NTP
National Toxicology Program

The National Toxicology Program
Toxicology in the 21st Century

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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 studied since program inception

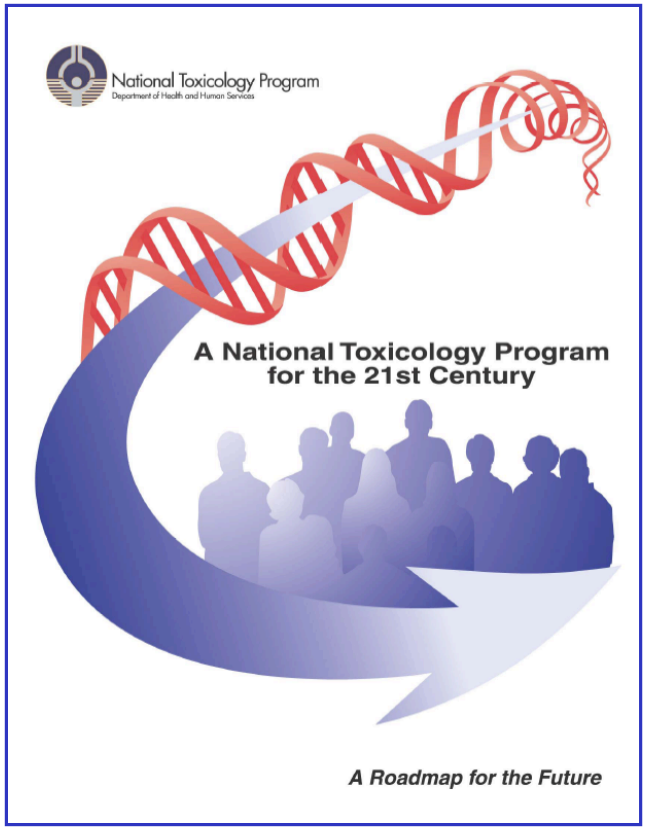
• Distinct test articles	2553
• Chronic rodent bioassays	616
• Genetically modified mice-cancer	13
• Genetic toxicity	2236
• Immunotoxicity	112
• Reproductive/developmental	242
• General and other toxicity	838

Current NTP research areas

- Cellular phone radiation
- Dietary supplements
- Combination HIV therapies
- DNA-based therapeutics
- Drinking water contaminants
- Endocrine disruptors
- Green chemistry
- Herbal medicines
- Nanoscale materials
- Occupational exposures
- Persistent organic pollutants
- Phototoxicants

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
- Improve the use of toxicokinetic information
- 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



Reexamine NTP Testing Strategies

Discontinue use of F344/N rat- select Wistar Han

Continue use of B6C3F1 mouse and sequence parental strains

(Frazer, K. *et al*. (2007) *Nature*, **448**:1050-1053)

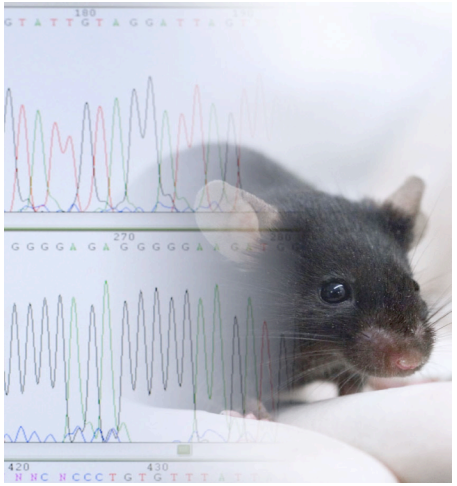
Utilize additional endocrine responsive endpoints in pre-chronic studies- include mammary whole mounts, measures of sexual development

Recognize importance of developmental programming- reexamine focus on young adult animals

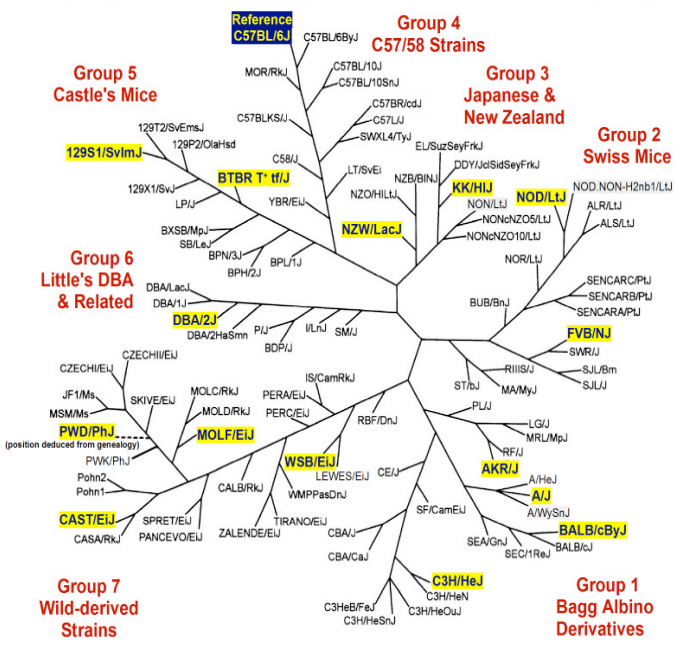
Identify biomarkers for pulmonary and cardiovascular injury, and altered carbohydrate/lipid metabolism and inflammation

- Dunnick, JK, Thayer, KA and Travlos, GS (2007) *Toxicol. Sci*.**100**:29-35
- Thayer, KA, Foster, PM (2007) *Environ. Health Perspect*. **115**:1351-1356.
- King-Herbert, A, Thayer, K, (2006) *Toxicol. Pathol*. **34**:802-805

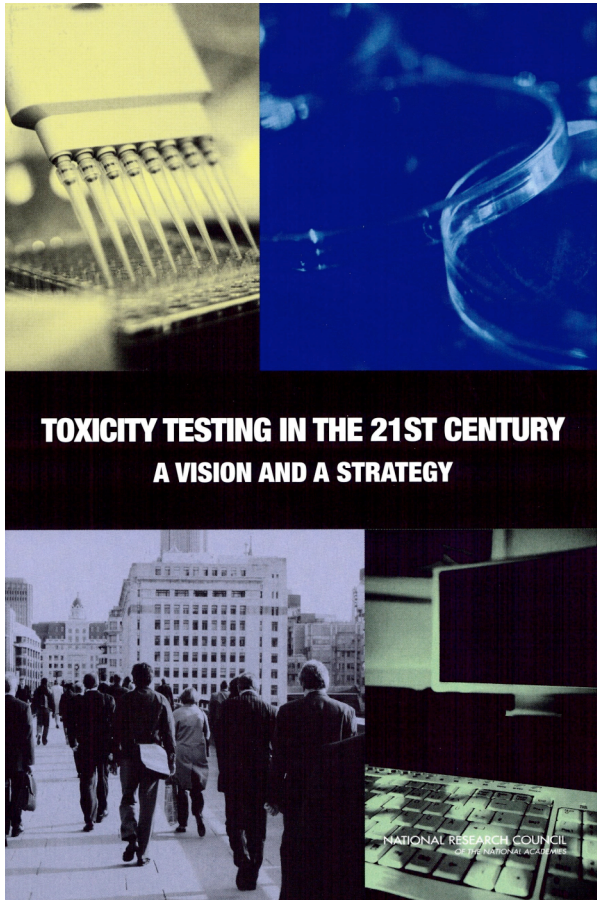
Host susceptibility initiative

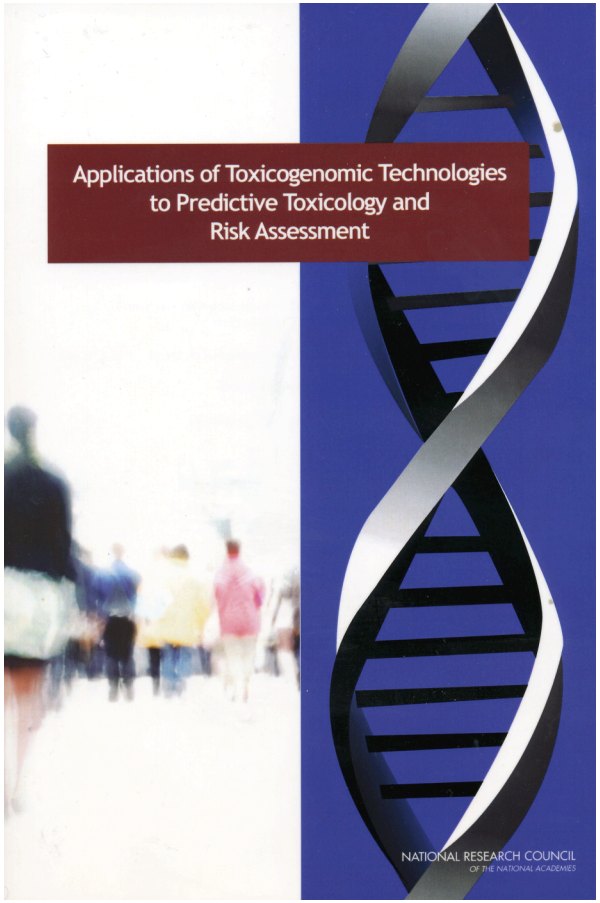


- Study the genetic basis underlying biological response
- Utilize multiple strains of inbred mice or GMMs
- Distinguish genetic from kinetic differences in response
- Exploit findings from NTP 15 isogenic mouse strain SNP analysis (> 8 million SNPs)
- Provide data to allow identification of QTLs with selected biological responses
- Partner with intramural and extramural scientists
- Current efforts focused on optimal design and staffing of program



National Research Council Reports (2007)





NTP Vision

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

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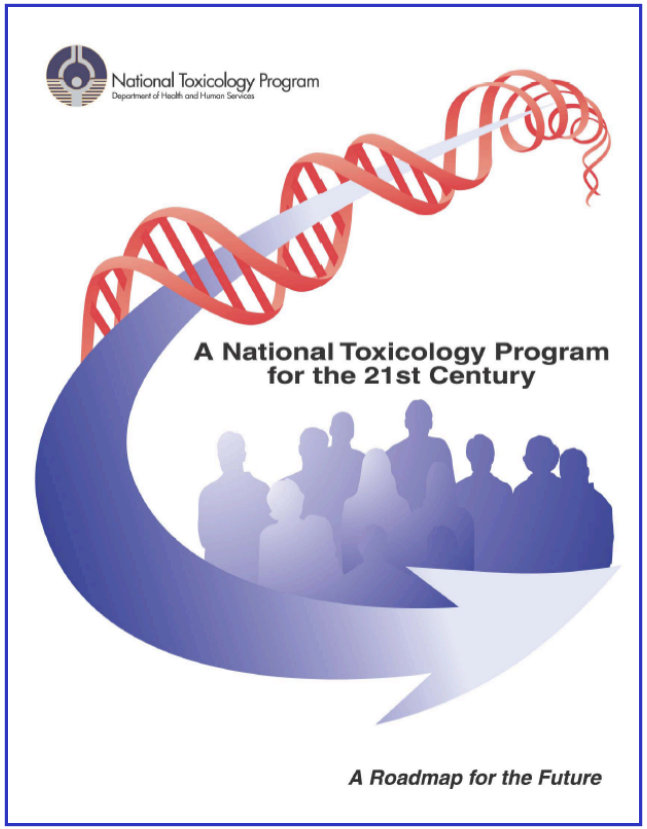


NTP in house *C. elegans* Screening Core activities

Develop methods to measure the toxicity of developmental and neurological toxicants

Expose to hundreds of potential developmental and/or neurological toxicants and determine changes in phenotypes (survival, size, growth, reproduction and movement)

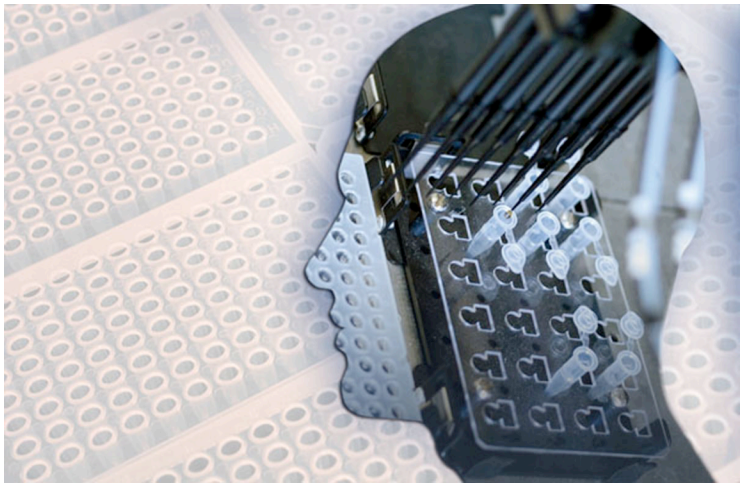
Create or obtain GFP-based, stress-responsive transgenic *C. elegans*

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

High throughput screening

- NTP Roadmap includes a major initiative to develop a high throughput screening 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 (HTS) 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 MLI
- NTP is developing tools to link data generated from HTS assays to data produced by the toxicology testing program
- Current focus is on critical pathways in immune function and cancer

Partnering with the NIH Molecular Libraries Initiative-cytotoxicity

- 1353 compounds tested for cytotoxicity in 13 human and rodent cell lines from liver, kidney, nerve, lung, skin, and blood
- Compounds limited to those soluble in DMSO, < 400 D MW, non volatile-assayed by luciferase fluoresence measure of [ATP], 1536 well format
- 15-point dose response curves, concentrations up to 92 μM, 40-hours
- Patterns of response were:
  - Reproducible
  - Some chemicals were uniformly toxic
  - Some were cell-type specific
  - Some not consistent in cells of similar tissue of origin from rodents and humans

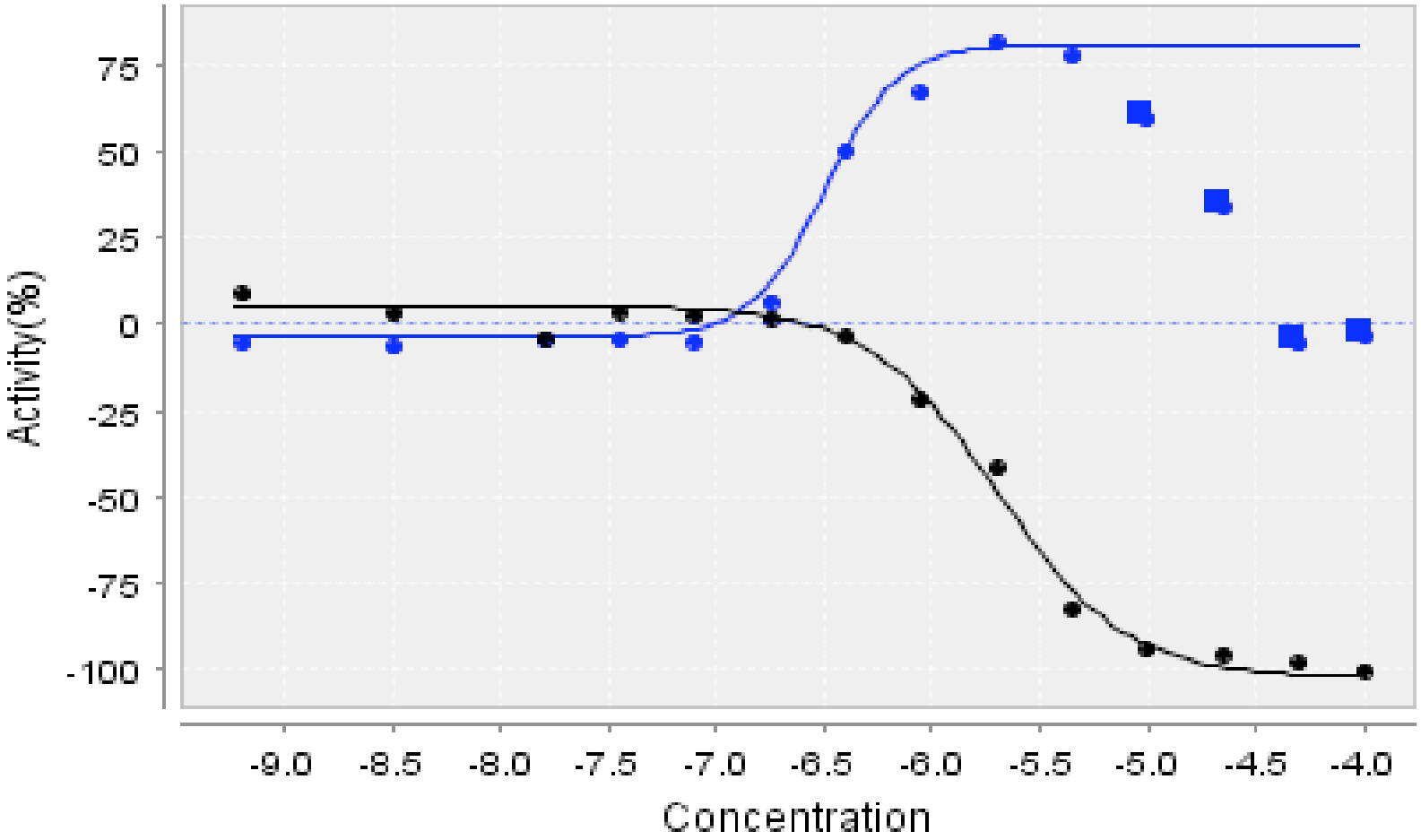
Xia, M, Huang, R, Witt, KL, Southall,N, Fostel,J, Cho,M, Jadhav,A, Smith,CS, Inglese, J, Portier, CJ, Tice, RR, Austin, CP (2007) Compound cytotoxicity profiling using quantitative high-throughput screening. *Environ. Health Perspect. (Online Nov. 22 2007).*

Partnering with the NIH Molecular Libraries Initiative-combining assays

- To probe toxicity pathways and distinguish mechanisms of action, patterns of response must be determined in batteries of assays
- Analysis techniques must include “supervised” and “unsupervised” approaches
- 1353 compounds tested for cytotoxicity vs caspase 3/7 activation in 13 human and rodent cell lines from liver, kidney, nerve, lung, skin, and blood
- 15-point dose response curves, concentrations up to 92 μM, 16-hours
- Patterns of response were complex as expected
- Mechanistic inferences that make biological sense were apparent in the data

Huang, R, Southall,N, Cho, M, Xia, M, Inglese, J, Austin, CP (2008) Characterization of diversity in toxicity mechanism using in vitro cytotoxicity assays in quantitative high throughput screening format. *Chem. Res. Toxicol.* (In press).

Cytotoxicity and caspase 3,7 activity of hexachloro-pentadiene in Jerkat Human Leukemia T cells



Interagency cooperation

- Memorandum of understanding on “High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings”
  - National Toxicology Program/ National Institute of Environmental Health Sciences
  - NIH Chemical Genomics Center/ National Human Genome Research Institute
  - Office of Research and Development/ US Environmental Protection Agency

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