

ECHA Workshop: Accelerating the Pace of Chemical Risk Assessment

John R. Bucher, PhD, DABT

NTP Associate Director

National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors

December 8, 2017





- A*STAR (Singapore)
- US Consumer Product Safety Commission
- California EPA
- US Environmental Protection Agency (EPA)*
- European Chemical Agency (ECHA)*
- European Food Safety Agency
- National Industrial Chemicals Notification and Assessment Scheme (NICNAS [Australia])
- National Institute for the Industrial Environment and Risks (INERIS [France])
- Joint Research Council (EU)
- Health Canada
- Safety and Health Technology Center (SAHTECH [Taiwan])
- National Institute for Public Health and the Environment (RIVM [Netherlands])
- Japanese Ministry of Health, Welfare and the Environment
- Korea Ministry of the Environment
- US National Toxicology Program
- Organisation for Economic Co-operation and Development (OECD)



- Why- Immediate drivers
 - TSCA reform act
 - REACH experience
 - Purpose
 - To “make the science of **new approach methodologies** (NAMS) work for common regulatory challenges”
 - “To bring together international regulators to discuss progress and barriers in applying new tools to prioritization, screening, and quantitative risk assessment of differing levels of complexity.”
- When and how-
 - Workshops to develop case studies
 - September 14-15, 2016 EPA Washington, DC
 - October 10-11, 2017 ECHA Helsinki, Finland
 - Periodic teleconferences



September 2016

- Using NAMS to address data poor, high exposure chemicals (ECHA)
- Use NAMS to improve chemical categories and biological activity groupings (EPA)
- In vitro bioactivity as a conservative PoD (EPA)
- New tools to predict exposures from various chemical structure and use categories (EPA)
- Develop multimedia exposure models to improve Pb mitigation efforts (EPA)
- Develop a range of validated NAMS to identify endocrine disruptors (France)
- Application of NAMS to perfluoroalkylated substances (EPA)
- Amphibian skin absorption models (EFSA)
- Develop reference doses from endocrine disruptors from in vitro assays (Korea)
- Medaka extended one generation reproduction assay (Japan)



September 2016

- Using NAMS to address data poor, high exposure chemicals (ECHA)
- Use NAMS to improve chemical categories and biological activity groupings (EPA)
- In vitro bioactivity as a conservative PoD (EPA)
- New tools to predict exposures from various chemical structure and use categories (EPA)
- Develop multimedia exposure models to improve Pb mitigation efforts (EPA)
- Develop a range of validated NAMS to identify endocrine disruptors (France)
- Application of NAMS to perfluoroalkylated substances (EPA)
- Amphibian skin absorption models (EFSA)
- Develop reference doses from endocrine disruptors from in vitro assays (Korea)
- Medaka extended one generation reproduction assay (Japan)



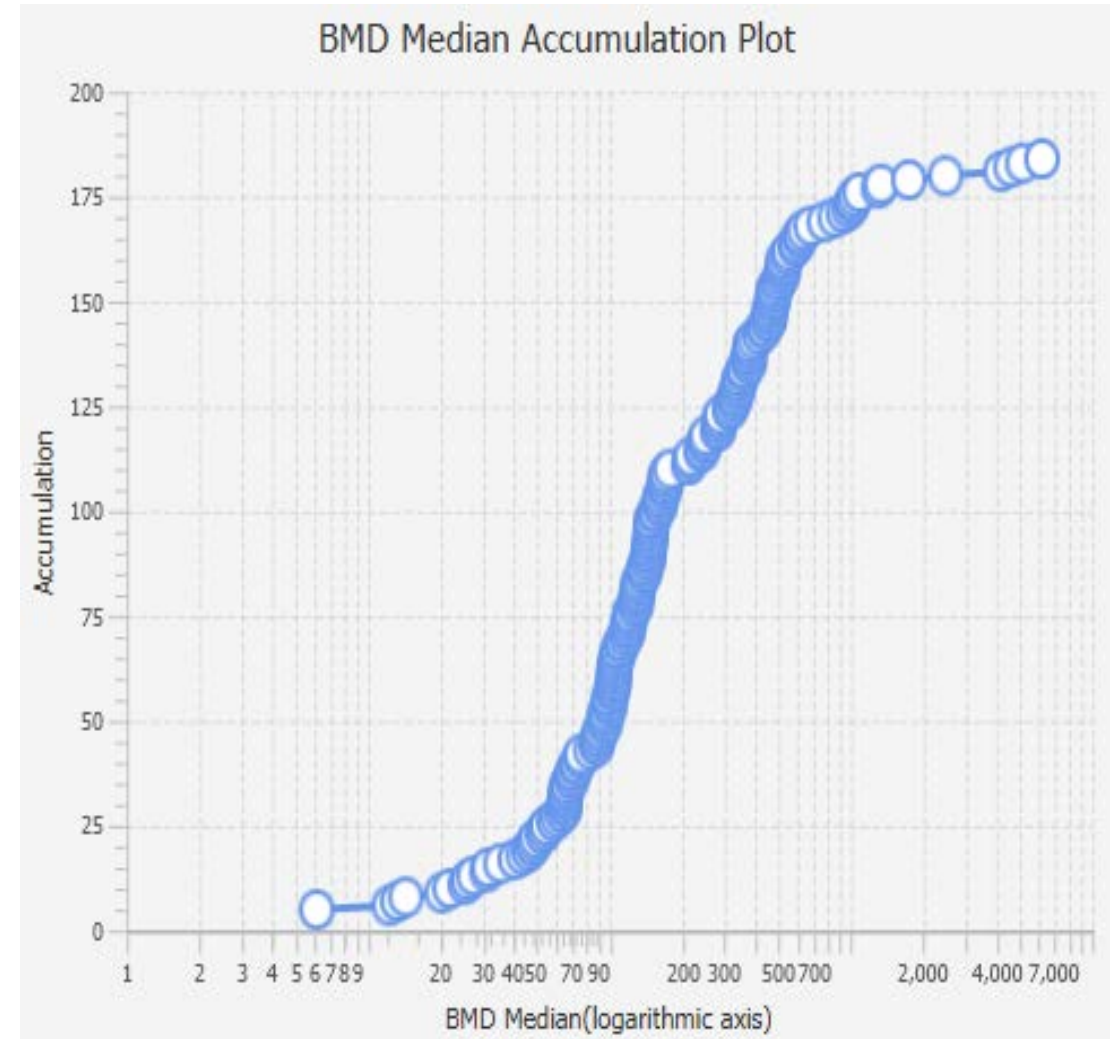
- The PoD for changes in hepatic gene expression is predictive of the PoD for biological effects in any organ in any length study.



- Administer chemical to male SD rats by oral gavage once per day for 4-5 days at 6 to 8 levels covering a wide dose range, from MTD downward to predicted NOEL
- Remove liver on day 5-6 for transcriptomic assessment- microarray or RNA seq with S1500+ gene set
- Load gene expression files and process according to preset criteria through BMDExpress2.0
- Perform benchmark dose modeling for both gene level and “pathway” level hepatic transcriptome changes
- Compare BMDs for gene or pathway expression changes with BMDs for any traditional toxicological response



- Plots either active genes or “pathways” in an ascending accumulative manner based on increasing median BMD or BMD_L
- Provides view of most to least dose sensitive gene or pathway
- Can identify genes or pathways by mouse click





p-Toluidine, *N,N*-dimethyl-*p*-toluidine 5-day genomics study

- *N,N*-dimethyl-*p*-toluidine (DNPT)
 - Hepatocellular tumors and liver toxicity in rats and mice
 - Nasal transitional epithelial adenoma/carcinoma and nasal toxicity in rats
- *p*-Toluidine (*p*-Tol)
 - Hepatocellular tumors in mice and liver toxicity in rats and mice
- Methemoglobinemia
 - Both chemicals through a postulated *p*-methyl phenyl hydroxylamine
- Compare transcriptomic profiles

Arch Toxicol
DOI 10.1007/s00204-016-1831-7



TOXICOGENOMICS

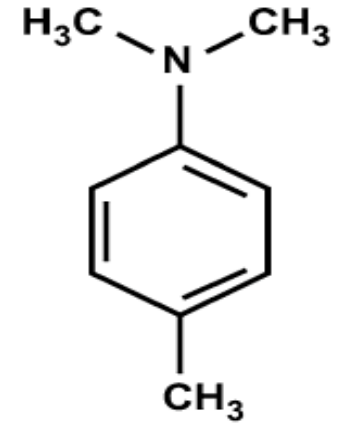
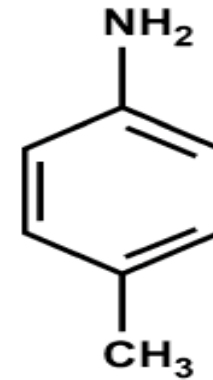
Hepatic transcriptomic alterations for *N,N*-dimethyl-*p*-toluidine (DMPT) and *p*-toluidine after 5-day exposure in rats

June K. Dunnick¹ · Keith R. Shockley² · Daniel L. Morgan³ · Amy Brix⁴ · Gregory S. Travlos⁵ · Kevin Gerrish⁶ · J. Michael Sanders⁷ · T. V. Ton⁵ · Arun R. Pandiri⁵



DMPT and *p*-toluidine 5-day genomics study design

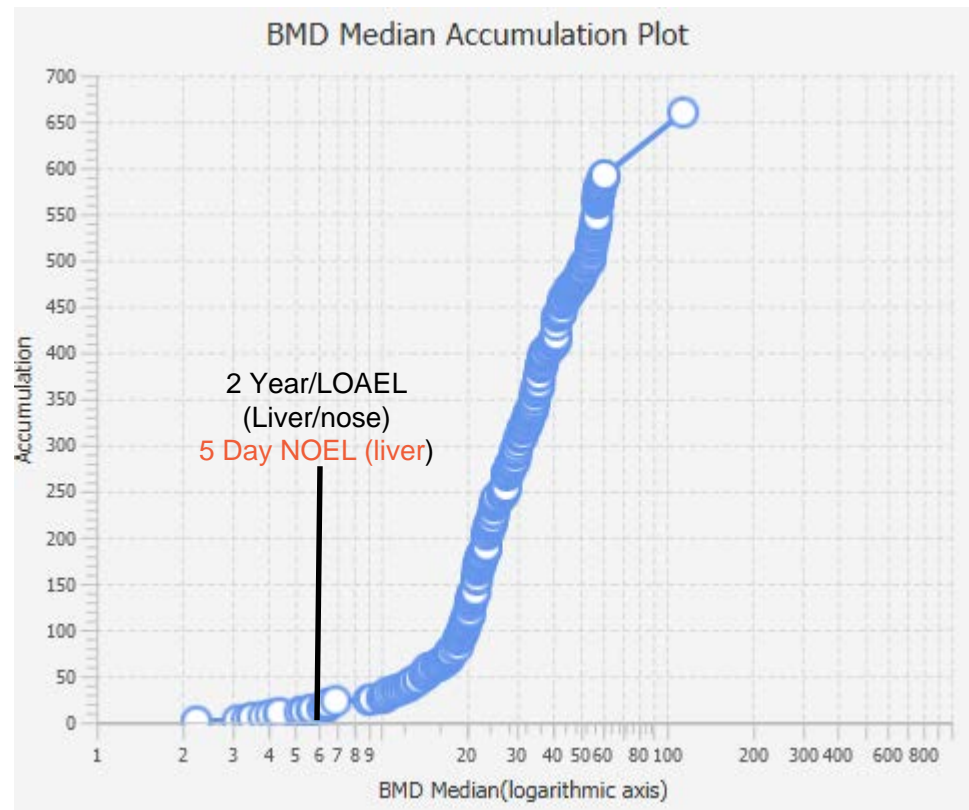
- **Model:** F344/N Rat (male)
- **Route:** Oral (corn oil gavage)
- **Dosing regiment:** 5 repeated doses, euthanize 24 hrs after last dose
- **Dose groups:** 6
 - 0, 1, 6, 20, 60, 120 mg/kg/day
- **Group size:** 5
- **Organ for transcriptomics:** Liver
- **Other endpoints:** Clinical observations, body and organ weights, clinical pathology



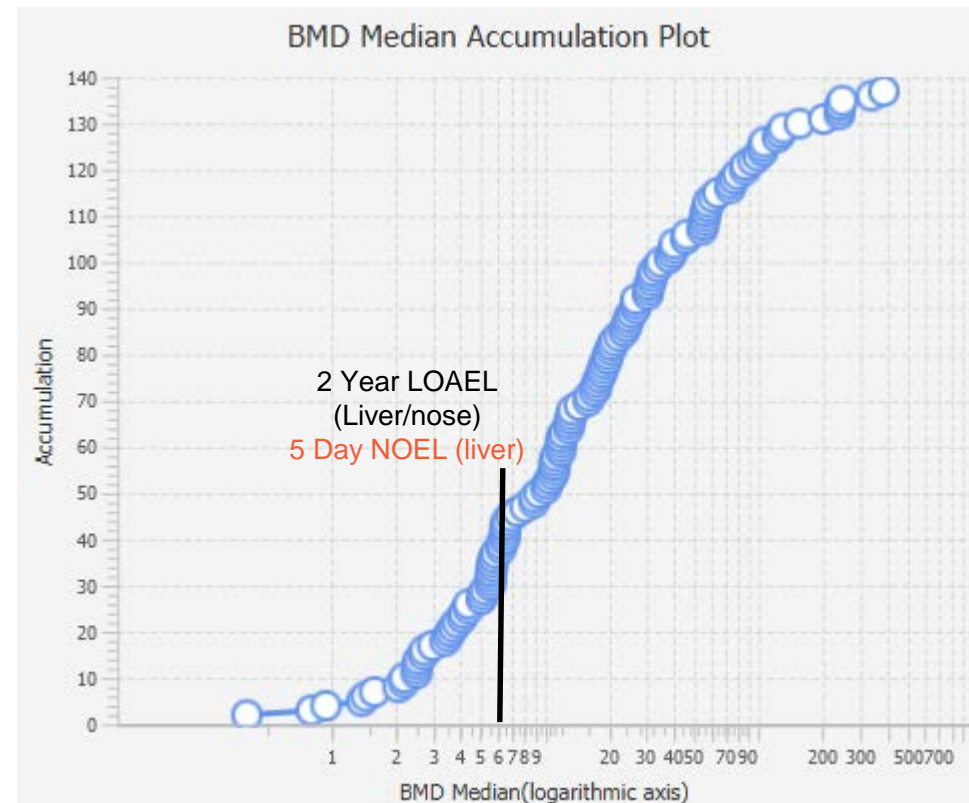


DMPT GO BP and gene BMD values

“Active” GO BP Terms

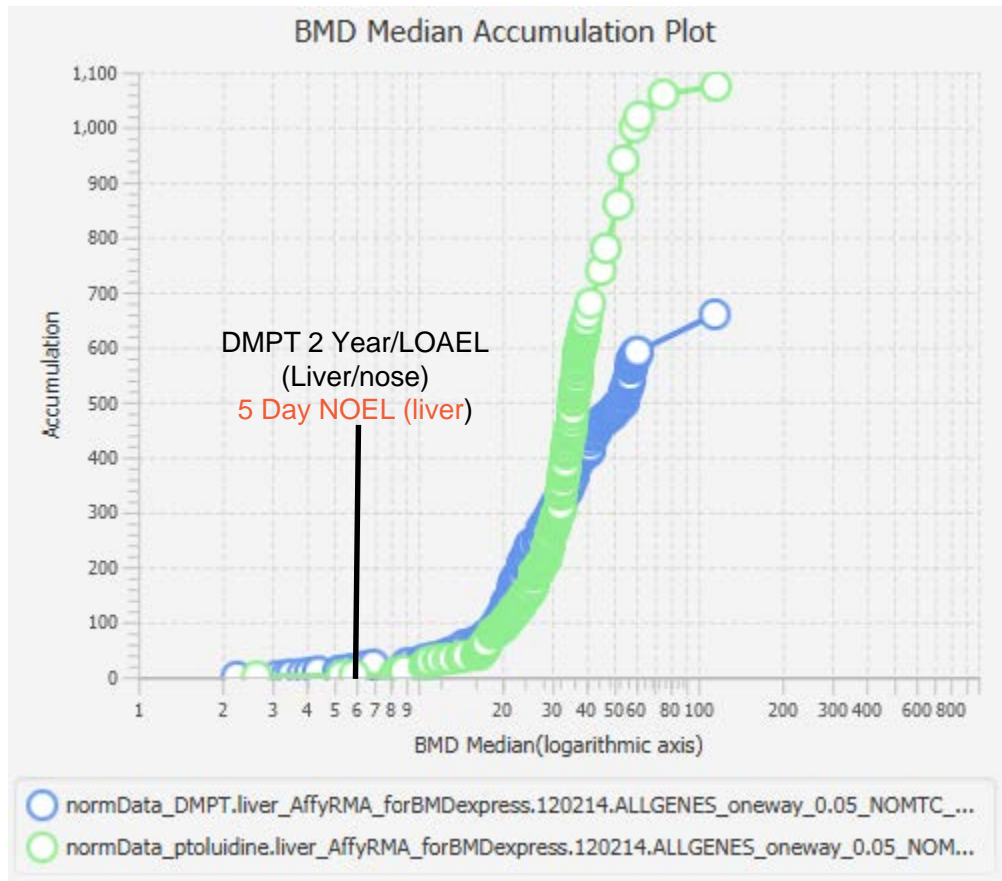


Genes

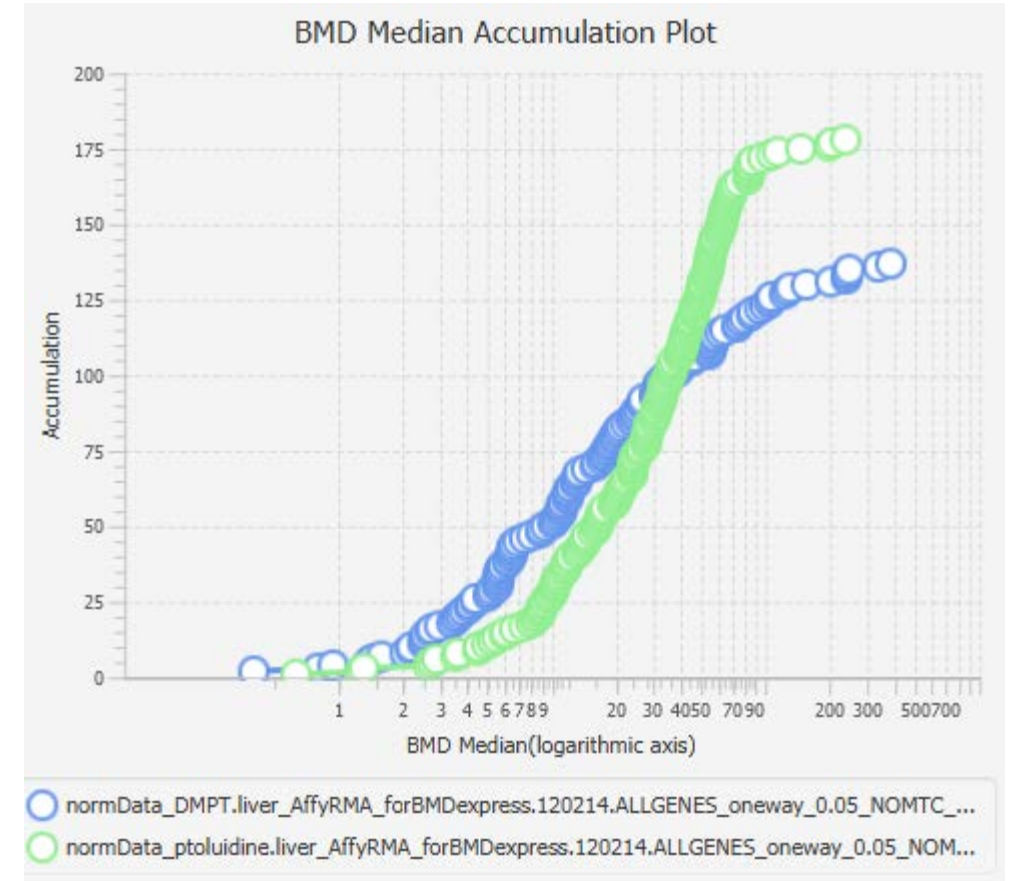


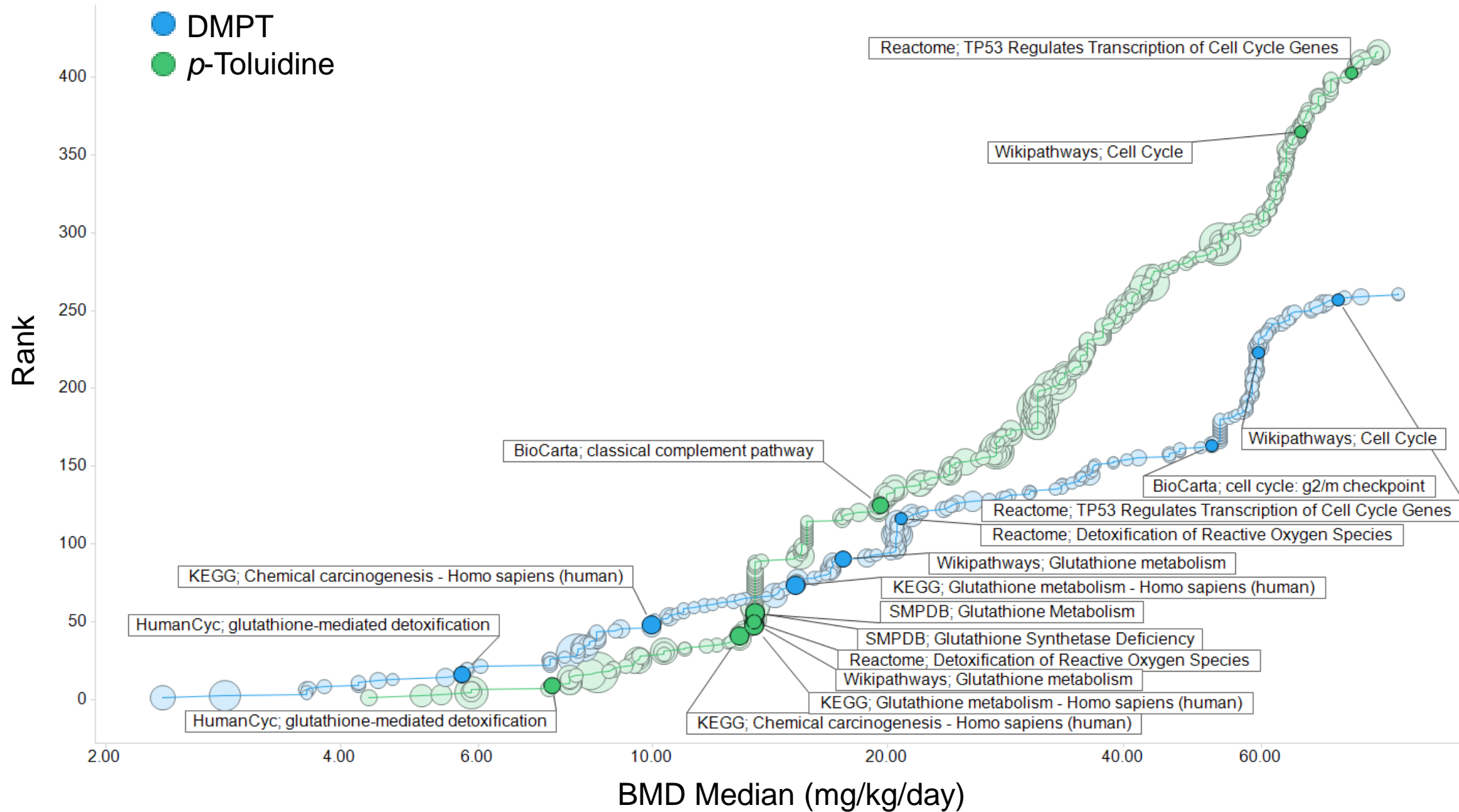


“Active” GO BP Terms



Genes







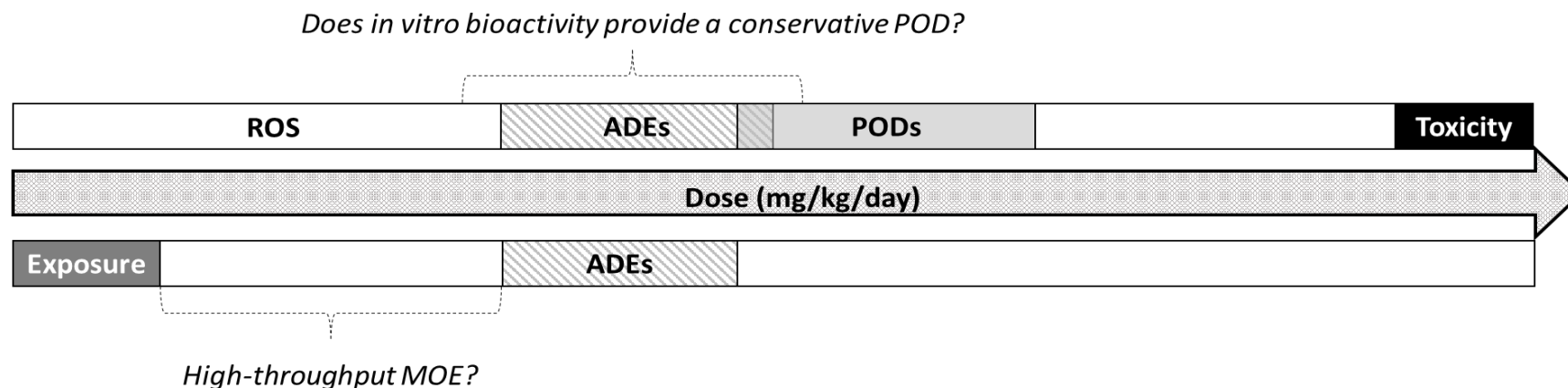
- What kinds of substances do we miss? Why?
- Do kinetic adjustments adequately accommodate bio-accumulative substances?
- Non toxic substances will produce gene expression changes- Do we care?
- Can this approach be used for more than prioritization?
- Can this bridge to *in vitro* transcriptomic-based risk assessment?



- Using NAMS to address data poor, high exposure chemicals (ECHA)
- Use NAMS to improve chemical categories and biological activity groupings (EPA)
- **In vitro bioactivity as a conservative PoD (EPA)**
- New tools to predict exposures from various chemical structure and use categories (EPA)
- Develop multimedia exposure models to improve Pb mitigation efforts (EPA)
- Develop a range of validated NAMS to identify endocrine disruptors (France)
- Application of NAMS to perfluoroalkylated substances (EPA)
- Amphibian skin absorption models (EFSA)
- Develop reference doses from endocrine disruptors from in vitro assays (Korea)
- Medaka extended one generation reproduction assay (Japan)



Examining the utility of *in vitro* bioactivity as a conservative point of departure



Use of high-throughput, *in vitro* bioactivity data in setting a conservative point-of-departure (POD) will require **greater confidence** that *in vitro* bioactivity data, in concert with high-throughput toxicokinetic information and reverse dosimetry, can be used to estimate administered dose equivalents (ADEs) at or below the PODs derived from traditional animal studies.

*Partner Agencies EPA, ECHA, EFSA, A*STAR, Health Canada, NTP*



Examining the utility of in vitro bioactivity as a conservative point of departure

<i>Partner</i>	<i>Primary roles/contributions</i>
<i>EPA-ORD [NCCT, NCEA, and CSS]</i>	<ul style="list-style-type: none"><i>• Lead/organizing partner</i><i>• Contributed high-throughput toxicokinetic information, high-throughput screening information (ToxCast/Tox21) and their corresponding administered dose equivalents, point-of-departure information from in vivo studies.</i><i>• Provided chemicals to A*STAR for additional screening in high-throughput assays.</i>
<i>ECHA</i>	<ul style="list-style-type: none"><i>• Compiling publicly available point-of-departure information from an IUCLID database of chemical registration information, with an emphasis on sharing information for chemicals with available high-throughput toxicokinetic information.</i>
<i>EFSA</i>	<ul style="list-style-type: none"><i>• Compiling point-of-departure and exposure information from registration dossiers with an emphasizing information for chemicals with available high-throughput toxicokinetic information.</i>
<i>A*STAR</i>	<ul style="list-style-type: none"><i>• Initiated bioactivity screen for 64 prioritized ToxCast chemicals in three organ-relevant (liver, kidney and lung) in vitro models.</i>
<i>Health Canada</i>	<ul style="list-style-type: none"><i>• Compiling exposure and point-of-departure information emphasizing information for chemicals with available high-throughput toxicokinetic information.</i>



Study flow (in development)

- Identify ~90 REACH-registered, “in vivo data-poor” chemicals with exposure information
- Identify ~40 “in vivo data-poor” chemicals with high throughput toxicokinetic data available
- Identify ~ 100 chemicals having 90-day or other repeated dose in vivo toxicity studies available with high throughput toxicokinetic data available
 - 35 chemicals with less conservative POD_{nam} estimates, POD_{traditional}:POD_{nam} ratio < 1;
 - 35 chemicals with a moderate POD_{traditional}:POD_{nam} ratio between 1 and 2 (assumed moderate level of protection)
 - 35 chemicals with an overly conservative POD_{nam} estimates, POD_{traditional}:POD_{nam} ratio > 2
- Select ~10 chemicals for in depth in vivo assessments by NTP
 - 90 day toxicity studies with toxicokinetic measurements
 - Sequential assessments of transcriptomics in selected organs



Thank you!

<https://niehs.nih.gov>





Ongoing 5-day studies

- Furan
 - Diethylhexyl phthalate
 - Acrylamide
 - Tris(chloropropyl) phosphate
 - Triclosan
 - Pentabromodiphenyl ether mixture
 - 3,3',4,4'- Tetrachloroazobenzene
- Thujone
 - Fenofibrate
 - Bromodichloroacetic acid
 - Hexachlorobenzene
 - Tetrabromobisphenol A
 - Pulegone
 - Methyleugenol
- Bisphenol AF
 - Coumarin
 - Perfluorooctanoic acid
 - Ethinyl estradiol
 - Ginseng
 - Milk thistle extract



Ongoing 5-day studies- data under review/reporting

- Decabromodiphenyl ether
 - 2,2',4,4'-tetrabromodiphenyl ether
 - Bis(2ethylhexyl) tetrabromophthalate
 - Tetrabromobisphenol A bis(2,3-dibromopropyl ether)
 - Hexachlorocyclopentadienyl-dibromocyclooctane
 - Decabromodiphenylethane
 - 2-Ethylhexyl diphenyl phosphate
 - Tert-butylphenyl diphenyl phosphate
 - Ginkgo biloba extracts (5)
- 2-Ethylhexyl-2,3,4,5-tetrabromobenzoate
 - Hexabromocyclododecane
 - Firemaster 680
 - Triphenyl phosphate
 - Isopropylated phenol phosphate
 - Tricresyl phosphate
 - Isodecyl diphenyl phosphate