EPA's Endocrine Disruptors Research Program: Unique Among Research Organizations

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EDCs STAR-EPA Workshop
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Outline

- Why is EPA studying EDCs?
- How is EPA's research program unique?
- What research is EPA conducting? What science questions are being addressed?
- How is the research evaluated? What information is gathered from the grantees and EPA scientists?
- What are the future activities?

Why is EPA studying EDCs?

- Evidence suggests that environmental exposure to chemicals that mimic hormones cause adverse effects in wildlife and may do so in humans

DENVER POST.

October 3, 2004

Mutant fish prompt concern

Study focuses on sewage plants

The Washington Post

Oct 15, 2004

Male Bass in Potomac Producing Eggs; Pollution Suspected Cause of Anomaly in River's South Branch

NIGHTLY NEWS WITH TOM BROKAW

FREE VIDEO

LAUNCH

Nov. 8: Researchers have discovered male fish that have developed female sexual organs because of estrogen and pollutants in the water. NBC's Tom Costello reports.

Nightly News

http://www.msnbc.msn.com/

Male fish becoming female?

Researchers worry about estrogen and pollutants in the water

Why is EPA studying EDCs?

- Evidence suggests that environmental exposure to chemicals that mimic hormones cause adverse effects in wildlife and may do so in humans
- Chemicals of concern (i.e., pesticides, industrial) are EPA's responsibility (e.g., TSCA, FIFRA, FQPA, SDWAA)
- Congressional mandates in 1996 raised scientific questions and research needs

EPA's Legislative Mandates (August 1996)

- **Food Quality Protection Act**
  - Must screen pesticides for estrogenic effects that may affect human health
  - Must use appropriate validated test systems or other scientifically relevant information
  - Can include other endocrine effects
- **Safe Drinking Water Act**
  - Can screen drinking water contaminants to which substantial numbers of persons are exposed

Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC)

- Chartered Oct 16, 1996 (www.epa.gov/scipoly/oscpendo)
- 39 members representing broad constituencies
- Recommendations proposed in 1998:
  - Estrogen, androgen and thyroid
  - Human and ecological effects
  - Broad universe of chemicals
  - Tier 1
    - *In vitro* and *in vivo* screens
    - Detect potential to interact with endocrine system
  - Tier 2
    - Multi-generation studies covering a broad range of taxa
    - Provide data for hazard assessment

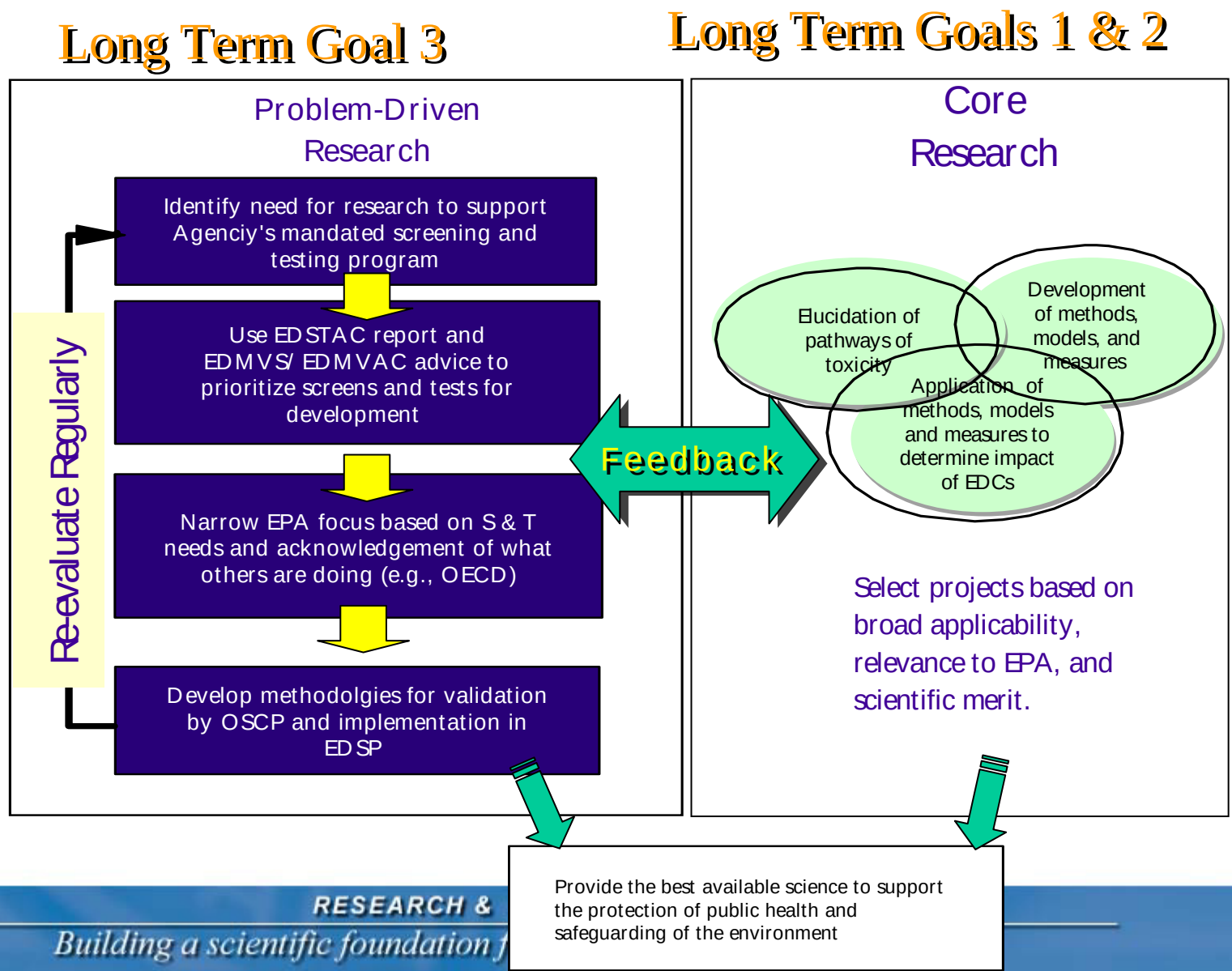
Why is EPA studying EDCs?

- Evidence suggests that environmental exposure to man-made chemicals that mimic hormones may cause adverse health effects in human and wildlife populations
- Chemicals of concern (i.e., pesticides, industrial) are EPA's responsibility (e.g., TSCA, FIFRA, FQPA, SDWAA)
- Congressional mandates in 1996 raised scientific questions and research needs
- Many uncertainties in our knowledge of endocrine disruptors
  - nature of effects (e.g., developmental/reproductive, cancer, neurobehavioral)
  - extent of the problem (e.g., declining wildlife populations, impacts on human male reproductive health)
  - dose-response relationships (e.g., which chemicals, what levels of exposure, shape of dose-response curve)

Diverse Nature of Research Program - Unique Among Research Organizations

- Multi-disciplinary set of research areas for both human health and wildlife – cuts across the risk assessment/risk management paradigm
- Research partners – bring diverse talents to address a science question
  - Across divisions within a lab
  - Across National Labs
  - With scientists from academia, other federal agencies, industry
- Research approaches
  - Computational, field, lab
  - Molecular to whole organisms
  - Invertebrates to humans
  - Biological, analytical, engineering

EDCs Research: Problem Driven & Core



Extramural Research Program

- Supported through STAR Program since '96
  - EPA-only RFAs in 1996, 1997, 2004, 2005, 2006
  - Multi-Agency participation in 1998/99 and 2000
  - EPA-only Computational Toxicology RFAs in 2002 and 2003
- Portfolio includes 48 grants (www.epa.gov/ncer)
  - Broad array of topics, species, chemicals
  - Support approximately $38 M total
    - PIs have received > $15.5 M in additional funding
  - Comp Tox supporting 7 grants ($4.8 M)
  - Grants awarded in other research programs - >18 (>$4.6 M)

Lessons Learned in EDCs Extramural Research Program

- Value of STAR Grantee Workshops with EPA and other scientists
  - 1998 (with NIEHS); 2002; 2004 (epidemiology); 2006
    - Current WS presenters from the 2000 (epidemiology), 2004 (low-dose) and 2005 (exposure) RFAs
- Topics of early RFAs were broad and cast a large net
  - Subsequent RFAs have been more targeted
- Where amenable, recently more awards have been made as cooperative agreements instead of grants
  - Provides a greater opportunity of sharing of data and leveraging resources
  - Appears to be a win-win situation

Multi-Year Plan (2000-2012): Long-Term Goals

- Provide a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors
- Determine the extent of the impact of endocrine disruptors on humans, wildlife, and the environment
- Support EPA's screening and testing program

Key Science Questions

- What are the chemical classes of interest and their potencies?
  - What are their mechanisms/modes of action
- What are the effects of exposure to multiple EDCs and will a TEF approach be applicable?

Examples of Research

LTG 1: Providing a Better Understanding of Science

- Determining classes of chemicals that act as EDCs and their potencies
  - (Anti)androgens, (anti)estrogens, antithyroids
- Investigating mode of action of certain EDCs
  - Results of studies on atrazine, a commonly used herbicide, and vinclozolin, a fungicide, were critical to improving the Agency's risk assessments and setting tolerances

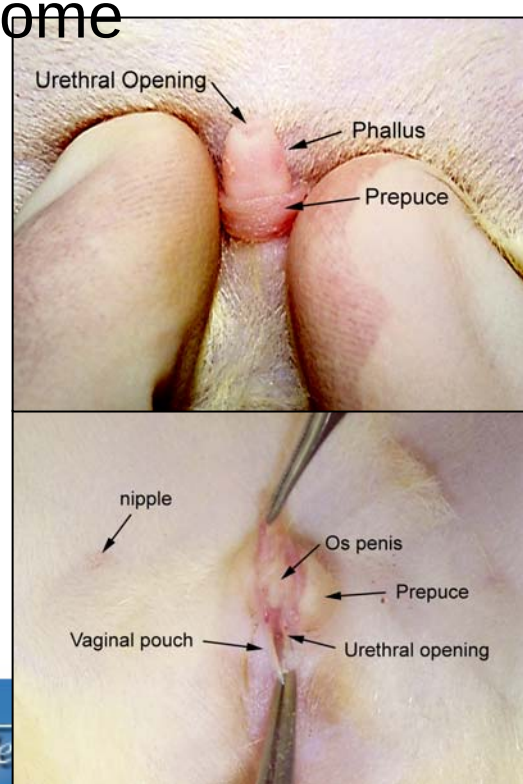
Examples of Research

LTG 1: Providing a Better Understanding of Science

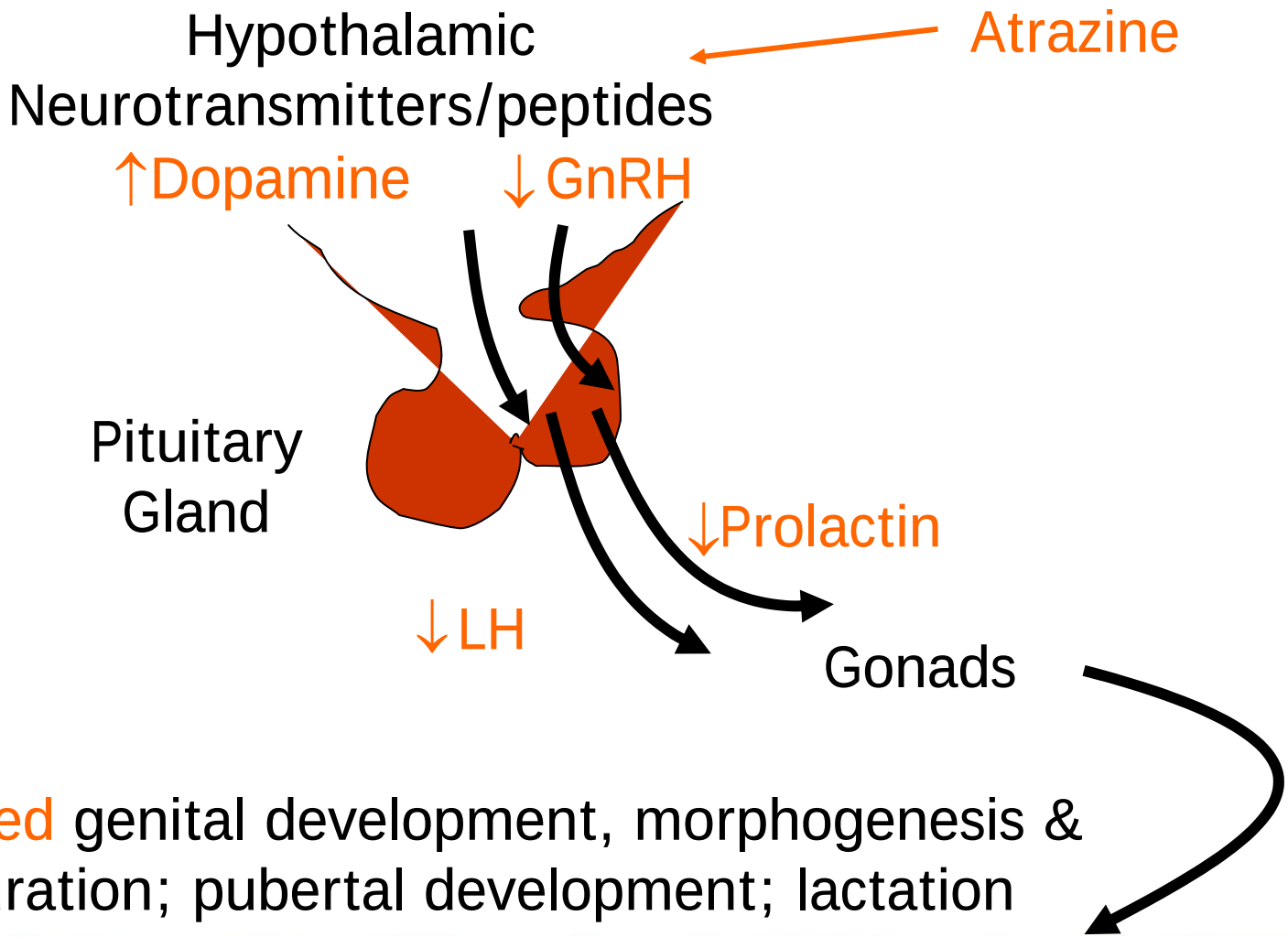
Pioneering research on androgens/anti-androgens

- Characterize chemicals that alter testosterone synthesis or androgen receptor function
- Differential effects predictive of pattern of outcome
  - AR inhibitors
  - Synthesis inhibitors with Leydig cell involvement
- Studying approaches to cumulative risk to EDCs – using chemicals that disrupt androgen signaling *in utero* as a model
  - Studies on mixtures of phthalates with & without AR antagonists
  - Effects appear dose additive

Gray et al. 2004, Adv Exp Med Biol 545: 217-241



Example: Neuroendocrine Influences of Exposures during Development



Examples of Research

LTG 1: Providing a Better Understanding of Science

- Characterizing cellular and molecular mechanisms of abnormal reproductive development
  - Effects of methoxychlor and vinclozolin during testis development and subsequent impact on male fertility – transgenerational epigenetic effects

Anway et al. 2005. Science 308:1466-9

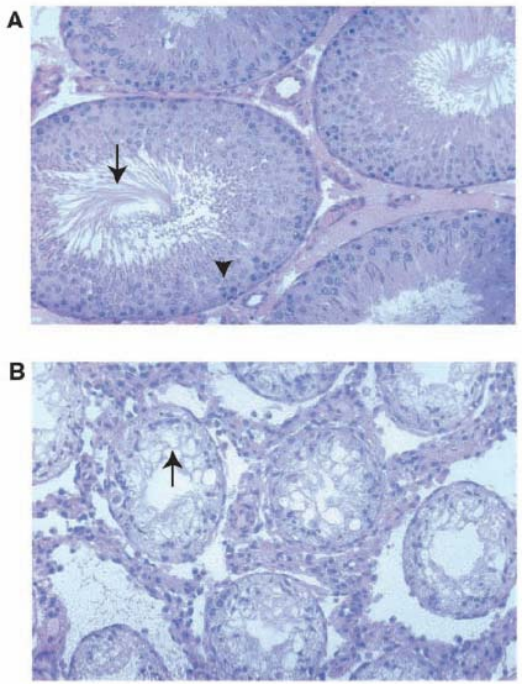
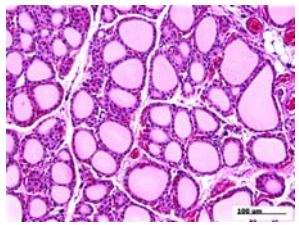


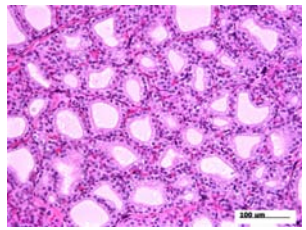
Fig. 2. Testis histology from control (A) and vinclozolin treatment (B) 100-day-old F_3 generation animals, ×200 magnification. The vinclozolin F_3 generation male is a representative infertile male. Arrow in (A) identifies the tails of elongate spermatozoa in the seminiferous tubule lumen; arrowhead labels spermatocytes in the tubule epithelial layer. Arrow in (B) identifies the lack of germ cells in the seminiferous tubule. Methods are provided in SOM.

Examples of Research

LTG 1: Providing a Better Understanding of Science

- Thyroid hormone homeostasis as a target for environmental chemicals





  - Perchlorate, PBDE-71, Linuron, PCBs, Dioxin-like chemicals, PTU, Methimazole, PFOS, PFOA

Key Science Questions

- What are the dose-response characteristics in the low dose region?

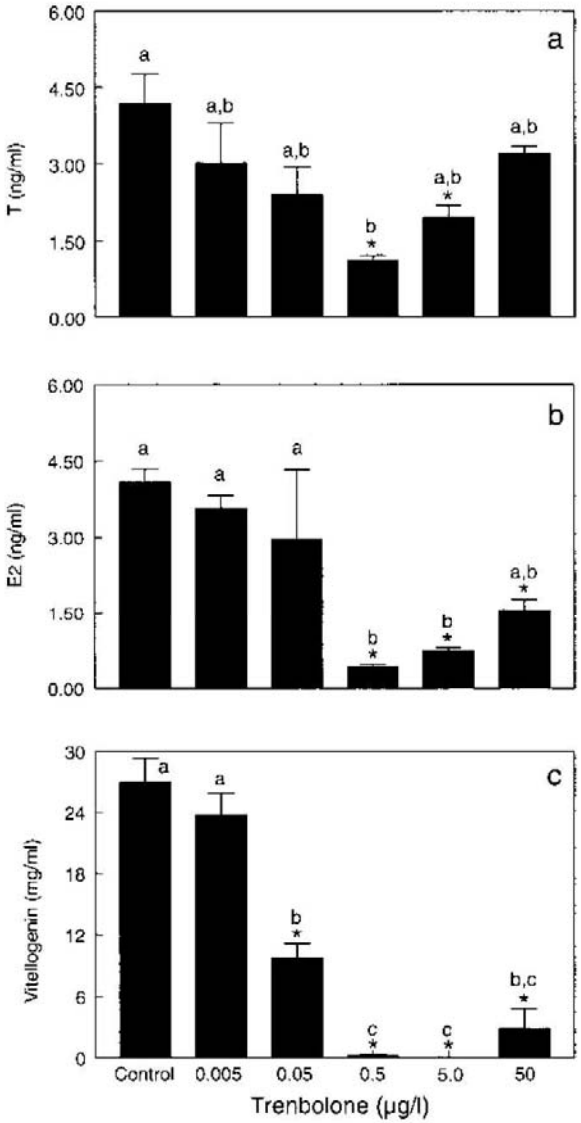
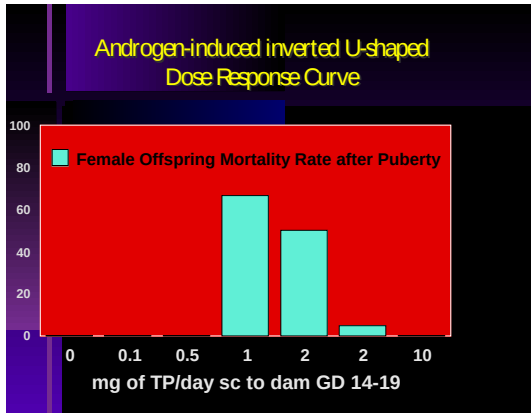


Fig. 9. Effects of 21-d exposure to β-trenbolone on fathead minnow female plasma concentrations of (a) testosterone (T), (b) β-estradiol (E_2), and (c) vitellogenin. Data are expressed as mean (standard error, $n = 3$). Values denoted by asterisks differed significantly from the control. Lettering above the bars indicates similarities and differences across the treatments based on multiple comparisons among all the groups (Tukey's test).

LTG 1: Providing a Better Understanding of Science

Determining the dose-response curves for EDCs at environmentally relevant concentrations

- *in vitro* and *in vivo*, U and inverted-U shaped dose response curves are not uncommon
- many curves appear linear in the low dose range while other responses to vinclozolin display a threshold



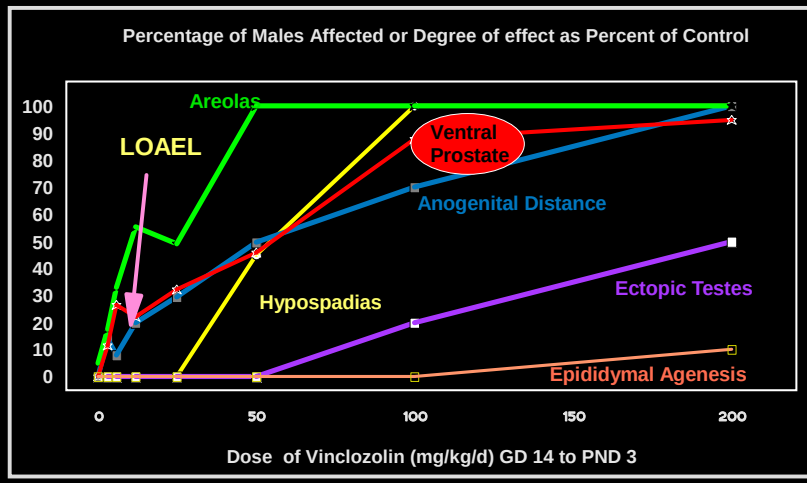
Awarded 3 grants in 2004 to focus on effects approaching environmentally relevant levels of exposure

Gray et al. 1999. Toxicol Ind Health 15(1-2): 48-64

Wolf et al. 2002. Toxicol Sci 65(1): 71-86

Ankley et al. 2003. Environ Toxicol Chem 22(6): 1350-60

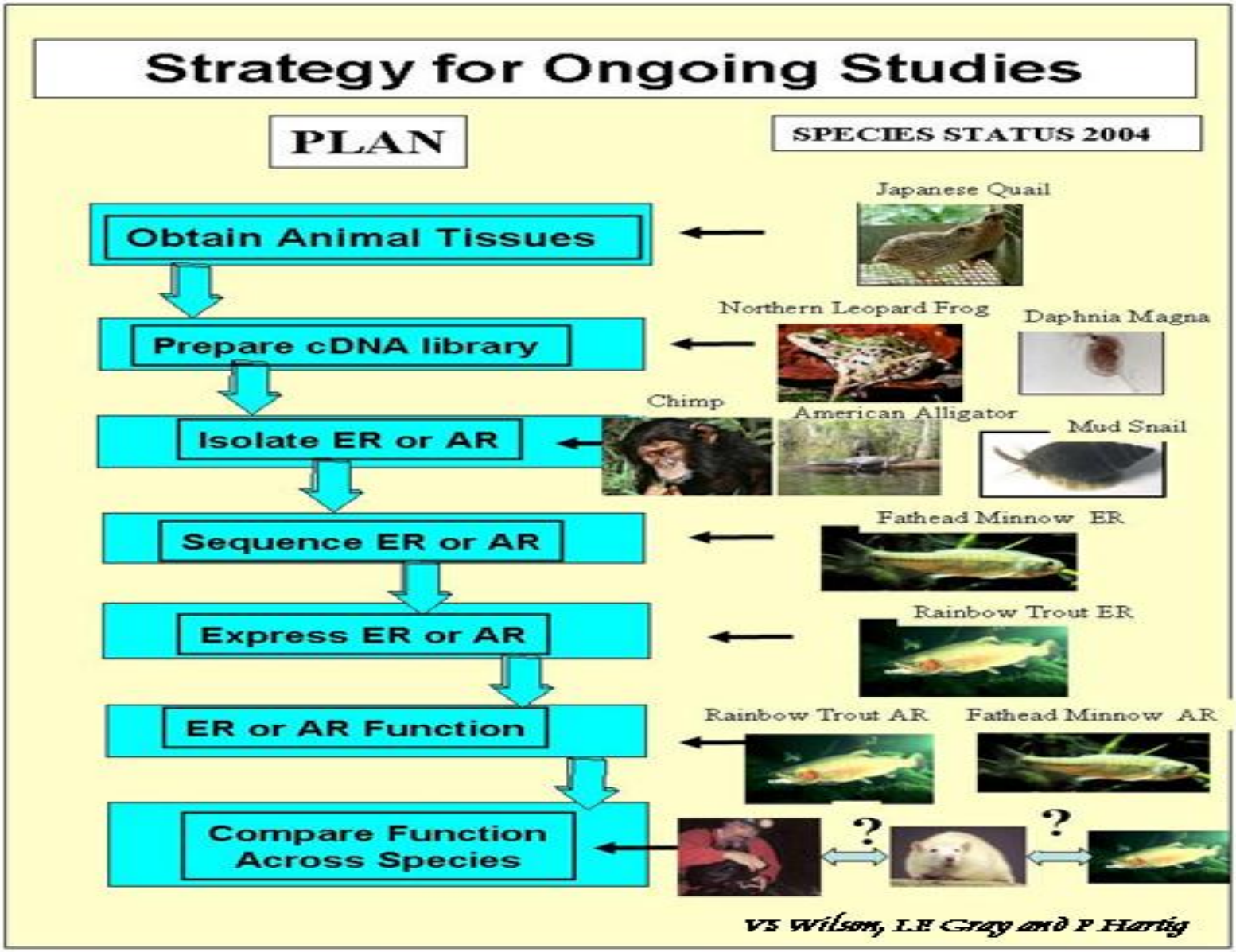
Data used by USEPA in their Risk assessment to set aPAD and limit acute dietary exposure to vinclozolin (Gray et al., 1999) including use of extra 10 x FQPA factor



Key Science Questions

- What extrapolation tools are needed?
  - To what extent can we extrapolate across species?

Examining the Ability to Extrapolate Across Species



Wilson et al., 2004. Environ Sci Technol 38(23):6314-21

Key Science Questions

- What are the major sources and environmental fates of EDCs?
- How and to what extent are human and wildlife populations exposed to EDCs?
- What effects are occurring in exposed humans and wildlife populations?
- What extrapolation tools are needed?
  - To what extent can we extrapolation from the individual to population level effects?
- How can unreasonable risks be managed?

Examples of Research

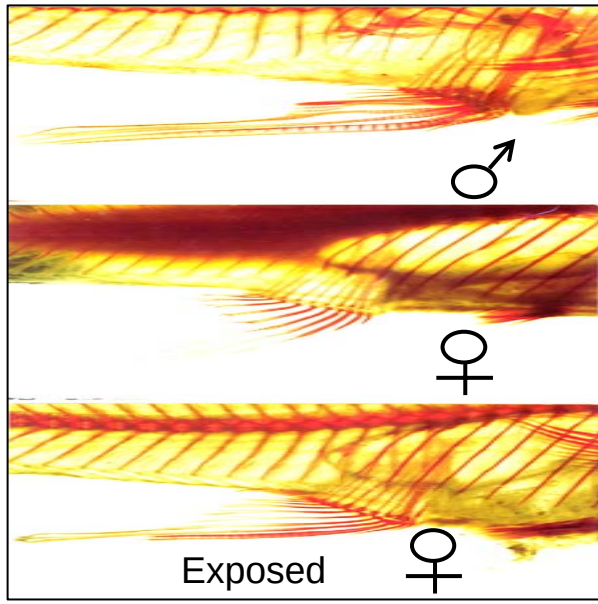
LTG 1: Providing a Better Understanding of Science

- Identifying major sources of EDCs entering the environment, focusing on:
  - wastewater treatment plants
  - drinking water treatment plants
  - confined animal feeding operations
- Developing tools to minimize exposures to EDCs

Examples of Research

LTG 2: Determining the Extent of the Impact of EDCs

- Paper Mill Effluents
  - Identified androgenic compounds and masculinization of female fish
- Waste Water Treatment Effluents
  - Identified feminization of male fish
  - 50 Effluents Study across 10 EPA Regions
- Drinking Water
  - Steroid hormones – analytical chemistry
- Developing novel methods to characterize exposures to mixtures



Mosquitofish

Buckeye Plant, Fenhalloway River, FL

Courtesy of Lou Guillette, UF

Examples of Research

LTG 2: Determining the Extent of the Impact of EDCs

- CAFOs – cross-laboratory effort
  - High levels of estrogens found in swine lagoons
  - Androgenic activity found in run-off from cattle farms
  - Characterized impact of exposures on current aquatic organisms and estimated future population-level effects
  - Determined temporal patterns of androgenic activity and concentrations of both α- and β-trenbolone (metabolites of trenbolone acetate implanted in cattle) in feedlot discharge
  - Topic of current RFA – potential for co-operative agreements

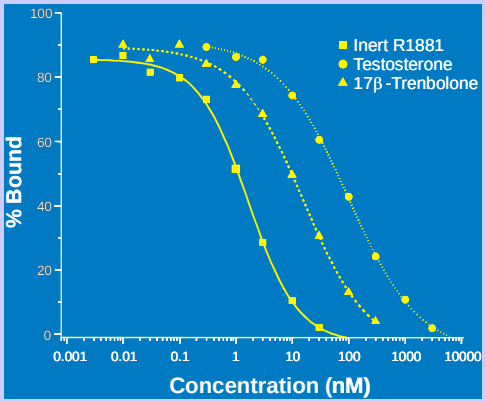
Examples of Research

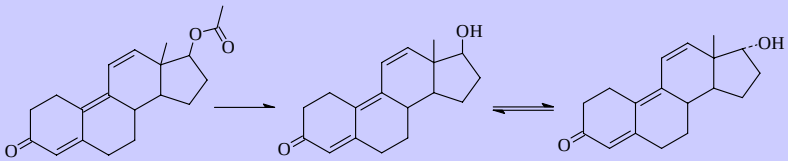
LTG 2: Determining the Extent of the Impact of EDCs

- Determining the magnitude of adverse impacts on wildlife
  - Evidence that EDCs are affecting wildlife at individual level
  - Evidence that EDC effects in individuals are causing population-level effects
  - What tools are needed to provide linkage between population level effects and diagnostic evidence of EDC impacts

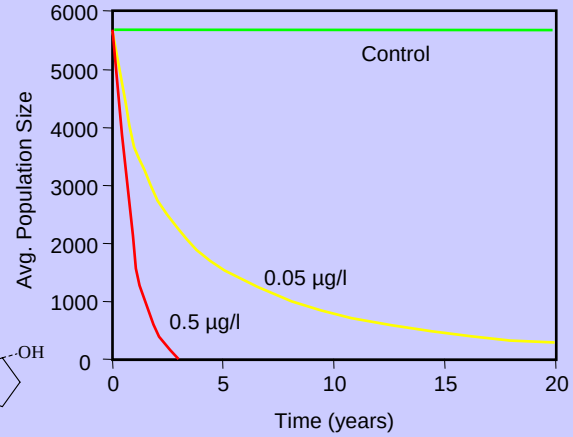
Linkage of Mechanistic Responses to Population-Level Effects: An Example

Androgen Receptor Binding

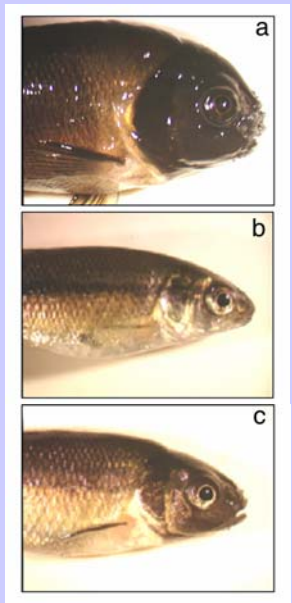




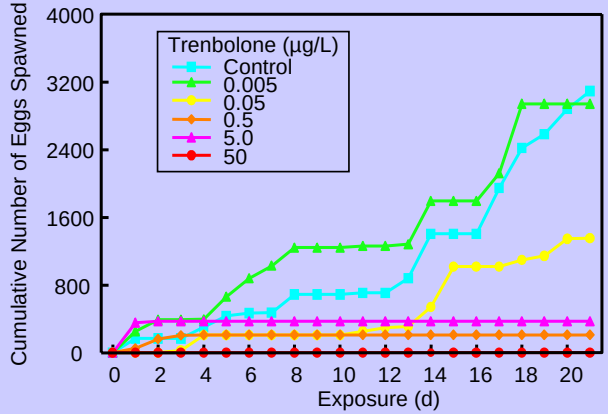
Population Dynamics



Masculinization by Trenbolone



Effects on Reproduction



Ankley et al. 2003, Env Tox Chem 22:1350-1360

Miller & Ankley. 2004. Ecotox Env Saf 59:1-9

Examples of Research

LTG 2: Determining the Extent of the Impact of EDCs

- Determining the magnitude of adverse impacts of EDCs on human health
  - Supporting 12 epidemiology studies across federal agencies
  - Exposure to high levels of PBBs prenatally and via breast milk may impact puberty in girls
  - Conducted large scale exposure studies to assess exposures of children to environmental chemicals, including some suspected EDCs

Key Science Questions

- Do our testing guidelines adequately evaluate potential endocrine-mediated effects?
  - How can we develop a battery of *in vitro* and *in vivo* screens and tests to address the mandates to implement a screening and testing program?

Endocrine Disruptors Screening Program Framework

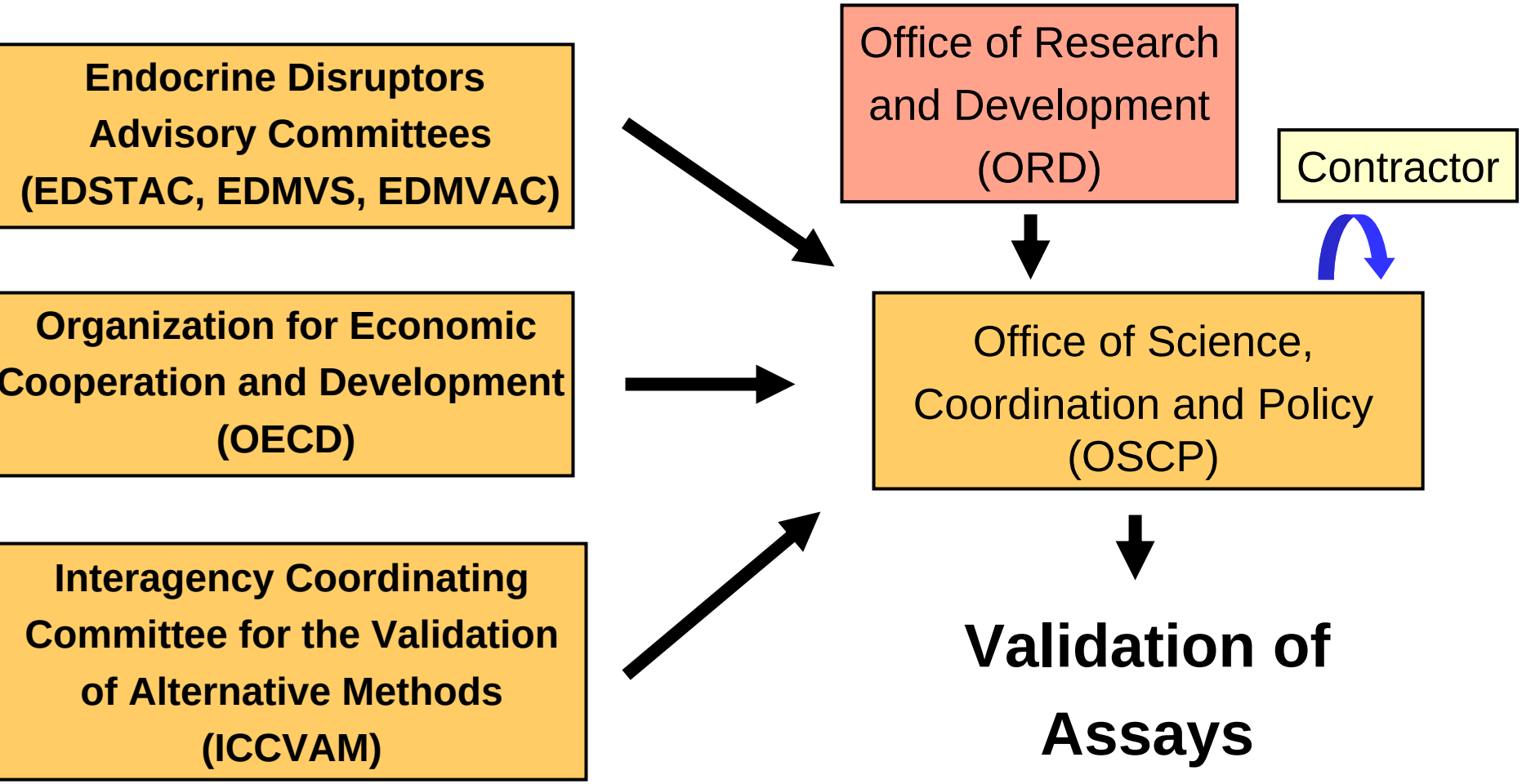
- **Initial Sorting**
- **Priority Setting –** e.g., QSAR, HTPS
- **Screening (Tier 1)**
  - Identifies substances for further testing
  - *In vitro* and *in vivo* assays
- **Testing (Tier 2)**
  - Identifies adverse effects and establishes dose-response relationship for hazard assessment
  - Multigenerational studies covering a broad range of taxa

Assays Under Development Under LTG 3: Supporting Agency's Screening and Testing Program

*T1 - **In vitro***	T1 - ***In vivo***	***T2 - In vivo***
ER (rat cytosol)	Hershberger	Mammalian 2-gen
hrER binding [a]	Uterotrophic	Avian 2-gen
AR (rat cytosol)	Pubertal (female)	Amphibian dev, repro
hrAR binding [a]	Pubertal (male)	Mysid Lifecycle
Steroidogenesis - rat sliced testes	Frog metamorphosis	Fish lifecycle
- H295R [a]	Fish screen	*In utero*/lactation -tier ? [a]
Aromatase - placenta		
- recombinant [a]		

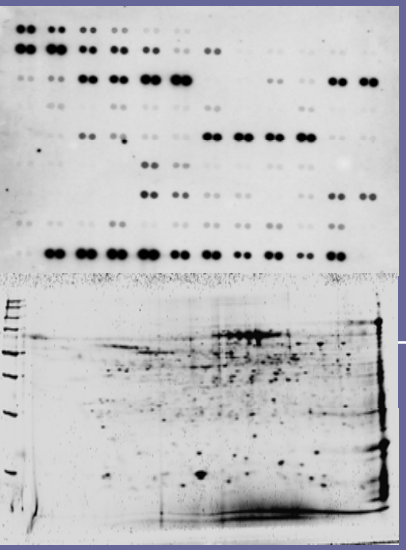
[a] alternate

EDSP Validation: Primary Contributors

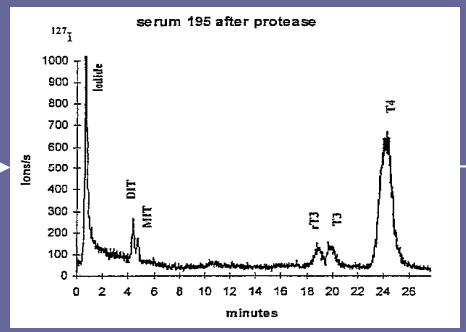


Xenopus Metamorphosis Model for Thyroid System Disruption

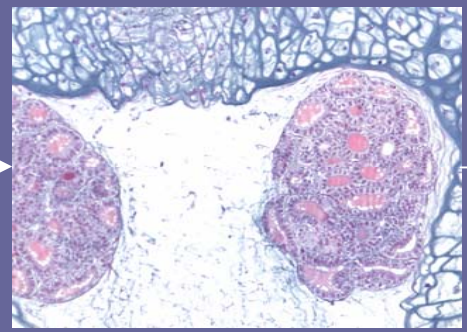
Molecular



Cellular



Tissue



Individual

Gene/Protein Expression

Circulating TH Status

Thyroid Histology

Altered Morphology

Hypothalamus
TRH (CRH) Release

Pituitary Gland
TSH Release

Thyroid Gland
Thyroid Hormone Synthesis

Peripheral Tissues
Deiodination
Morphology

Tietge Laboratory,
MED/NHEERL/ORD/USEPA

Coordinating Research Across US Federal Agencies and Collaborating Internationally

- Endocrine Disruptors Interagency Working Group –1995-2000; 2003-
  - Representation: 10-14 federal agencies
  - Research needs document; Inventory of federal research; Established national priorities
  - Co-sponsored two multi-agency requests for applications (RFAs) for extramural grants
  - Identifying new projects – e.g., workshops on impacts to aquatic organisms and humans, BPA, brain as a target
  - Evaluation of advances made in agency research programs to address national priorities
- Provide support for Administrator for G-8 Environmental Ministers Meetings since 1997
- Chaired IPCS/WHO/OECD Steering Committee
  - Developed Global Endocrine Disruptors Research Inventory
  - Developed a “Global State of the Science” report (WHO, 2002)
- Collaborate with EU and Japan
- Participate on OECD work groups
- Participate in US-EU Science and Technology Meetings
- Collaborate with GWRC
- Exploring establishing Global Endocrine Disruptors Working Group

Overall Assessment of EDC Research Program by BOSC – December 2004

- Design - goals and scientific questions of the Research Program deemed appropriate; multi-disciplinary set of research areas for both human health and wildlife that cuts across the risk assessment/risk management paradigm
- Relevance – of direct relevance to legislation that EPA administers and that it serves the Program Offices well
- Progress – research has been productive and of high scientific quality; of particular note is the excellent progress under LTG 3
- Leadership - nationally and internationally recognized; research is disseminated in top-tier scientific journals; scientists at the forefront of EDC research in screening and testing methodologies
- Resources – resources have been used efficiently; astute in leveraging with other federal agencies; continuation of extramural grants program is vital

Summary of BOSC Recommended Changes to MYP

- Clarify focus of EDCs research
- Application of new technologies (link to other MYPs)
  - Predictive tools
  - 'Omics, systems biology, and computational toxiciology
- Leverage with partners
  - Wildlife toxicology
  - Variability of species
  - Sources, exposures, pharmaceuticals
- Common ground for ecological and human health
- Summarize (link) accomplishments to date

Bibliography

- In peer reviewed journals
  - LTG 1 – 203 (111)
  - LTG 2 – 257 (216)
  - LTG 3 – 94 (67)
- 2004 Analysis used the Thomson Essential Science Indicators (ESI) and Journal Citation Report (JCR) as benchmarks
  - 24 ESI fields
  - JCR benchmarks – impact factor and immediacy index
- Important measure of progress and success monitored by BOSC and OMB

Bibliometric Analysis

- Papers covered 11 of the 24 ESI fields
- 10 papers appeared in top 1% in 4 fields
- Ratio of average cites to expected sites exceeds 1 in all but one field
- JCR Impact Factor - 44% of papers appear in the top 10% of journals
- JCR Immediacy Index - 20% of papers appear in the top 10% of journals

Trainees (as of December 2004)

- Current Postdocs – 20
- Current Predocs – 35
- Former Postdocs – 35
- Former Predocs – 22
- Former Master's and Undergraduate Students - 20

What's in the Future?

- Updating Multi-Year Plan
  - Taking into consideration recommendations by BOSC Program Review
    - Mid-cycle review in 2007
  - Continuing to develop new methods/tools and applying them to environmentally relevant issues – e.g., WWTP, CAFOs, pharmaceuticals
- Interest in expanding our partnerships and collaborations
- Communicating results
  - Informal interactions with client offices within EPA
  - Workshops
  - Developing a website

Summary

- There is global concern regarding exposures to some environmental agents that interfere with endocrine systems
- EPA has developed a research program that has three Long Term Goals and is addressing specific key science questions
- EPA's program is unique among research organizations
  - Human health and wildlife
  - Effects, exposure, risk management
  - Intramural and extramural research
  - Core and problem-driven
  - Leveraged with collaborators in other federal agencies, academia, and industry