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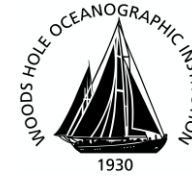
The AHR Ligand Binding Domain - Role in Predicting Sensitivity of Avian Species to Dioxin-like Compounds

Sean W. Kennedy

**National Wildlife Research Centre (NWRC)
Environment Canada**

***SETAC North America 30th Annual Meeting
New Orleans, LA, November 19 – 23, 2009***





- **Environment Canada & University of Ottawa**
 - Sean Kennedy, Reza Farmahin, Jessica Hervé, Jessica Head, Lukas Mundy, Doug Crump, Stephanie Jones, Dongmei Wu, Brian Collins
- **Michigan State University**
 - Matthew Zwiernik, Steven Bursian, Andrew Cohen-Barnhouse, Tim Fredericks, Jane Link, Rita Seston, Sarah Coefield, Dustin Tazelaar, Patrick Bradley
- **University of Saskatchewan**
 - John Giesy, Steve Wiseman, Paul Jones, Yi Wan, Yinfei Yang
- **Woods Holes Oceanographic Institution**
 - Mark Hahn, Sibel Karchner, Diana Franks
- **ENTRIX Inc.**
 - Denise Kay, John Newsted, Shaun Roark



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Funding for Tittabawasee River Avian Research

- The Dow Chemical Company
 - Un-restricted grant to Michigan State University, University of Saskatchewan and University of Ottawa
- Environment Canada – STAGE Program
- le Fond Québécois de la Recherche sur la Nature et les Technologies
- Other funds to Michigan State University, University of Saskatchewan and Woods Hole Oceanographic Institution



SETAC 2009

Tittabawasee River Avian Research Presentations

MICHIGAN STATE
UNIVERSITY



- **Field studies**

- Seston *et al.* Abstract 248
- Fredricks *et al.* Abstract 249
- Fredricks *et al.* Abstract MP 179
- Williams *et al.* Abstract MP 180
- Fredricks *et al.* Abstract MP 181
- Tazelaar *et al.* Abstract MP 182
- Coefield *et al.* Abstract MP 183
- Seston *et al.* Abstract MP 184



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SETAC 2009

Tittabawasee River Avian Research Presentations



- **Laboratory studies**

- Kennedy *et al.* Abstract 247
- Yang *et al.* Abstract 537
- Cohen-Barnhouse *et al.* Abstract MP 177
- Cohen-Barnhouse *et al.* Abstract MP 178



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This Presentation

- The Tittabawasee River avian research projects:
 - The goals of our field and laboratory studies
- Laboratory studies with chicken, pheasant and Japanese quail:
 - A few highlights from:
 - egg injection studies
 - *in vitro* hepatocyte studies
 - The aryl hydrocarbon receptor (AHR) research:
 - objective
 - recent results and future plans

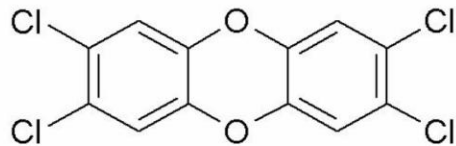


The Tittabawasee River Avian Research Project

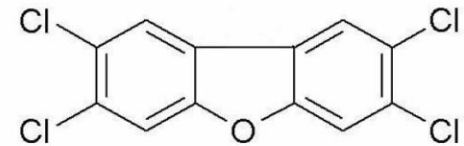
- General goal is to determine the effects of environmental exposure to dibenzo-*p*-dioxins and dibenzofurans in wild birds
- The research includes:
 - Field studies with several avian species
 - Chemical residue analysis
 - Biomarker analysis
 - Laboratory studies (egg injection and *in vitro* studies)
- **Problem** – there are large differences in sensitivity to ‘dioxins and furans’ among avian species – major challenge to risk assessments
- **Solution** – develop methods to predict species sensitivity



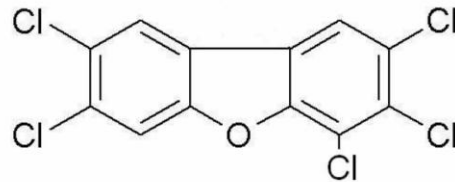
Dioxins and Furans of Particular Interest in the Avian Laboratory Studies



2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

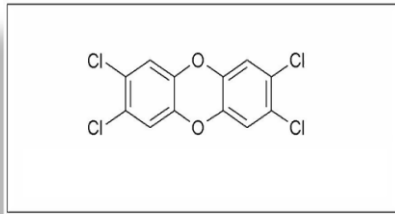


2,3,7,8-tetrachlorodibenzofuran (TCDF)

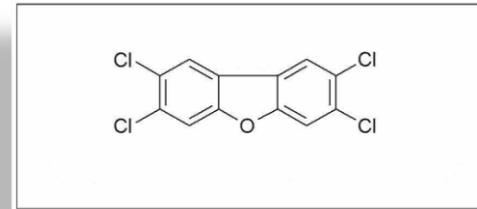


2,3,4,7,8-pentachlorodibenzofuran (PeCDF)

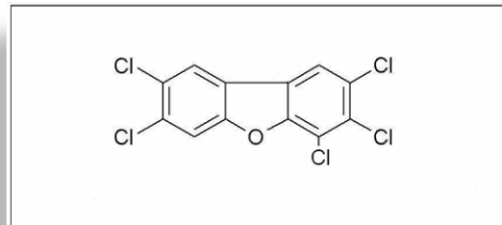
Current Opinion of the World Health Organization



2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)



2,3,7,8-tetrachlorodibenzofuran (TCDF)

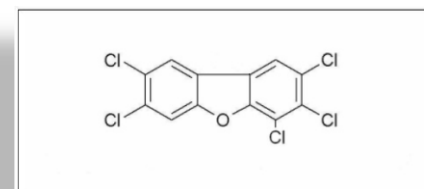
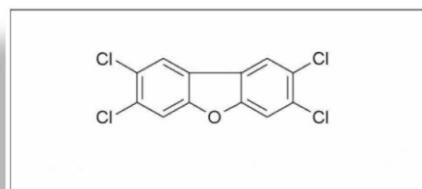
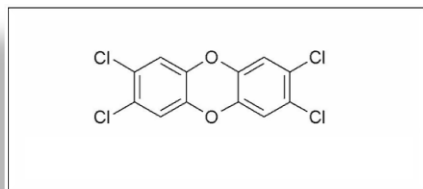


2,3,4,7,8-pentachlorodibenzofuran (PeCDF)

These three DLCs are Equipotent Toxicants in Birds
(i.e., TEFs for TCDD, TCDF and PeCDF = 1.0)



The Laboratory Studies



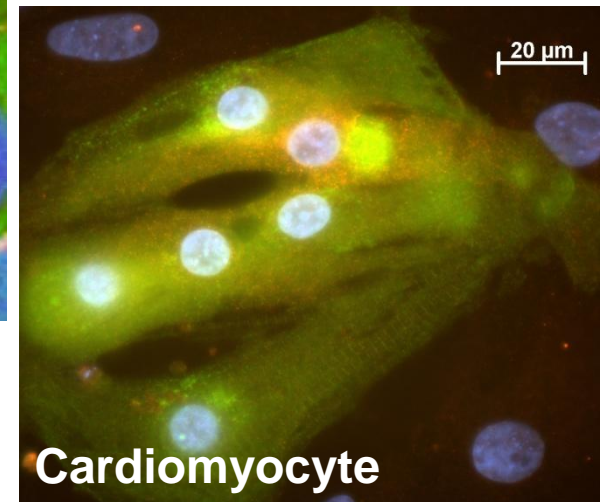
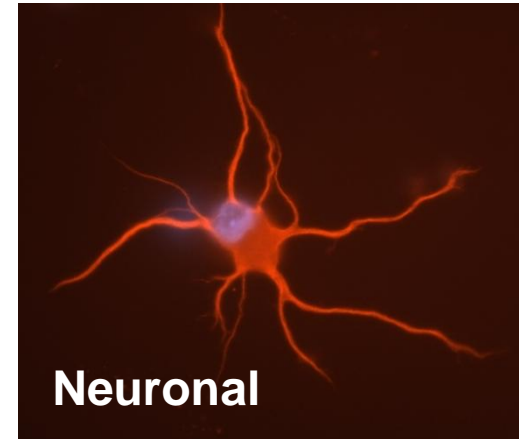
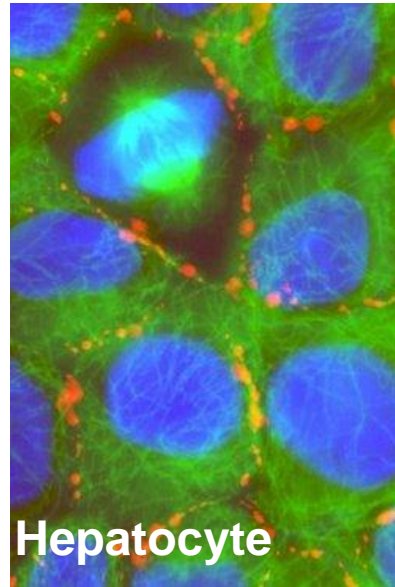
- Egg injection and *in vitro* studies
- Three species: chicken, pheasant, Japanese quail
- *In vitro* studies include:
 - CYP1A induction in primary hepatocyte cultures
 - Aryl hydrocarbon receptor (AHR) sequencing
 - Binding affinity of TCDD, PeCDF and TCDF to the AHR
 - AHR expression



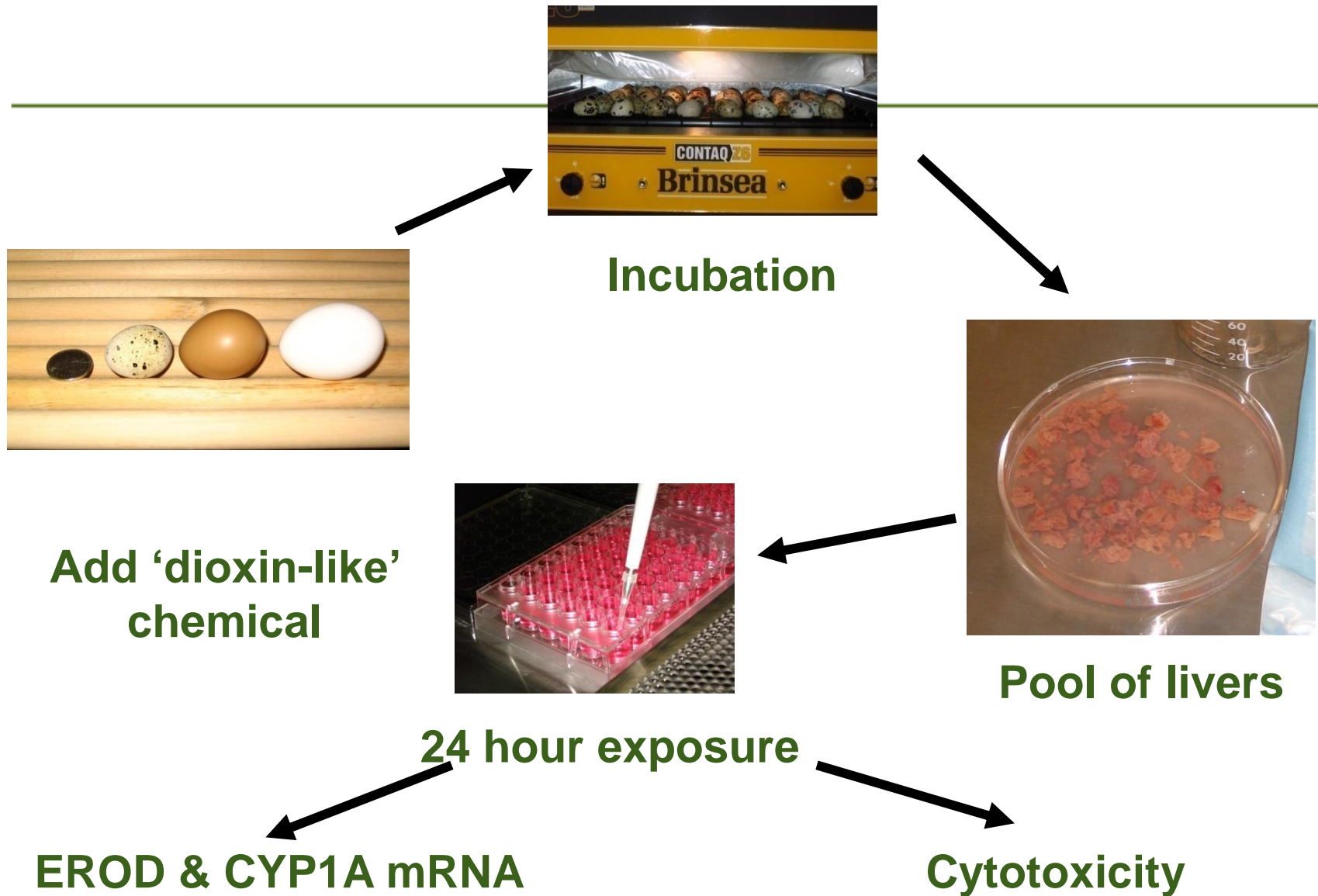
Egg Injection and Cell Culture



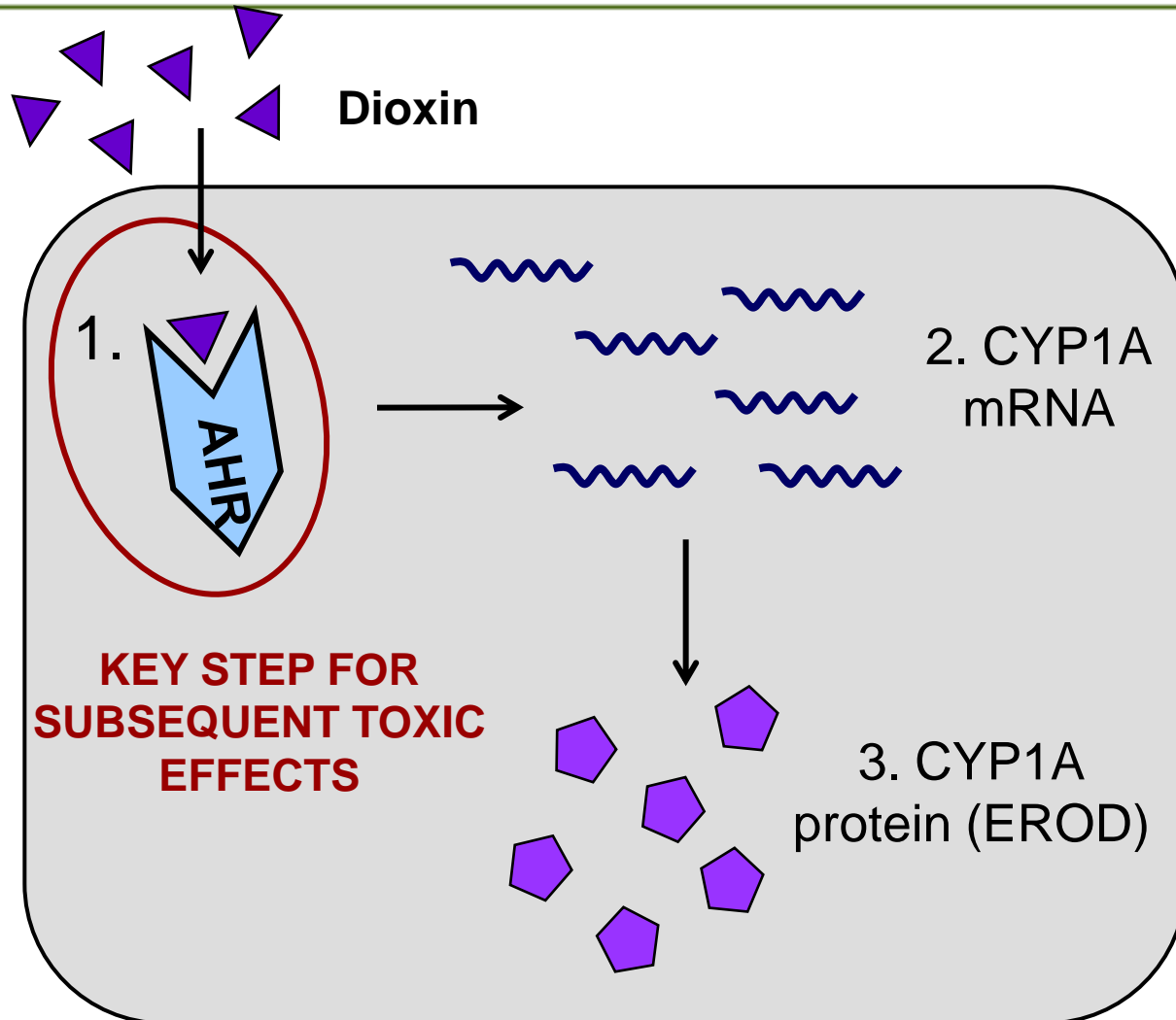
Primary Cell Cultures



CYP1A Induction in Hepatocyte Cultures



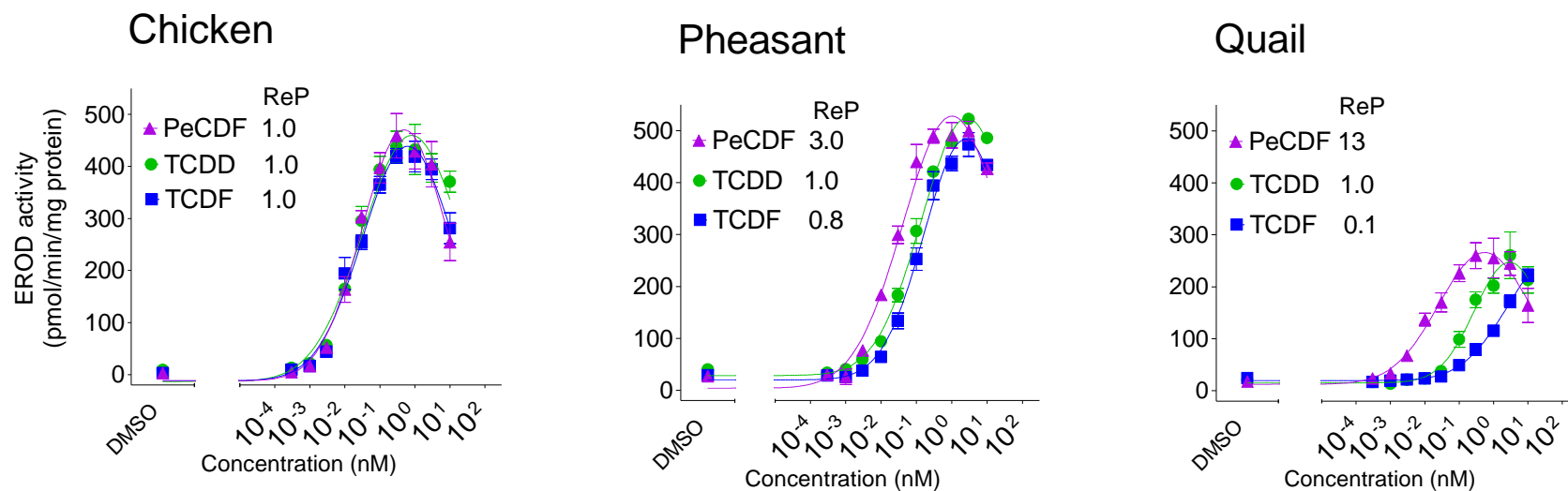
Simplified Dioxin Response Pathway



1. Dioxin binds and activates AHR
2. CYP1A mRNA increases
3. CYP1A protein increases



Concentration-Dependent Effects of TCDD, PeCDF and TCDF on EROD Activity



Hervé *et al.* *ToxSci Advance Access*, Nov. 1, 2009



CYP1A Induction in Hepatocyte Cultures

- When exposed to TCDD or TCDF

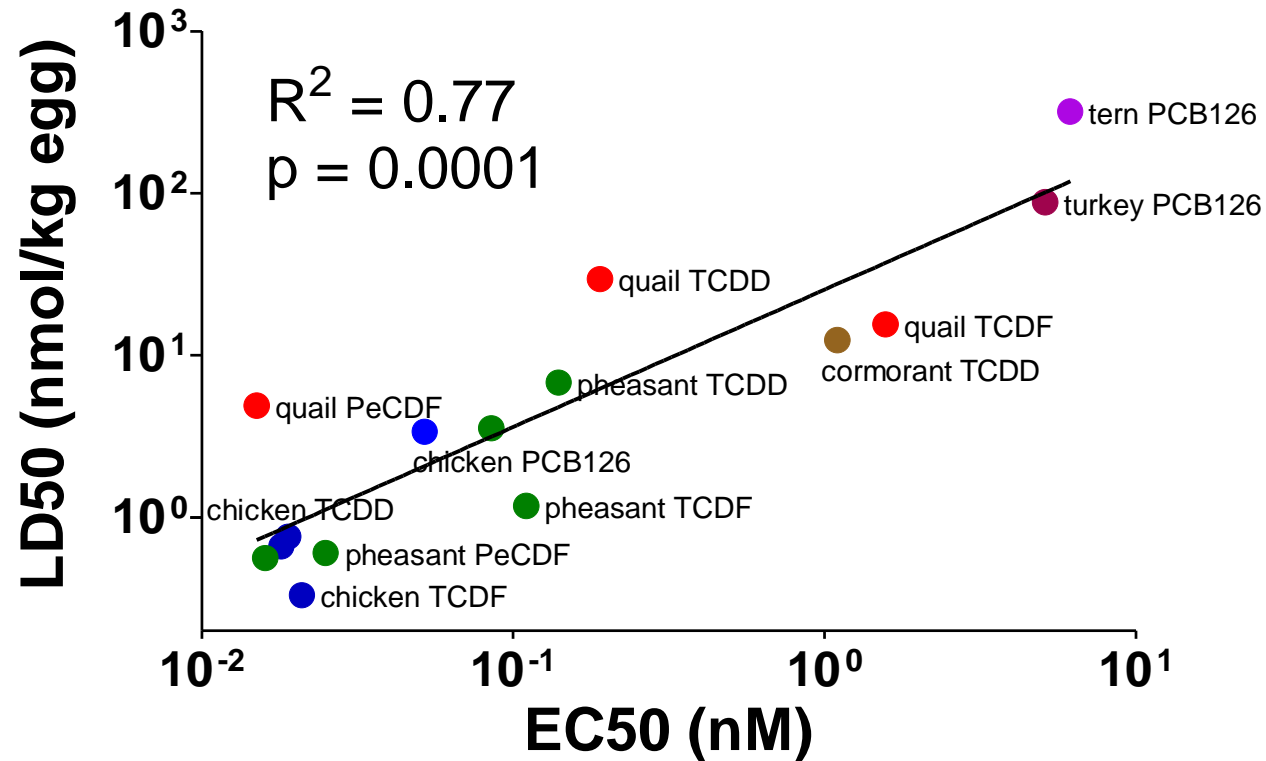


Hervé *et al.* ToxSci Advance Access, Nov. 1, 2009

- When exposed to PeCDF



Egg Injection Toxicity Data Compared to CYP1A Induction in Hepatocyte Cultures



LD₅₀ data: Cohen-Barnhouse; Head & Kennedy (*Ecotoxicology*, 2009)

EC₅₀ data: Hervé *et al.* (*Tox. Sci.*, 2009); Head & Kennedy (*Ecotoxicology*, 2009)



Avian Potency Values - Reloaded

- TCDD *is not* the most potent dioxin/furan in all avian species
- The LD50 of PeCDF *is 6 times lower* than the LD50 of TCDD in pheasant and quail
- PeCDF is a *more potent CYP1A inducer* than TCDD in pheasant hepatocytes (3-5 fold) and quail hepatocytes (13-30 fold) cultures

Abstracts MP 177 and MP 178
Hervé *et al.* *Tox. Sci.* (2009) in press



Possible implications to avian TEF values



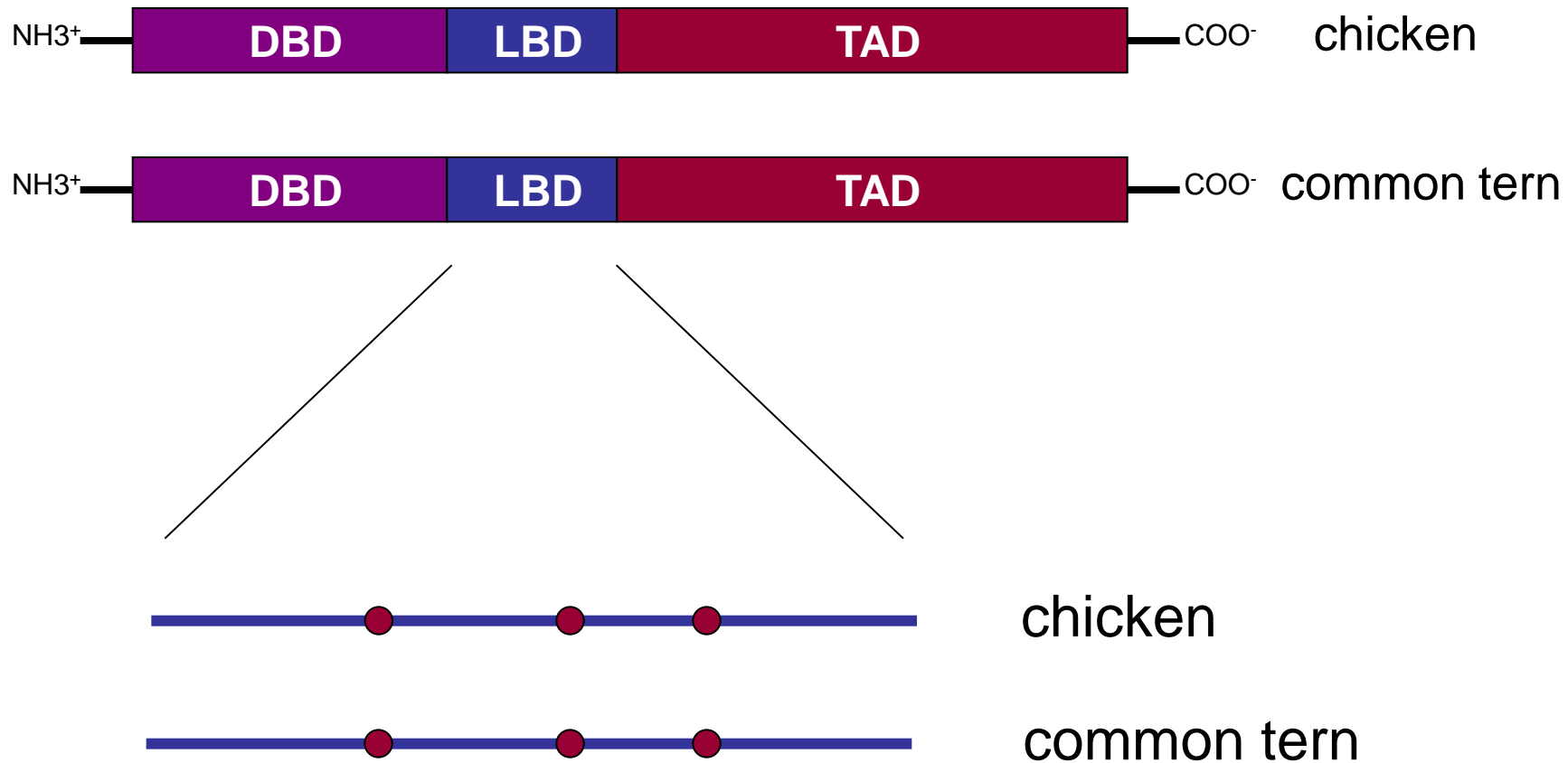
The Avian AHR Genotyping Project

Goal:

- Develop a method to determine the sensitivity of **any** avian species to the toxic and molecular effects of 'dioxin-like' compounds that is based upon AHR sequence
 - Karchner *et al.*, *PNAS* (2006)
 - Head *et al.* *Environ. Sci. Technol.* (2008)
 - Farmahin *et al.* (2010)
 - Head *et al.* (2010)
 - Kennedy *et al.* (2010)






The Key Role of the Ligand Binding Domain (LBD) Within the Avian AHR Gene



Avian AHR Amino Acids # 324 and # 380

Position 324

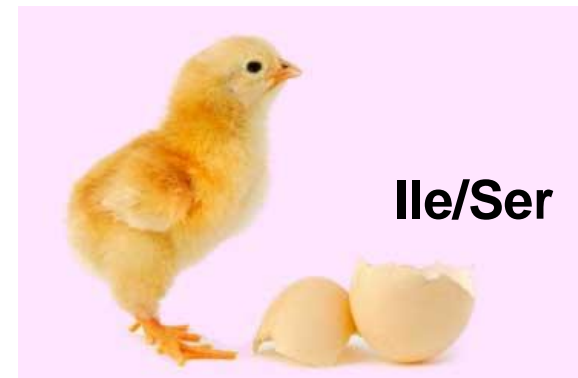
Position 380

	Ala	Ser
Val	 <p>Insensitive</p>	<p>Moderately sensitive?</p>
Ile	 <p>Moderately sensitive?</p>	 <p>Very sensitive</p>

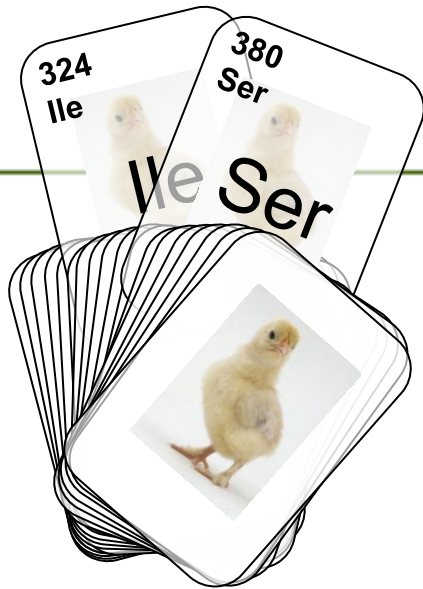
Insensitive



Very sensitive



Site-directed Mutagenesis



JQ-V324I



JQ-V380S



EC50(nM)

18

0.22

1.5

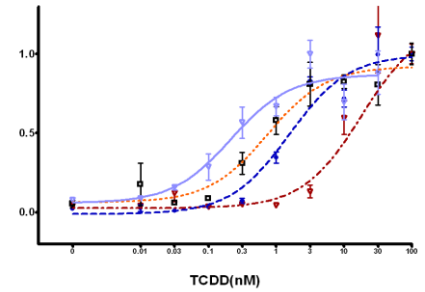
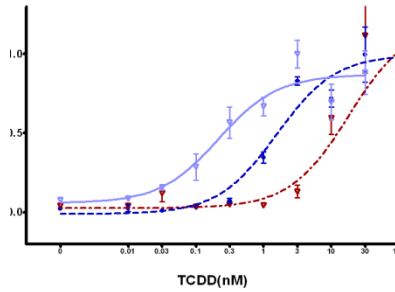
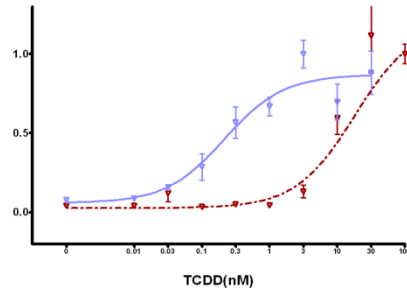
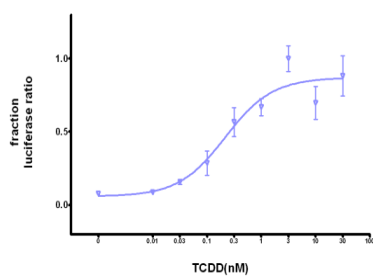
0.7

chicken

J.quail

JQ-V324I

JQ-A380S

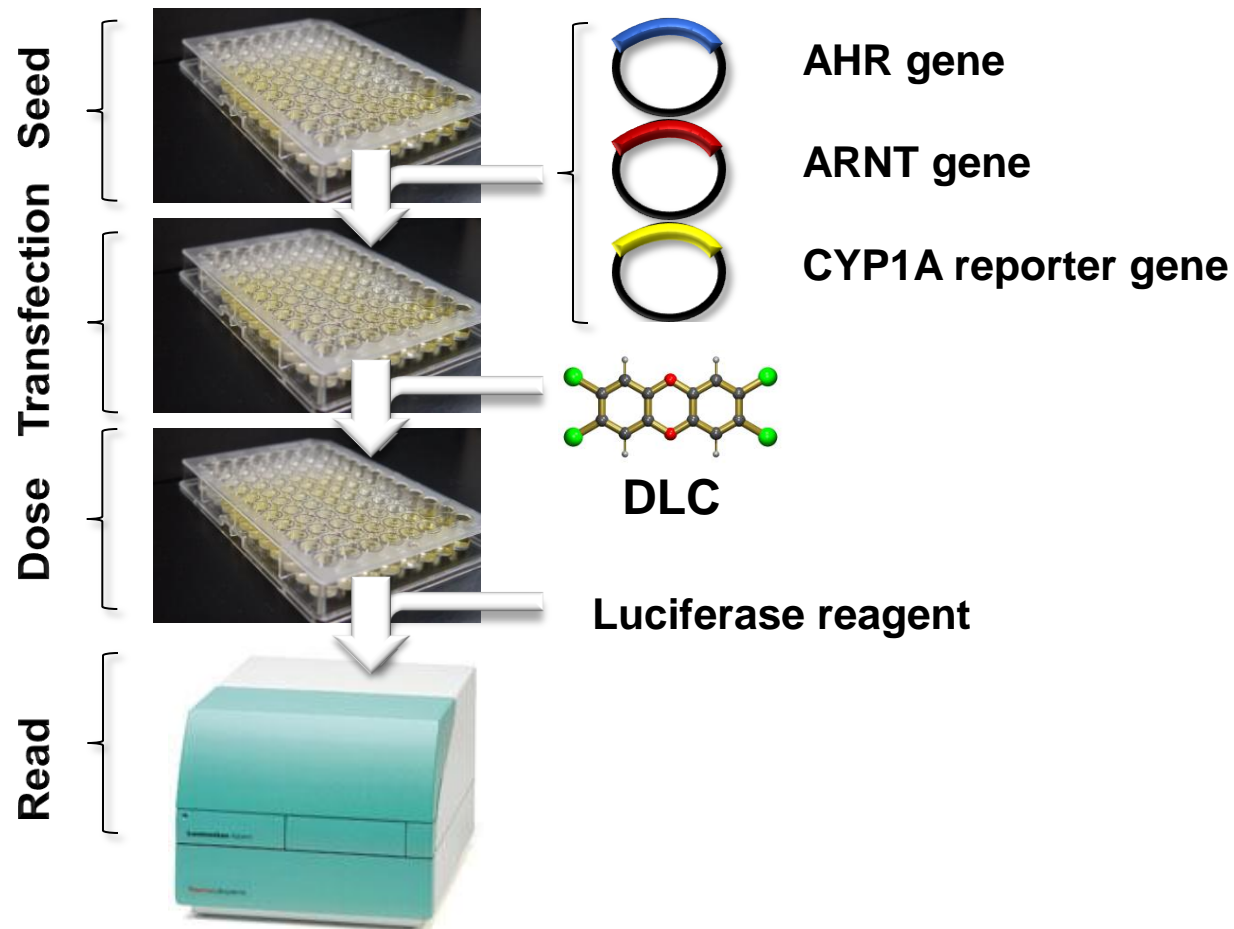


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CYP1A Expression in COS-7 Cells

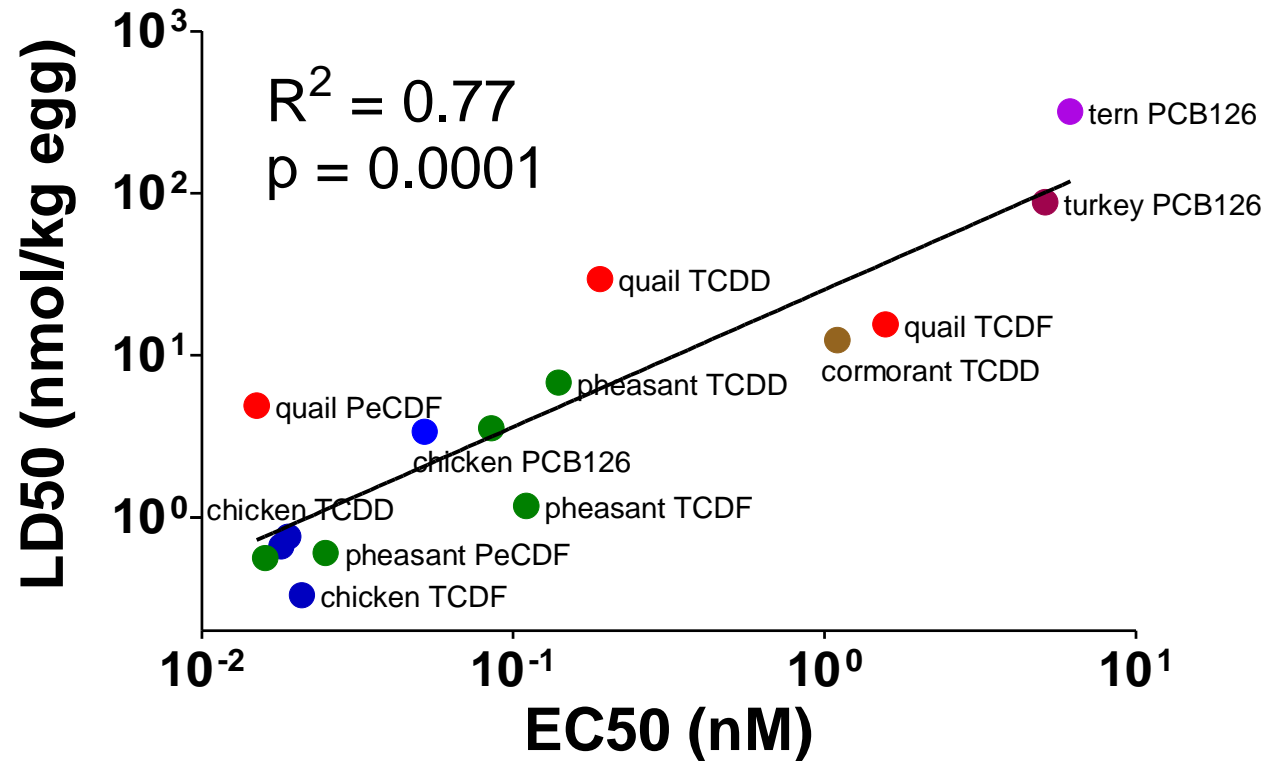


The AHR Genotyping Project - Current Research

- Ligand binding domain sequenced for 72 wild avian species
- Using site-directed mutagenesis ('card tricks') all sub-types of constructs were prepared
- The predicted sensitivity of all sub-types of AHR constructs is being tested with TCDD, PeCDF and TCDF
- Methods to predict avian species sensitivity to dioxin-like chemicals based upon AHR genotype will be available soon



Egg Injection Toxicity Data Compared to CYP1A Induction in Hepatocyte Cultures



LD₅₀ data: Cohen-Barnhouse; Head & Kennedy (*Ecotoxicology*, 2009)

EC₅₀ data: Hervé *et al.* (*Tox. Sci.*, 2009); Head & Kennedy (*Ecotoxicology*, 2009)



Costumes & Card Tricks

NWRC Molecular Toxicology Lab



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