NTP
National Toxicology Program

Tox21: A U.S. Federal Collaboration to Improve the Human Hazard Characterization of Chemicals

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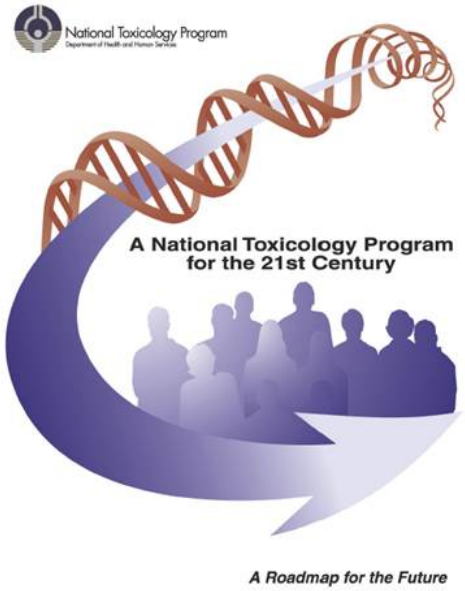
Topics

- Background
- Tox21 Phase I - Proof of principle
- Tox21 Phase II - Expanded compound screening
- Tox21 Phase III - Improving on biological coverage and relevance

2004 NTP Vision and Roadmap for the 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



To meet the challenges of 21st century toxicology, the 2004 NTP Roadmap included a major initiative to develop a high throughput screening (HTS) program with 3 main goals:

- To prioritize chemicals for further in-depth toxicological evaluation
- To identify mechanisms of toxicity (*characterize toxicity pathways, facilitate cross-species extrapolation, provide input to models for low-dose extrapolation*)
- To develop predictive models for *in vivo* biological response in humans

2004 NTP Vision and Roadmap for the 21st Century

Roadmap Activities: High-Throughput Screening (HTS)

Short-term Activities

- Catalogue available assays
- Convene working groups to provide advice on selection of assays
- Develop assays
- Identify initial set of chemicals for testing

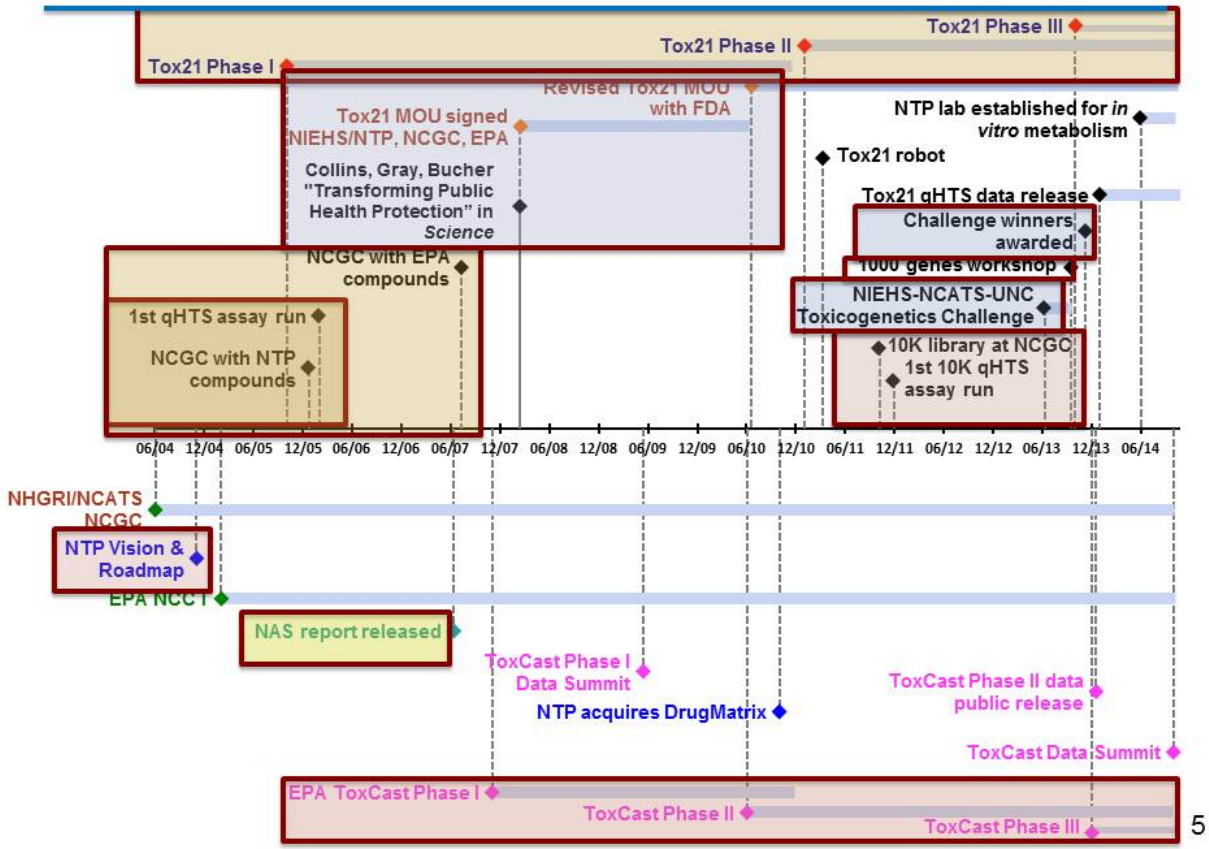
Mid-term Activities

- Continue assay development
- Validate individual assays
- Develop methods for analysis of data
- Develop HTS database
- Review effectiveness

Long-term Activities

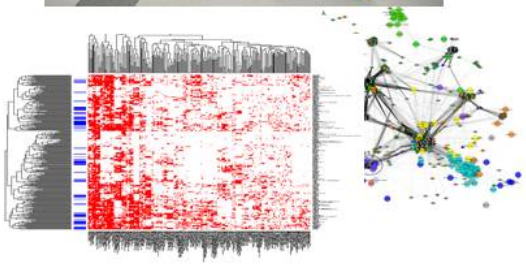
- Develop mechanisms to make chemical sets and tissue banks available for external researchers
- Evaluate HTS data for predictability of toxicity
- Develop a communication plan
- Review effectiveness

Tox21 Timeline



Tox21 Goals

- Identify patterns of compound-induced biological response in order to:
  - Characterize toxicity/disease pathways
  - Facilitate cross-species extrapolation
  - Model low-dose extrapolation
- Prioritize compounds for more extensive toxicological evaluation
- Develop predictive models for biological response in humans



Tox21

Agency Points of Contact

FDA – Suzanne Fitzpatrick Ph.D.
NCGC/NCATS –Anton Simeonov, Ph.D.
EPA/NCCT –Russell Thomas, Ph.D.
NIEHS/NTP - Raymond Tice, Ph.D.

Assays & Pathways Working Group

Co-Chairs
Kevin Gaido, Ph.D. (FDA)
Keith Houck, Ph.D. (EPA)
Kristine Witt, M.S. (NTP)
Menghang Xia, Ph.D. (NCGC)

- Identify toxicity pathways & corresponding assays
- Review nominated assays and prioritize for use at the NCGC

Chemical Selection Working Group

Co-Chairs
William Leister, Ph.D. (NCGC)
Donna Mendrick, Ph.D. (FDA)
Ann Richard, Ph.D. (EPA)
Suramya Waidanatha, Ph.D. (NTP)

- Establish compound libraries for qHTS (10K, mixtures, water-soluble)
- Establish QC procedures for compound identity, purity, concentration, and stability

Informatics Working Group

Co-Chairs
Ruili Huang, Ph.D. (NCGC)
Richard Judson, Ph.D. (EPA)
Jennifer Fostel, Ph.D. (NIEHS)
Weida Tong, Ph.D. (FDA)

- Evaluate assay performance
- Develop prioritization schemes and prediction models
- Make all data publicly accessible

Targeted Testing Working Group

Co-Chairs
Michael DeVito, Ph.D. (NTP)
David Gerhold, Ph.D. (NCGC)
Timothy Shafer, Ph.D. (EPA)
Robert Sprando, Ph.D. (FDA)

- Evaluate relevance of prioritization schemes & prediction models
- Extrapolate *in vitro* concentration to *in vivo* dose

Tox21 Phase I – Proof of Principle (2005 – 2010)

- EPA via ToxCast™ screened 320 compounds (309 unique, primarily pesticide actives and some endocrine active compounds) in ~550 assays.
  - Data made public via ACToR (Aggregated Computational Toxicology Resource; http://epa.gov/actor)
- NCGC screened 1408 compounds (1353 unique) from NTP and 1462 compounds (1384 unique, includes ToxCast Phase I compounds) from EPA at 15 concentrations in 140 qHTS assays representing 77 predominantly cell-based reporter gene endpoints.
  - Data made public via PubChem (http://pubchem.ncbi.nlm.nih.gov/) and CEBS (Chemical Effects in Biological Systems; http://www.niehs.nih.gov/research/resources/databases/cebs/)

Tox21 Phase I qHTS

- **Phenotypic readouts**
  - Cytotoxicity
  - Apoptosis: caspase 3/7, 8, 9
  - Membrane integrity: LDH, protease release
  - Mitochondrial toxicity (membrane potential)
- **Genetox:** p53, ATAD5, DNA damage repair deficient lines (chicken DT40 lines)
- **Cell signaling**
  - Stress response: ARE, ESRE, HSP, Hypoxia, AP-1
  - Immune response: IL-8, TNFα, TTP
  - Other: AP-1, CRE, ERK, HRE, JNK3, NFkB
- **Epigenetics**
  - Locus DeRepression (LDR)
- **Drug metabolism**
  - CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4
- **Target specific assays**
  - Nuclear receptors: AR, AhR, ERα, FXR, GR, LXR, PPARα, PPARδ, PPARγ, PXR, RXR, TRβ, VDR, RORα, RORγ
  - hERG channel
  - Isolated molecular targets: 12hLO, 15hLO1, 15hLO2, ALDH1A1, HADH560, HPGD, HSD17b4, APE1, TDP1, DNA polymerase III, RECQ1 helicase, RGS4, BRCA, IMPase, O-Glc NAc Transferase, Caspase-1/7, CBFβ-RUNX1, PK, Tau, Cruzain, β-Lactamase, PRX, YjeE , NPS, Proteasome, SF1, SMN2, beta-globin splicing, Anthrax Lethal Factor, TSHR
- **Genetic variability:** 87 HapMap CEPH Panel

Conclusions from Tox21 Phase I

- The qHTS platform can be used to screen libraries of environmental compounds for *in vitro* biological activity.
- High quality data are essential for automated data analysis and interpretation.
- Reference compounds are needed to demonstrate the biological relevance of results.
- Chemical QC (ID, purity, stability) is essential.
- The lack of xenobiotic metabolic capability is a significant limitation.

Reviewed in Tice et al. EHP 121:756–765 (2013)

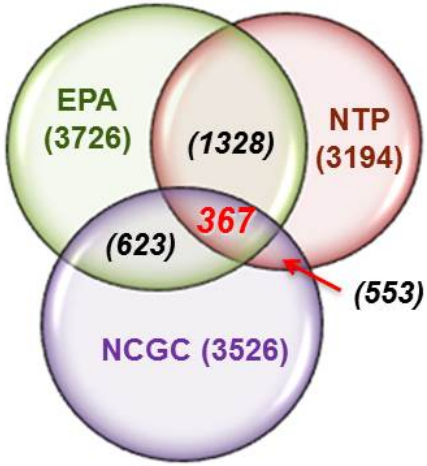
Tox21 Phase II – Expanded Compound Screening (2011 – 2014)

- EPA's ToxCast™ Phase II: ~700 compounds in ~700 assays, ~1000 compounds in endocrine activity assays
  - Data available at http://www.epa.gov/ncct/toxcast/data.html as well as via the Chemical Safety for Sustainability Dashboard (http://actor.epa.gov/dashboard/)
- NCGC qHTS Phase II:
  - 10K compound library screened 3 x at 15 concentrations in qHTS assays that focused on:
    - Nuclear receptor activation or inhibition
    - Induction of cellular stress response pathways
    - Data released in PubChem (88 entries to date at https://www.ncbi.nlm.nih.gov/pcassay?term=tox21)
  - The 1000 genomes project

Tox21 10K Compound Library

88 single-sourced compounds in duplicate on each plate to assess technical variability

All substances being evaluated for identification, purity and stability under conditions of use



Unique	EPA	NTP	NCGC	Total	Total Unique	
GSIDs	3726	3194	3524	10444	8307	unique substances
Tox21 IDs	3729	3210	3733	10672	10496	unique solution IDs
wells	4224	3726	4224	12174	12174	total number of test cmpd wells

2255 replicate substances (GSIDs) across 3 inventories

Compound identity and structures available at http://www.epa.gov/ncct/dsstox/sdf_tox21s.html

Phase II Nuclear Receptor/Related qHTS Assays*

hAhR full length receptor in HepG2 cells	
hAR full length receptor in MDA kb2 cells; partial receptor in HEK293 cells	
hERα full length receptor in BG1 cells; partial receptor in HEK293 cells	
hFXR partial receptor in HEK293 cells	
hGR full length receptor in HeLa cells	**NR assays conducted in agonist and antagonist modes**
hPPARδ partial receptor in HEK293 cells	
hPPARγ partial receptor in HEK293 cells	
hPXR full length receptor in HepG2 cells	
hRORγ partial receptor in CHO cells	
rTRβ full length receptor in GH3 cells; partial human receptor in HEK293 cells	
hVDR partial receptor in HEK293 cells	
Inhibition of aromatase using MCF-7 cells	
Retinol Signaling Pathway in C3H10T1/2 cells	

**Bolded text indicates completed assays*

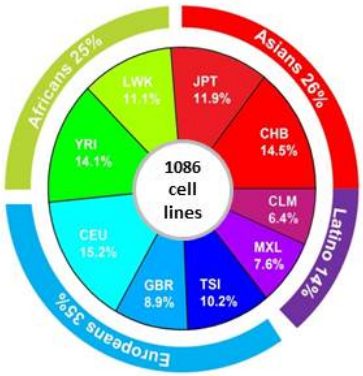
Phase II Stress Response qHTS Assays

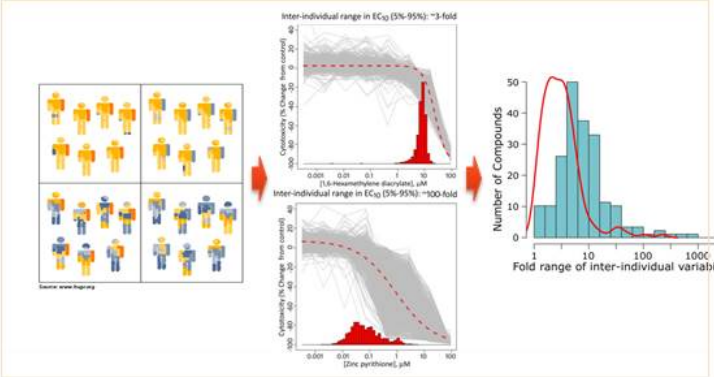
Endoplasmic Reticulum stress	**EndoR (lipid damage) in HeLa cells**
Genotoxic stress	**P53 activation in HCT-116 colon cancer cells**
	ATAD5 levels in HEK293 cells (ATPase family AAA domain-containing protein 5 – a DNA damage response element)
	DT40 (DNA-repair mutant isogenic chicken cell clones) (Rev3 (-/-), rad54/ku70 (-/-), wild type)
	pH2AX induction in CHO cells
Heat shock	Hsp70 in HeLa or HepG2 cells
Hypoxia	HRE (HIF-1α) in ME-180 cervical carcinoma cells
Inflammation	**NFκB in ME-180 cells**
Oxidative stress	**ARE/Nrf2 in HepG2**
Multiple stresses, cell death, specific toxicities	AP-1 activation in ME-180 or HepG2 cells
	Caspase 3/7 activation
	LDH release, ATP levels to assess cytotoxicity
	mitochondrial membrane potential in HepG2 cells
	hERG (ion channel effects) in U2OS cells
	Cell death/viability kinetic studies in 4 cell types

**Bolded text indicates completed assays*

The 1000 Genomes qHTS Toxicity Screening Project

Population-wide study design (Collaboration with I. Rusyn at UNC-CH)





1086 Human lymphoblastoid cell lines representing 9 racial groups

179 compounds **(9 duplicates)**

8 concentrations (0.33 nM - 92 μM)

1-3 plate replicates

1 assay (CellTiterGLO® - ATP production)

= ~2,400,000 data points + 2-5x10^6 SNPs

NIEHS-NCATS-UNC DREAM
Toxicogenetics Challenge
(June 10 – September 16, 2013)

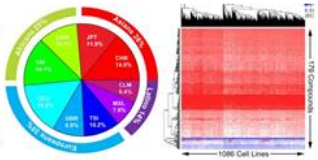
Goal: Use crowdsourcing to better predict the toxicity of chemicals

(1) Use the biological data (SNPs, basal gene expression) to develop a model that accurately predicts ***individual responses*** to compound exposure

(2) Use the intrinsic chemical properties to develop a model that accurately predicts how a particular ***population*** will respond to ***certain types of chemicals***

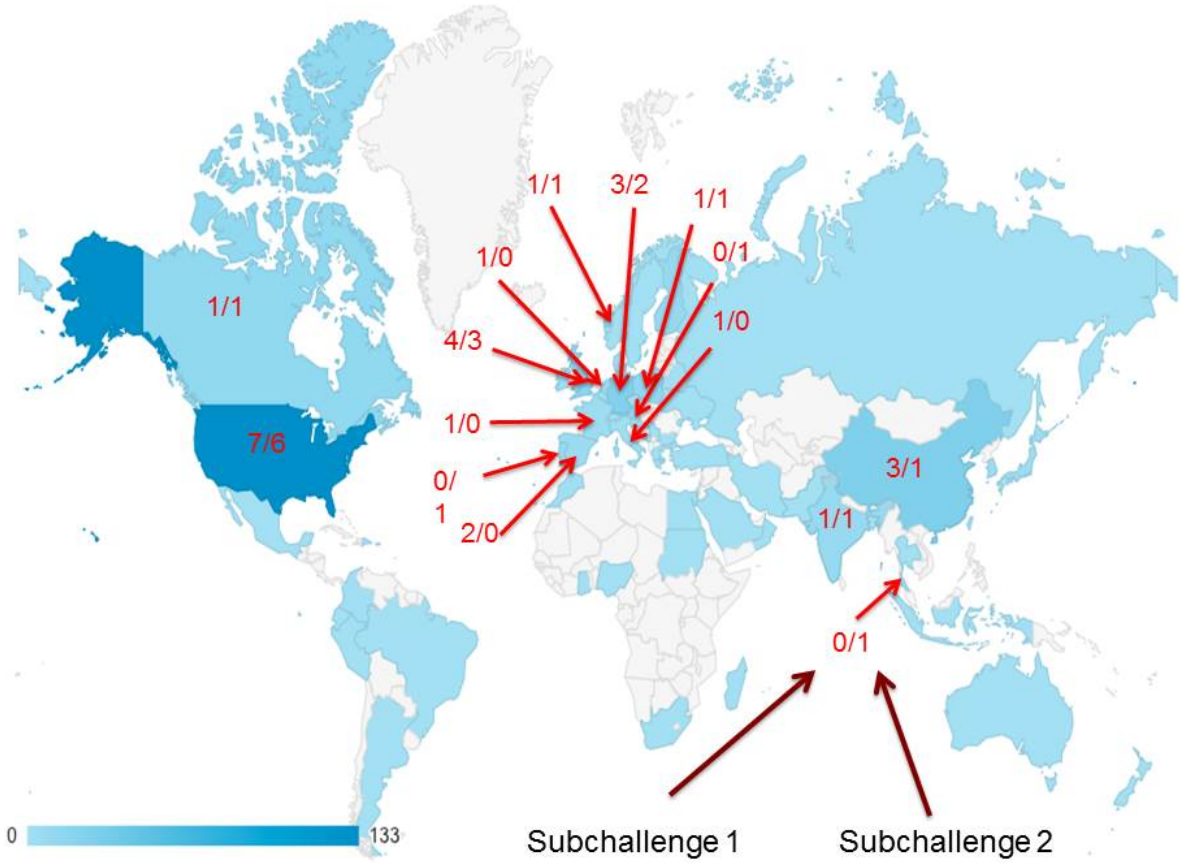
How it works:

Data set: genomic, cytotoxicity and chemical properties data from ~1000 cell lines and ~200 chemicals



Subchallenge 1: 34 teams submitted 99 prediction models
Subchallenge 2: 24 teams submitted 85 prediction models

Winner: Quantitative Biomedical Research Center
(UT Southwestern Medical Center, Dallas, TX)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0/1
1/1
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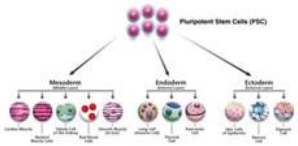
4/3
1/0
7/6
0/
1
2/0
3/1
1/1
0/1
0
133
Subchallenge 1
Subchallenge 2

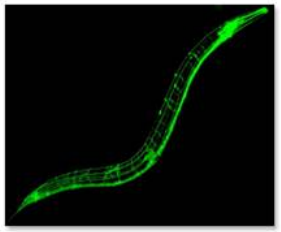
Tox21 Phase II qHTS Limitations

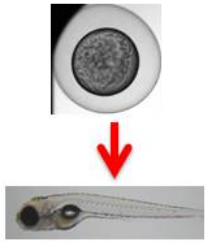
- Chemical space
- Biological space
- Xenobiotic metabolism
- Acute exposure
- Simple biological systems
- Tools to analyze “big” data
- Screening single compounds

Tox21 Phase III – Improving on Biological Coverage and Relevance (2013 - ?)

- Focus on more physiologically-relevant *in vitro* cell systems (e.g., human stem cell derived differentiated cell populations).
- Include cell types (e.g., HepaRG in 2D and 3D models) that incorporate xenobiotic metabolism/allow for longer-term exposures.
- Increase the use of computational models to predict metabolism/toxicity.
- Increase the testing of compounds in alternative animal models.
- Develop and implement a high throughput transcriptomics platform for human, rat, mouse, zebrafish, and *C. elegans.*







5 days

Tox21: A collaboration of many…….

Biomolecular Screening Branch and Adjuncts