

Species-Specific Endocrine Disruption: PCB- and PAH-Induced Estrogen Effects

Project Scope

Accumulating evidence suggests that chemicals and complex mixtures are capable of eliciting endocrine-disrupting activities that adversely affect human health, and affect wildlife. Adverse effects of exposure to endocrine disrupting chemicals (EDCs) have been demonstrated in numerous species, resulting in compromised reproductive fitness. In addition, significant increases in the incidence of hormone-dependent cancers have been observed in humans. It has been proposed that some of these effects may be mediated by the estrogen receptor binding activities of the EDCs.

Estrogens and the estrogen receptor (ER) play major roles in a number of normal physiological processes such as the regulation of developmental processes, homeostasis and fertility, as well as in pathological conditions such as hormone-dependent diseases. Although the function and activities of estrogen and the ER are conserved between species, the amino acid sequences of the ligand-binding domains of the ERs are less conserved. This suggests that species may exhibit different responses and sensitivities to non-traditional estrogenic ligands and that a single test species may not be sufficient to identify and to assess the risks of environmental estrogens to human and wildlife health.

The main objective of this research was to examine, for several species, the proposed estrogen receptor-mediated activities of a structurally diverse set of compounds using a battery of species-based *in vitro* and *in vivo* assays. This research project examined the estrogenic activities of polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) and their effects in a number of species including humans, rodents, birds, fish and amphibians. The studies were performed to define the limitations of using a single surrogate species (e.g., a rodent) to identify and assess estrogen effects on human and wildlife health, based on the divergence in amino acid sequences and binding activities.

To address this objective, an integrative approach was used that involved a series of *in vitro* and *in vivo* assays using estrogen receptors from a number of species. Species-specific differences in ligand preference and binding affinities initially identified using *in vitro* assays (i.e., receptor binding, gene expression) were then verified *in vivo* by monitoring effects on gene expression (e.g., vitellogenin).

Grant Title and Principal Investigator

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Key Findings and Implications

- Competitive ligand binding and reporter gene assays identified several compounds that exhibited differential interactions with recombinant estrogen receptors.
- There were substantial differences in relative binding affinities between species.
- There was poor correlation between the *in vitro* and *in vivo* activities and striking differences in efficacy and potency between the species examined.
- Results do not preclude the use of a single surrogate species for screening purposes, but the ability to predict *in vivo* activity based on *in vitro* data is questionable.

Publications include eight peer reviewed journal articles.

Project Period: January 1998 to December 2000

Relevance to ORD's Multi-Year Research Plan

This project contributes to ORD's Multi-Year Plan long-term goal of providing a better understanding of the science of underlying effects of endocrine disruptors. The study concentrates on the approaches needed to assess risks in humans and wildlife, with a focus on the use of a single surrogate species. The importance to risk assessment is in determining if a single surrogate species can be used when various species may exhibit different responses and sensitivity to chemicals.

Project Results and Implications

Initial species comparisons of ER binding involved 44 PCBs, 9 hydroxylated PCBs, and 8 Aroclor PCB mixtures at exposure concentrations ranging from 1 nM to 10 uM. Further investigations were conducted with 35 structurally diverse synthetic and natural estrogenic compounds (e.g., steroids, pharmaceuticals, phytoestrogens, mycotoxins, organochlorine compounds, polyaromatic hydrocarbons, and other industrial chemicals). Competitive ligand binding and reporter gene assays identified several compounds that exhibited differential interactions with recombinant ERs consisting of the D, E, and F domains of the human, mouse, chicken, green anole lizard, frog (*Xenopus*), and rainbow trout ERs (Fig. 1). Qualitatively, all receptor fusion proteins bound the same set of compounds. However, there were substantial differences in relative binding affinities between species. In general, reporter gene expression data correlated with competitive binding results. Intriguingly, the rainbow trout ER ligand binding and reporter gene transactivation were found to be temperature dependent and functioned more effectively closer to the physiological temperature. Comparison of the amino acids that lined the binding pocket of the rainbow trout and human ERs identified two conservative substitutions (substitutions that do not change the overall electrostatic charge on the protein), which were found to play a substantial role in this temperature dependence.

Three compounds that exhibited differential activity *in vitro*, β -zearalenol, genistein, and 4-tert-octylphenol, were then examined *in vivo* to determine their ability to induce vitellogenin messenger ribonucleic acid (mRNA) levels in juvenile trout, frog, and Japanese quail. There was a poor correlation between the *in vitro* and *in vivo* activities and striking differences in efficacy and potency between the species examined. Overall, these results suggest that there general consistency with regard to ER binding of EDCs across the species tested, but the ability to predict the full range of *in vivo* activity of EDCs based on *in vitro* data from a single species is questionable.

Figure 1. Summary of the Ability of Polychlorinated Biphenyls to Compete with [3H]17 β -Estradiol for Binding to Recombinant Fusion Proteins Containing the Human, Green Anole and Rainbow Trout Estrogen Receptor D, E, and F Domains^{a,b}

PCB	Human	Anole	Rainbow Trout
<i>Mono-ortho</i>			
58	non	non	Weak
60	non	non	Weak
68	non	non	Weak
<i>Di-ortho</i>			
18	non	non	weak
41	non	non	>10 μ M
44	non	non	weak
47	non	non	>10 μ M
49	non	non	weak
87	non	non	non
99	non	non	weak
101	non	non	weak
112	non	non	weak
115	non	non	>10 μ M
128	non	non	weak
138	non	non	weak
153	non	non	weak
<i>Tri-ortho</i>			
45	non	non	>10 μ M
51	non	non	>10 μ M
84	non	non	weak
91	non	non	>10 μ M
95	non	non	weak
143	non	non	>10 μ M
149	non	non	weak
151	non	non	weak
173	non	non	>10 μ M
177	non	non	>10 μ M
178	non	non	weak
183	non	non	weak
187	non	non	weak
<i>Tetra-ortho</i>			
54	non	weak	>10 μ M
104	>10 μ M	>10 μ M	1.3 +/- 0.6 μ M
184	>10 μ M	>10 μ M	0.4 +/- 0.1 μ M
188	>10 μ M	>10 μ M	1.3 +/- 1.2 μ M
<i>Co-planar</i>			
126	non	non	non
169	non	non	weak

a. Source: Abstracted from Table 3 of Matthews and Zachareski (2000) (permission being sought)

b. "non" = no discernable displacement of estrogen at any concentration tested, "weak" = < 10 percent estrogen displacement at highest concentration tested, " >10 μ M" = calculated IC₅₀ > 10 μ M from three experiments

Investigators

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For More Information**Investigator Website:**

<http://www.bch.msu.edu/~zacharet/>

NCER Project Abstract and Reports:

http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/173/report/0