EPA United States Environmental Protection Agency

Genomic Dose Response: The BIG Picture

NTP Genomic Dose Response Modeling Expert Panel Meeting
October 23, 2017

Russell Thomas
Director
National Center for Computational Toxicology

Scott May Want To Rethink Asking Me To Be The “Big Picture Guy”…

It is a Well Known Fact that Toxicology Continues to Have a Data Problem

Toxicity Testing

Strategies to Determine Needs and Priorities

Steering Committee on Identification of Toxic and Potentially Toxic Chemicals for Consideration by the National Toxicology Program

Board on Toxicology and Environmental Health Hazards

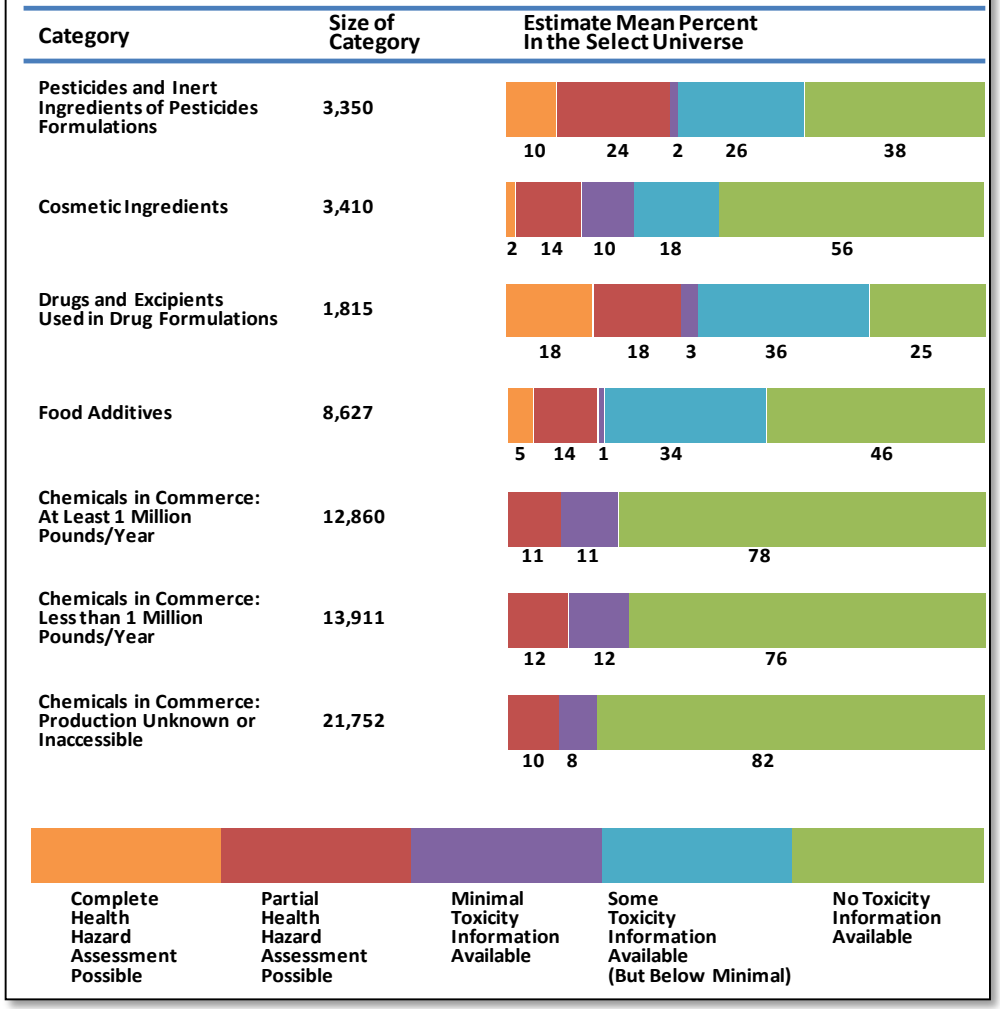
Commission on Life Sciences

National Research Council

- Major challenge is too many chemicals and not enough data
- Total # chemicals = 65,725
- Chemicals with no toxicity data of any kind = ~46,000

NATIONAL ACADEMY PRESS
Washington, D.C. 1984

US National Research Council, 1984



It is a Well Known Fact that Toxicology Continues to Have a Data Problem

The Toxicity Data Landscape for Environmental Chemicals

Richard Judson,[1] Ann Richard,[1] David J. Dix,[1] Keith Houck,[1] Matthew Martin,[1] Robert Kavlock,[1] Vicki Dellarco,[2] Tala Henry,[3] Todd Holderman,[3] Philip Sayre,[3] Shirlee Tan,[4] Thomas Carpenter,[5] and Edwin Smith[6]

[1]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [2]Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency, Arlington, Virginia, USA; [3]Office of Pollution Prevention and Toxics and [4]Office of Science Coordination and Policy, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, USA; [5]Office of Water, Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency, Washington, DC, USA; [6]Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, Illinois, USA

OBJECTIVE: Thousands of chemicals are in common use, but only a portion of them have undergone significant toxicologic evaluation, leading to the need to prioritize the remainder for targeted testing. To address this issue, the U.S. Environmental Protection Agency (EPA) and other organizations are developing chemical screening and prioritization programs. As part of these efforts, it is important to catalog, from widely dispersed sources, the toxicology information that is available. The main objective of this analysis is to define a list of environmental chemicals that are candidates for the U.S. EPA screening and prioritization process, and to catalog the available toxicology information.

DATA SOURCES: We are developing ACToR (Aggregated Computational Toxicology Resource), which combines information for hundreds of thousands of chemicals from > 200 public sources, including the U.S. EPA, National Institutes of Health, Food and Drug Administration, corresponding agencies in Canada, Europe, and Japan, and academic sources.

DATA EXTRACTION: ACToR contains chemical structure information; physical–chemical properties; *in vitro* assay data; tabular *in vivo* data; summary toxicology calls (e.g., a statement that a chemical is considered to be a human carcinogen); and links to online toxicology summaries. Here, we use data from ACToR to assess the toxicity data landscape for environmental chemicals.

DATA SYNTHESIS: We show results for a set of 9,912 environmental chemicals being considered for analysis as part of the U.S. EPA ToxCast screening and prioritization program. These include high- and medium-production-volume chemicals, pesticide active and inert ingredients, and drinking water contaminants.

CONCLUSIONS: Approximately two-thirds of these chemicals have at least limited toxicity summaries available. About one-quarter have been assessed in at least one highly curated toxicology evaluation database such as the U.S. EPA Toxicology Reference Database, U.S. EPA Integrated Risk Information System, and the National Toxicology Program.

KEY WORDS: ACToR, carcinogenicity, database, developmental, hazard, HPV, MPV, pesticide, reproductive, toxicity. *Environ Health Perspect* 117:685–695 (2009). doi:10.1289/ehp.0800168 available via *http://dx.doi.org/* [Online 22 December 2008]

The U.S. Environmental Protection Agency (EPA) has a significant interest in developing more efficient and informative toxicity determination approaches in part because of the large number of chemicals under its jurisdiction. Ultimately, it would be beneficial to characterize the toxicologic profiles of all chemicals in use in the United States. However, the size of this chemical universe [in excess of 75,000 chemicals, which is the estimated number in the Toxic Substances Control Act (TSCA 1976) inventory (U.S. EPA 2004b)] makes this goal too difficult using current approaches to toxicity characterization that rely on extensive animal testing, cost millions of dollars, and can take 2–3 years per chemical. The International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) recently released several reports describing a more focused, tier-based approach for toxicity testing of agricultural chemicals, which would ultimately lead to the use of fewer animals (Burton et al. 2006; Carmichael et al. 2006). The National Research Council (NRC) recently released a report titled *Toxicity Testing in the 21st Century: A Vision and a Strategy* that outlines a much more ambitious and long-term vision for developing novel *in vitro* approaches to chemical toxicity characterization and prediction (NRC 2007) that would largely eliminate animal testing. The NRC report addresses several concerns about the current testing methods, specifically, the desire *a*) to reduce the number of animals used in testing, *b*) to reduce the overall cost and time required to characterize each chemical, and *c*) to increase the level of mechanistic understanding of chemical toxicity. The U.S. EPA and the National Institutes of Health (NIH) are actively pursuing approaches to implement ideas outlined in the NRC report (Collins et al. 2008).

Regardless of the level of quality of toxicology data on environmental chemicals, many chemicals lack significant amounts of data. In the United States and Canada, an estimated 30,000 chemicals are in wide commercial use, based on U.S. EPA and Environment Canada data (Muir and Howard 2006). The European Union's Registration, Evaluation, and Authorization of Chemicals (REACH) program has recently released its first set of registered substances, which contains > 140,000 entries (REACH 2008). The exact number of chemicals in use is, in a sense, unknowable because it depends on where one sets the threshold of use and because use changes over time. The major point is that the number is relatively large and that only a relatively small subset of these chemicals have been sufficiently well characterized for their potential to cause human or ecologic toxicity to support regulatory action. This "data gap" is well documented (Allanou et al. 1999; Applegate and Baer 2006; Birnbaum et al. 2003; Guth et al. 2005; NRC 2007; U.S. EPA 1998).

The high cost and lengthy times associated with the use of animal testing to determine a chemical's potential for toxicity make this strategy impractical for evaluating tens of thousands of chemicals, hence the large inventories of existing chemicals for which few or no test data are available. An alternative approach is to attempt to assess much larger numbers of chemicals by employing more efficient *in vitro* methods. One strategy applies a broad spectrum of relatively inexpensive and rapid high-throughput screening

Address correspondence to R. Judson, U.S. Environmental Protection Agency, 109 T.W. Alexander Dr. (B205-01), Research Triangle Park, NC 27711 USA. Telephone: (919) 541-3085. Fax: (919) 541-1194. E-mail: judson.richard@epa.gov

We acknowledge significant contributions from members of the U.S. EPA Aggregated Computational Toxicology Resource (ACToR) development team: T. Cathey, T. Transue, and R. Spencer of Lockheed Martin, and F. Ellouraí, D. Smith, J. Vail, and K. Daniel. We also acknowledge the significant contribution of M. Wolf (Lockheed Martin) in relation to the U.S. EPA's Distributed Structure-Searchable Toxicity Data Network structure inventory incorporated into ACToR.

This article has been reviewed by the U.S. EPA and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

The authors declare they have no competing financial interests.

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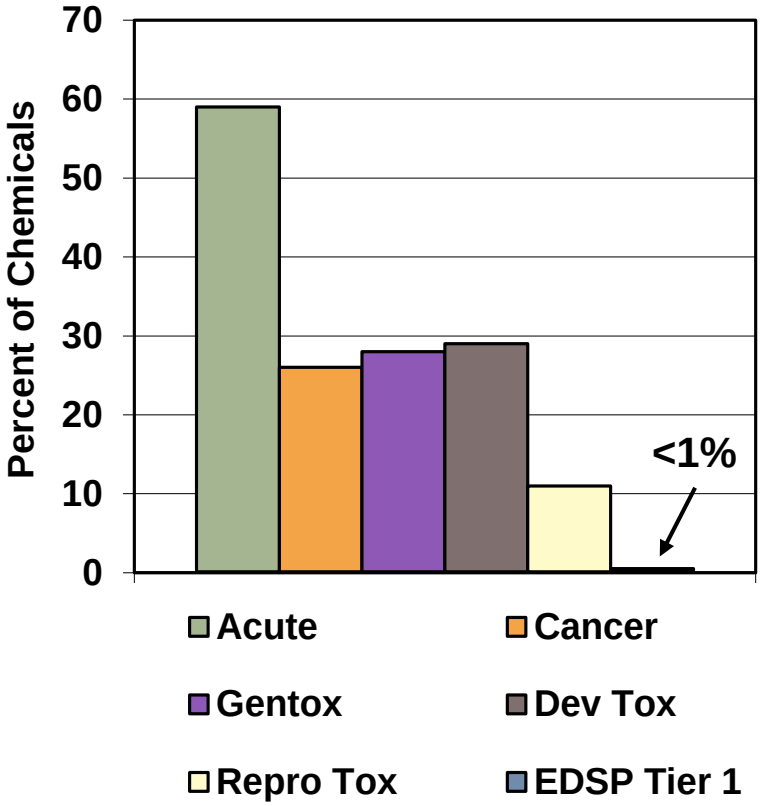


Modified from Judson *et al.*, EHP 2009

For Those With Data, Have We Been Truly Predictive or Just Protective?

…data compiled from 150 compounds with 221 human toxicity events reported. The results showed the true positive human toxicity concordance rate of 71% for rodent and nonrodent species, with nonrodents alone being predictive for 63% of human toxicity and ***rodents alone for 43%.***

Regulatory Toxicology and Pharmacology 32, 56–67 (2000)
doi:10.1006/rtph.2000.1399, available online at http://www.idealibrary.com on IDEAL®

Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals

Harry Olson,[1] Graham Betton,[2] Denise Robinson,[3] Karluss Thomas,[3] Alastair Monro,[1] Gerald Kolaja,[4] Patrick Lilly,[5] James Sanders,[6] Glenn Sipes,[7] William Bracken,[8] Michael Dorato,[9] Koen Van Deun,[10] Peter Smith,[11] Bruce Berger,[12] and Allen Heller[13]

[1]*Pfizer Inc., Groton, Connecticut;* [2]*AstraZeneca Pharmaceuticals, Macclesfield, England;* [3]*ILSI-HESI, Washington, DC, 20036;* [4]*Pharmacia & UpJohn, Kalamazoo, Michigan;* [5]*Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut;* [6]*Rhone-Poulenc Rorer, Collegeville, Pennsylvania;* [7]*University of Arizona, Tucson, Arizona;* [8]*Abbott Laboratories, Abbott Park, Illinois;* [9]*Eli Lilly and Co., Greenfield, Indiana;* [10]*Janssen Research Foundation, Beerse, Belgium;* [11]*Monsanto-Searle Laboratories, Skokie, Illinois;* [12]*Sanofi-Synthelabo, Inc., Malvern, Pennsylvania; and* [13]*Bayer Corporation, West Haven, Connecticut*

Received January 22, 2000

Table 2-2. Uncertainty/safety factors for various reference values

Reference value	UF[a]				FQPA[b]
	U_A	U_H	U_L	U_D	
ARE	1, 3, 10	1, 3, 10	1, 3, 10	ND	NA
AEGL	1, 3, 10	1, 3, 10	3[c]	ND[d]	NA
OPP acute and intermediate RfDs	10	10	3, 10	ND[e]	10±
OW HAs	1, 3, 10	1, 3, 10	1, 3, 10	case-specific	NA
ATSDR MRLs	1, 3, 10	1, 3, 10	1, 3, 10	ND[d]	NA

[a] Uncertainty factors: U_A = animal-to-human; U_H = within-human variability; U_L = LOAEL-to-NOAEL; U_D = database deficiency.
[b] Additional safety factor required under FQPA.
[c] Endpoint = lethality, not really a LOAEL-to-NOAEL adjustment in this case.
[d] Database deficiencies considered, and a factor may be included for intermediate RfDs if, for example, there is no reproduction and fertility study.
[e] Overlaps with the FQPA safety factor (see U.S. EPA, 2002b)

ND = not done
NA = not applicable

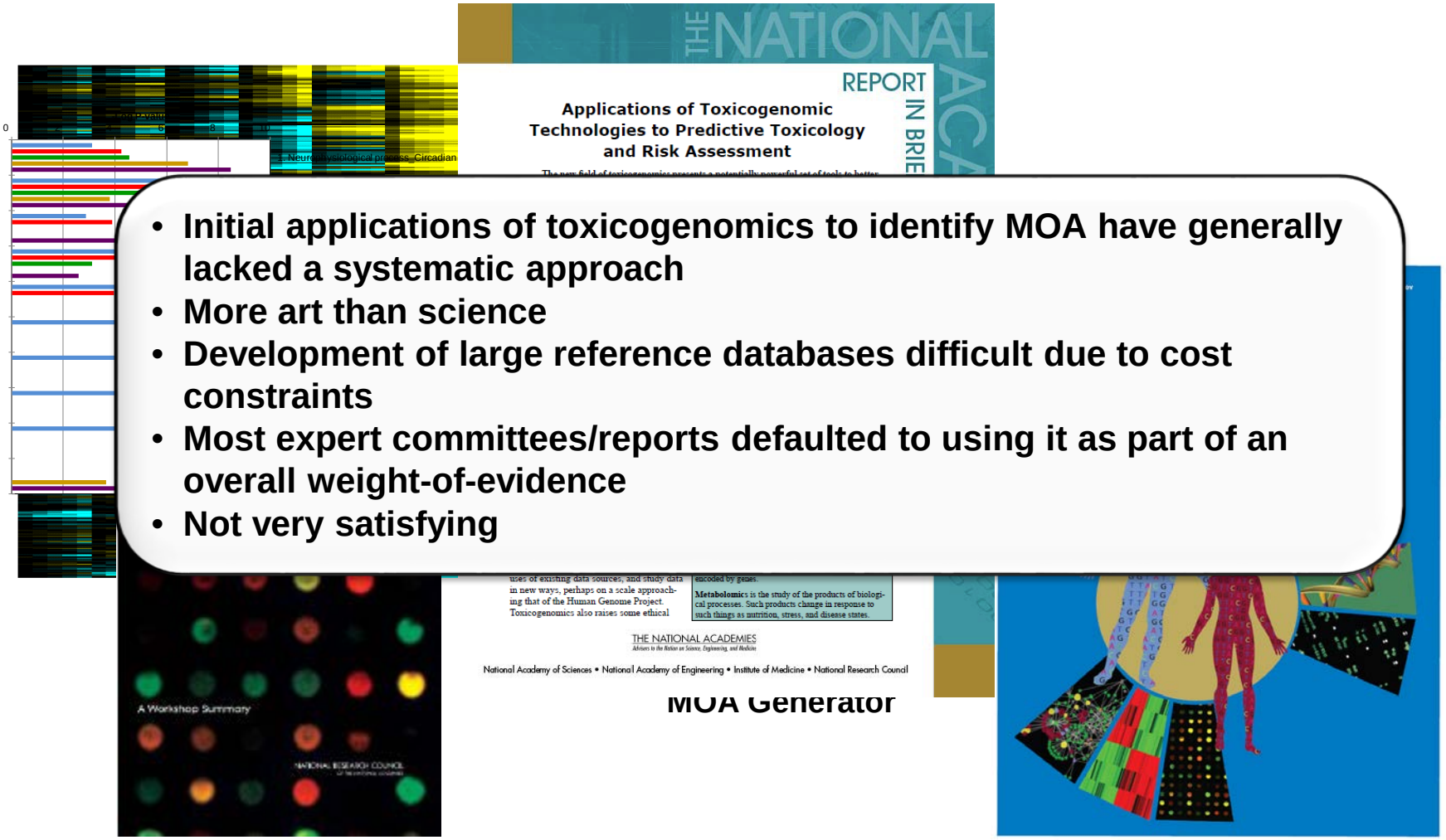
EPA/630/P-02/002F
December 2002
Final Report

A REVIEW OF THE REFERENCE DOSE AND REFERENCE CONCENTRATION PROCESSES

Prepared for the
Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC

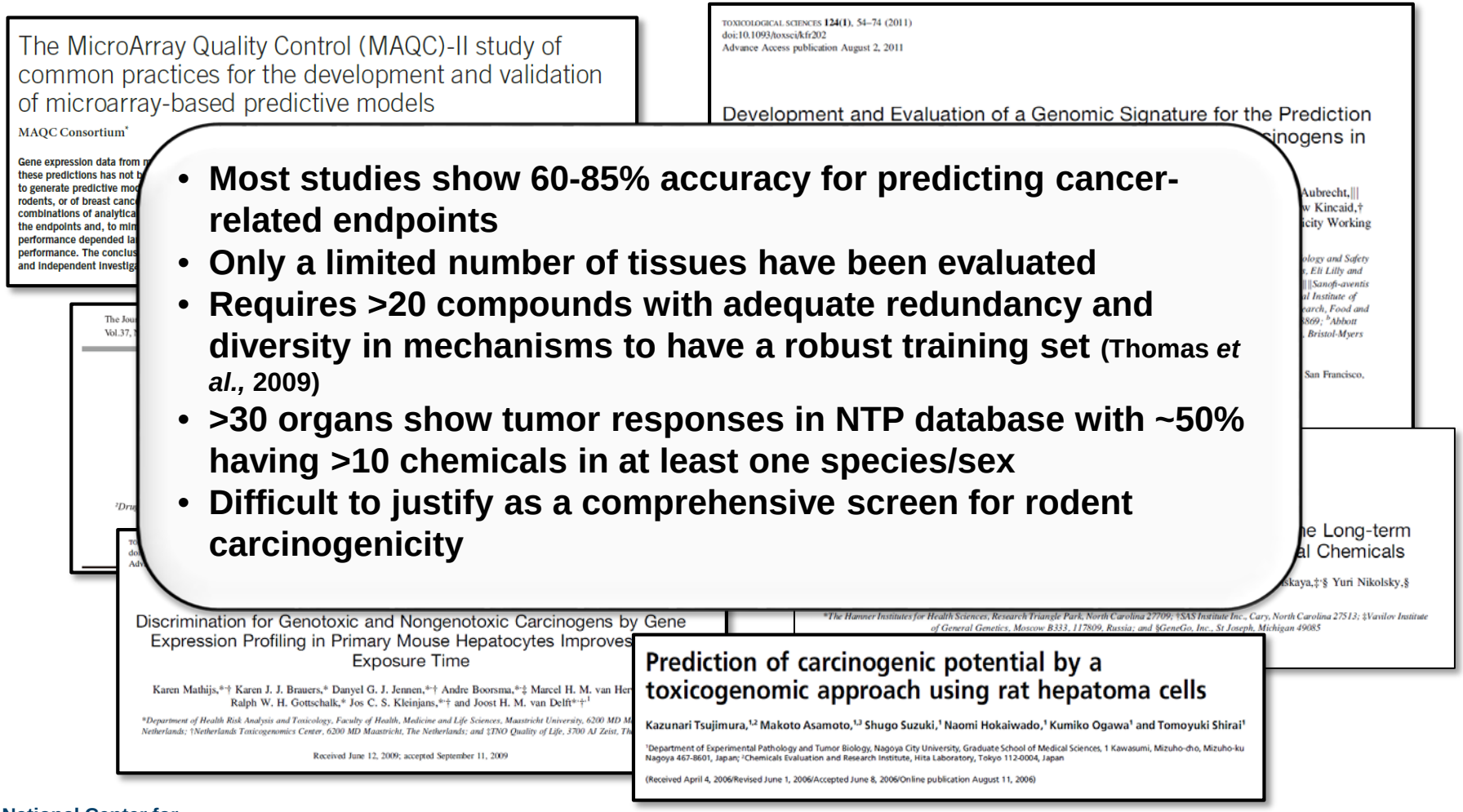
Budding Field of Toxicogenomics Promised to Change All That…

Initial Focus of Toxicogenomics Was on Inferring MOA



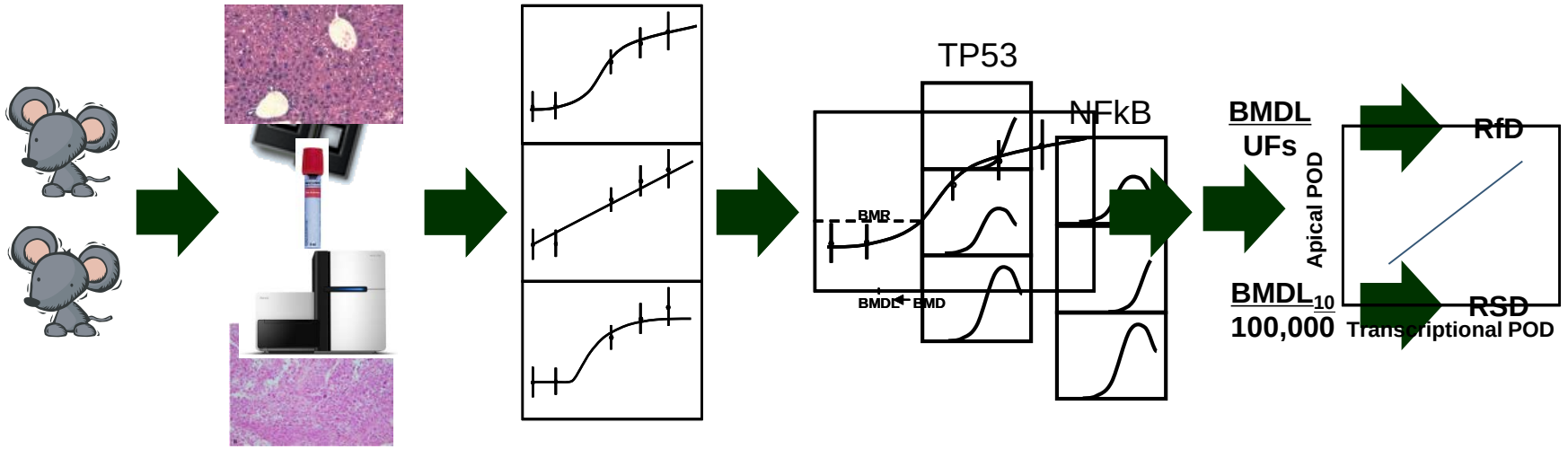
- **Initial applications of toxicogenomics to identify MOA have generally lacked a systematic approach**
- **More art than science**
- **Development of large reference databases difficult due to cost constraints**
- **Most expert committees/reports defaulted to using it as part of an overall weight-of-evidence**
- **Not very satisfying**

Focus Shifted Towards Supervised Classification Approaches



- **Most studies show 60-85% accuracy for predicting cancer-related endpoints**
- **Only a limited number of tissues have been evaluated**
- **Requires >20 compounds with adequate redundancy and diversity in mechanisms to have a robust training set (Thomas *et al.*, 2009)**
- **>30 organs show tumor responses in NTP database with ~50% having >10 chemicals in at least one species/sex**
- **Difficult to justify as a comprehensive screen for rodent carcinogenicity**

Continued Evolution Toward More Quantitative Applications



Short-term Animal Studies

Chronic Animal Studies

Transcriptional Responses

Most Sensitive Pathological Response Considered Adverse

Fit Data with Statistical Models

Identify Point of Departure (i.e., BMD)

Identify Pathways Significantly Perturbed

Summarize Pathway and Calculate Summary Value (usually the median pathway BMD)

Derive Safe Exposure Level

Compare *In Vivo* Apical and Transcriptional PODs

Thomas *et al.*, *Tox Sci.*, 2011
Thomas *et al.*, *Mut Res.*, 2012
Thomas *et al.*, *Tox Sci.*, 2013

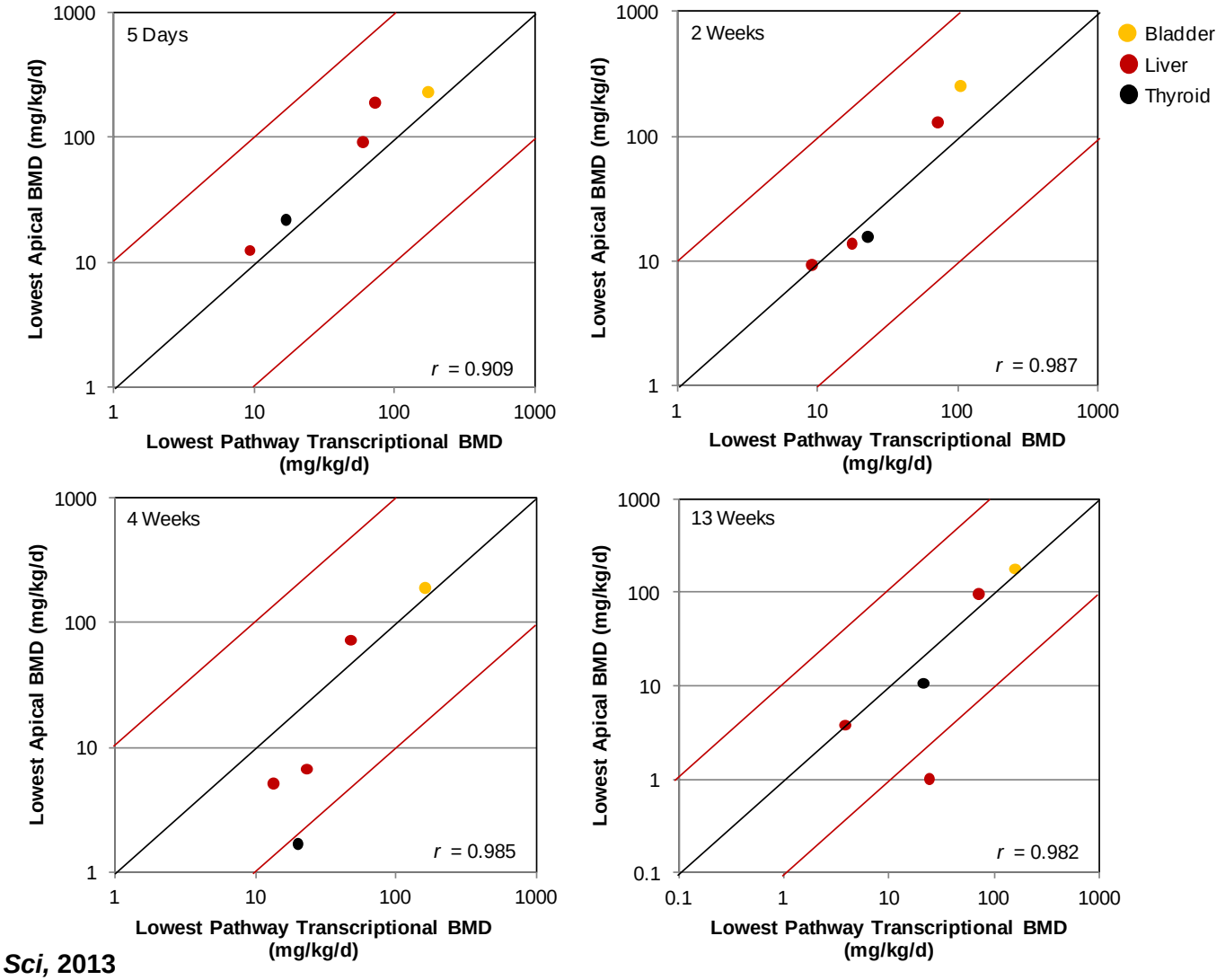
In Vivo Study to Assess Transcriptional and Apical Correlation

Chemical	Route	Doses[c]	Rodent Model	Time Point	Target Tissue
1,2,4-Tribromobenzene[a]	Gavage	2.5, 5, 10, 25, 75 mg/kg	Male Sprague Dawley rats	5 d, 2, 4, 13 wks	Liver
Bromobenzene[a]	Gavage	25, (50), 100, 200, 300, 400 mg/kg	Male F344 rats	5 d, 2, 4, 13 wks	Liver
2,3,4,6-Tetrachlorophenol[a]	Gavage	10, 25, 50, 100, 200 mg/kg	Male Sprague Dawley rats	5 d, 2, 4, 13 wks	Liver
4,4'-Methylenebis (N,N-dimethyl) benzenamine[b]	Feed	50, 200, 375, 500, 750 ppm	Male F344 rats	5 d, 2, 4, 13 wks	Thyroid[b]
N-Nitrosodiphenylamine[b]	Feed	250, 1000, 2000, 3000, 4000 ppm	Female F344 rats	5 d, 2, 4, 13 wks	Bladder[b]
Hyd					
1,4-					
Propylene glycol mono-t-butyl ether[b]	Inhalation	25, 75, 300, 800, 1200 ppm	Female B6C3F1 mice	13 wks	Liver
1,2,3-Trichloropropane[b]	Gavage	2, 6, 20, 40, 60 mg/kg	Female B6C3F1 mice	13 wks	Liver
Methylene Chloride[b]	Inhalation	100, 500, 2000, 3000, 4000 ppm	Female B6C3F1 mice	13 wks	Liver, Lung
Naphthalene[b]	Inhalation	0.5, 3, 10, 20, 30 ppm	Female B6C3F1 mice	13 wks	Lung
1,4-Dichlorobenzene[b]	Gavage	100, 300, 400, 500, 600 mg/kg	Female B6C3F1 mice	13 wks	Liver

Measured apical (histological and organ weight; n = 10) and gene expression changes (n = 5) at each dose and time point in the target tissue.

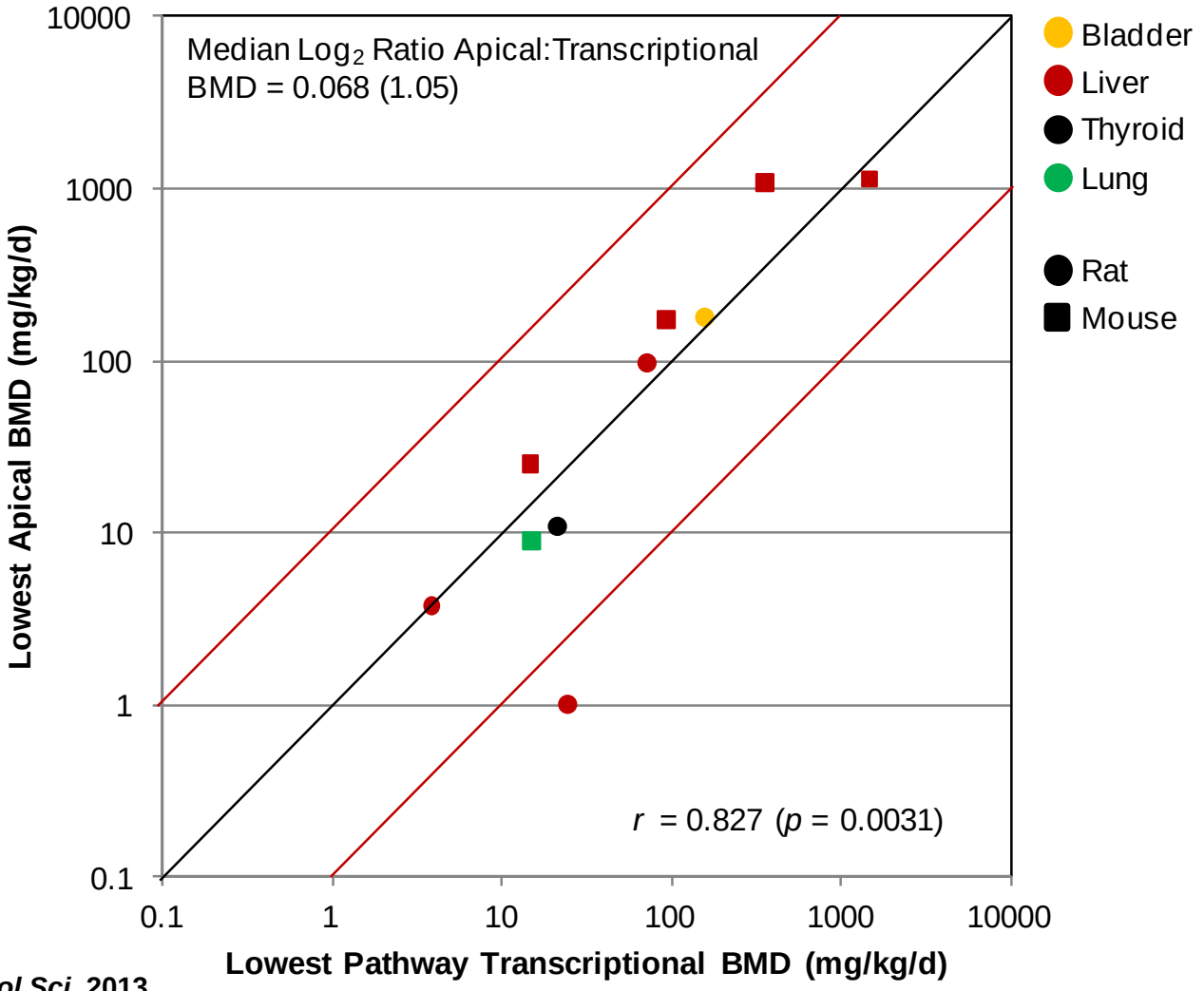
[a]Chemicals in IRIS database for non-cancer endpoints only
[b]Chemicals previously tested by the U.S. National Toxicology Program
[c]Underlined doses used in NTP two-year rodent bioassay or IRIS database

Temporal Changes Between Transcriptional and Non-Cancer PODs



Thomas *et al.*, *Toxicol Sci*, 2013

Combined Correlation Between Non-Cancer and Transcriptional PODs



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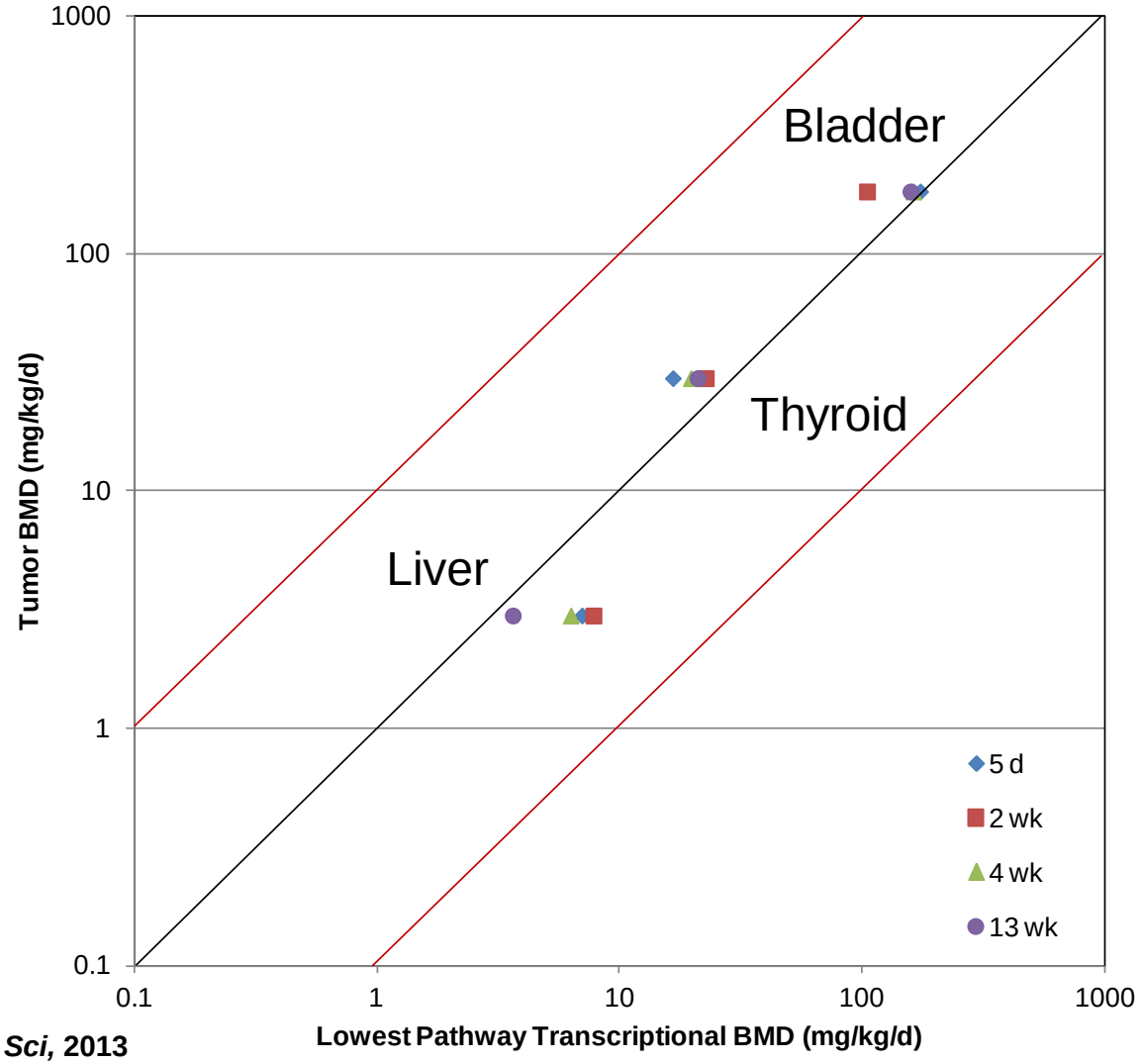
Temporal Changes Between Transcriptional and Cancer PODs



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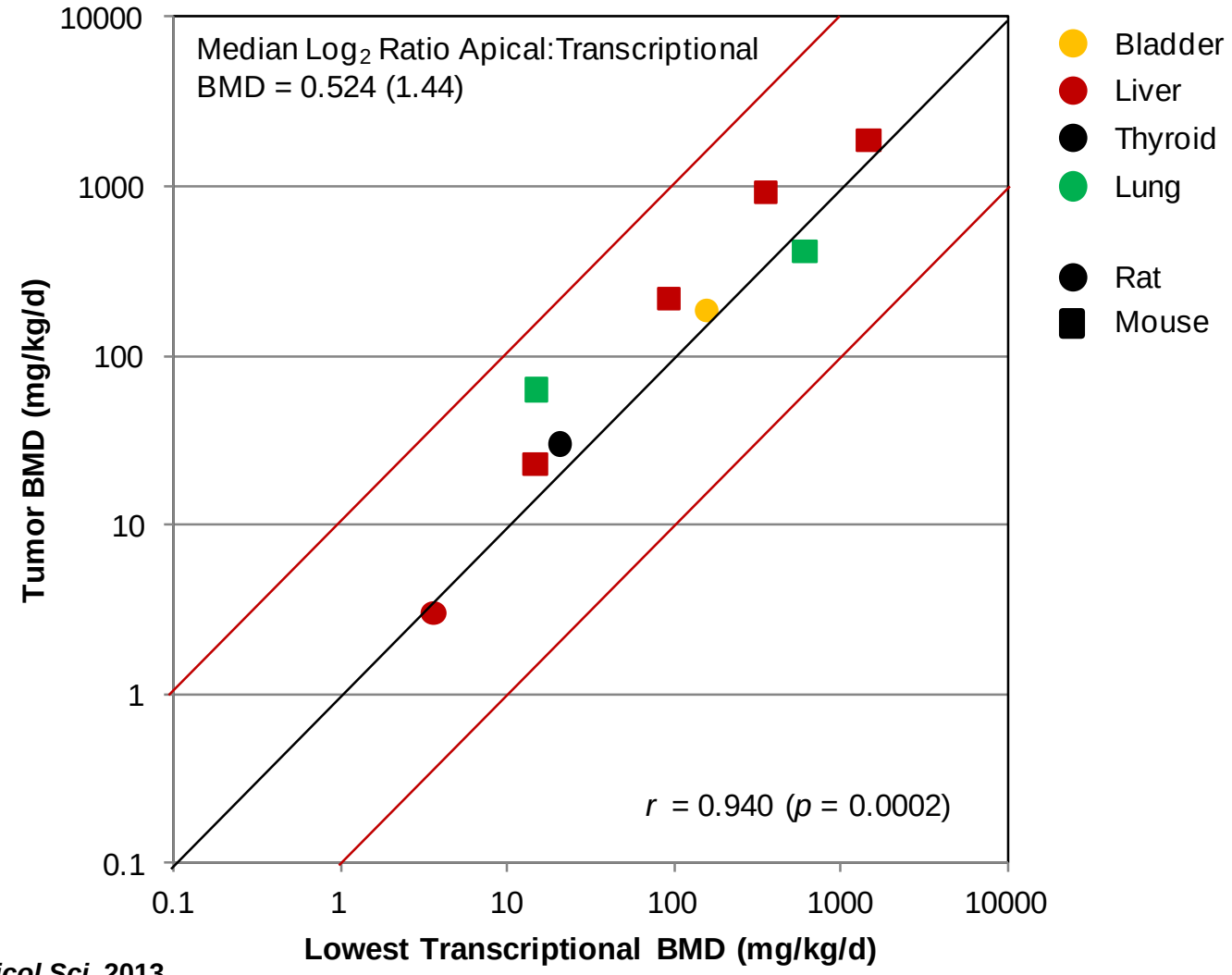
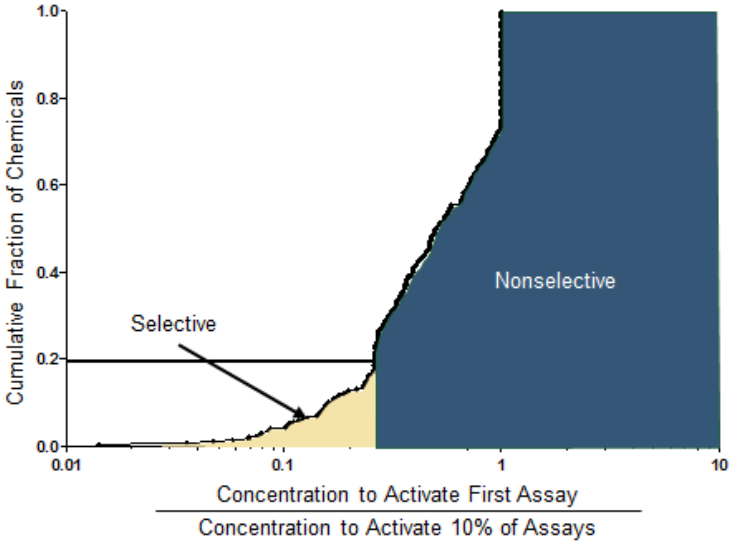


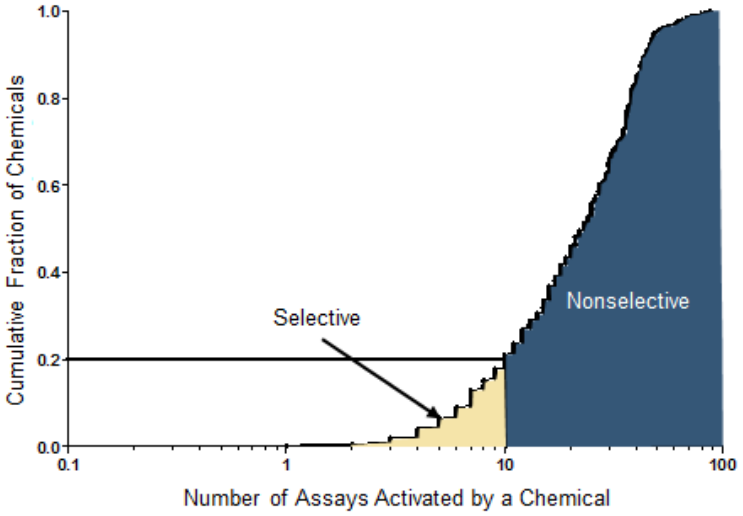
Thomas *et al., Toxicol Sci,* 2013

Why Could this Be True?

- Most histological changes do not occur without upstream or downsream changes in the transcriptome

- Most environmental chemicals are highly non-selective in their interactions with biological systems





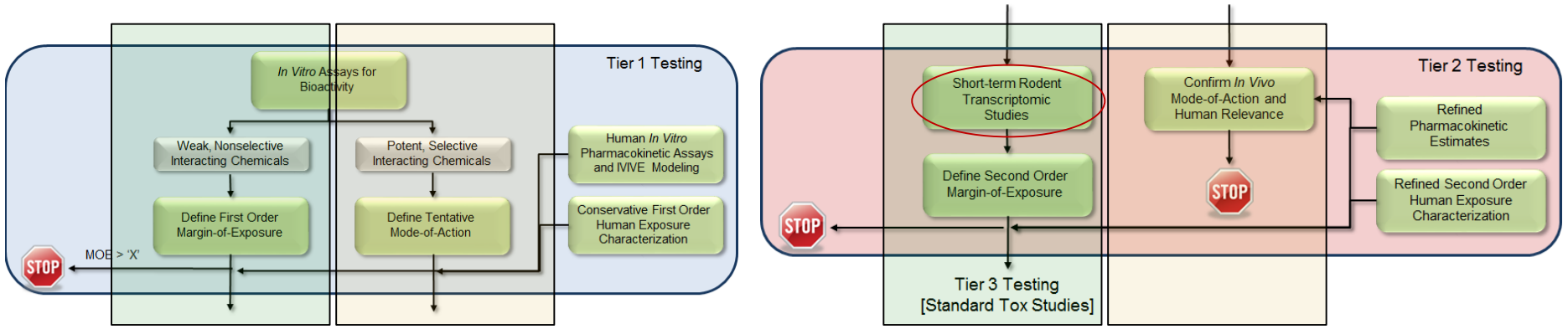
Thomas *et al., Tox Sci.*, 2013

Integration of TGX in a Tiered 21st Century Toxicity Testing Framework

TOXICOLOGICAL SCIENCES 136(1), 4–18 2013
doi:10.1093/toxsci/kft178
Advance Access publication August 19, 2013

Incorporating New Technologies Into Toxicity Testing and Risk Assessment: Moving From 21st Century Vision to a Data-Driven Framework

Russell S. Thomas,*,1 Martin A. Philbert,† Scott S. Auerbach,‡ Barbara A. Wetmore,* Michael J. Devito,‡ Ila Cote,§ J. Craig Rowlands,¶ Maurice P. Whelan,‖ Sean M. Hays,‖‖ Melvin E. Andersen,* M. E. (Bette) Meek,‖‖‖ Lawrence W. Reiter,# Jason C. Lambert,** Harvey J. Clewell III,* Martin L. Stephens,†† Q. Jay Zhao,** Scott C. Wesselkamper,** Lynn Flowers,§ Edward W. Carney,¶ Timothy P. Pastoor,‡‡ Dan D. Petersen,** Carole L. Yauk,§§ and Andy Nong§§

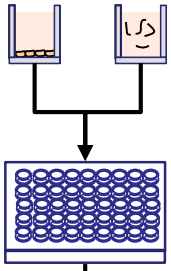


Protective Predictive Protective Predictive

The Next Frontier for Toxicogenomics

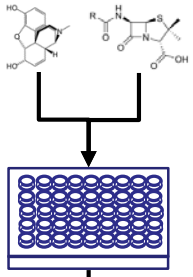
ToxCast
~600 assays

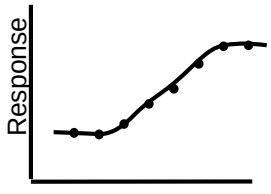
Tox21
~50 assays



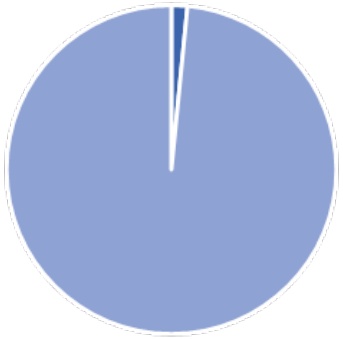
ToxCast
~1,000
chemicals

Tox21
~10,000
chemicals





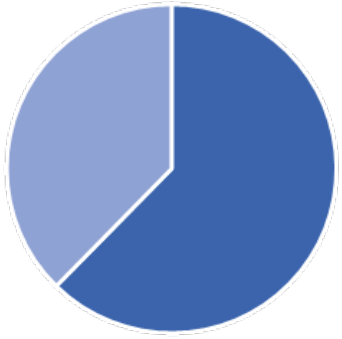
Gene Coverage



ToxCast

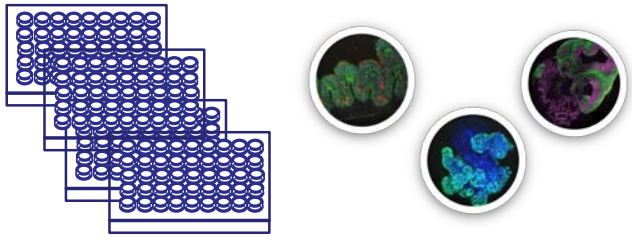
Not in ToxCast

Pathway Coverage*



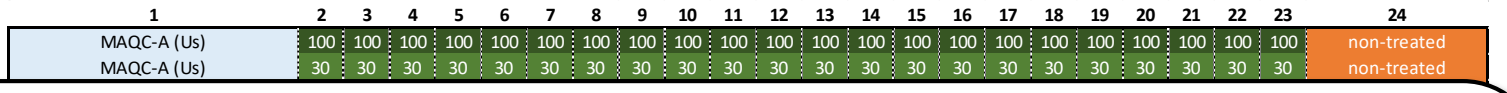
*At least one gene from pathway represented

Developing a Portfolio of High-Throughput Toxicogenomic Tools



High-Throughput Transcriptomic Screening Platform (TempOSeq)

- Low cost, 384-well, cell lysate compatible
- Whole transcriptome (EPA), S1500+ (NTP)
- Workflow integration of reference materials and controls, development of performance standards
- Portable platform/workflow for collaborative data generation



Platform

Tech

IN PROGRESS

- Half way through first large scale screen of 2,200 chemicals (ToxCast I/II/III) in single cell type
- Additional screens across multiple cell types/lines
- Tox21 cross-partner project to add reference chemical database
- Genetic perturbations (RNAi/CRISPR/cDNA) for targets/pathways with no reference chemicals

Scre

- Cytog… characterized cells
- *Functional Performance*
- Parallel HCI screen
- RNA and lysate reference materials
- Positive and negative controls

Standard Treatment Protocols

CMAP Match 4/5 (80%)

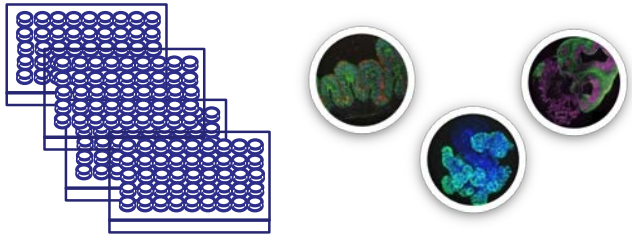
Standard Analysis Protocols

Randomized treatment

Data Repository

Josh Harrill, Unpublished

Developing a Portfolio of High-Throughput Toxicogenomic Tools



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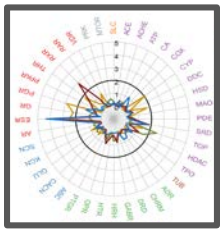
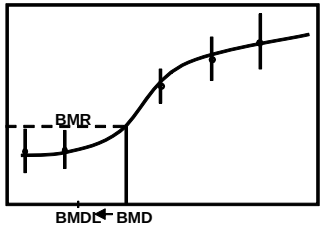


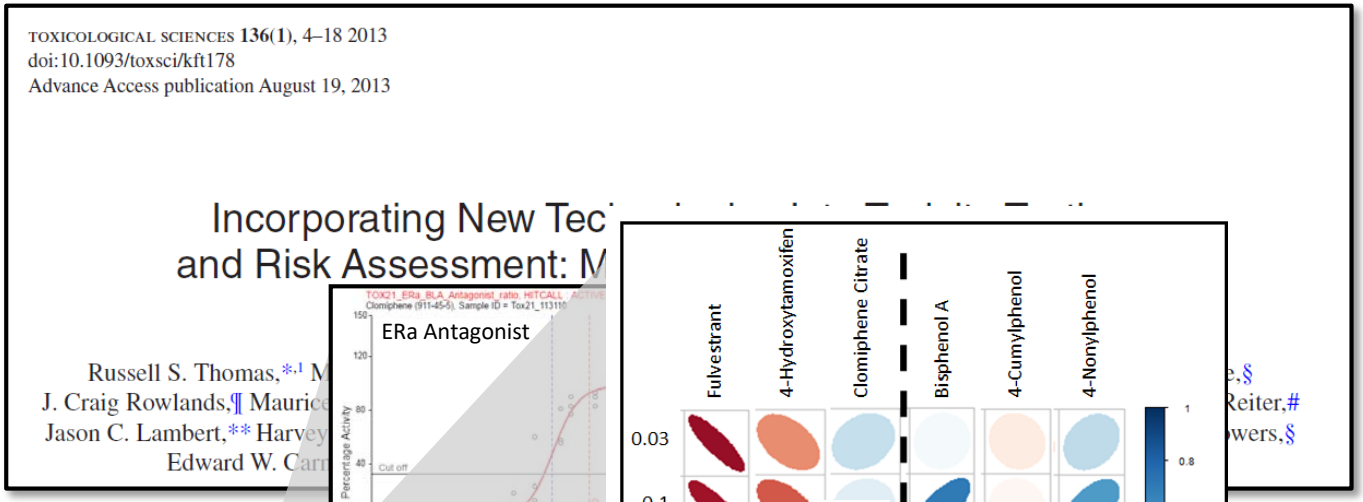
Karmaus, Unpublished

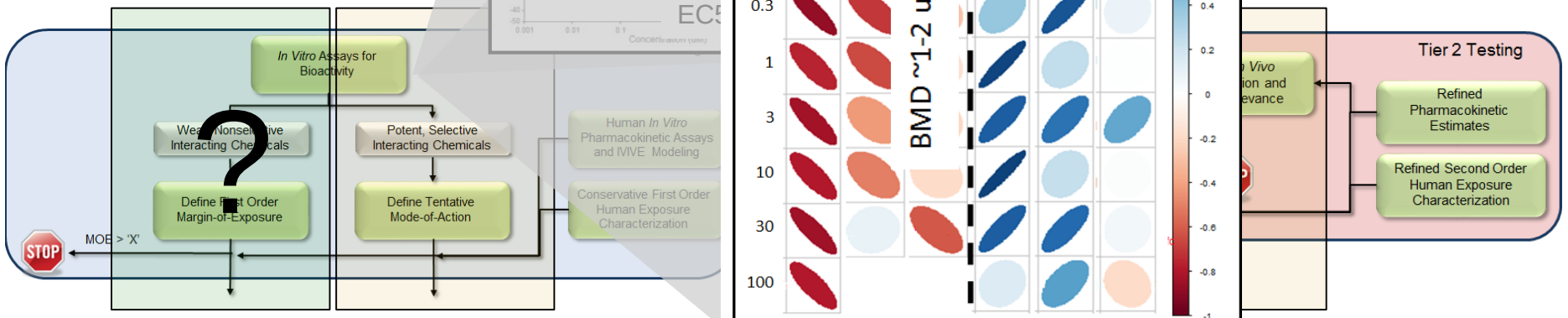
Mode of Action/MIE Analysis
- Refined CMAP tool and machine learning approaches
- Curating reference chemical database for MIE and directional response
- >60 MIEs and growing



Concentration Response Analysis
- BMDExpress 2.0
- Tcpl

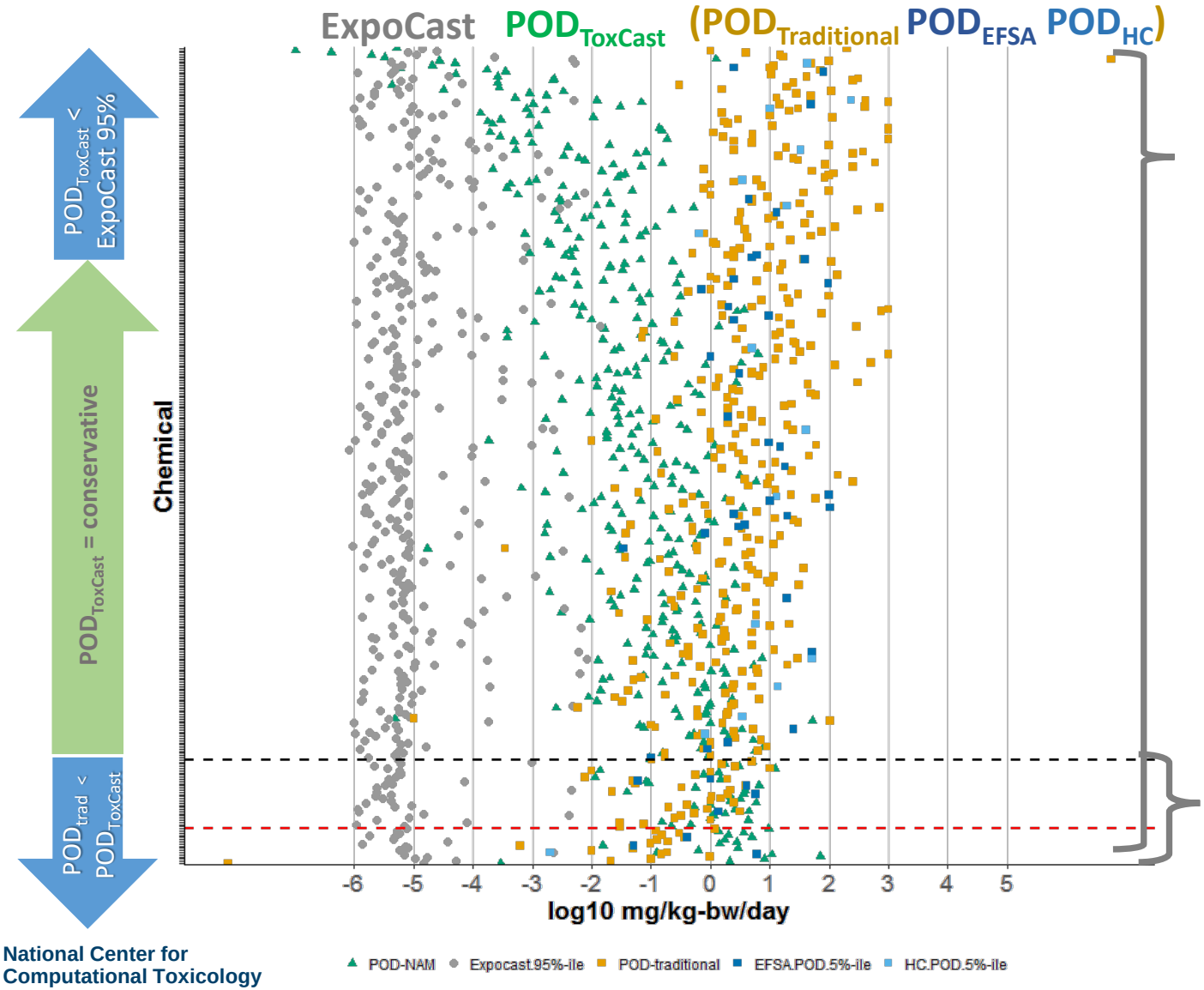
Expanding the Tiered 21st Century Toxicity Testing Framework





Protective Predictive Protective Predictive

International Case Study Evaluating Bioactivity as a Conservative POD



Total =

379 chemicals

httk, ToxCast data, and POD value(s) currently available

For ~87% of the chemicals, $POD_{ToxCast}$ was conservative.

Missing an important component of biology?

What is the Regulatory Need?

H. R. 2576

One Hundred Fourteenth Congress
of the
United States of America

AT THE SECOND SESSION

Begun and held at the City of Washington on Monday, the fourth day of January, two thousand and sixteen

An Act

To modernize the Toxic Substances Control Act, and for other purposes.

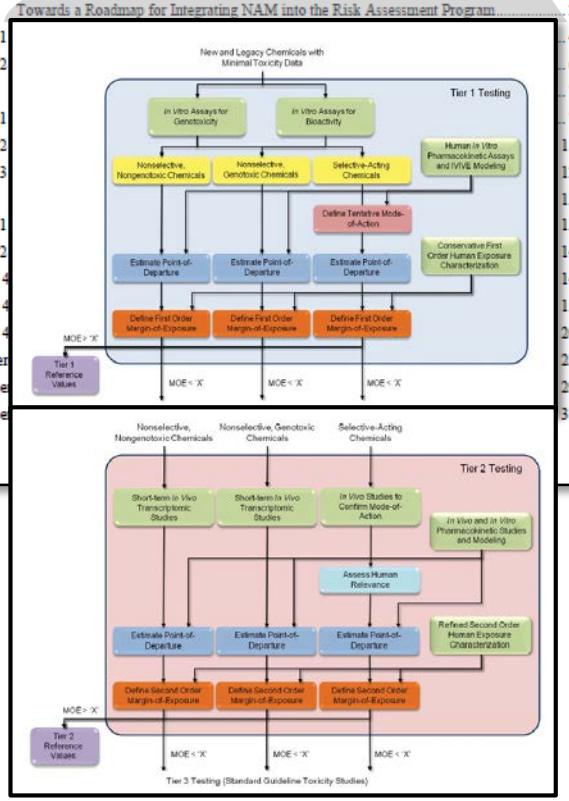
Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

"(1) PRIORITIZATION FOR RISK EVALUATIONS.—

"(A) ESTABLISHMENT OF PROCESS.—Not later than 1 year after the date of enactment of the Frank R. Lautenberg Chemical Safety for the 21st Century Act, the Administrator shall establish, by rule, a risk-based screening process, including criteria for designating chemical substances as high-priority substances for risk evaluations or low-priority substances for which risk evaluations are not warranted at the time. The process to designate the priority of chemical substances shall include a consideration of the hazard and exposure potential of a chemical substance or a category of chemical substances (including consideration of persistence and bioaccumulation, potentially exposed or susceptible subpopulations and storage near significant sources of drinking water), the conditions of use or significant changes in the conditions of use of the chemical substance, and the volume or significant changes in the volume of the chemical substance manufactured or processed.

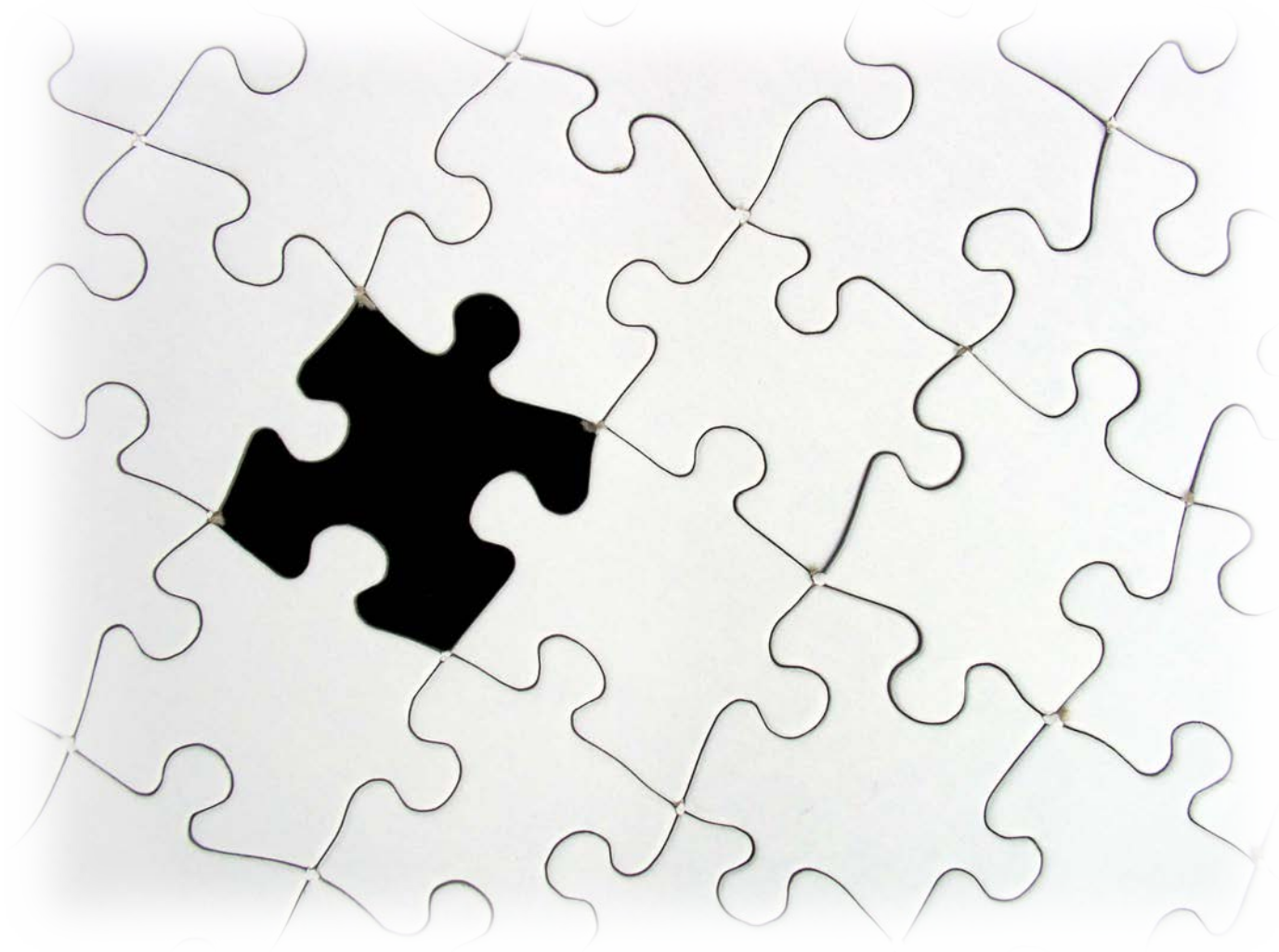
Chemicals Management Plan (CMP) Science Committee
Objectives Paper
Meeting #5; 16–17 November 2016
Integrating New Approach Methodologies within the CMP:
Identifying Priorities for Risk Assessment,
Existing Substances Risk Assessment Program

Contents



EPA United States Environmental Protection Agency

But, A Necessary Piece Is Convergence and Acceptance of Analysis Approaches



Acknowledgements and Questions

Tox21 Colleagues:
- NTP
- FDA
- NCATS

EPA Colleagues:
- NERL
- NHEERL
- NCEA

Collaborative Partners:
- Unilever
- A*STAR
- ECHA
- EFSA
- Health Canada

EPA's National Center for Computational Toxicology