



“...to integrate modern computing and information technology with molecular biology to improve Agency prioritization of data requirements and risk assessment of chemicals”

Computational Toxicology Program Overview

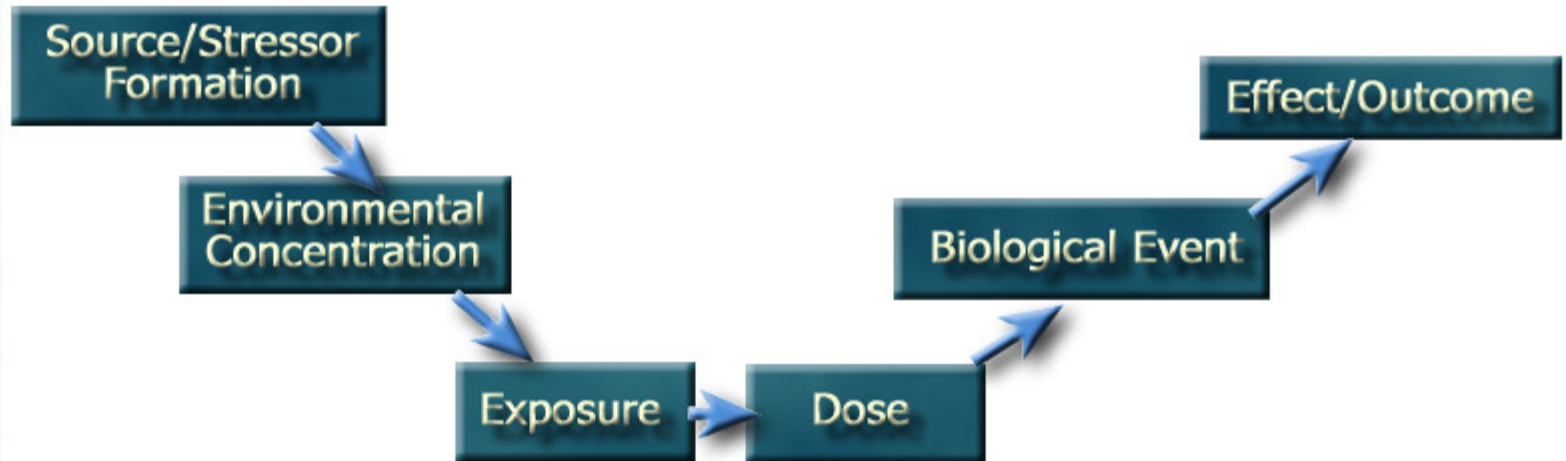
**BOSC Site Visit
RTP, NC
April 25-26 2005**

Overview

- Regulatory Context
- Program Development
- Framework
 - ▶ Contents
 - ▶ SAB Review
 - ▶ Workshop
 - ▶ Implementation
- National Center
- Summary Comments

**RESEARCH &
DEVELOPMENT**

*Building a
scientific
foundation
for sound
environmental
decisions*



EPA Context: Quantitative Risk Assessment/ Risk Management for Priority Pollutants

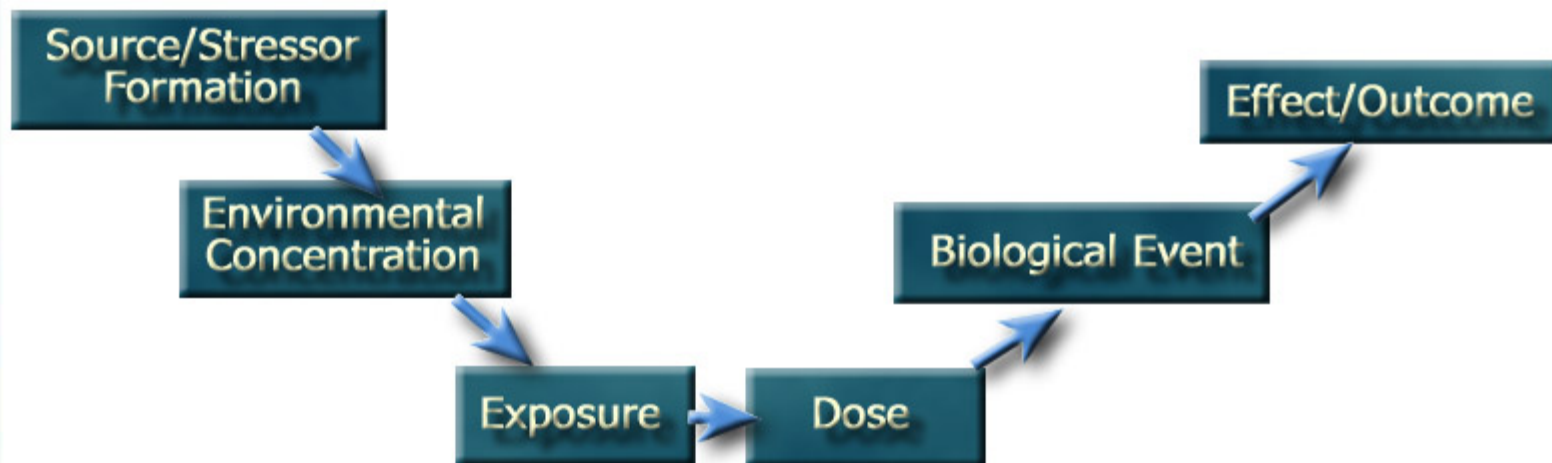
- Methods to Detect & Characterize
- Evaluate Single Chemical at a Time

COMPUTATIONAL TOXICOLOGY



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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)

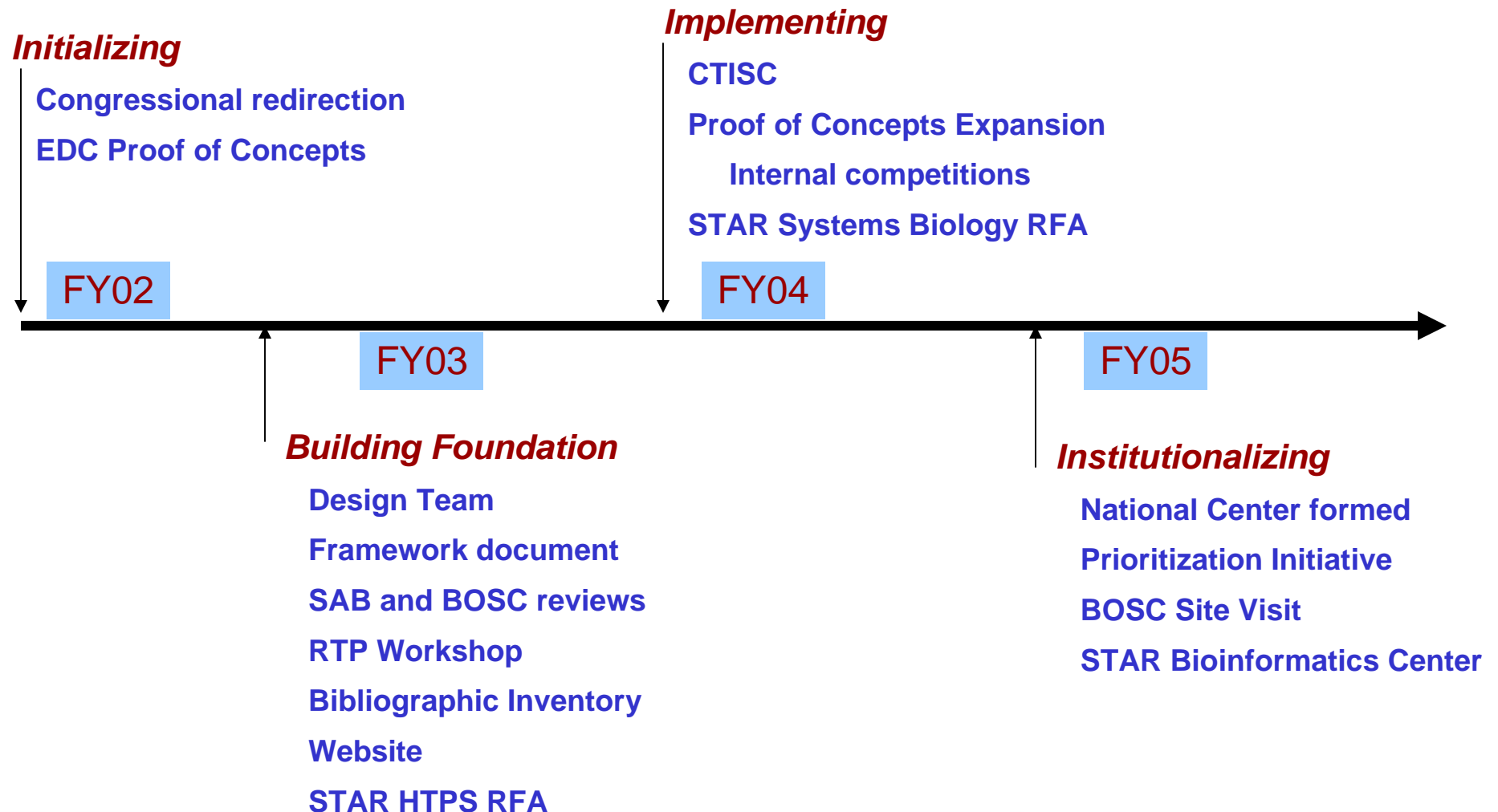
COMPUTATIONAL TOXICOLOGY



An Example - Pesticidal Inerts

- **Constituents added to the active ingredient of a registered pesticide**
- **Legislative mandate to re-issue registrations**
 - “tolerance exemptions”
- **Based on MOE estimates**
 - Anticipated exposure level/NOAEL
- **Legal Burden of Proof**
 - “Reasonable certainty of no harm”
- **THE PROBLEM**
 - Approximately 850 entities
 - No routine testing requirements
 - August, 2006 deadline
 - Similar situation for the ~350 non food use anti-microbials, as well as later on the ~3000 food use inerts

Program Development



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Phase I:
***Initializing Computational
Toxicology***

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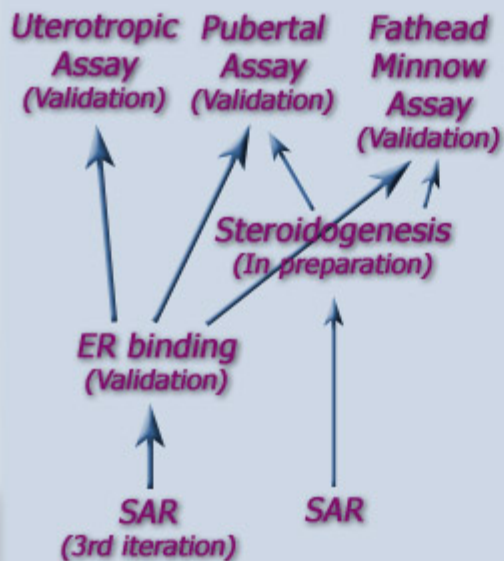
In vivo

In vitro

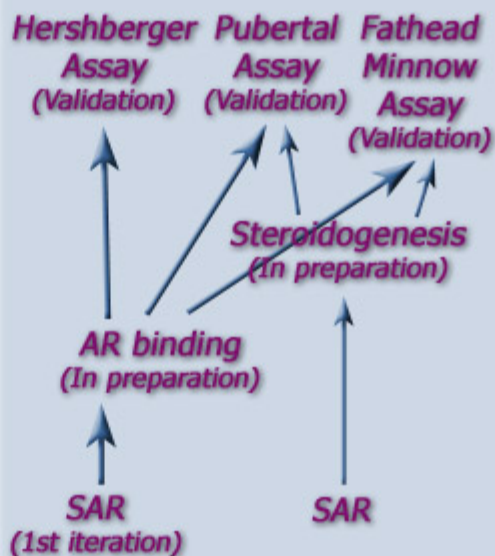
In silico

Impaired Reproduction/Development

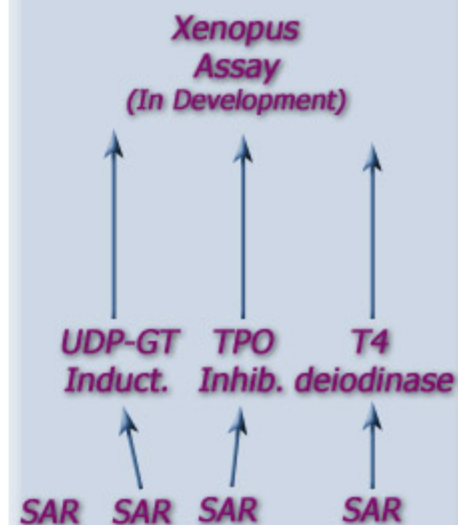
Estrogen Pathways



Androgen Pathways



Thyroid Pathways



Initial Proof of Concept Studies

- ▶ ER Binding data refinement
 - ✦ Ki vs IC50s to
 - ✦ WFU, Near completion
- ▶ ER QSAR Enhancement
 - ✦ Data quality, chemical domain, iterative improvements
 - ✦ Internal thru MED/NHEERL
- ▶ Steroid docking model studies
 - ✦ In silico approach using crystal structures of receptors
 - ✦ Internal thru ECD/NHEERL (now NCCT),
- ▶ H295R Assay evaluation
 - ✦ In vitro assay for steroidogenesis using a human cell line
 - ✦ Contract, Entrix, 3yr project ending 04/05
- ▶ Hypothalamic-Pituitary-Gonadal Axis Systems model
 - ✦ Internal thru RTD/NHEERL, long term, ongoing

H295R Development

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

Chemical	CYP17	StAR	CYP11A	CYP19	CYP21	HMGR	17 β HSD1	3 β HSD1	3 β HSD2	CYP11B2	CYP11B1
Inducers											
8-Br-cAMP		↑		↑↑	↑↑↑			↑	↑↑↑	↑↑↑	↑↑↑
PMA	↓↓↓	↑		↑↑	↑↑↑		↓	↑	↑↑↑	↑↑↑	↑↑
forskolin	↑	↑	↑	↑↑	↑↑↑	↑		↑↑	↑↑↑	↑↑↑	↑↑↑
lovastatin	↑	↑						↓		↑↑	↑
Inhibitors											
spironolactone				↑							↑
DL-aminoglutethimide					↓		↑	↑	↓		↑↑↑ Δ
daidzein		↓			↑		↑ Δ	↑ Δ	↑ Δ	↑ Δ	↑↑↑ Δ
ketoconazole			↑ Δ		↑ Δ		↑ Δ	↑ Δ		↑	↑
spironolactone				↑							↑
DL-aminoglutethimide					↓		↑	↑	↓		↑↑↑ Δ
daidzein		↓			↑		↑ Δ	↑ Δ	↑ Δ	↑ Δ	↑↑↑ Δ
ketoconazole			↑ Δ		↑ Δ		↑ Δ	↑ Δ		↑	↑

^a Symbols indicate fold difference relative to control; ↑=2-fold or more; ↑↑=5-fold or more; ↑↑↑=10-fold or more. Δ= 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.

Expanded Proof of Concepts

- **ER and AR Transcription Assay scale up**
 - ▶ To use NHEERL developed cell lines, or equivalent
 - ▶ Contract being let ~05/05, to last ~1 year, ~100 chemicals
- **ICONIX Predictive Toxicogenomics evaluation**
 - ▶ Hepatic gene changes following acute exposure in rat
 - ▶ Contract let 01/05, results in 9 months, 5 chemicals
- **STAR RFAs - long term research**
 - ▶ HTPS ('03; n=4)
 - ✦ Yeast, *Daphnia*, fish models
 - ▶ Systems Biology ('04, n=3)
 - ✦ Fish models, uterotrophic model

Phase II:

Building a Foundation

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ORD Design Team (2003)

NCEA

K. Hammerstrom
J. Swartout

NCER

E. Francis

NERL

J. Blancato
T. Collette
G. Toth
E. Weber

NHEERL

G. Ankley
J. Fowle
E. Gray
R. Kavlock, Chair
L. Reiter, Exec. Lead
H. Tilson
G. Veith
D. Wolf

NRMRL

D. Young

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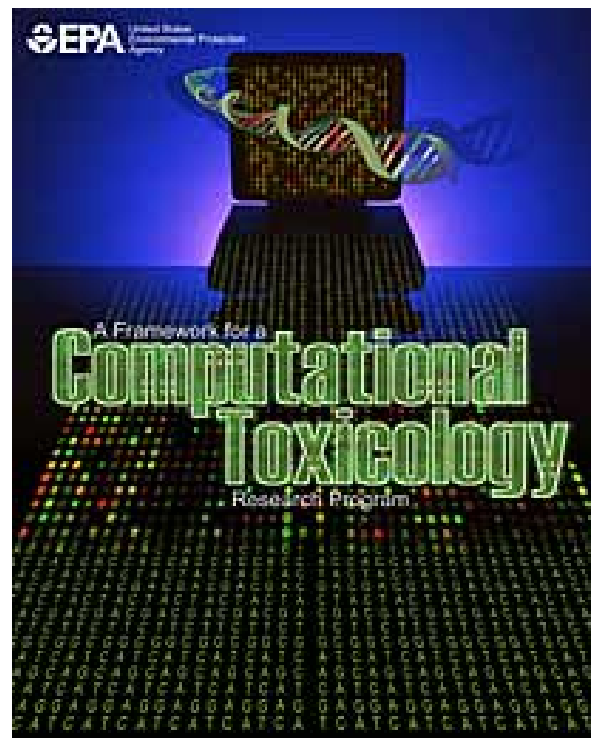
ORD's Computational Toxicology Research Program

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

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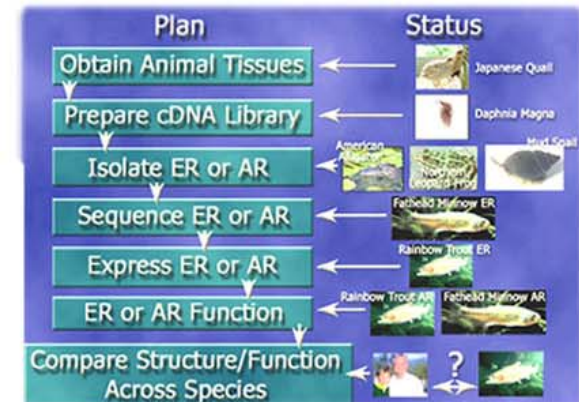
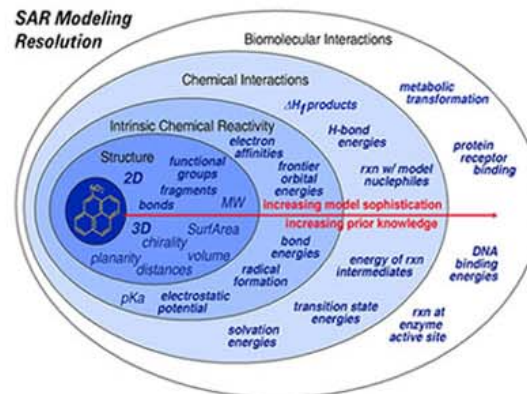
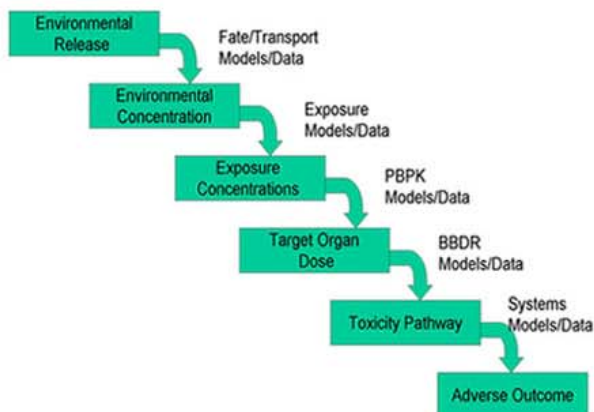
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OBJECTIVES OF THE PROGRAM

1. Improve the Linkages in the Source to Outcome Paradigm

2. Provide Predictive Models for Hazard Identification

3. Enhance Quantitative Risk Assessment



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I. Source to Outcome Linkages

- Chemical transformation and metabolism
- Exposure Indicators
- Dose metrics
- Characterization of Toxicity Pathways
- Metabonomics
- Systems Biology
- Modeling Frameworks

II. Predictive Models

- QSAR approaches
- Pollution Prevention Strategies
- High Throughput Screening

III. Enhancing Quantitative RA

- Applying computational methods in quantitative risk assessments
 - ▶ Validation and development of protocols
 - ▶ Defining importance of responses
 - ▶ Modifying Uncertainty factors
- Dose response assessments
- Cross species extrapolations
- Mixtures

SAB Consult – Charge

September 2003

- Soundness of organizing principles?
- Addresses major issues of concern?
- Captures key scientific uncertainties?
- Suggest priorities?
- Additional issues?
- Feasibility of proposed process?

SAB Consult - Key Points

- Generally a well constructed document
- Sound strategy and a good starting point for the program
- Reasonable proof-of-concept beginning
- Must engage policy arms of Agency in moving forward
- Next steps must be more clearly defined

SAB Comments

- Suggested priorities
 - ▶ Metabonomics
 - ▶ Mixtures
- Acknowledge
 - ▶ Validation need for QSARs, *et al*
 - ▶ Different states of maturity of technologies
 - ▶ Need for strong LIMs
 - ▶ Need for translational research

SAB Comments

- Consider
 - ▶ Incorporating exposure models into the framework
 - ▶ Proof of concept for a risk assessment application
 - ▶ Defining what constitutes an adverse effect
 - ▶ Participation in CEBs
 - ▶ Holding additional workshops with stakeholders
- Cross reference
 - ▶ Children's health
 - ▶ Molecular epidemiology
 - ▶ Harmonization of cancer/non-cancer
 - ▶ Integrated Eco/Human health assessment

ORD Workshop, 9/03

- Introduction to the Framework
- Related research strategies from other organizations
- Highlighted approaches
 - ▶ Diagnostic indicators, high throughput screening, toxicity pathway identification, metabonomics and systems biology
- Regulatory needs
 - ▶ OPPTS, OPP, FDA
- Breakout group discussions
 - ▶ “top down” and “bottom up”



Available online at www.sciencedirect.com

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Reproductive Toxicology 19 (2005) 265–280

Reproductive
Toxicology

www.elsevier.com/locate/reprotox

Workshop report

Computational Toxicology: Framework, Partnerships, and Program Development

September 29–30, 2003, Research Triangle Park, North Carolina

Robert Kavlock*, Gerald T. Ankley, Tim Collette, Elaine Francis, Karen Hammerstrom, Jack Fowle, Hugh Tilson, Greg Toth, Patricia Schmieder, Gilman D. Veith, Eric Weber, Douglas C. Wolf, Doug Young

Office of Research and Development, US Environmental Protection Agency, Washington, DC, USA

Received 16 February 2004

Available online 20 July 2004

1. Overview

Computational toxicology is a new research initiative being developed within the Office of Research and Development (ORD) of the US Environmental Protection Agency (EPA). Operationally, it is defined as the application of mathematical and computer models together with molecular chemistry and biological approaches to improve our understanding of the key toxicological issues faced by the regulatory program offices of EPA. A two-day workshop on the topic was held on September 29–30, 2003, at the EPA's Research Triangle Park campus in North Carolina.

The focus of the workshop was on a proposal entitled *A Framework for a Computational Toxicology Research Program in ORD* (available with ancillary information at www.epa.gov/comptox), which identifies research needs to provide the basis for a focused and integrated research effort utilizing modern computing, chemistry, and molecular biology tools for developing in silico models that can predict the ecological and human health risk of potentially toxic chemicals. Traditional risk assessment of chemicals relies primarily on laboratory testing on a chemical-by-chemical basis to obtain data about adverse effects and the quantitative relationship between doses and likelihood of response. In human health risk assessment, these laboratory data are extrapolated to predict the likelihood of an adverse effect and to estimate risk to humans. The large number of chemicals in commerce for which assessments need to be made and the expense of testing limits our ability to apply stan-

dard toxicity testing methods to relatively few of the vast array of chemicals of interest and necessitates new scientific approaches to the problem. The proposal puts forth three strategic objectives of the emerging computational toxicology program in ORD to address this situation: (1) to improve linkages in the source to outcome paradigm used for risk assessment by EPA, (2) develop predictive models for hazard identification, and (3) improve quantitative risk assessment. The first objective is largely technology based, and is intended to develop the tools that will enable advances in the remaining two goals. The objectives are designed to enhance EPA's ability to prioritize and screen chemicals for toxicity for testing, and to develop accurate risk assessments more economically and efficiently. Overall, successful completion of these objectives would allow the EPA to more efficiently screen, test, and evaluate the toxicity of chemicals. To meet these objectives, a number of activities need to be initiated; these are detailed in subsequent sections of this report.

At the beginning of the workshop, Dr. Paul Gilman, Assistant Administrator for ORD, and Dr. Lawrence Reiter, Director of the National Health and Environmental Effects Research Laboratory (NHEERL), highlighted the importance of the emerging area of computational toxicology to risk assessment at EPA and to the larger scientific community. EPA scientists then presented information on ORD's current capabilities and ongoing research in computational toxicology. Scientists from other agencies and research organizations and academia were invited to present related organizational strategies and computational toxicology approaches under development at their institutions. Representatives of the US Food and Drug Administration, EPA's Office of Prevention, Pesticides, and Toxic Substances (OPPTS), and ORD's Office of Science Policy and

* Corresponding author. Present address: National Health and Environmental Effects Research Laboratory (E0305-01), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA.

Workgroup Recommendations

- Tools for prioritization and ranking
- Identifying optimal testing approaches
- Predicting metabolism
- Mixture assessments
- Extrapolation across species
- Application to diagnostic risk assessments

Phase III:

***Implementing
Computational Toxicology***

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CTISC (Jan 04 – present)

- ORD

- ▶ NCEA
 - Karen Hammerstrom
 - Ines Pagan
- ▶ NCER
 - Elaine Francis
 - David Mustra
- ▶ NERL
 - Tim Collette
 - Greg Toth
 - (Eric Weber)
- ▶ NHEERL
 - Gerald Ankley
 - Jack Fowle
 - Robert Kavlock
 - Doug Wolf
- ▶ NHSRC
 - TBN
- ▶ NRMRL
 - Jorge Santo Domingo
 - Doug Young

- OPPTS

- ▶ Vickie Dellarco
- ▶ Yin-Tak Woo

- OAR

- ▶ Dennis Pagano

- OW

- ▶ Steve Kueberuwa
- ▶ Clifton Townsend

- Regions

- ▶ David Macarus (Reg 5)

PoCs, Augmentations & New Starts

Amphibian Systems Model

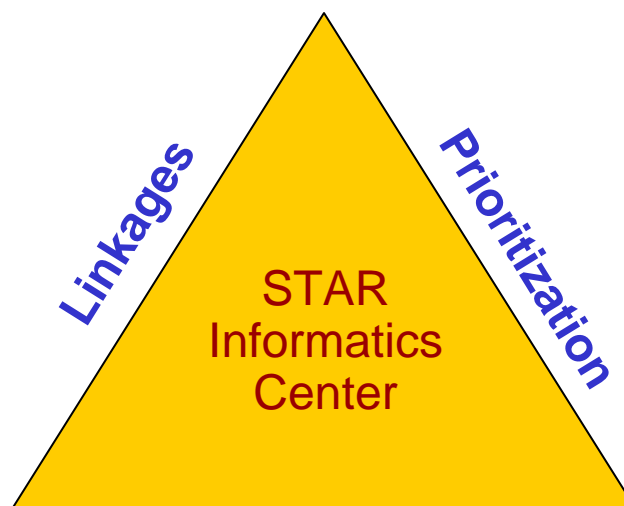
Fish Proteomics
Fish Toxicogenomics
Fish metabonomics

Conazole MOA

Children's Health
Pulmonary Biomarkers

Microbial Metagenomics

STAR Systems Models



DSSTox

Metabolic Simulator

ASTER

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

STAR HTPS RFA

QRA

Diesel Particles

HPG Axis Model

Pellston, SOT and NCEA Workshops

Denotes Center Staff Participation

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Workshops

- WTEC Systems Biology Survey
- SOT Probabilistic Risk Assessment
- Toxicology Forum
- Pellston on Ecogenomics in risk assessment
- NCEA Impact of Genomics on HHRA

Partnerships

- Department of Energy
- Department of Defense
- NIEHS
- NCTR
- IBM
- Affymetrix
- CIIT Centers for Health Research
- ITSC (Former Soviet Union)
- SBIR Solicitation
 - ▶ Released March 24 2005; closes May 25 2005
 - ▶ Exposure diagnostics, biotransformations, docking models, QSAR databases and models, molecular signatures, 'omic integration

Phase IV:

***Institutionalizing
Computational Toxicology***

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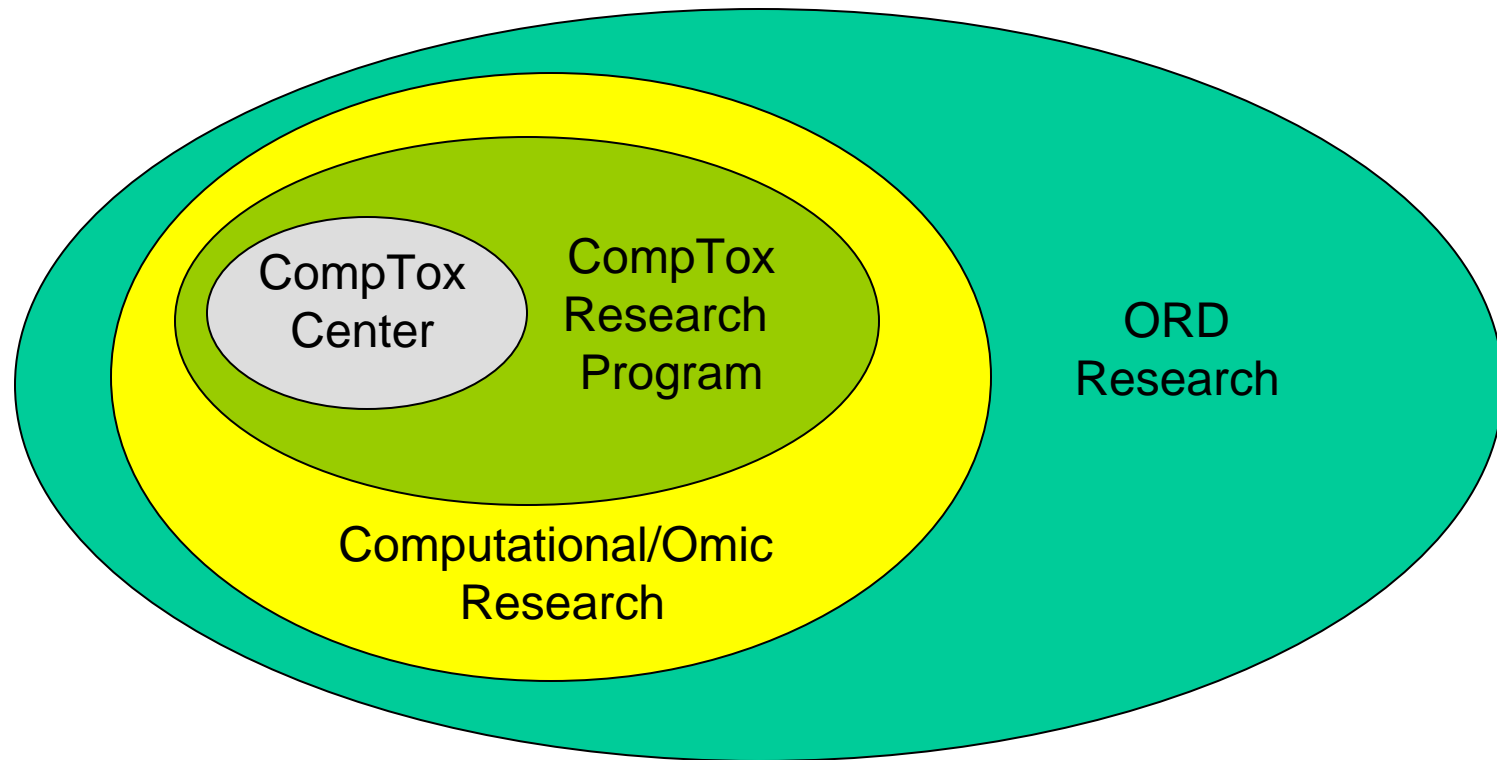
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NCCT Mission

- To provide **scientific expertise and leadership** related to the application of mathematical and computational tools and models
- To **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

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Organizational Development

- Creation of Center announced, 10/04
- All day retreat, 12/02/04
- Biweekly “inventory” sessions
 - Share backgrounds
 - Identify common areas
- Formal start, 2/20/05
- Memorandum of Agreement with NERL and NHEERL
- Engaging the National Computer Center
- Outreach to NTP/NIEHS
- Staffing Up
- STAR Center for Environmental Bioinformatics
- Implementation Plan, 09/05

STAR Environmental Bioinformatics Center

(closed February 24 2005; Review June-July 2005)

- The successful applicant will describe a scientifically sound multidisciplinary approach to development and application of computational methods that target multiple points along source-to-outcome continuum;
- Center presents opportunity for investigators with differing expertise to work together on larger issues than could be addressed in a single grant or cooperative agreement proposal;
- Applicants should focus proposals on computational area for which they have demonstrated expertise;
- Proposed Centers are encouraged to bring together institutions with strengths in different computational disciplines;
- Centers should discuss application and development of bioinformatics standards and nomenclature, which will increase the portability and usability of Center-developed resources and tools.

Staffing Status

- 19 FTEs
 - ▶ Admin (4 of 4)
 - ▶ Systems Modelers (4 of 8)
 - ▶ Computational Chemists (5 of 5)
 - ▶ Bioinformaticians (0 of 2)
- Recruitment Actions
 - ▶ S/T Recruit (Rory Conolly) - on board May 1st
 - ▶ Bioinformatics - August
 - ✦ One senior (14/15), one junior (12/13)
 - ▶ High throughput screening – August
 - ▶ Systems biology modelers (2) - Sept
 - ▶ Ecological modeling (?)
- Postdoctoral Fellows and Details

Current Staffing

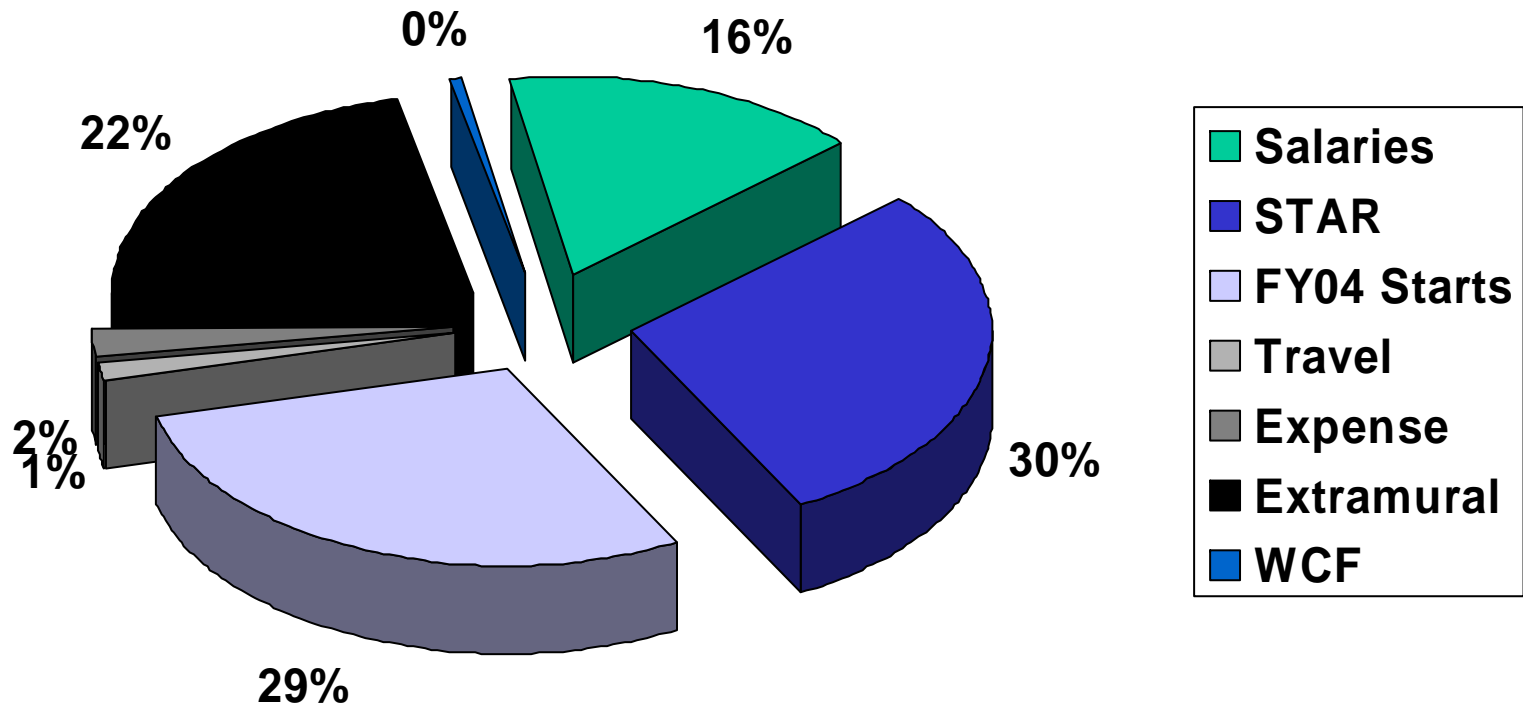
Barton, Hugh	PBPK/PD models
Blancato, Jerry	PBPK models, Deputy
Conolly, Rory	Systems biology
Cohen Hubal, Elaine	Exposure models
Dean, Karen	Program Analyst
Kavlock, Robert	Prioritization, Director
Little, Steve	Computational Chemistry
Pasquinelli, Melissa (R)	Computational Chemistry
Rabinowitz, Jim	Computational Chemistry
Richard, Ann	Chemoinformatics
Roberts, Sandy	Executive Secretary
Setzer, Woody	PK/PD models (statistical)
*Tornero, Mike (R)	Computational Chemistry
Weber, Eric	Computational chemistry
Wolf, Marty (LM)	Chemoinformatics
Zager, Mike (UNC)	PBPK/PD Models

R=EPA Post Doc, LM=Lockheed Martin Contract, UNC Trainee COOP

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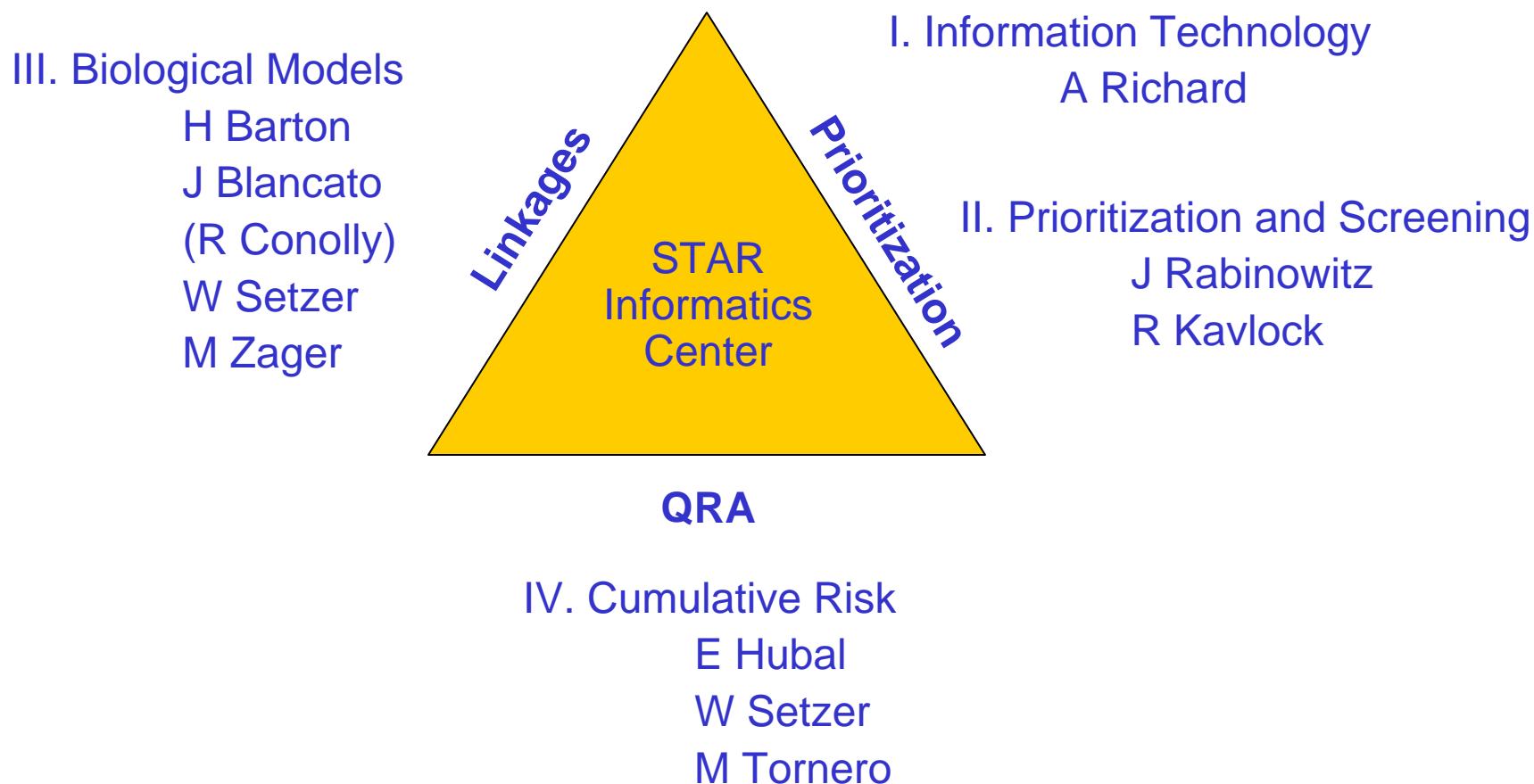
Computational Toxicology FY05 Operating Budget (~\$12M, 19 FTEs)



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Emerging Focal Areas for the NCCT



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Scientific Systems and Application Center

- Chemical Structure Annotation and Development of an Agency-wide Chemical Structure-browser
 - Ann Richard, Lead
- Enhancing Portability of Computational Models
 - H Barton, Lead
- Understanding Molecular Targets via Computational Modeling
 - J Rabinowitz, Lead
- Visual Analytical Application to Exposure Modeling
 - Elaine Hubal, Lead

Why a Center?

- Commitment by ORD
- Regulatory Need and Support
- Visibility and Stature
- Freshness
- Critical Mass
- Cross-cutting Problems and Expertise
- Focus
- Dedicated Resources
- Urgency



Computational Toxicology



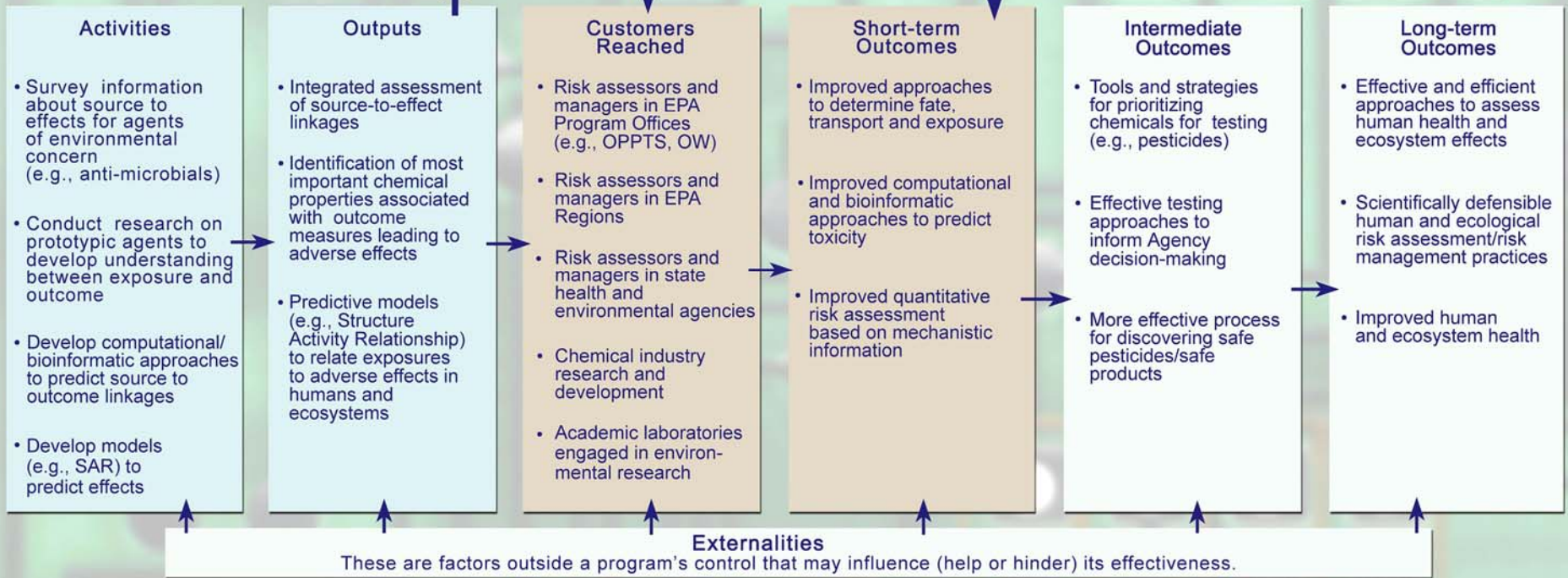
Outreach

Communication, tech transfer, training, and feedback are essential to enable clients to apply outputs and achieve outcomes.

Performance Measurement

e.g., effective transfer of information, findings, and results to customers, partners, and the public

Environmental Indicators



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Summary Comments

- Solid start at implementing program
 - ▶ Responsive to input
 - ▶ Addresses human and ecological health
- Enhanced by establishment of the Center
 - Talented, motivated and enthusiastic staff
 - Solid understanding of critical issues
 - Establishing focal areas
 - Developing working relationships
 - Delivering interim products
- Build confidence in predictions and extrapolations
- Adding value to ORD efforts