U.S. EPA’s Research on the Ecological Exposure and Effects of Endocrine Disruption Chemicals & Pharmaceuticals
Principal Investigators & Contributors

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Earl Gray and Vickie Wilson, NHEERL-RTP – Mammalian In Vitro Assays

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Mitch Kostich, NERL-Cincinnati - Bioinformatics/Chemistry/Ecotoxicogenomics

Marc Mills, NRMRL- Cincinnati- Wastewater/Chemistry

Christian, Daughton, Tammy Jones-Lepp, Lantis Osemwengie, NERL-Las Vegas – PPCPs Wastewater Fate Chemistry

Susan Glassmeyer, NERL-Cincinnati - Drinking Water Fate Chemistry
Outline

• Ecological Effects, Ecotoxicogenomics and Bioinformatic Projects

• Wastewater and Chemistry Fate Projects

• Drinking Water/Chemistry Fate Research Projects
Ecological Effects Projects

Fathead Minnow Short-Term Reproduction Assay - Gary Ankley – NHEERL-Duluth 218 529 5147

Medaka 2-Generation Test Protocol for Assessing the Effects of Endocrine-Disrupting Chemicals
Rodney Johnson – NHEERL Duluth 218 529 5117

Xenopus laevis Short-Term Metamorphosis Assay - Joe Tietge, Sig Degitz – NHEERL-Duluth 218 529 5176

Mammalian In Vitro Cell Assays. – Vickie Wilson, Earl Gray – NHEERL – RTP 919 541 3559

Genomic and proteomic basis for interspecies extrapolations based upon estrogen and androgen receptor structure and function among animals. – Vickie S Wilson, C Rider, M Cardon, LE Gray, Jr, GA LeBlanc, LJ Guillette, P. Hartig, NHEERL – RTP 919 541 3559
Ecotoxicogenomics and Bioinformatic Projects

**Whole Lake Experiment EE2 Additions. Robert Flick**, Jim Lazorchak, Greg Toth, NERL-Cincinnati, Karen Kidd DFO – 513 569 7394

**Ohio River EDC Instream Effects. Adam Biales (NERL)**, Eric Emery (ORSANCO), Brent Johnson (NERL), Marc Mills (NRMRL), Jim Lazorchak (NERL), Karen Blockson (NERL), Amy Bergdale (R3), Lou Reynolds (R3), Frank Borsuk (R3), Vicki Blazer (USGS) 513 569 7094

**Proteomics Study of protein populations of one cell versus another**
**David Lattier**, Adam Biales, David Bencic – NERL-Cincinnati – 513 569 7976

**Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabonomics in Small Fish Models - David Bencic**, Rong Lin Wang, Iris Knoebl, David Lattier, Jim Lazorchak, NERL-Cincinnati, Gary Ankley, Dan Villeneuve, NHEERL-Duluth, Tim Collette, NERL-Athens, Partnerships: STAR Grant; Joint Genome Institute; Sandia National Laboratory – 513 569 7201

**An informatic approach to prioritizing eco-pharmaceutical research and Measuring relevant ecological exposures to Pharmaceuticals. - Mitch Kostich**, Jim Lazorchak, Greg Toth, NERL-Cincinnati – 513 569 7645

**RESEARCH & DEVELOPMENT**
*Building a scientific foundation for sound environmental decisions*
Fathead Minnow Short-Term Reproduction Assay

Gary Ankley – NHEERL- Duluth

Purpose
Detect chemical’s ability to interfere with sex steroid axis resulting in reproductive effects

- Estrogens, androgens, anti-estrogens, anti-androgens
- Altered steroid metabolism

Test Overview
- Initiated with mature, spawning fish
- 14-21 day pre-exposure followed by > 21 day chemical exposure
  - Behavior
  - Fecundity
  - Fertility
  - Hatch
  - Secondary sex characteristics
  - Gonadal status (GSI, histology)
  - Plasma vitellogenin
  - Plasma steroids (E2, T, KT)
<table>
<thead>
<tr>
<th>Chemical</th>
<th>MOA</th>
<th>Nominal Concentrations</th>
<th>Spawning Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxychlor</td>
<td>ER Agonist</td>
<td>0.5 and 5 µg/L</td>
<td>4/2</td>
</tr>
<tr>
<td>Methyltestosterone (12-d exposure due to mortality)</td>
<td>AR Agonist</td>
<td>0.2 and 2 mg/L</td>
<td>4/2</td>
</tr>
<tr>
<td>β-Trenbolone</td>
<td>AR Agonist</td>
<td>0.005, 0.05, 0.5, 5, and 50 µg/L</td>
<td>4/2</td>
</tr>
<tr>
<td>α-Trenbolone</td>
<td>AR Agonist</td>
<td>0.003, 0.01, 0.03, and 0.1 µg/L</td>
<td>1/1</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>AR Antagonist</td>
<td>200 and 700 µg/L</td>
<td>1/1</td>
</tr>
<tr>
<td>Flutamide</td>
<td>AR Antagonist</td>
<td>50 and 500 µg/L</td>
<td>4/2</td>
</tr>
<tr>
<td>Fadrozole</td>
<td>Aromatase Inhibitor</td>
<td>2, 10, and 50 µg/L</td>
<td>4/2</td>
</tr>
<tr>
<td>PFOS (14 d exposure at 1.0 mg/L due to mortality)</td>
<td>Aromatase Inhibitor</td>
<td>0.03, 0.1, 0.3 and 1.0 mg/L</td>
<td>1/1</td>
</tr>
<tr>
<td>Prometon</td>
<td>Aromatase Inhibitor</td>
<td>15, 50, 250, and 1250 µg/L</td>
<td>1/1</td>
</tr>
<tr>
<td>Fenarimol</td>
<td>Aromatase Inhibitor, ER Agonist, AR Antagonist</td>
<td>0.1 and 1.0 mg/L</td>
<td>1/1</td>
</tr>
<tr>
<td>Prochloraz</td>
<td>Aromatase Inhibitor, AR/ER Antagonist</td>
<td>0.03, 0.1, and 0.3 mg/L</td>
<td>1/1</td>
</tr>
</tbody>
</table>
Medaka 2-Generation Tests for Assessing the Effects of Endocrine-Disrupting Chemicals

Rodney Johnson – NHEERL Duluth

Objectives

- Determine the relationships between short- and long-term tests
- Evaluate potential for important long-term population-level effects
  - Sex reversals and intersex effects
  - Fecundity and fertility
  - Sex ratios
- Evaluate and, if observed, determine basis for trans-generational effects
- Evaluate relative sensitivities of activational (reproductive) vs. developmental life-stages to EDCs

Comparative Life-stage/Endpoint Assessments

<table>
<thead>
<tr>
<th>Development endpoints, (F₁ &amp; F₂)</th>
<th>Reproductive endpoints (F₁ &amp; F₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight and length</td>
<td>Fecundity</td>
</tr>
<tr>
<td>Secondary sex characters</td>
<td>Fertility</td>
</tr>
<tr>
<td>Genotypic sex</td>
<td>Reproductive Behavior</td>
</tr>
<tr>
<td>Phenotypic sex</td>
<td>Embryo mortality</td>
</tr>
<tr>
<td>Vitellogenin</td>
<td>Hatch</td>
</tr>
<tr>
<td>Histopathology</td>
<td>E2 and Trenbolone</td>
</tr>
</tbody>
</table>

RESEARCH & DEVELOPMENT
Building a scientific foundation for sound environmental decisions
Xenopus laevis Short-Term Metamorphosis Assay
Joe Tietge, NHEERL-Duluth

Purpose
- Detect chemical’s ability to interfere with thyroid hormone axis resulting in developmental effects.

Test Overview
- Initiated with tadpoles at onset of thyroid function
- 14-21 days exposure
  - Developmental stage
  - Thyroid histology
## Summary of Studies

<table>
<thead>
<tr>
<th>Chemical</th>
<th>MOA</th>
<th>Results</th>
<th>Developmental Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methimazole</td>
<td>Thyroid peroxidase inhibitor</td>
<td>+</td>
<td>Retarded</td>
</tr>
<tr>
<td>PTU</td>
<td>Thyroid peroxidase inhibitor</td>
<td>+</td>
<td>Retarded</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>Iodide uptake inhibitor</td>
<td>+</td>
<td>Retarded</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>UDPGT inducer</td>
<td>+</td>
<td>Retarded</td>
</tr>
<tr>
<td>Pregnenolone-16α-carbonitrile</td>
<td>UDPGT inducer</td>
<td>+/-</td>
<td>Retarded</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Corticosteroid receptor agonist</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>Corticosteroid receptor agonist</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>Estrogen receptor agonist</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>17β-Trenbolone</td>
<td>Androgen receptor agonist</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroid hormone receptor agonist</td>
<td>+</td>
<td>Accelerated</td>
</tr>
<tr>
<td>T3</td>
<td>Thyroid hormone receptor agonist</td>
<td>+</td>
<td>Accelerated</td>
</tr>
<tr>
<td>Iopanoic Acid</td>
<td>Deiodinase Inhibitor</td>
<td>+</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>
**17b-Trenbolone - AR Coordinated Research RTP & Duluth** Vickie Wilson and Earl Gray NHEERL/RTP

Methods:
CV-1 Transcriptional Activation

I. Cell Culture Medium made With Effluent

II. Measure Luciferase Activity

RESEARCH & DEVELOPMENT
Building a scientific foundation for sound environmental decisions

Trenbolone induced tubercle formation in females


Trenbolone dose response in MDA-kb2 stable cell line
Genomic and proteomic basis for interspecies extrapolations based upon estrogen and androgen receptor structure and function among animals.

VS Wilson, C Rider, M Cardon, LE Gray, Jr, LJ Guillette, GA LeBlanc, P. Hartig

OBJECTIVE

The Agency has a regulatory mandate to protect both human and ecosystem health.

The research described herein is designed to determine how similar ER and AR structure and function are among species from different classes.

This research is in response to GPRA Goal 8.2. and is detailed in Theme aims 6a and 6b.
Whole Lake Experiment EE2 Additions
Robert Flick, NERL-Cincinnati

Conducted by Fisheries and Oceans Canada
In collaboration with US EPA

Major Findings

- Fathead minnows and pearl dace showed elevated whole body concentrations of vitellogenin within 7 weeks of EE2 additions to Lake 260.
- Egg development delayed in fathead minnows and pearl dace; testes development impaired; testes-ova observed in males.
- Reproductive failure was observed in both of these minnow species during the second year of additions.
Ohio River EDC Instream Effects
Adam Biales, NERL-Cincinnati

Objective:
• Establish linkages between EDC occurrence and negative effects in wild fish
• Characterize the impact of EDCs from WWTP effluents on indigenous and deployed fish
• Determine the extent of EDC exposure/effects using probabilistic study design in a Great River system
• Integrate molecular/cellular indicators with traditional water quality metrics

Experimental Design
At selected POTW locations on the Ohio River, sampling stations upstream and downstream of discharge will be selected for the following:
• Collect water for lab exposure and gene expression assays
• Collect indigenous fish for measuring gene expression and histopathology
• Deploy FHM for gene expression assay
• Collect water samples for chemical characterization of hormones
• Evaluate passive sampling devices for characterizing EDC exposures for deployed and indigenous fish
Proteomics
David Lattier, David Bencic, Adam Biales NERL-Cincinnati

Yellow = Similar level of protein
Blue = Down-regulated protein
Red = Up-regulated protein

*Similar to microarrays – global expression changes – but is anonymous and no sequence information is necessary to measure changes.
Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabonomics in Small Fish Models
David Bencic – NERL-Cincinnati

Increasing Diagnostic (Screening) Utility

- **Phase 1:** FHM
- **Phase 2:** ZF
- **Phase 3:** FHM

Increasing Ecological Relevance

- **Molecular**
- **Cellular**
- **Organ**
- **Individual**
- **Population**

Long-term, ecologically-relevant whole-organism endpoints in FHM systems will be linked to short-term, diagnostic (molecular) endpoints in FHM systems by using zebrafish, and the numerous genomic tools available for this species, as a surrogate.

Systems modeling

Population modeling

Population modeling
Phase 2: Microarray Results

<table>
<thead>
<tr>
<th>Gene Expression</th>
<th>30 ng/L EE₂ Testis (48 h)</th>
<th>30 ng/L EE₂ Testis (96 h)</th>
<th>3.0 µg/L TRB Ovary (96 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up-regulated</td>
<td>492 ± 339</td>
<td>875 ± 356</td>
<td>856 ± 955</td>
</tr>
<tr>
<td>Down-regulated</td>
<td>556 ± 380</td>
<td>822 ± 356</td>
<td>847 ± 759</td>
</tr>
</tbody>
</table>

Total number of differentially-expressed genes in the testes (48 and 96 h) or ovaries of zebrafish exposed to 30 ng/L EE₂ or 3.0 µg/L TRB, respectively, as determined by microarray analyses. Numbers are mean ± S.D. (n=4 or 5) with differential expression set as p<0.001 and absolute fold change>1.3.
The model: data flow

Sales data for 939 products

Sum mass over 397 distinct active ingredients

Top 50: mass lo / dose
Top 50: mass hi / dose

Union of 2 top 50 lists = Top 65

List sorted by ascending L/dose

PEC / Min daily dose => L/dose

Mass X EA% => excreted activity (PEC)
### Top 65: mechanism of action

#### BETA-BLOCKERS
- atenolol
- metoprolol
- propranolol
- carvedilol

#### ANGIOTENSIN ANTAGONISTS
- lisinopril
- ramipril
- valsartan

#### ANTI-DIABETICS
- metformin
- insulin
- glipizide
- glyburide

#### ESTROGENS
- conjugated estrogens
- ethinyl estradiol
- estradiol

#### BETA-BLOCKERS
- carvedilol
- propranolol
- metoprolol
- atenolol

#### PEPTIDYL-TRANSFERASE INHIBITORS
- amoxicillin
- penicillin v

#### STATINS
- simvastatin
- atorvastatin
- rosuvastatin

#### H1 ANTIHISTAMINES
- meclizine
- promethazine
- cetirizine

#### ANTI-DIABETICS
- glyburide
- glipizide
- insulin
- metformin

#### NO AGONISTS
- nitroglycerin
- isosorbide mononitrate

**RESEARCH & DEVELOPMENT**

*Building a scientific foundation for sound environmental decisions*
Wastewater and Chemistry Projects

National Wastewater Treatment Plant (WWTP) Endocrine Disrupting Chemicals (EDCs) Screening Study - Jim Lazorchak, NERL-Cincinnati Marc Mills, Greg Sayles, NRMRL, 513 569 7076

Monitoring for Human-Use Pharmaceuticals and Drugs of Abuse in WWTPs and Source Waters, Tammy L Jones-Lepp, NERL/Las Vegas 702 798 2144

Origin, Transport and Fate of Synthetic Musk Compounds in the Las Vegas Basin Lantis Osemwengie, NERL/Las Vegas 702 798 2513
Collaborators NRMRL, Regions, States, WWTPs
Grab or Composite samples Collected from 50 Effluents, in 9 Regions/23 states

• Fathead Minnow (FHM) exposed to effluents for 24 hours
• Vg measured by Quantitative Polymerase Chain Reaction (QPCR) on RNA extracted from livers of exposed fish
• Chemical analyses for natural and synthetic estrogens –NRMRL

Findings
• 26% of effluents caused up-regulation of Vg expression in male FHMs Estrogenic exposure
• 4% of effluents caused down-regulation of Vg expression in female FHMs Androgenic exposure
• Chemical analysis confirmed estrogenic compounds in WWTP effluents
Monitoring for Human-Use Pharmaceuticals and Drugs of Abuse in WWTPs and Source Waters
Tammy L Jones-Lepp, NERL/Las Vegas

SPE – solid phase extraction – provides quick, accurate, and convenient method for grab sampling.

POCIS- Polar Organic Integrative Sampling – provides time-weighted average concentration of chemicals that can be related to risk assessments.


Data for methamphetamine and MDMA represent the first-ever report of these abused/illicit drugs as pollutants. MDMA = 3,4-methylenedioxymethamphetamine or “Ecstasy”.

NOTICE: Although this work was reviewed by EPA and approved for publication, it may not accurately reflect official Agency policy.
Origin, Transport & Fate of Synthetic Musk Compounds in the Las Vegas Basin

Lantis Osemwengie – NERL/Las Vegas

Galaxolide: polycyclic class

35 – 152 ppt

0.06 – 1.02 ppt

Musk Xylene: Nitro musk class

1400-4500 ppt
Drinking Water/Chemistry Research Projects

Use of Pharmaceuticals and Other Wastewater Chemicals as Indicators of Human Fecal Contamination
Susan Glassmeyer NERL-Cincinnati in collaboration with USGS 513 569 7526

Persistence of Wastewater Compounds During Drinking Water Treatment: Removal and Potential Exposure
Susan Glassmeyer NERL-Cincinnati in collaboration with USGS 513 569 7526
Use of Pharmaceuticals and Other Wastewater Chemicals as Indicators of Human Fecal Contamination

Susan Glassmeyer NERL-MCEARD

**Overall Objective**: Evaluate the utility of chemicals as indicators of impacts on water by *human* fecal material

- **Phase 1**: *Wastewater treatment plant (WWTP) study*. Collected samples downstream from 10 WWTPs to determine which compounds survive through treatment, and estimate environmental persistence. (*ES&T* 2005, 39, 5157-5169)

- **Phase 2**: *Lagrangian study*. Used dye tracer to get time of travel to sampling points downstream from 2 WWTPs. With this data, able to calculate pseudo first-order rate constants to quantify the decreases in concentration.

- **Phase 3**: *Epidemiological study*. Participated in the National Epidemiological and Environmental Assessment of Recreational (NEEAR) Water Study to determine if there is a link between these chemicals and negative health impacts.
Persistence of Wastewater Compounds During Drinking Water Treatment: Removal and Potential Exposure

Susan Glassmeyer NERL-MCEARD

Objective: Examine drinking water facilities impacted by human wastewater (due to proximity to WWTP discharges, or reclaimed water facilities) to determine the “worst case scenario” of persistence of wastewater compounds (esp. pharmaceuticals) through drinking water treatment.

USGS will be developing two new methods. The first will incorporate pharmaceuticals not currently included in their methods; the second will focus on disinfection/ degradation by products of compounds known to be present in the raw/ source waters (FY 06)

Sampling will occur in two rounds. First Round: The raw and finished water of 10-15 drinking water treatment facilities will be sampled to determine gross removal. Second Round: At least quarterly for one year, 2-4 drinking water treatment facilities will be sampled throughout the treatment process to gauge the effectiveness of each step, and determine any effects of seasonality on the compounds found in the water (FY 07-09)
Collaborations with EPA Regions, Other Federal Agencies, and Academia Using Omic Markers.

• Region 7 & U.S. FWS Missouri River at Omaha, NE. – Assessing potential for estrogenic exposure of endangered species – Pallid Sturgeon.

• Region 3 Smallmouth Bass projects South Branch Potomac and Main Stem Potomac River – USGS/WVA/USFWS

• Region 3/5 ORSANCO - EDC Genomic indicators and biocriteria metric development - Ohio River Project (Erich Emery, Lou Reynolds Reg 3)

• Region 5 Criteria and Water Quality Standards collaborations Nonylphenol Projects with St Cloud State University and University of Wisconsin Superior (Peter Howe, Heiko Schoenfuss, Al Alwan, Larry Zintek)

• Region 8 Review of 104 (b) Proposal and Collaboration: South Platte/Boulder Creek Project (David Norris)

• Region 9 Methods Development and Enhancing Region 9’s ability to assess estrogens in California streams. UC - Davis Trout/Fathead Minnow Vg project (Dan Riordan, Victor de Vlaming)

• Support for Office of Water CAFO Permits Program and Regions 4,5 and 6. Concentrated Animal Feedlot Operations