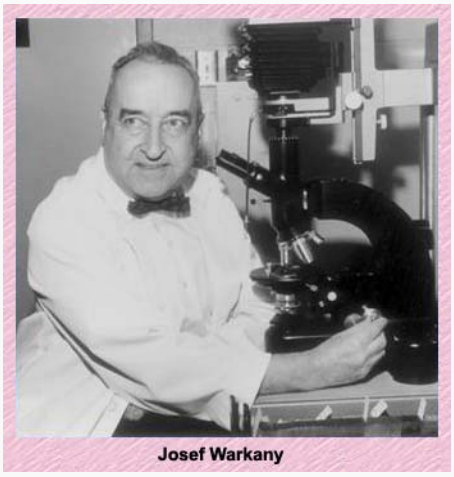
A Random Walk Through Teratology (and Beyond)

Robert Kavlock
Deputy Assistant Administrator for Science
Office of Research and Development
US Environmental Protection Agency

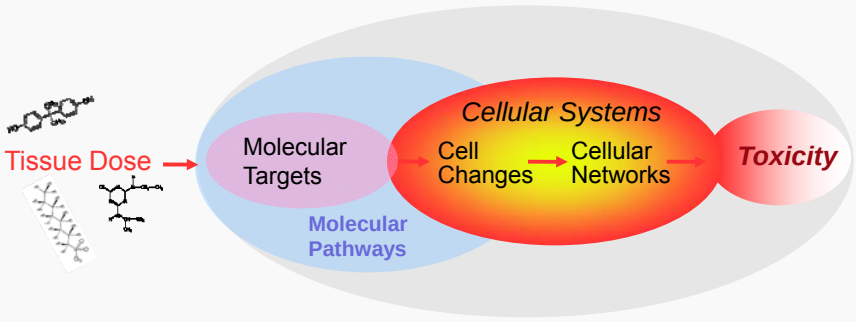
Warkany Lecture – 53rd Teratology Annual Meeting
Tuscon, Arizona
23 June, 2013



Josef Warkany

36 years, 231 publications and 463 co-authors later…..

Predicting Human Toxicity: A Grand Challenge



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The Case for Change

- Data poor chemicals with limited recourse under TSCA
- Thousands of chemicals queued for endocrine disruptor screening
  - 11 tests in current screen, per chemical cost exceeds $750k
- Poor predictive value of rodent toxicology studies
  - High cost of late failures in drug development
- Safer design of chemicals (green chemistry)
- Legislative mandates in the EU
- And most of all, need for improved inclusion of mechanism of action in risk assessment
  - That results in a new system that is as least as protective of human health as current paradigm

The Fetotoxic Potential of Municipal Drinking Water in the Mouse

NEIL CHERNOFF, ELLEN ROGERS, BRENDA CARVER, ROBERT KAYLOCK AND EARL GRAY
U. S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina 27711

TERATOLOGY (1979) *19:* 165-170.

TABLE 3

Maternal and fetal effects analyzed by two-way ANOVA with multiple covariance

	Month	Water	Month X water
Maternal liver/body weight	+++	ns	ns
No. implants	++	ns	ns
No. live	ns	ns	ns
No. dead	ns	ns	+
Average fetal weight	++	ns	ns
No. sternal ossif. centers	+	ns	ns
No. caudal ossif. centers	ns	ns	ns
% supernumerary ribs	ns	++	ns
% enlarged renal pelvis	++	ns	ns

ns not significant.
+ significant, $p < 0.05$.
++ significant, $p < 0.01$.
+++ significant, $p < 0.001$.

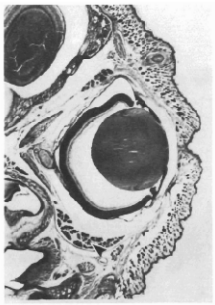
6

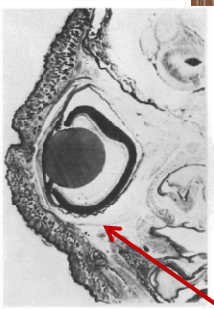
POSTNATAL EVALUATION OF MORPHOLOGICAL AND FUNCTIONAL EFFECTS OF PRENATAL EXPOSURE TO NITROFEN IN THE LONG-EVANS RAT

Robert J. Kavlock, L. Earl Gray, Jr.

Developmental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

Journal of Toxicology and Environmental Health, 11:679–690, 1983





Prenatal Exposure to the Fungicide Dinocap Causes Behavioral Torticollis, Ballooning and Cleft Palate in Mice, but not Rats or Hamsters

L. Earl Gray, Jr., John M. Rogers, Robert J. Kavlock, Joseph S. Ostby, Janet M. Ferrell, and Katrina L. Gray

Teratogenesis, Carcinogenesis, and Mutagenesis 6:33–43 (1986)

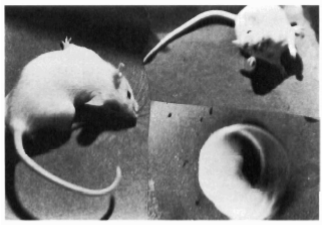


Fig. 2. The picture on the upper right shows the tilted-head posture of a dinocap-treated mouse with torticollis. The same mouse is shown spinning in the lower right and on the left. The picture on the lower right was taken at a slow shutter speed to display the motion and on the left at a faster shutter speed to stop the action.

Functional Teratogens of the Rat Kidney

II. Nitrofen and Ethylenethiourea

GEORGE P. DASTON,[1] BLAIR F. REHNBERG, BRENDA CARVER, AND ROBERT J. KAVLOCK

Department of Biological Sciences, University of Wisconsin, Milwaukee, Wisconsin 53201, and Developmental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

FUNDAMENTAL AND APPLIED TOXICOLOGY 11, 401–415 (1988)

TABLE 1

EFFECTS OF MATERNAL ETU EXPOSURE ON TERM FETUSES

Dose (mg/kg)	No. dams inseminated	No. pregnant at term	Avg. no. implants	Avg. % resorbed	Avg. fetal wt (g)	% Fetuses w/kidney score > 5[a]	% Fetuses w/nonrenal defects[b]
0	6	6	11.0 ± 1.8	1.05 ± 1.15	4.16 ± 0.10	0	0
10	6	6	10.7 ± 1.8	12.23 ± 4.81	4.35 ± 0.24	7.5	0
20	6	5	13.0 ± 1.2	9.68 ± 9.07	4.18 ± 0.16	8.5	0
40	6	6	10.2 ± 1.8	14.70 ± 8.54	4.31 ± 0.29	21.8	3.6
80	6	5	14.2 ± 1.6	9.62 ± 6.38	4.36 ± 0.13	48.4	39.1
160	5	5	9.4 ± 1.3	9.28 ± 4.56	4.07 ± 0.21	82.4[c]	95.2

Developmental Toxicity and Structure–Activity Relationships of Aliphatic Acids, Including Dose–Response Assessment of Valproic Acid in Mice and Rats[1,2]

MICHAEL G. NAROTSKY,* ELAINE Z. FRANCIS,† AND ROBERT J. KAVLOCK‡

FUNDAMENTAL AND APPLIED TOXICOLOGY 22, 251–265 (1994)

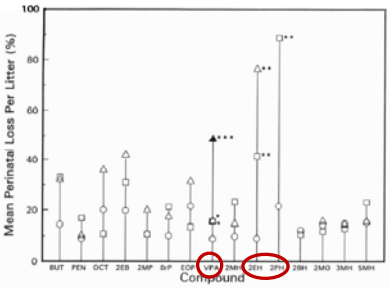


FIG. 2. Comparison of perinatal loss for dams treated with short-chain aliphatic acids. Each data point represents the mean value per litter for each dose level: O, control; □, low dose; △, high dose. For VPA: △, 400 mg/kg; ▲, 500 mg/kg. *, **, and ***, significantly different from concurrent controls at the 0.05, 0.01, and 0.001 levels.

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Nonadditive Developmental Toxicity in Mixtures of Trichloroethylene, Di(2-ethylhexyl) Phthalate, and Heptachlor in a 5 × 5 × 5 Design[1]

MICHAEL G. NAROTSKY,*[,2] EDIE A. WELLER,†[,3] VERNON M. CHINCHILLI,†[,4] AND ROBERT J. KAVLOCK‡

FUNDAMENTAL AND APPLIED TOXICOLOGY 27, 203–216 (1995)

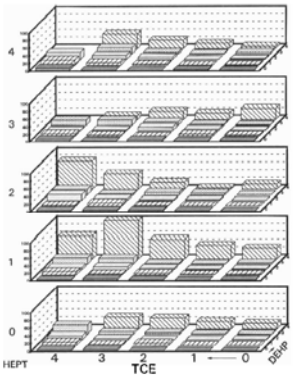


FIG. 10. Mean incidence of pups per litter (%) with anophthalmia or microphthalmia; values are represented by pillar height. Each plot represents a different level of HEPT. The origin (Group 000) is in the lower right corner. The level of TCE increases from 0 to 4 moving to the left, DEHP increases moving back, and HEPT increases moving up.

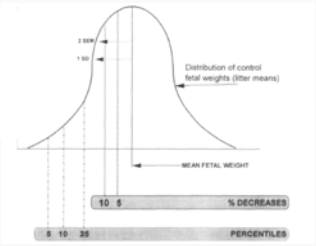
10

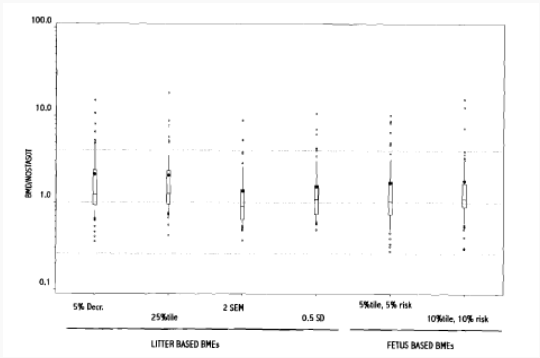
Dose–Response Assessments for Developmental Toxicity

IV. Benchmark Doses for Fetal Weight Changes[1]

ROBERT J. KAVLOCK,* BRUCE C. ALLEN,† ELAINE M. FAUSTMAN,‡ AND CAROLE A. KIMMEL§

FUNDAMENTAL AND APPLIED TOXICOLOGY 26, 211–222 (1995)





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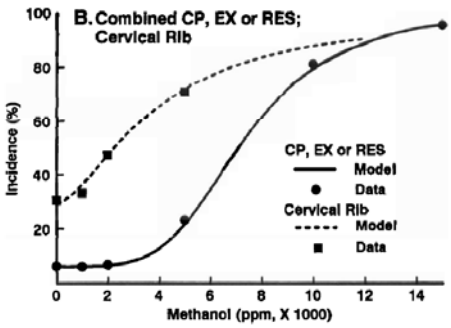
The Developmental Toxicity of Inhaled Methanol in the CD-1 Mouse, With Quantitative Dose–Response Modeling for Estimation of Benchmark Doses

JOHN M. ROGERS, M. LEONARD MOLE, NEIL CHERNOFF, BRENDA D. BARBEE, CHRISTINE I. TURNER, TINA R. LOGSDON, AND ROBERT J. KAVLOCK

TERATOLOGY 47:175–188 (1993)



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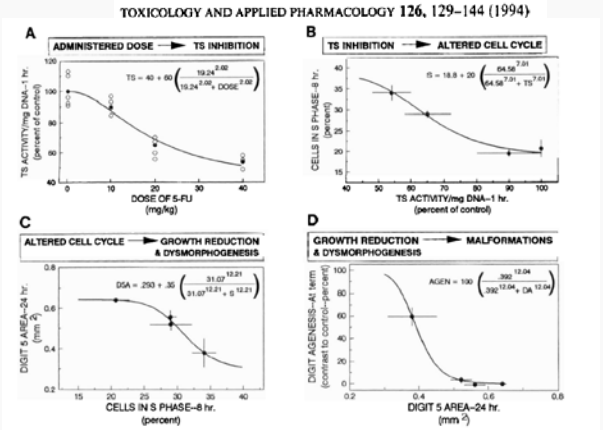
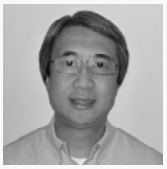
Biologically Based Dose–Response Modeling in Developmental Toxicology: Biochemical and Cellular Sequelae of 5-Fluorouracil Exposure in the Developing Rat[1]

DANA L. SHUEY,*[,2] CHRISTOPHER LAU,† TINA R. LOGSDON,‡ ROBERT M. ZUCKER,‡ KENNETH H. ELSTEIN,‡ MICHAEL G. NAROTSKY,‡ R. WOODROW SETZER,§ ROBERT J. KAVLOCK,† AND JOHN M. ROGERS†[,3]

TOXICOLOGY AND APPLIED PHARMACOLOGY 126, 129–144 (1994)





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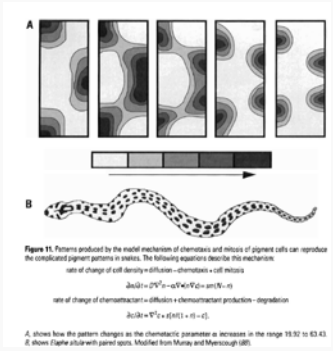
The Road to Embryologically Based Dose–Response Models

Robert J. Kavlock and R. Woodrow Setzer

National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

The goal of researchers working in the area of developmental toxicology is to prevent adverse reproductive outcomes (early pregnancy loss, birth defects, reduced birth weight, and altered functional development) in humans due to exposures to environmental contaminants, therapeutic drugs, and other factors. To best achieve that goal, it is important that relevant information be gathered and assimilated in the risk assessment process. One of the major challenges of improved risk assessment is to better use all pertinent biological and mechanistic information. This may be done qualitatively (e.g., demonstrating that the experimental model is not appropriate for extrapolation purposes); semiquantitatively (using information to reduce the degree of uncertainty present under default extrapolation procedures), or quantitatively (formally describing the relationships between exposure and adverse outcome in mathematical forms, including components that directly reflect individual steps in the overall progression of toxicity). In this paper we review the recent advances in the risk assessment process for developmental toxicants and hypothesize on future directions that may revolutionize our thinking in this area. The road to these changes sometimes appears to be a well-mapped course on a relatively smooth surface; at other times the path is bumpy and obscure, while at still other times it is only a wish in the eye of the engineer to cross an uncharted and rugged environment. — Environ Health Perspect 104(Suppl 1):107–121 (1996)

Key words: risk assessment, RfD, developmental toxicity, teratogencity, benchmark dose, biologically based dose–response models, BBDR, homeobox, skeletal development, limb development, pattern formation, mathematical models



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An Aha Moment

SOCIETY OF TOXICOLOGY

37th Annual Meeting

PROGRAM

MARCH 1-5, 1998

SEATTLE, WASHINGTON

Washington State Convention Center

MONDAY, MARCH 2

8:30 AM - 9:30 AM
WSCC: BALLROOMS 6B-C
PLENARY LECTURE: THE TOYOTA PRINCIPLE, LECTURER: JUDGE ALEX KOZINSKI

9:30 AM - 11:30 AM
WSCC: EXHIBIT HALL A
POSTER SESSION FOR VISITING STUDENTS

9:30 AM - 11:30 AM
WSCC: BALLROOMS 6B-C
BURROUGHS WELLCOME TOXICOLOGY SCHOLAR AWARD LECTURES: MECHANISMS OF CHEMICAL TOXICITY: THE DARK SIDE OF THE IMMUNE SYSTEM, LECTURER: DEBRA L. LASKIN & MAMMALIAN DNA ALKYLATION REPAIR, LECTURER: LEONA D. SAMSON

12:00 PM - 1:15 PM
WSCC: BALLROOMS 6B-C
MRC LECTURE: GENE AND GENOMES: DECIPHERING THE PERIODIC TABLE OF LIFE, LECTURER: LEROY HOOD

5:00 PM OR 5:30 PM - 6:30 PM (CHECK ON-SITE CALENDAR)
THE SHERATON HOTEL
SPECIALTY SECTION MEETINGS: FOOD SAFETY, *IN VITRO*, MOLECULAR BIOLOGY, REPRODUCTIVE AND DEVELOPMENTAL, AND VETERINARY

6:30 PM - 8:00 PM
CHECK ON-SITE CALENDAR FOR MEETING LOCATION
REGIONAL CHAPTER MEETINGS

7:00 PM - 9:00 PM
CHECK ON-SITE CALENDAR FOR MEETING LOCATION
BREATHLESS IN SEATTLE PUBLIC FORUM

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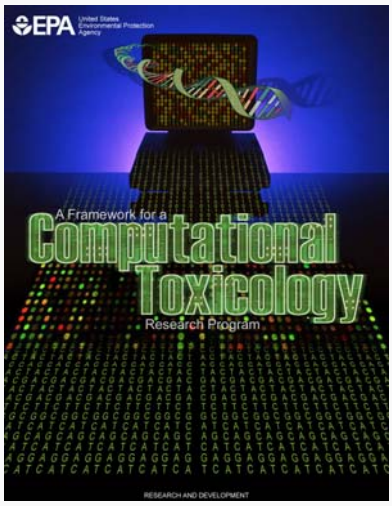
EPA's Computational Toxicology Research Program (2003)

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/ncct

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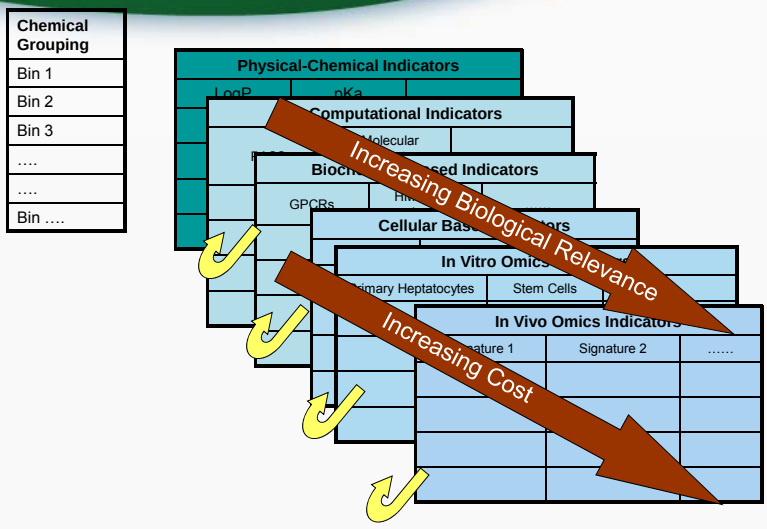
ToxCast Information Domains



Circa 2005

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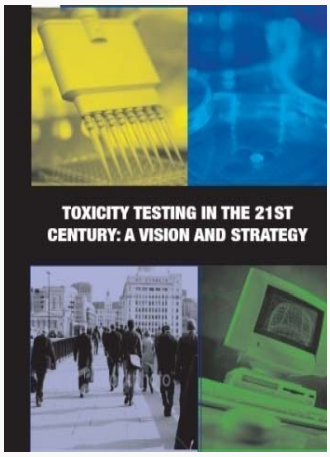
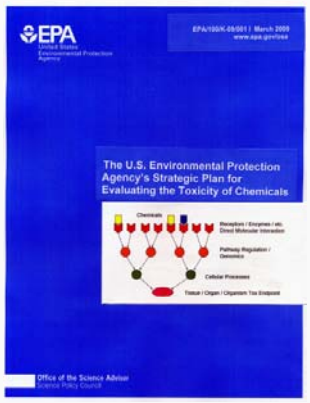
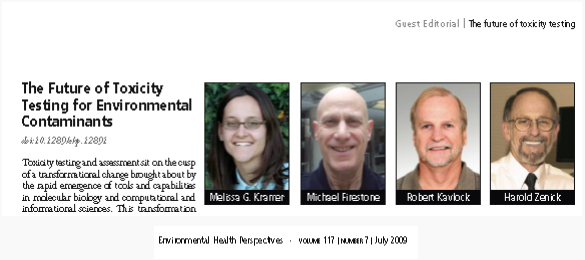
National Academy Report (2007)



Design a 'modern' toxicity testing program to assess potential human risks posed by exposures to environmental agents over a broad range of doses and compounds and to be in a position to use this information in quantitative human health risk assessment.





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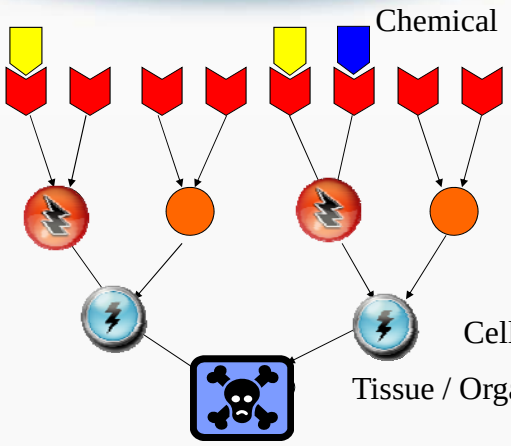
Toxicity Pathways



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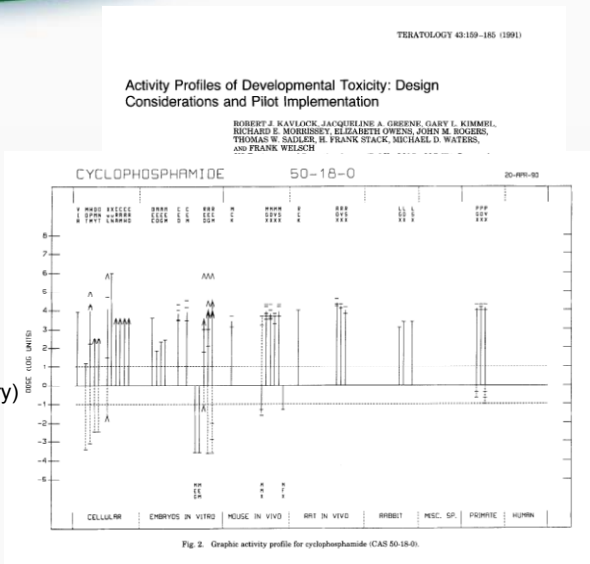
So Haven't We Been Here Before???

- Post Ames test era push
  - "Smith List" of teratogens
  - Chernoff/Kavlock assay
  - In vitro assays
    - HEPM (Pratt)
    - MOT (Braun)
    - µMass (Flint)
    - CRA (Daston)
    - Hydra (Johnson)
    - Drosophila(Bournias-Vardiabasis)
    - Neuroblastoma (Mummery)
    - FETAX (Sabourin)
    - WEC (New)
    - …….
    - …….


TERATOLOGY 43:159–185 (1991)

Activity Profiles of Developmental Toxicity: Design Considerations and Pilot Implementation

ROBERT J. KAVLOCK, JACQUELINE A. GREENE, GARY L. KIMMEL, RICHARD E. MORRISSEY, ELIZABETH OWENS, JOHN M. ROGERS, THOMAS W. SADLER, H. FRANK STACK, MICHAEL D. WATERS, AND FRANK WELSCH

CYCLOPHOSPHAMIDE 50-18-0

CELLULAR | EMBRYOS IN VITRO | MOUSE IN VIVO | RAT IN VIVO | RABBIT | MISC. SP. | PRIMATE | HUMAN

Fig. 2. Graphic activity profile for cyclophosphamide (CAS 50-18-0).

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So What's Different This Time?

- Molecular, Cellular and Systems Biology
- High Throughput Screening
- Information Technology and Management
- Major Government Investments

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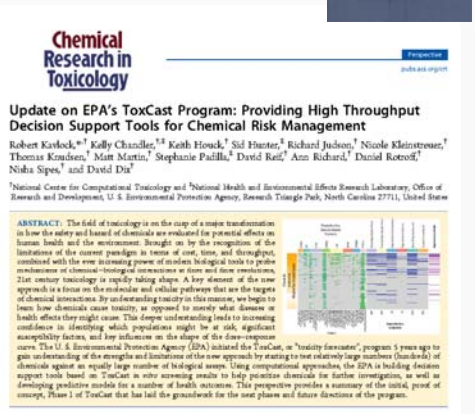
CompTox Approach

- Identify targets or pathways linked to toxicity
  - Chemicals perturbing these can lead to adverse events
- Obtain assays for these targets or pathways
  - Assays probe "Molecular Initiating Events" or "Key Events"
- Screen large numbers of chemicals, starting with those we have a lot of toxicological information on
- Develop predictive models: *in vitro → in vivo*
  - "Toxicity Signature"
- Use signatures:
  - Prioritize chemicals for targeted testing ("Too Many Chemicals" problem)
  - Suggest / distinguish possible AOP / MOA for chemicals



Chemical Research in Toxicology

Perspective

Update on EPA's ToxCast Program: Providing High Throughput Decision Support Tools for Chemical Risk Management

Robert Kavlock,*[,†] Kelly Chandler,[†,§] Keith Houck,[†] Sid Hunter,[‡] Richard Judson,[†] Nicole Kleinstreuer,[†] Thomas Knudsen,[†] Matt Martin,[†] Stephanie Padilla,[‡] David Reif,[†] Ann Richard,[†] Daniel Rotroff,[†] Nisha Sipes,[†] and David Dix[†]

[†]National Center for Computational Toxicology and [‡]National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

ABSTRACT: The field of toxicology is on the cusp of a major transformation in how the safety and hazard of chemicals are evaluated for potential effects on human health and the environment. Brought on by the recognition of the limitations of the current paradigm in terms of cost, time, and throughput, combined with the ever increasing power of modern biological tools to probe mechanisms of chemical−biological interactions at more and more resolutions, 21st century toxicology is rapidly taking shape. A key element of the new approach is a focus on the molecular and cellular pathways that are the targets of chemical interactions. By understanding toxicity in this manner, we begin to learn how chemicals cause toxicity, as opposed to merely what diseases or health effects they might cause. This deeper understanding leads to increasing confidence in identifying which populations might be at risk, significant susceptibility factors, and key influences on the shape of the dose−response curve. The U.S. Environmental Protection Agency (EPA) initiated the ToxCast, or "toxicity forecaster", program 5 years ago to gain understanding of the strengths and limitations of the new approach by testing to test relatively large numbers (hundreds) of chemicals against an equally large number of biological assays. Using computational approaches, the EPA is building decision support tools based on ToxCast in vitro screening results to help prioritize chemicals for further investigation, as well as developing predictive models for a number of health outcomes. This perspective provides a summary of the initial, proof of concept, Phase 1 of ToxCast that has laid the groundwork for the next phases and future directions of the program.

Kavlock et al, Chemical Research in Toxicology (2012)

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Intergovernmental Innovation

MEMORANDUM OF UNDERSTANDING

ON

High Throughput Screening, Toxicity Pathway Profiling, and Biological Interpretation of Findings

XI. APPROVAL

National Toxicology Program

Linda S. Birnbaum, Ph.D., DABT, ATS — Date: 5.25.10
Director
National Institute of Environmental Health Sciences
National Institutes of Health

NIH Chemical Genomics Center

Eric D. Green, M.D., Ph.D. — Date: 6/3/10
Director
National Human Genome Research Institute
National Institutes of Health

U.S. Environmental Protection Agency

Paul T. Anastas, Ph.D. — Date: 4 June 2010
Assistant Administrator
Office of Research and Development

Food and Drug Administration

Janet Woodcock, MD — Date: 5/24/10
Director
Center for Drug Evaluation and Research

Reactions We Get

- Biology is too complicated to addressed by this reductionist approach
- You will miss toxicities expressed due to emergent properties of cells and tissues
- You don't have feedback loops that could afford resiliency
- We will never know all the toxicity pathways, so this is doomed to failure
- Your approach does not have liver
- Assay (x) in your battery did not get the right answer for my chemical
- My assay disagrees with assay (x), so your approach is flawed
- You can't test my chemical because of your limitations
- Everything is going to get tagged hazardous because of a positive in vitro response
- You don't consider dose-response
- How we can we be sure about protectiveness for human health
- Finally someone is tackling the problem, let's give them a chance

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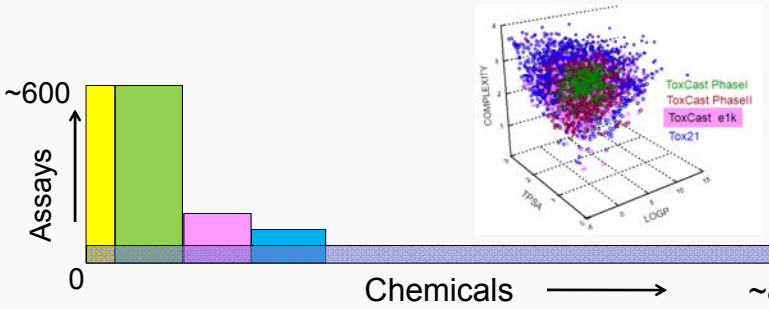
Testing under ToxCast and Tox21

Set	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~1100	2011	Now
ToxCast Phase II	767	~600	~1100	03/2013	09/2013
ToxCast Phase IIIa	1001	~100	~100	Just starting	2014
E1K (endocrine)	880	~50	~120	03/2013	09/2013
Tox21	8,193	~25	~50	Ongoing	Ongoing



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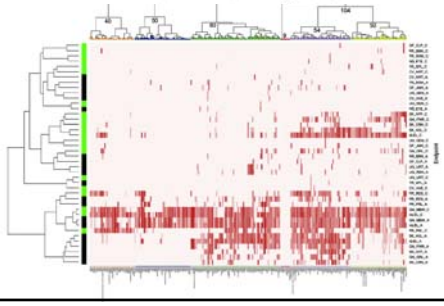
Reproductive Toxicology 28 (2009) 209–219
Contents lists available at ScienceDirect
Reproductive Toxicology
journal homepage: www.elsevier.com/locate/reprotox
ELSEVIER

Profiling the activity of environmental chemicals in prenatal developmental toxicity studies using the U.S. EPA's ToxRefDB☆

Thomas B. Knudsen[a,*], Matthew T. Martin[a], Robert J. Kavlock[a], Richard S. Judson[a], David J. Dix[a], Amar V. Singh[b]

[a] National Center for Computational Toxicology (NCCT), Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, United States
[b] Lockheed Martin, contractor to NCCT, Research Triangle Park, NC, United States



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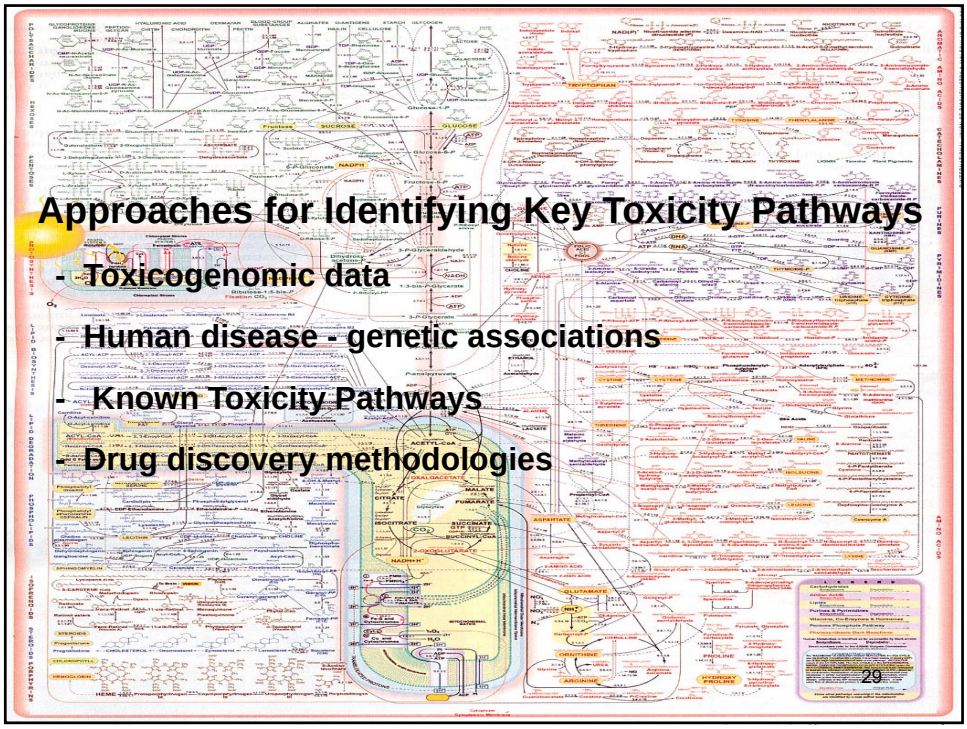

ToxCast HTS Assays: >1100 readouts / effects

Assay Provider
ACEA
Apredica
Attagene
BioSeek
CellzDirect
NCGC/Tox21
NHEERL MESC
NHEERL NeuroTox
NHEERL Zebrafish
NovaScreen
Odyssey Thera

Biological Response
cell proliferation and death
cell differentiation
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor activity
receptor binding

Target Family
Response Element
Transporter
Cytokines
Kinases
Nuclear Receptor
CYP450 / ADME
Cholinesterase
Phosphatases
Proteases
XME metabolism
GPCRs
Ion Channels

Assay Design
viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Readout Type
Single
Multiplexed
Multiparametric

Cell Format
Cell free
Cell lines
Primary cells
Complex cultures
Free-living embryos

Species
Human
Rat
Mouse
Zebrafish
Sheep
Boar
Rabbit
Cattle
Guinea pig

Tissue Source
Lung, Breast
Liver, Vascular
Skin, Kidney
Cervix, Testis
Uterus, Brain
Intestinal, Spleen
Bladder, Ovary
Pancreas, Prostate
Inflammatory, Bone

Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrayscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

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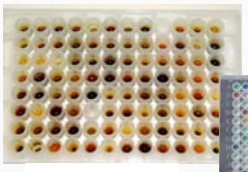
Screening Throughput

c

r

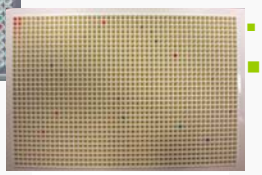


- 8 rows x 12 columns
- 88 test samples

96-well plate

- 16 rows x 32 columns
- 352 test samples

384-well plate
4 x 96-well plates



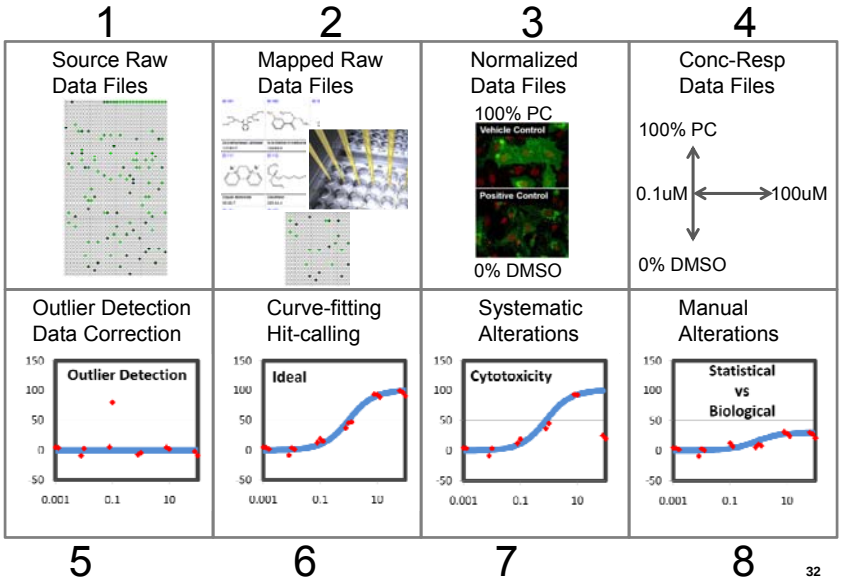
- 32 rows x 48 columns
- 1,408 test samples

1536-well plate
16 x 96-well plates

If @ 100 microtiter plates per day:

Plate format	samples§/day (wells/day)	Time to screen 1 M samples
96-well	8,800 (9,600)	4 months
384-well	35,200 (38,400)	4 weeks
1536-well	**140,800** (153,600)	**7 days**

ToxCast Data Analysis Pipeline



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Analysis of Eight Oil Spill Dispersants Using Rapid, In Vitro Tests for Endocrine and Other Biological Activity

RICHARD S. JUDSON,*,† MATTHEW T. MARTIN,† DAVID M. REIF,† KEITH A. HOUCK,† THOMAS B. KNUDSEN,† DANIEL M. ROTROFF,† MENGHANG XIA,‡ SRILATHA SAKAMURU,‡ RUILI HUANG,‡ PAUL SHINN,‡ CHRISTOPHER P. AUSTIN,‡ ROBERT J. KAVLOCK,† AND DAVID J. DIX†

National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, and NIH Chemical Genomics Center, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland 20892

Received June 25, 2010. Revised manuscript received June 30, 2010. Accepted June 30, 2010.

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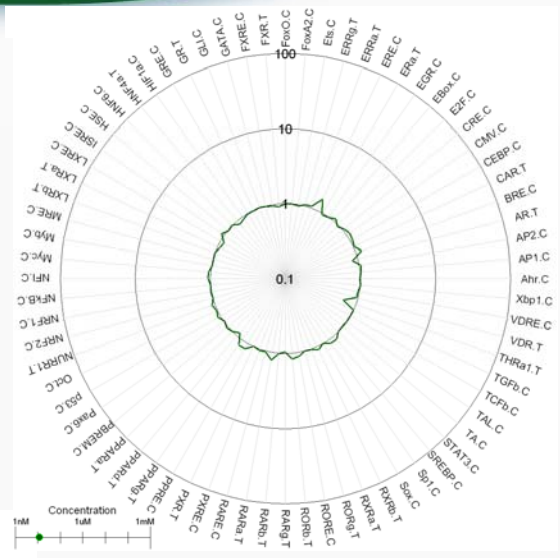
Effect of Concentration Response on Polypharmacology



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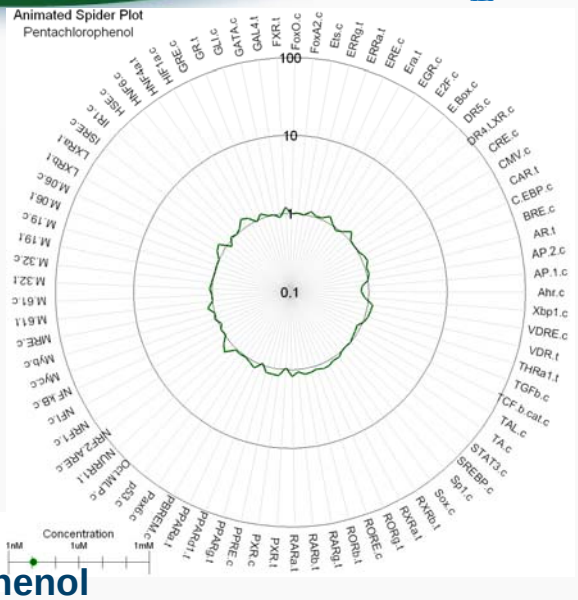
Pharma_X

Effect of Concentration Response on Polypharmacology



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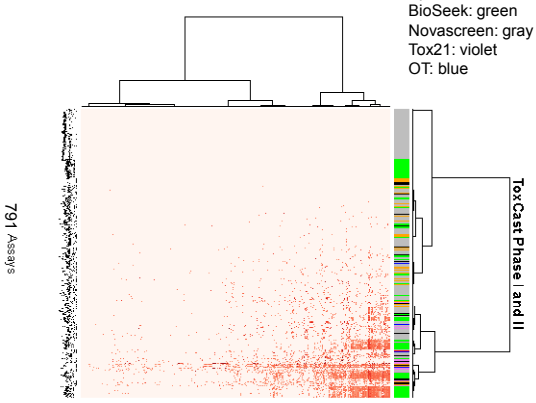
Pentachlorophenol

Circa 2013: 831,341 AC50s

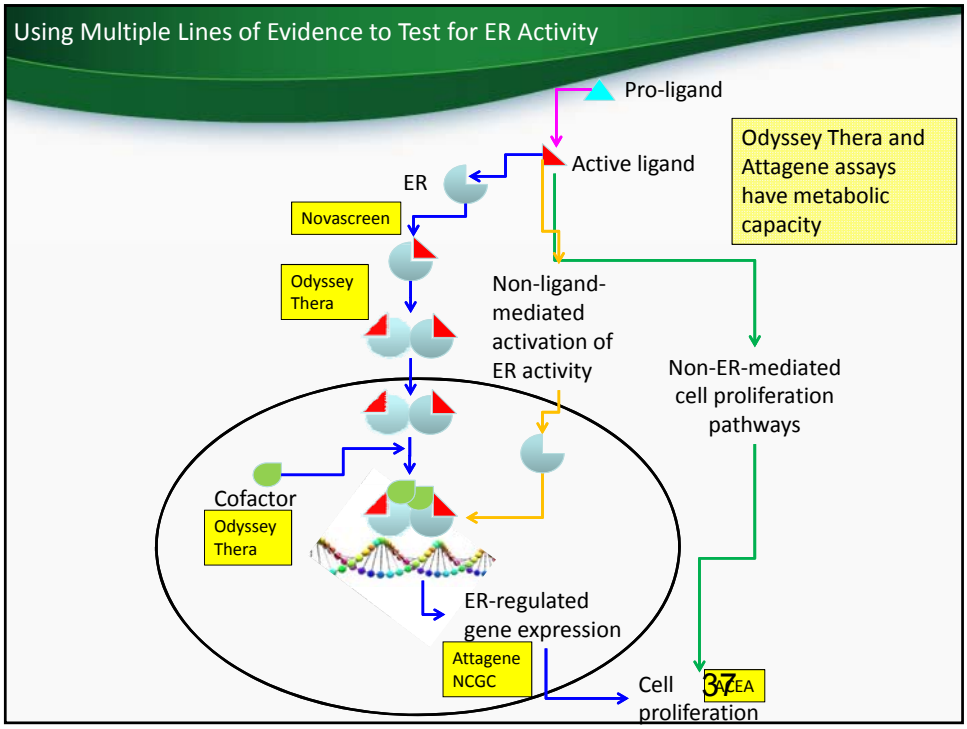
February 20, 2010

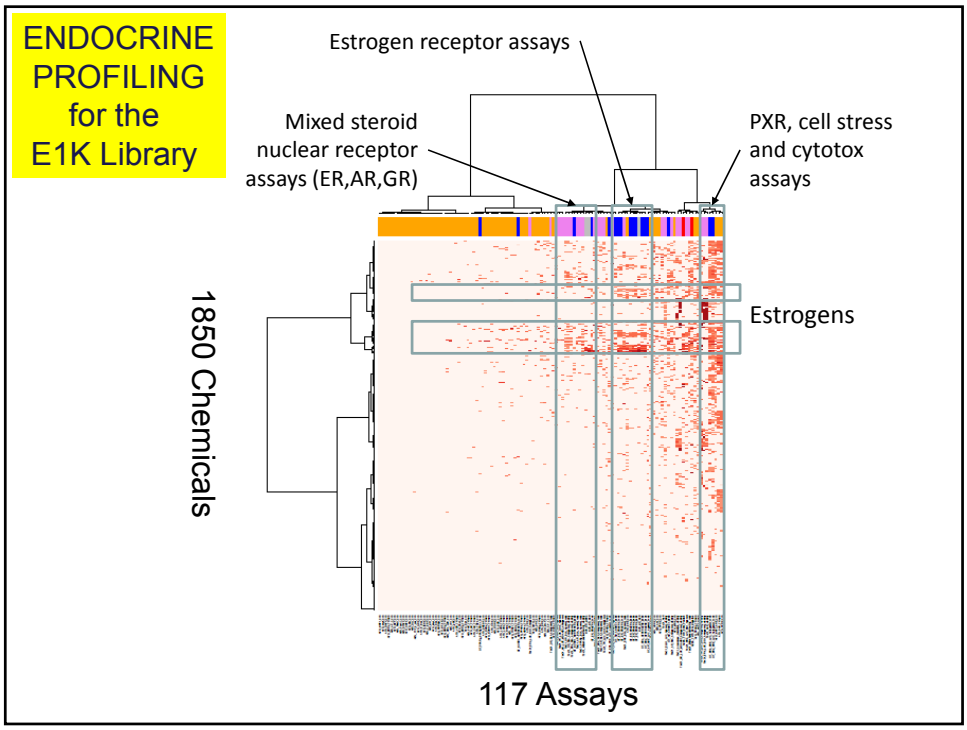
June 19, 2013

ACEA: red
Attagene: orange
Apredica: black
BioSeek: green
Novascreen: gray
Tox21: violet
OT: blue



1051 Chemicals


Using Multiple Lines of Evidence to Test for ER Activity
Pro-ligand
Active ligand
Odyssey Thera and Attagene assays have metabolic capacity
ER
Novascreen
Odyssey Thera
Non-ligand-mediated activation of ER activity
Non-ER-mediated cell proliferation pathways
Cofactor
Odyssey Thera
ER-regulated gene expression
Attagene NCGC
Cell proliferation
CEA


ENDOCRINE PROFILING for the E1K Library
Estrogen receptor assays
Mixed steroid nuclear receptor assays (ER,AR,GR)
PXR, cell stress and cytotox assays
Estrogens
1850 Chemicals
117 Assays

Predictive Toxicity Models

- **Endpoints**
  - Liver tumors: Judson et al. 2010, Env Hlth Persp 118: 485-492
  - Hepatocarcinogenesis: Shah et al. 2011, PLoS One 6(2): e14584
  - Rat fertility: Martin et al. 2011, Biol Reprod 85: 327-339
  - Rat-rabbit prenatal devtox: Sipes et al. 2011, Toxicol Sci 124: 109-127
  - Zebrafish development: Sipes et al. 2011, Birth Defects Res C 93: 256-267
- **Pathways**
  - Endocrine disruption: Reif et al. 2010, Env Hlth Persp 118: 1714-1720
  - Microdosimetry: Wambaugh and Shah 2010, PLoS Comp Biol 6: e1000756
  - mESC differentiation: Chandler et al. 2011, PLoS One 6(6): e18540
  - HTP risk assessment: Judson et al. 2011, Chem Res Toxicol 24: 451-462
  - Angiogenesis: Kleinstreuer et al. 2011, Env Hlth Persp 119: 1596-1603
  - Cancer Hallmarks: Kleinstreuer et al. 2012, Toxicol Sci, 131:40-55
  - Endocrine activity: Rotroff et al. 2013, Env Hlth Persp 121:7-14

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Critical Tox21 Issues

- Cells don't get disease
- Not all compounds can be screened in HTS
- Incorporation of metabolic capabilities
- Interactions between different cell types
- Range of human variability
- Extrapolation from acute to chronic exposure conditions
- Interpretation of effective *in vitro* concentrations

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Validation/Qualification

- Definition
  - A process to determine the relevance, reliability and fitness for purpose of a test
- Relevance
  - Assay must test an aspect of biology that will help assess the safety of a chemical. A positive result in the assay should be indicative of perturbations to or interactions with the target or pathway the assay is designed to test. (Evaluate with reference compounds)
- Reliability
  - Assay must produce similar results over time, across reagent batches, etc. (Evaluate with reference compounds)
- Fitness for Purpose
  - For prioritization application, an HTS assay should provide sufficient positive and negative predictive power so that the prioritized chemicals are significantly enriched in positives when run in the guideline test.

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Some Current Activities

•The Hamner Institute efforts in pathway modeling

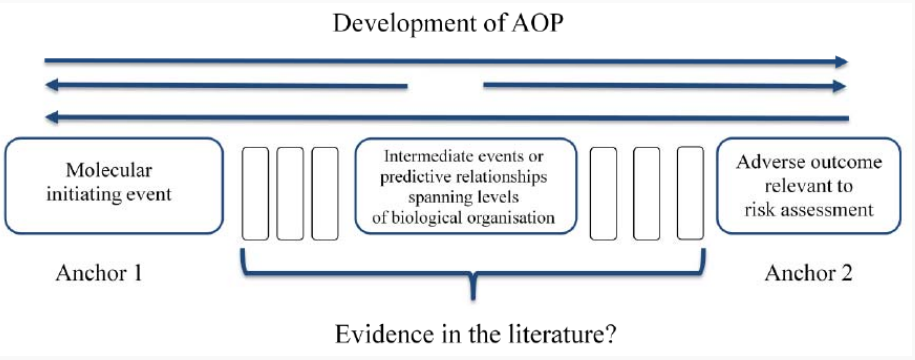
• The Johns Hopkins Humane Toxome project

• DARPA/NIH/FDA microphysiological systems projects
  - •Wyss Institute and MIT, $35m each
  - •Ten human organs on a chip within 5 years

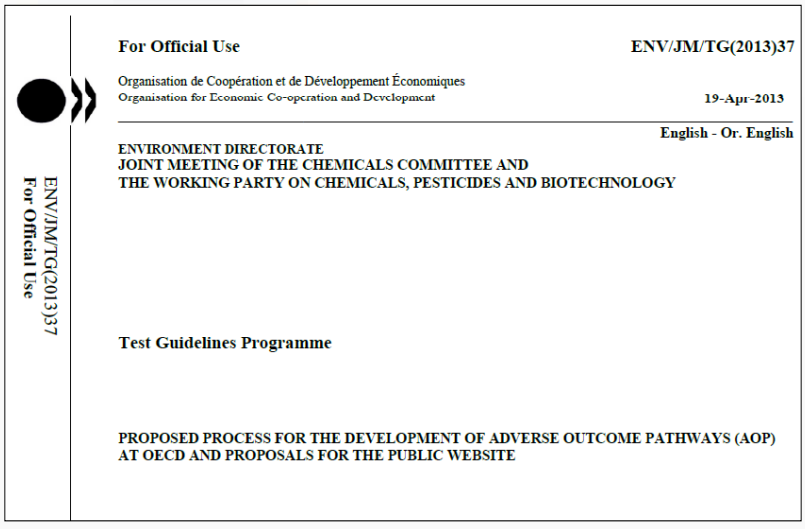
• EU Funded Projects
  - •ReproTect, AXLR8, eTOX, SEURAT, HeCaTos

• OECD Adverse Outcome Pathway codification

• ToxCast Data Summit, May 2014



OECD Template and Guidance on developing and assessing the completeness of Adverse Outcome Pathways



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Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

19-Apr-2013

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

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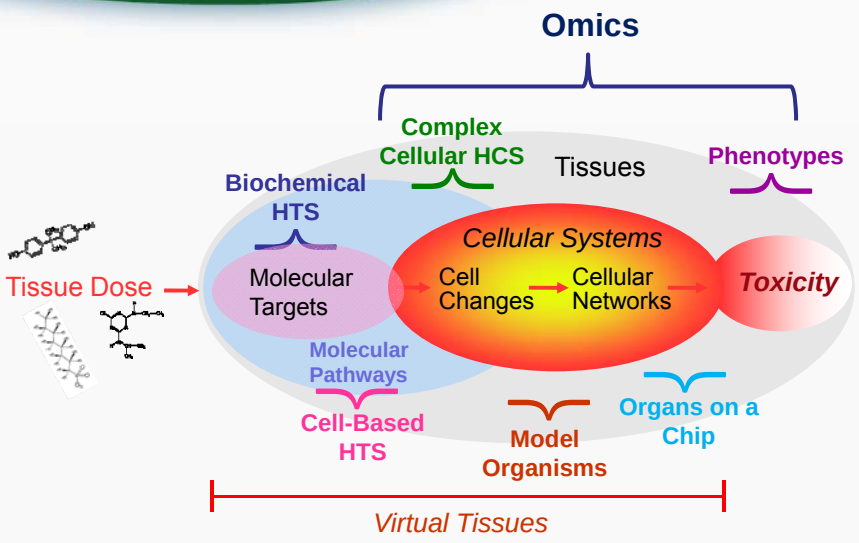
Test Guidelines Programme

PROPOSED PROCESS FOR THE DEVELOPMENT OF ADVERSE OUTCOME PATHWAYS (AOP) AT OECD AND PROPOSALS FOR THE PUBLIC WEBSITE

Future needs

- More chemicals, more pathways, more informatics
  - IVIVE, metabolic competency
- International coordination, data sharing and transparency
  - EU (REACH, AXLR8, Seurat, IMI (eTOX))
  - TSCA, Canadian DSL, Australian NICNAS
- Tools of high throughput exposure estimates
  - More use based than volume based
- Computational systems models for emergent properties
- Fit for purpose acceptance
  - Translation into Applications
    - Prioritization
    - Animal Refinement (Integrated Test Strategies)
    - High throughput risk assessment methodologies
    - National Emergencies

Predicting Human Toxicity: A Grand Challenge



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Related Presentations at this Meeting:

Chandler et al, Stem Cells, #7, Sunday 10:30am

AOP Symposium, Sunday 3-6:30pm

Sipes et al, Computational Embryology, #26 Tuesday 3:15pm

Kleinstreuer et al, Genetic Models, #28, Tuesday 4pm

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Thanks for Listening

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