From Molecular Mechanisms of Action to Ecological Risk Assessment:

The Aryl Hydrocarbon Receptor - Birds

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Environment Canada
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Aryl hydrocarbon receptor (AHR)

- Ligand-activated transcription factor
- Activated by naturally-occurring and xenobiotic ligands
  - Dioxin-like compounds (DLCs): polychlorinated dibenzo-p-dioxins (PCDDs) and furans (PCDFs), and some PCBs
- Regulates expression of several genes (e.g. cytochrome P4501A; CYP1A)
- Mediates most, if not all, **adverse outcomes** of DLCs
Adverse Outcomes Mediated by Activation of AHR1 in Birds

- Liver toxicity
- Cardiovascular toxicity
- Embryonic deformities (bills, limbs)
- Immuno-suppression
- Edema
- Porphyria
- Embryo-lethality
Relative Sensitivity (ReS) to TCDD Embryotoxicity

**Chicken**
- Most Sensitive
  - LD50 = 0.21 ug/kg

**Pheasant**
- LD50 = 1.2 ug/kg

**J. Quail**
- Least Sensitive
  - LD50 = 9.7 ug/kg

*Cohen-Barnhouse et al. (2011) Tox. Sci. 119: 93-103*
The Question Chickens Ask:

Why are we so sensitive to dioxin?
Large Differences in Sensitivity to Dioxins, Furans and PCBs – The Role of AHR1
The Avian AHR1 Genotyping Project

Goal:

• Develop methods to determine the sensitivity of any avian species to the toxic and molecular effects of any DLC that are based upon:
  
  – subtle differences in the AHR1 among species
DLCs We Have Studied to Date

TCDD

PeCDF

TCDF

HCB

PCB 126

PCB 77

PCB 105

PCB 118
For More Details

• Gillian Manning
  – PCBs (77, 126, 105, 118) and TCDD
  – Relative potencies in all known AHR1 constructs
  – More detail on concentration-response curves
  – Wednesday, 1:55 PM; Room 208

• Sean Kennedy
  – More details & hexachlorobenzene ‘story’
  – Thursday, 3:10 PM; Room 207
Amino Acids at Sites 324 and 380 Avian AHR1 are IMPORTANT

Karchner et al. (2006) PNAS 103: 6252 – 6257
Head et al. (2008) ES&T 42: 7535 – 7541
Farmahin et al. Submitted for Publication
Avian AHR1 Receptors – The LBD

- Homology Model
- Template – Holo X-ray structures of HIF-2α PAS B domain co-crystallized with ligands (Motto et al. In press)
The Chicken AHR1 Receptor

Chicken AHR1

Ile 324

Ser 380
The Chicken AHR1 Receptor
ring-necked pheasant HAH R1
Luciferase Reporter Gene (LRG) Assay

- AHR gene
- ARNT gene
- CYP1A5 reporter gene (firefly luciferase)
- Control plasmid (*Renilla* luciferase)

**Procedure**

1. **DLC**
2. Firefly luciferase reagent
3. **Read**
4. **Dose**
5. Transfection
6. **Seed**

**Reactions**

- **Beetle Luciferin**
  - **Oxyluciferin**
  - $\text{Beetle Luciferin} + \text{ATP} + \text{O}_2 \rightarrow \text{Oxyluciferin}$
  - **Recombinant Firefly Luciferase**
  - $\text{Recombinant Firefly Luciferase} + \text{Mg}^{2+} \rightarrow \text{Oxyluciferin}$

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**Canada**
Relative Sensitivity (ReS) Calculations

- ReS refers to *inter-species* comparisons
- The ReS of all AHR1 constructs were compared to the sensitivity of the *chicken AHR1 construct*

*For example:*

\[
\frac{\text{Chicken TCDD EC}_{50}}{\text{Quail TCDD EC}_{50}} = \frac{0.21 \text{ nM}}{21 \text{ nM}}
\]

Quail ReS = 0.01
Relative Sensitivity (ReS) Values

![Graphs showing relative sensitivity values for TCDD, PeCDF, TCDF, and PCB-126 in Chicken, Pheasant, and Quail.](image-url)
Relative Potency (ReP) Calculations

• ReP refers to *inter-compound* comparisons
• Because the **maximum responses** (Y-axis) and **slopes** were not always identical, the approach used by Villeneuve *et al.* was used to determine ReP values

\[
\text{Chicken TCDD EC}_{50} \div \text{Chicken PeCDF } Y_{50}
\]

\[
0.21 \text{ nM} \div 0.20 \text{ nM}
\]

PeCDF ReP = 1.0
Relative Potency (ReP) Values

Chicken

Pheasant

Quail
Site-Directed mutagenesis
The AHR1 LBD in 75 Avian Species

- There are amino acid differences at 6 sites (#324, #380 and 4 other sites), but only 324 and 380 are important

- **Type 1** (chicken-like)
  4/75 species (5%)
  TCDD potency = PeCDF potency

- **Type 2** (pheasant-like)
  39/75 species (52%)
  PeCDF potency > TCDD potency

- **Type 3** (Japanese quail-like)
  32/75 species (43%)
  PeCDF potency > TCDD potency
Egg Injection & LRG Assay Comparison
(Using all known egg injection LD50 data)
Binding Affinity to Chicken AHR1

[Graph showing the binding affinity of TCDD, PeCDF, and TCDF with varying concentrations of cold ligand (nM).]
Chicken AHR1-TCDD

Ile 324

Ser 380
Chicken AHR1-PeCDF

Ile 324

Ser 380
Will this Research be Useful for Ecological Risk Assessments?

YES

- Knowing the sensitivity of wild avian species to DLCs could be useful in many locations (e.g., U.S. Superfund sites)
- For PCDD/Fs and ‘dioxin-like’ PCBs, our data suggest that there are likely only 3 major genotypes of birds:
  - Chicken-like (Type 1)
  - Pheasant-like (Type 2)
  - Japanese quail-like (Type 3)
- The genotype can be determined for any avian species using tissue, blood and, probably, eggshell samples
The ‘Adverse Outcome Pathway’ Paradigm for Ecotoxicology – A Personal View

• Of use for multiple reasons
  – Scientifically sound – incorporates the best ideas and methods of modern toxicology
  – The AOP paradigm and methods are stimulating to the next generation of eco-toxicologists

• But:
  – Ensure that one’s research allows time to focus on a few pathways (DNA microarray data overload!)
  – Beware of too much information that one cannot assimilate and understand (DNA microarray data overload!)
Co-Authors

- **Reza Farmahin** – PhD student – dioxins/furans/PCBs
- **Gillian Manning** – MSc student - PCBs
- Doug Crump
- Stephanie Jones
- Lukas Mundy
- Steven Bursian
- Timothy Fredricks
- John Giesy
- Matthew Zwiernik
- Sibel Karchner
- Mark Hahn
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• US Environmental Protection Agency
• Environment Canada
• Various grants to students and professors at:
  – University of Ottawa
  – Michigan State University
  – University of Saskatchewan
  – Woods Hole Oceanographic Institution
Homology Modeling

- Chicken AHR1 LBD (residues 235-402) was searched using PSI-BLAST against the protein data bank (PDB)

- the most significant alignments to PAS domain structures of AHR LBD were within the C-terminal PAS domain of HIF2α

- Holo X-ray structures (co-crystallized with THS ligands; PDB ID: 3F1O, 3H7W, 3H82) of the HIF-2α PAS B domain were used as templates similar to that used by Motto et al.¹

- the model for chicken AHR1 was generated using Easy Modeller version 2.1²


The ProSA z-scores values were -4.07, within the range of values typical for native protein structures of similar size.
Model validation

- PROCHECK
  - 91.5% of residues reside in the most favoured regions
    - a good quality model would be expected to have over 90% in the most favoured regions
Avian TEFs - Reloaded

- TCDD is not the most toxic DLC in all avian species

- PeCDF is more potent than TCDD in pheasant and quail

- PeCDF is likely more potent than TCDD in all ‘Type 3’ wild avian species

Possible implications to avian TEF values
AHR1 Activation → Adverse Outcomes

- Dioxin-Like Compound (DLC)

Aryl hydrocarbon receptor (AHR1)

CYP1A4

CYP1A5

Aryl hydrocarbon receptor nuclear translocator (ARNT)

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