Report of the NTP Associate Director

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National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors
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Topics

- Recent and upcoming meetings and events
- Senior staff changes
- Review of 2004 Roadmap accomplishments
- Current and new directions

Recent Meetings and Events

- Events since the Dec. 2012 Board of Scientific (BSC) meeting
  - Office of Health Assessment and Translation (OHAT) systematic review approach presented at:
    - NAS IRIS review, first meeting Irvine, CA - Dec 13, 2012
    - EPA IRIS arsenic stakeholder meeting, RTP - Jan 8, 2013
    - SOT exhibitor-hosted session: Implementing Systematic Review at the NTP: Mar 12, 2013, (San Antonio, TX)
    - Editorial on implementation in NTP, *EHP*, Apr 2013
  - Report on Carcinogens (RoC)
    - NAS reviews of styrene and formaldehyde, joint panel kickoff - Mar 19, 2013
    - Peer review of the draft RoC monographs on 1-bromopropane and cumene - Mar 21-22, 2013 (RTP, NC); BSC liaison: Dr. Dale Hattis
    - Information-gathering webinar on pentachlorophenol synthesis contaminants and human cancer studies - Apr 11, 2013
    - D.C. District court dismisses SIRC styrene lawsuit challenging listing of styrene in the 12th RoC - May 15, 2013

Upcoming Meetings

- Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) - Sept 24, 2013, RTP
  - ICCVAM changes in directions and procedures
  - Adverse outcome pathways for skin sensitization and integrated testing strategies
  - Tox 21 update
- NTP Technical Reports peer review meeting

Oct 29, 2013	Feb 2014
Cobalt metal	Bromodichloroacetic acid
Glycidamide	Cimstar 3800 (metalworking fluid)
Tetrabromobisphenol A	Green tea extract
Vinylidine chloride	Indole-3-carbinol

Staff Changes

- Retire

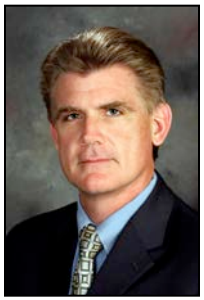
  None – yea!

- Hire

  Dr. Stephen Ferguson
  Biomolecular Screening Branch

- Changes

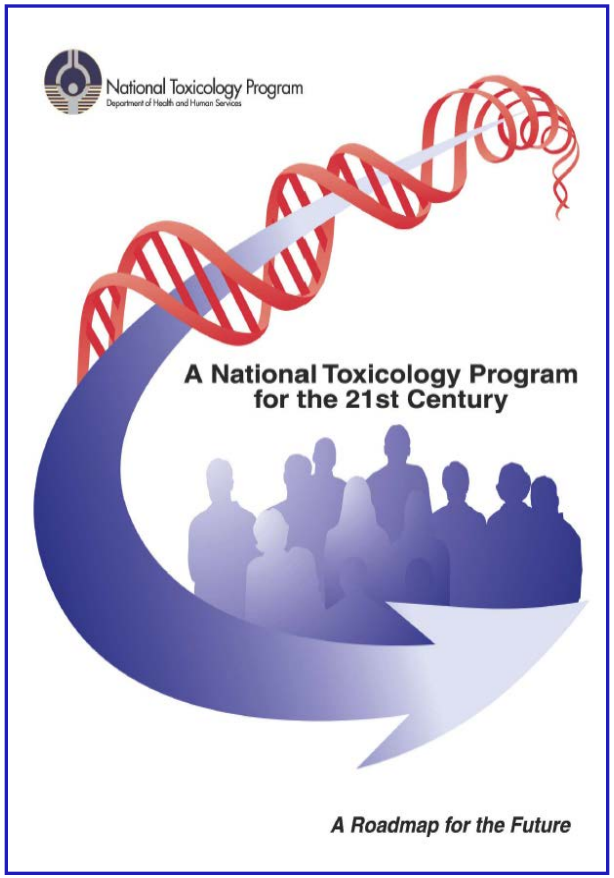
Dr. Michelle Hooth
Acting Chief
Program Operations Branch



Dr. Warren Casey
Acting Director
NICEATM

Organizing Principles of NTP Roadmap 2004

- Refine traditional toxicology assays
- Develop rapid mechanism-based predictive screens for environmentally induced diseases
- Improve the overall utility of NTP products for public health decision making
  - NTP Vision - public meeting 2004
  - NTP Roadmap retreat - 2004



Refine Traditional Toxicology Assays

- Workshops on:
  - Genetically-modified rodent models for cancer hazard identification - 2003
  - Species and strains for cancer studies - 2005
  - Hormonally induced reproductive tumors - relevance of rodent bioassays - 2006
  - Biomarkers for toxicity studies - 2006
- Modified one generational study design
- Perinatal dosing as a default approach
- Diversity Outbred mouse model
- Mouse methylome project
- Molecular mining of archival tissues
- *Need to further utilize and ultimately evaluate value of these investments*

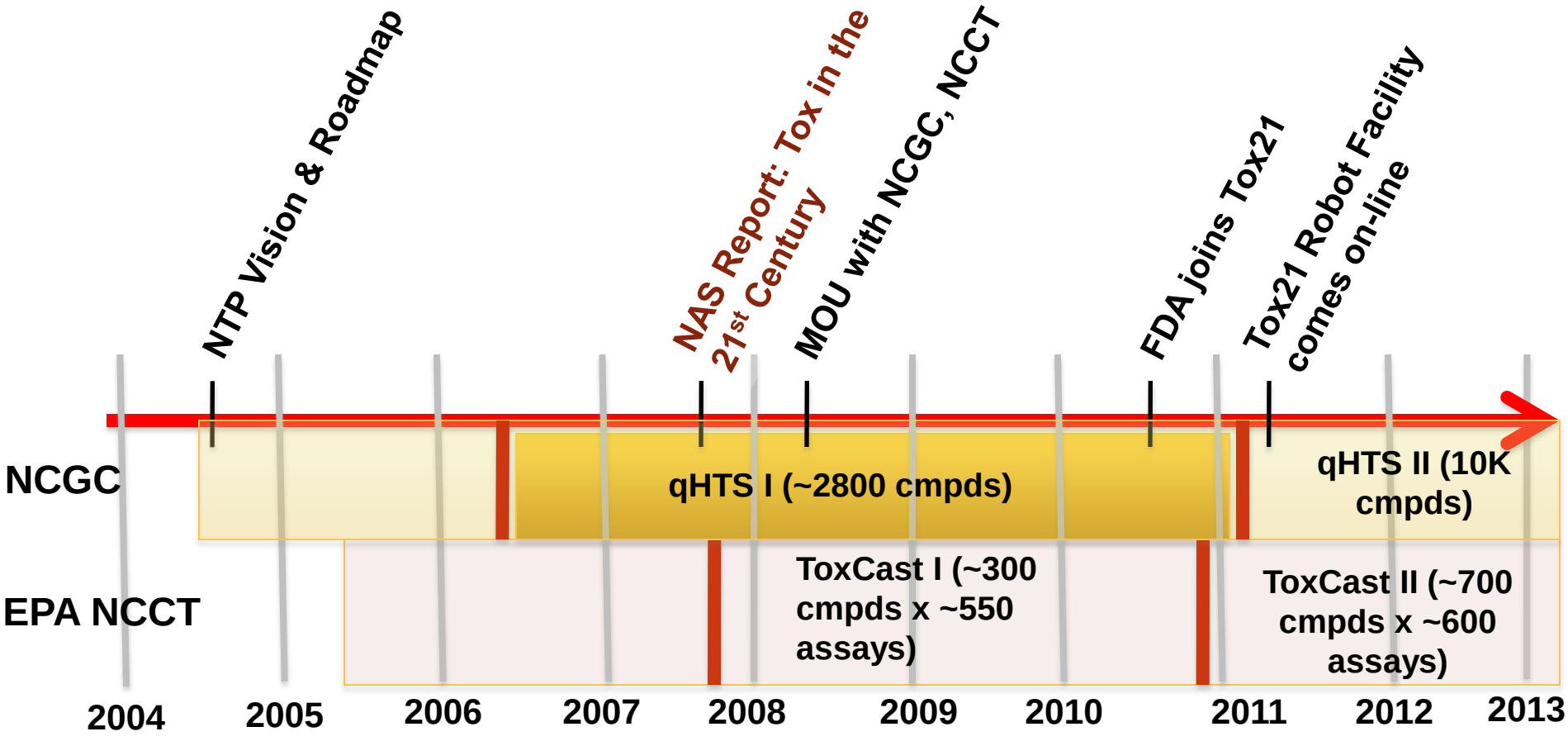
Develop Rapid Mechanism-based Predictive Screens for Environmentally Induced Diseases

- Workshops on:
  - HTS Assays - 2005
  - Chemical Genomics - 2006
  - HTS vendor meeting - 2008
- MoU with NIEHS/NTP, EPA/ORD, NHGRI/NIH Chemical Genomics Center - 2008
- Collins *et al.* Transforming public health protection, *Science* - 2008
- NTP BSC Review of the DNTP Biomolecular Screening Branch - 2010
- Evolution of Tox21 - 2008 to present; FDA joins Tox21 - 2010
- Dedicated robot for Tox21 at NCGC - 2011
- *NCGC moves to larger facility - Sept 2013*
- *Need a significant effort to move findings into the testing program*

Improve Utility of NTP Products for Public Health Decisions

- Reproduction and Development Criteria Working Group - 2008
- Immunotoxicology Criteria Working Group - 2008
- Redesign of the RoC listing process - 2011
- Development of systematic review and process for data stream (human, animal) integration - 2011-present - OHAT
- *Currently exploring methods for assessing the quality of collective in vitro, rapid screening, and "other" information that supports mechanistic understanding*
  - *Need for "risk of bias" tools for in vitro or "other" data similar to those for human or experimental animal data*
  - *Need to establish metrics for "confidence" in the data*
  - *Need to develop in concert with ICCVAM*

The Tox21 Timeline



Tox21 - a "Community Resource" Project

Tox21 Phase II Human Nuclear Receptor and Related qHTS Assays*

AhR full length receptor in HepG2 cells
AR full length receptor in MDA kb2 cells and partial receptor in HEK293 cells
ERα full length receptor in BG1 cells and partial receptor in HEK293 cells
FXR partial receptor in HEK293 cells
GR full length receptor in HeLa cells
PPARδ partial receptor in HEK293 cells
PPARγ partial receptor in HEK293 cells
PXR full length receptor in HepG2 cells
TRβ full length receptor in GH3 cells and partial receptor in HEK293 cells
VDR partial receptor in HEK293 cells
Inhibition of aromatase using MCF-7 cells

All NR assays conducted in agonist and antagonist modes

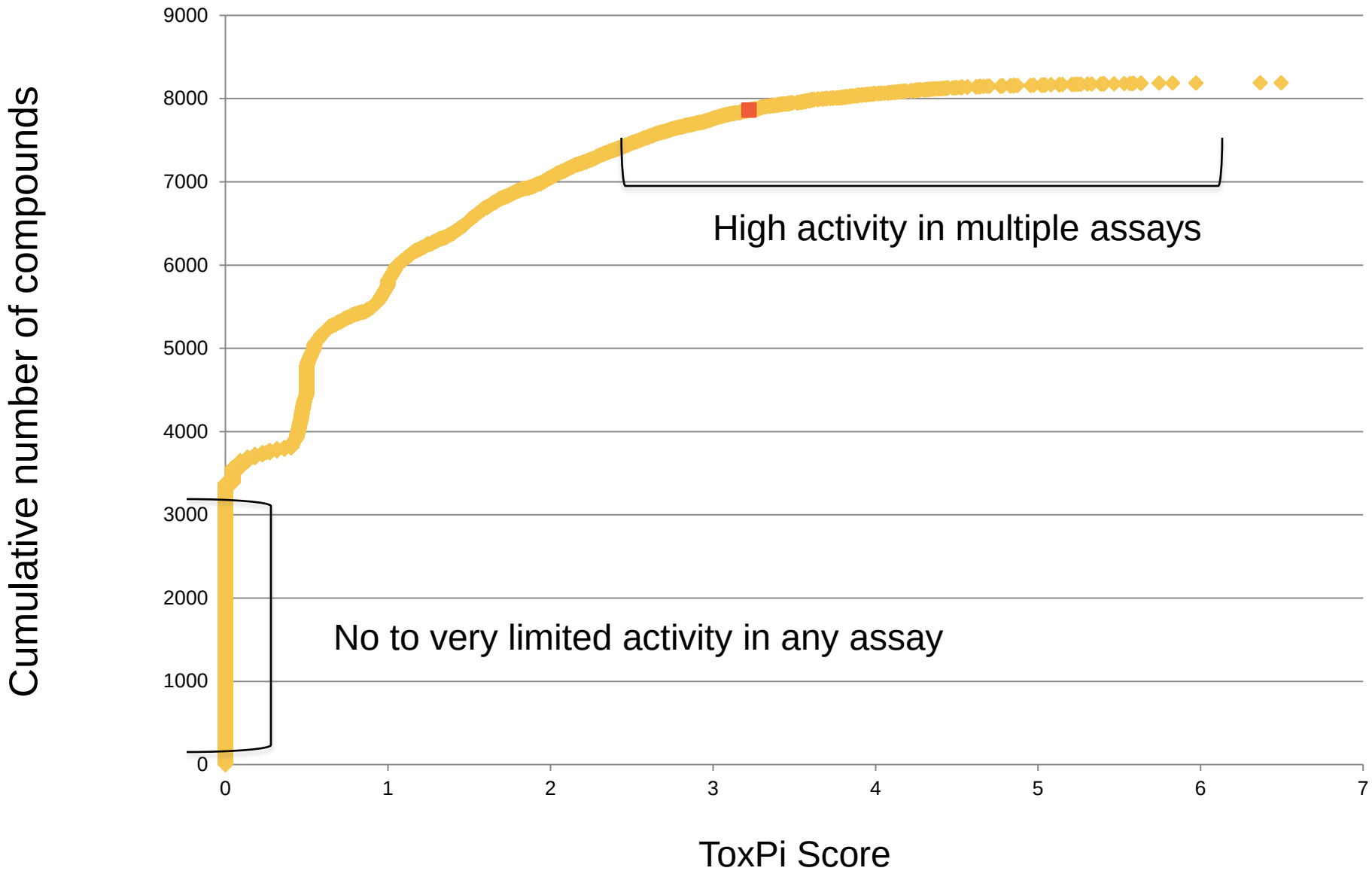
**Bolded text indicates completed assays*

Tox21 Phase II Stress Response and Other qHTS Assays*

Endpoint	Assay
Endoplasmic reticulum stress	Induction of lipid damage in HeLa cells
Genotoxic stress	**p53 activation in HCT-116 colon cancer cells**
	ATAD5 activation (DNA damage response element) in HEK293 cells
	Increased cytotoxicity in isogenic DNA-repair deficient chicken DT40 cell clones (Rev3 (-/-), rad54/ku70 (-/-) vs wild type
Heat shock	**Hsp70 induction in HeLa cells**
Hypoxia	Induction of hypoxia inducible factor 1α in ME-180 cervical carcinoma cells
Inflammation	Induction of NFκB in ME-180 cells
Oxidative stress	**Induction of antioxidant response element Nrf2 in HepG2 cells**
Other	Activator protein-1 activation in ME-180 cells
	Caspase 3/7 activation in multiple cell lines
	Cytotoxicity (LDH release, ATP levels) in multiple cell lines
	Mitochondrial membrane potential in HepG2 cells

**Bolded text indicates completed assays*

Activity Rankings for Tox21 Compounds across All Assays



General Approach to 10K Compound Analysis

Identify AC50 values for robust and consistent responses in **all** assays

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Convert the AC50 values to negative log (i.e., pAC50) values

↓

Directionally adjust pAC50 values based on assay specific +/- response

↓

Run Pearson correlation in Partek on direction adjusted pAC50 data

↓

Set correlation threshold and draw network in Cytoscape

↓

Annotate networks with assay specific results, predicted/known toxicities, etc

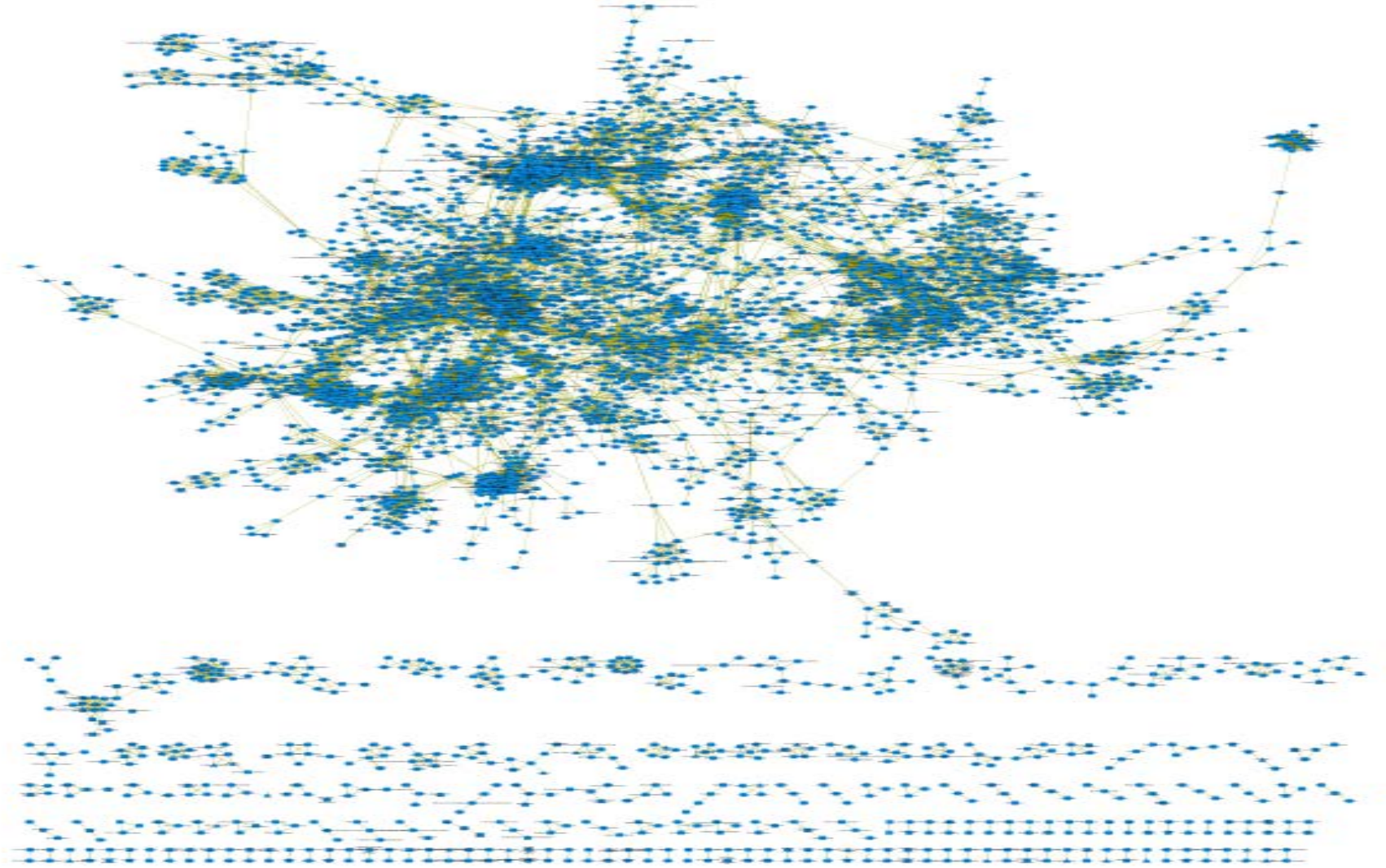
↓

Identify networks associated with selected compound or compounds

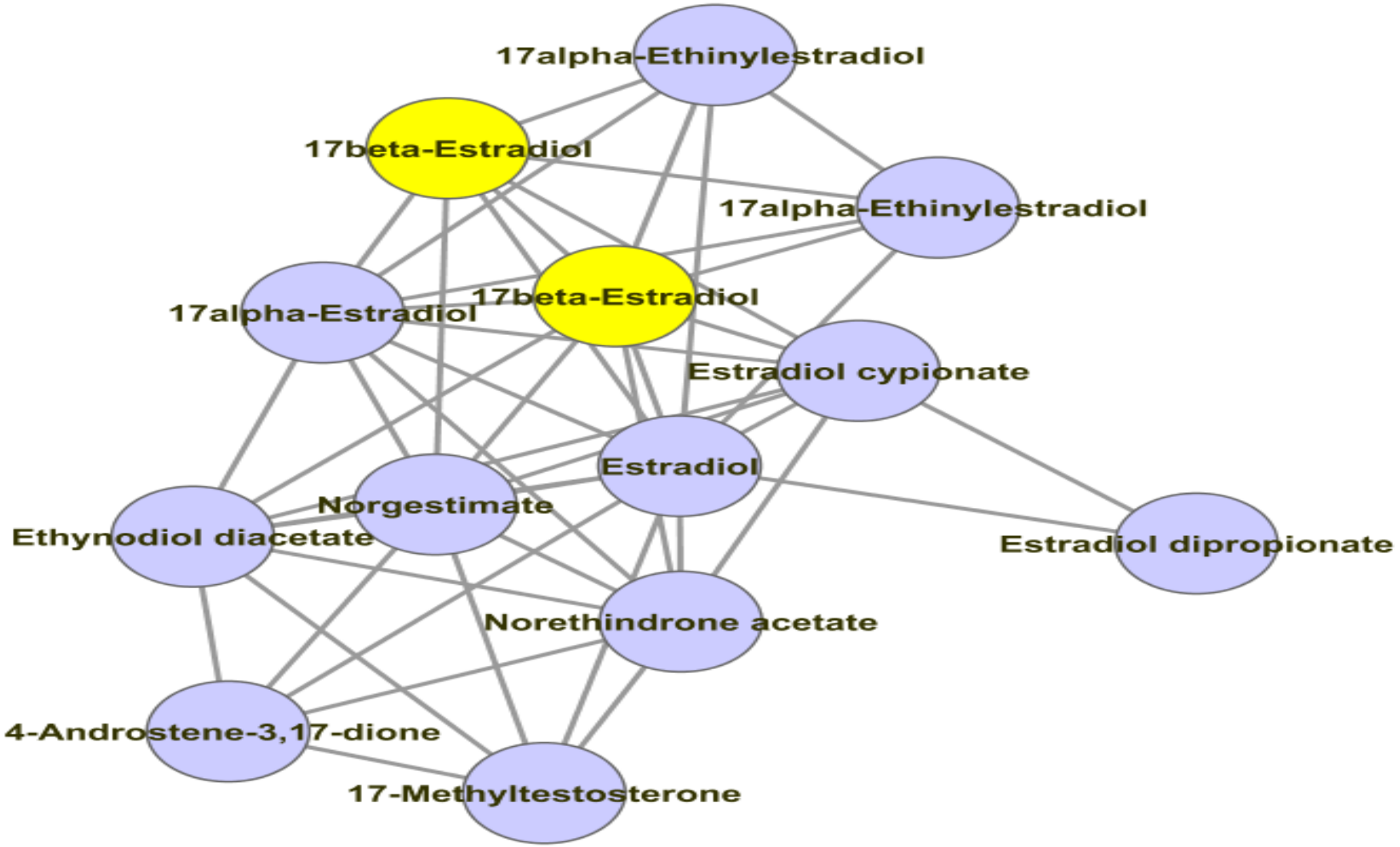
↓

Evaluate relevance of response and identify compounds of interest

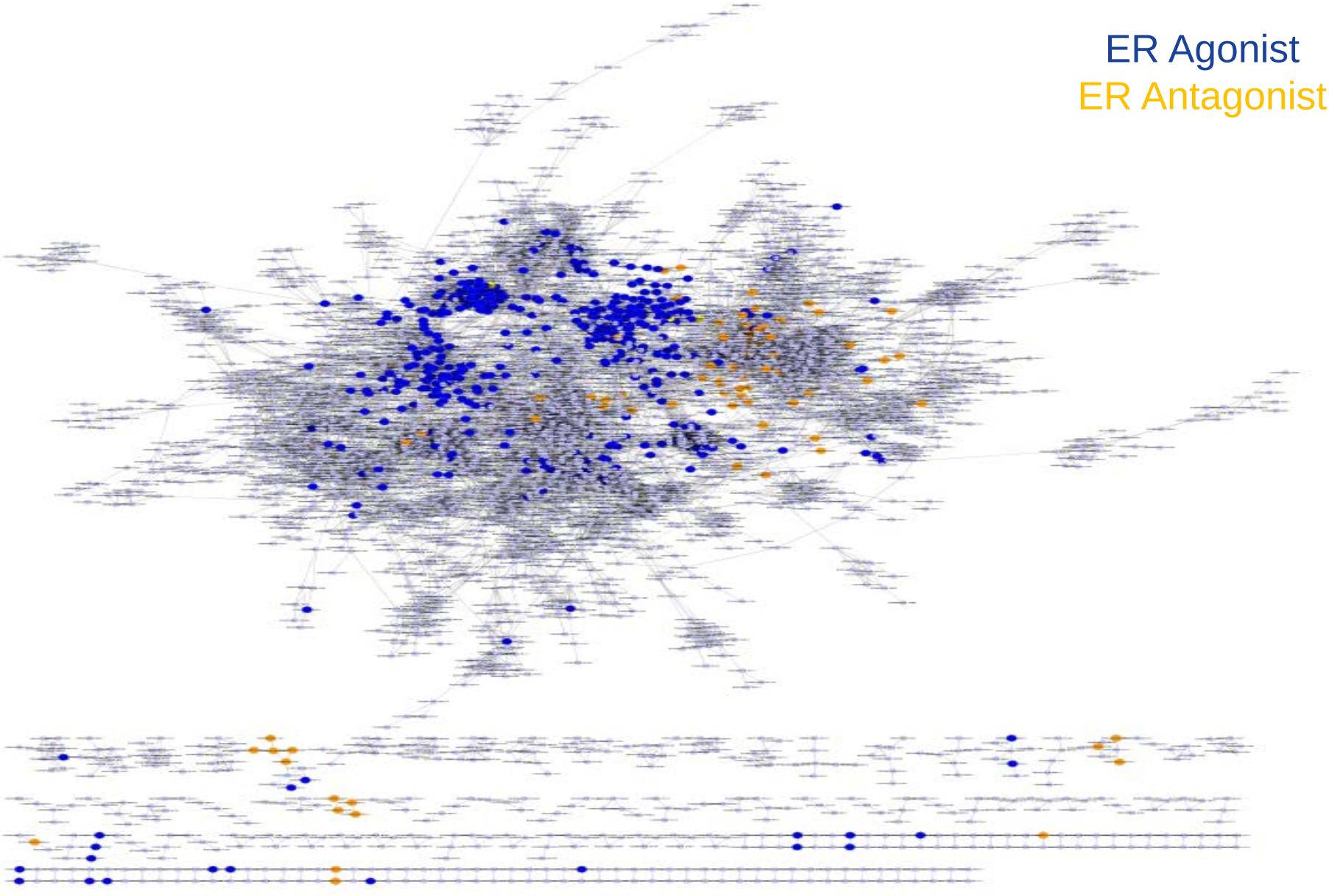
pAC50 with Pearson Correlation >0.7 Connectivity Network for all Assays to Date



17β-Estradiol (Pearson >0.7) Nearest Neighbors



ER Actives (pAC50 with Pearson Correlation >0.7) Connectivity Network for all Assays to Date with ER "Painting"



Tox21 Challenges and Questions

- Major challenges and areas under development:
  - Metabolism
  - Multiplexed endpoints
  - Higher order cell and tissue interactions
- Major questions:
  - How predictions from Tox21 relate to current test method results
  - How Tox21 results can inform traditional studies and vice versa
  - Whether identification of affected pathways can predict disease
  - How Tox21 data integrate into DNTP analysis activities
  - How Tox21 data can be best used to protect public health

Current State of Roadmap Activities

- Accomplished many of the goals outlined in the 2004 NTP Roadmap
- Refined traditional animal-based methods
- Developed new data streams to address toxicological issues
- Developed improved ways to transparently evaluate environmental health data and translate findings for public health protection
- *Working to provide new scientific opportunities and ways to address agency and public concerns and provide answers in a more relevant time frame*