Endocrine Disruptor Mechanisms: Beyond Receptor Binding

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ENDOCRINE DISRUPTION

“...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.”

Kavlock et al., 1996. Research needs for the assessment of environmental effects of endocrine disruptors: a report of the USEPA-sponsored workshop
Maintaining Homeostasis is a complex process

- Involves many signal transduction pathways
- Many alternative pathways
- Many enzymes involved
- Rate limiting steps unknown
- Cybernetic feedback loops
ENDOCRINE DISRUPTION

• Direct - (Mimics)
  – Agonists
  – Antagonists
  – Partial Agonists

• Environmental estrogens are only one mimic

• Indirect
  • Induction of Enzymes that Directly or Indirectly Affect Hormone Concentrations
  • Alteration of signal transduction pathways
Mechanism of Action for ER-Activation

- Estrogen or xenoestrogen

- Protein Phosphorylation of ER

- DNA Binding

- Nuclear Factors

- mRNA

Ligand-Independent Activation

- Estrogenic Effects

ER-Responsive Genes
ENDOCRINE DISRUPTION

- Hormone Pathway Disruption - Indirect
  - Tissue Damage
  - Substrate Changes
  - Cascade of Effects
  - Behavior
  - Interaction - Nutrition
  - Signal transduction cross-talk
Endocrine Disruption

• In the United States current attention is focused primarily on compounds that can affect steroid hormones

• Reauthorization of the clean Water Act

• Food Safety Protection Act

• Endocrine Disrupter Screening and Testing Committee (EDSTAC)
  – Estrogen receptor (ER)
  – Androgen Receptor (AR)
  – Thyroid Receptor (TR)
U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program
http://www.epa.gov/oscpmont/oscpendo/index.htm

Initial Sorting
↓
Priority Setting
↓
Tier 1 Screening
↓
Tier 2 Testing
Endocrine Disruptor Screening Program

Tier 1 Screening
- detect chemical substances capable of interacting with the estrogen, androgen, and thyroid hormonal systems
- combination of *in vitro* and *in vivo* assays
- weight of evidence approach will be used to determine if substance warrants further testing or placed in “HOLD” box

Tier 2 Testing
- designed to determine if substance has effects similar to naturally occurring hormones
- designed as a battery of *in vivo* testing that encompass critical life stages and processes, a broad range of doses, and administration by a relevant route of exposure
- data will be used as the basis of the dose response characterization for risk assessment purposes
Proposed Tier 1 Screening Battery

• *In Vitro* Screens
  – ER Binding / Reporter Gene Assay*
  – AR Binding / Reporter Gene Assay*
  – Steroidogenesis Assay with minced testis

• *In Vivo* Screens
  – Rodent 3-day Uterotrophic Assay (sc)
  – Rodent 20-day Pubertal Female Assay with Thyroid
  – Rodent 5-7 day Hershberger Assay
  – Frog Metamorphosis Assay
  – Fish Reproduction Screening Assay

* These assays are in the HTPS

**Alternate assays have also been proposed**
Proposed TIER 2 Testing Battery

- multigenerational reproduction and development studies in:
  - rodents
  - birds
  - frogs
  - fish
  - shrimp
Limitations of Screening Methods

• If used in a sequential decision process
  – False negatives
  – If negative in the binding assay, may still be positive as an endocrine disrupting compound
Binds to Estrogen Receptor or is predicted to do so from QSAR

Further Testing because compound may be an endocrine disruptor

The proposed sequential testing provides useful information for designing additional testing, but does not allow for a sorting of compounds or assist in prioritizing compounds for additional testing.
Effects on steroidogenic enzymes

• At level of expression
  – measure mRNA levels: RT-PCR

• Effects on enzyme concentrations
  – measure catalytic activities: selective substrates

• Effects on metabolism of steroid hormones
  – measure steroid hormone concentrations
H295R cell line

- Human female adrenocortical carcinoma
- Produces many steroid hormones
  - gluco- & mineralocorticoids, progestins, androgens & estrogens
- Expresses (inducible) steroidogenic cytochrome P450 (CYP) enzymes
  - CYP11A, CYP11B, CYP17, CYP19, CYP21
Example: Triazine Herbicides

Do not bind to ER

Result in estrogenic effect in vitro

Induction of aromatase by triazines

- Atrazine
- Simazine
- Propazine

Triazine concentration (µM)

Aromatase activity (pmole/h/mg Protein)
Induction of CYP19 mRNA by triazines

Amplification response ratio of CYP19/beta-actin (% of control ratio)

- DMSO
- Atrazine
- Simazine
- Propazine
- 8Br-cAMP

Control 30 µM 100 µM
Proposed Mechanisms of Action for Triazines

- Aromatase induction / promotion
  - via protein kinase A pathway
  - via steroidogenic pathways
- Inhibition of phosphodiesterase
- Results in less conversion of c-AMP to AMP so that AMP increases
- c-AMP increases signal transduction of Protein Kinase A
- Protein kinase A increases CREB and SF-1
- Aromatase m-RNA is up-regulated such that more aromatase protein is formed and aromatase activity increases
Protein Kinase A Signaling Pathway

Ligands (ACTH, LH, FSH, GnTH) → Membrane bound receptors

- Adenylyl cyclase → cAMP
- G-protein

- phosphodiesterase
- cAMP → AMP
- STAR

- Protein Kinase A
- CREB
- SF-1
- MIS

- Cholesterol-esteresterase
- cholesterol
- aromatase

Zoology Dept. & National Food Safety and Toxicology Center
Michigan State University
Effects of other compounds on Aromatase activity  H295R cells

• Imidazole-type fungicides decrease aromatase activity. Competitive inhibitors
  – imazalil
  – prochloraz
  – difenoconazole
  – penconazole
  – propiconazole

Effects of other compounds on Aromatase activity H295R cells

- Several imidazole-type fungicides and the structurally similar fungicide, vinclozolin induced aromatase activity
  - Diclobutrazole
  - Tricyclazole
  - Paclobutrazole
  - Nuarimol

Vinclozolin and diclobutrazole increase cAMP 150%, whereas forskolin increases cAMP 300%
Cross-Talk of AhR-Mediated Processes

- Compounds that do not bind to the AhR can affect ARNT-requiring pathways, thus affecting AhR-mediated pathways without binding to the AhR
  - Example: Hypoxia-inducable factor (HIF)

Interference between AhR and Hypoxia Signaling Pathways

TCDD

Hypoxia, CoCl₂, Dfx
Indirect effects on steroid hormones and thyroid hormone

- **CYP** gene expression can be altered
  - increased hormone clearance
- **UDPGT-activity** increased resulting in decreases in thyroid hormone
Questions ????????
Thank You

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