U.S. EPA's Research on the Ecological Exposure and Effects of Endocrine Disruption Chemicals & Pharmaceuticals

Principal Investigators & Contributors

Jim Lazorchak, NERL-EERD – Blue Collar Toxicologist!

Gary Ankley, Joe Tietge, Rodney Johnson, Sig Degitz NHEERL-Duluth – Ecological Effects

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

Bob Flick, Adam Biales, David Lattier, David Bencic, NERL-Cincinnati - Ecotoxicogenomics

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

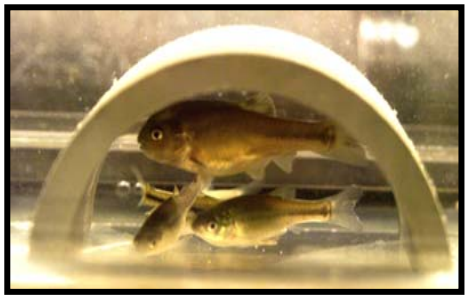
Whole Lake Experiment EE2 Additions. Robert Flick, Jim Lazorchak, Greg Toth, NERL-Cincinnati, Karen Kidd DFO – **513 569 7394**

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**



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)

List of EDCs/Pharmaceuticals Tested at Duluth

Chemical	MOA	Nominal Concentrations	Spawning Ratio
Methoxychlor	ER Agonist	0.5 and 5 μg/L	4/2
Methyltestosterone (12-d exposure due to mortality)	AR Agonist	0.2 and 2 mg/L	4/2
β-Trenbolone	AR Agonist	0.005, 0.05, 0.5, 5, and 50 μg/L	4/2
α-Trenbolone	AR Agonist	0.003, 0.01, 0.03, and 0.1 μg/L	1/1
Vinclozolin	AR Antagonist	200 and 700 μg/L	1/1
Flutamide	AR Antagonist	50 and 500 μg/L	4/2
Fadrozole	Aromatase Inhibitor	2, 10, and 50 μg/L	4/2
PFOS (14 d exposure at 1.0 mg/L due to mortality)	Aromatase Inhibitor	0.03, 0.1, 0.3 and 1.0 mg/L	1/1
Prometon	Aromatase Inhibitor	15, 50, 250, and 1250 μg/L	1/1
Fenarimol	Aromatase Inhibitor, ER Agonist, AR Antagonist	0.1 and 1.0 mg/L	1/1
Prochloraz	Aromatase Inhibitor, AR/ER Antagonist	0.03, 0.1, and 0.3 mg/L	1/1

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

Development endpoints, (F_1 & F_2)
Weight and length
Secondary sex characters
Genotypic sex
Phenotypic sex
Vitellogenin
Histopathology

Reproductive endpoints (F_1 & F_2)
Fecundity
Fertility
Reproductive Behavior
Embryo mortality
Hatch

E2 and Trenbolone

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

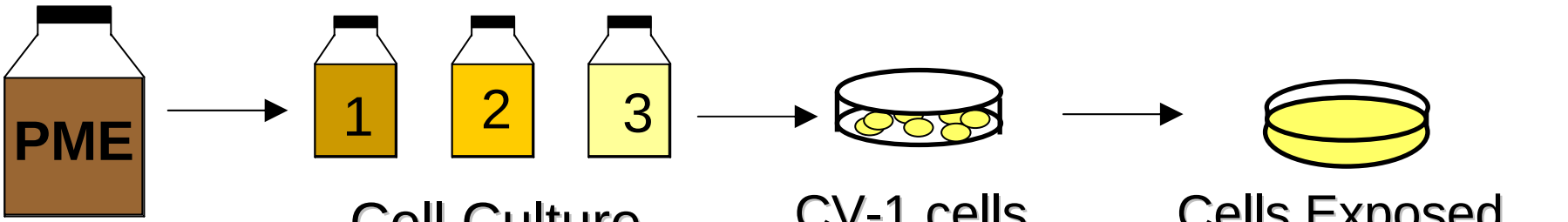
Chemical	MOA	Results	Developmental Effects
Methimazole	Thyroid peroxidase inhibitor	+	Retarded
PTU	Thyroid peroxidase inhibitor	+	Retarded
Perchlorate	Iodide uptake inhibitor	+	Retarded
Phenobarbital	UDPGT inducer	+	Retarded
Pregnenolone-16α –carbonitrile	UDPGT inducer	+/-	Retarded
Dexamethasone	Corticosteroid receptor agonist	-	None
Corticosterone	Corticosteroid receptor agonist	-	None
17β-Estradiol	Estrogen receptor agonist	-	None
17β-Trenbolone	Androgen receptor agonist	-	None
T4	Thyroid hormone receptor agonist	+	Accelerated
T3	Thyroid hormone receptor agonist	+	Accelerated
Iopanoic Acid	Deiodinase Inhibitor	+	Abnormal

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

CV-1 cells hAR MMTV-luciferase

Cells Exposed 1) CAFO in media 2) As above + OH-F

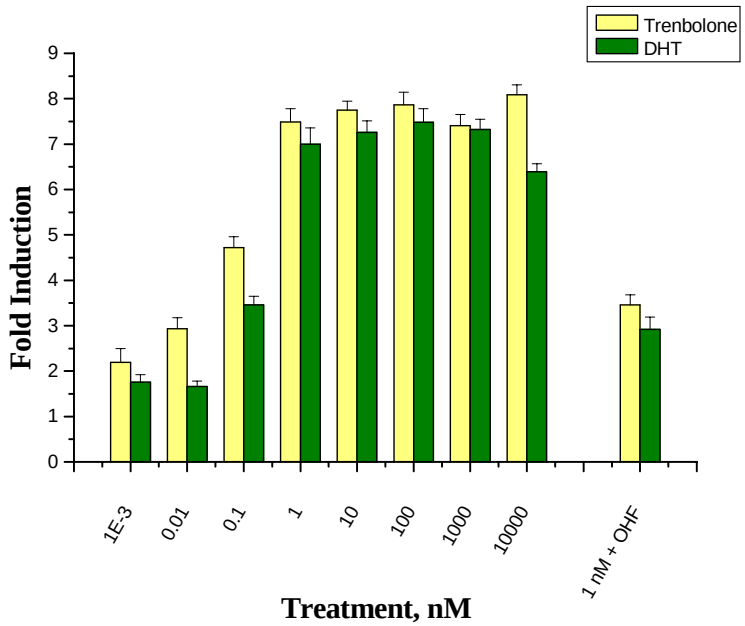
II.

Measure Luciferase Activity

17b-Trenbolone - Coordinated Research
RTD & Duluth

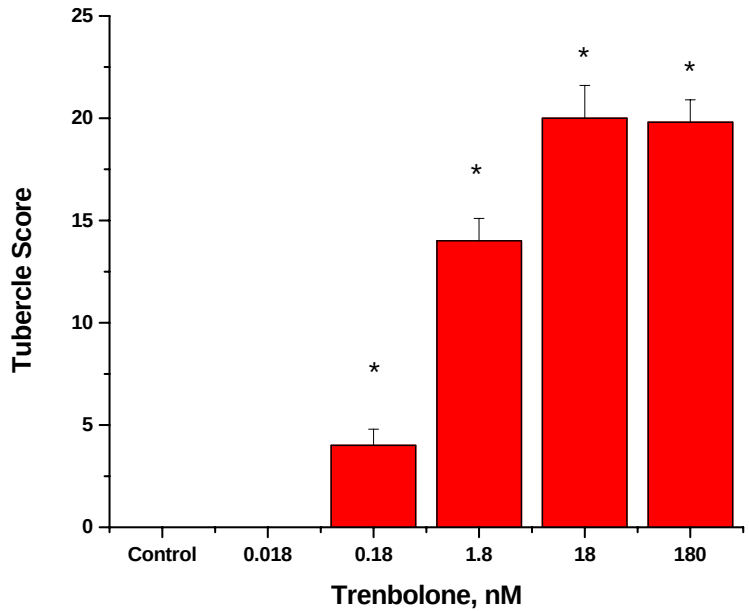
Wilson, et al, 2002, Tox. Sci , 70:202

Trenbolone dose response in MDA-kb2 stable cell line



Ankley, et al, 2003, Env. Tox Chem, 22:1350.

Trenbolone induced tubercle formation in females



Genomic and proteomic basis for interspecies extrapolations based upon estrogen and androgen receptor structure and function among animals.

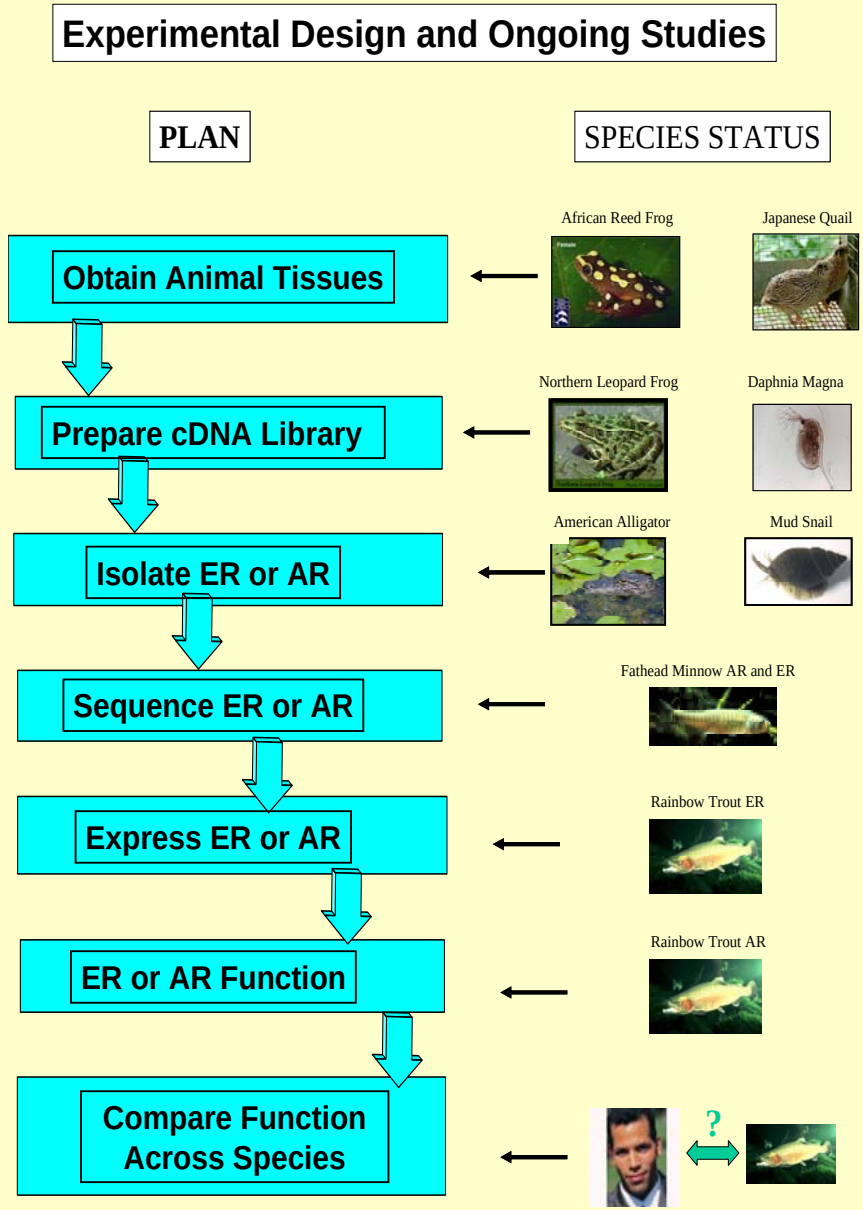
VS Wilson, C Rider, M Cardon, LE Gray, Jr, LJ Guillette, GA LeBlanc, P. Hartig

OBJECTIVE

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

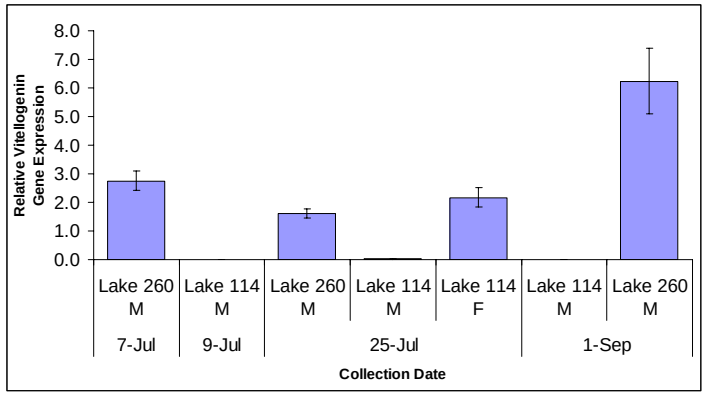
The research described herein is designed to determine how similar ER and AR structure and function are among species from different classes.

This research is in response to GPRA Goal 8.2. and is detailed in Theme aims 6a and 6b.



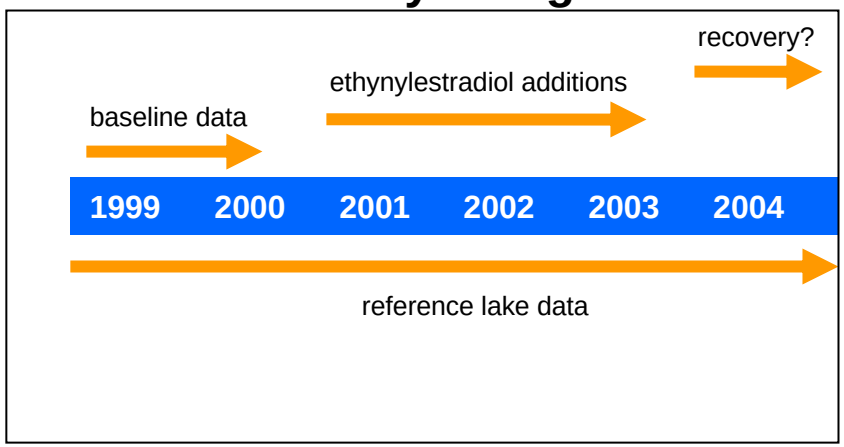
Whole Lake Experiment EE2 Additions

Robert Flick, NERL-Cincinnati



Vitellogenin gene expression in male fathead minnows

Study Design



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

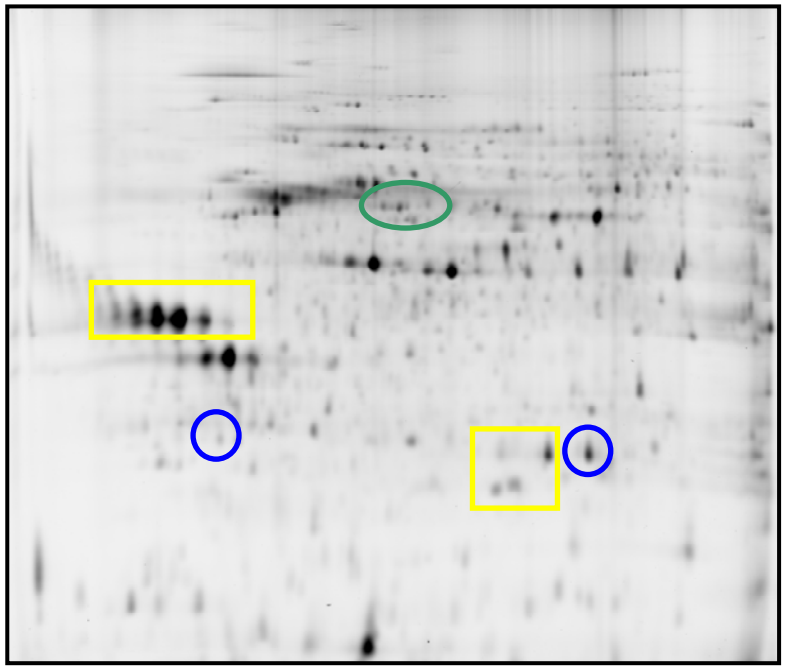
At selected POTW locations on the Ohio River, sampling stations upstream and downstream of discharge will be selected for the following:

- **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**

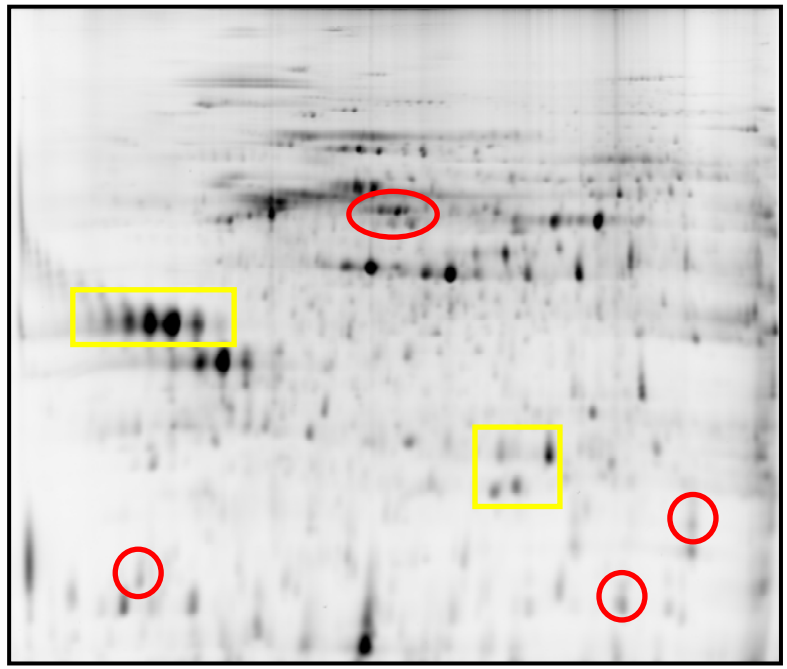
Proteomics

David Lattier, David Bencic, Adam Biales NERL-Cincinnati

FHM Control Brain



FHM Treated Brain



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

- Molecular
- Cellular
- Organ
- Individual
- Population

Increasing Ecological Relevance

Phase 1: FHM

Population modeling

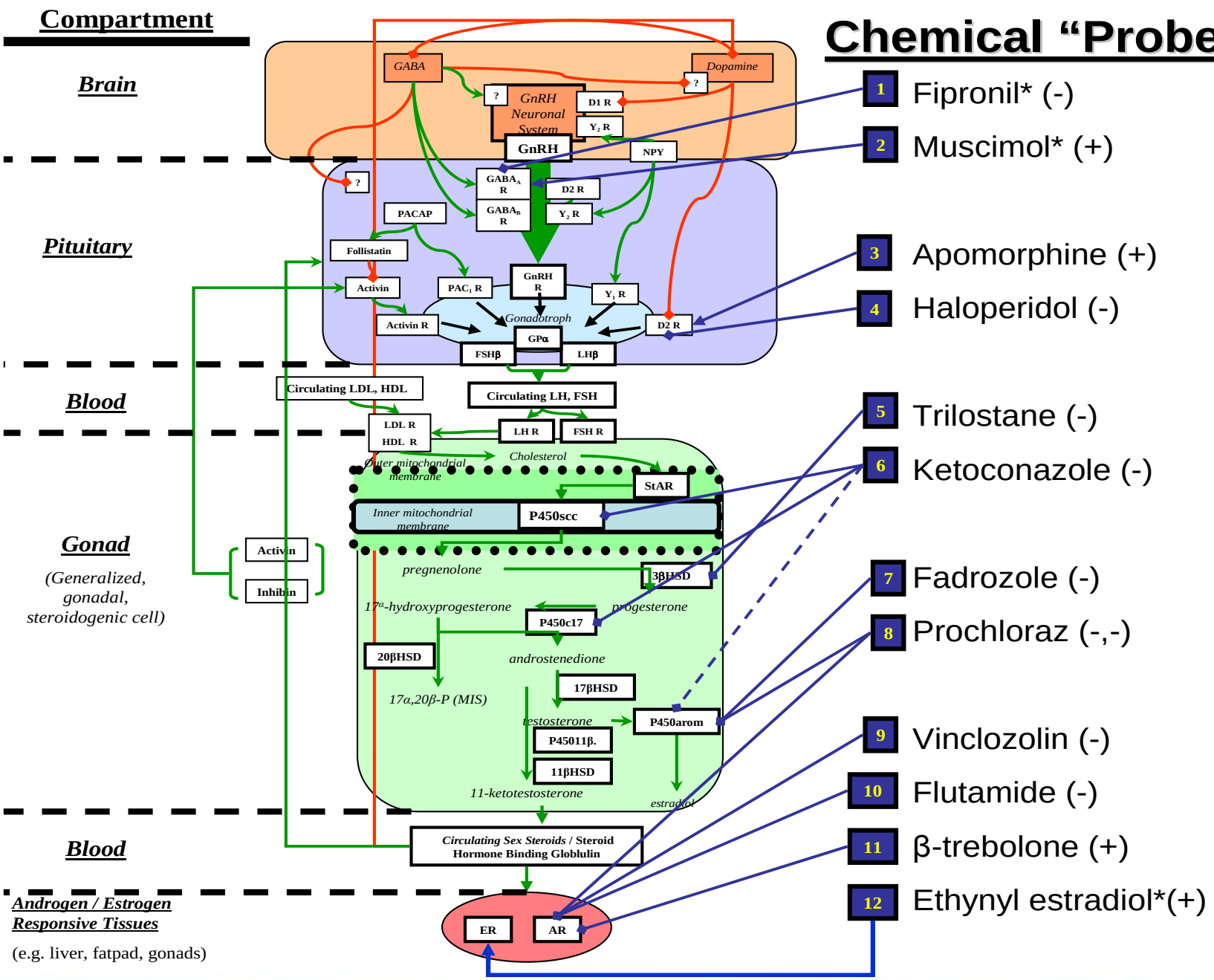
Phase 2: ZF

Systems modeling

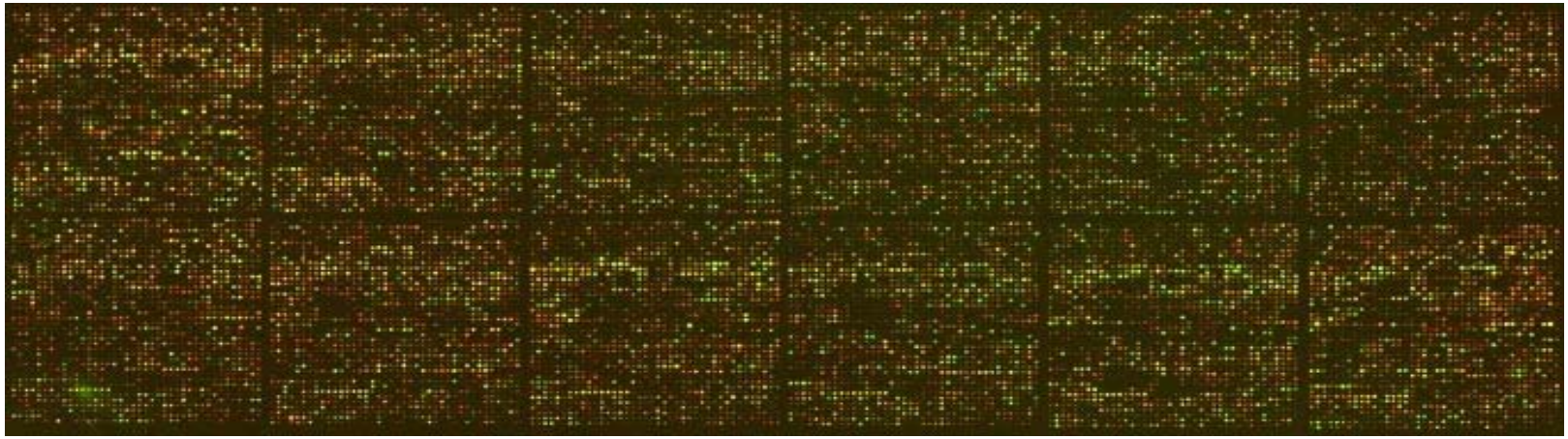
Phase 3: FHM

Systems modeling

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


Compartment
Brain
Pituitary
Blood
Gonad
(Generalized, gonadal, steroidogenic cell)
Blood
Androgen / Estrogen Responsive Tissues
(e.g. liver, fatpad, gonads)
GABA
Dopamine
GnRH Neuronal System
GnRH
D1 R
Y2 R
NPY
GABAA R
GABAB R
D2 R
PACAP
Follistatin
Activin
PAC1 R
GnRH R
Y1 R
Activin R
Gonadotroph
GPα
FSHβ
LHβ
Circulating LDL, HDL
Circulating LH, FSH
LDL R
HDL R
LH R
FSH R
Cholesterol
Outer mitochondrial membrane
StAR
Inner mitochondrial membrane
P450scc
Activin
Inhibin
pregnenolone
3βHSD
17α-hydroxyprogesterone
progesterone
P450c17
20βHSD
androstenedione
17βHSD
17α,20β-P (MIS)
testosterone
P450arom
P45011β.
11βHSD
11-ketotestosterone
estradiol
Circulating Sex Steroids / Steroid Hormone Binding Globlulin
ER
AR
Chemical "Probes"
1 Fipronil* (-)
2 Muscimol* (+)
3 Apomorphine (+)
4 Haloperidol (-)
5 Trilostane (-)
6 Ketoconazole (-)
7 Fadrozole (-)
8 Prochloraz (-,-)
9 Vinclozolin (-)
10 Flutamide (-)
11 β-trebolone (+)
12 Ethynyl estradiol*(+)

Phase 2: Microarray Results

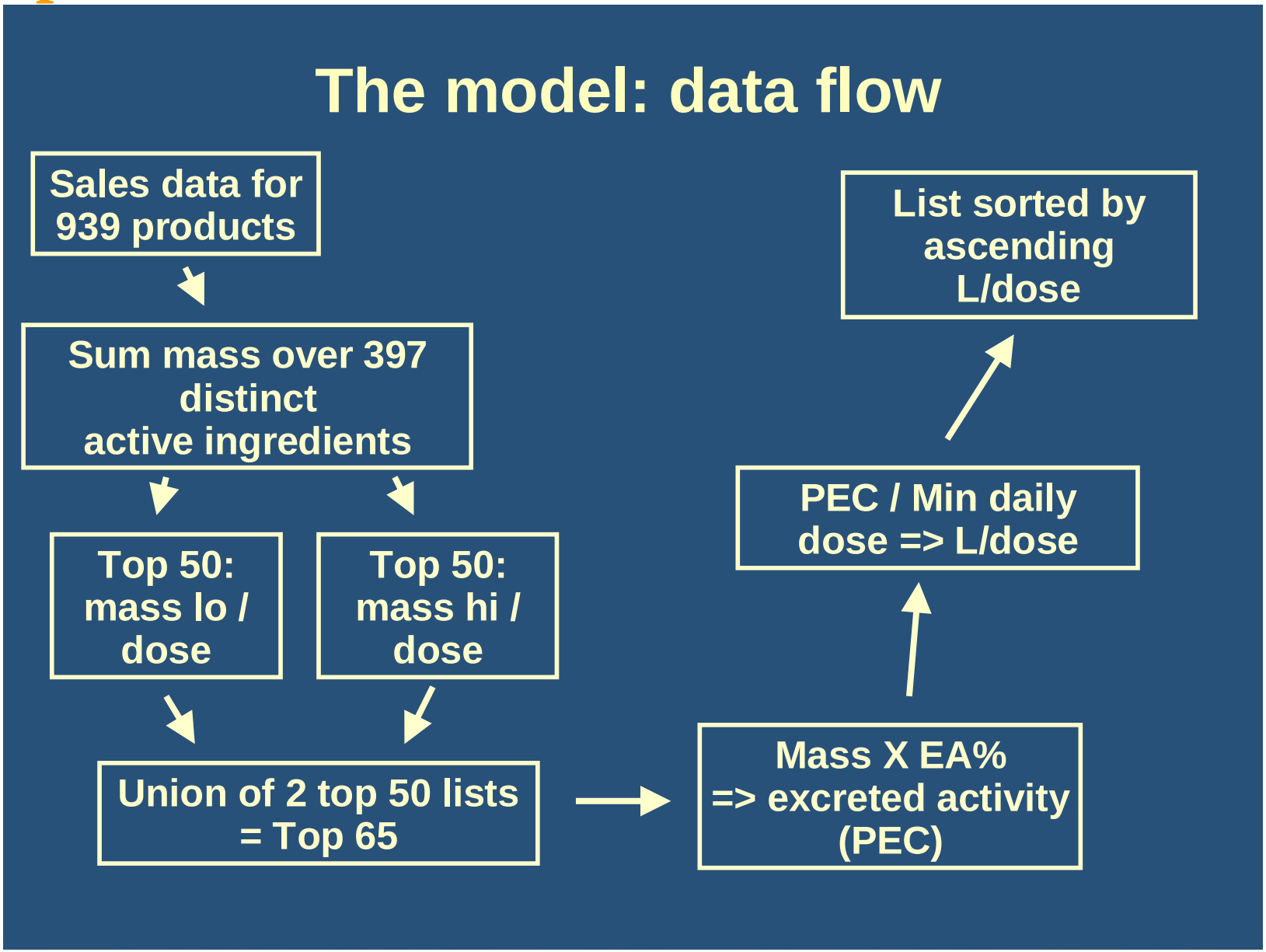


Gene Expression	30 ng/L EE_2 Testis (48 h)	30 ng/L EE_2 Testis (96 h)	3.0 µg/L TRB Ovary (96 h)
Up-regulated	492 ± 339	875 ± 356	856 ± 955
Down-regulated	556 ± 380	822 ± 356	847 ± 759

Total number of differentially-expressed genes in the testes (48 and 96 h) or ovaries of zebrafish exposed to 30 ng/L EE_2 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.

Eco-pharmaceutical research

Mitch Kostich, NERL-EERD



Top 65: mechanism of action

BETA-BLOCKERS
atenolol
metoprolol
propranolol
carvedilol

ANTI-DIABETICS
metformin
insulin
glipizide
glyburide

ESTROGENS
conjugated estrogens
ethinyl estradiol
estradiol

ANGIOTENSIN ANTAGONISTS
lisinopril
ramipril
valsartan

H1 ANTIHISTAMINES
cetirizine
promethazine
meclizine

STATINS
simvastatin
atorvastatin
rosuvastatin

NO AGONISTS
nitroglycerin
isosorbide mononitrate

PEPTIDYL-TRANSFERASE INHIBITORS
amoxicillin
penicillin v

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

National Wastewater Treatment Plant (WWTP) Endocrine Disrupting Chemicals (EDCs) Screening Study

Jim Lazorchak, NERL-Cincinnati

Collaborators NRMRL, Regions, States, WWTPs
Grab or Composite samples Collected from 50 Effluents, in 9 Regions/23 states

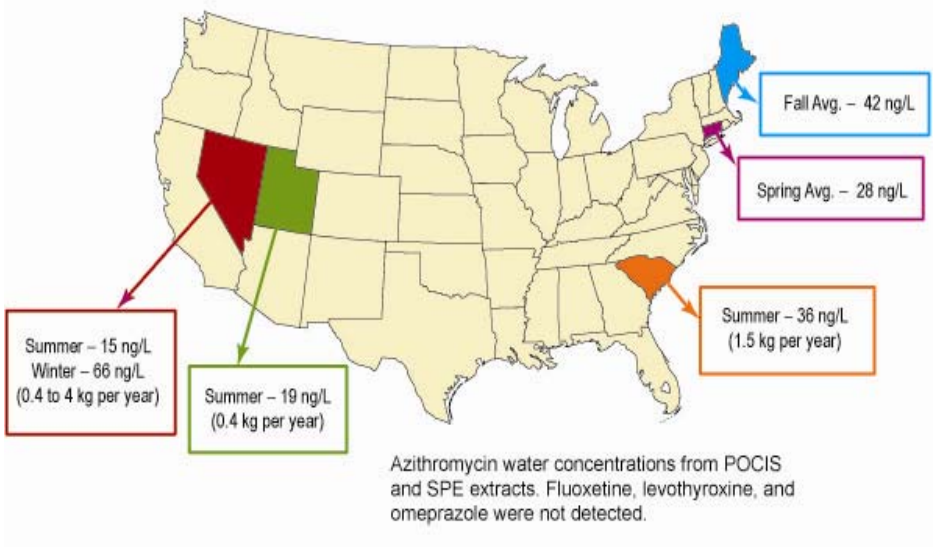
- Fathead Minnow (FHM) exposed to effluents for 24 hours
- Vg measured by Quantitative Polymerase Chain Reaction (QPCR) on RNA extracted from livers of exposed fish
- Chemical analyses for natural and synthetic estrogens –NRMRL

Findings

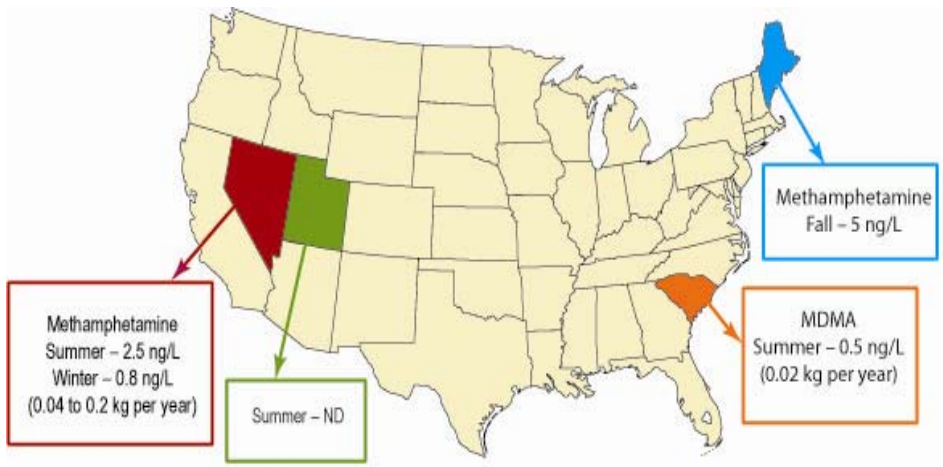
- 26% of effluents caused up-regulation of Vg expression in male FHMs Estrogenic exposure
- 4% of effluents caused down-regulation of Vg expression in female FHMs Androgenic exposure
- Chemical analysis confirmed estrogenic compounds in WWTP effluents

Monitoring for Human-Use Pharmaceuticals and Drugs of Abuse in WWTPs and Source Waters

Tammy L Jones-Lepp, NERL/Las Vegas



Azithromycin water concentrations from POCIS and SPE extracts. Fluoxetine, levothyroxine, and omeprazole were not detected.



Detection of methamphetamine and MDMA corresponds to regions of heightened drug usage according to the U.S. Drug Enforcement Agency.

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

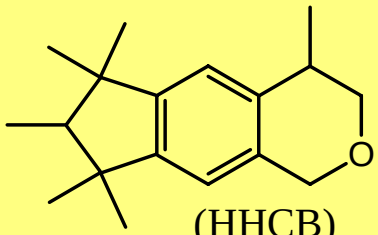
POCIS- Polar Organic Integrative Sampling – provides time-weighted average concentration of chemicals that can be related to risk assessments.

u-LC-ESI/ITMS – micro-liquid chromatography-electrospray ionization/ion trap mass spectrometry – provides determinative method for accurate identification and quantitation of unknowns.

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

Origin, Transport & Fate of Synthetic Musk Compounds in the Las Vegas Basin

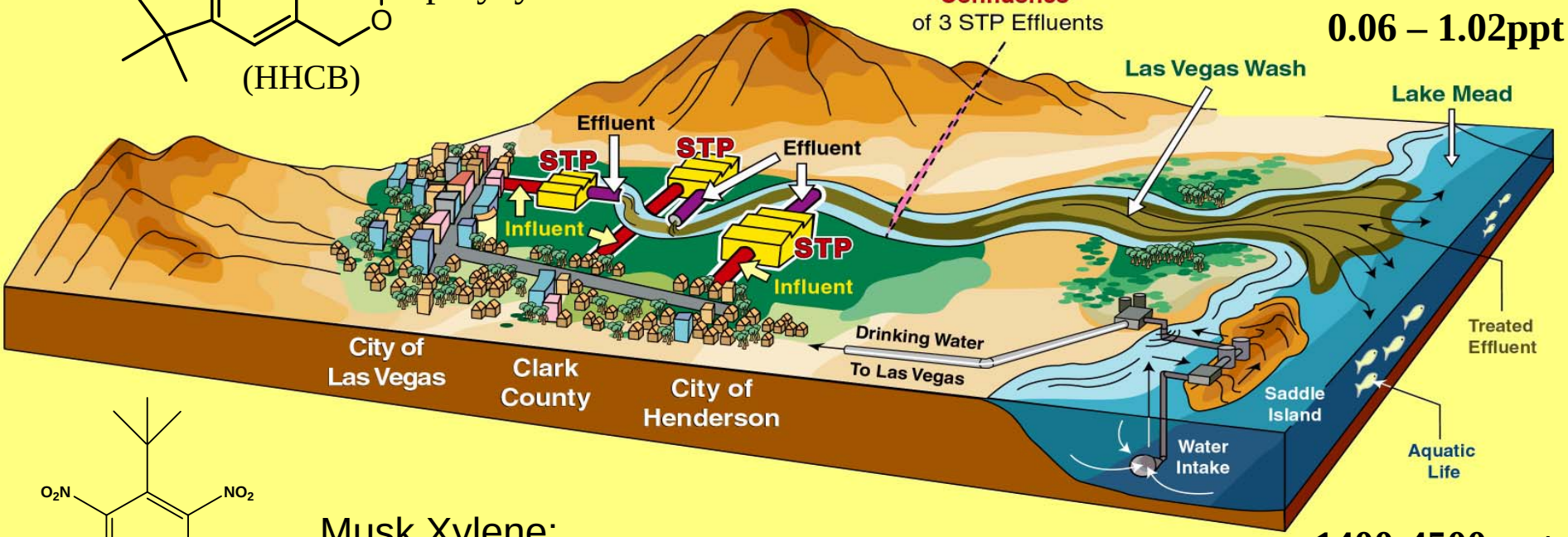
Lantis Osemwengie – NERL/Las Vegas



(HHCB)

Galaxolide:
polycyclic class

35 – 152 ppt



0.06 – 1.02ppt

MX

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

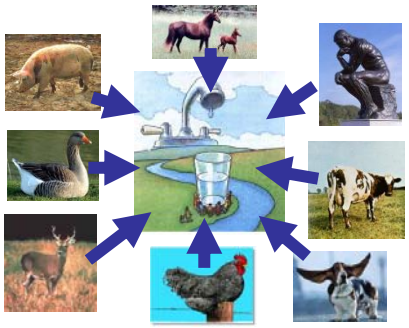
Persistence of Wastewater Compounds During Drinking Water Treatment: Removal and Potential Exposure

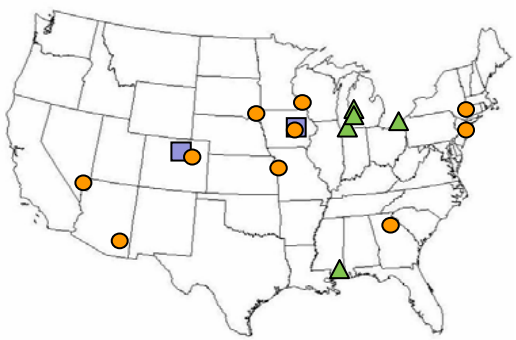
Susan Glassmeyer NERL-Cincinnati in collaboration with USGS

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

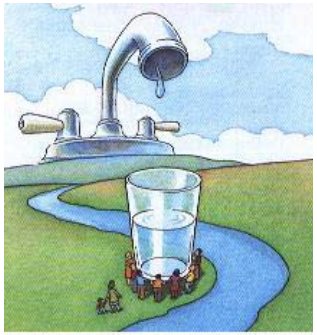
●Phase 1: **Wastewater treatment plant (WWTP) study.** Collected samples downstream from 10 WWTPs to determine which compounds survive through treatment, and estimate environmental persistence. (*ES&T* **2005**, *39*, 5157-5169)

■ 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**