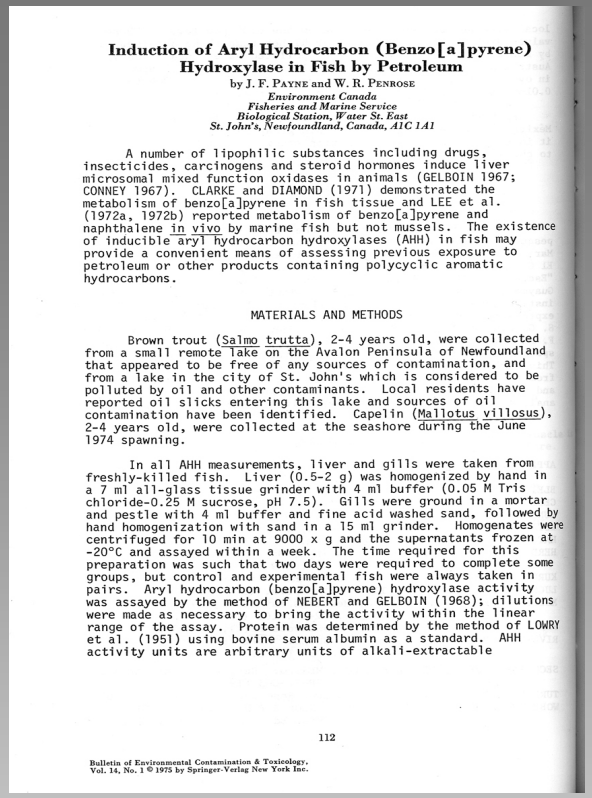
Evolution of the Science of Ecotoxicology: A 30 (Plus) Year Retrospective*

Gary Ankley, ERLD *or* MED *or* GLTED, ORD, EPA

*Content does not necessarily reflect EPA position or policy



Induction of Aryl Hydrocarbon (Benzo[a]pyrene) Hydroxylase in Fish by Petroleum

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A number of lipophilic substances including drugs, insecticides, carcinogens and steroid hormones induce liver microsomal mixed function oxidases in animals (GELBOIN 1967; CONNEY 1967). CLARKE and DIAMOND (1971) demonstrated the metabolism of benzo[a]pyrene in fish tissue and LEE et al. (1972a, 1972b) reported metabolism of benzo[a]pyrene and naphthalene in vivo by marine fish but not mussels. The existence of inducible aryl hydrocarbon hydroxylases (AHH) in fish may provide a convenient means of assessing previous exposure to petroleum or other products containing polycyclic aromatic hydrocarbons.

MATERIALS AND METHODS

Brown trout (Salmo trutta), 2-4 years old, were collected from a small remote lake on the Avalon Peninsula of Newfoundland that appeared to be free of any sources of contamination, and from a lake in the city of St. John's which is considered to be polluted by oil and other contaminants. Local residents have reported oil slicks entering this lake and sources of oil contamination have been identified. Capelin (Mallotus villosus), 2-4 years old, were collected at the seashore during the June 1974 spawning.

In all AHH measurements, liver and gills were taken from freshly-killed fish. Liver (0.5-2 g) was homogenized by hand in a 7 ml all-glass tissue grinder with 4 ml buffer (0.05 M Tris chloride-0.25 M sucrose, pH 7.5). Gills were ground in a mortar and pestle with 4 ml buffer and fine acid washed sand, followed by hand homogenization with sand in a 15 ml grinder. Homogenates were centrifuged for 10 min at 9000 x g and the supernatants frozen at -20°C and assayed within a week. The time required for this preparation was such that two days were required to complete some groups, but control and experimental fish were always taken in pairs. Aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity was assayed by the method of NEBERT and GELBOIN (1968); dilutions were made as necessary to bring the activity within the linear range of the assay. Protein was determined by the method of LOWRY et al. (1951) using bovine serum albumin as a standard. AHH activity units are arbitrary units of alkali-extractable

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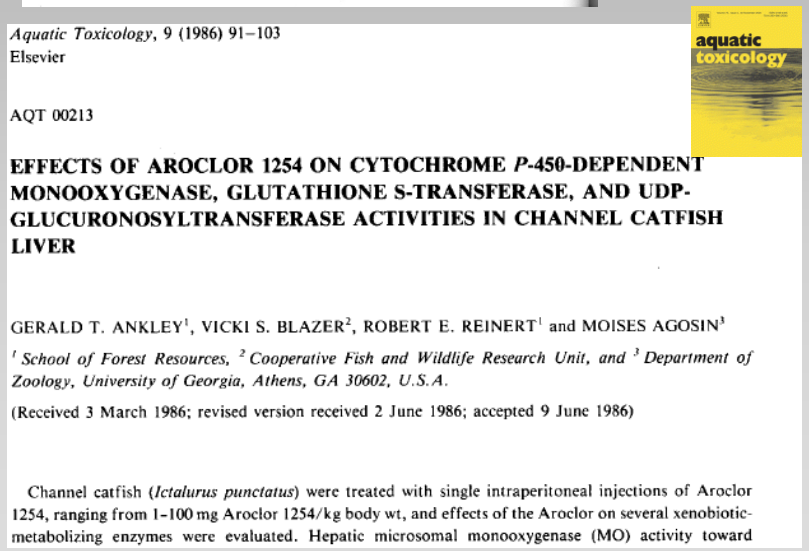

Aquatic Toxicology, 9 (1986) 91–103
Elsevier

AQT 00213

EFFECTS OF AROCLOR 1254 ON CYTOCHROME *P*-450-DEPENDENT MONOOXYGENASE, GLUTATHIONE S-TRANSFERASE, AND UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES IN CHANNEL CATFISH LIVER

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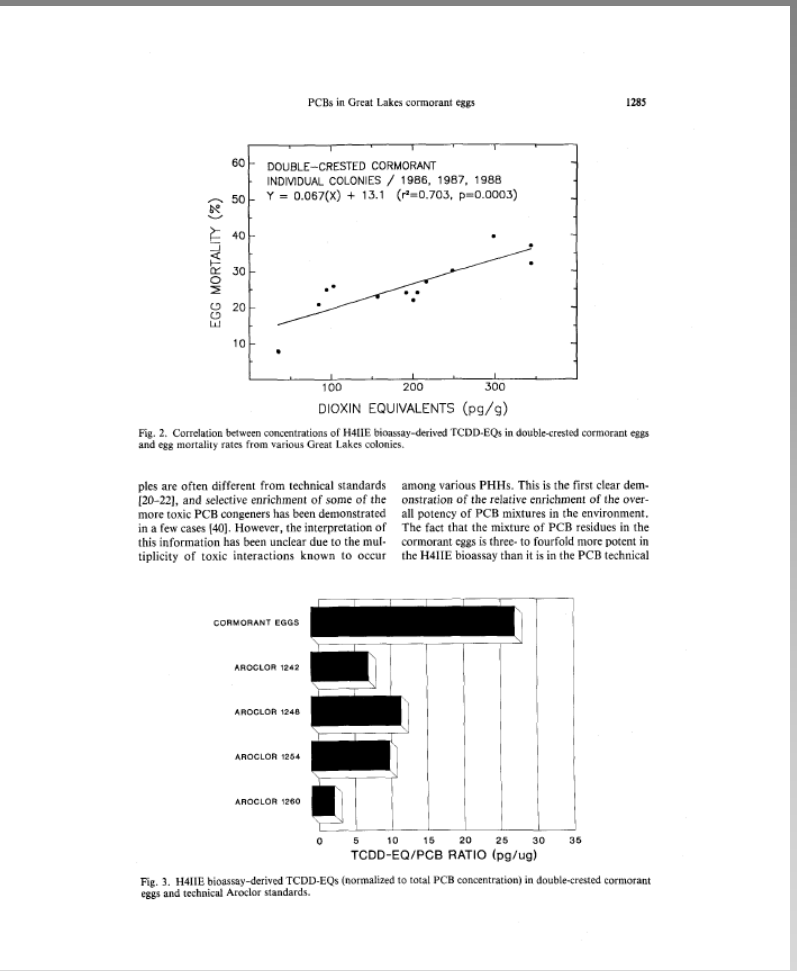
Channel catfish (*Ictalurus punctatus*) were treated with single intraperitoneal injections of Aroclor 1254, ranging from 1-100 mg Aroclor 1254/kg body wt, and effects of the Aroclor on several xenobiotic-metabolizing enzymes were evaluated. Hepatic microsomal monooxygenase (MO) activity toward

Graduated from MSU with BS in Fisheries and Wildlife, 1982—good jobs with MI DNR pretty uncommon—onto grad school!

Interested in fish physiology/biochemistry and environmental pollution so fascinated by work showing specific changes in MFOs by PAHs

Spent 5 years at UGA studying effects of environmental factors on Phase 1 and 2 xenobiotic-metabolizing enzymes in fish

Became an “expert” in the area, but wasn’t sure what any of this actually meant or what should come next!



PCBs in Great Lakes cormorant eggs 1285

Fig. 2. Correlation between concentrations of H4IIE bioassay-derived TCDD-EQs in double-crested cormorant eggs and egg mortality rates from various Great Lakes colonies.

ples are often different from technical standards [20–22], and selective enrichment of some of the more toxic PCB congeners has been demonstrated in a few cases [40]. However, the interpretation of this information has been unclear due to the multiplicity of toxic interactions known to occur among various PHHs. This is the first clear demonstration of the relative enrichment of the overall potency of PCB mixtures in the environment. The fact that the mixture of PCB residues in the cormorant eggs is three- to fourfold more potent in the H4IIE bioassay than it is in the PCB technical

Fig. 3. H4IIE bioassay-derived TCDD-EQs (normalized to total PCB concentration) in double-crested cormorant eggs and technical Aroclor standards.

Moved to postdoctoral position in Dept. of Fisheries and Wildlife at MSU in 1987

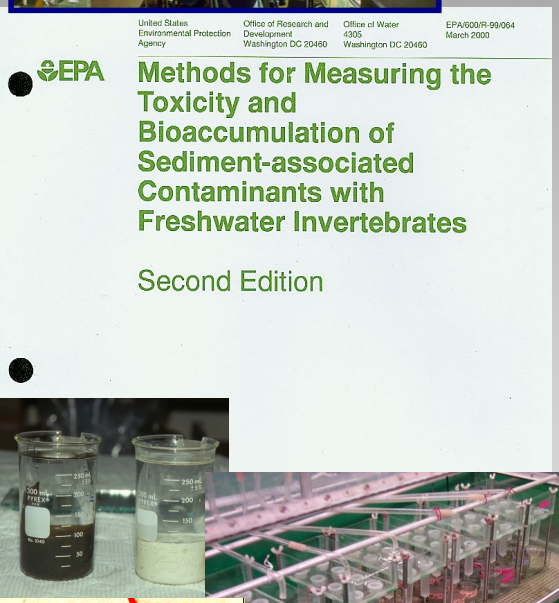
Joined dynamic (and eclectic) group of young scientists engaged in projects ranging from contaminated sediments to effects of organochlorines on Great Lakes fish/birds

Started to appreciate critical role of applied research and “roles” of measurements at different biological levels of organization

Emphasis on Ah-receptor signaling pathways and apical adverse effects

Still looking for a “real” job!

Tillitt, D.E., G.T. Ankley, J.P. Giesy, J.P. Ludwig, H. Kurita, D.V. Weseloh, C.A. Bishop, J. Larson, and T.J. Kubiak. 1992. PCB residues and egg mortality in double-crested cormorants from the Great Lakes. Environ. Toxicol. Chem. 11, 1281-1288.



Started at EPA ORD lab in Duluth May, 1988

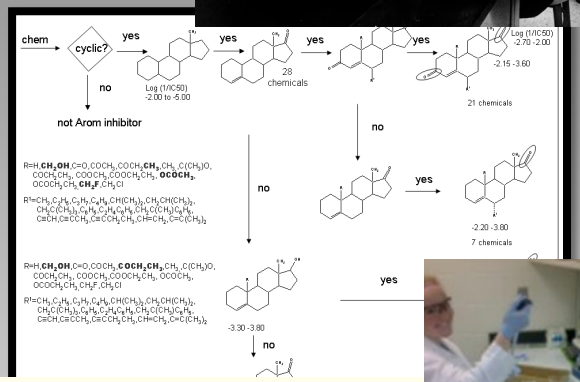
Considered by many to be the premier ecotox research lab in world

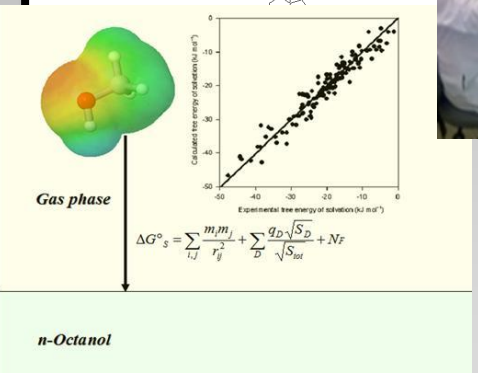
Joined effluent program developing TIE methods to support WET-based regulation

Serves as Duluth lead for emerging EPA initiative-development of tests and criteria for contaminated sediments

Effluent/sediment testing programs arguably most applied/client-driven work conducted by ORD in that era

Sediment effort, especially, highlighted importance of extensive, multi-partner consortiums to address tough environmental challenges





Duluth lab also renown for pioneering work in the area of predictive ecotoxicology

QSAR models for predicting narcosis toxicity and chemical bioaccumulation

Identification of likely toxic mode-of-action based on structure

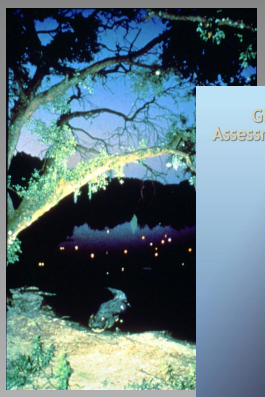
Development of mechanism-based bioconcentration/bioaccumulation models

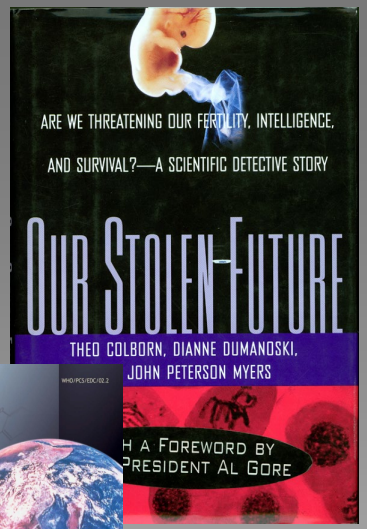
Surprisingly little interaction between the “empirical” and “predictive” toxicologists at the lab; working in both areas considered a bit odd…

Scientific Developments and Regulatory Challenges at the Turn of the Century

- Unprecedented breakthroughs in biological knowledge/tools
  - Sequencing of human genome spawns data, concepts, and tools to support multi-faceted 'omics
  - High-throughput technologies revolutionize in vitro testing
  - Bioinformatic techniques enable mining of high-content data and support systems-based modeling

- New requirements and a changing landscape for regulatory (eco)toxicology
  - Legislated requirements for data for many more chemicals, species, and endpoints than in the past (e.g., REACH)
  - Need to understand not only apical outcomes, but mechanistic basis of outcomes
  - Desire for more information tempered by increasingly limited testing resources/animal welfare concerns





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DESCRIPTION AND EVALUATION OF A SHORT-TERM REPRODUCTION TEST WITH THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*)

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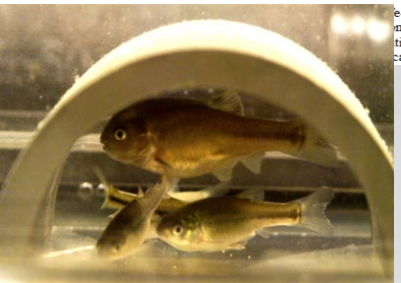
e-cycle testing, most current toxicity tests with fish do not explicitly
n early life-stage survival and development. However, some classes
tions below those that affect development. Further, estimates of the
cal to the ecological risk assessment process. In this manuscript, we



EDCs “poster child” for new challenges facing the field

Unprecedented in terms of public visibility

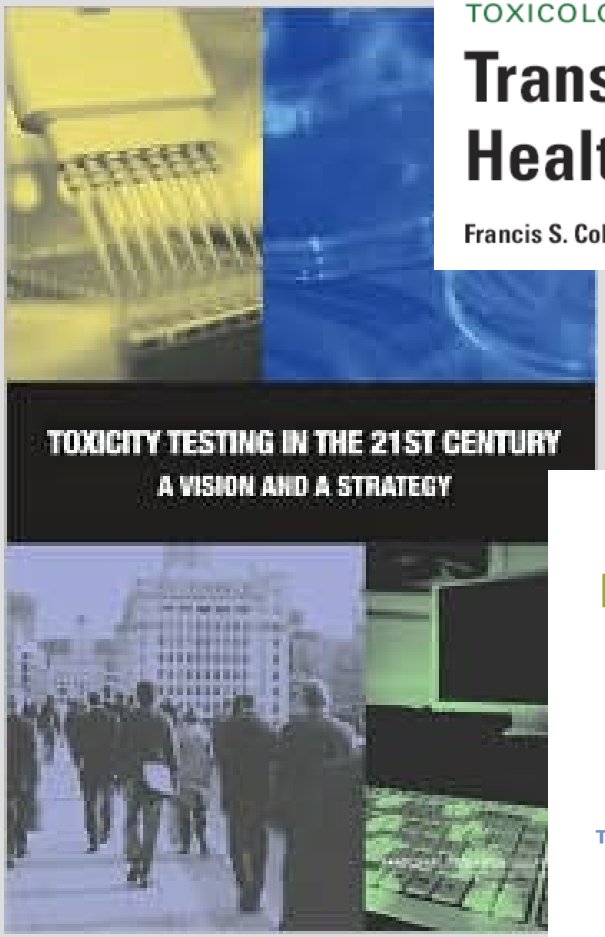
Desire to assess 1000’s of chemicals as possible EDCs in prospective/retrospective settings with few new resources

Need to link perturbation of specific pathways (EAT) to adverse apical outcomes, requiring development of tests with both mechanistic and apical endpoints

Scope of the issue world-wide, compelling international communication/collaboration (e.g., OECD)

Personally invigorating, but intimidating…

Fueling A Paradigm Shift in Regulatory Toxicology



POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

Francis S. Collins,[1*†] George M. Gray,[2*] John H. Bucher

15 FEBRUARY 2008 VOL 319 SCIENCE www.sciencemag.org

Meeting *the* Scientific Needs *of* Ecological RISK Assessment *in* a Regulatory Context

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PROCTER & GAMBLE SERVICES COMPANY NV/SA (BELGIUM)

CORNELIS J. VAN LEEUWEN
EUROPEAN COMMISSION

Three strategies could move both science and regulation forward.

During the past decade, the field of ecological risk assessment has progressed considerably. Advances have come from such international bodies as

Increasing efficiency, cost-effectiveness, and focus

Risk assessment is a tiered process distinguished by levels of increasing complexity, beginning with the preliminary

Intelligent Testing Strategies in Ecotoxicology: Mode of Action Approach for Specifically Acting Chemicals

Technical Report No. 102

Predicting Chemical Toxicity with Limited Data

- Identify “normal” biological pathways whose perturbation results in adverse responses to chemicals
- Determine chemical characteristics that enable them to perturb these pathways
- Develop mechanism-based approaches to measure these characteristics
  - *In silico* (computational) methods (e.g., QSAR)
  - *In vitro* pathway-based measures of bioactivity
  - Short-term *in vivo* tests with pathway-specific, biomarker-type endpoints
- Translate these mechanistic data into transparent depictions of potential risk/hazard

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Hazard/Risk Assessment

ADVERSE OUTCOME PATHWAYS: A CONCEPTUAL FRAMEWORK TO SUPPORT ECOTOXICOLOGY RESEARCH AND RISK ASSESSMENT

GERALD T. ANKLEY,* RICHARD S. BENNETT, RUSSELL J. ERICKSON, DALE J. HOFF, MICHAEL W. HORNUNG, RODNEY D. JOHNSON, DAVID R. MOUNT, JOHN W. NICHOLS, CHRISTINE L. RUSSOM, PATRICIA K. SCHMIEDER, JOSE A. SERRRANO, JOSEPH E. TIETGE, and DANIEL L. VILLENEUVE

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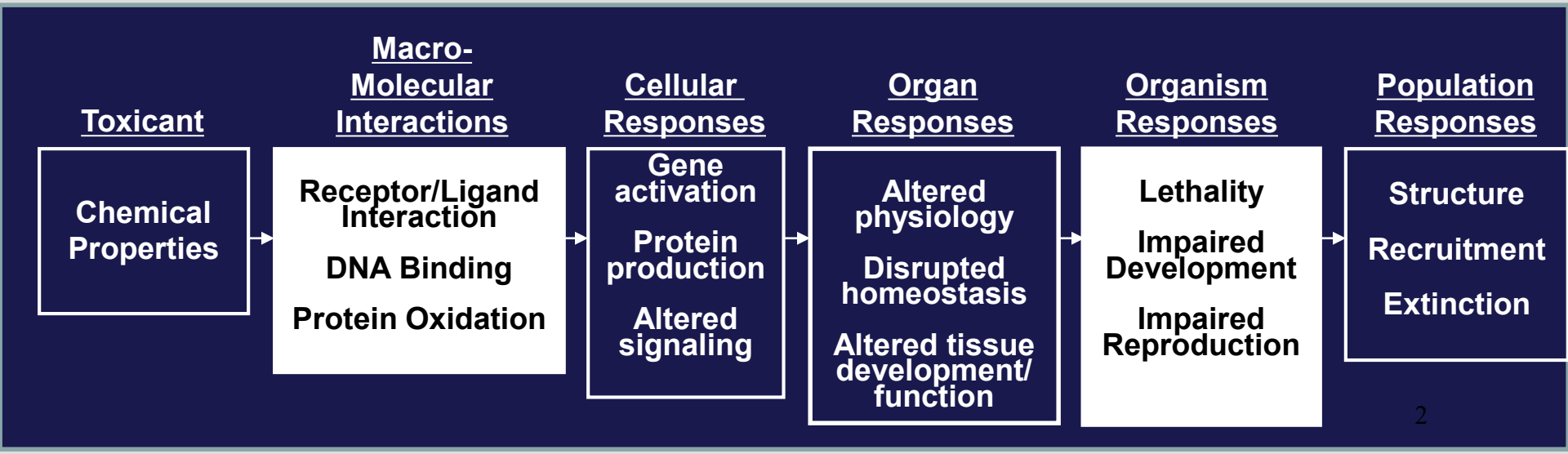
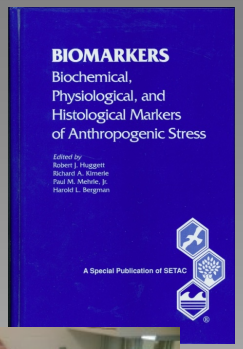
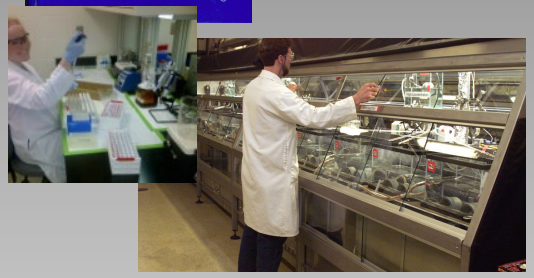
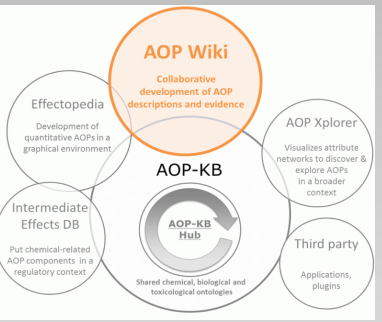
An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment.



AOP Framework: Meeting the Challenges of Integration, Translation and Communication







- Builds on existing concepts (e.g., MOA) to help address decades-old dilemma of making meaningful linkages across levels of organization
- Essentially “ties” empirical to predictive toxicology
  - Neither is better—both are essential
- Concurrent development of AOP wiki enables new approaches to exploring/accessing toxicological data
- Applications to both prospective and retrospective assessments
  - Assessing EDCs
  - Prioritizing testing
  - Complex mixture toxicity

Changes (or not?) Over Last 30+ Years

- Always something new to engage us…but typically with no guarantee of new resources
- While the challenges we face often seem daunting…they are not insurmountable
- Require innovative, cross-disciplinary application of concepts/tools
- Collaboration is a must—cannot allow boundaries, scientific or regional, to act as impediments

Acknowledgements

SETAC Meeting Photo Forthcoming