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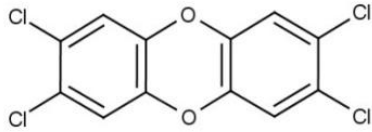
***In Vitro* Assays and AHR Sequence Predict *in Vivo* Sensitivity to Dioxin- like Compounds in Birds**

Sean W. Kennedy

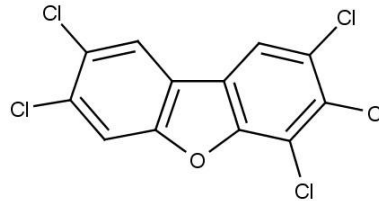
Environment Canada

SETAC, Boston, November 17, 2011

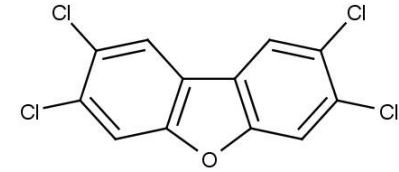
Some Dioxin-Like Compounds (DLCs)



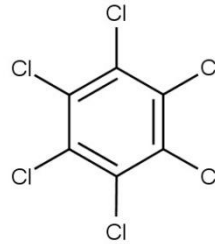
TCDD



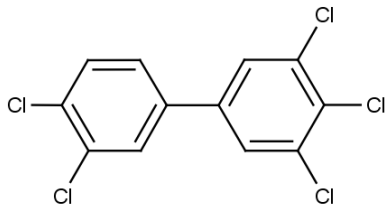
PeCDF



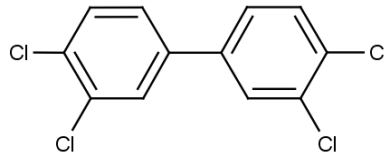
TCDF



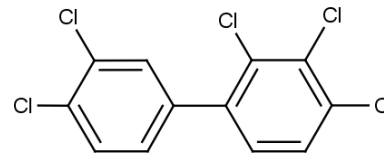
HCB



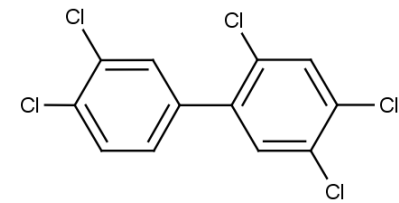
PCB 126



PCB 77



PCB 105



PCB 118



Major Toxic Effects of DLCs in Birds

- Liver toxicity
- Cardiovascular toxicity
- Embryonic deformities (bills, limbs)
- Immuno-suppression
- Edema
- Porphyria
- Embryo-lethality



Egg Injection Studies

- × 3,200 Japanese quail eggs
 - 100 eggs/dose
- × 1,760 Common pheasant eggs
 - 80 eggs/dose
- × 2,200 White Leghorn chicken eggs
 - 100 eggs/dose



Relative Sensitivity (ReS) to TCDD Embryotoxicity

Chicken



Most Sensitive

LD50 = 0.21 ug/kg

Pheasant



LD50 = 1.2 ug/kg

J. Quail



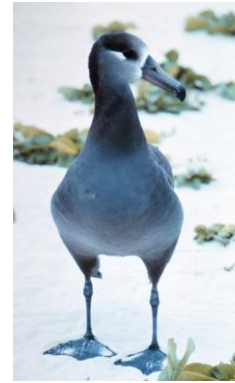
Least Sensitive

LD50 = 9.7 ug/kg

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



Large Differences in Sensitivity to Dioxins, Furans and PCBs = Major Problem for Risk Assessments



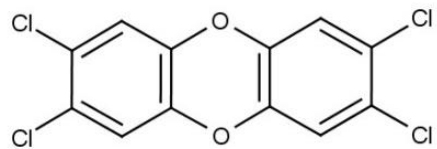
Methods to Predict the Sensitivity of Avian Species to DLCs

- 1. Primary Cultures of Avian Hepatocytes**
- 2. Luciferase Reporter Gene (LRG) Assay**
- 3. The Avian AHR1 Genotyping Project**

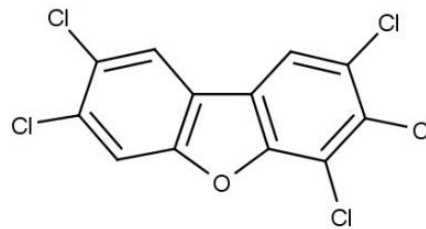


Aryl hydrocarbon receptor (AHR)

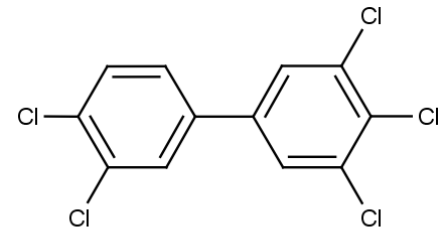
- Ligand-activated transcription factor
- Activated by dioxin-like compounds (DLCs): polychlorinated dibenzo-*p*-dioxins (PCDDs) and furans (PCDFs), and some PCBs



TCDD



PeCDF



PCB 126

- Regulates expression of several genes (e.g. cytochrome P4501A; CYP1A)
- Mediates most, if not all, toxic effects of DLCs

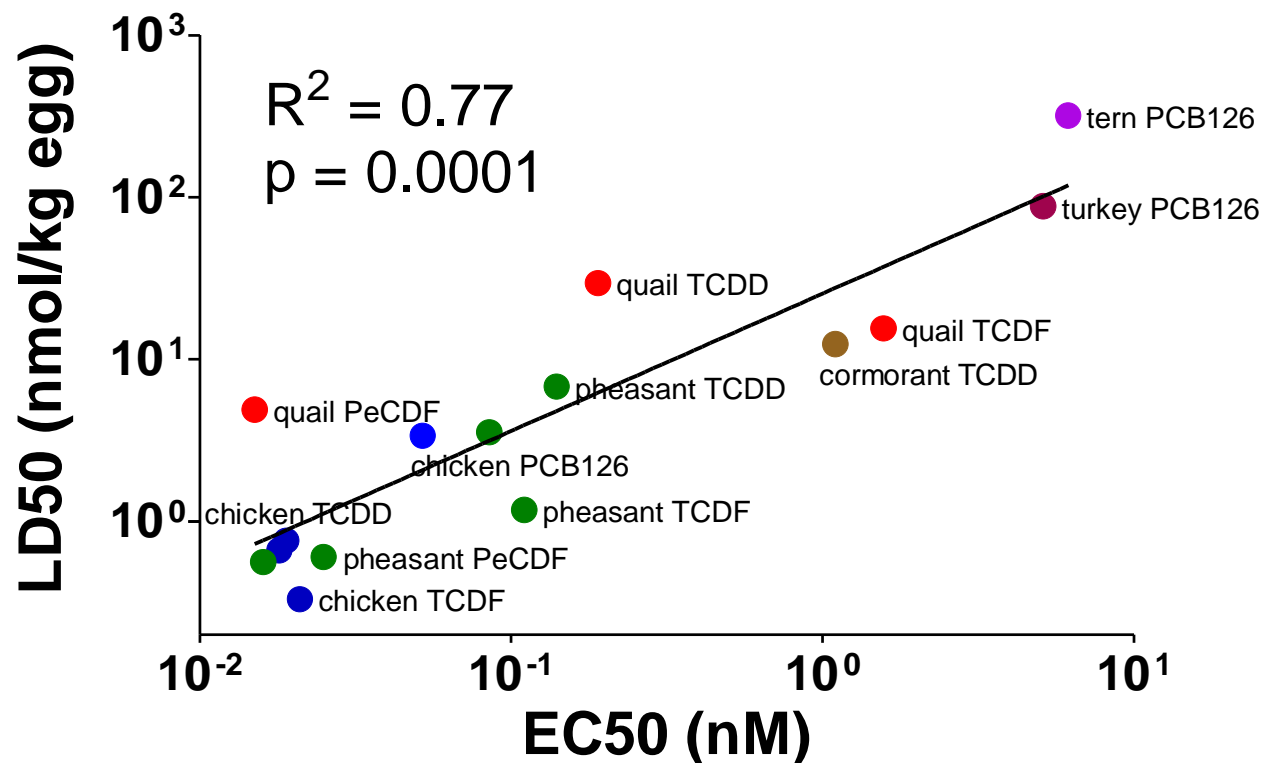


1. Primary Cultures of Avian Hepatocytes

- **Reliable methods for many species were developed 15 years ago**
 - **Late-stage embryos are usually used**
 - **CYP1A4 induction measured:**
 - **Ethoxyresorufin O-deethylase (EROD)**
 - **Messenger RNA (mRNA)**
 - **Several species (12-15) have been studied at the National Wildlife Research Centre (Ottawa) and elsewhere**



Egg Injection Toxicity Data Compared to EROD Induction in Hepatocyte Cultures



LD₅₀ data: Cohen-Barnhouse *et al.*; Head & Kennedy (*Ecotoxicology*, 2010)

EC₅₀ data: Hervé *et al.* (*Tox. Sci.*, 2010); Head & Kennedy (*Ecotoxicology*, 2010)



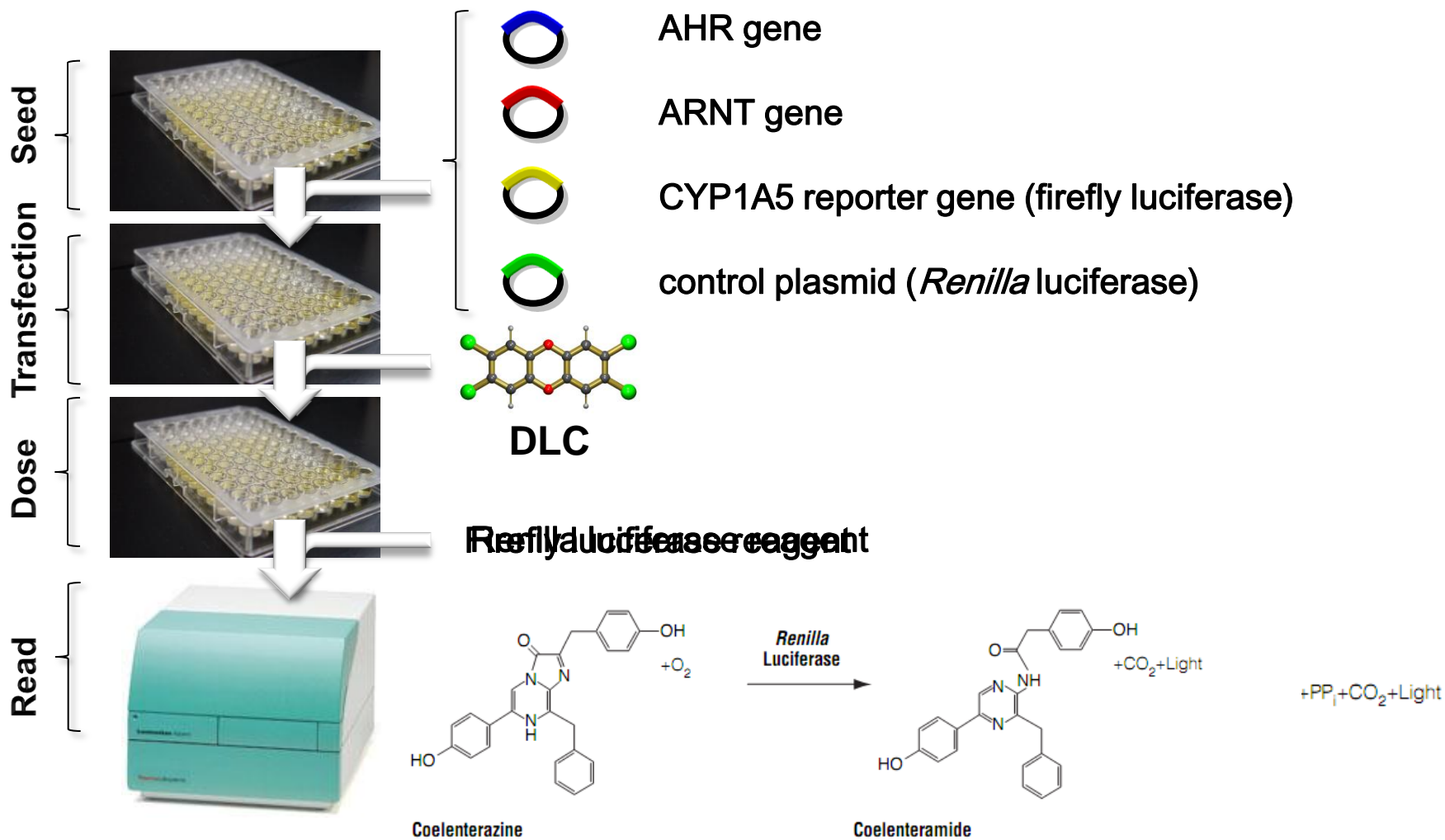
2. Luciferase Reporter Gene (LRG) Assay

- **An improved method was recently developed at Environment Canada**
- **Based on a method developed in the Hahn lab (Woods Hole, MA) and in the Iwata lab (Matsuyama, Japan)**
- **96-well format; rapid**
- **Can likely be used to predict the LD50 of *any* avian species exposed to *any* PCDD/F or PCB**

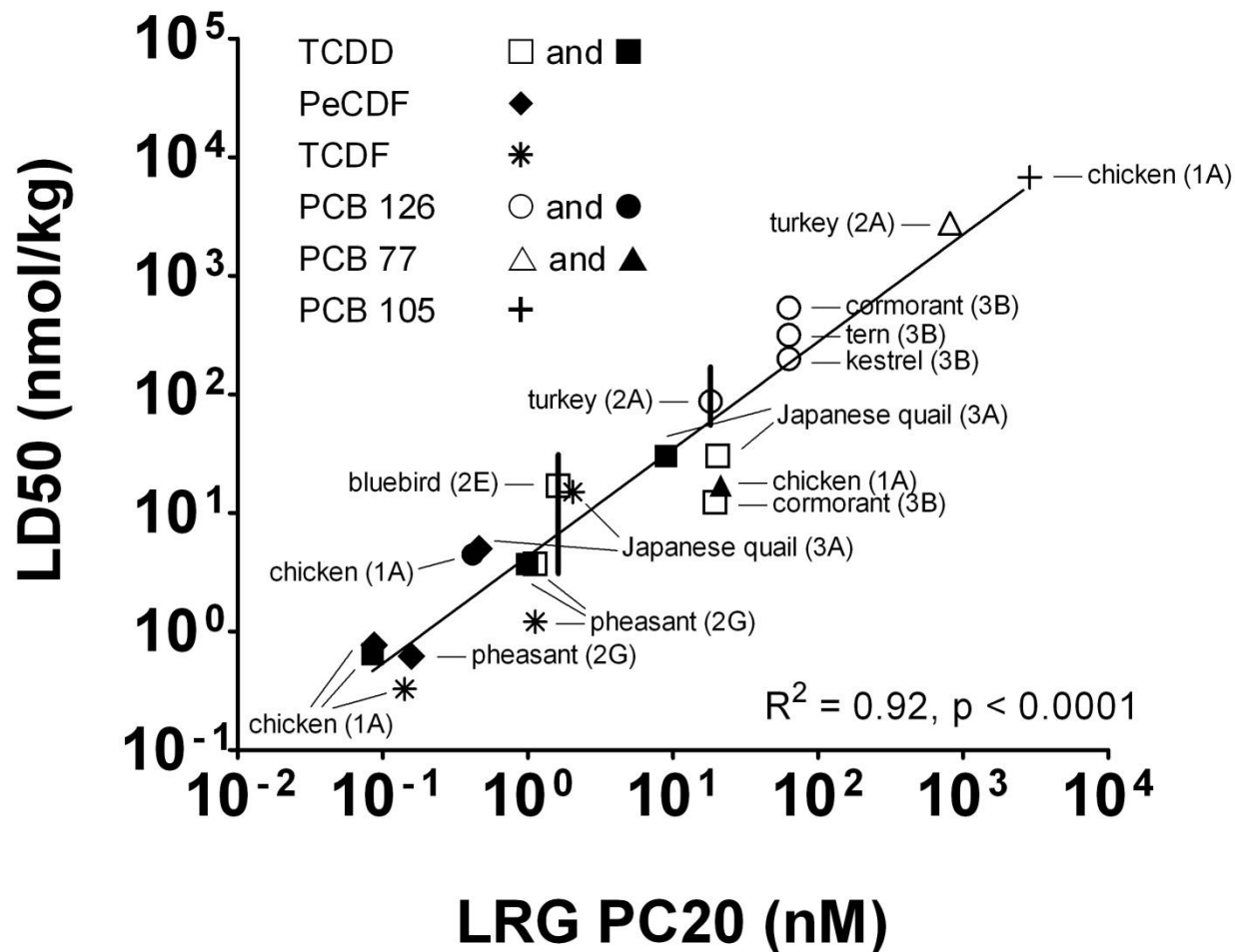
Karchner, Franks, Kennedy, Hahn (2006) *PNAS*
Farmahin *et al.* Submitted for publication



Luciferase Reporter Gene (LRG) Assay



Egg Injection & LRG Assay Comparison (Using all known egg injection LD50 data)



3. The Avian AHR1 Genotyping Project

The Goal:

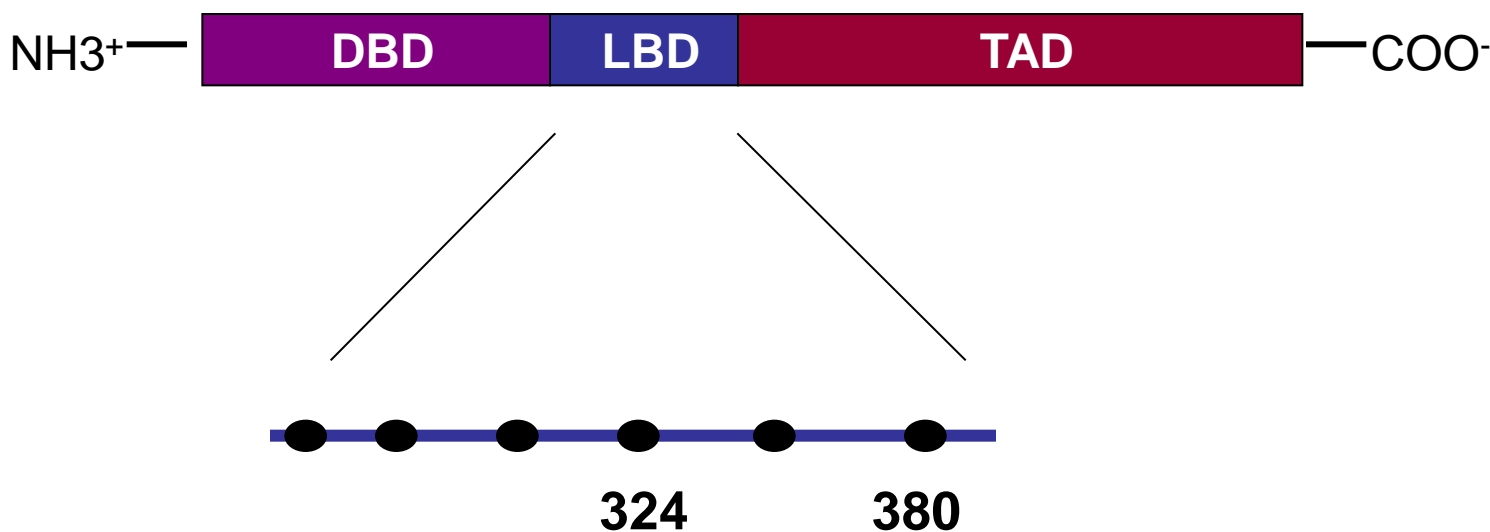
Develop methods to determine the sensitivity of **any** avian species to the toxic and molecular effects of **any** DLC that is based upon subtle differences in AHR1 sequence among species.

The Vision:

No need for LRG assay or other *in vitro* assays in the future.



Amino Acids at Sites 324 and 380 Avian AHR1 are IMPORTANT



Karchner *et al.* (2006) PNAS **103**: 6252 – 6257

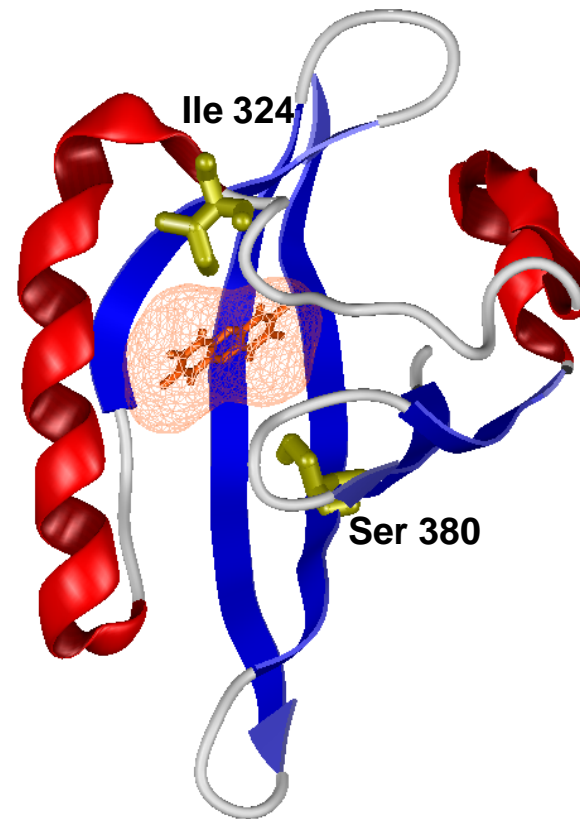
Head *et al.* (2008) ES&T **42**: 7535 – 7541

Farmahin *et al.* Submitted for Publication

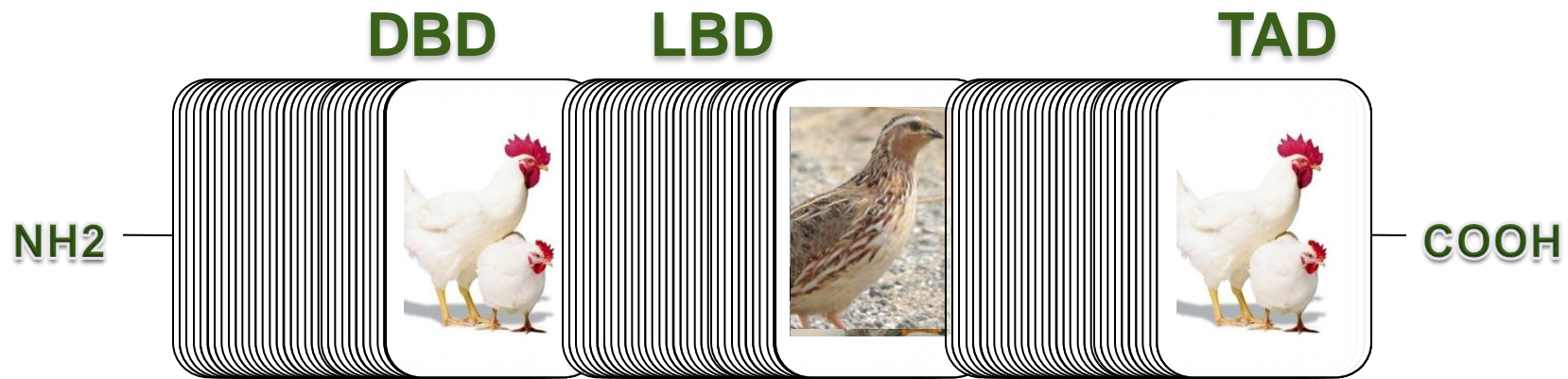


Chicken AHR1-TCDD

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



Site-Directed mutagenesis



Jeep bluebird



The AHR1 LBD in 75 Avian Species

Only sites 324 and 380 affect the sensitivity to AHR1 activation by PCDD/Fs and PCBs in the LRG assay

- **Type 1** (chicken-like)
4/75 species (5%)
- **Type 2** (pheasant-like)
39/75 species (52%)
- **Type 3** (Japanese quail-like)
32/75 species (43%)



HCB Poisoning in Turkey 1955 - 1960



4000 – 5000 cases of Porphyria
Cutanea Tarda



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U.S. Magnesium Facility



Great Salt Lake, Utah

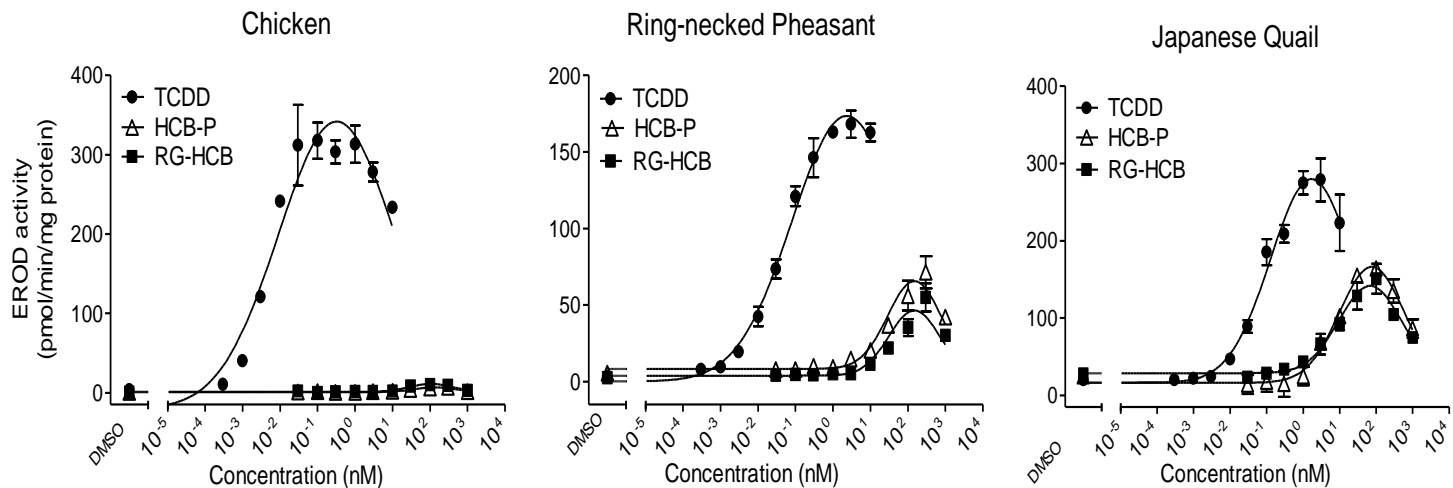


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CYP1A Induction by HCB in Avian Hepatocyte Cultures



Un-predicted Potency and Efficacy Differences

Mundy *et al.* (2010) *Toxicol. Appl. Pharmacol.* **248**: 185-193

Mundy *et al.* Submitted for publication



Will this Research be Useful for Ecological Risk Assessments?

YES

- Knowing the sensitivity (i.e., relative LD50 values) of wild avian species to PCDD/Fs and PCBs could be useful in many locations (e.g., U.S. Superfund sites)
- The genotype can be determined for **any avian species** using tissue, blood and, probably, eggshell samples



Co-Authors

- **Reza Farmahin** – PhD student – dioxins/furans/PCBs
- **Gillian Manning** – MSc student – PCBs
- **Lukas Mundy** – former MSc student - HCB
- Doug Crump
- Stephanie Jones
- Lukas Mundy
- Steven Bursian
- Timothy Fredricks
- John Giesy
- Matthew Zwiernik
- Sibel Karchner
- Mark Hahn



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 - Michigan State University
 - University of Saskatchewan
 - Woods Hole Oceanographic Institution

