Molecular Methods in Contaminants Studies:

Profiling of gene induction in response to contaminants or other treatments

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The "–omics" and toxicology

- Tools for screening for toxic effects and gaining mechanistic insight
  - Genomics (transcriptomics) – mRNA, DNA
    - Toxicogenomics, ecotoxicogenomics
  - Proteomics- proteins
  - Metabolomics – intermediary metabolites, small molecules, peptides

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Traditional vs. "–omic" Toxicology

- Identify chemicals of potential concern
  - doses used to determine adverse effects
    - selected to ensure that tissue level or whole animal toxic responses can be identified
    - molecular/biochemical changes
- Limited vs. multiple endpoints
  - Traditional methods = whole-organism responses (mortality, growth, reproduction)
    - minimal understanding of the mechanisms

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Traditional vs. "–omic" Toxicology

- Genomics technologies simultaneously examine a number of response pathways
  - Offers the opportunity to understand possible mode(s) of action
  - Improve mixtures analysis
- Extrapolation from sentinel species
- Standardization
  - Handling and interpreting large amounts of data

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Biomarkers vs. signature profiles

- Biomarkers
  - e.g. HSPs, MT, Cyt P450, GSH
- Signature profiles
  - cellular receptors, modifying enzymes, repair enzymes, cell cycle control factors, regulators of cell division and death

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Toxicogenomic Methods

- Combination of toxicology, genetics, and molecular biology
- Expression – DNA microarrays, qRT-PCR, expression libraries (SSH)
- Genetic variations – sequencing, qPCR (SNPs)……

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Why measure at the molecular level?

- Total concentrations in tissues are insufficient criteria for making toxicologically relevant judgments (Scheuhammer et al. 1998)
  - susceptible populations and life stages
  - effects of mixtures
- Tissue concentrations do not always reflect the susceptibility of a species to a contaminant
  - more efficient repair systems = higher resistance
  - sensitive species = detrimental effects at significantly lower levels of exposure

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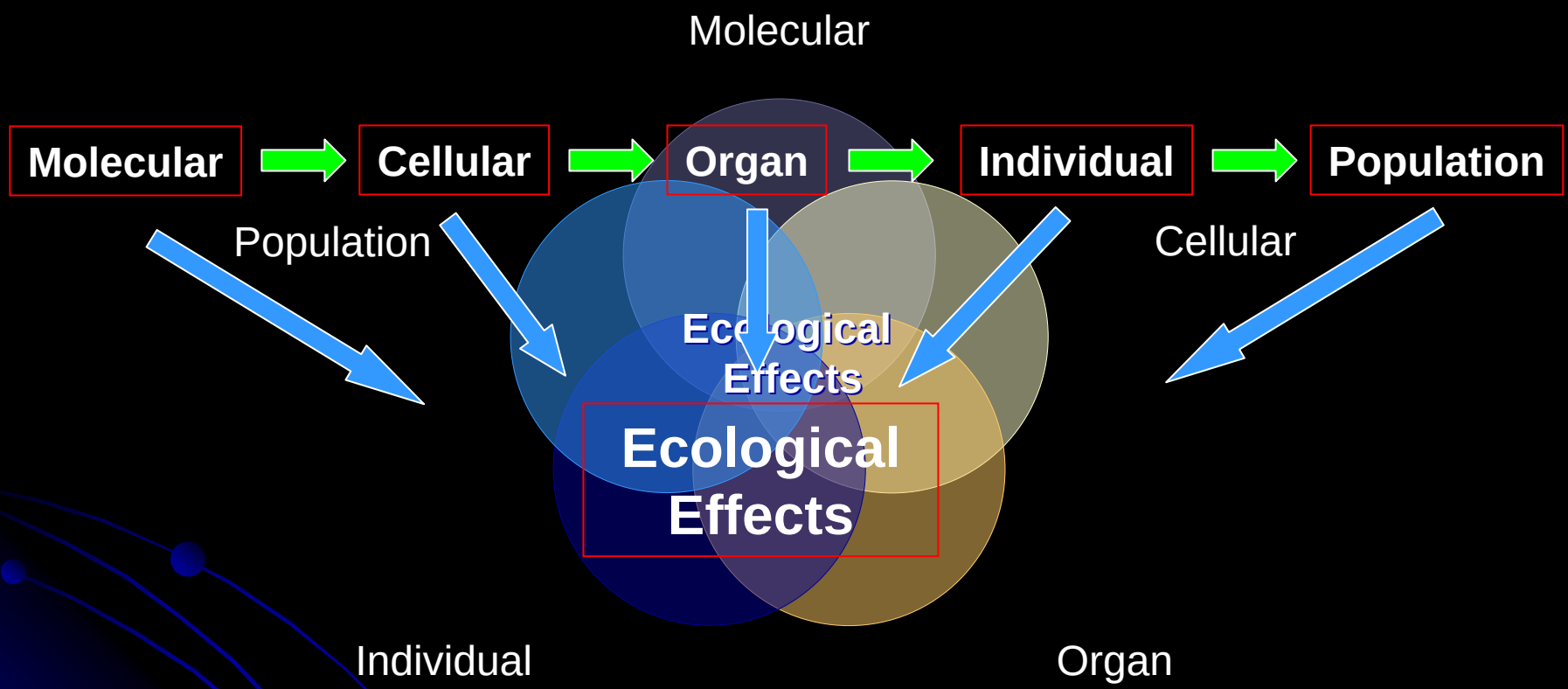
Can we predict ecological effects?



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Applications for Genomics Data

- Genomics technologies are likely to contribute significantly to defining a chemical's mode of action, in evaluating effects on susceptible populations and life stages, and in assessing exposure to and effects of chemical mixtures

- Genomics will not fundamentally alter risk assessment process but will serve as a more powerful tool for evaluating the exposure to, and effects of stressors

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U.S. EPA Interim Policy on Genomics, 2002;
Genomics: Applications, Challenges, and Opportunities for the U.S. EPA, 2006

Role for Genomics

- Genomics data alone are currently insufficient as a basis for risk assessment and management decisions
  - Links between molecular events and adverse ecological outcomes are not yet established

- Useful in a weight-of-evidence approach for wildlife, human health and ecological risk assessments

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Current Research

- Profiling Mercury Responsive Genes in Bird Embryos as an Indicator of Species Sensitivity

- Genotoxicity In Avian Species Inhabiting a Mercury-contaminated River System

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Profiling Mercury Responsive Genes

- CalFed – Gary Heinz, Dave Hoffman
  - provide information that will lead to a reduction of mercury in resident fish tissues to protect humans and wildlife
- Previous analyses conducted at Patuxent found that embryos of different bird species vary in their sensitivity to methylmercury (Heinz, 2003).
- Minimal information available on molecular mechanisms that account for sensitivity differences

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Profiling Mercury Responsive Genes

- Challenge – predicting responses across phylogenetic groups
  - extrapolate from a limited set of test species
- Are observed residue levels harmful?
- What causes differences in sensitivity?

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Species-specific toxicity

- Genetic variability (polymorphisms) - associated with altered efficiency of a biological pathway and with risk for adverse effects
  - affects sensitivity to contaminants
- Certain genes have a greater than average influence over susceptibility
- Study of toxic effects cannot be separated from the study of the genetic mechanisms underlying variations in individual susceptibilities.

Previous Studies

- Alterations in gene expression due to Hg
  - immune response
  - nervous system function
  - reproduction, including egg fertilization and development
  - mammals, fish, and amphibians

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Profiling Mercury Responsive Genes

- Dosing of wild bird eggs with MeHg
  - measuring each species' sensitivity to mercury
  - laughing gulls, grackles, kestrels, osprey, tree swallows
- Objectives
  - Identify differentially expressed genes in embryos exposed to methyl mercury
    - Compare gene profiles in liver, brain, and kidney from control and dosed embryos
    - Evaluate association between gene expression, mercury dose, and the level or types of deformities observed
    - Future studies? Compare profiles in highly sensitive with less sensitive sp., SNPs

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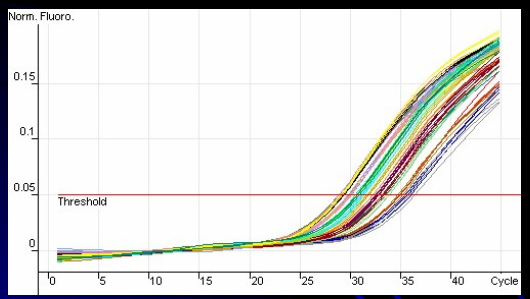
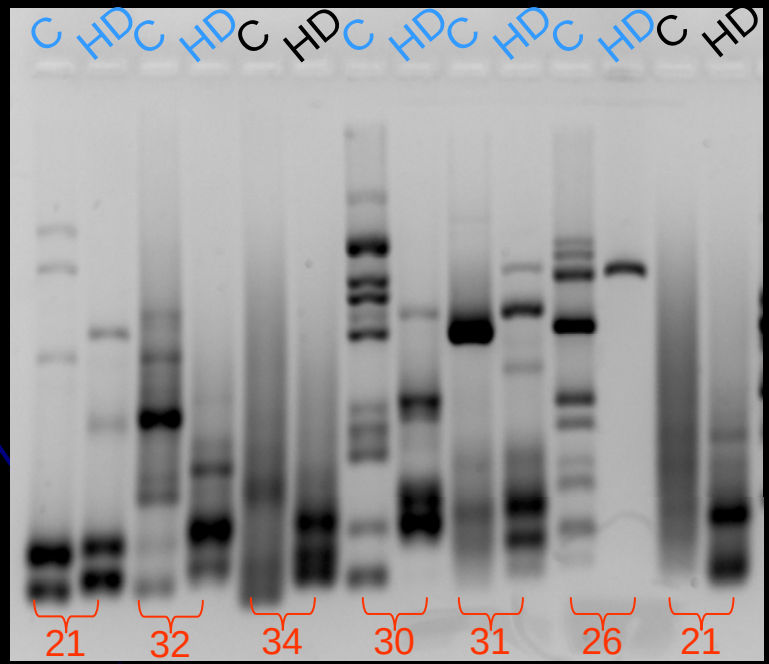
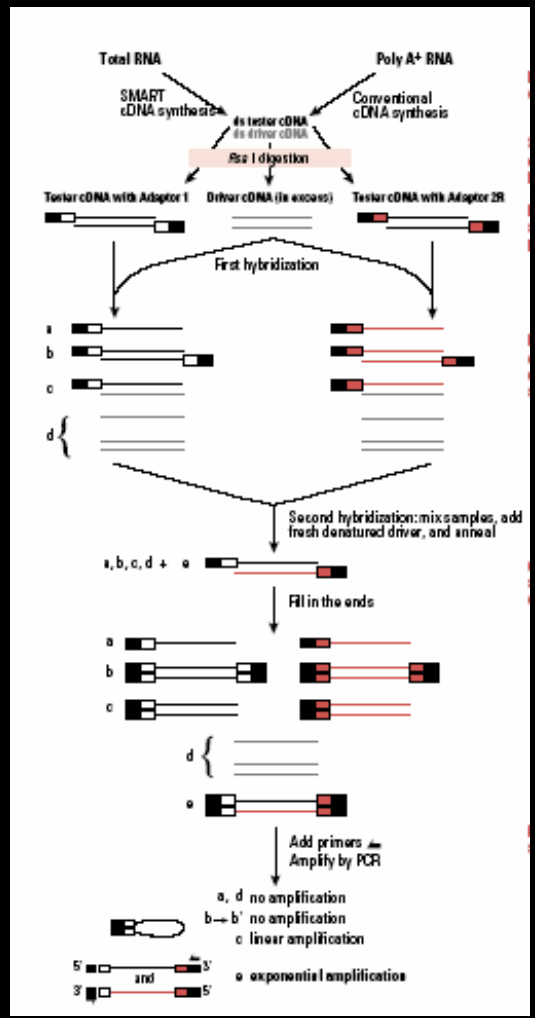
Mercury responsive genes

- mRNA Profiling
  - Subtractive Hybridization
  - Differential Display PCR
  - qRT-PCR







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Genotoxicity In a Mercury-Contaminated River System

- The potential of a stressor to cause damage to a cell's DNA
  - not limited to exogenous environmental pollutants
- Genotoxicants may bind directly to DNA or act indirectly, leading to DNA damage by affecting enzymes involved in DNA replication or repair
  - genotoxic substances are not necessarily carcinogenic
- Genotoxic Disease Syndrome
  - genetic changes that are detrimental to the fitness of an individual exposed to xenobiotics

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Genotoxicity In a Mercury-Contaminated River System

- Weight of evidence approach
- Multiple end points important for understanding the magnitude of injury
  - Are the levels of Hg in birds having genotoxic effects?
- Provides mechanistic data in the assessment of bird health
  - Improves totality of injury assessment

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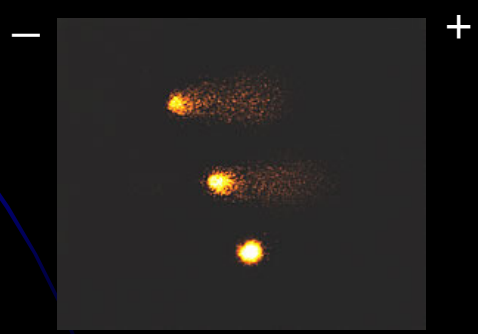
Comet Assay

- Sensitive single cell assay that detects DNA strand breaks and unwinding via electrophoresis.

- ‘Comet tail’ visualized under fluorescence microscopy.
  - damaged DNA migrates during electrophoresis from the nucleus towards the anode forming a head (cell nucleus with intact DNA) and a tail (relaxed and broken DNA).



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Other Genetic Methods

- Polymorphic variants (SNPs)
  - individual susceptibility
- Population/Community Analysis
  - Microsatellites, mtDNA, AFLP, TRFLPs, RAPDs

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