ORDs Computational Toxicology Research Program

Briefing for Region 6

January 10, 2006

Robert Kavlock
National Center for Computational Toxicology
US Environmental Protection Agency
Research Triangle Park, NC

Enabling ("Omic") Technologies

Cell Biology

DNA

Transcription

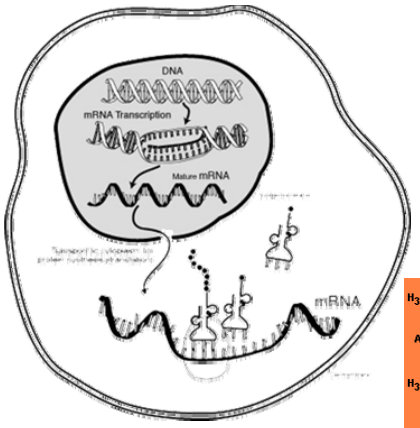
mRNA

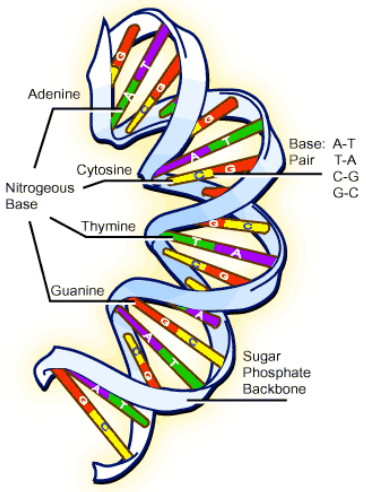
Translation

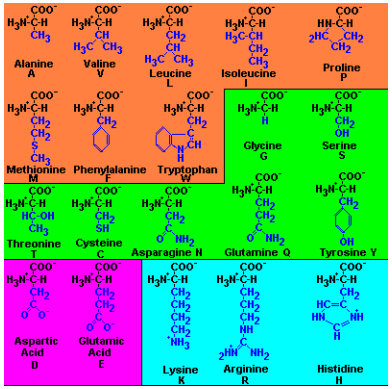
Protein

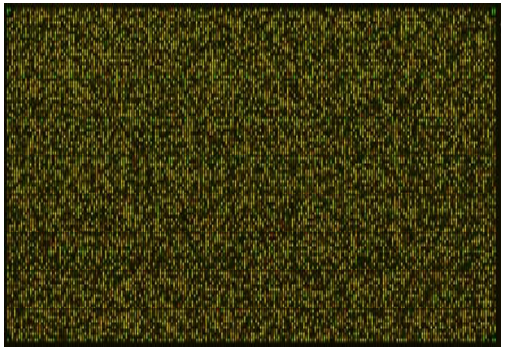
Metabolism

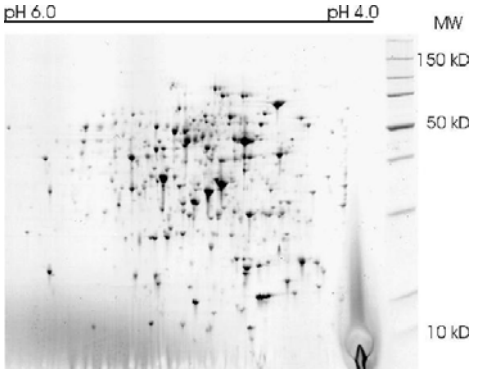
Metabolites

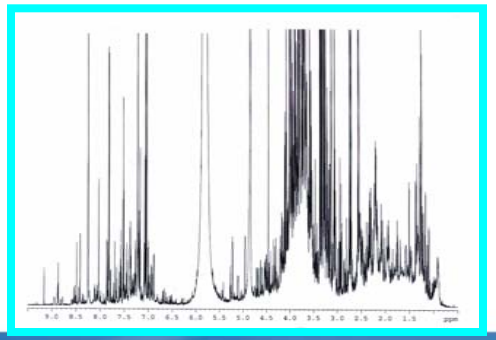






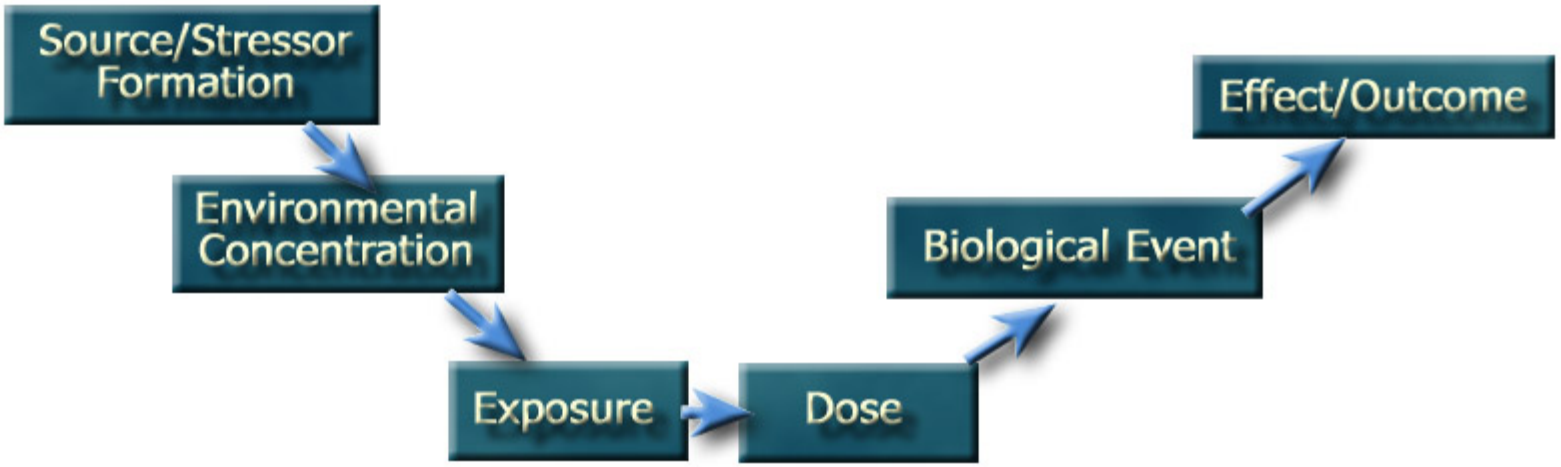






RESEARCH & DEVELOPMENT

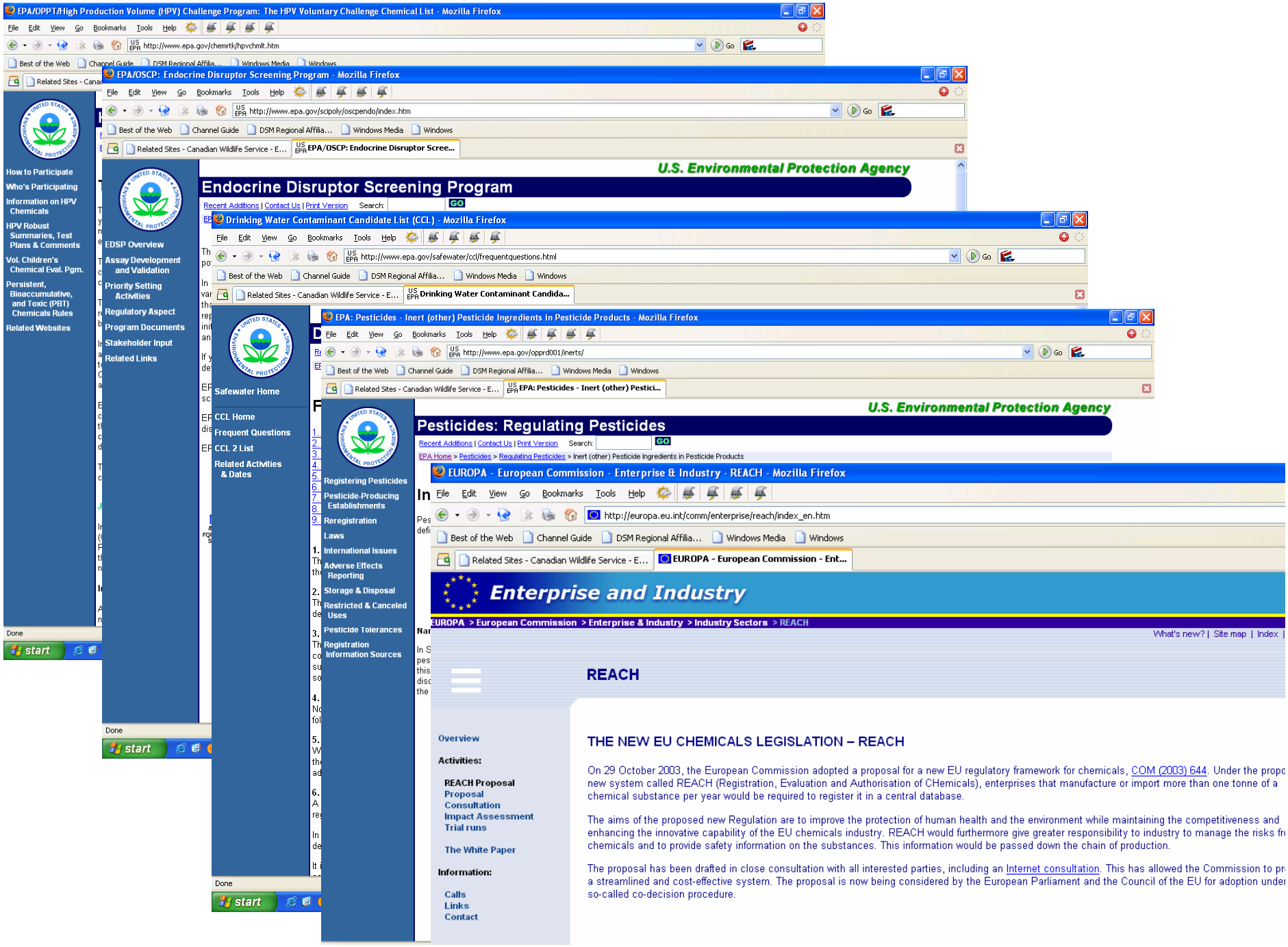
Building a scientific foundation for sound environmental decisions



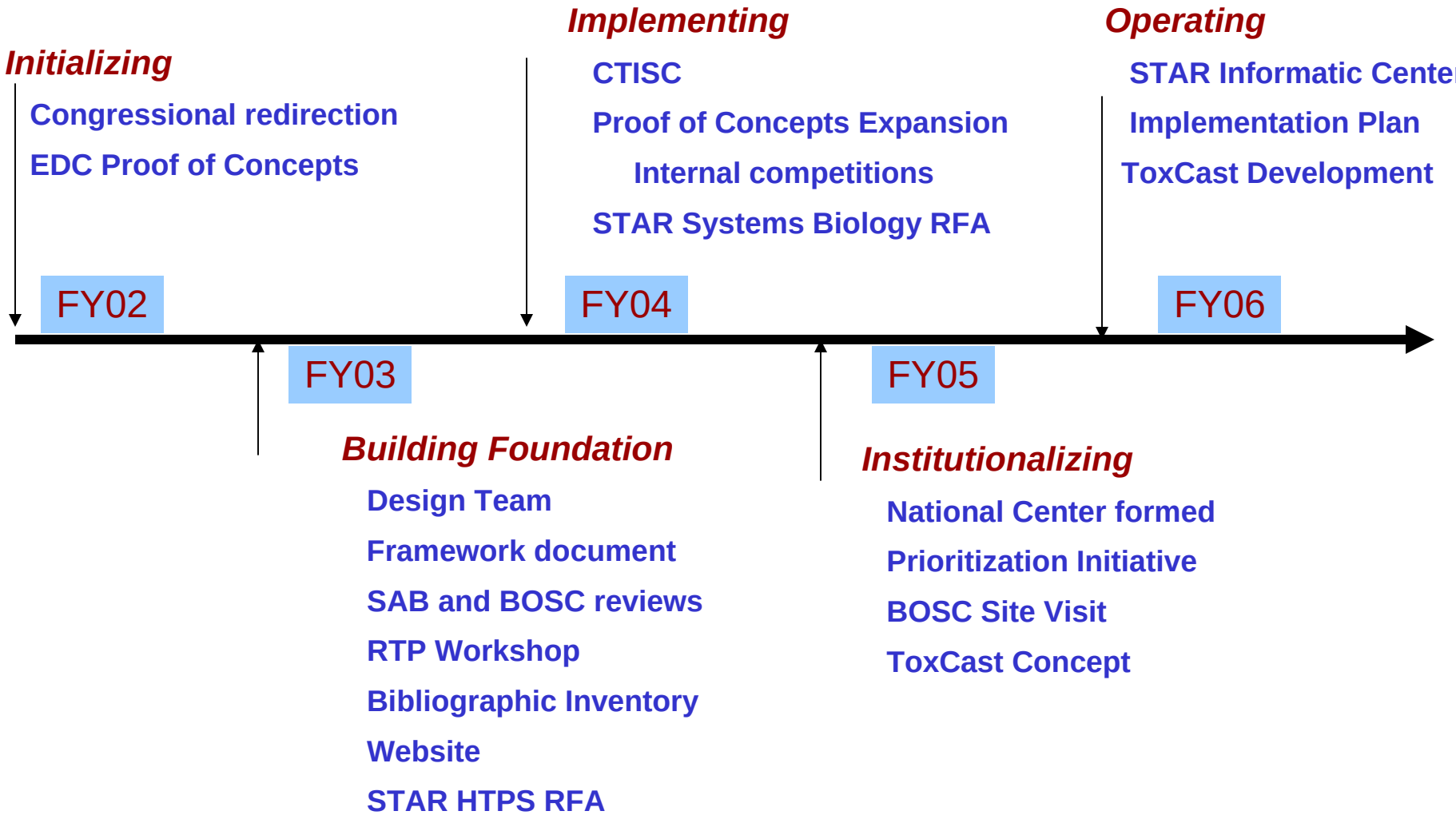
PROGRAMMATIC CHALLENGES

- Many Priority Lists Already in Queue (e.g., EDC's, Pesticide Inerts, HPV's, CCL) with No Risk-Based Criteria for Setting Testing Priorities
- Different Authorities – Different Testing Requirements with No Scientific Basis for Flexible Testing Approaches
- Lack Data Needed to Reduce Uncertainties by Quantitative Risk Assessments (e.g., extrapolations)

COMPUTATIONAL TOXICOLOGY


EPA/OPPT/High Production Volume (HPV) Challenge Program: The HPV Voluntary Challenge Chemical List - Mozilla Firefox
http://www.epa.gov/chemrtk/hpvchmlt.htm
How to Participate
Who's Participating
Information on HPV Chemicals
HPV Robust Summaries, Test Plans & Comments
Vol. Children's Chemical Eval. Pgm.
Persistent, Bioaccumulative, and Toxic (PBT) Chemicals Rules
Related Websites
EPA/OSCP: Endocrine Disruptor Screening Program - Mozilla Firefox
http://www.epa.gov/scipoly/oscpendo/index.htm
U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program
EDSP Overview
Assay Development and Validation
Priority Setting Activities
Regulatory Aspect
Program Documents
Stakeholder Input
Related Links
Drinking Water Contaminant Candidate List (CCL) - Mozilla Firefox
http://www.epa.gov/safewater/ccl/frequentquestions.html
Safewater Home
CCL Home
Frequent Questions
CCL 2 List
Related Activities & Dates
EPA: Pesticides - Inert (other) Pesticide Ingredients in Pesticide Products - Mozilla Firefox
http://www.epa.gov/opprd001/inerts/
Pesticides: Regulating Pesticides
Registering Pesticides
Pesticide-Producing Establishments
Reregistration
Laws
International Issues
Adverse Effects Reporting
Storage & Disposal
Restricted & Canceled Uses
Pesticide Tolerances
Registration Information Sources
EUROPA - European Commission - Enterprise & Industry - REACH - Mozilla Firefox
http://europa.eu.int/comm/enterprise/reach/index_en.htm
Enterprise and Industry
EUROPA > European Commission > Enterprise & Industry > Industry Sectors > REACH
REACH
Overview
Activities:
REACH Proposal
Proposal
Consultation
Impact Assessment
Trial runs
The White Paper
Information:
Calls
Links
Contact
THE NEW EU CHEMICALS LEGISLATION – REACH
On 29 October 2003, the European Commission adopted a proposal for a new EU regulatory framework for chemicals, COM (2003) 644. Under the propo
new system called REACH (Registration, Evaluation and Authorisation of CHemicals), enterprises that manufacture or import more than one tonne of a
chemical substance per year would be required to register it in a central database.
The aims of the proposed new Regulation are to improve the protection of human health and the environment while maintaining the competitiveness and
enhancing the innovative capability of the EU chemicals industry. REACH would furthermore give greater responsibility to industry to manage the risks fr
chemicals and to provide safety information on the substances. This information would be passed down the chain of production.
The proposal has been drafted in close consultation with all interested parties, including an Internet consultation. This has allowed the Commission to pr
a streamlined and cost-effective system. The proposal is now being considered by the European Parliament and the Council of the EU for adoption under
so-called co-decision procedure.

Program Development



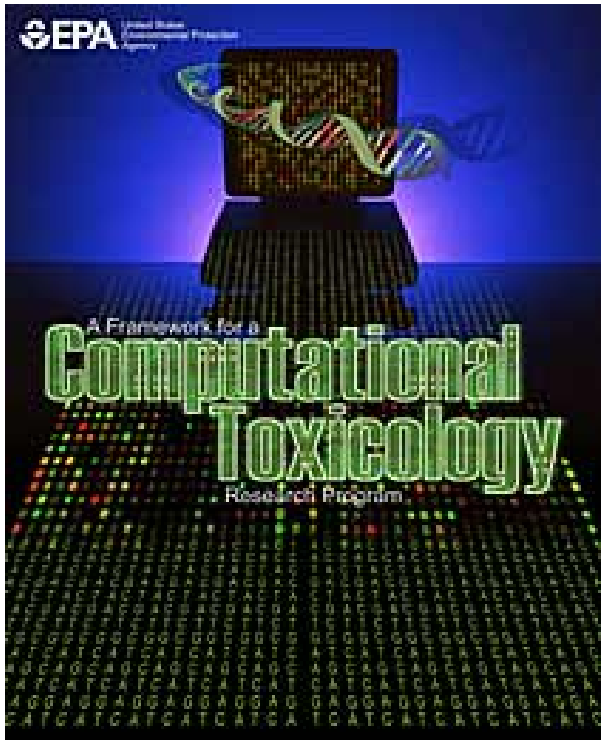
ORD's Computational Toxicology Research Program

Themes:

- A technology-based, hypothesis-driven effort to increase the soundness of risk assessment decisions within EPA
- Build the capacity to prioritize, screen and evaluate chemicals by enhancing the predictive understanding of toxicity pathways

Success:

- Measured by ability to produce faster and more accurate risk assessments for less cost relative to traditional means and to classify chemicals by their potential to influence molecular and biochemical pathways of concern



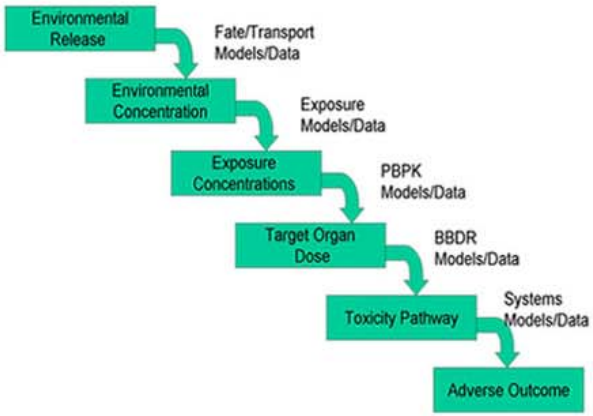
www.epa.gov/comptox

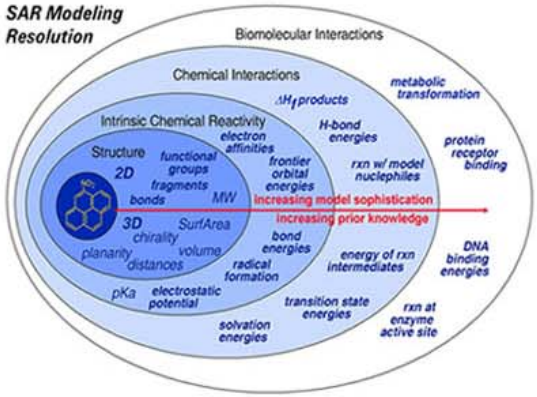
OBJECTIVES OF THE PROGRAM

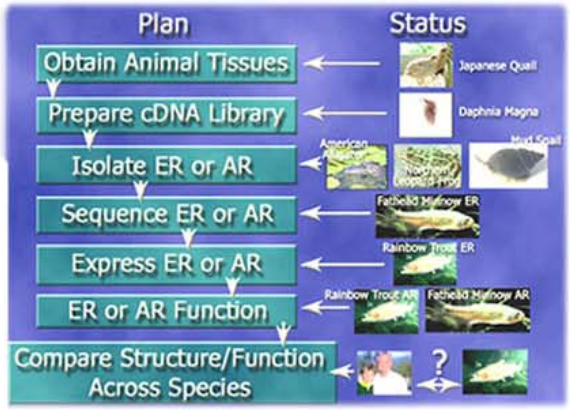
1. Improve the Linkages in the Source to Outcome Paradigm

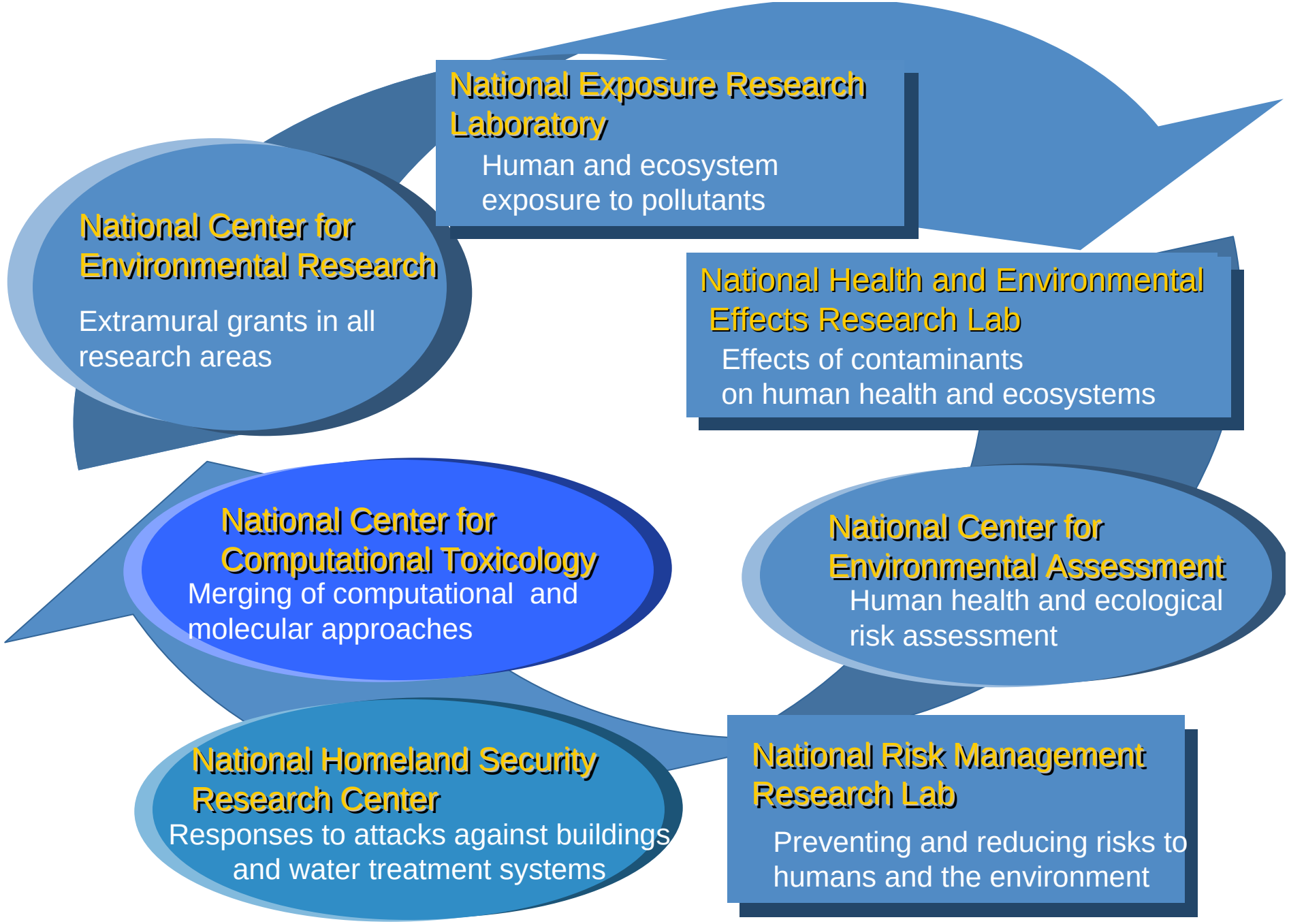
2. Provide Predictive Models for Hazard Identification

3. Enhance Quantitative Risk Assessment







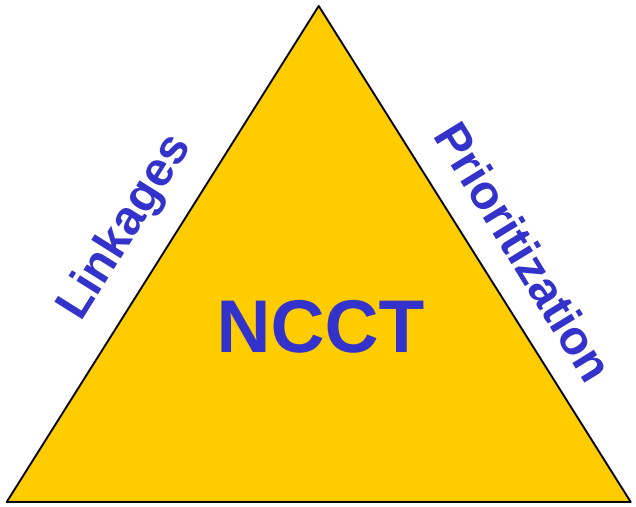

National Exposure Research Laboratory
Human and ecosystem exposure to pollutants
National Center for Environmental Research
Extramural grants in all research areas
National Health and Environmental Effects Research Lab
Effects of contaminants on human health and ecosystems
National Center for Computational Toxicology
Merging of computational and molecular approaches
National Center for Environmental Assessment
Human health and ecological risk assessment
National Homeland Security Research Center
Responses to attacks against buildings and water treatment systems
National Risk Management Research Lab
Preventing and reducing risks to humans and the environment

NCCT Mission

- To provide **scientific expertise and leadership** related to the application of mathematical and computational tools and models
- T**o improve the predictive capabilities** of the methods, models and measurements that constitute the input materials to the computational models.
- To **conduct and/or sponsor research** to provide models for fate and transport of chemicals, environmental exposures to humans and wildlife, delivery of the chemical to the target site of toxicity, molecular and cellular pathways of toxicity, and ultimately systems level understanding of biological processes and their perturbation
- Maintain a strong emphasis on the **development of partnerships** with other government and private organizations

Program Development: Formation and Staffing of a National Center for Computational Toxicology

III. Biological Models
- H Barton
- J Blancato
- M Breen*
- R Conolly
- R Garcia *
- C Rodriquez *
- TBN (Title 42)
- TBN
- TBN*
- TBN*



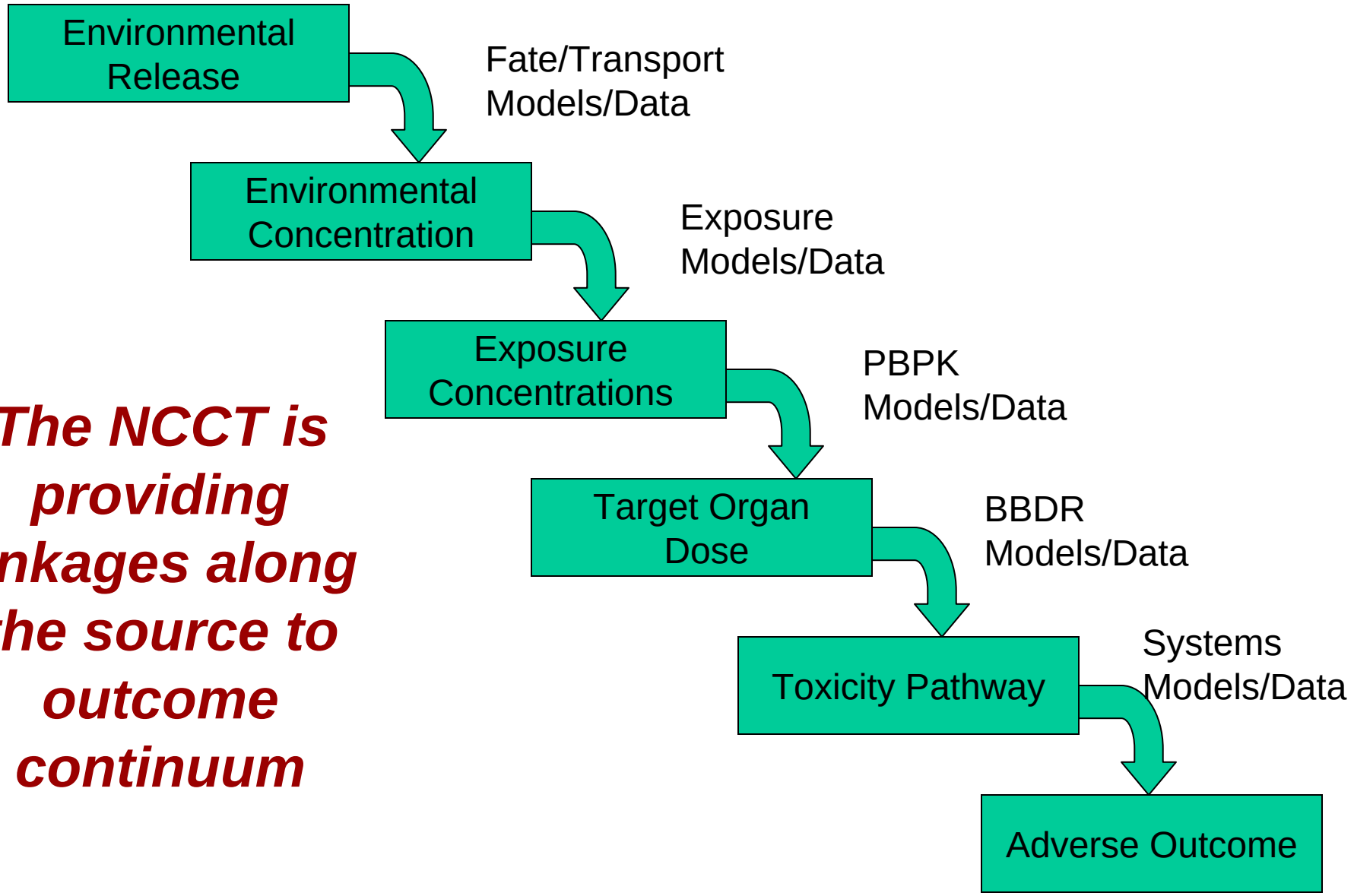
I. Information Technology
- A Richard
- TBN (Title 42)
- TBN
- TBN *

II. Prioritization and Screening
- D Dix
- R Goldsmith *
- K Houck
- R Kavlock
- M Martin (intern)
- S Little
- M Pasquinelli*
- J Rabinowitz

IV. Cumulative Risk
- E Hubal
- W Setzer
- TBN*

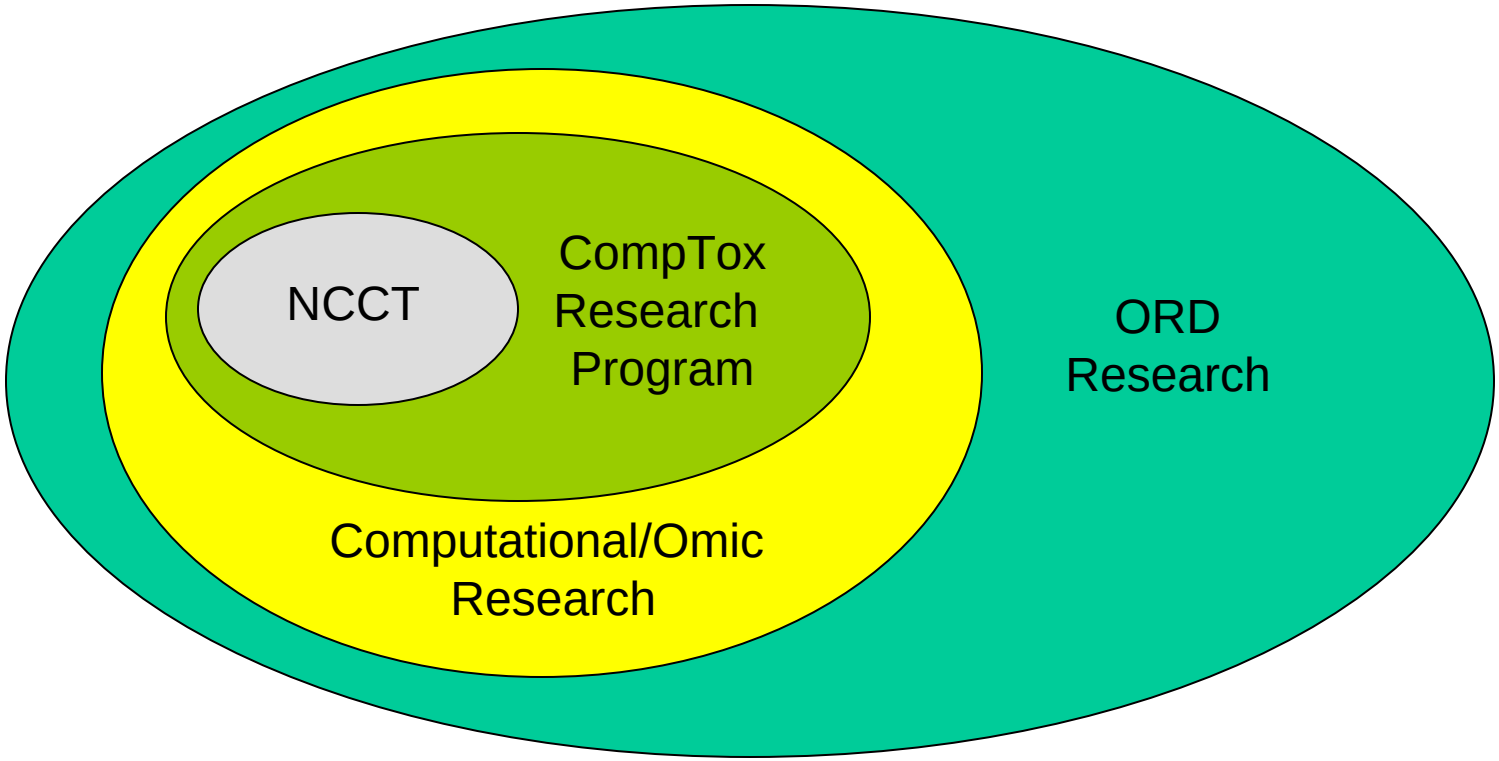
Administrative Support
- Karen Dean, Program Analyst
- Sandra Roberts, Exec Secretary

*** = Postdoc**



The NCCT is providing linkages along the source to outcome continuum

The NCCT is part of a larger whole…



Computational Toxicology Implementation and Steering Committee

(Jan 04 – present)

- **NCEA**
  - **Ines Pagan**
  - **Paul White**
- **NCER**
  - **Susan Laessig**
  - **David Mustra**
- **NERL**
  - **Tim Collette**
  - **Greg Toth**
- **NHEERL**
  - **Gerald Ankley**
  - **Jack Fowle**
  - **Doug Wolf**
- **NHSRC**
  - **Chandrika Moudgal**
- **NPD**
  - **Elaine Francis**
- **NRMRL**
  - **Jorge Santo Domingo**
  - **Doug Young**
- **OPPTS**
  - **Vickie Dellarco**
  - **Yin-Tak Woo**
- **OAR**
  - **Scott Jenkins**
- **OW**
  - **Steve Kueberuwa**
  - **Clifton Townsend**
- **Regions**
  - **David Macarus (Reg 5)**

Program Development: Implementing a Cross-ORD Research Portfolio

Amphibian Systems Model

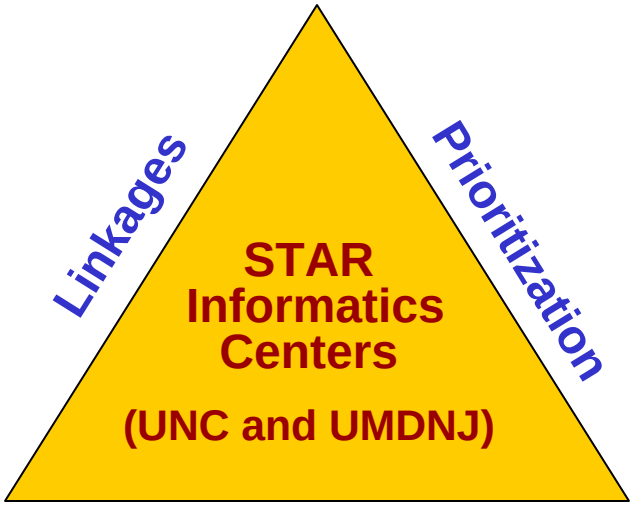
Fish Proteomics
Fish Toxicogenomics
Fish metabonomics

Conazole MOA

Children's Health
Pulmonary Biomarkers

Microbial Metagenomics

STAR Systems Models



QRA

Diesel Particles
HPG Axis Model
Pellston, SOT and NCEA Workshops

DSSTox

Metabolic Simulator

ASTER

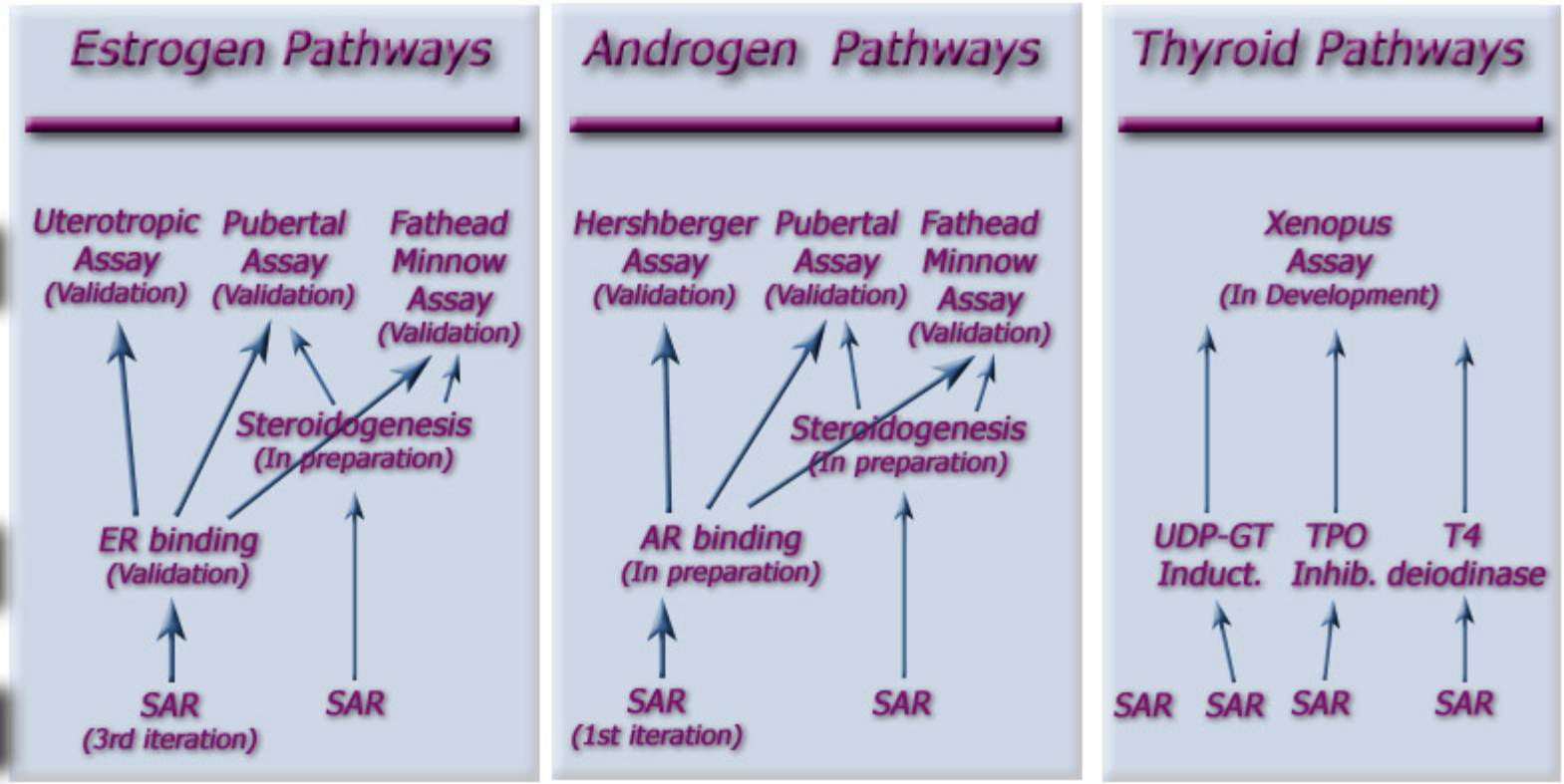
ER Binding Data
Molecular Docking
ER/AR Scale up
H295R Assay
Iconix Contract

STAR HTPS RFA

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Impaired Reproduction/Development



COMPUTATIONAL TOXICOLOGY

Proof of Concept Studies

- ER Binding data refinement
- ER QSAR Enhancement
- Steroid docking model studies
- H295R Assay evaluation
  - In vitro assay for steroidogenesis using a human cell line
- ER and AR Transcription Assay scale up
  - To use NHEERL developed cell lines, or equivalent
- Predictive Toxicogenomics evaluation
  - Hepatic gene changes following acute exposure in rat
  - Groups of chemicals from 2 classes of hepatotoxicants

H295R Development

TABLE 4. Fold Differences in Gene Expression for H295R Cells Exposed to Model Chemicals[a]

Chemical	CYP17	StAR	CYP11A	CYP19	CYP21	HMGR	17βHSD1	3βHSD1	3βHSD2	CYP11B2	CYP11B1
Inducers											
8-Br-cAMP		↑		↑↑	↑↑↑			↑	↑↑↑	↑↑↑	↑↑↑
PMA	↓↓↓	↑		↑↑	↑↑↑		↓	↑	↑↑↑	↑↑↑	↑↑
forskolin	↑	↑	↑	↑↑	↑↑↑	↑		↑↑	↑↑↑	↑↑↑	↑↑↑
lovastatin	↑	↑						↓		↑↑	↑
Inhibitors											
spironolactone				↑							↑
DL-aminoglutethimide					↓		↑	↑	↓		↑↑↑ Λ
daidzein		↓			↑		↑ Λ	↑ Λ	↑ Λ	↑ Λ	↑↑↑ Λ
ketoconazole			↑ Λ		↑ Λ		↑ Λ	↑ Λ		↑	↑
spironolactone				↑							↑
DL-aminoglutethimide					↓		↑	↑	↓		↑↑↑ Λ
daidzein		↓			↑		↑ Λ	↑ Λ	↑ Λ	↑ Λ	↑↑↑ Λ
ketoconazole			↑ Λ		↑ Λ		↑ Λ	↑ Λ		↑	↑

[a] Symbols indicate fold difference relative to control; ↑=2-fold or more; ↑↑=5-fold or more; ↑↑↑=10-fold or more. Λ= response recovered at highest concentration. All other differences are less than 2-fold.

Zhang, X., R. Yu, P. D. Jones, J. L. Newsted, T. Gracia, M. Hecker, K. Hilscherova, J. T. Sanderson, R. Wu, and J. P. Giesy. 2005. Quantitative RT-PCR Methods for Evaluating Toxicant-Induced Effects on Steroidogenesis Using the H295R Cell Line. *Environ. Sci. Technol.* 39:2777-2785.

STAR Environmental Bioinformatics Centers

- RFA - The successful applicant will:
  - Provide a multidisciplinary approach to development and application of computational methods that target multiple points along source-to-outcome continuum;
  - Presents opportunities for investigators with differing expertise to work together on larger issues than could be addressed in a single grant or cooperative agreement proposal;
  - Discuss application and development of bioinformatics standards and nomenclature, which will increase the portability and usability of Center-developed resources and tools.
  - Funding via a Cooperative Agreement
  - Five year duration
- Recipients (October 2006)
  - UMDNJ and UNC
- David Mustra, NCEA, Project Officer

Partnerships

- Internal EPA
  - Communities of Practice
    - **Chemoinformatics, Prioritization, Biological Modeling**
- Federal
  - Department of Energy
  - Department of Defense
  - FDA/NCTR
  - NIEHS/NTP
  - NIH/MLI
- Private Sector
  - IBM
  - Affymetrix
  - CIIT Centers for Health Research
  - SBIR Solicitations
- International
  - ITSC (Former Soviet Union weapons scientists)

EPA Data Challenges:

- “Electronification” of historical data
- Structure-annotation
- Data standardization & integration

- InChI text annotation
- EPA Structure-browser
- Collaborations with ATSDR, ECB, FDA, NIEHS

Distributed Structure-Searchable Toxicity (DSSTox) Public Database Network

http://www.epa.gov/nheerl/dsstox

DSSTox

The Distributed Structure-Searchable Toxicity (DSSTox) Database Network is a project of EPA's Computational Toxicology Program, helping to build a public data foundation for improved structure-activity and predictive toxicology capabilities. The DSSTox website provides a public forum for publishing downloadable, standardized toxicity data files that include chemical structures. More

Recent Additions: 1Mar05

***New Database Additions:**

- FDA Maximum (Recommended) Daily Dose Database (FDAMDD) of 1217 pharmaceuticals - 1Mar05

***Expanded and modified versions:**

- Consolidated, updated Carcinogenic Potency Database - All Species (CPDBAS), 1451 compounds: 91 new records added to v2a
- CAS registry numbers added to EPAFHM and DBPCAN

***New Standard Fields added to all DSSTox files:**

- INChI (IUPAC/NIST Chemical Identifier) unique structure-text codes
- IUPAC systematic chemical names (generated by ACD/Name)
- Standard Toxicity Fields: StudyType, Species, Endpoint fields

***New Features of Site:**

- FTP Download Instructions for easy access to archived and new DSSTox data files
- New information pages: INChI, DSSTox Standard Toxicity Fields
- Links to External Public Databases adopting DSSTox standards: ISSCAN *new*

- DSSTox Graphic Flowchart
- DSSTox Project Goals
- DSSTox Publications

DSSTox Databases:

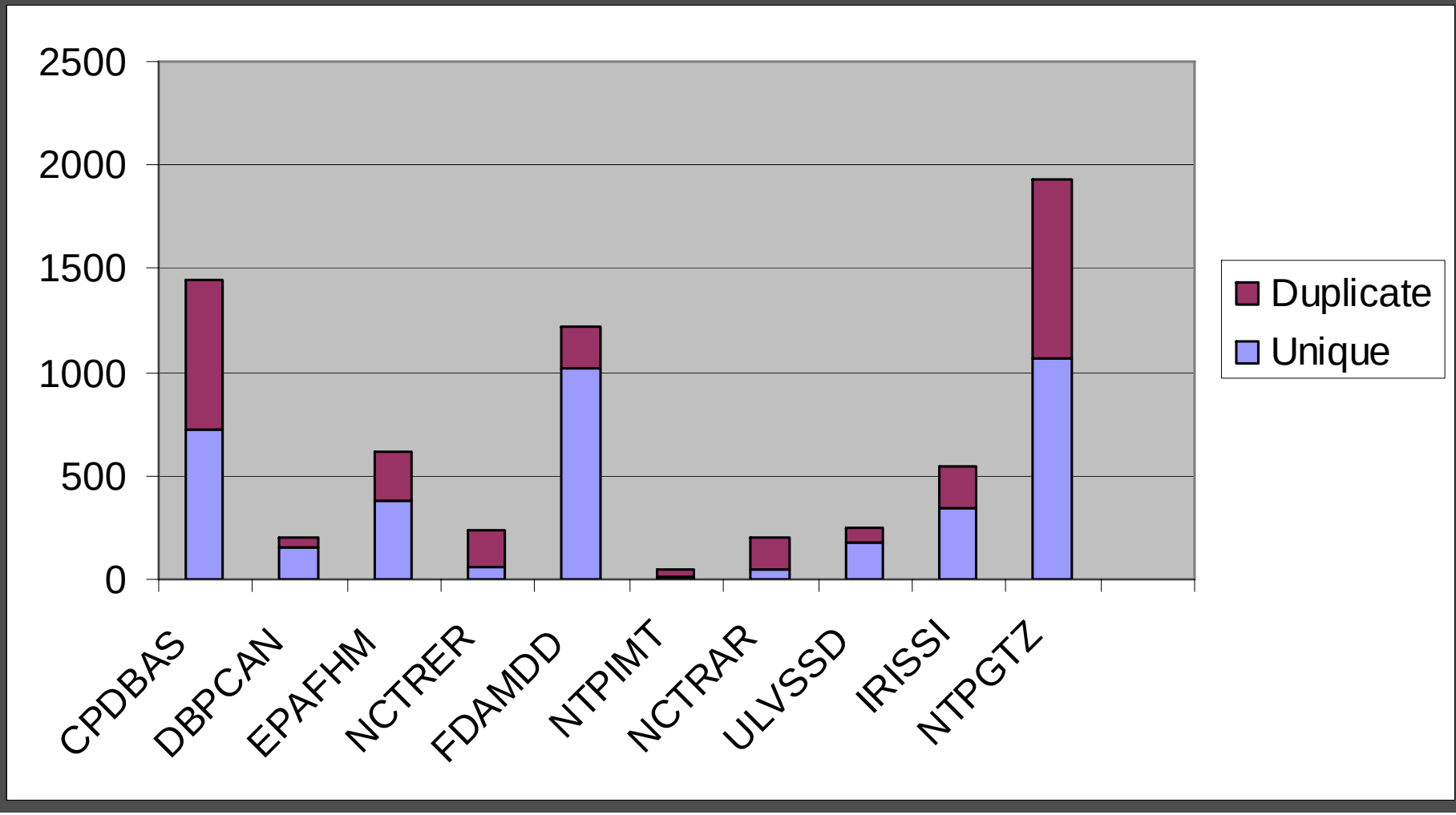
CPDBAS_v2a_1451_1Mar05
DBPCAN_v2a_209_1Mar05
EPAFHM_v2a_617_1Mar05
FDAMDD_v1a_1217_1Mar05**
NCTRER_v2a_232_1Mar05

** *new addition*

DSSTox Chemoinformatics

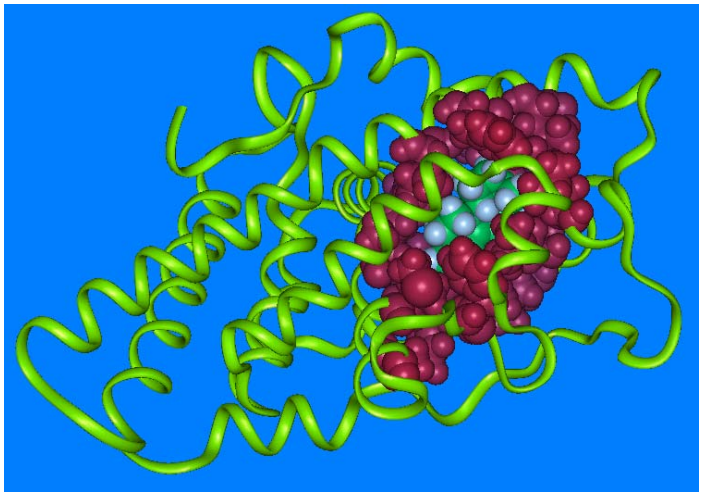
Total Records: 6625

Total Unique Records: 3967 (no replicates)



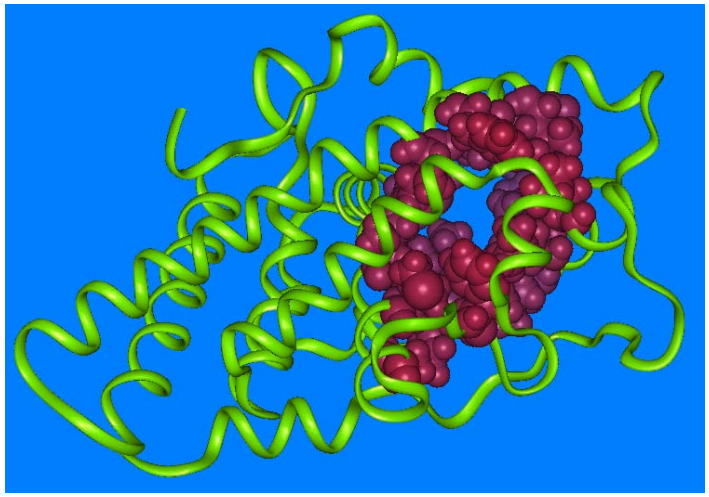
Molecular Docking

Crystal structure
from the literature



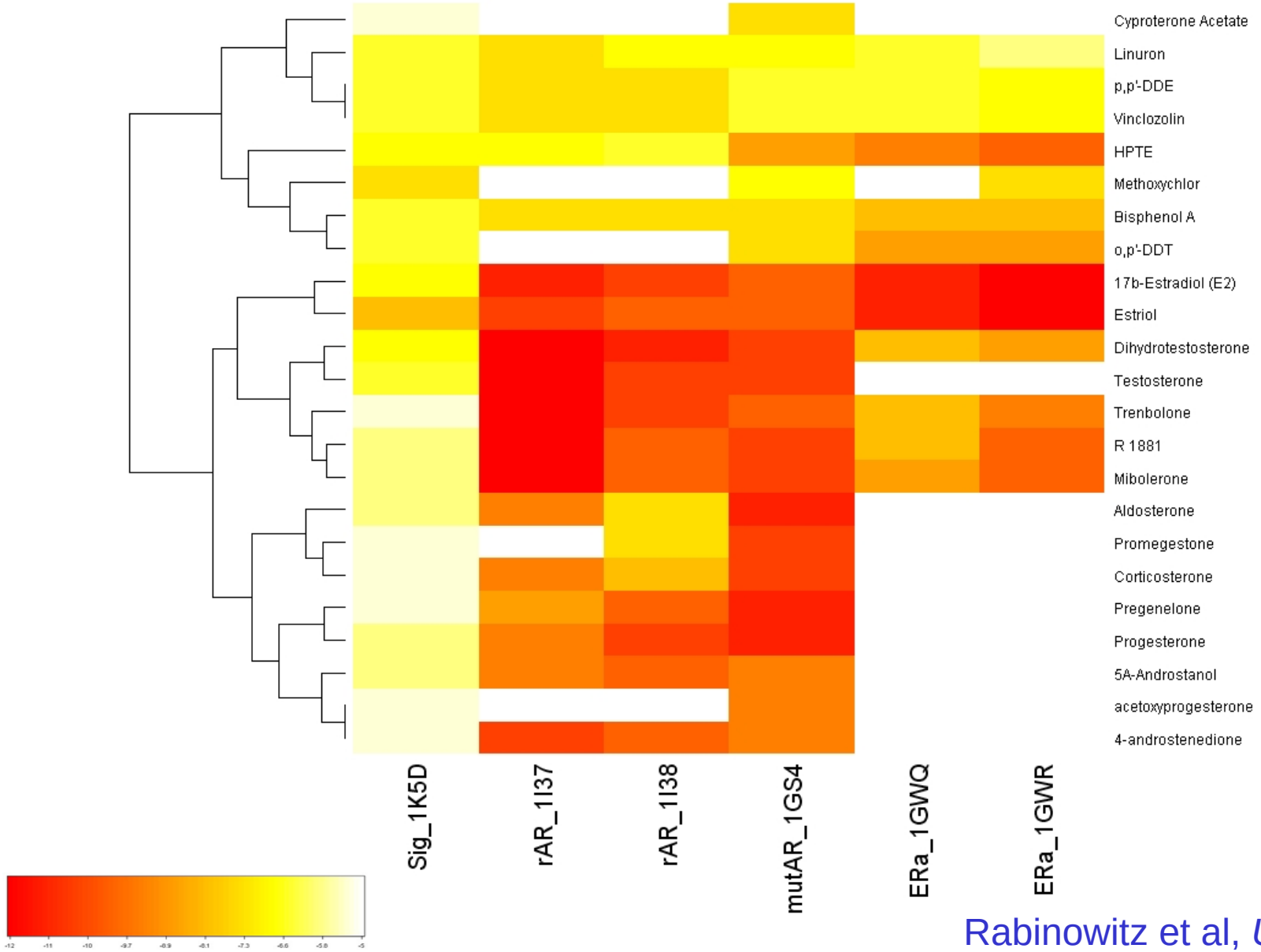
1E3G Human Androgen Receptor
Ligand Binding Domain with
Ligand Metribolone (R1881)

Computationally
created target



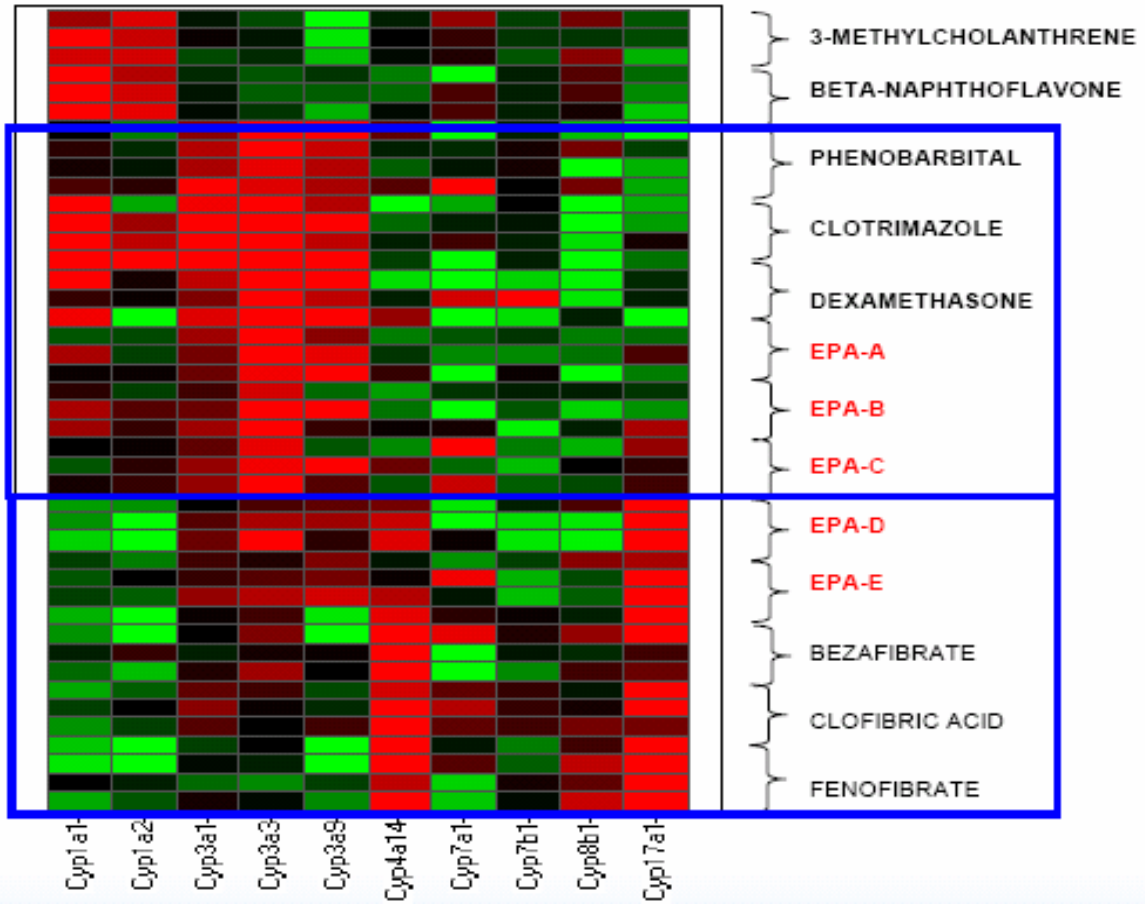
1E3G Human Androgen Receptor
Ligand Binding Domain with
Ligand removed computationally

Dendrogram of Steroid Receptor Ligands against multiple Nuclear Receptors



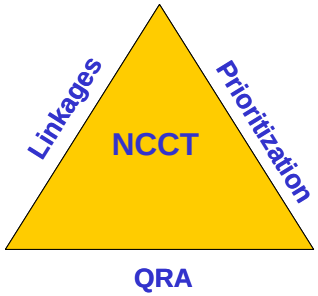
Rabinowitz et al, *Unpublished*

Induction of Cytochrome P450s by EPA-A/B/C Similar to PXR/CAR Agonists, EPA-D/E Similar to PPAR-α Agonists



Heat map showing the perturbation of nuclear receptor-regulated metabolism genes by EPA compounds and and typical ligands

ICONIX PHARMACEUTICALS



ToxCast– A Prioritization Concept

- **Assumptions**
  - Prioritization/Categorization is needed
  - Prioritization is not equivalent to screening
  - Need broad coverage of potential outcomes
  - Outcomes mediated by chemical-biological interactions
  - There is no current model
  - Technological advances can be employed (e.g., HTS)
  - Cost is a factor in acceptance
- **Pharmaceutical experience is helpful, but caveats**
  - Focused on targets
  - Accepts a high false negative rate
  - “Activity” levels higher than for environmental chemicals
- Build upon examples where mode/mechanism of action has already, or is being, employed in hazard or risk assessment

Enabling HTS Technologies Developed in the Search for Bioactive Compounds

- 96 to 384 to 1536 robotics revolution
- Compound/chemical libraries >10^6
- Assay development and miniaturization
- Computational tools for management and analysis of large volumes of data

Biological spectra analysis: Linking biological activity profiles to molecular structure

PNAS **January 11, 2005** vol. 102 no. 2 **261–266**

CHEMISTRY PHARMACOLOGY

Anton F. Fliri*, William T. Loging, Peter F. Thadeio, and Robert A. Volkmann*†

Pfizer Global Research and Development, Groton, CT 06340

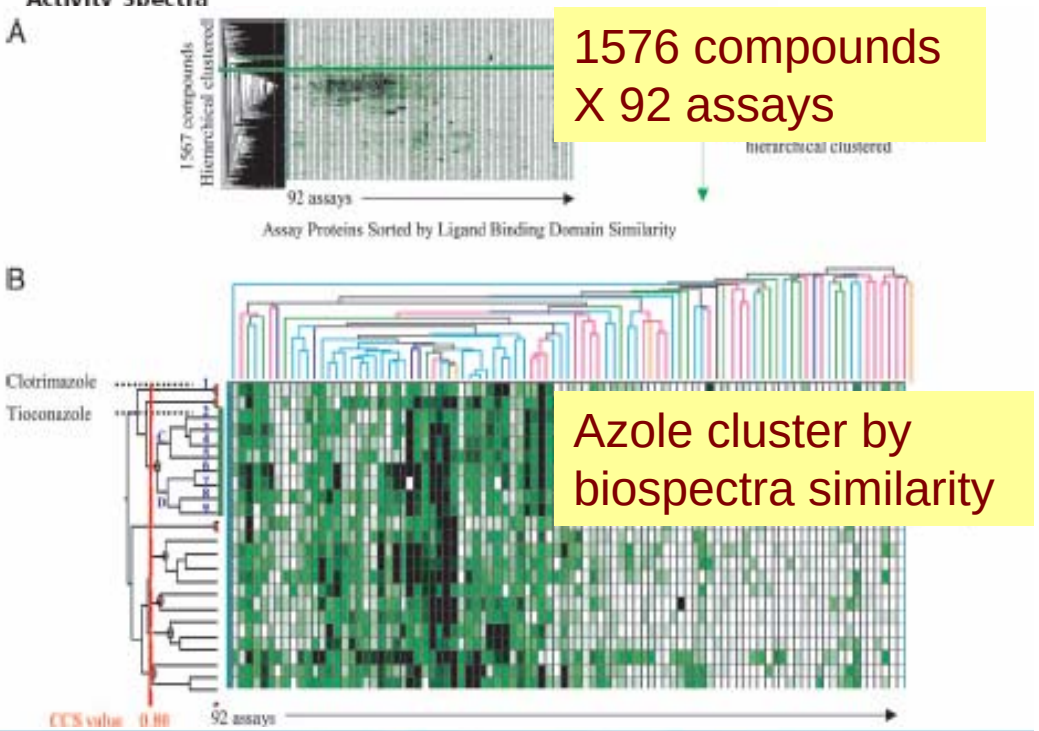
Communicated by Larry E. Overman, University of California, Irvine, CA, October 25, 2004 (received for review September 4, 2004)

Establishing quantitative relationships between molecular structure and broad biological effects has been a longstanding challenge in science. Currently, no method exists for forecasting broad biological activity profiles of medicinal agents even within narrow boundaries of structurally similar molecules. Starting from the premise that biological activity results from the capacity of small organic molecules to modulate the activity of the proteome, we set out to investigate whether descriptor sets could be developed for measuring and quantifying this molecular property. Using a 1,567-compound database, we show that percent inhibition values, determined at single high drug concentration in a battery of *in vitro* assays representing a cross section of the proteome, provide precise molecular property descriptors that identify the structure of molecules. When broad biological activity of molecules is represented in spectra form, organic molecules can be sorted by quantifying differences between biological spectra. Unlike traditional structure–activity relationship methods, sorting of molecules by using biospectra comparisons does not require knowledge of a molecule's putative drug targets. To illustrate this finding, we selected as starting point the biological activity spectra of clotrimazole and tioconazole because their putative target, lanosterol demethylase (CYP51), was not included in the bioassay array. Spectra similarity obtained through profile similarity measurements and hierarchical clustering provided an unbiased means for establishing quantitative relationships between chemical structures and biological activity spectra. This methodology, which we have termed biological spectra analysis, provides the capability not only of sorting molecules on the basis of biospectra similarity but also of predicting simultaneous interactions of new molecules with multiple proteins.

biospectra | proteome | structure–function relationships

differences in biological environments (8). Considering the complexity of this requirement, computational solutions that precisely link molecular structure to broad biological response are currently not possible (9, 10). We report here an approach to structure–function studies that is based on measurements of the capacity of molecules to interact with the proteome (11).

Translation of Chemical Property Information into Biological Activity Spectra

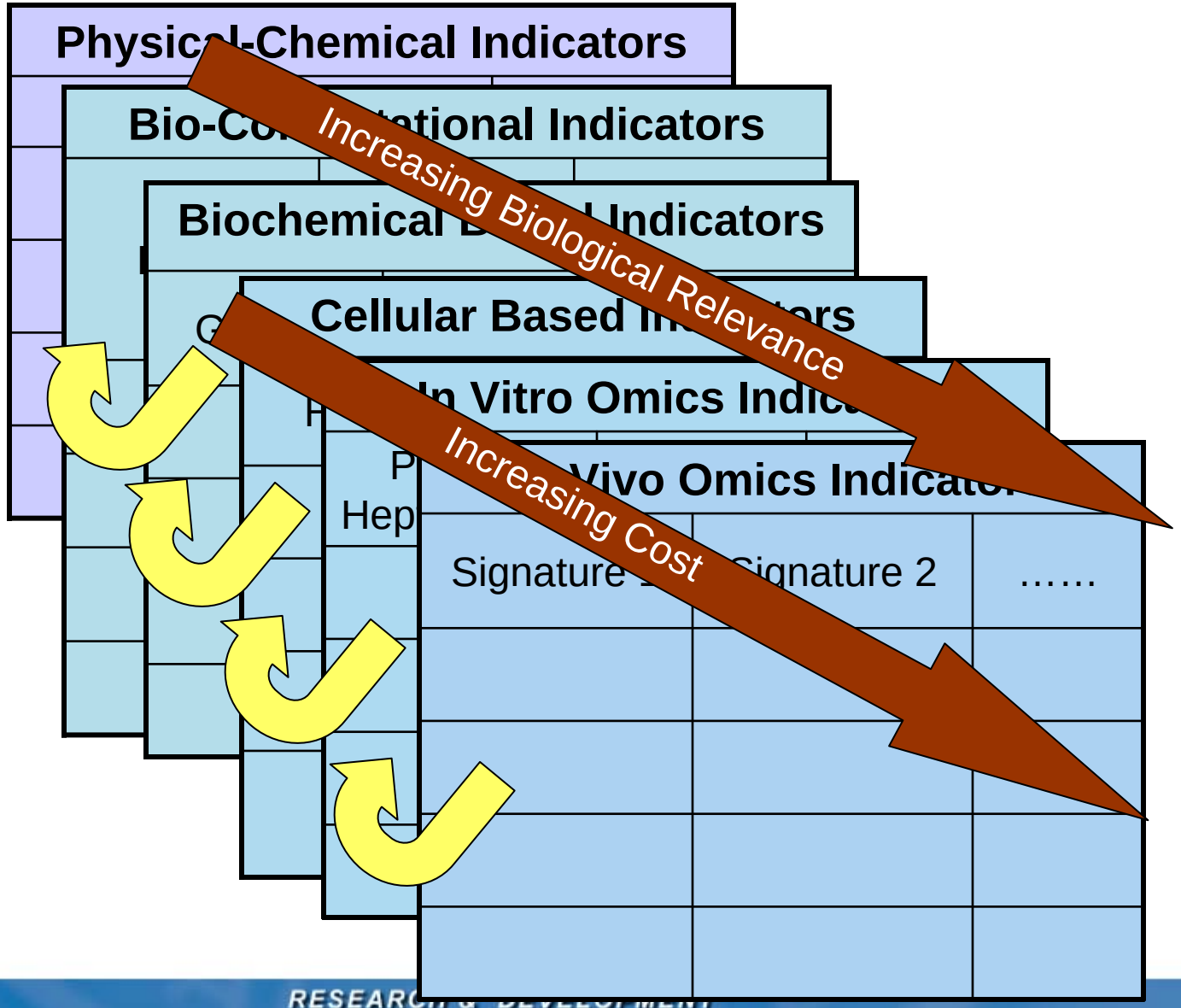


1576 compounds
X 92 assays

Azole cluster by biospectra similarity

ToxCast Information Domains

Chemical Grouping
Bin 1
Bin 2
Bin 3
….
….
Bin ….



Potential Outcomes

- Ability to categorize or prioritize chemicals
  - Tool box of indicators across information domains
  - Cost effective approach for assessing potential to be biologically active agents
  - Potential targeting of outcomes of concern
- Flexibility
  - Adaptable to technological advances
  - Refinement of key indicators with experience
- Development of predictive models as database enlarges
- More effective and efficient use of animals in testing

U.S. Department of Health and Human Services
NIH News
National Institutes of Health

NIH Creates Nationwide Network of Molecular Libraries Screening Centers To Accelerate Study of Human Biology and Disease

"Roadmap" Grants Will Establish Nine New Centers in Seven States

BETHESDA, Md., Wed., June 15th, 2005 - The National Institutes of Health (NIH) today announced it is awarding $88.9 million in grants to nine institutions over three years to establish a collaborative research network that will use high-tech screening methods to identify small molecules that can be used as research tools. Small molecules have great potential to help scientists in their efforts to learn more about key biological processes involved in human health and disease.

"This tremendous collaborative effort will accelerate our understanding of biology and disease mechanisms," said Elias A. Zerhouni, M.D., NIH director. "More importantly, it will, for the first time, enable academic researchers to explore novel ideas and enable progress on a broad front against human disease."

For example, the broad-based screening effort will eventually enable researchers to explore the hundreds of thousands of proteins believed to be encoded by the approximately 25,000 genes in the human genome. To date, only a few hundred human proteins have been studied in detail using small molecule probes.

Certain small organic chemical compounds, also referred to as small molecules, can be valuable tools for understanding the many important cellular events involved in health and disease, which is key to identifying possible new targets for diagnosis, treatment and prevention. To date, most useful small molecules have been found serendipitously. The molecular libraries screening program is an effort by NIH to take an efficient, high-throughput approach toward the discovery of many more useful compounds.

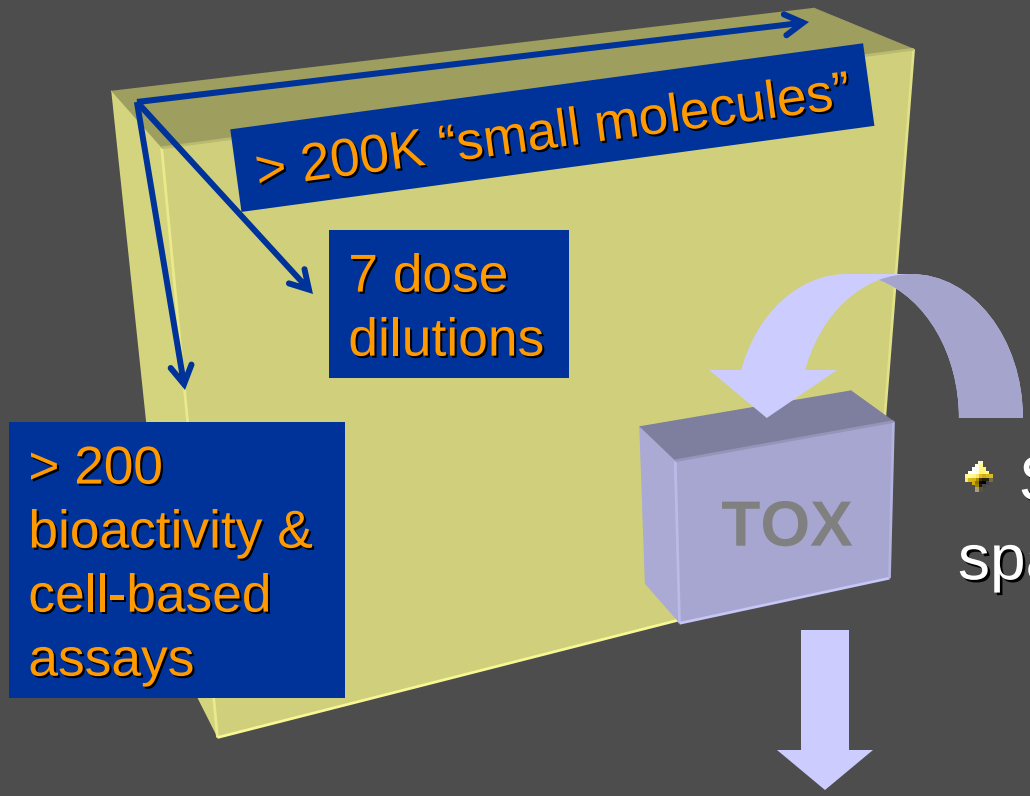
The Molecular Libraries Screening Centers Network (MLSCN) is being developed through the NIH Roadmap for Medical Research. Specifically, the network is part of the Roadmap's "New Pathways to Discovery" initiative, which has set out to advance the understanding of biological systems and build a better "toolbox" for medical researchers in the 21st century. The network is funded by all of the institutes of the NIH and co-administered by the National Institute of Mental Health (NIMH) and the National Human Genome Research Institute (NHGRI) on behalf of NIH. The operation of the network will be overseen by a project team made up of staff from NIH's 27 institutes and centers.

Data generated from the high-throughput assays conducted at the screening centers will be made available to researchers in both the public and private sectors through the PubChem database at (PubChem), created and managed by the National Library of Medicine at NIH. The network's first screening center, the NIH Chemical Genomics Center (NCGC), was established in June 2004 by the NHGRI's intramural program to jumpstart the roadmap effort. Another critical component of the network is the Molecular Libraries Small Molecule Repository, located in San Francisco at Discovery Partners International, a drug discovery research firm. The repository houses the collection of small molecules that will be used for screening by the centers.

NIH/NCGC Roadmap:
Small Molecules High-Throughput Screening Initiative

nihroadmap.nih.gov

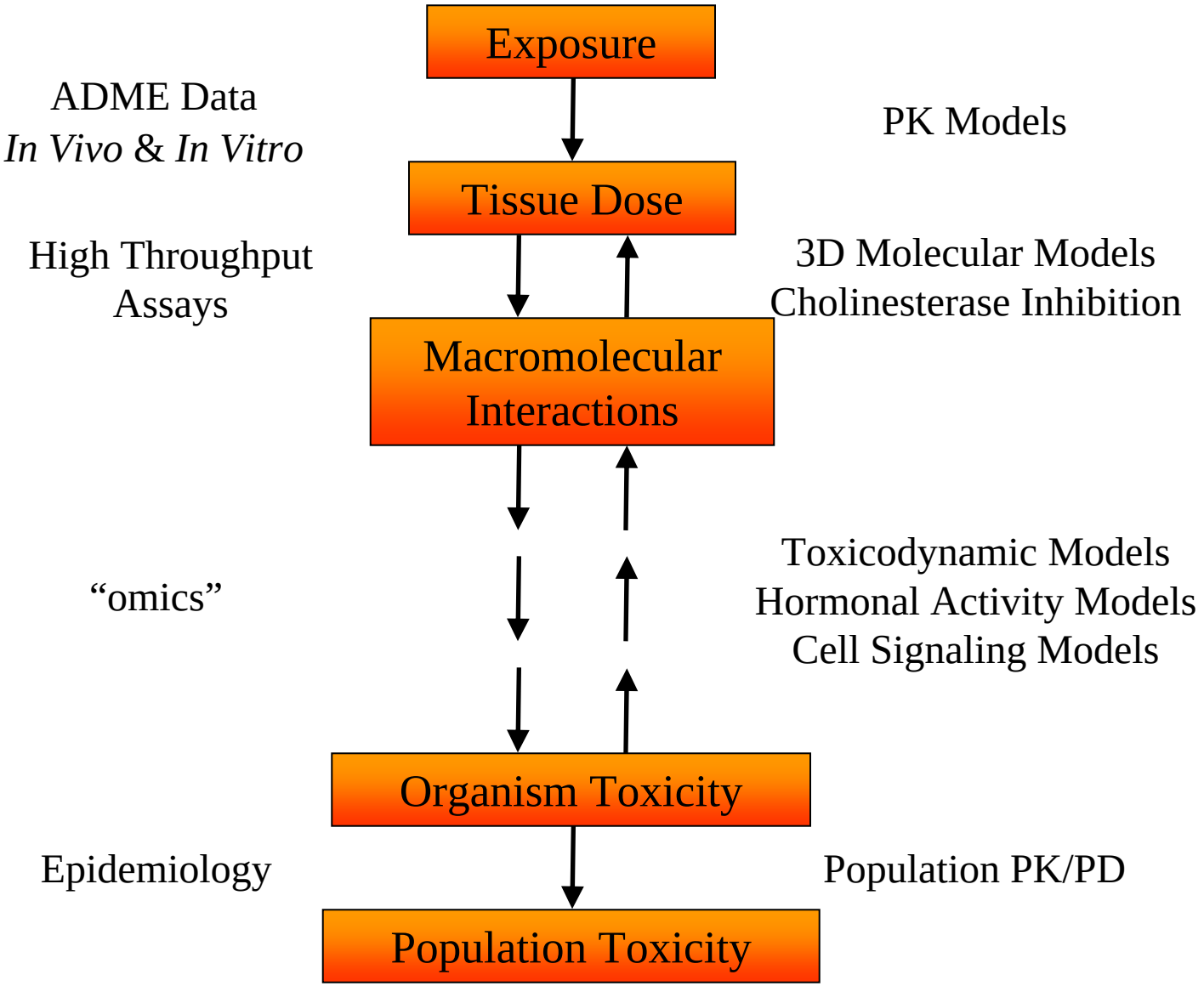
NIH Chemical Genomics Center



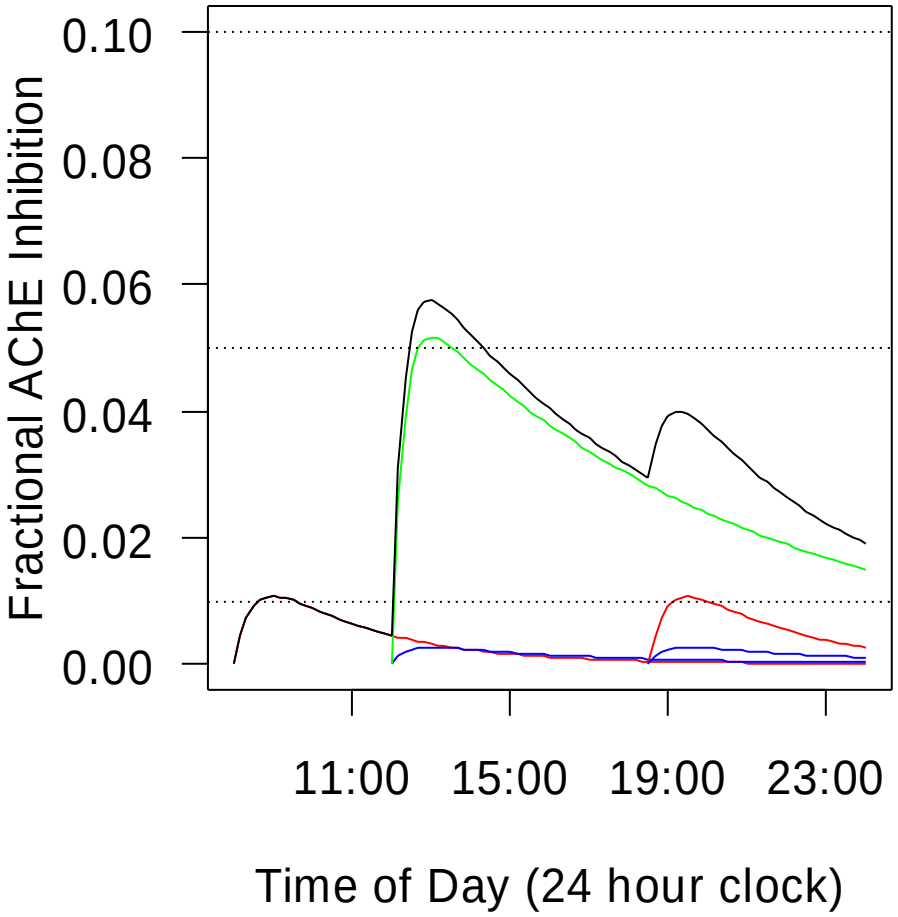
- Sample "toxicity" chemical space:
  - NTP chemicals
  - EPA pesticides, inerts
  - EPA HPV Chemicals
  - DSSTox
  - NCI/ChemNavigator

- Reference dataset of toxicity-related chemicals with structures & bioactivity profiles

Systems Biology Modeling



PK/PD Model of N- methyl Carbamate Activity



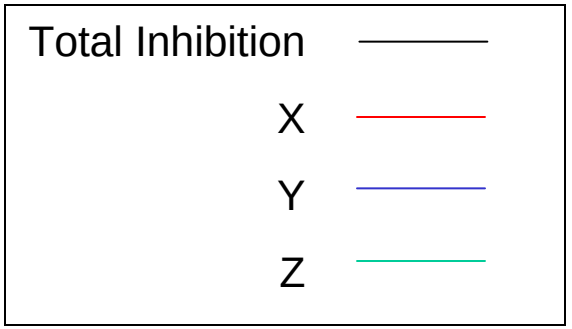
Example Response Metrics

Maximum inhibition: 5.8%

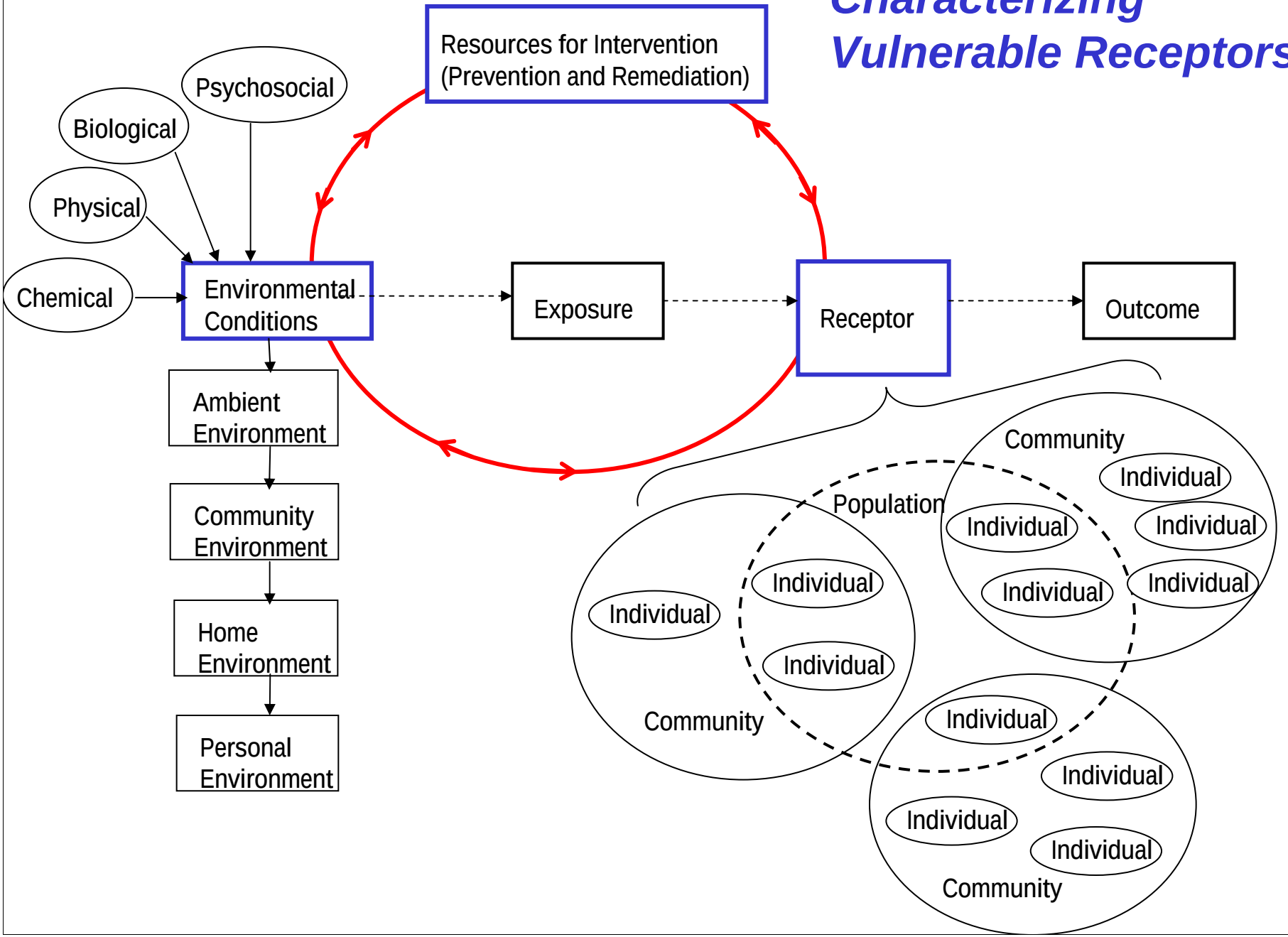
Fraction of time > 10%: 0%

Fraction of time > 5%: 7.6%

Fraction of time > 1%: 54%



Characterizing Vulnerable Receptors



NCCT - Near Term Objectives

- Development and implementation of advanced biological models
  - Statistical methods for parameter estimation
  - Probabilistic modeling framework
- Information technology development and application
  - Expansion of DSSTox, with sub-structure searching
- Prioritization method development and application
  - Flexible docking models for nuclear receptors
  - ToxCast proof of concept demonstration
- Advanced computational approaches for cumulative risk assessment
  - Visual analytical approach to exposure-body burden relationships


COMPUTATIONAL TOXICOLOGY