NTP
National Toxicology Program

ECHA Workshop: Accelerating the Pace of Chemical Risk Assessment

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NTP Board of Scientific Counselors
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Who

- 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, when, how

- 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

Initial proposed case studies

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)
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NTP’s contributing hypothesis

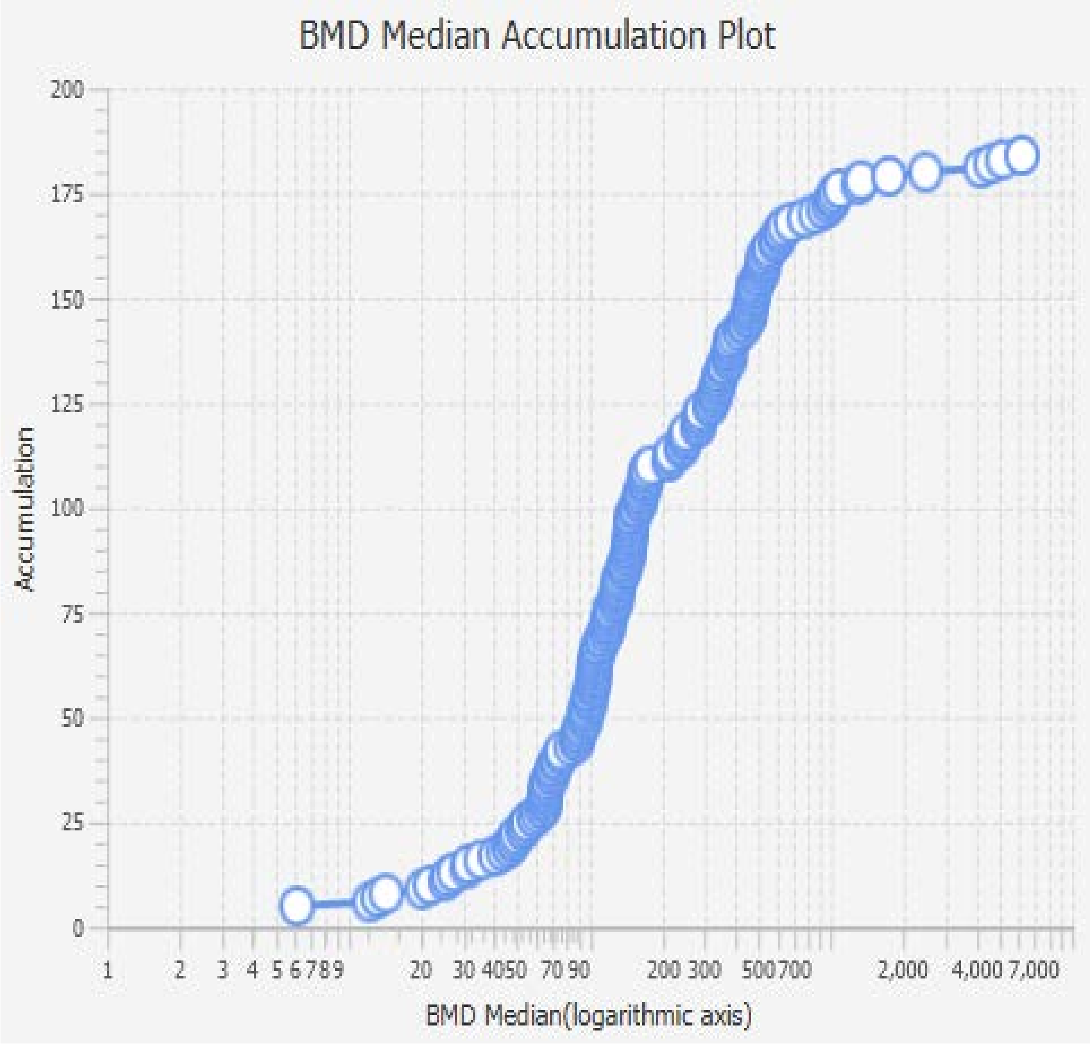
- The PoD for changes in hepatic gene expression is predictive of the PoD for biological effects in any organ in any length study.

Study design

- 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

Accumulation plots

- Plots either active genes or “pathways” in an ascending accumulative manner based on increasing median BMD or BMDL
- 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

CrossMark

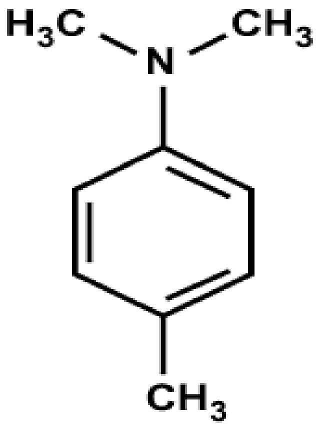
TOXICOGENOMICS

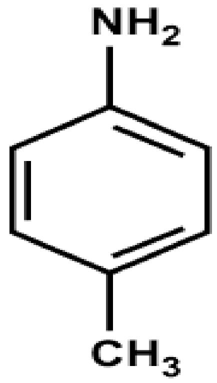
Hepatic transcriptomic alterations for *N,N*-dimethyl-*p*-toluidine (DMPT) and *p*-toluidine after 5-day exposure in rats

June K. Dunnick[1] · Keith R. Shockley[2] · Daniel L. Morgan[3] · Amy Brix[4] · Gregory S. Travlos[5] · Kevin Gerrish[6] · J. Michael Sanders[7] · T. V. Ton[5] · Arun R. Pandiri[5]

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

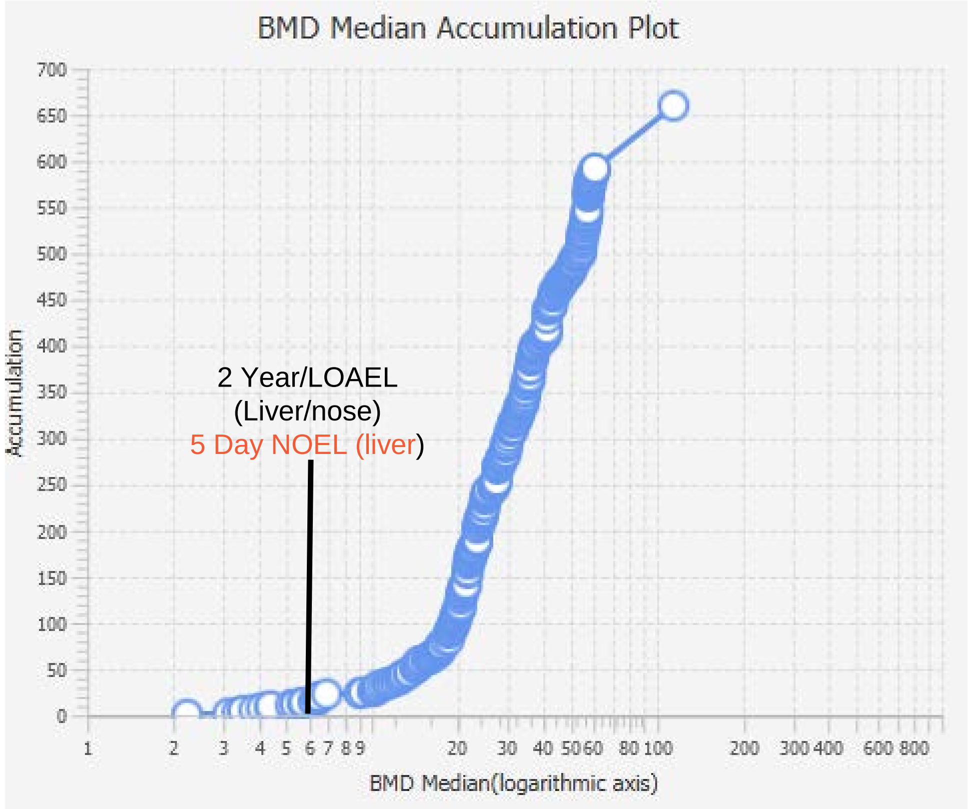
- **Model**: F344/N Rat (male)
- **Route**: Oral (corn oil gavage)
- **Dosing regiment**: 5 repeated doses, euthanize 24 hrs after last dose
- **Dose groups:** 6
  - 0, 1, 6, 20, 60, 120 mg/kg/day
- **Group size:** 5
- **Organ for transcriptomics**: Liver
- **Other endpoints**: Clinical observations,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



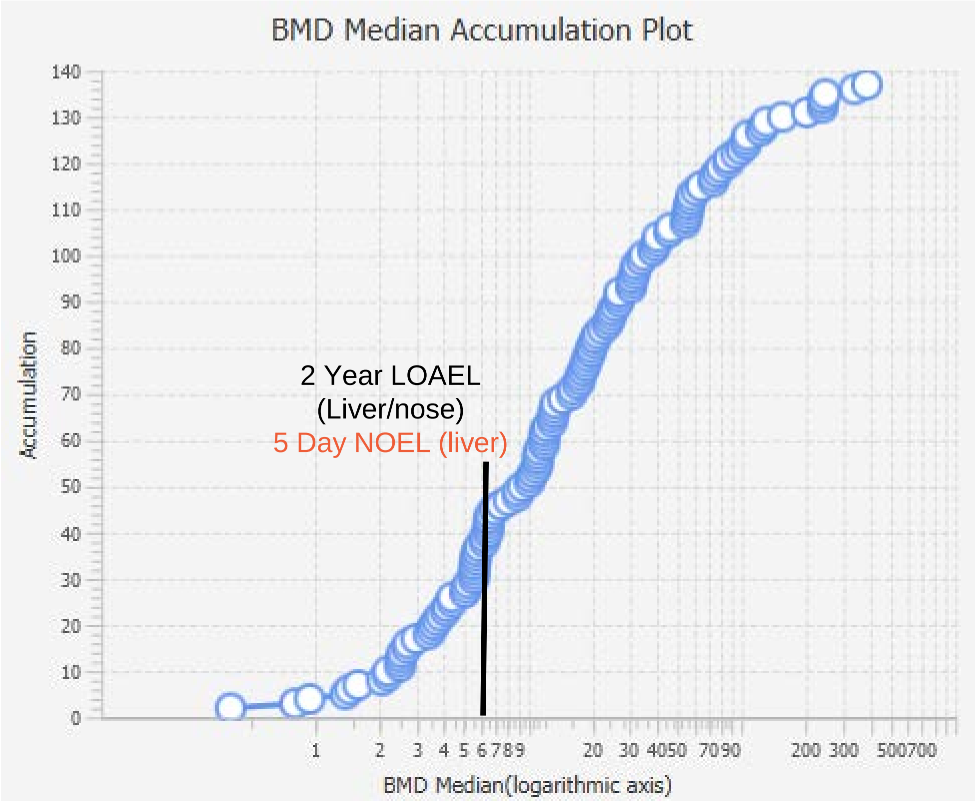


DMPT GO BP and gene BMD values

“Active” GO BP Terms

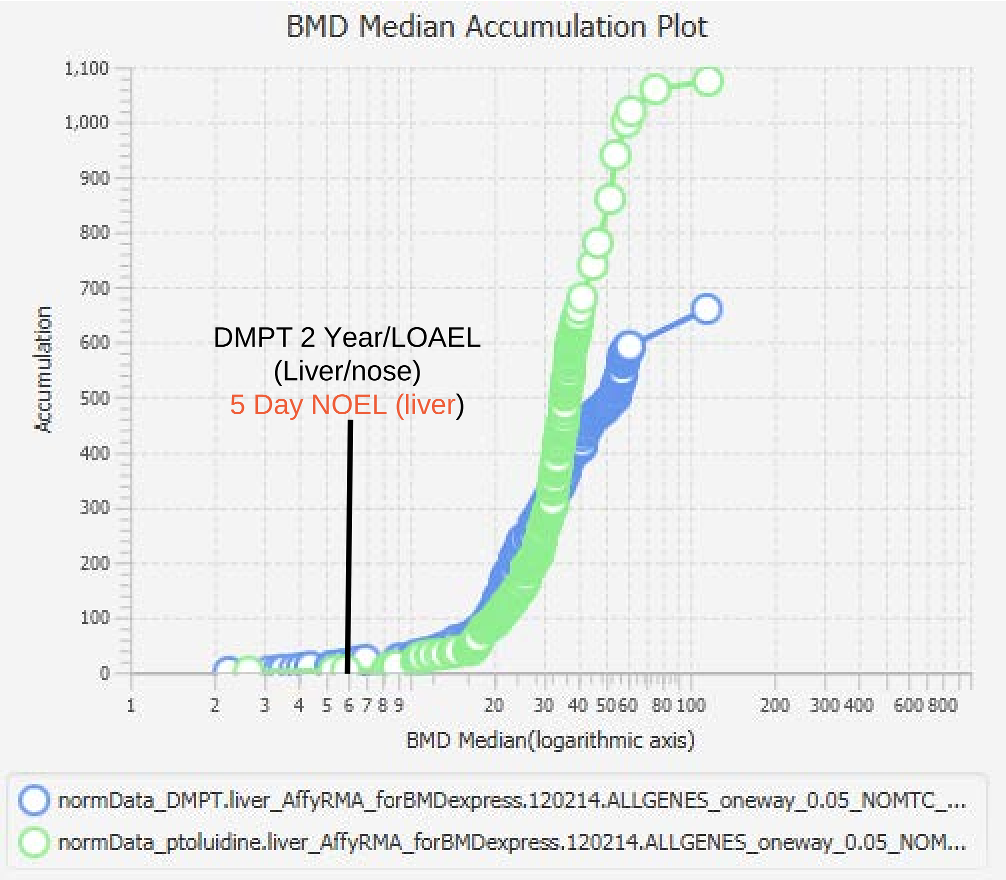


Genes

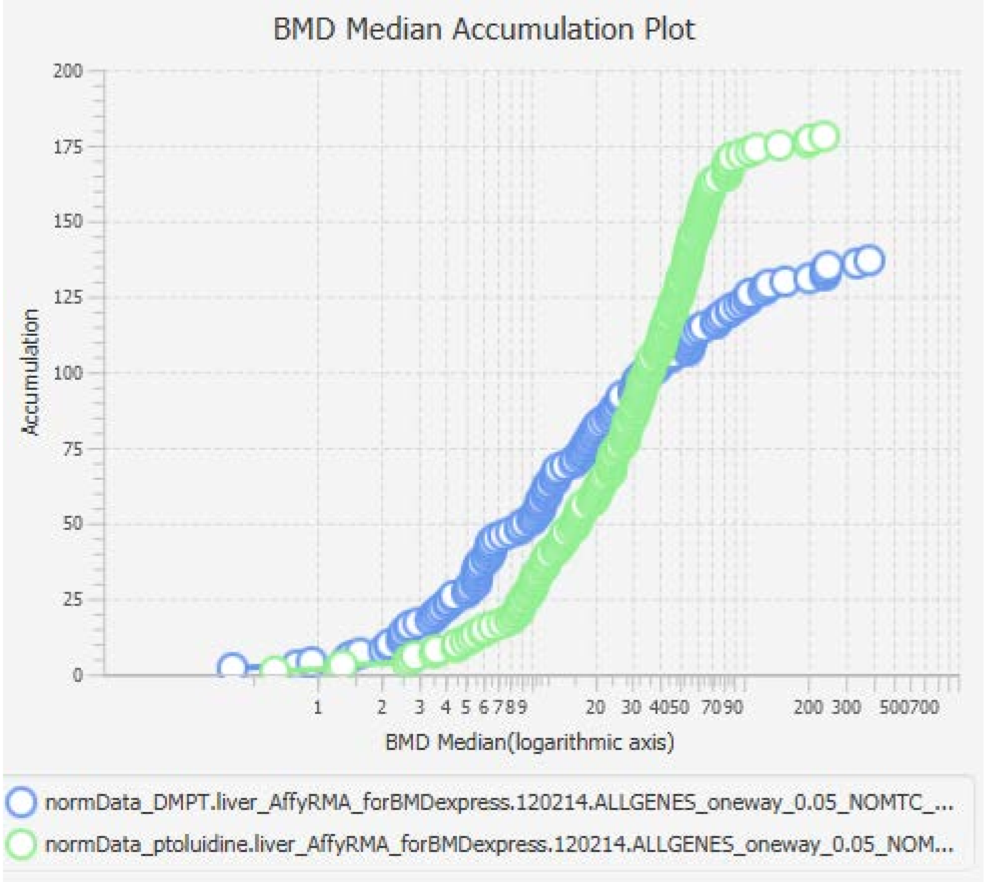


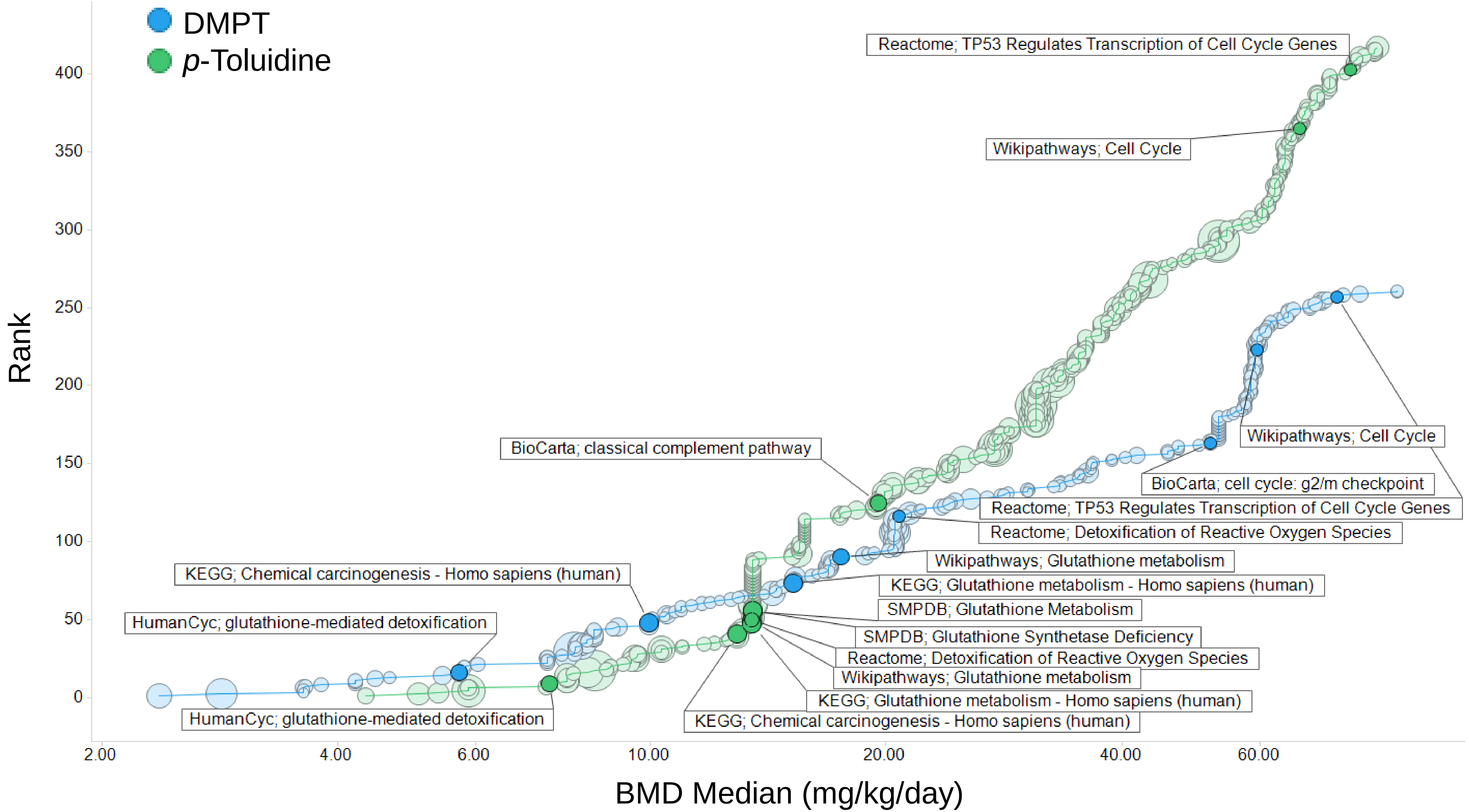
DMPT vs *p*-toluidine

“Active” GO BP Terms



Genes




DMPT
p-Toluidine
Rank
400
350
300
250
200
150
100
50
0
2.00
4.00
6.00
10.00
20.00
40.00
60.00
BMD Median (mg/kg/day)
Reactome; TP53 Regulates Transcription of Cell Cycle Genes
Wikipathways; Cell Cycle
BioCarta; classical complement pathway
Wikipathways; Cell Cycle
BioCarta; cell cycle: g2/m checkpoint
Reactome; TP53 Regulates Transcription of Cell Cycle Genes
Reactome; Detoxification of Reactive Oxygen Species
Wikipathways; Glutathione metabolism
KEGG; Glutathione metabolism - Homo sapiens (human)
SMPDB; Glutathione Metabolism
SMPDB; Glutathione Synthetase Deficiency
Reactome; Detoxification of Reactive Oxygen Species
Wikipathways; Glutathione metabolism
KEGG; Glutathione metabolism - Homo sapiens (human)
KEGG; Chemical carcinogenesis - Homo sapiens (human)
KEGG; Chemical carcinogenesis - Homo sapiens (human)
HumanCyc; glutathione-mediated detoxification
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Issues to address

- 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?

APCRA case studies

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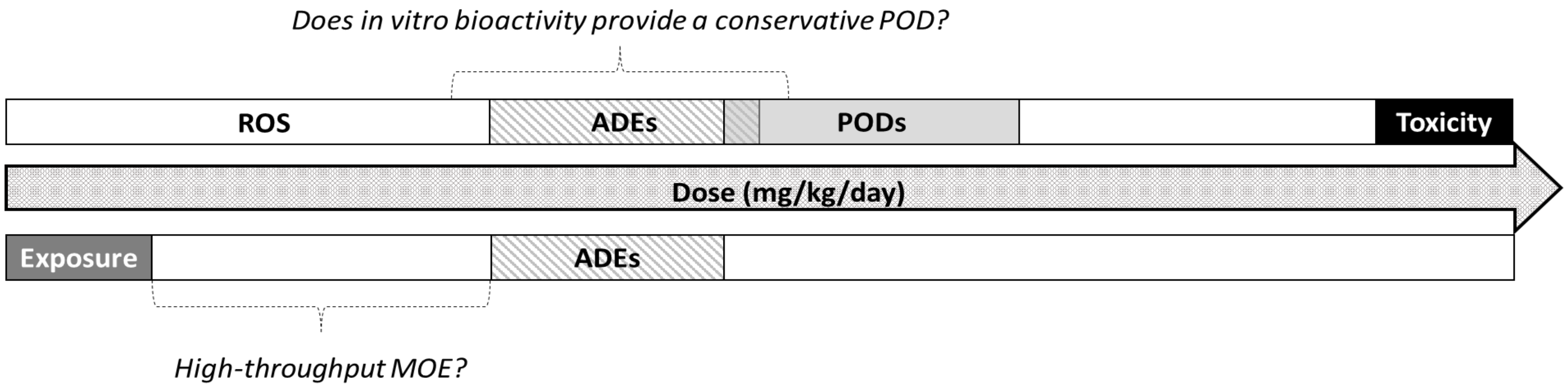
Examining the utility of in vitro bioactivity as a conservative point of departure



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

Partner	*Primary roles/contributions*
EPA-ORD [NCCT, NCEA, and CSS]	• *Lead/organizing partner* • *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.* • *Provided chemicals to A*STAR for additional screening in high-throughput assays.*
ECHA	• *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.*
EFSA	• *Compiling point-of-departure and exposure information from registration dossiers with an emphasizing information for chemicals with available high-throughput toxicokinetic information.*
*A*STAR*	• *Initiated bioactivity screen for 64 prioritized ToxCast chemicals in three organ-relevant (liver, kidney and lung) in vitro models.*
Health Canada	• *Compiling exposure and point-of-departure information emphasizing information for chemicals with available high-throughput toxicokinetic information.*

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 PODnam estimates, PODtraditional:PODnam ratio < 1;
  - 35 chemicals with a moderate PODtraditional:PODnam ratio between 1 and 2 (assumed moderate level of protection)
  - 35 chemicals with an overly conservative PODnam estimates, PODtraditional:PODnam 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

NIH National Institute of Environmental Health Sciences

National Toxicology Program
U.S. Department of Health and Human Services

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