>0.0485</td>
<td>1100</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>0.0571</td>
<td>4300</td>
<td>12</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>0.05</td>
<td>5400</td>
<td>14</td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

$^a$ Apparent BCF was calculated as the concentration in fish at the end of the exposure phase divided by the average water concentration

$^b$ BCFK was estimated as $K_u/K_d$
### Chronic Ecotoxicology (Fresh water)

Based on the laboratory toxicity studies, PFOS is known to be slightly chronically toxic to aquatic organisms.

<table>
<thead>
<tr>
<th>Trophic Level</th>
<th>Test Species</th>
<th>Test Duration</th>
<th>Endpoint</th>
<th>NOEC</th>
<th>LOEC</th>
<th>LC50</th>
<th>EC50</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalgae</td>
<td>Ankistrodesamia flos-aquae</td>
<td>96 h</td>
<td>Growth</td>
<td>0.3</td>
<td>0.5</td>
<td>&gt;3.2</td>
<td>&gt;3.2</td>
<td>Boudreau et al. 2003</td>
</tr>
<tr>
<td></td>
<td>Daphnia pulex</td>
<td>96 h</td>
<td>Survival</td>
<td>0.3</td>
<td>0.5</td>
<td>&gt;0.15</td>
<td>&gt;0.15</td>
<td>McIvor et al. 1994</td>
</tr>
<tr>
<td></td>
<td>Daphnia magna</td>
<td>96 h</td>
<td>Growth</td>
<td>1.1</td>
<td>1.2</td>
<td>15.6</td>
<td>15.6</td>
<td>Palmer and Krueger 2001</td>
</tr>
<tr>
<td>Amphipods</td>
<td>Ostracods</td>
<td>96 h</td>
<td>Survival</td>
<td>0.3</td>
<td>0.5</td>
<td>3.2</td>
<td>3.2</td>
<td>Desjardins et al. 2001</td>
</tr>
<tr>
<td>Fish</td>
<td>Pimephales promelas</td>
<td>48 h</td>
<td>Survival</td>
<td>20</td>
<td>30</td>
<td>130</td>
<td>130</td>
<td>Robertson et al. 1986</td>
</tr>
<tr>
<td></td>
<td>Pimephales salomonis</td>
<td>48 h</td>
<td>Survival</td>
<td>20</td>
<td>30</td>
<td>130</td>
<td>130</td>
<td>Robertson et al. 1986</td>
</tr>
<tr>
<td></td>
<td>Pimephales promelas</td>
<td>48 h</td>
<td>Immobility</td>
<td>0.8</td>
<td>1.2</td>
<td>67.2</td>
<td>67.2</td>
<td>Robertson et al. 2003</td>
</tr>
<tr>
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<td>Pimephales salomonis</td>
<td>48 h</td>
<td>Immobility</td>
<td>0.8</td>
<td>1.2</td>
<td>67.2</td>
<td>67.2</td>
<td>Robertson et al. 2003</td>
</tr>
</tbody>
</table>

### Bioconcentration and Accumulation (Field)

- However big differences exist between laboratory and field measured results
- Bioaccumulation calculated in the field ranges greatly (6,300 to 125,000 for the common shiner), and is often much higher than what is predicted in the laboratory
- Reasons for the difference include: interspecies variability, sex-dependent variables, diet over the entire life span, and precursors being metabolized to PFOS
- More data is needed to evaluate bioconcentration and bioaccumulation under environmental conditions

### Chronic Ecotoxicology (Marine)

There is limited chronic marine toxicological data available, but in general it appears that marine microorganisms and invertebrates behave similarly to their freshwater relatives.

### Acute Ecotoxicology (Fresh water)

In general based on the laboratory toxicity studies, PFOS is known to be moderately acutely toxic to aquatic organisms.

<table>
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<tr>
<th>Trophic Level</th>
<th>Test Organism/Species</th>
<th>Test Duration</th>
<th>Endpoint</th>
<th>NOEC</th>
<th>LOEC</th>
<th>LC50</th>
<th>IC50</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroalgae</td>
<td>Lemna gibba</td>
<td>72 h</td>
<td>Survival</td>
<td>15</td>
<td>30</td>
<td>100</td>
<td>100</td>
<td>Desgirardes et al. 2001</td>
</tr>
<tr>
<td>Invertebrates</td>
<td>Daphnia pulex</td>
<td>48 h</td>
<td>Biomass</td>
<td>6.6</td>
<td>31.1</td>
<td>110</td>
<td>110</td>
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<td>Survival</td>
<td>33.1</td>
<td>110</td>
<td>110</td>
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<td>Immobility</td>
<td>0.8</td>
<td>1.2</td>
<td>67.2</td>
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<td>0.8</td>
<td>1.2</td>
<td>67.2</td>
<td>67.2</td>
<td>Robertson et al. 2003</td>
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<tr>
<td>Amphipods</td>
<td>Euphausia krill</td>
<td>96 h</td>
<td>Survival</td>
<td>3.2</td>
<td>4.4</td>
<td>15.6</td>
<td>15.6</td>
<td>Drotter and Knueger 1996</td>
</tr>
<tr>
<td></td>
<td>Euphausia krill</td>
<td>96 h</td>
<td>Survival</td>
<td>7.8</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>Robertson et al. 1986</td>
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<tr>
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<td>96 h</td>
<td>Survival</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
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<td>Survival</td>
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<td>8.9</td>
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<td>8.9</td>
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<td>8.9</td>
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<td>8.9</td>
<td>8.9</td>
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</tbody>
</table>

### Acute Ecotoxicology (Marine)

Limited marine toxicity data exists, and the Sheepshead minnow (Cyprinodon variegatus) study reports a value above the solubility of PFOS in salt water because they added 0.05% methanol to increase PFOS solubility.

<table>
<thead>
<tr>
<th>Trophic Level</th>
<th>Test Organism/Species</th>
<th>Test Duration</th>
<th>Endpoint</th>
<th>NOEC</th>
<th>LOEC</th>
<th>EC50</th>
<th>EC100</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertebrates</td>
<td>Artemia salina</td>
<td>48 h</td>
<td>Survival</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>Robertson et al. 1986</td>
</tr>
<tr>
<td></td>
<td>Daphnia pulex</td>
<td>48 h</td>
<td>Survival</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>Robertson et al. 1986</td>
</tr>
<tr>
<td></td>
<td>Daphnia pulex</td>
<td>96 h</td>
<td>Survival</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>Robertson et al. 1986</td>
</tr>
<tr>
<td>Amphipods</td>
<td>Mandrillus spicatum</td>
<td>96 h</td>
<td>Survival</td>
<td>1.1</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>Drotter and Knueger 1996</td>
</tr>
<tr>
<td></td>
<td>Euphausia krill</td>
<td>96 h</td>
<td>Survival</td>
<td>1.1</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>Drotter and Knueger 1996</td>
</tr>
<tr>
<td>Fish</td>
<td>Ostracods</td>
<td>96 h</td>
<td>Survival</td>
<td>1.1</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>Drotter and Knueger 1996</td>
</tr>
<tr>
<td></td>
<td>Ostracods</td>
<td>96 h</td>
<td>Survival</td>
<td>1.1</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>Drotter and Knueger 1996</td>
</tr>
<tr>
<td></td>
<td>Cyprinodon variegatus</td>
<td>96 h</td>
<td>Survival</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>Palmer et al. 2002</td>
</tr>
</tbody>
</table>

### Ecotoxicology for Perfluorobutanesulfonate (PFBS)

PFBS was chosen because it is one of the main replacement chemicals now used instead of PFOS.
Quantitative Structure Activity Relationship (QSAR)

- Shorter than 6 or 7 carbons do not tend to accumulate and bioconcentration factors are usually less than 1.0
- Bioconcentration tends to go up by a factor of about 100 with the addition of 2 carbons for PFCs C4 to C8
- Chain-lengths greater than 12 appear to have reduced toxicity
- Length does not appear to be as important for fluorotelomer alcohols

Water Quality Criteria for PFOS

- Purpose: To derive water quality values for those perfluorinated compounds (PFCs) that have sufficient and appropriate toxicity data
- Used the US EPA Great Lakes Initiative methodology because it provided specific procedures and methods for utilizing toxicity data to derive water quality values protective of aquatic life
- OVERALL GOAL: To derive toxicity reference values that are protective of aquatic life

Quantitative Structure Activity Relationship (QSAR)

Chain-length not functional group makes the difference

Water Quality Criteria (PFCs)

- Log Scale
- 121 mg/L CMC for PFBS
- 25 mg/L CMC for PFOA
- 21 mg/L CMC for PFOS
- 47 mg/L AWV for PFOS
- 57 mg/L AWV for PFBS

CMC: criteria maximum concentration
CCC: criteria continuous concentration
AWV: avian wildlife value

Conclusions

- Based on the GLI a protective water concentration of PFOS was calculated to be 0.46mg PFOS/L for chronic exposure and 0.78mg PFOS/L for acute exposure.
- In most cases chain-length appears to be the most important factor determining PFC toxicity
- There are big differences between BCF calculated in the field and what has been calculated in the laboratory
- There are still many knowledge gaps and more aquatic toxicity data is needed

Quantitative Structure Activity Relationship (QSAR)

- Limited Toxicological data available for many PFCs, so the use a Quantitative Structure Activity Relationship was developed to estimate toxicological data where no measured data is available
- Results show that chain-length is the most important factor in determining toxicity, although functional head group and the addition of an amide group can also be important
Thank You!
Sensitivity of chicken, ring-necked pheasant and Japanese quail embryo hepatocyte cultures to ethoxyresorufin O-deethylase (EROD) induction upon exposure to TCDD, PeCDF and TCDF

J.C. Hervé 1,2, S.P. Jones 3, L. Mundy 4, M.J. Zwierink 5, S. Burris 6, J.P. Giesy 1, P.D. Jones 7, Y. Wan 8 and S.W. Kennedy 1,2


RESULTS

Relative potency and relative sensitivity based on EROD induction

- In chicken hepatocytes, the EROD inducing potencies of TCDD, PeCDF and TCDF were similar, but in pheasant and quail hepatocytes, PeCDF was more potent than TCDD (Fig. 2, Tables 2 and 3).
- When exposed to TCDD and TCDF, chicken hepatocytes were more sensitive to EROD induction than PeCDF, but quail and pheasant hepatocytes were less sensitive to TCDD and PeCDF, species differences observed (Tables 2 and 3).

Comparison of EC50- and EChr-based methods

- Both TCDD and PeCDF were more potent EROD inducer than TCDF in pheasant and quail hepatocytes, but quail and pheasant hepatocytes had very similar EC50 values (Fig. 2).

DISCUSSION

Intercompartmental comparisons: relative potency as EROD inducers

The findings that PeCDF is a more potent EROD inducer than TCDD in pheasant and quail eggs is consistent with the previous work, but not with the relative potencies of the AhR agonists determined by pregnant female rats (PeCDF > TCDD), suggesting that the different AhR agonists active in the two species.

Table 3: Relative EROD-inducing potencies and sensitivities. A) Relative potency (ReP) values and B) relative sensitivity (ReS) values. Standard errors are shown in brackets.

<table>
<thead>
<tr>
<th>Species</th>
<th>EC50 (pmol/min/mg)</th>
<th>EChr (nM)</th>
<th>ECthr (nM)</th>
<th>EROD activity (pmol/min/mg)</th>
<th>Maximal EROD activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>460 (36)</td>
<td>0.018</td>
<td>0.00041</td>
<td>0.00032</td>
<td>443 (17)</td>
</tr>
<tr>
<td>PeS</td>
<td>525 (6)</td>
<td>0.0051</td>
<td>(0.01)</td>
<td>0.0011</td>
<td>486 (12)</td>
</tr>
<tr>
<td>Quail</td>
<td>248 (31)</td>
<td>0.19</td>
<td>0.02</td>
<td>0.0073</td>
<td>285 (24)</td>
</tr>
</tbody>
</table>

Table 4: Rank order of A) relative potencies and B) relative sensitivities based on EC50 or EChr. ReP and ReS values are shown in brackets.

<table>
<thead>
<tr>
<th>B) Relative sensitivities of species</th>
<th>A) Relative potencies of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken (1) &gt; PeS (0.2) &gt; Quail (0.04)</td>
<td>EROD based ReP</td>
</tr>
<tr>
<td>TCDD (1) &gt; PeCDF (0.9) &gt; TCDF (0.8)</td>
<td>EROD based ReS</td>
</tr>
<tr>
<td>TCDF (1) &gt; PeCDF (0.9) &gt; EROD based ReS</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Cytochrome P450 (1) &gt; EROD (0.9)</td>
<td></td>
</tr>
<tr>
<td>PeS (0.2) &gt; Quail (0.04)</td>
<td></td>
</tr>
</tbody>
</table>

OBJECTIVE

To determine the relative sensitivities of chicken, pheasant and quail hepatocyte cultures to EROD induction by TCDD, PeCDF and TCDF.

METHODS

Qual, pheasant and chicken eggs were incubated until 1 to 3 days prehatch.

Livers were dissected, pooled and digested, hepatocytes were plated.

Cells were exposed to serial dilutions of TCDD, PeCDF or TCDF for 24 hours.

CYP1A1 activity (EROD) was measured and EC50, EChr, ReP and ReS values were calculated.

RESULTS

Figure 2: EROD concentration-response curves for chicken, pheasant or quail embryo hepatocytes exposed to PeCDF (A), TCDF (B) or TCDD (C) for 24 h. Points represent mean EROD activity caused by a particular concentration on three replicate cell culture plates; bars represent standard errors and values before the axis break indicate EROD activity observed for control (DMSO-treated hepatocytes).

<table>
<thead>
<tr>
<th>Species</th>
<th>EC50 (pmol/min/mg)</th>
<th>EChr (nM)</th>
<th>ECthr (nM)</th>
<th>EROD activity (pmol/min/mg)</th>
<th>Maximal EROD activity</th>
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<td>525 (6)</td>
<td>0.0051</td>
<td>(0.01)</td>
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</tr>
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<td>Chicken (1) &gt; PeS (0.2) &gt; Quail (0.04)</td>
</tr>
<tr>
<td>TCDD (1) &gt; PeCDF (0.9) &gt; TCDF (0.8)</td>
</tr>
<tr>
<td>TCDF (1) &gt; PeCDF (0.9) &gt; EROD based ReS</td>
</tr>
<tr>
<td>TCDF (1) &gt; PeCDF (0.9) &gt; EROD based ReS</td>
</tr>
<tr>
<td>Cytochrome P450 (1) &gt; EROD (0.9)</td>
</tr>
<tr>
<td>PeS (0.2) &gt; Quail (0.04)</td>
</tr>
</tbody>
</table>

Figure 1: EROD concentration-response curves for chicken and component 2 (C2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>EROD activity (pmol/min/mg)</th>
<th>Maximal EROD activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCD2</td>
<td>0.03</td>
<td>1 (0.01)</td>
</tr>
<tr>
<td>PeS</td>
<td>0.008</td>
<td>4 (0.01)</td>
</tr>
<tr>
<td>Quail</td>
<td>0.001</td>
<td>1 (0.01)</td>
</tr>
</tbody>
</table>

To determine the relative sensitivities of chicken, pheasant and quail hepatocyte cultures to EROD induction by TCDD, PeCDF and TCDF.
Molecular mechanisms underlying differences in sensitivity of avian species to embryotoxic effects of chlorinated dioxins and furans- Recent advances in the characterization of aryl hydrocarbon receptor 1 (AHR1) in birds

Reza Farmahin1,2, Steven J. Bursian3, Doug Crump2, John P. Giesy3, Mark E. Hahn5, Jessica A. Head6, Stephanie P. Jones2, Lukas Mundy1, Matthew J. Zwiernik4, Sean W. Kennedy1,2

BACKGROUND AND RATIONALE
Chlorinated dibeno-p-dioxins, dibenzofurans, non-ortho substituted polyhalogenated biphenyls and other 'dioxin-like compounds' (DLCs) are lipophilic environmental contaminants that are toxic to most vertebrates. Responsiveness to the toxic effects of DLCs varies among species and strains. For example, some species of birds are 10 to 1000 fold less sensitive to the embryotoxic effects of these chemicals than the highly sensitive domestic chicken. It has been postulated that differential sensitivity is caused by differences in genes expression that occur subsequent to the binding of a DLC to the aryl hydrocarbon receptor (AHR). Binding affinity to the AHR is dependent on the confirmation of the ligand-binding domain (LBD) and the orientation of gene specific primary (GSF), 2’ and 3’ RACE fragments. The basic residues (Leu, Ser, Thr) are AHR specific and dem. A and B repeats are indicated.

The sequences of the LBD of AHR1 have been determined for >70 species of birds (Kennedy et al., 2009). Among these species, there are a total of six differences in amino acid residues (a324, a380 and four others) within the LBD. To determine the possible influence of all six amino acids on the binding affinities of DLCs to AHR1 we will be carrying out site-directed mutagenesis and other studies similar to the work conducted by Karchner et al. (1) on all variants of AHR1 LBD in birds. Some of these studies will be conducted with the three Galliforma mentioned above (chicken, ring-necked pheasant and Japanese quail) as model organisms. Here we present data on the full-length sequences of AHR1 for these species and outline some of our plans for future studies. This research is part of a larger project that includes egg injection studies (Poster #WP149), hepatic cytochrome P450 and field based exposure and response studies (Poster #WP222). The purpose is to identify the molecular and physiological reasons that underlie avian species differences and phenotypic responses to DLCs. The ultimate goal is to develop molecular and cell culture methods that can be used to characterize aryl hydrocarbon receptor 1 (AHR1) in birds

RESULTS
Full-length cDNA sequences for ring-necked pheasant and Japanese quail AHR1 were obtained and aligned with chicken AHR1 (accession # NM_204418) are shown in Fig. 3

Table 1: The number of amino acid sequences and their similarities in chicken, ring-necked pheasant and Japanese quail. Two allele variants that include J.quail AHR1*1 and J.quail AHR1*2 were used (see Figs. 3 and 6).

Figure 2: Clopping strategy for ring- necked pheasant and Japanese quail amino acid sequences. Sequences were aligned using ClustalW, and gene specific primers (GSP) and a 5’ and 3’ RACE fragments were isolated. The basic residues (Leu, Ser, Thr) are AHR specific and dem. A and B repeats are indicated.

Figure 4: Insertion deletion (insdel) in two allelic variants of Japanese quail AHR1 (Liquit AHR1*1 and j.quail AHR1*2) are shown.

Figure 5: Comparison of the ligand binding domains of AHR1 in chicken (accession: NM_204418), ring-necked pheasant and Japanese quail. Differences in amino acids at sites 257, 281, 324, and 380 are indicated. Identical sequences at these positions will be called out.

Table 1: Amino acid sequences of chicken, ring-necked pheasant, and Japanese quail (two allele variants; J.quail AHR1*1 and J.quail AHR1*2) were aligned using ClustalV2. Amino acids that are identical in two or more sequences are highlighted in the table. The percentage of identity is given. Positions 324 and 380 within the ligand binding domain of AHR1 (2).

Table 1:

Name | Length | N 257 | Length | Percent identity
--- | --- | --- | --- | ---
Chicken AHR1 | 555 | R 257 | 329 | 95%
Duck AHR1 | 555 | F 257 | 329 | 95%
Ring-necked pheasant AHR1 | 555 | J 257 | 329 | 87%
Ring-necked pheasant J.quail AHR1*1 | 555 | J 257 | 329 | 87%
Ring-necked pheasant J.quail AHR1*2 | 555 | J 257 | 329 | 87%
Japanese quail AHR1*1 | 555 | J 257 | 329 | 87%
Japanese quail AHR1*2 | 555 | J 257 | 329 | 87%

Funded by an unrestricted grant from Dow Chemical Company, Environment Canada’s Wildlife Toxicology and Disease Division, Environment Canada’s STAGE (Strategic Applications of Genomics for the Environment) program and a WHO Sea Grant.

REFERENCES

ACKNOWLEDGMENTS
1. Department of Biology, University of Ottawa, Ottawa, ON, Canada.
2. NVIRC, Environment Canada, Ottawa, ON, Canada.
3. Western University, London, ON, Canada.
4. University of Saskatchewan, Saskatchewan, SK, Canada.
5. Department of Biology, University of Ottawa, ON, Canada.
6. University of Michigan, Ann Arbor, MI, USA.
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Full-length cDNA sequences for ring-necked pheasant and Japanese quail AHR1 were obtained and aligned with chicken AHR1 (accession: NM_204418) are shown in Fig. 3

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5. Department of Biology, University of Ottawa, ON, Canada.
6. University of Michigan, Ann Arbor, MI, USA.
Abstract
A real time polymerase chain reaction (RT-PCR) array was developed for studying chemical-induced effects on gene expression of selected endocrine pathways along the hypothalamic-pituitary-gonadal (HPG) axis of the small, oviparous fish, the Japanese medaka (Oryzias latipes). The Japanese medaka HPG PCR array combines the quantitative performance of SYBR® Green-based real-time PCR with the multiple gene profiling capabilities of a microarray to examine expression profiles of 36 genes associated with endocrine pathways in brain, liver and gonad. A pathway-based approach was implemented to analyze and visualize time-dependent or concentration-dependent mRNA expression in the HPG axis of Japanese medaka. The performance of the Japanese medaka HPG PCR array was evaluated by examining effects of five model compounds. The organ-specificity and concentration-specific gene expression profiles derived by the Japanese medaka HPG axis RT-PCR array provides a powerful tool to delineate chemical-induced modes of action. In addition, the medaka real time PCR array demonstrate potential to quantitatively predict the adverse effects on reproduction.

Introduction
Endocrine disruptors
An exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior

Small fish model
- Conservation of basic aspects of the HPG axis across vertebrates
- Small body size & relatively rapid life-cycle
- 4 month generation time from embryos to adults
- Genomic sequence (www.ensembl.org)

Methods
Exposure
- Animal: 4 month adult medaka
- Exposure: 5 model chemicals
- Sex: 5 male; 5 female per tank
- Vehicle control: DMSO
- RNA isolation: brain, liver & gonads

Results
Fish fecundity

Discussion
1. Application of the medaka HPG PCR array facilitated mechanistic understanding of environmental EDCs
2. Molecular response at mRNA has potential to quantitatively evaluate chemical-induced adverse effects on reproduction
3. The medaka HPG axis model has potential to be an effective ecotoxicological screening tool for EDCs

References:
Species-specific accumulation of polychlorinated dibenzop-dioxins (PCDDs), dibenzofuran (PCDFs), and coplanar polychlorinated biphenyls (PCBs) in fishes from the Tittabawassee and Saginaw Rivers (Michigan, USA)

Yi Wan1, Paul D. Jones2, Ryan R. Holmen3, Jong Seong Khim1, Denise P. Kay3, Shaun A. Roark4, John L. Newsted5, and John P. Glesby2

1Department of Biomedical Veterinary Science and Toxicology Centre, University of Saskatchewan, Saskatoon, SK S7N 5B3, Saskatchewan, Canada
2ENTRIX, East Lansing, MI, USA

ABSTRACT

Factors causing differential accumulation of contaminants among species are a major focus of ecotoxicology and environmental chemistry studies. In this study, polychlorinated dibenzop-dioxins (PCDDs), dibenzofurans (PCDFs), and nonortho-substituted (coplanar, dioxin-like) polychlorinated biphenyl (PCB) congeners were analyzed in twelve fish species from the Tittabawassee and Saginaw rivers in Michigan, USA. Based on stable isotope determination the trophic levels for all fishes, excluding migratory walleye and white sucker, ranged from 2.0±0.26 (carp) to 3.2±0.34 (largemouth bass). The greatest PCDD/F concentrations were found in carp (n=50) followed by channel catfish (n=49). PCDD/Fs, PCBs and ΣTEQs in carp were approximately 30-, 9- and 30- fold greater than the least concentrations in other fishes. 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF and 2,3,7,8-TCDD were the predominant congeners found in all fishes, but relatively small proportions of 2,3,7,8-TCDF and greater proportions of more-chlorinated congeners were found in carp and channel catfish. Positive relationships were found between lipid content, body weight and concentrations of ΣPCDD/Fs, ΣPCBs and ΣTEQs, but negative relationships were found between trophic levels and all the above parameters. Multiple regression analysis demonstrated that lipid content and trophic level were important determining factors for PCBs, but lipid content and body weight were the strongest predictors for PCDD/Fs and ΣTEQs. Furthermore, Biotransfer Accumulation Factors (BSAFs) indicated that differences in bioavailability among chemicals are the main reason for the different patterns of relative concentrations among species.

INTRODUCTION

• A number of reports have highlighted the importance of chemical and biological factors in the bioaccumulation and trophic transfer of persistent organic pollutants in aquatic ecosystems
• No research has addressed the factors influencing differences in bioaccumulation of dioxins among species in river ecosystems
• The Saginaw River and its largest tributary, the Tittabawassee River, have been contaminated by various organic pollutants including PCDD/Fs and dioxin-like PCBs by historic industrial activities
• Nine PCDDs, eleven PCDFs and twelve non-ortho- and mono-ortho PCB congeners in twelve species of fish (314 individuals) collected from the Tittabawassee and Saginaw Rivers were studied to assess the factors influencing trophic transfer of dioxins in a river ecosystem, and provide site-specific concentrations for human health risk assessment.

MATERIALS AND METHODS

• The fishes studied were those preferentially harvested by anglers and the selection of species was based on information such as creel surveys conducted by state and federal agencies as well as local fishery experts.
• Fish were collected, using standard electro-fishing equipment (Smith-Root) and techniques, from six distinct river reaches, four in the Tittabawassee River and two in the Saginaw River.
• PCDD/Fs and PCBs were analysed following EPA methods 1613 and 1688 respectively.
• Stable nitrogen isotope analysis was used to quantitatively determine the habitat and trophic levels of these fishes.
• WA=walleye, WS=white sucker, WB=white bass, SB=smallestmouth bass, LB=largemouth bass, CC=channel catfish, CA=carp, FD=freshwater drum, BC-black crappie, NP=northern pike, BG-bluegill, and SS=green sunfish.

RESULTS

• The δ15N value is an indicator of the origin of nutrients and migratory behaviors, increasing values from freshwater to marine ecosystems
• The δ15N values of walleye and white sucker were greater than those of other fishes, which are probably due to their habitats, since these two fishes live in Saginaw Bay, Lake Huron, and migrate up to the river systems to spawn
• The δ15N value is used to calculate the trophic levels of the fishes. TL = δ15Nfish + δ15Nwater / 3.4
• The trophic level of carp was the least followed by channel catfish, which is consistent with the bottom feeding behavior, the top predator among the biological samples in this study is large mouth bass.
• The δ15N value is significantly correlated with the origin of nutrients and migratory behaviors, increasing values from freshwater to marine ecosystems

• Statistically significant correlations were observed between trophic level, lipid content, body weight and tissue contaminant concentrations.
• Multiple linear regressions were used to further assess the effects of the biological factors on accumulations of dioxins for all local fishes.

• Log(ΣPCBs) = 0.439log(ΣTEQ)(p=0.001, 28.7%) - 0.304TL(p=0.001, 10.4%) + 5.304
• Lipid content and trophic level were important factors for PCBs, and the percentage contributions were 28.7% and 10.4%, respectively. Negative correlations between concentrations of dioxin-like PCBs and trophic level were due to the bentivorous fishes occupying lower trophic levels and consequently having an extra uptake pathway, sediment ingestion.

• Log(ΣPCDD/Fs)=0.634log(ΣTEQ)(p=0.001, 13.7%) + 0.464log(BW)(p=0.001, 37.0%) - 0.976
• Log(ΣTEQs)= 0.482log(BW)(p=0.001, 10.4%) + 0.471log(WL)(p=0.001, 20.2%) - 0.633
• Lipid content and body weight were strong predictors of concentrations of PCDD/Fs and ΣTEQs, explaining 17.7% and 37.0% of the variance for PCDD/Fs, respectively, and 10.4% and 20.2% of the variance for ΣTEQs, respectively.
Assessment of Toxicity of Upper Danube River Sediments Using a Combination of Chemical Fractionation, the Danio rerio Embryo Assay and the Ames Fluctuation Test

Eric Higley\(^1\), Stefanie Grund\(^1\), Thomas B.-Seiler\(^1\), Urte Lubcke-von Varel\(^1\), Werner Brack\(^1\), Tobias Schulz\(^1\), Jan Wölz\(^2\), Hanno Zielke\(^2\), John Giesy\(^3\), Henner Hollert\(^2\), Markus Hecker\(^4\)

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Introduction

The world’s river systems provide fresh water to people and support thousands of species. However, many of the great rivers have been polluted in the past decades. Possible sources of such pollution include effluents from domestic sewage plants (i.e. urine and feces, detergents, pharmaceuticals), industry (i.e. PCBs, dioxins, and metals), agricultural runoff (i.e. pesticides and fertilizers), and storm water runoff from urban areas (i.e. salts, oil, and antifreeze). Severely contaminated sediments from many rivers and lakes have been shown to be acutely and chronically toxic to fish and benthic invertebrate species. For example, sediment samples from the Upper Danube River that were analyzed in six separate assays were found to have considerable geno-toxic, cytotoxic, mutagenic, embryo-toxic and estrogenic effects. It has been hypothesized that decline in fish stocks in the Upper Danube River since the early 1990s may be associated with this pollution. Here, we report on the results of a study conducted to determine the toxicity of extracts from sediments of the Danube River by means of the Danio rerio embryo assay, and by assessing lethal and sub-lethal endpoints. In addition, mutagenicity was assessed using the Ames fluctuation assay. For the sediment samples that revealed toxicity, fractionation of each sample was performed by separating compounds according to their polarity, planarity, and the size of the aromatic ring system. 18 fractions for each sediment sample were tested separately in the Ames fluctuation assay and Danio rerio embryo assay to assess which group of chemicals within the sediment sample caused the original toxicity.

Objectives

1. Assess the toxicity of raw sediment extracts from four locations along the Upper Danube River using the Danio rerio Embryo Assay and Ames Fluctuation Assay
2. Evaluate which groups of chemicals caused the measured toxicities using new chemical fractionation techniques that separate the raw sediment extracts into 18 different chemical fractions.
3. Analyze all 18 chemical fractions using the Danio rerio Embryo Assay and Ames Fluctuation Assay.

Methods

Sampling and extraction

• Sediments were sampled (top 5cm) at four locations along the Upper Danube River using a Van Veenen grabber in January 2006 (Figure 1)
• Samples were extracted and fractionated into different chemical groups using a new technique by Varel et al., 2008 that uses 3 HPLC columns and separates the sample into 18 fractions according to their polarity, planarity and the size of their aromatic system
• Crude sediment extracts and all 18 fractions were analyzed for their toxicity using the Ames fluctuation assay and Danio rerio egg assay

Results

...fractional increases in the number of mutations compared to the controls as determined by the Ames Fluctuation Assay. TA98 Bacteria measures frame shift mutations and TA100 Bacteria measures base pair substitutions.

Table 1. Fractions (3 – 17) showing significant increases in the number of mutagenic revertants compared to the controls as determined by the Ames Fluctuation Assay. TA98 Bacteria measures frame shift mutations and TA100 Bacteria measures base pair substitutions. Sig=Sigmaringen, Opf=Opfingen, Lau=Lauchert, Lau ref=Lauchert Reference.

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Chemical fractions that showed effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig</td>
<td>X XX</td>
</tr>
<tr>
<td>Lau</td>
<td>X X</td>
</tr>
<tr>
<td>Lau ref</td>
<td>XXX XX</td>
</tr>
<tr>
<td>Opf</td>
<td>XX</td>
</tr>
<tr>
<td>Sig ref</td>
<td>X X</td>
</tr>
</tbody>
</table>

Conclusions

• Mortality of Danio rerio embryos increased in a dose-dependent manner when exposed to whole sediments collected at Opfingen and Sigmaringen, but none of the fractionated samples were toxic. These results indicate that the observed toxicity was likely due to the combination of groups of chemicals in the whole sediment samples.
• Toxicity was observed for whole sediments from Sigmaringen, Opfingen and Lauchert in the Ames Fluctuation Assay only when TA98 bacteria with S9 were used. Toxicity was also found in the fractionated samples in both bacterial strains, although the pattern was inconsistent.
• However, toxicity was measured in fractions 10 and 15 of every sediment sample except Lauchert Reference. Previous work has found that fraction 10 can contain six-ringed PAHs (i.e. benzo(a)pyrene or benzo(k)fluoranthene) and fraction 15 can contain more non-polar chemicals like benzo(c)fluoranthene. Further work using other analytical techniques may identify which chemicals caused the observed toxicity.

References:
Perfluorinated Compounds in Environmental Samples Collected from Inner-Mongolia, China

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ABSTRACT

Inner-Mongolia is a region of China which historically has seen little development and industry. Sediment (n=7), water (n=8), and biological (n=12) samples were collected from Inner-Mongolia to determine the extent of perfluorinated compound (PFC) pollution in a less industrialized region and to shed light on their long-range transport and ultimate fate of these compounds. Our results indicate that PFCs are only moderately concentrated in sediments and water samples. Some biological samples contained detectable concentrations of several PFCs including PFOS (0.48-1.13 ng/g) and PFOA (0.09-1.2 ng/g); however, their concentrations were mostly lower than the detection limit. There is currently some debate as to whether soil and sediment are the ultimate sink for PFCs as they are for many neutral organic compounds. PFCs detected in these environmental samples from Inner-Mongolia likely represent background globally distributed concentrations in China. Overall, the detection of PFCs and their precursors in various environmental matrices from remote regions suggest their long range transport and distribution.

RESULTS

- PFOS and PFOA were detected in all environmental samples collected from China.
- Concentrations of PFOS and PFOA were lower in water samples compared to sediment and biological samples.
- The distribution of PFOS and PFOA varied across different regions within China.
- The highest concentrations were observed in freshwater and coastal marine environments.

CONCLUSIONS

- PFOS was detected in all but one of the water samples.
- PFOA was detected in all water samples and was consistently found at the highest concentrations relative to the other PFCs monitored for.
- Water concentration of PFOS found in Inner Mongolia are similar to or lower than most other regions of China.
- Sediment and biological samples collected from Inner Mongolia, China do not appear to be heavily contaminated with PFCs.
- Concentrations are consistent with other remote environments.

ONGOING and FUTURE WORK

- Monitoring and Assessment of Exposure and Potential Biological Effects of Perfluorinated Compounds in the Yellow Sea Region of China and Korea
- Find environmental levels of target persistent and toxic contaminants and if appropriate determine loadings and sources
- Determine distribution and source characteristics (and possibly fate, transport, or food web) of target contaminants
- Address potential biological effects associated with samples
- Identify potential toxic chemicals based on a TIE and mass balance approach
- Establish background monitoring data for target contaminants and possibly develop site-specific environmental quality guidelines
- Prioritize the site-specific contaminants of concern in the study area

REFERENCES

Perfluorinated Compounds in Sediment and Water from Bohai Bay and Its Vicinity, China

Jong Seong Khim1*, Tieyu Wang2, Wentao Jiao2, Jonathan E. Naile1, Jing Geng2, Chunli Chen2, Yonglong Lu2, Yi Wan1, Paul D. Jones1, John P. Giesy1,3,4

1 Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK S7N 5B3, Saskatchewan, Canada
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3 Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824, USA
4 Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong, SAR China

INTRODUCTION

- Bohai Bay and its vicinity (north coast of China) contains several industrial complexes and a large commercial harbor.
- Despite the fact that perfluorinated compounds (PFCs), are known to have been used extensively in the region with potential for intentional and accidental release, little was known regarding the current status of PFCs concentrations in this region.
- The present study was one of the first efforts to examine the concentrations, distribution, and potential ecological effect of PFCs in this area of China.
- We present the results of instrumental analyses on the distribution of 17 PFCs including perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in sediment and water from Bohai Bay and Guanting Reservoir.

MATERIALS & METHODS

1. Study Area

- Sediment and water samples were collected from Bohai Bay and its vicinity city of Tianjin (Fig. 2) and from Guanting Reservoir (Fig. 3).
- Soil samples were also collected, but soil PFCs data presented elsewhere.

2. Instrumental Analysis

- PFCs were concentrated from sediment and water by use of solid phase extraction.
- PFCs were identified and quantified by liquid chromatography interfaced with a triple quadrupole tandem mass spectrometer (LC-MS/MS).

3. Comparison to Environmental Quality Criteria

- Concentrations of neither PFOS nor PFOA in water samples exceeded concentrations thought to be protective of aquatic life both.
- Overall, the PFCs detected in environmental samples from these areas were relatively low to moderate compared to other studies in Asia and likely represent background globally distributed concentrations of these compounds.

RESULTS & DISCUSSIONS

1. Occurrence & Concentrations of PFCs

- Of 17 PFCs measured, PFOS and PFOA were found to be the predominant compounds in both sediment and water.
- Concentrations of PFOS and PFOA in sediment were as great as 2.15 ng/g DW (mean=0.46, n=15) and 0.74 ng/g DW (mean=0.31, n=19), respectively.
- Concentrations of PFOS (mean=1.79 ng/L, n=15) and PFOA (mean=4.18 ng/L, n=15) in water samples were generally three orders of magnitude less than corresponding sedimentary concentrations.
- There is currently some debate as to whether soil and sediment are the ultimate sink for PFCs as they are for many other organic compounds.

Table 1. Concentrations of PFOS and PFOA in sediment (ng/g DW) and water (ng/L) samples from Bohai Bay and Guanting Reservoir, China

<table>
<thead>
<tr>
<th>Location</th>
<th>Samples</th>
<th>PFOS</th>
<th>PFOA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean=0.125</td>
<td>mean=0.15</td>
</tr>
<tr>
<td></td>
<td>(mean=0.34)</td>
<td>(mean=0.31)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean=0.15</td>
<td>mean=0.31</td>
</tr>
<tr>
<td></td>
<td>(mean=0.35)</td>
<td>(mean=0.33)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean=0.15</td>
<td>mean=0.25</td>
</tr>
<tr>
<td></td>
<td>(mean=0.32)</td>
<td>(mean=0.30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean=0.20</td>
<td>mean=0.29</td>
</tr>
<tr>
<td></td>
<td>(mean=0.30)</td>
<td>(mean=0.32)</td>
<td></td>
</tr>
</tbody>
</table>

2. Spatial Distribution of PFCs

- PFOS was detected in sediment from nearly all locations in Bohai Bay, while PFOS was detectable in only two locations from Guanting Reservoir.
- PFOA in sediments from Bohai Bay and Guanting Reservoir were found to be similar, indicating widespread contamination of PFOA throughout the study area.
- PFOA was detected in water from all locations from both Bohai Bay and Guanting Reservoir, which suggests widespread distribution of PFOA.
- Relatively greater PFOS concentrations were observed in water from Bohai Bay than Guanting Reservoir, which could be explained by industrial activities in Bohai Bay and nearby Tianjin.

3. Comparison to Environmental Quality Criteria

- Concentrations of neither PFOS nor PFOA in water samples exceeded concentrations thought to be protective of aquatic life both.
- Overall, the PFCs detected in environmental samples from these areas were relatively low to moderate compared to other studies in Asia and likely represent background globally distributed concentrations of these compounds.

Fig. 4. Sample preparation and extraction sequence (e.g, sediment samples)

Fig. 5. PFCs in Bohai Bay vs. Water Quality Criteria
ABSTRACT

Perfluorinated compounds (PFCs), such as perfluorooctanesulfonate (PFOS) and related compounds, have recently been identified in the environment and have become the subject of increasingly intense environmental research. Despite their detection both in biota and in aqueous media, little attention has been paid to their possible presence in soils. The limited available data indicates that some PFCs such as PFOS and perfluorooctanoic acid (PFOA) may strongly sorb to solids, thus soil may be suspected to be an important sink for PFCs as they are for many other neutral organic compounds. In the present study, the concentrations and distribution of 5 PFCs were quantified in soil samples (n=18) collected from the Beijing and Tianjin regions of China, the latter being the biggest industrialized coastal city of Bohai Bay. Among the PFCs measured, PFOS and PFOA were found to be the most predominant compounds with the greatest concentrations. PFOS and PFOA concentrations in soil ranged from 0.01 to 6.43 ng/g and from 0.12 to 2.77 ng/g, on a dry weight basis, respectively. Other PFCs showed relatively lower concentrations compared to PFOS and PFOA and most were below the detection limits. PFCs concentrations detected in this study were not sufficient to induce ecological or human health effects, however, the present data does provide some insight into the potential sources of PFCs in Chinese industrialized coastal areas. Further studies are needed to elucidate the occurrence, exposure and possible sources of PFCs in different environmental media in these areas.

METHODS and QA/QC

• Samples were collected during August of 2007 and were stored frozen until analysis
• Samples were extracted using a modified Solid Phase Extraction (SPE) method to optimize recovery and minimize contamination
• Recoveries for all 8 compounds were greater than 70% thus concentrations were not corrected
• Negative ES‐HPLC‐MS/MS operated in MRM was used for data analysis
• The use of Teflon related materials were avoided during all steps of sample collection and analysis
• A second column was inserted directly upstream of the HPLC injector port to separate any possible contamination coming from the eluents or instrument

RESULTS

Average Concentration of PFCs Detected in Soil Samples from northeastern China

- Bohai Bay, Tianjin
- Guanting, Beijing

Occurrence & Concentrations of PFCs

• Of the 17 PFCs measured 5 were routinely found about the limit of detection
• PFOS and PFOA were found to be the predominant compounds present in the soils around Bohai Bay and Guanting reservoir respectively
• Concentrations of PFOS in soil were as great as 4.7 ng/g DW (mean=0.88, n=8) and as low as 0.09 ng/g DW
• There is currently some debate as to whether soil and sediment are the ultimate sink for PFCs as they are for many other organic compounds.

CONCLUSIONS

• Soil samples collected from the Bohai Bay region of China do not appear to be heavily contaminated with PFCs
• PFOS was found at concentrations above the limit of detection (0.1 ng/g) only 50% of the time
• The soils around Bohai Bay and Guanting reservoir do not appear to be a substantial sink for PFCs and may suggest that sediment, water, or biota are the ultimate sink
• Concentrations of PFCs found in the soil are not great enough that toxicological effects would be expected
• Overall, the PFC concentrations detected in soil samples from this area were relatively low to moderate when compared to the other few studies that have looked at soil concentrations of these compounds.

ONGOING and FUTURE WORK

Monitoring and Assessment of Exposure and Potential Biological Effects of Perfluorinated Compounds in the Yellow Sea Region of China and Korea

• Find environmental levels of target persistent and toxic contaminants and if appropriate determine loadings and sources
• Determine distribution and source characteristics (and possibly fate, transport, or food web) of target contaminants
• Address potential biological effects associated with samples
• Identify potential toxic chemicals based on the TE and mass balance approach
• Establish background monitoring data for target contaminants and possibly develop the site‐specific environmental quality guidelines
• Prioritize the site‐specific contaminants of concern in the study area

REFERENCES

Abstract

Poor recruitment of white sturgeon Acipenser transmontanus in the Columbia River has been documented since the 1970s. There are many possible causes for this phenomenon, including water pollution (e.g., wastewater metals released by a metallurgical facility and other industrial and municipal facilities). In general, little is known about the potential toxicity of metals such as Cu, Cd, and Zn to white sturgeon and their potential influence on survival of eggs and/or juveniles. The purpose of this study was to establish baseline laboratory toxicity data for the exposure of early life-stages of white sturgeon to Cu, Cd, and Zn that can be used in risk assessments, and, in combination with field experiments conducted in a parallel study (see A. Tompsett et al.; White sturgeon hatch and survival after exposure to Columbia River surface water at two sites in British Columbia, Canada: SETAC), to assess the potential toxicity of these metals in waters of the Columbia River. Embryos, larvae, and fry were exposed to increasing concentrations of dissolved Cu, Cd, and Zn for 65 days using laboratory-based flow-through exposure systems. In addition, 96hr LC50 static toxicity tests were conducted for each metal in order to calculate water effect ratios (WER) between laboratory and flow-through systems (see above). Preliminary results indicate that early life-stages of white sturgeon are more sensitive to Cu and Zn during the first 20 days post hatch compared to Cd which had a greater impact during staged exposure.

Introduction

There is evidence that adult white sturgeons are spawning and depositing viable eggs in certain areas of the Canadian reach of the Columbia River, especially at Waneta, Eddy located just north of the U.S.-Canada border, but only limited numbers of young of the year (YOY) have been found in habitats considered suitable for this life stage (Golder Associates Ltd., 2007). It has been reported, however, that year old juveniles released into the Columbia River as part of a recovery initiative exhibit good survival, growth rates and body condition. Habitat alteration, varying flow regime, poor nutrition, genetic bottlenecks, predation and pollution have all been suggested as possible explanations. Presently, little toxicity data exist characterizing the sensitivity of white sturgeon to metals such as Cu, Cd, Zn.

Objectives

1. Develop a species-specific dose–response relationship for Cu, Cd and Zn that will be used to establish metal toxicity threshold values for white sturgeon.
2. Collect information that will be used along with metal speciation models to predict thresholds for effects of these metals on eggs and larvae of white sturgeon under field conditions.

Methods

• Continuous flow-through exposure systems were designed and used to test 5 different exposure concentrations per metal based upon environmentally relevant concentrations found in the Columbia River and concentrations expected to produce toxic effects (Fig.1):
  - Cu: 0.2 µg/L (ppb) – 260 µg/L
  - Cd: 0.02 µg/L (ppb) – 82 µg/L
  - Zn: 1 µg/L (ppb) – 1000 µg/L

• Fertilized white sturgeon eggs were obtained from the Kootenay Trout Hatchery, Fort Steele, B.C.

• Embryos, larvae and juveniles were exposed for 65 days and the surviving juveniles were euthanized, measured, weighed and fixed in formalin.

• 96hr static renewal LC50 tests were conducted with 6 day old larvae.

• Further morphological analyses are currently being conducted at the University of Saskatchewan Toxicology Centre.

Results

• 100% mortality occurred between hatch and day 10 for the two highest doses of Cu (Fig.2) and the highest dose of Cd and Zn (Fig.3,4).

• Cd 4 (10.24 µg/L) treatment experienced greater mortality near the end of the exposure period (day 40) compared to other treatments.

• 96hr LC50 values for Cu, Cd and Zn were 74.3 µg/L, 15.3 µg/L and 156 µg/L, respectively.

• Water effects ratios (WER) indicate a 4 fold factor for Cd and Zn and a 0.5 fold factor for Cu between Columbia River water and standard laboratory water for early life-stages of white sturgeon (Table 1).

Discussion

• Copper affects sodium regulation across the gills and appears to affect early life-stages of white sturgeon during initial exposure, especially at the higher doses (Fig.2,5).

• Cadmium is known to disrupt calcium uptake but has also been found to bioaccumulate within the kidneys and liver. In the present study, cadmium appears to have a pronounced acute effect at the highest dose at an early stage and a more chronic effect in the second to highest dose towards the end of the exposure period (Fig.3).

• Zinc is an essential nutrient and most fish can tolerate relatively high concentrations.

• A sensitive transition period from yolk sac to exogenous feeding (day 20-35) was discovered within the controls and all treatment groups (except the high metal doses where 100% mortality occurred prior to feeding) that promoted fish mortality (Fig.2,3,4).

• The drastic increase in mortalities across all groups during the transition feeding stage has raised the question of whether it may be more appropriate to test early life-stages of white sturgeon at independent time intervals, excluding this period of time that is characterized by a naturally greater mortality.

• A significant dose-response relationship is apparent in the copper treatment when examining day 1-20, the period prior to the sensitive transition feeding stage (Fig.5).

• Early life stages of white sturgeon appear to be less sensitive to Cd and Zn in Columbia River water compared to standard laboratory water and relatively more sensitive to Cu (Fig.6).

• Complexation of metals with organic materials decreases bioavailability and in turn toxicity to fish and could explain the lower toxicity of Cd and Zn in river water.

• The decrease in Cd toxicity in laboratory water compared to river water is surprising and merits further investigation.

• Environment Canada’s water quality guidelines (WQG) for Cu, Cd and Zn in the Columbia River are displayed in Table 1. The LC50 values for the metals of concern for early life-stages of white sturgeon are well above the set water quality guidelines.

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Hecker et al., 2007. Relative sensitivity of bull trout (Salvelinus confluentus) and rainbow trout (Onchorhyncus mykiss) to acute exposures of cadmium and zinc. Env. Tox. and Chem. Vol 21, pp 67-75.

White sturgeon hatch and survival after exposure to Columbia River surface water at two sites in British Columbia, Canada

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Abstract

The subpopulation of white sturgeon (Acipenser transmontanus) that resides in the Columbia River between the Hugh L. Keenleydale dam in British Columbia, Canada, and the Grand Coulee Dam in Washington state, USA, has suffered nearly 30 consecutive years of poor recruitment. Factors such as altered flow regime due to damming, loss of critical habitat, predation, and pollution have been suggested as causes for the lack of recruitment, but none has been convincingly linked with the disappearance of young-of-the-year sturgeon. In the current study, surface water toxicity up- and downstream of a large metal smelter was examined as possible contributors to the life-stage specific bottleneck in the white sturgeon population. Hatchery fertilized eggs from wild brood stock were exposed to Columbia River surface water from 8 hr to 60 d post-fertilization at two sites, one upstream and one downstream from the smelter effluent outfalls. A filtered city water control group was also examined to characterize any effects of inputs upstream of the study area not related to the smelter. The exposures took place in mobile laboratories outfitted with flow-through exposure chambers that allowed the white sturgeon to be exposed to the river water in real-time, a close representation of the natural exposure scenario. Preliminary data suggests that neither hatch nor survival through 60 d was affected by river water exposure. Evaluation of growth rates and histological endpoints in larvae are ongoing.

Introduction

- Poor recruitment of white sturgeon in the trans-boundary region of the Columbia River
- Adult sturgeon spawn and lay fertilized eggs that successfully hatch
- However, few young-of-the-year have been found in habitats considered suitable for this life stage
- Hatchery-reared juveniles released to the river exhibit good survival and growth
- Effluent inputs from a metal smelter in Trail, BC, Canada have been suggested as a contributor to reproductive failure

Project Objectives

- Expose early life-stages of white sturgeon to Columbia River surface water at 2 sites
- Filtered city water control also evaluated
- Evaluation of hatch, survival, growth, and morphology at each site

Method

- Experiments performed riverside in retrofitted commercial trailers
- Flow-through systems with continuous renewal and recirculation (Figure 3)
- Experiments performed riverside in retrofitted commercial trailers
- Eggs hatched and larvae grown to 60 d post-fertilization
- Dead eggs and larvae collected, counted, and preserved daily
- Biweekly water samples for metal analysis
- Larvae euthanized, weighed, measured, and preserved for subsequent analysis at exposure termination

Results

Survival to Hatch

- Percent hatch was not significantly different between control and downstream treatments
- Lower hatch rates in river water treatments were due to fungal growth
- Hatch rates ranged between 76–82%
- Percent mortality of control larvae was significantly greater than river water treatments at exposure termination

Cumulative Mortality of White Sturgeon Larvae

Days 12-60

- Percent mortality of control larvae was significantly greater than river water treatments at exposure termination
- However, cumulative mortality curves show similar trends across treatments (Figure 5) –except from 28-37 d
- -magnitude of mortality rate greater in controls
- -Period of greatest mortality (28-37 d) coincides with transition to exogenous feeding

Discussion

- River water from downstream of the metal smelter had no adverse effect on survival to 60 d, but outside of the transition period, cumulative mortality curves were similar across treatments.
- Number of mortalities was highly dependent upon initial stocking density and all densities were below ASTM recommendations
- Future studies with white sturgeon should use lower stocking densities
- Number of fish surviving to 60 d post-fertilization did not differ by treatment
- River water from downstream of the metal smelter probably had no adverse effect on survival to 60 d, but more advanced statistical analysis to correct for stocking density is required to confirm this conclusion.

Future Work

- Analysis of survival, growth, and morphology data
- Analysis of water samples for trace metals
- Histological and molecular analysis of selected samples
- Expansion of project in 2009 to includes sites in both Canada and USA.

Acknowledgements

This research was funded by an unrestricted grant from Teck. The authors would like to thank A. Jonas, S. Sedgwick, K. Smyth, E. Higley, J. Naile, and J. Duquette for laboratory assistance. R. Ek and the Kootenay Trout Hatchery provided fertilized white sturgeon eggs and invaluable guidance. We would also like to thank the City of Trail, Selkirk College, USEPA, and Teck (B. D. Duncan and K. Brown).
EFFECTS OF POLYCHLORINATED DIBENZOFURANS ON MINK

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INTRODUCTION

Mink are often predicted to have the greatest potential for adverse effects in multi-species risk calculations for sites with a substantial aquatic habitat where polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and other dioxin-like compounds are the contaminants of concern (COCA). This is because mink:

- are apex carnivores
- consume small quantities of food relative to their body mass
- are among the mammals that are more sensitive to any hydrocarbon receptor (AhR)-mediated effects

Thus, remedial criteria are often derived for mink in situations where risks are predicted to occur due to chemical exposure. It is important that exposure concentrations at which adverse effects are predicted to occur be as accurate as possible to properly protect wildlife and other species from adverse effects due to chemical exposure but also to protect from habitat destruction due to remediation based on misunderstanding of critical effect concentrations.

Considerable toxicological information is available on the effects of PCBs and PCDDs on mink, but limited toxicological information is available for PCDFs. This report compares the toxic effects reported for laboratory and field studies on mink with both mixed and single dioxin-like congener exposures and demonstrates that exposure concentrations at which adverse effects occur cannot be determined reliably for complex mixtures in which PCDFs dominate the total calculated TEQ values, thereby suggesting that the values of the mammalian-specific TEFs suggested by the WHO may overestimate the toxic potency of PCDFs in mink.

RESULTS

Table 1. Estimated average first-order elimination rate constants, based on data from both 90- and 180-d time points, for 2,3,7,8-TCDF and 4-PCDF by dose group. N=6 except where noted.

<table>
<thead>
<tr>
<th>Dose daily TEQ (ng kg⁻¹ d⁻¹)</th>
<th>First order rate constant, d⁻¹ Mean (S.D.)</th>
<th>Estimated half-life, d Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.98</td>
<td>1.6 (0.6)</td>
<td>0.43</td>
</tr>
<tr>
<td>3.8</td>
<td>2.7 (0.7)</td>
<td>0.27</td>
</tr>
<tr>
<td>8.2</td>
<td>4.1 (0.6)</td>
<td>0.17</td>
</tr>
<tr>
<td>2,3,7,8-PCDF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.62</td>
<td>0.086 (0.012)</td>
<td>8.1</td>
</tr>
<tr>
<td>2.2</td>
<td>0.095 (0.008)</td>
<td>7.3</td>
</tr>
<tr>
<td>9.5</td>
<td>0.087 (0.019)</td>
<td>8.0</td>
</tr>
<tr>
<td>Mixture: 2,3,7,8-PCDF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.084 (0.006)</td>
<td>7.4</td>
</tr>
</tbody>
</table>

The apparent disparity between predicted and observed relative potency for 2,3,7,8-TCDF as compared to TCDD- and PCB 126-containing mixtures may be in part due to dissimilar metabolic transformation and elimination.

Table 2. Reproductive outcomes resulting from mink dietary exposure to PCB 126 and 2,3,7,8-TCDF.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
<th>Reproductive outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 126</td>
<td>240 TEQ/kg diet</td>
<td>Complete reproductive failure</td>
</tr>
<tr>
<td>2,3,7,8-TCDF</td>
<td>240 TEQ/kg diet</td>
<td>Whelping rate not different from control (80%)</td>
</tr>
</tbody>
</table>

The most comprehensive comparison of mixture and congener toxic potency was made by comparing all the laboratory dose response relationships between concentrations of TCDD and occurrence of squamous epithelial cell proliferation or jaw lesions. Jaw lesions are a sensitive response of mink to 2,3,7,8-TCDD, PCB 126, and mixtures of dioxin-like compounds.

The environmental mixtures that resulted in jaw lesions had great proportions of non-ortho PCBs, specifically PCB 126 (Table 3). There was no clear relationship between the presence of jaw lesions and the total concentration of TEQs, contributed by PCDD or PCDF, 2,3,7,8-TCDF or non-ortho PCBs. This does not indicate that there was no dose response for these compounds but rather the data set is limited.

CONCLUSION

The results of this study suggest that the values of the mammalian-specific TEFs suggested by the WHO overestimate the toxic potency of PCDFs to mink. Therefore, hazard cannot be accurately predicted by making comparisons to TCDD-derived exposure studies conducted with PCDDs or PCBs in situations where mink are exposed to TEQ mixtures dominated by PCDDs.

ACKNOWLEDGEMENTS

Funding for the field study described herein was provided through an unrestricted grant from The Dow Chemical Company to Michigan State University. The laboratory animal exposure to 2,3,7,8-TCDF was funded in part by a grant from the Michigan Great Lakes Protection Fund. The toxicokinetic study was funded and supported by The Dow Chemical Company.

REFERENCES

A comparison of methods for estimating wildlife dietary exposure concentration using measured dietary items

Shaun A. Roark1, Denise P. Kay1, John L. Newsted1, Matthew J. Zwiernik2, and John P. Giesy1,3

ABSTRACT

In ecological risk assessment, US EPA guidance recommends characterizing exposure with measures of central tendency (CT) and reasonable maximum (RM). However, the choice of parameter to represent these measures often depends on specific guidance and on characteristics of the data. To address this issue, methods were compared for estimating parameters to describe dietary concentration based on measured concentrations of dioxin and furan TEQs (WHO 2000) in dietary items for mink on the Tributaries River (Michigan, USA). The first approach was to estimate each parameter (median, mean, 95th percentile) independently for each category of dietary item. The second approach was similar to the first, but data were log-transformed. The third approach was to bootstrap sample data from the log-normal distribution. The fourth approach, a modification of the third, was to fit a distribution to the data in each dietary category and use transformed data for each category. The approach was to calculate summary statistics (mean, median, etc.) for each distribution output. The approach was repeated 10,000 times, and a distribution of potential dietary concentrations was generated. Results from each approach were compared as follows:• Use of a resampling procedure (Approach 3) avoids assumptions about the distribution of the data, and therefore may provide the best characterization dietary concentration based on measured molecular concentrations in dietary items. • Use of unbounded Monte Carlo procedures (Approach 4) can overestimate the maximum but the 95th centile is representative of reasonable maximum. • Use of unbounded distribution has potential for a large overestimate – although this did not occur here with 10,000 randomly drawn samples from each fitted distribution. DISCUSSION

To estimate exposure using measured concentrations in dietary items, the choice of the best approach to describe CT and RM exposure can be unclear, yet may influence conclusions regarding risk. It is difficult to accurately characterize variation in the dietary concentration estimate based on summary statistics for each dietary category (Approaches 1 & 2). The use of a resampling procedure (Approach 3) avoids assumptions about the distribution of the data, and therefore may provide the best characterization dietary concentration based on measured molecular concentrations in dietary items. Use of unbounded Monte Carlo procedures (Approach 4) can overestimate the maximum but the 95th centile is representative of reasonable maximum. Use of unbounded distribution has potential for a large overestimate – although this did not occur here with 10,000 randomly drawn samples from each fitted distribution. QUESTIONS FOR FURTHER STUDY

Concentrations for fish used here were based on composite samples – would the use of individual samples change the results?

The data set used here is robust – what is the effect of reduced sample size on accuracy and uncertainty of these approaches?

Is the gain in apparent accuracy of the resampling approach outweighed by the uncertainty in other parts of the model (e.g., dietary proportions, groupings of species in dietary categories)?

REFERENCES


ACKNOWLEDGEMENTS

This research is the collaborative effort of the Michigan/Indiana team, which includes the Michigan State University Feline Environmental Research Team, the US Environmental Protection Agency’s Great Lakes National Program Office (U.S. EPA-1995 Unit) and the Michigan Department of Environmental Quality (MDEQ) for their support and assistance. This work was partially supported by a National Science Foundation Research Grant (0622405).
An evaluation of 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in tissues of wild game from the floodplains of the Tittabawassee and Saginaw Rivers (MI, USA)

Ryan R. Holem1, John J. Matousek1, Patrick W. Bradley1, John L. Newsted1, Denise P. Kay1, Alan L. Blankenship1,2, Shaun A. Roark1, Melissa S. Shotwell1, and John P. Giesy3

Abstract
The Tittabawassee River is located in central Michigan and flows southeast through Midland and into the Saginaw River and eventually to the Saginaw Bay of Lake Huron. Precious studies have reported elevated soil, sediment, and fish concentrations of dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) downstream of Midland. In addition, elevated polychlorinated biphenyls (PCB) concentrations have been found in soils, sediments, and fish from the Saginaw River. It was suspected that wild game residing in this area may contain detectable concentrations of these contaminants. To evaluate this, white-tailed deer, wild turkey, fox squirrel, cottontail rabbit, Canada goose, and wood duck were collected from several locations along the Tittabawassee and Saginaw Rivers (Figure 1). Edible tissues from these animals were analyzed for PCDDs and PCDFs, and some were also analyzed for dioxin-like PCBs. Results based on concentrations of 237 polychlorinated dibenzo-p-dioxin equivalents (2006 WHO TEs) have been summarized and are reported in this paper.

Methods
• Wild game species commonly pursued by hunters can be found throughout the Tittabawassee and Saginaw River floodplains
• The degree of exposure of floodplain residing wild game to PCDDs, PCDFs, and PCBs had not been evaluated

Results
• Overall, the least TEQ concentrations (range of means: 0.1–0.9 ng/kg ww D/F TEQ, ND=1/2 DL) were observed in muscle tissue from deer, rabbit, and squirrel
• TEQ concentrations were greatest in livers of white-tailed deer and skin-on tissues of wild turkey and wood duck (range of means: 0.2 – 43 ng/kg ww D/F TEQ, ND=1/2 DL)
• In birds, TEQ concentrations in skin-off samples were less than in skin-on samples (Figure 3)
• The greatest TEQ concentrations were observed in tissues of animals from the central sampling location, IPA (Figure 2, 4)
• D/F TEQ values for deer muscle and liver, squirrel, and turkey were comparable (mean TEQ values ≤0.5 ng TEQ/kg, ND=1/2 DL) in animals from the upstream reference (SF) and downstream (CISGA) sampling locations (Figure 2, 4)
• PCB contribution to total TEQ was minimal with the exception of wood duck tissues in which PCB contribution to total TEQ ranged from 30-50% (Figure 3, ND=0)

Discussion & Conclusions
• Average total TEQ concentrations in wild game tissues were less than average total TEQ in fillets of many species of fish collected from the Tittabawassee and Saginaw Rivers
• Wood ducks and wild turkeys commonly feed on invertebrates, which likely increases exposure to PCDDs, PCDFs, and PCBs through ingestion of sediment/solls
• Greater concentrations observed in skin-on bird tissues compared to skin-off due to the high lipid content of skin
• Greater concentrations observed in skin-on bird tissues compared to skin-off due to the high lipid content of skin
• Animals were collected from locations upstream and downstream of Midland (Figure 1) by use of traps and firearms in 2003 and 2007
• Whole animals were removed from collection locations (i.e., entrails removed at laboratory) to prevent contamination from other media such as floodplain soils
• Tissues commonly consumed by humans were removed from each animal and freeze-fractured (i.e., cryogenic homogenization)
• Tissue samples were analyzed for 17 PCDD/PCDFs; some also for 12 dioxin-like PCBs
• Tissue samples were analyzed for 17 PCDD/PCDFs; some also for 12 dioxin-like PCBs
• Tissue samples were analyzed for 17 PCDD/PCDFs; some also for 12 dioxin-like PCBs

References

Acknowledgements
This work was funded by The Dow Chemical Company

Figure 1. Sampling locations along the Tittabawassee and Saginaw Rivers.
Effects of TCDD, Arochlor 1254, and PeCDF Injected Into the Air Cell of Japanese Quail (Coturnix japonica) Prior to Incubation


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Abstract

Amino acid substitutions in the ligand-binding domain of the aryl hydrocarbon receptor (AhR) have been proposed to determine the molecular basis for differential sensitivity of birds to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-like compounds (Farman et al., MP3). The results of recent studies have suggested that birds can be classified into one of three TCDD-sensitivity categories: very sensitive (chicken), moderately sensitive (ringneck pheasant), and sensitive (Japanese quail). A series of egg injection studies have been conducted to confirm the proposed avian sensitivity classification. The effect of TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and 2,3,4,7,8-pentachlorodibenzo-p-dioxin (PeCDF) on hatchability of Japanese quail eggs and growth and survival of hatchlings is reported here. Doses ranging from 0.07 to 91 ng/g were injected into the air cell prior to incubation. Hatchlings were maintained for 12 d to assess growth and survivability. A sample of the chicks was weighed, euthanized, and necropsied conducted on the day of hatching and at 12-d of age. Selected tissues from 12-d-old chicks were removed, weighed, and processed for histological assessment. Subsamples of liver were processed for determination of concentrations of the target compounds as well as induction of cytochrome P450 enzymes. LD-50 values based on hatchability data were determined to be 11.25 (0 – 20), 95% confidence intervals, 3.11 (2.02 – 5.75) and 1.13 (0.27 – 1.53) ng/g egg, for TCDD, TCDF, and PeCDF, respectively. The relative potencies (RePs) of TCDF and PeCDF compared to TCDD were 3.4 and 13.3, respectively. The ReP of PeCDF based on hatchability of Japanese quail eggs is similar to a ReP value of 12-30 based on induction of CYP1A activity in cultured Japanese quail hepatocytes determined in a complimentary study. To our knowledge, this is the first study indicating that a TCDD-like compound is substantially more toxic to birds than TCDD. It would be of interest to determine if dietary exposure of Japanese quail to TCDD-like compounds results in similar ReP values as determined in the in vitro induction and egg injection studies.

Methods

- Injection of 0.1 µg/g egg into the air cell of Japanese quail eggs with:
  - Trisulfine
  - TCDD: 11 doses (0.072 to 91 ng/g)
  - PeCDF: 10 doses (0.64 to 16 ng/g)
  - TCDF: 10 doses (0.128 to 9.42 ng/g)

- Eggs incubated for 19 d (23°C, 32% humidity)

- Unhatched eggs (if any) opened to determine:
  - Age of embryo at death
  - Presence and type of deformities

- Hatchlings transferred to battery

- 12-d-old chicks

- Weighted

- Tissues removed and weighed

- Liver:
  - CYP1A4 and CYP1A5 mRNA abundance (Wiseman et al., WP229)
  - Brain, heart, bursa and spleen

Results

- TCDD Embryos Mortality

- Cranial Deformities

- Bill Deformities

- Trunk Deformities

- Limb Deformities

- There were no consistent changes in body mass or relative organ mass in TCDD-, PeCDF- and TCDF-exposed quail when compared to vehicle control

Conclusions

- The relative potencies (RePs) of TCDF and PeCDF compared to TCDD were 3.4 and 13.3, respectively

- The ReP of PeCDF based on hatchability of Japanese quail eggs is similar to a ReP value of 12-30 based on induction of CYP1A activity in cultured Japanese quail hepatocytes determined in a complimentary study (Herve et al., MP33)

- To our knowledge, this is the first in vivo study indicating that a TCDD-like compound is substantially more toxic in an avian species than TCDD

Funding for this project was provided through a non-restricted grant from The Dow Chemical Co., Midland, MI.

Poster Number: WP210
Multiple Lines of Evidence Risk Assessment of Great Horned Owls (Bubo virginianus) exposed to PCDF/DDs in Midland, MI

Coefield, S.J. 1, Zwierink, M.J. 1, Fredricks, T.B. 1, Seston, R.M. 1, Nadeau, M.W. 1, Tazelaar, D.L. 1, Moore, J.N. 1, Shotwell, M.S. 2, Kay, D.P. 3, Glesy, J.P. 1, 3
1. Michigan State University, East Lansing, MI, USA 2. ENTRIX, Inc. East Lansing, MI, USA 3. Veterinary Biomedical Sciences & Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada.

INTRODUCTION

The Tittabawassee River floodplain and watershed in Midland, MI are contaminated with polychlorinated dibenzo-p-dioxins and dioxin-like compounds (PCDDs and PCDFs). The main sources of contamination are the Dow Chemical Company and the Midland Power Plant. The primary objective of the research team was to study the risk of this contamination to wildlife, specifically Great Horned Owls (GHO). To assess this risk, the team undertook a multiple lines of evidence (MLE) approach, which involves the use of a combination of different types of data and evidence to assess environmental contamination and its impact on wildlife. In this study, GHO dietary composition was assessed through diet analysis, and contaminants were measured in plasma samples of nestling GHOs. The results of this study are expected to contribute to our understanding of the environmental health risk to great horned owls from dioxin-like compounds in the Tittabawassee River floodplain.

RESULTS AND DISCUSSION:

```
1Dietary Exposure

The average daily intake (ADI) of contaminants was calculated as the product of the total concentration of a given compound in the diet and the dry matter content of the diet, divided by the body weight of the GHO (the ADI was calculated as: ADI = (C*DM/BD)/BW, where C is the concentration of the contaminant in the diet, DM is the dry matter content of the diet, BD is the body weight of the GHO, and BW is the body weight of the GHO).

2Tissue-based Exposure

The concentrations of PCDD/DDs in the GHO's tissues were measured using the method of measurement plasma samples were extracted by the method of measurement (2,3,7,8-TCDD = 7.8 ng/g, 2,3,7,8-PCDF = 0.6 ng/g).

3Productivity and Abundance

The productivity of adult GHOs in the study area was significantly greater in the downstream area downstream of The Dow Chemical Co. (0.31 responses/river km) compared to the upstream area (0.11 responses/river km). This suggests that the downstream area may be more suitable for GHO productivity.

4Discussion

The results of this study indicate that the GHOs in the Tittabawassee River floodplain area are exposed to dioxin-like compounds, which may pose a risk to their health and survival.

REFERENCES:


ACKNOWLEDGMENTS

Funding was provided through an unrestricted grant to The Dow Chemical Company to Michigan State University.
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Abstract

Ethoxyresorufin-O-deethylase (EROD) and methoxyresorufin-O-dealkylase (MROD) activity was assayed in several avian species nesting in the Tittabawassee River and Chippewa River floodplains near Midland, Michigan, to examine the exposure and potential effects of polychlorinated dibenzofurans (PCDFs) and dibenzofuran (PCDDs). Concentrations of PCDD/PCDFs in biota have been found to be 10- to 20-fold greater downstream (study areas) of Midland when compared to upstream (reference areas) but the toxicological significance of these differences relative to avian population health downstream of Midland is still being investigated. Tree swallow (TS), eastern bluebird (EB), house wren (HW), and belted kingfisher (BK) were chosen as species of interest. In this study, maximum EROD and MROD activity was assayed utilizing a kinetic assay in liver tissue collected from nestlings prior to fledging. Seventeen 2,3,7,8-substituted PCDF and PCDD congeners were measured in whole body homogeneous and converted to TCDD using WHOeydm TEF values. EROD and MROD activities (μmol product/mg microsomal protein/min) were greater at downstream study areas and varied by species (EROD: EB>KF>TS>HW; MROD: EB>BF>KF>HW=TS). Avian TCDDs at study areas were similar for EB, BF, and HW while TS had slightly greater nesting whole body accumulation. The study areas congeners profiles for whole body nesting homogenates were dominated by 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF (80-90%), but varied with species by the EB profile dominated by PeCDD, Ts profile dominated by TCDF and the KF and HW profile were a mixture of PeCDD and TCDF. Enzyme induction levels reported here, primarily from fumon exposure, are greater than or equal to previously reported induction levels for TS exposed to similar TCDD-dominant TEQ levels. Despite greater enzyme activity and WHOeydm TCDDs at study areas compared to reference areas overall productivity measurements are comparable between sites.

Introduction

Next box trail established on both study and reference areas in the Tittabawassee River floodplain south of Midland, MI, USA (Figure 1) in 2004 and has since been consistently monitored. Study areas included 3 passerine species: TREE SWALLOW, TS; HOUSE WREN, HW; EASTERN BLUEBIRD, EB; and the BELTED KINGFISHER (BK).

Objectives

- Quantify both enzyme activity and residue concentrations in field collected samples from the Tittabawassee floodplains for several avian species.
- Compare induction levels between these primarily fumon exposed birds and existing literature values.

Results

EROD and MROD maximum induction was 5- to 4-fold greater in study areas for belted kingfisher compared to reference areas (Figure 3).

Discussion

Conclusions

- Based on these results fumon induction of CYP1A activity is greater for these species than previously reported for both TCDD and PCDF field based exposure.

- TEQs and EROD/MROD positively correlated for all species studied.

- Further studies with additional avian species are needed to compare against present maternal induction and total TCDDs (14).

- Incorporation of data from overall comparison of labs to field studies should prove extremely interesting.
Exposure of American robins (Turdus migratorius) to PCDF and PCDD on the Tittabawassee River floodplain, MI, USA.

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1Michigan State University, 2Department of Zoology / 3Department of Animal Science / National Food Safety and Toxicology Center, East Lansing, MI, USA; 4Entrix, Inc., East Lansing, MI, USA; 5Department of Biomedical Veterinary Sciences and Toxicology Centre University of Saskatchewan, Saskatoon, Saskatchewan, Canada

ABSTRACT

Polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzodioxin (PCDD) concentrations in the tissues of receptor species are important assessment endpoints in evaluations of ecological risk. During the spring and summer of 2005, 2006 and 2007, American robin eggs, 30 nestlings and 12 adults were collected from the Tittabawassee River floodplain from upstream reference sites and study sites downstream of the city of Midland, MI, USA. Previous studies have indicated that study sites had concentrations of PCDF and PCDD that were greater than in nearby reference areas. Concentrations of the 17 2,3,7,8-substituted PCDDs and PCDFs were quantified in American robin tissues and normalized to 2,3,7,8-dibenzo-p-dioxin using WHO avian 1998 TEFs. Preliminary American robin egg TEQs ranged from 2.4 ng/kg ww to 1.5 x 10^4 ng/kg ww in reference areas and 2.5 x 10^4 ng/kg ww to 1.7 x 10^5 ng/kg ww wet weight in study areas, while preliminary nestling tissue TEQs ranged from 1.0 ng/kg ww to 2.1 x 10^4 ng/kg ww in reference areas and 4.7 x 10^4 ng/kg ww to 5.6 x 10^5 ng/kg ww in study areas.

METHODS AND MATERIALS

• American robin tissues were collected in 2005, 2006, 2007 and 2008 from nests located within the floodplains of target and reference areas of the Tittabawassee River floodplain
• Fresh egg samples were collected randomly prior to or during incubation
• Added egg samples were collected opportunistically following hatch date or nest failure
• Egg sample TEQ concentrations based on calculated fresh mass minus the mass of shell (Hoyt, 1978)
• Nestling samples were collected approximately 12 d following hatch date
• Nestling samples homogenized following removal of feathers, bill and legs below the tibiotarsus
• Soil samples and dietary samples collected from the Tittabawassee River floodplain 2003-2006
• Concentrations of TEQ in soil are expressed as ng/kg on a dry weight basis and ng/kg wet weight for tissues
• Chemical extraction EPA methods 3540C and 3541
• Analyses of the 17 2,3,7,8-substituted PCDF/D congeners concentrations in samples are conducted at AgriQuality Limited (Lower Hurt, New Zealand) using EPA method 8290
• All TEQ values based on avian World Health Organization toxicity equivalency factors (Van den Berg et al., 1998)
• The TEQ concentrations are calculated by assigning a proxy value of ½ the detection limit (DL) for congeners below the DL

RESULTS

Figure 1. Map of sampling locations in the Tittabawassee River floodplain in Michigan, USA.

Table 1. Concentrations of TEQ-avian (ng/kg ww) measured in diet items of the Tittabawassee River and composition.

<table>
<thead>
<tr>
<th>Dietary Item</th>
<th>Ref. Item</th>
<th>Ref. max</th>
<th>Study Item</th>
<th>Study max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td>0.6</td>
<td>1.8</td>
<td>2.7</td>
<td>13</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>3.3</td>
<td>16</td>
<td>410</td>
<td>1900</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>1.0</td>
<td>1.5</td>
<td>42</td>
<td>98</td>
</tr>
<tr>
<td>Misc.</td>
<td>1.2</td>
<td>4.5</td>
<td>23</td>
<td>380</td>
</tr>
<tr>
<td>Earthworm</td>
<td>1.4</td>
<td>2.4</td>
<td>220</td>
<td>530</td>
</tr>
</tbody>
</table>

Figure 2. Congener contribution of avian TEQs for American robin tissues in the Tittabawassee River floodplain.

Table 2. Concentrations of TEQ-avian (ng/kg dw) measured in surface soil of the Tittabawassee River floodplain.

<table>
<thead>
<tr>
<th>Reference N</th>
<th>Study N</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>27</td>
<td>6.7</td>
<td>4478</td>
</tr>
<tr>
<td>6</td>
<td>425</td>
<td>3.95</td>
<td>11800</td>
</tr>
</tbody>
</table>

Table 3. Concentrations of TEQ-avian (ng/kg ww) in American robin egg and nestlings collected in the Tittabawassee River floodplain.

<table>
<thead>
<tr>
<th>Reference Area</th>
<th>Study Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median 95% UCL</td>
<td>Median 95% UCL</td>
</tr>
<tr>
<td>Egg</td>
<td>Nestling</td>
</tr>
<tr>
<td>7.2 (16)</td>
<td>183 (357)</td>
</tr>
<tr>
<td>9.2 (8)</td>
<td>157 (253)</td>
</tr>
</tbody>
</table>

ACKNOWLEDGMENTS

We would like to thank all field/laboratory personnel that helped with this project, especially the following: Jeremy Moore, Michael Nadeau, Anna Boegelhoid, Michelle Hodges, Clay Manutz, Cheslie Groberman, Nathan Hubbard, Melanie Collins, William Folland, Megan Barker, Michael Szor, Aranne Neigh, Karl Strouse, Breton and Carrie Joldensena, Cynus Park, Mike Fales, Megan Mikessell, Ben Nessia, Jinchan Ge, Lam Wong, Mick Kramer, Patrick Bradley, Nozomi Ikeda, Emily Koppel, Melissa Palmer, Cassie Stieler, Bethany Opperman, William Sterling, Lucy Sharrard, Sandy Mazzoni, and Kelly Winchell. Additionally, this study would not have been possible without the dedicated team of the employees at Entrix, Inc., East Lansing, MI and wonderful support staff at Michigan State University. Funding was provided through an unrestricted grant from The Dow Chemical Company to Michigan State University.

REFERENCES


CONCLUSIONS

• Median soil TEQ concentrations are more than 600 times greater in study locations than in reference locations.
• Median invertebrate diet TEQ concentrations are more than 1 order of magnitude greater in study areas than in reference areas.
• 2,3,4,7,8-PeCDF, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD and 2,3,7,8-TCDD account for more than 90% of the TEQs in American robin tissues in both reference and study area
• 2,3,4,7,8-PeCDF predominates the congener profiles of both eggs and nestlings collected from the study area
• 1,2,3,7,8-PeCDD and 2,3,7,8-TCDD account for the greatest percentage of reference egg congener profiles while nestling reference profiles are predominately 2,3,7,8-TCDF.
• American median robin egg TEQ concentrations are more than 25 times greater in study locations than in reference locations.
• American median American robin nestling TEQ concentrations are more than 40 times greater in study locations than in reference locations.
• American robin tissue TEQ concentrations comparable to those in tree swallow (Tachycineta bicolor) tissues where hatch success was negatively associated with concentrations of 2,3,7,8-TCDD in eggs of the Woonasquatucket River, Rhode Island, USA.

TEQ concentrations in diet items are greater in study areas than in reference areas. Median TEQ concentrations in reference areas are as great as 3.3 ng/kg ww while study area median TEQ concentrations are as great as 4.0 ng/kg ww relative to the same taxonomic order, Coleoptera, or beetles, than in reference areas. Concentrations measured in reference area soils are as little as 4.0 ng/kg dw while study location soils exhibit concentrations as great as 1.9 x 10^4 ng/kg dw (Table 2). Conger profiles vary between reference and study areas with 2,3,7,8-PeCDF contributing approximately 80% to the total TEQs in eggs and 70% in nestlings in study areas, whereas 2,3,4,7,8-PeCDF contributes less than 20% to the total TEQs in eggs and less than 10% in nestlings in reference areas (Figure 2). Median TEQ concentrations in American robin tissues are greater in study areas than in reference areas. Median TEQs are as little as 3.6 ng/kg ww in reference nestlings and as great as 1.8 x 10^4 ng/kg ww in study area eggs (Table 3).
Impact of TCDD, PeCDF and TCDF Exposure on Hepatic Cyp1A4 Transcript Abundance in Japanese Quail and Ring-Necked Pheasant in Ovo

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Results 1: Impact of DLCs on Cyp1A4 Transcript Abundance

Japanese Quail

Ring-Necked Pheasant

Results 2: Regression Analysis of DLC Impact on Cyp1A4 Transcript Abundance

Japanese Quail

Ring-Necked Pheasant

Results 3: LOEC and Potency Values for Cyp1A4 Induction

Japanese Quail

Ring-Necked Pheasant

Legend:

LOEC Values:

- TCDD
- PeCDF
- TCDF

Potency Values:

- Relative potencies of TCDD, PeCDF and TCDF based on Cyp1A4 induction

Methods

App Injection

Assay

Figure 1: Effect of A) TCDD, B) PeCDF, and C) TCDF on Cyp1A4 transcript abundance in Japanese Quail and Ring-Necked Pheasant. Significant changes (denoted by *) in transcript abundance (P < 0.1, Mann Whitney U test) relative to the control were observed within each DLC treatment group. Data is shown as percent control, however statistical analysis was performed on mean normalized expression values where j actin was used as the reference gene. Concentrations of DLCs are represented as administered doses calculated from nominal concentrations of prepared injection solutions.

Figure 2: Linear regression analysis of changes in Cyp1A4 transcript abundance in response to A) TCDD, B) PeCDF, C) TCDF in Japanese Quail and Ring-Necked Pheasant. 95% Confidence intervals were plotted to the regression line. The point on the x-axis where the lower confidence interval transects the axis was estimated as the Lowest Observed Effective Concentration (LOEC) of chemical that induced Cyp1A4 transcript. LOEC values are represented in figure 3A. Concentrations of DLCs are administered doses calculated from nominal concentrations of prepared injection solutions.

Figure 3: (A) In Ovo LOEC values for TCDD, PeCDF and TCDF based on Cyp1A4 induction as determined by linear regression analysis (Figure 2). (B) Relative potencies of TCDD, PeCDF and TCDF in Japanese Quail and Ring-Necked Pheasant.

Results and Conclusions

Intra-species Comparisons
- Ranking order of Cyp1A4 LOEC Values:
  - Japanese Quail: TCDD > PeCDF > TCDF
  - Ring-Necked Pheasant: PeCDF > TCDD > TCDF
- Ring-Necked Pheasant: PeCDF is 15x more potent as an inducer of Cyp1A4 than TCDD.

Findings from Ring-Necked Pheasant, but not Japanese Quail, are in accordance with Herve et al. (Poster #MP33), who show that PeCDF is a more potent inducer of EROD than TCDD in vitro.

Interspecies Comparisons
- Assuming Cyp1A4 transcript abundance can be used as an indicator of sensitivity to DLCs, our results suggest that Ring-Necked Pheasant is more sensitive to each chemical than Japanese Quail.
- Based on relative potencies PeCDF is approximately 18x more potent as an inducer of Cyp1A4 in Ring-Necked Pheasant than in Japanese Quail.

Ongoing Research and Future Directions

A complementary study is currently being performed in Chicken. Cyp1A4 transcript abundance will be assayed in samples from this study. Analysis of Cyp1A4 transcript abundance and EROD activity is being conducted for each species. Tissue levels of TCDD, PeCDF and TCDF will be quantified in all species. This data will be utilized when preparing data for manuscript preparation.

The utility of the mRNA approach as an indicator of species sensitivity and DLC impact will be assessed in wild birds from contaminated sites (See Fredricks, poster # WP222).

Related Posters

Readers interested in this research are encouraged to view posters WP210 (Cohen-Brownhouse), WP33 (Herve et al.), WP222 (Fredricks et al.), and MP36 (Parnakh et al.).

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