NexGen

# Linking Research to Risk Assessment

**Dr. Linda Birnbaum**

NIH, National Institute of Environmental Health Sciences

EPA United States Environmental Protection Agency

NIEHS National Institute of Environmental Health Sciences

NCGC NIH Chemical Genomics Center

CDC Centers for Disease Control and Prevention

NIEHS
National Institute of Environmental Health Sciences

# Linking Research with Risk Assessment

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National Toxicology Program

NexGen Conference
Tuesday, February 15, 2011

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

NIEHS
National Institute of Environmental Health Sciences

# Our Commitment : *Translating Bench Science into Environmental Public Health*

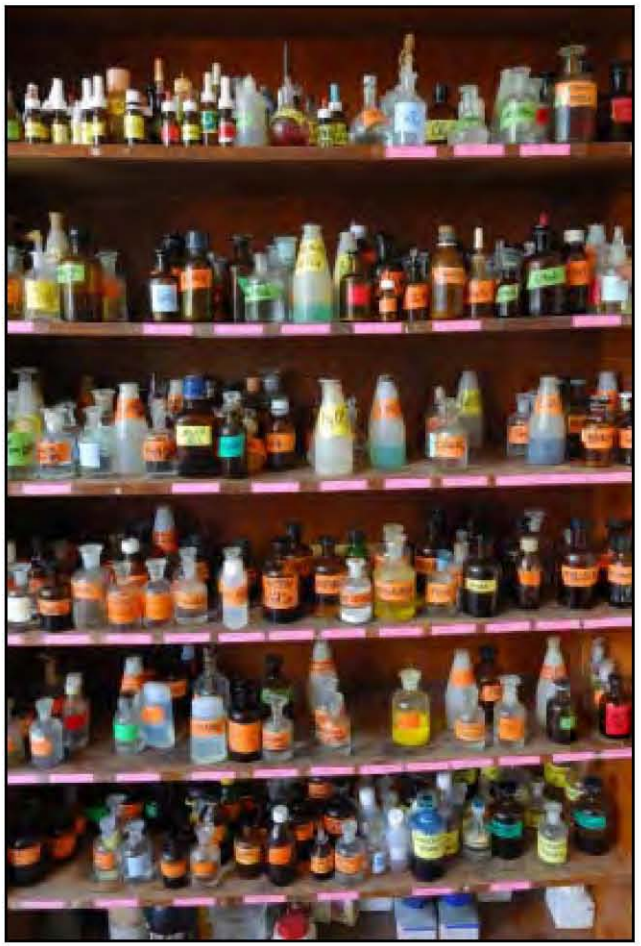
# Environmental Health Research: Poised to Advance Research and Protect Public Health

## The Challenge

- How do we come together to think strategically about the breadth, scope, participants, and goals for Environmental Health Research, a multi-science discipline primed for significant impact on human health in the 21st Century?
- How do we create an Environmental Health Research Strategy that provides the data and information needed by the multiple audiences who use EHS data?
- Do we have the right tools and methods to do Environmental Health Research in the 21st Century?
- How can we integrate better new methods and technologies into science policy?

# Priority Areas in Environmental Health Sciences

- Low Dose
- Windows of Exposure
- Toxicology Screening
- Mixtures
- Routes of Exposure
- Emerging Hazards
- Human Health Effects of Climate Change
- Clinical Research

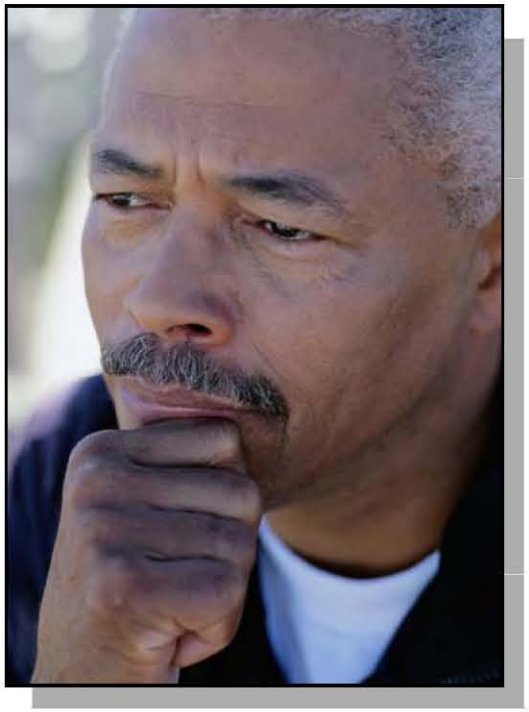


# Conceptual Shift for Environmental Health Sciences

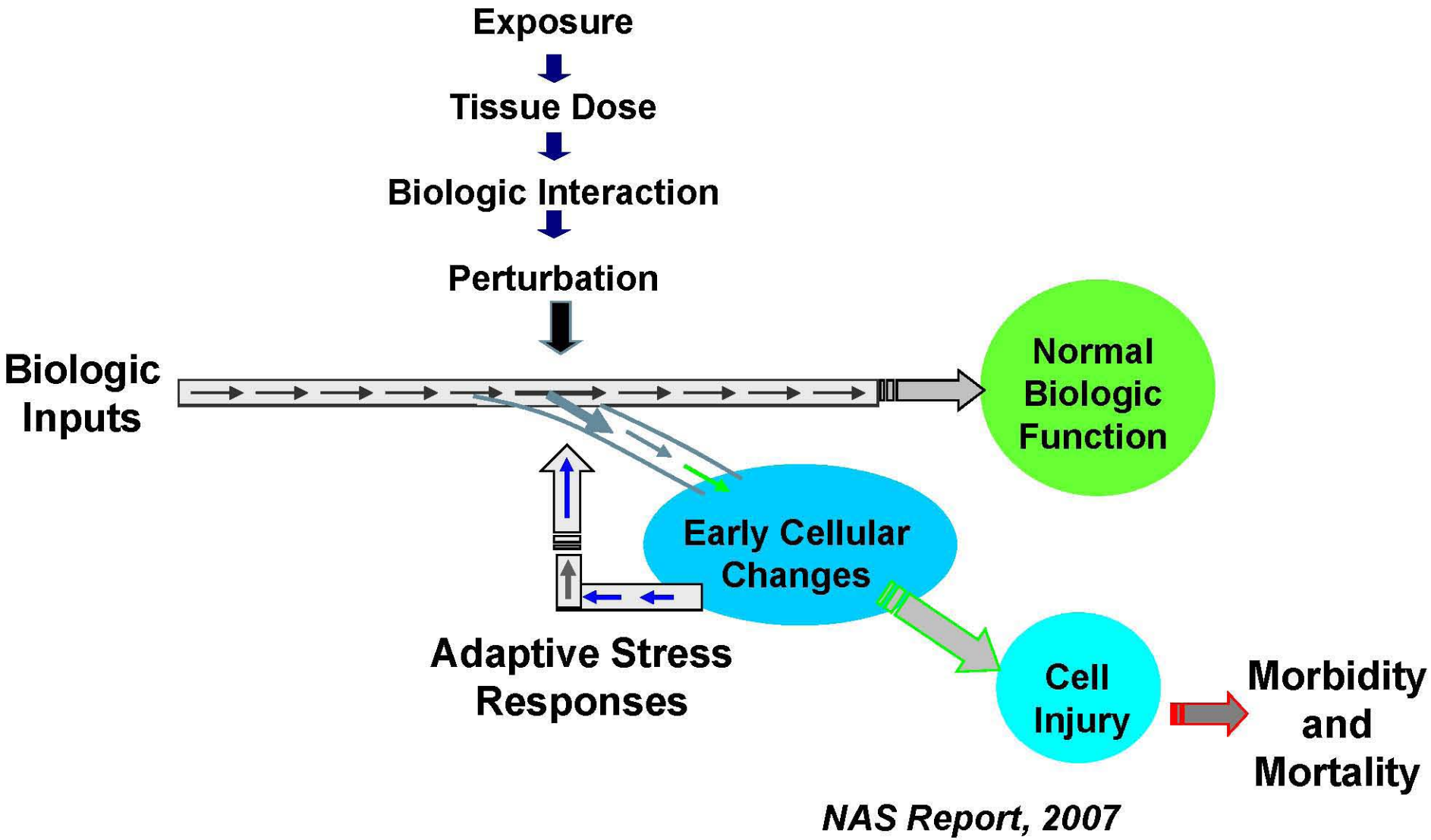
**OLD…** chemicals act by overwhelming the body's defenses by brute force at very high doses

**NEW…** chemicals can act like hormones and drugs to disrupt the control of development and function at very low doses to which the average person is exposed

**NEW…** susceptibility to disease persists long after exposure (**epigenetics)**



# Conceptual Shift
# Activation of Toxicity Pathway




*NAS Report, 2007*

# NAS Implementation Strategy Requirements

- comprehensive suite of *in vitro* tests, preferably based on human cells, cell lines, or components
- targeted animal tests to complement *in vitro* tests
- computational models of toxicity pathways to support application of *in vitro* test results in risk assessments
- infrastructure changes to support basic and applied research needed to develop the tests and pathway models
- validation of tests and test strategies
- evidence justifying that toxicity-pathway approach is adequately predictive of adverse health outcomes to use in decision-making

***NAS Report, 2007***

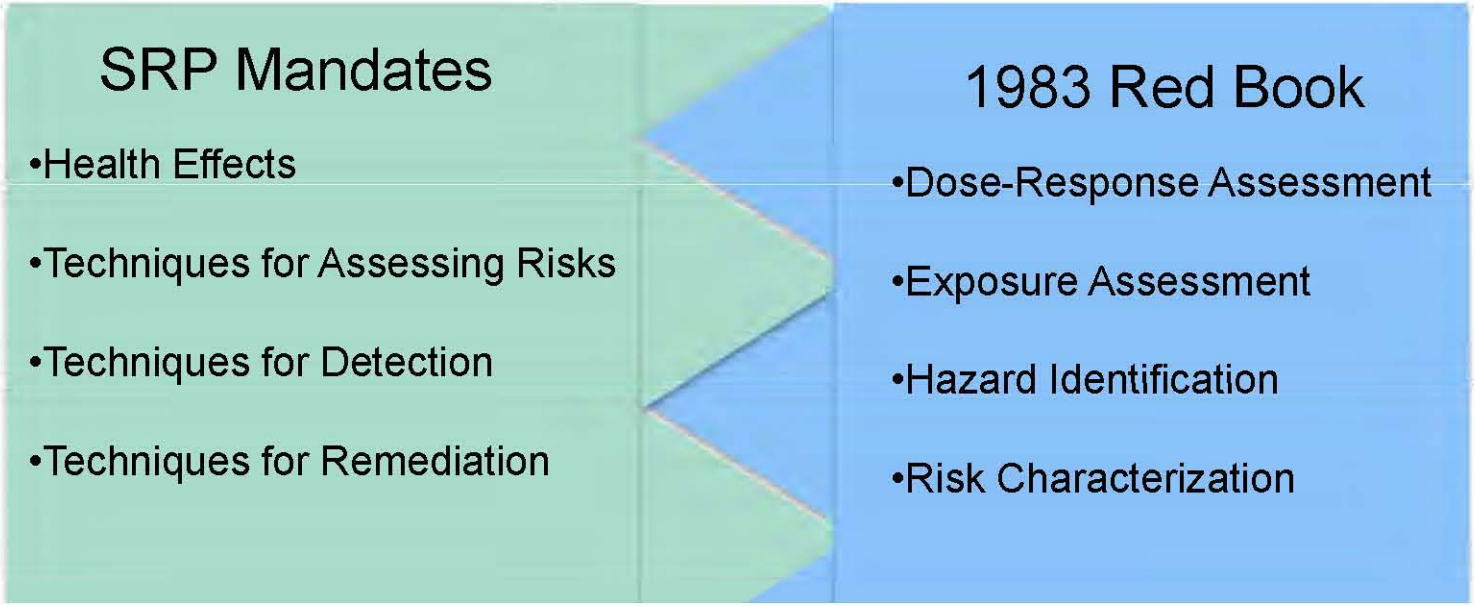
# NIEHS Center for Risk & Integrated Sciences

- Directs a translational research program including bioengineering, integrated systems, computational methods, validation of biomarkers, and application of innovative ‘omics research.
- Represents the NIEHS in areas of advanced technologies, bioengineering, nanosciences and bioinformatics, and identifies opportunities for applying these tools to exposure assessment, disease etiology, and risk reduction.
- Assesses and evaluates progress and effectiveness of the Center's programs

# SRP Develops Reliable and Relevant Scientific Data – a Major Determinant of the Quality of any Risk Assessment

The SRP is a university-based basic research program established in 1986 under Superfund Amendments Reauthorizatic (SARA) to Research:

The 1983 Red Book described the four key stages in the risk-assessment process as:




**The SRP Mandates fold into the key stages in the risk assessment process**

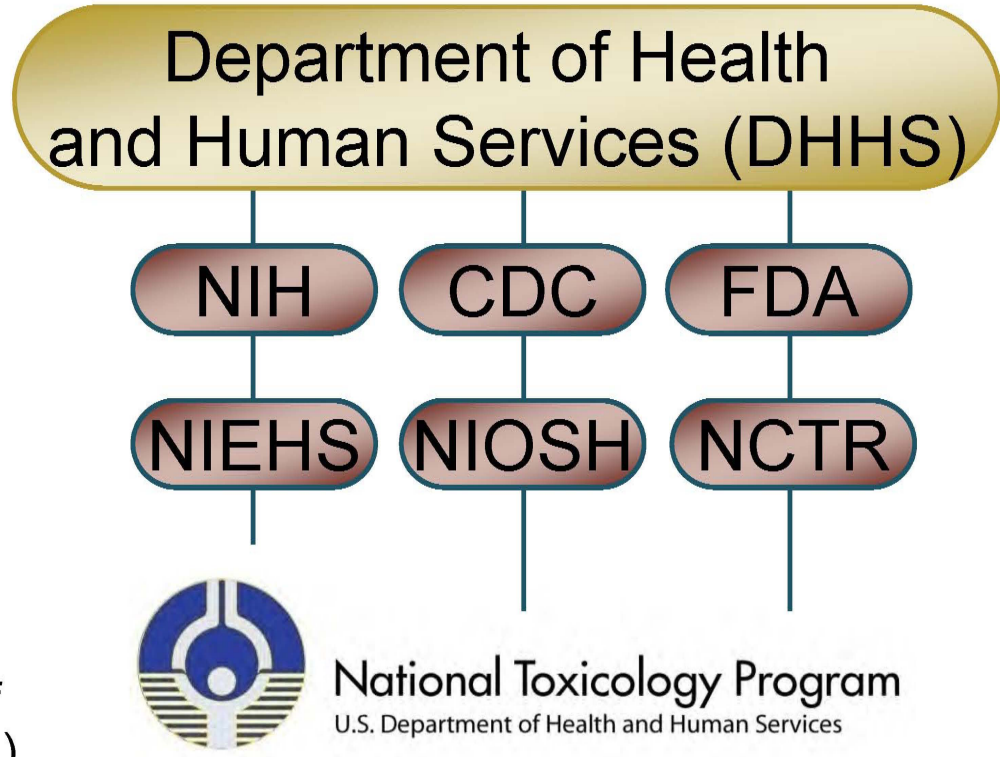
# *"The Critical Final Process in Risk Assessment is Ultimately Communication."* – *National Research Council 2009*

The SRP proactively communicates it's scientific accomplishments to the public. Grantees at Dartmouth College produced a 10 minute film, *In Small Doses: Arsenic*, to educate private well owners about the importance of testing their wells for arsenic.

- The film is publically available on the Dartmouth Toxic Metals Program website and **insmalldoses.org**.
- Broad dissemination of the film is underway--it has been shown at the SRP Annual Meeting, the Northeast Private Well Water Symposium, and the 2009 NH Joint Water and Watershed Conference.
- Postcards promoting the movie and the website were produced and distributed at each conference.

# National Toxicology Program

- Interagency program
  - Established in 1978 to coordinate toxicology research across the Department of Health and Human Services (DHHS)
  - Headquartered at NIEHS
- Research on "nominations"
  - Thousands of agents evaluated in comprehensive toxicology studies
  - Results communicated through technical reports, scientific publications and the web
- Analysis activities
  - Report on Carcinogens (RoC)
  - Center for the Evaluation of Risks to Human Reproduction (CERHR)
  - NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)




http://ntp.niehs.nih.gov

# New and Renewed Areas of Emphasis for NTP

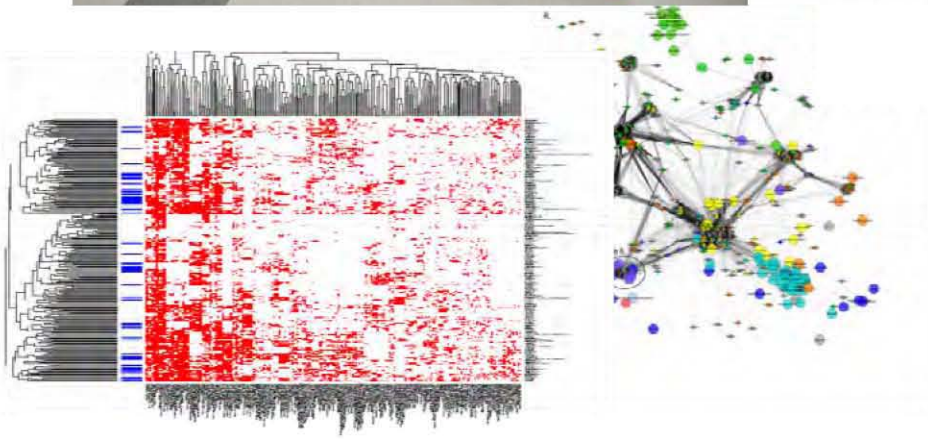
- Better coordination across the Federal government
- Develop new methods for efficient, thorough toxicological assessments
- Increase understanding of exposure-response relationships and issues of dosimetry
- Integrate results from new "data rich" techniques (i.e. genomics, high through-put screening) with traditional toxicology data to provide public health context
- Toxicity for the 21st Century or "Tox21"
  - MOU between NTP, EPA, NHGRI and FDA
  - High throughput, robotic testing of toxic compounds in cell and molecular assays
  - Using knowledge of biological response to identify toxicity pathways
  - Prioritization for further testing

# An Expanded U.S. Tox21 Community

- Revised MoU on "High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings" released on **July 19, 2010** by:
  - **National Toxicology Program:** Linda S. Birnbaum, Ph.D., DABT, ATS
    Director, National Institute of Environmental Health Sciences, NIH
  - **NIH Chemical Genomics Center:** Eric D. Green, M.D., Ph.D.
    Director National Human Genome Research Institute
  - **U.S. Environmental Protection Agency:** Paul T. Anastas, Ph.D.
    Assistant Administrator Office of Research and Development
  - **Food and Drug Administration:** Janet Woodcock, M.D.
    Director, Center for Drug Evaluation and Research

# Tox21 Goals

- Research, develop, validate, and translate innovative compound testing methods that characterize toxicity pathways
- Identify compounds, assays, informatic tools, and targeted testing needed for the innovative testing methods
- Prioritize compounds for more extensive toxicological evaluation
- Identify mechanisms of compound-induced biological activity in order to characterize toxicity pathways, facilitate cross-species extrapolation, and provide input to models for low-dose extrapolation
- Develop predictive models for biological response in humans

| Areas of Expertise | NTP | NCGC | EPA | FDA |
|---|---|---|---|---|
| Historical Toxicology Data | ✓ | | ✓ | ✓ |
| Human Toxicological Data | | | | ✓ |
| Experimental Toxicology | ✓ | | ✓ | ✓ |
| qHTS | | ✓ | | |
| Low to Mid Throughput Assays | ✓ | ✓ | ✓ | ✓ |
| Lower Organism Systems | *C. elegans* | | Zebrafish | Zebrafish/ *C. elegans* |
| *In Vitro* 3-D Model Systems | ✓ | | ✓ | ✓ |
| Effect of Human/Animal Genetic Background on Toxic Effects | ✓ | ✓ | | |
| Computational Toxicology | ✓ | ✓ | ✓ | ✓ |
| Human Exposure Assessment | | | ✓ | |
| Validation Experience | ✓ | ✓ | ✓ | ✓ |

# Assays screened at NCGC against the Tox21Collection

- Phenotypic readouts
  - Cytotoxicity
    - Cell viability assay (measures ATP)
  - Apoptosis
    - Caspase assays (measure activity of Caspase 3/7, 8, 9)
  - Membrane integrity
    - LDH release
    - Protease release
  - Mitochondrial toxicity
    - Mitochondrial membrane potential
  - Gene tox
    - P53, ELG1, DNA damage repair (chicken DT40 lines and mouse lines)
- Cell Signaling
  - Stress response: ARE, ESRE, HSP, Hypoxia, NFkB (agonist), AP-1 (agonist)
  - Immune response: IL-8, TNFα, TTP
  - Other: AP-1, CRE, ERK, HRE, JNK3, NFkB, TSH, LDR, NPS, Proteasome, SF1, SMN2, Thalassemic (beta-globin splicing), Anthrax Lethal Factor
- Target specific assays
  - Nuclear receptors: AR, AhR, ERα, FXR, GR, LXR, PPARα, PPARδ, PPARγ, PXR, RXR, TRβ, VDR, RORα, RORγ
  - hERG channel
  - Isolated molecular targets: 12hLO, 15hLO1, 15hLO2, ALDH1A1, HADH560, HPGD, HSD17b4, α-Glucosidase, α-Galactosidase, Glucocerebrosidase, APE1, TDP1, DNA polymerase III, RECQ1 helicase, RGS4, BRCA, IMPase, O-Glc NAc Transferase, Caspase-1and -7, CBFβ-RUNX1, PK, Tau, Cruzain, β-Lactamase, PRX, YjeE
- Drug metabolism
  - CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4
- Genetic variation
  - 40 Lymphoblastoid twin cell lines
  - 87 HapMap lines (selected compounds)

# Toxicity Testing in the 21st Century

- Advancing Technology
  - In vitro screening
    - Human tissues more readily available
    - Increases in through-put
      - From 10's to 100,000 chemicals/year/assay
  - Omics
    - Genomics, Proteomics, Metabolomics
      - From single endpoints to high content data (10's of endpoints to 10,000,s)
  - Bioinformatic advances and challenges
    - How do we use all this high content data?
      - Development of databases linking genomic signatures with pathologies
      - Development of predictive signatures of pathological and toxicological concerns

# Grant and Contract-related NTP Activities Supporting Tox21

- Support the development of assays and informatic tools through the NIEHS SBIR/STTR program
  - qHTS (e.g., gap junctions, ROS)
  - *in vitro 3D tissue models (skin, lung, kidney)*
  - lower organism models (*C. elegans, zebrafish)*
  - informatic tools
  - NexGen tools for archived tissues

NIEHS
National Institute of Environmental Health Sciences

# The Genes, Environment, and Health Initiative

## *Genetic Susceptibility - Linking Exposure to Disease*

### Exposure Biology Program

Identify genetic variants in animal models, develop technology and biomarkers

- Diet
- Physical Activity
- Environmental Exposures
- Psychosocial Stress and Addictive Substances

Genetics/Epigenetics

Environment

GxE

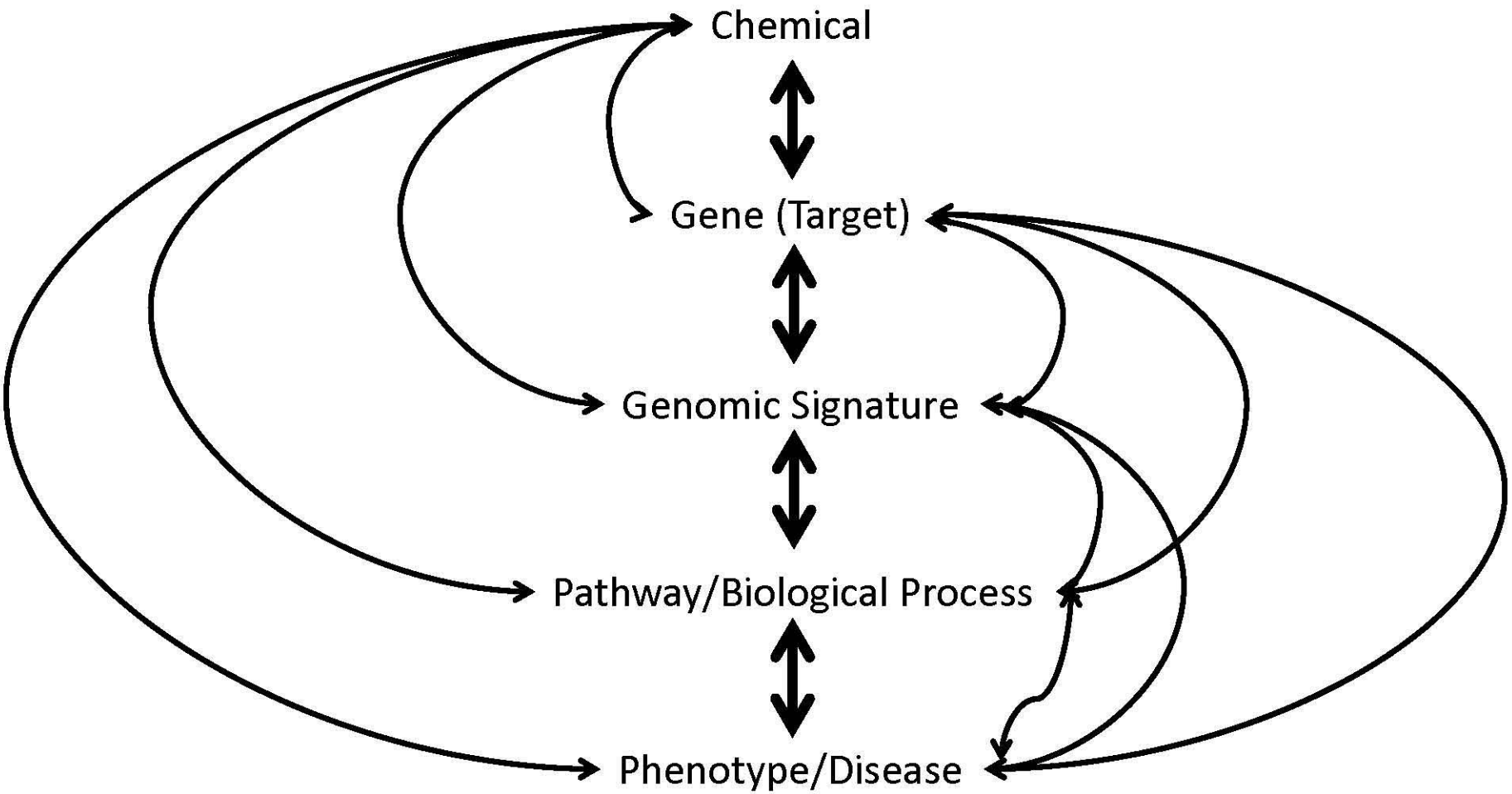
Time

### Human Genetics Program

Identify genetic variants

- GWA Studies
- Data Analysis
- Replication
- Sequencing
- Database
- Function
- Translation

# Relationships Evaluated in Tox 21



# How do we identify Pathways

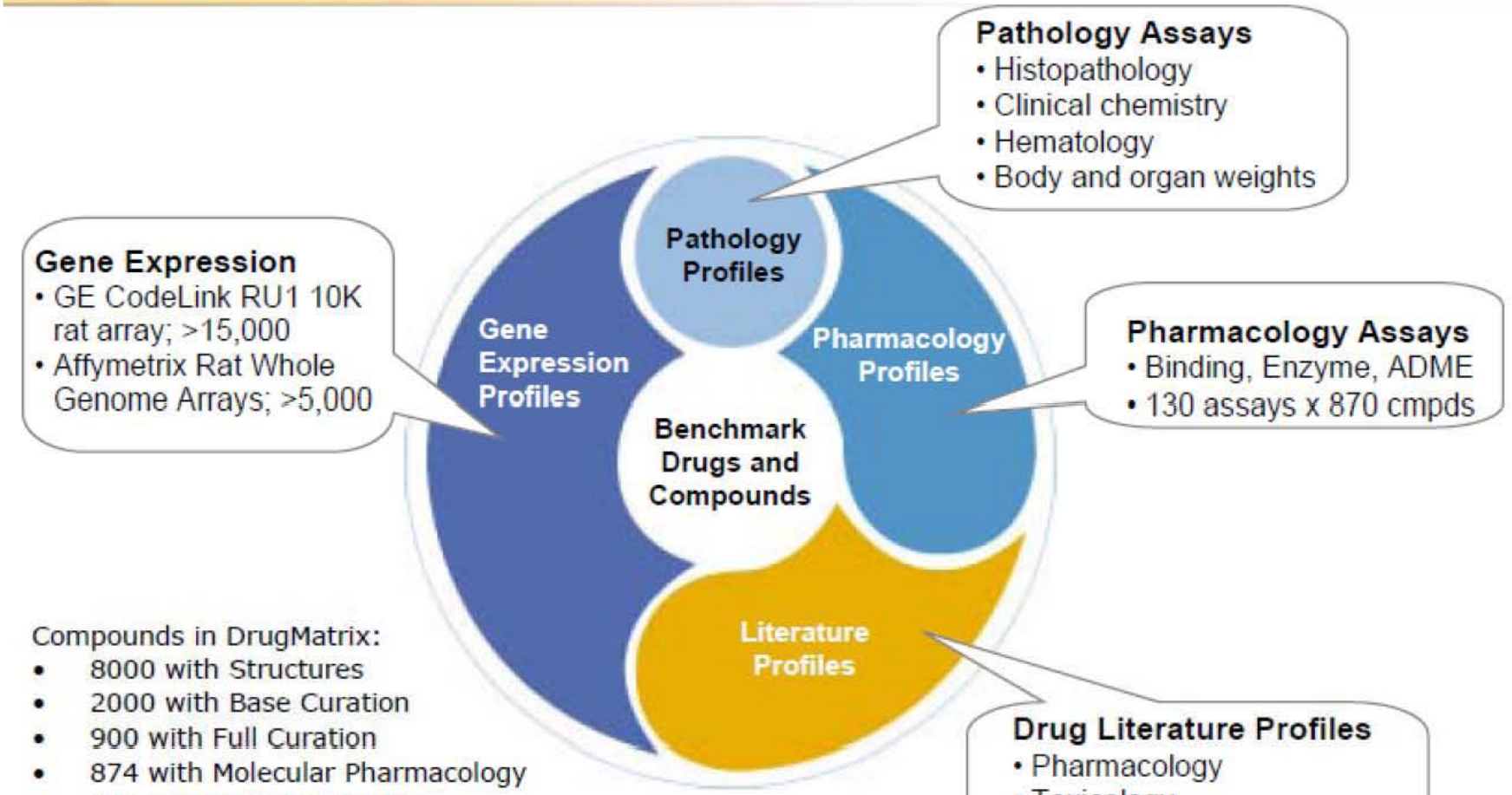
- Toxicity Pathway – These are **genes** associated with chemical-initiated events
- Disease Pathway – These tend to be **genes** that are associated with diseases through genetic studies.
  - Linking these two pathways/networks can strengthen the association of a toxicity pathway to adverse effects.

# Database Resources

| Relationships | Number |
|---|---|
| Chemicals to Pathways | 21 |
| Chemicals to Genomic Signatures | 5 |
| Chemicals to Pathways | 6 |
| Chemicals to Phenotype/Disease | 15 |
| Genes (Targets) to Genomic Signatures | 5 |
| Genes (Targets) to Pathways/Biological Processes | 6 |
| Genes (Targets) to Disease | 13 |
| Genomic Signatures to Pathways | 6 |
| Genomic Signatures to Disease | 6 |
| Pathways to Disease | 6 |
| Chemicals to Genomic signatures | 5 |

# NTP Acquisition of DrugMatrix

## The design of DrugMatrix: A rat toxicogenomic database




Compounds in DrugMatrix:

- 8000 with Structures
- 2000 with Base Curation
- 900 with Full Curation
- 874 with Molecular Pharmacology
- 637 in Expression Studies

# NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity

## January 11-13, 2011

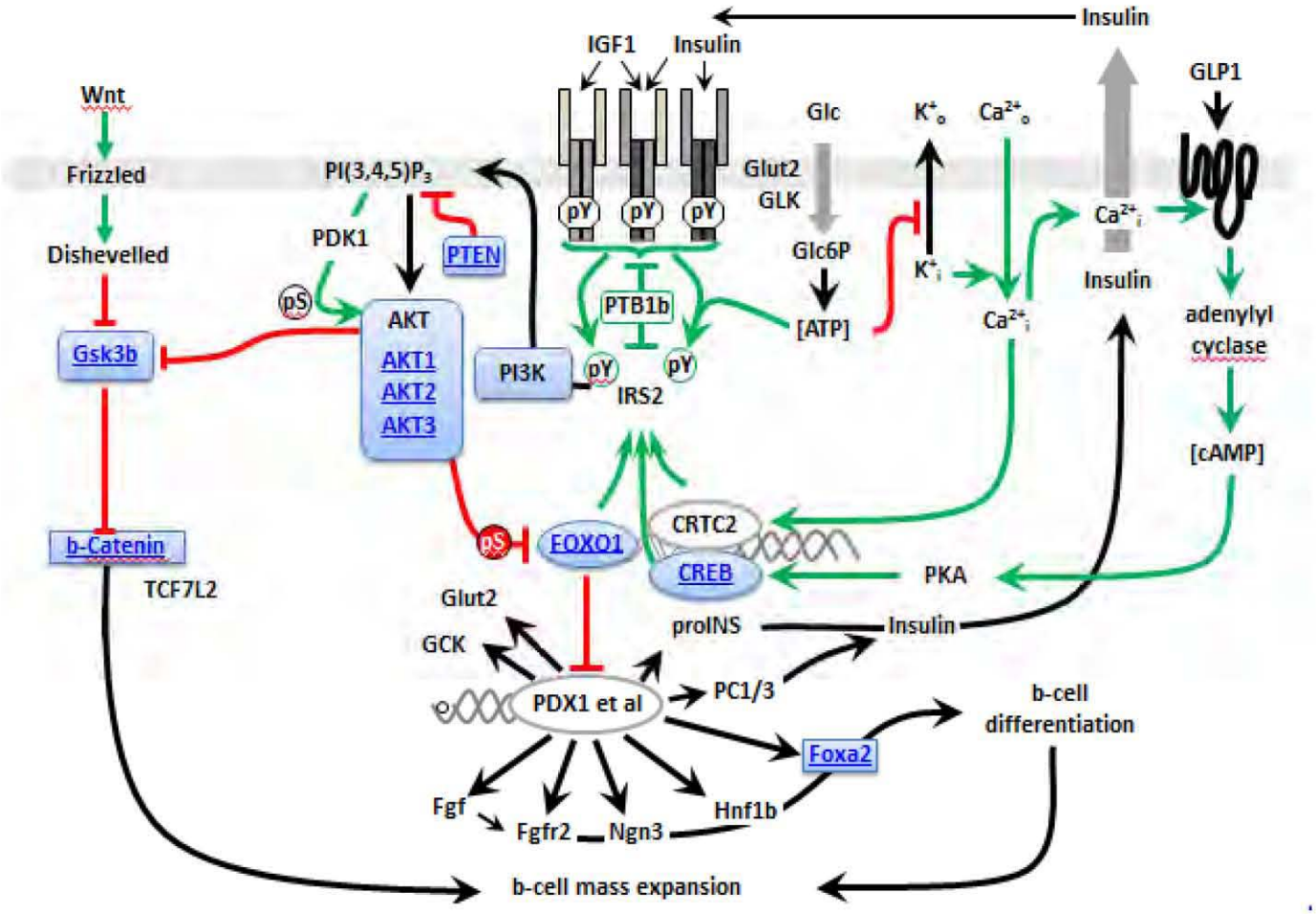
Brought together experts on diseases, toxicology, epidemiology and HTS/bioinformatics

Identified toxicity pathways (AhR, PPAR, CAR, PXR, others) associated with diabetes and obesity in studies of arsenic, phthalates, organotins, nicotine, etc

Identified disease pathways (insulin signaling, adipocyte differentiation, feeding behavior etc.) associated with diabetes and obesity

Identified some critical future HTS targets to better understand associations between environmental exposures and these diseases.

# Disease Pathway for Type I and Type II Diabetes



# Top Insulin-Sensitivity Gene Targets not in Tox21 (Morris White)

- Insulin receptor substrate-1 (IRS1)
- Insulin receptor substrate-2 (ISR2)
- Transcription factor 7-like 2 (TCF7L2)
- Phosphatidylinositol 3-kinase (PI3K)
- Phosphatase and tensin homolog (pTEN)
- Glucose transporter 2 (GLUT2)

# In Conclusion: Linking Research and Risk Assessment

- Partnerships between research and regulatory agencies
- Risk and Safety – 2 sides of same coin
- New issues and technologies
- Complex issues require both individual and team approaches

- Improve integration across research disciplines
- Improve translation and communication of basic science findings into human health protection
  - Stop talking and start listening

# Thank you!

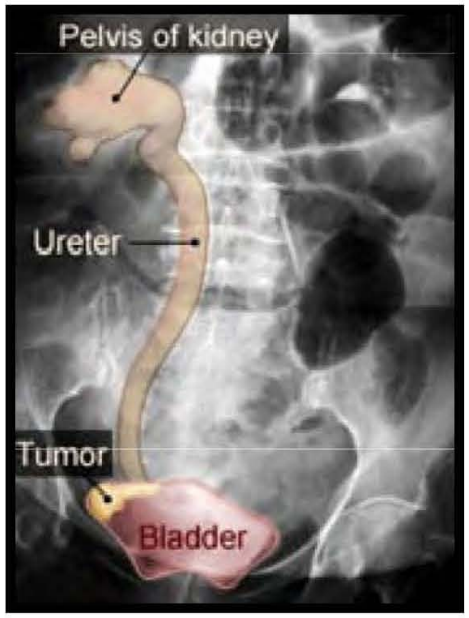
NIEHS
National Institute of
Environmental Health Sciences

National Toxicology Program
U.S. Department of Health and Human Services

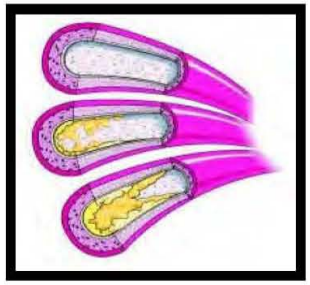
Srp
Superfund
Research Program

# Dose-Response Assessment: Arsenic - How Much is TOO much?

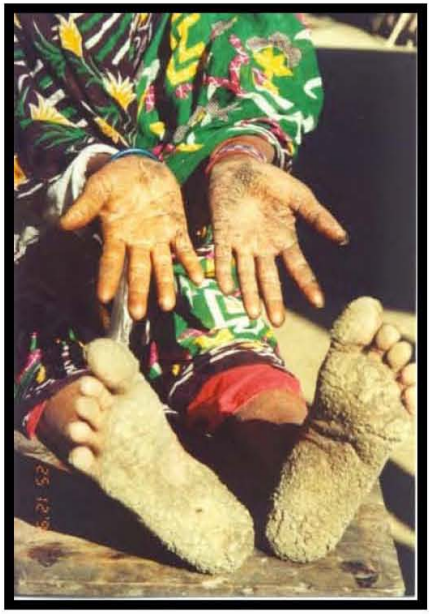
The previous U.S. EPA arsenic water standard was **50 µg/L (ppb)**. However, the EPA issued a new standard of **10 µg /L (ppb)** in response to studies that suggest 50 µg/L (ppb) could still cause disease. 84 studies found in EPA's IRIS draft document for arsenic were written by SRP-funded researchers.




Bladder Cancer
Liver Cancer
Kidney Cancer



Hypertension
Developmental effects
Diabetes



Skin Cancer
Keratosis
Melanosis
Blackfoot Disease

NIEHS
National Institute of Environmental Health Sciences

# Exposure Assessment

## DERMAL EXPOSURE ASSESSMENT

- The ability to quantify dermal exposure and evaluate its contribution to total exposure and internal dose is critical to the development of exposure and risk assessment models.
- UNC-Chapel Hill SRP researcher Dr. Nylander-French developed and validated:
  - a non-invasive sampling technique, coupled with analytical methods (GC/MS; ELISA), to quantify dermal exposure.
  - a mathematical model to quantify the penetration of chemicals deposited on the skin surface into its deeper layers

## SPATIAL AND TEMPORAL ANALYSES FOR EXPOSURE ASSESSMENT

- Researchers at the SRP at Boston University (BU) have used Geographic Information System (GIS) data and statistical methods to examine the geographical distribution of disease, which can provide important clues to exposures and the origins of the disease.
- The researchers integrated groundwater modeling, residential mobility, and information about public water systems in GIS to assess exposure to drinking water impacted by wastewater effluent.

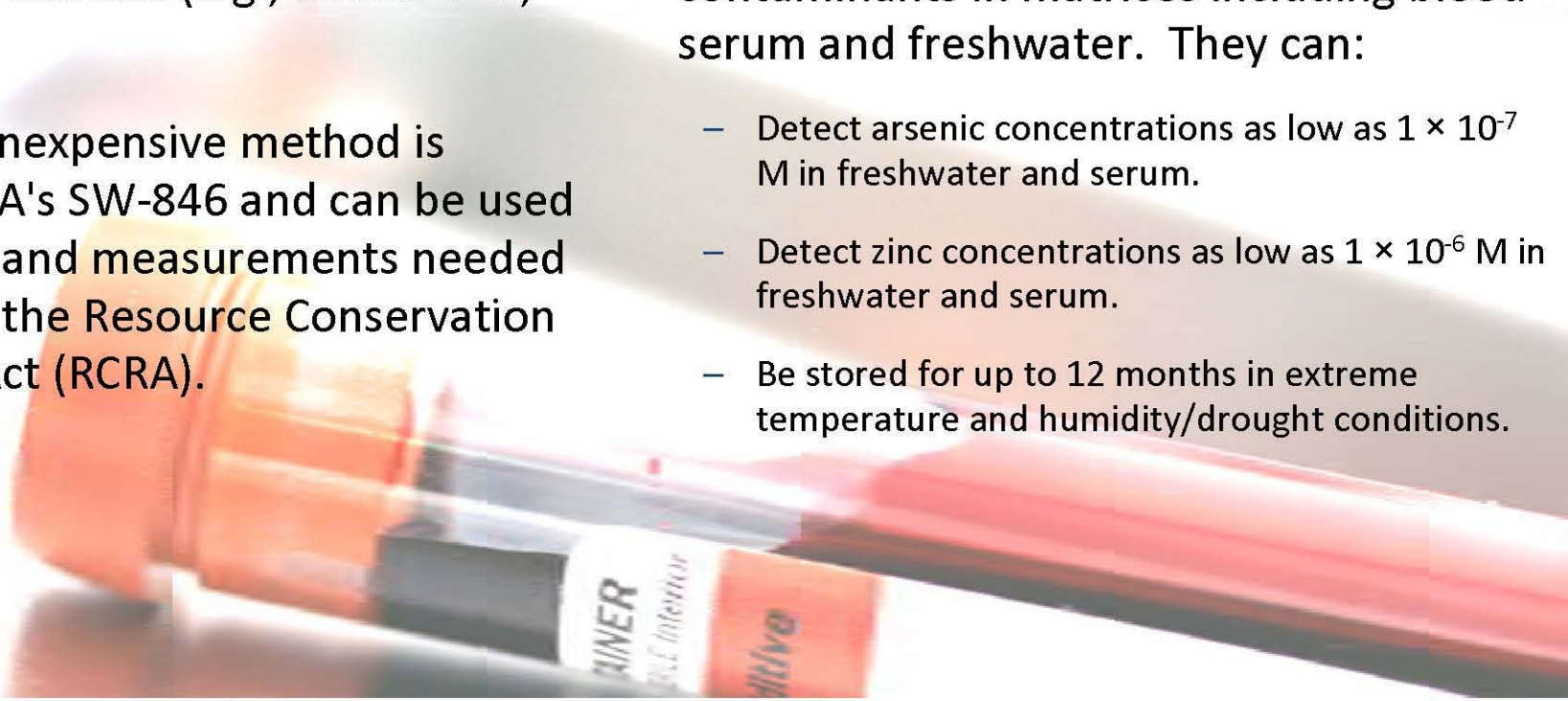
# Hazard Identification – SRP Research Examples

## XDS-CALUX

- SRP-funded researcher Dr. Michael Denison, UC-Davis, developed and validated CALUX: a bioassay to detect dioxins, furans, and PCBs in biological matrices (e.g., blood, breast milk, tissues); environmental matrices (.g., water, soil, ash); and food matrices (e.g., animal fats, milk).
- This rapid and inexpensive method is published in EPA's SW-846 and can be used for evaluations and measurements needed to comply with the Resource Conservation and Recovery Act (RCRA).

## SPORE-BASED BIOSENSORS

- Dr. Sylvia Daunert, University of Kentucky SRP developed whole-cell sensing systems with spore-forming bacteria.
- These resilient systems can be used for on-site applications to test for hazardous contaminants in matrices including blood serum and freshwater. They can:
  - Detect arsenic concentrations as low as $1 \times 10^{-7}$ M in freshwater and serum.
  - Detect zinc concentrations as low as $1 \times 10^{-6}$ M in freshwater and serum.
  - Be stored for up to 12 months in extreme temperature and humidity/drought conditions.



# Risk Characterization

## **B**iological **R**esponse **I**ndicator **D**evices for **G**auging **E**nvironmental **S**tressors (BRIDGES)

- Dr. Kim Anderson at the Oregon State University SRP combines passive sampling devices plus an embryonic zebrafish developmental model to assess the toxicity of bioavailable contaminant mixtures present in the environment.
- The "BRIDGES" bio-analytical tool links bioavailable contaminant concentrations to biological responses, providing an interface between environmental exposure and aquatic/human health risk.
- Dr. Anderson believes the BRIDGES bio-analytical tool could be used as a complementary tool for environmental and risk assessment in conjunction with chemical characterization of sites.
- BRIDGES could inform management actions by ***providing site-specific mixture toxicity*** data that could be used to validate management actions or suggest the need to further investigate or reassessment.

# SRP Research Informs Uncertainty Characterizations in the Risk Assessment Process

- Benzene is carcinogenic, but ***must be metabolized to exert its toxicity***.
- Drs. Stephen Rappaport and Martyn Smith (UC-Berkeley) are investigating human metabolism of benzene and identified a previously unrecognized enzyme active at low benzene concentrations.
- Applying their two-enzyme model, it is reasonable to conclude that *current risk assessments would likely underestimate leukemia risks at ambient air concentrations of benzene by a factor of about three* for nonsmoking women.

# SRP Research Informs Uncertainty Characterizations in the Risk Assessment Process

- **Arsenic Just as Risky Ingested as Inhaled**
- Cancer is the leading cause of death associated with arsenic exposures, exceeding mortality from bladder cancer, kidney cancer, and cardiovascular disease.
- While it seems logical to expect that the risks from inhalation with direct exposure to lung cells would be much higher than the risks from ingestion, Dr. Allan Smith at the UC-Berkeley SRP found that lung cancer risks are ***not dependent upon the exposure pathway,*** but rather on the absorbed dose.

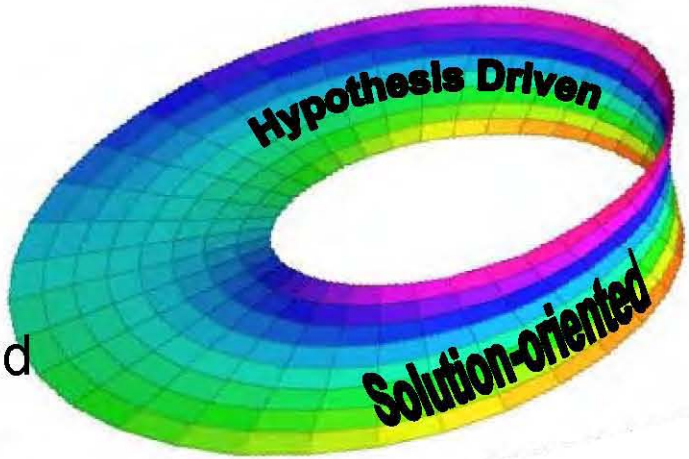
# Superfund Research Program (SRP) History and Structure

- Created in 1986 under the Superfund Amendments and Reauthorization Act (SARA)
- Mandates: To fund and support the development of
  - Advanced techniques for detection, assessment, and evaluation of effects on human health of hazardous substances
  - Methods and technologies to detect hazardous substances in the environment
  - Methods to assess the human health risks presented by hazardous substances
  - Basic biological, chemical, and physical methods to reduce the amount and toxicity of hazardous substances

Superfund Research Program

# Guiding Principles

- Accountable - to stakeholders and taxpayers
- Coordinated – among other research and training programs
- Transparent – open communication




Srp
Superfund
Research Program

# A University-Based Grant Program

- Multidisciplinary Research
  - Collaborative research supports projects covering the spectrum of environmental health
  - Links researchers specializing in molecular toxicology, biomarker development, remediation tools, engineering, epidemiology, and more
- Training
  - Over 400 graduate and postdoctoral trainees
  - The education and training delivered is interdisciplinary, making trainees competitive in their respective fields
- Community engagement
  - Community driven
  - Draws from expertise of program investigators

# The Grantees

- Three Grant Mechanisms
  - Multi-project Grants
    - 19 Universities
    - 208 Research Projects & Cores
    - 100 Collaborating Institutions
  - Individual Project Grants
    - 11 Grantees
  - SBIR/STTR Grants
    - 3 grantees
    - 13 within the past 2 years

- Statistics
  - ~250 researchers
  - ~400 trainees
  - >200 hazardous waste sites visited by SRP researchers

Srp Superfund Research Program

# Research Our Grantees Conduct

- Biomedical
- Bioavailability
- Fate and transport
- Remediation methods and technologies
- Analytical/monitoring
- Epidemiology

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# Research Products

- Over 6000 publications
- 55 patents
- Enrichment materials (fact sheets, videos)
- Research Briefs
- Webinars
- Workshops
- Conferences


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NIEHS
National Institute of Environmental Health Sciences

# Superfund Research and Worker Training Program

## Research

- Detect hazardous substances in the environment
- Evaluate the risk of hazardous substances on human health
- Develop basic biological, chemical, and physical methods to reduce the toxicity of hazardous substances

**NIEHS**

Use environmental sciences to understand human biology and human disease

**EPA**

Regulate to protect human health and the environment

**ATSDR**

Prevent harmful exposures and diseases related to toxic substances

Srp
Superfund Research Program

# In Conclusion: A New Vision for NIEHS and NTP

- A strong desire to partner with our sister institutes and other federal agencies: EPA, CDC, FDA, DOE….
- Health and Environment is a priority
- New issues and technologies are emerging
- We need the best individual and team science to address complex diseases and complex environmental impacts
- We need to improve integration across research disciplines and with all partners
- We need to improve our translation and communication of basic science findings into human health protection