NTP
National Toxicology Program

NIEHS/NTP Activities Related to the NICEATM-ICCVAM Five-Year Plan

John Bucher, Ph.D.

National Toxicology Program

National Institute of Environmental Health Sciences

National Institutes of Health

NTP Goals

- *Coordinate toxicological testing programs within the Department of Health and Human Services*
- *Strengthen the science base in toxicology*
- *Develop and validate improved test methods*
- *Provide information about potentially hazardous substances to health regulatory and research agencies, scientific and medical communities, and the public*

Number of Substances and Endpoints Studied since NTP Inception

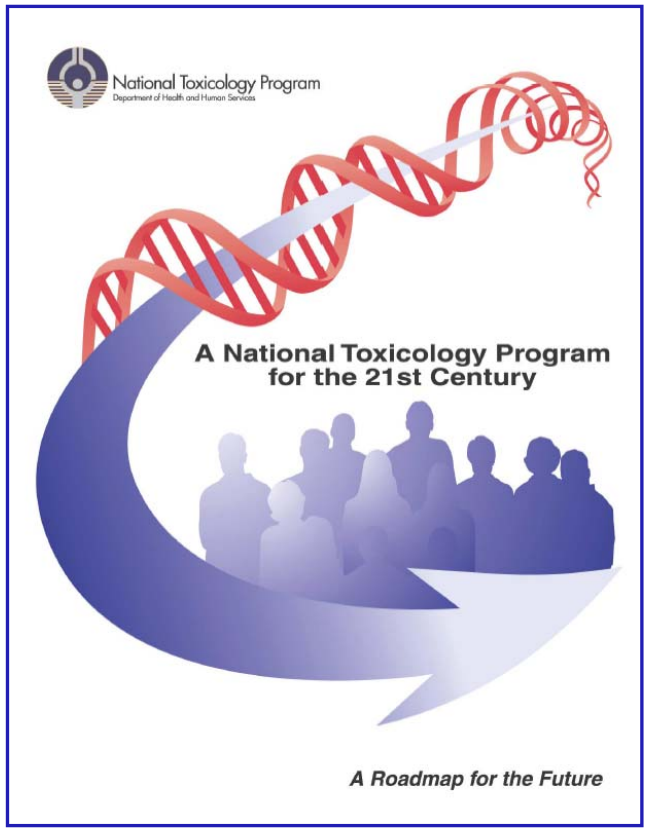
• Distinct test articles	2553
• Chronic rodent bioassays	616
• Genetically modified mice-cancer	13
• Genetic toxicity	2236
• Immunotoxicity	112
• Reproductive/developmental toxicity	242
• General and other toxicity	838

NTP Vision for 21st Century

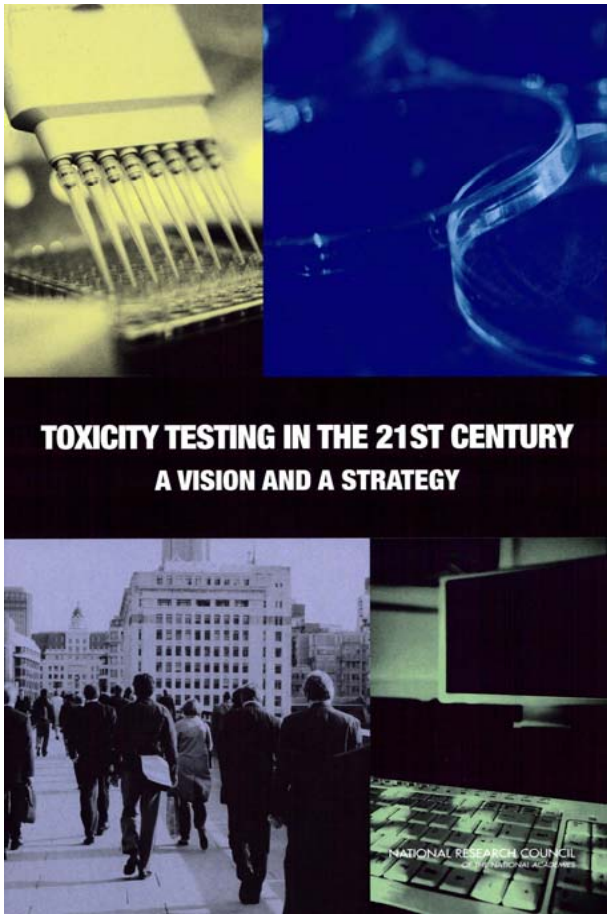
To support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad array of target specific, mechanism-based, biological observations

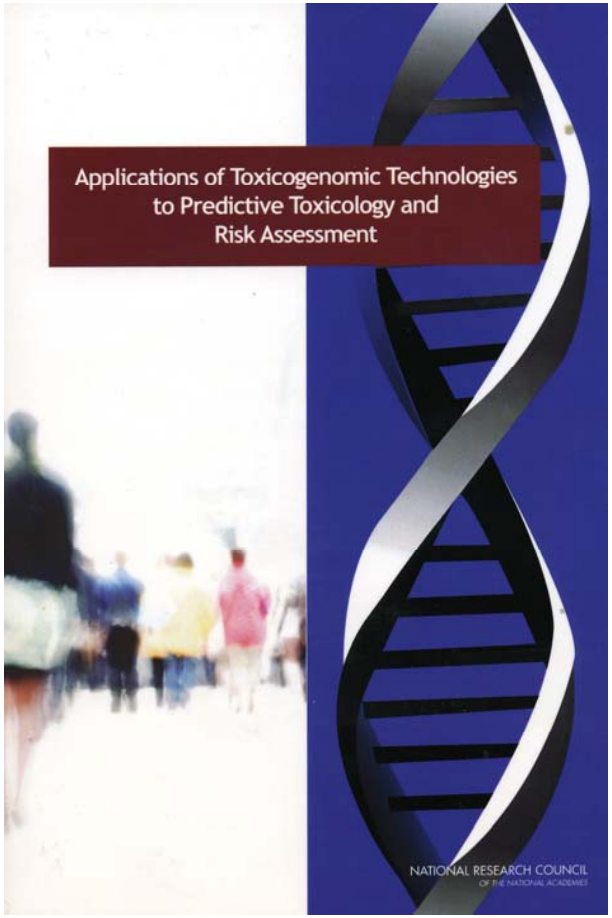
Essential Elements of the NTP Roadmap

- Reexamine current research protocols and models to ensure optimal utility and sensitivity
- Expand endpoints targeted in *in vivo* studies
- Further evaluate and refine the use of non-mammalian animal models
- Develop high throughput capabilities for assessing mechanistic targets *in vitro*
- Create analytic capabilities to integrate diverse toxicology information to add value and understanding



National Research Council Reports (2007)





High Throughput Screening (HTS)

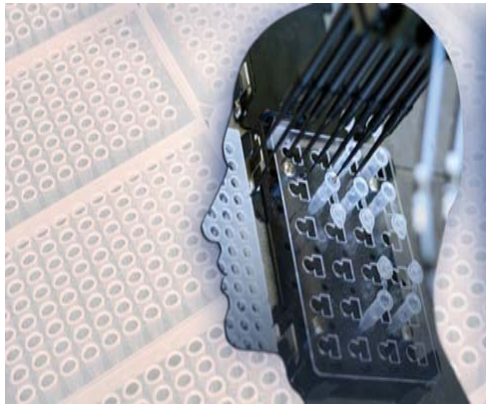
- NTP Roadmap includes a major initiative to develop a HTS program with 3 main goals:
  - Identify mechanisms of action
  - Develop predictive models for *in vivo* biological response
  - Prioritize substances for further in-depth toxicological evaluation

Partnering with the NIH Molecular Libraries Initiative (MLI)

- The MLI uses high throughput methods to identify small molecules to probe functions of genes, cells, and biochemical pathways
- In 2005, NTP established a collaboration with the NIH Chemical Genomics Center (NCGC)
  - Identify batteries of cell-based and biochemical assays to probe toxicity pathways
  - NTP supplied chemicals, assays and financial support to the NCGC
  - NTP is developing tools to link data generated from HTS assays to data produced by the toxicology testing program
  - Current focus is on toxicity pathways in immune function and cancer

Biomolecular Screening Branch- October 2007

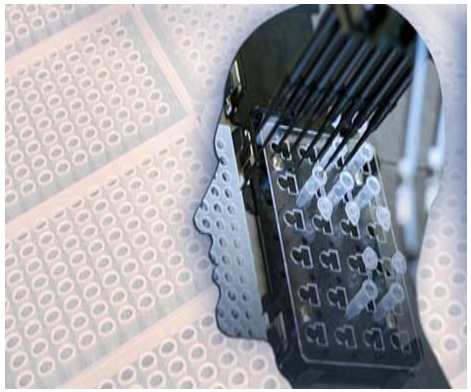
- Develops NTP research and testing activities in high* and medium-throughput screening assays for rapid detection of biological activities of significance to toxicology and carcinogenesis
- Carries out the NTP automated screening assays with C. *elegans*
- Develops analysis tools and approaches to allow an integrated assessment of HTS endpoints and associations with findings from traditional toxicology and cancer models
- Dr. Ray Tice, Acting Branch Chief
- Permanent Branch Chief and bioinformatics recruits underway



*Collaborations with EPA ToxCast Program and NIH Chemical Genomics Center, Molecular Libraries Initiative

Biomolecular Screening Branch - Activities

- First chemical set screened in most of the 96 assays currently at NCGC
- Additional ~ 4000 chemicals being selected
- Assay selection continues
  - Discussions with commercial suppliers: public meeting Sept. 11-12, 2008
  - Targets on Toxicity Pathways* are being identified
- IAG with NCGC funding increased
- MOU with EPA and NCGC signed
- Co-sponsor 3-D tissue models NIH Roadmap Initiative for FY '09



* *Toxicity Testing in the 21st Century: A Vision and a Strategy*, National Research Council, National Academies Press, August, 2007

NTP Interagency Agreement with the NCGC

- NTP Assays
  - Cytotoxicity (ATP levels, LDH release)
  - Apoptosis (3/7, 8, 9)
  - p53 (positives retested in a knockout cell line created with RNAi)
  - Antioxidant response pathway in HepG2 cells
  - Gadd 45a
  - Cytotoxicity in chicken cell lines deficient in various DNA repair pathways
  - Ion channel assay (hERG)
- NCGC Assays (96)
  - Toxicity (11 of 11)
  - Cellular signaling (10 of 25)
  - Isolated molecular targets (22 of 43)
  - Profiling (2 of 7)
  - EPA (10 nuclear receptors - AR, Era, FXR, GR, LXRb, PPARd, PPARg, RXR, TRb, VDR, RORa)

Interagency Cooperation

- Memorandum of Understanding: “High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings”
  - Signed February 14, 2008
  - Agreement between:
    - National Institute of Environmental Health Sciences/ National Toxicology Program
    - NIH National Human Genome Research Institute/ Chemical Genomics Center
    - US Environmental Protection Agency/ Office of Research and Development

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What the Partners Bring to the MOU

Activities	NTP	NCGC	EPA
Historical Tox Data	X		X
Experimental Toxicology	X		
High-Throughput Testing		X	
Mid-throughput Systems			X
Lower organism model System	*C. elegans*		Zebrafish (developing embryo)
In Vitro 3-D Model Systems	X (NIH Roadmap)		X
Effect of Human/Animal Genetic Background on Toxic Effects	X		
Computational Toxicology		X (limited)	X
Validation Experience	X (NICEATM-ICCVAM)		X (endocrine disruptor)

MOU Focus Groups

- **Pathways/Assays Group - K. Witt (NTP), M. Xia (NCGC), K. Houck (EPA)**
  - Identify key toxicity pathways and suitable assays (focus on human cells) for those pathways
    - Include biotransformation capacity in assays
    - Evaluate assay reliability
- **Compound Group - C. Smith (NTP), N. Southall (NCGC), D. Dix (EPA)**
  - Define ~7600 compound library (will include NCGC Drug Library of ~2000 compounds)
  - Establish a library of water soluble compounds
  - Mixtures
- **Bioinformatics - TBN (NTP), R. Huang (NCGC), R. Judson (EPA)**
  - Define positive vs negative call
  - Evaluate reproducibility across related endpoints
  - Evaluate patterns of response and relationship to adverse health outcomes in experimental animals and humans
- **Targeted Testing - J. Bucher (NTP), S. Edwards (EPA), J. Inglese (NCGC)**
  - Evaluate alternative assay platforms or species (e.g. *C. elegans*, *D. rerio*, *D. melanogaster*) as a resource for secondary screening
  - Integrate data from *in vitro* screening with existing and new data generated from traditional *in vivo* toxicity testing protocols to aid in the interpretation of the output from HTS

Number of Candidate Chemicals

Universe	13,116
With structures	9,968
Not in current plates	7,670
Plausible P-chem, relevant to NTP and EPA	6,870

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National Toxicology Program

ToxCast Website: www.epa.gov/ncct/toxcast

National Center for Computational Toxicology

Contact Us Search: All EPA This Area Go

You are here: EPA Home » National Center for Computational Toxicology » ToxCast™ Program

The EPA Web site will be unavailable on Sunday, March 2, 2008 from 8:00 pm until 10:00 pm ET.

ToxCast™ Program

Predicting Hazard, Characterizing Toxicity Pathways, and Prioritizing the Toxicity Testing of Environmental Chemicals

Introduction

In 2007, EPA launched ToxCast™ in order to develop a cost-effective approach for prioritizing the toxicity testing of large numbers of chemicals in a short period of time. Using data from state-of-the-art high throughput screening (HTS) bioassays developed in the pharmaceutical industry, ToxCast™ is building computational models to forecast the potential human toxicity of chemicals. These hazard predictions will provide EPA regulatory programs with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations, and lead to more efficient use of animal testing.

In its first phase, ToxCast™ is profiling over 300 well-characterized chemicals (primarily pesticides) in over 400 HTS endpoints. These endpoints include biochemical assays of protein function, cell-based transcriptional reporter assays, multi-cell interaction assays, transcriptomics on primary cell cultures, and developmental assays in zebrafish embryos. Almost all of the compounds being examined in Phase 1 of ToxCast™ have been tested in traditional toxicology tests, including developmental toxicity, multi-generation studies, and sub-chronic and chronic rodent bioassays. ToxRefDB, a relational database being created to house this information, will contain nearly $1B worth of toxicity studies in animals when completed. ToxRefDB is integrated into a more comprehensive data management system developed by NCCT called ACToR (Aggregated Computational Toxicology Resource), that manages the large-scale datasets of ToxCast™. ACToR is comprised of several independent data repositories linked to a common database of chemical structures and properties, and to tools for development of predictive HTS and genomic bioactivity signatures that strongly correlate with specific toxicity endpoints from ToxRefDB. These ToxCast™ signatures will be defined and evaluated by their ability to predict outcomes from existing mammalian toxicity testing, and identify toxicity pathways that are relevant to human health effects.

The second phase of ToxCast™ will screen additional compounds representing broader chemical structure and use classes, in order to evaluate the predictive bioactivity signatures developed in Phase I. Following successful conclusion of Phases I and II, ToxCast™ will provide EPA regulatory programs an efficient tool for rapidly and efficiently screening compounds and prioritizing further toxicity testing.

ToxCast™ Navigation

- Introduction
- ToxCast™ Chemicals
- ToxCast™ Assays
- ToxCast™ Information Management
- ToxCast™ Partnerships
- ToxCast™ Contractors
- ToxCast™ Presentations
- ToxCast™ Publications
- ToxCast™ News

ToxCast Contracts for Generating HTS and Genomics Data

Receptors, Enzymes

NOVASCREEN
a Caliper Life Sciences company

BioFocus DPI
A Galápagos Company

Cell Signaling

BioSeek

Transcription Factors

attagene
The Home of TFomics™

Metabolic Transformation

iVAL

Cell Function

CELLUMEN

ACEA Biosciences, Inc.

EXPRESSION ANALYSIS

PHYLONIX
Zebrafish Assays for Drug Screening

In vitro Genomics

Cell Function

Alternative Species

Phased Development of ToxCast

Phase	No. of Chemicals	Chemical Criteria	Purpose	No. of Assays	Cost per Chemical	Target Date
I	320	Data rich (pesticides)	Signature development	>400	$20k	FY07-08
IIa	>300	Data rich chemicals	Validation	>400	$15-20k	FY09
IIb	>100	Known human toxicants	Extrapolation	>400	$15-20k	FY09
IIc	>300	Expanded structure and use diversity	Extension	>400	$15-20k	FY10
III	Thousands	Data poor	Prediction and prioritization	???	$10-15k	FY11-12

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WormTox: Development of toxicological screening tools using *Caenorhabditis elegans*

Jonathan H. Freedman, Ph.D.

Laboratory of Molecular Toxicology, NIEHS

Windy Boyd, Ph.D.

National Toxicology Program Screening Core

Characteristics of *C. elegans*

- Non-parasitic nematode
- ~ 1 mm in length
- 10 day life span
- 3.5 day developmental cycle
- 959 somatic cells
- Highly differentiated digestive, reproductive, muscular and nervous (chemo- and mechano-sensory) systems
- Cell and developmental biology are understood in exceptional detail.
- Cell lineages are known for the entire developmental program
- Transgenic nematodes (*lacZ*, GFP)
- Orthologous gene expression

QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.

What Can You Monitor in Medium Throughput Format?

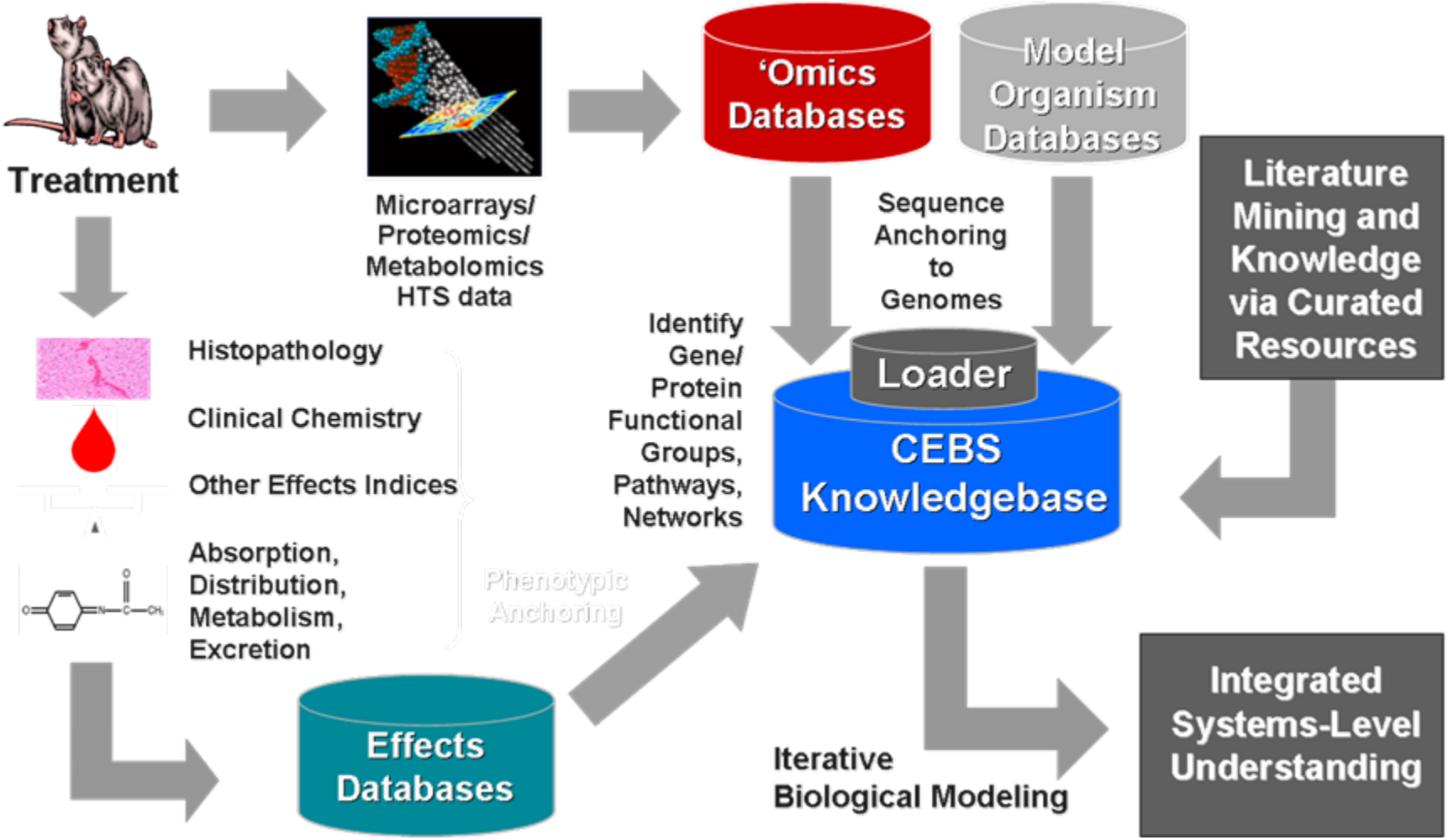
- Growth
- Movement
- Feeding
- Reproduction
- Size
- Shape
- Gene expression
- Development
  - Whole organism
  - Specific cells

- Screen using
  - Wild-type *C. elegans*
  - Genetic mutations
  - Transgenic nematodes
  - RNAi Library

NTP In-house *C. elegans* Screening Core Activities

- Develop methods to measure the toxicity of developmental and neurological toxicants
- Expose to hundreds of potential toxicants and determine changes in phenotypes (survival, size, growth, reproduction and movement)
- Create or obtain GFP-based, stress-responsive transgenic *C. elegans*
- Use *C. elegans* microarray analysis to evaluate a subset of chemicals
- Adapt methods for high throughput analysis to assess the toxicological responses in *C. elegans* in which each gene has been inactivated using RNA interference

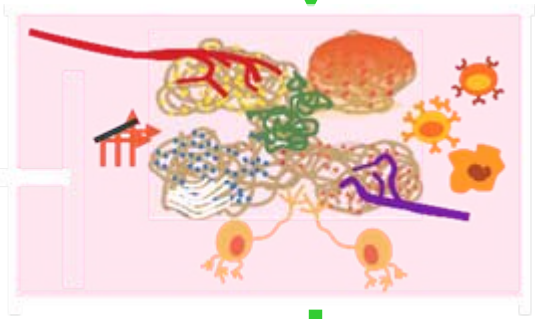
CEBS Knowledgebase – Systems Level Data Integration



3-D Tissue Models: NIH Roadmap

Unique NIH contribution:
from life and physical sciences for biomedical applications

Trans-NIH:
multi-use platforms



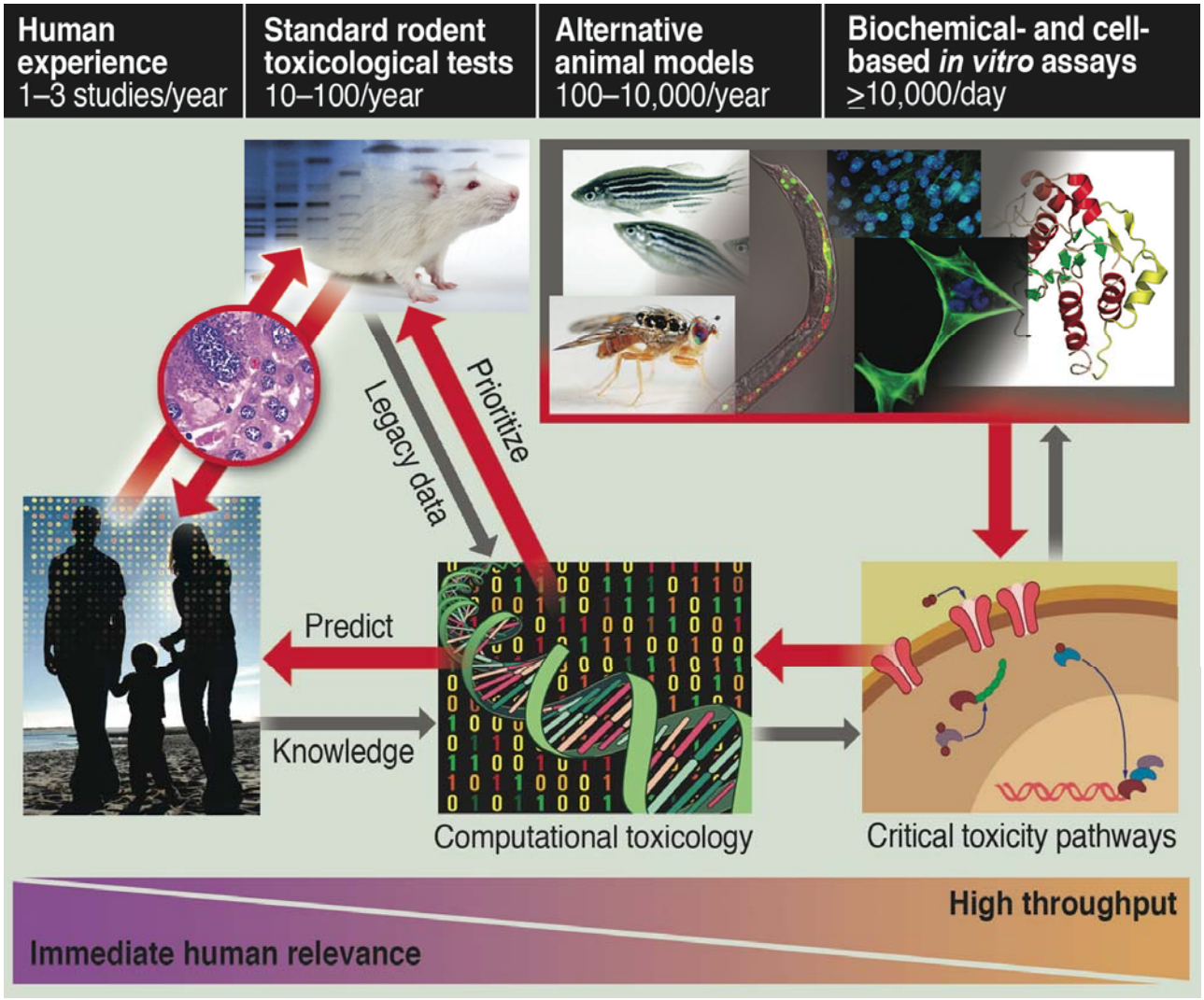
Advances IC Missions:
novel platforms for research, therapeutic discovery, toxicology screening

Transformative:
Mimic in situ complexity to obtain physiologically relevant data

Institute of Biomedical Imaging and Bioengineering (NIBIB)
hunzikerr@mail.nih.gov

Transforming Environmental Health Protection

- Collins, FS, Gray, GM, Bucher, JR* (2008) *Science* 319:906-7
  * With much appreciation: R. Tice, K.Witt, C. Smith, C. Austin, C. Portier, B. Kavlock, *et. al*



A National Toxicology Program 21st Century Model

1. Toxicants are initially examined using cell-based HTS technology (in vitro)
2. Candidate toxicants are then tested using model organisms (*C. elegans*, zebrafish, Drosophila)
3. Compounds are then considered for mammalian “in life” studies
4. Iterative process to develop predictive models

Goals and Timeline

Short-term goals (2-3 years)

- Continue to develop tools and approaches
- Characterize and probe toxicity pathways
- Further develop and populate relational databases
- Develop capabilities for metabolism
- Prioritize chemicals for more in-depth and targeted testing and carry out iterative testing

Long-term goals (5 years)

- Inform cross-species and low-dose extrapolation
- Develop predictive models for human diseases
- Establish role in regulatory science (ICCVAM-NICETAM)

NTP Expectations for 21st Century Methods and Data

- Continue to refine traditional methods and develop new methods to provide basic toxicology information for public health protection
  - Mechanistic information
  - Exposure-response information
  - Predictive of toxicity to humans, animals and the environment
  - Life stage susceptibility
  - Genetic susceptibility
- Results from new “data rich” techniques: genomics, proteomics, HTS, must be reconciled with existing testing information for **conceptual validation**
- Approaches to accomplish **formal validation** of new methods for human hazard and risk estimations must be concurrently developed