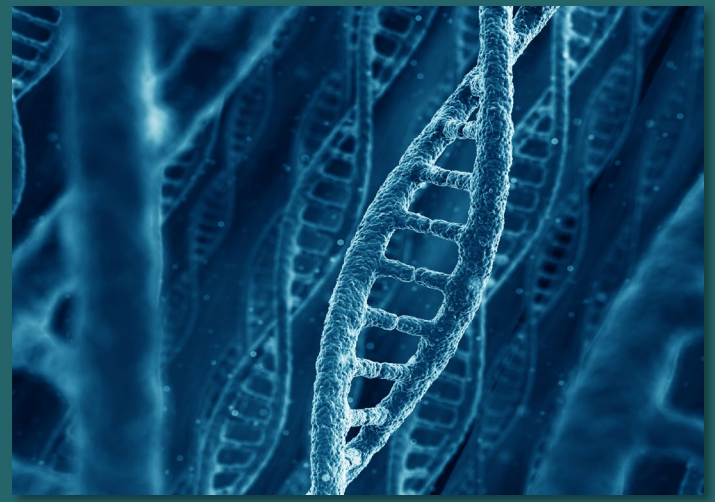
# Transcriptomics in Dose Response Assessment Literature Review

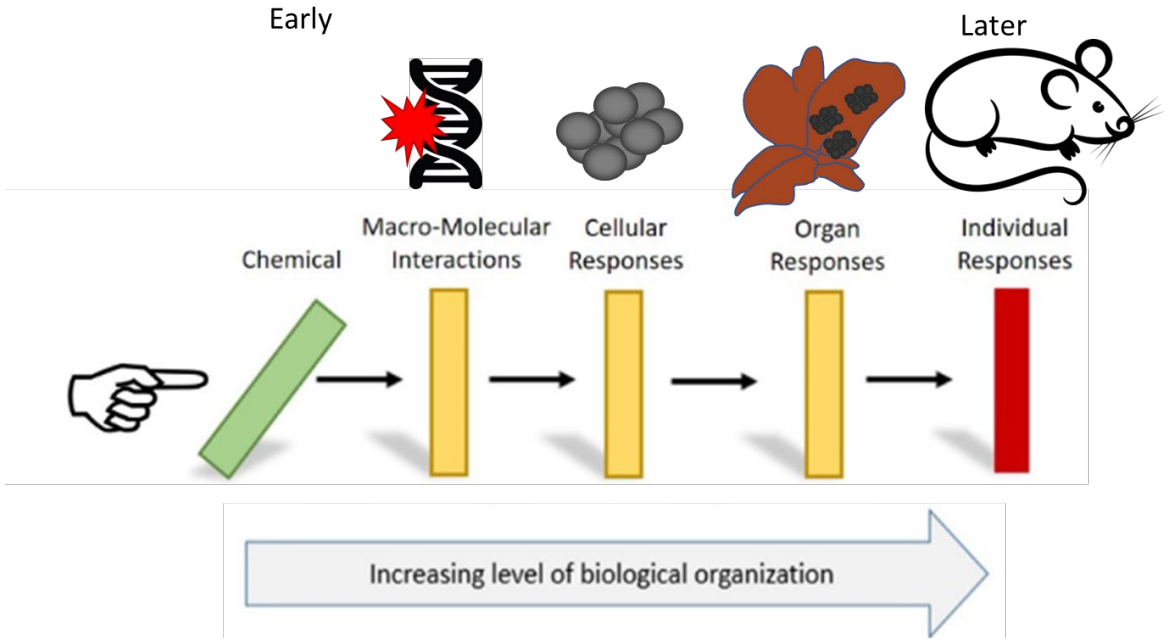
Leah Wehmas, Ph.D.
Center for Computational Toxicology and Exposure



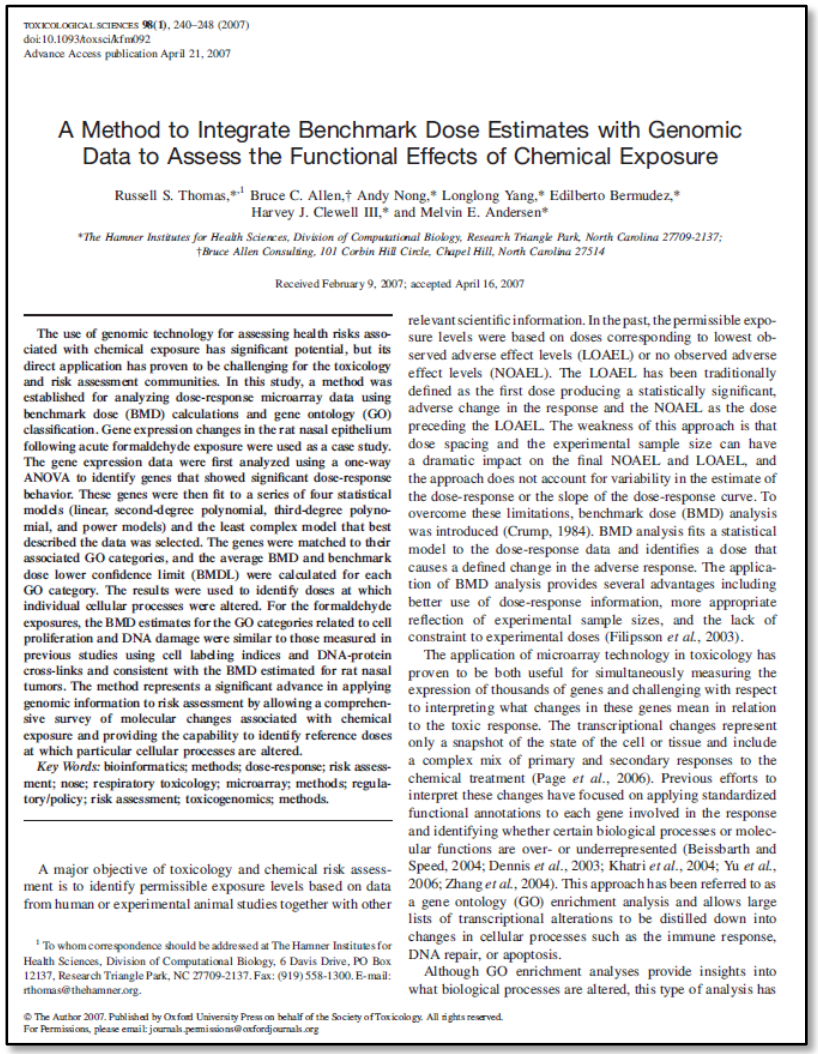
*The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. EPA*

# Rationale for application of dose response assessment to transcriptomics

- Gene expression changes occur with chemical exposure
- Changes precede apical adverse effects
- These changes can be measured and are dose dependent
- Changes in gene response can indicate chemical potency



# Initial application of dose response assessment to transcriptomics



TOXICOLOGICAL SCIENCES 98(1), 240–248 (2007)
doi:10.1093/toxsci/kfm092
Advance Access publication April 21, 2007

A Method to Integrate Benchmark Dose Estimates with Genomic Data to Assess the Functional Effects of Chemical Exposure

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*The Hamner Institutes for Health Sciences, Division of Computational Biology, Research Triangle Park, North Carolina 27709-2137; †Bruce Allen Consulting, 101 Corbin Hill Circle, Chapel Hill, North Carolina 27514

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The use of genomic technology for assessing health risks associated with chemical exposure has significant potential, but its direct application has proven to be challenging for the toxicology and risk assessment communities. In this study, a method was established for analyzing dose-response microarray data using benchmark dose (BMD) calculations and gene ontology (GO) classification. Gene expression changes in the rat nasal epithelium following acute formaldehyde exposure were used as a case study. The gene expression data were first analyzed using a one-way ANOVA to identify genes that showed significant dose-response behavior. These genes were then fit to a series of four statistical models (linear, second-degree polynomial, third-degree polynomial, and power models) and the least complex model that best described the data was selected. The genes were matched to their associated GO categories, and the average BMD and benchmark dose lower confidence limit (BMDL) were calculated for each GO category. The results were used to identify doses at which individual cellular processes were altered. For the formaldehyde exposures, the BMD estimates for the GO categories related to cell proliferation and DNA damage were similar to those measured in previous studies using cell labeling indices and DNA-protein cross-links and consistent with the BMD estimated for rat nasal tumors. The method represents a significant advance in applying genomic information to risk assessment by allowing a comprehensive survey of molecular changes associated with chemical exposure and providing the capability to identify reference doses at which particular cellular processes are altered.

*Key Words:* bioinformatics; methods; dose-response; risk assessment; nose; respiratory toxicology; microarray; methods; regulatory/policy; risk assessment; toxicogenomics; methods.

A major objective of toxicology and chemical risk assessment is to identify permissible exposure levels based on data from human or experimental animal studies together with other relevant scientific information. In the past, the permissible exposure levels were based on doses corresponding to lowest observed adverse effect levels (LOAEL) or no observed adverse effect levels (NOAEL). The LOAEL has been traditionally defined as the first dose producing a statistically significant, adverse change in the response and the NOAEL as the dose preceding the LOAEL. The weakness of this approach is that dose spacing and the experimental sample size can have a dramatic impact on the final NOAEL and LOAEL, and the approach does not account for variability in the estimate of the dose-response or the slope of the dose-response curve. To overcome these limitations, benchmark dose (BMD) analysis was introduced (Crump, 1984). BMD analysis fits a statistical model to the dose-response data and identifies a dose that causes a defined change in the adverse response. The application of BMD analysis provides several advantages including better use of dose-response information, more appropriate reflection of experimental sample sizes, and the lack of constraint to experimental doses (Filipsson *et al.*, 2003).

The application of microarray technology in toxicology has proven to be both useful for simultaneously measuring the expression of thousands of genes and challenging with respect to interpreting what changes in these genes mean in relation to the toxic response. The transcriptional changes represent only a snapshot of the state of the cell or tissue and include a complex mix of primary and secondary responses to the chemical treatment (Page *et al.*, 2006). Previous efforts to interpret these changes have focused on applying standardized functional annotations to each gene involved in the response and identifying whether certain biological processes or molecular functions are over- or underrepresented (Beissbarth and Speed, 2004; Dennis *et al.*, 2003; Khatri *et al.*, 2004; Yu *et al.*, 2006; Zhang *et al.*, 2004). This approach has been referred to as a gene ontology (GO) enrichment analysis and allows large lists of transcriptional alterations to be distilled down into changes in cellular processes such as the immune response, DNA repair, or apoptosis.

Although GO enrichment analyses provide insights into what biological processes are altered, this type of analysis has

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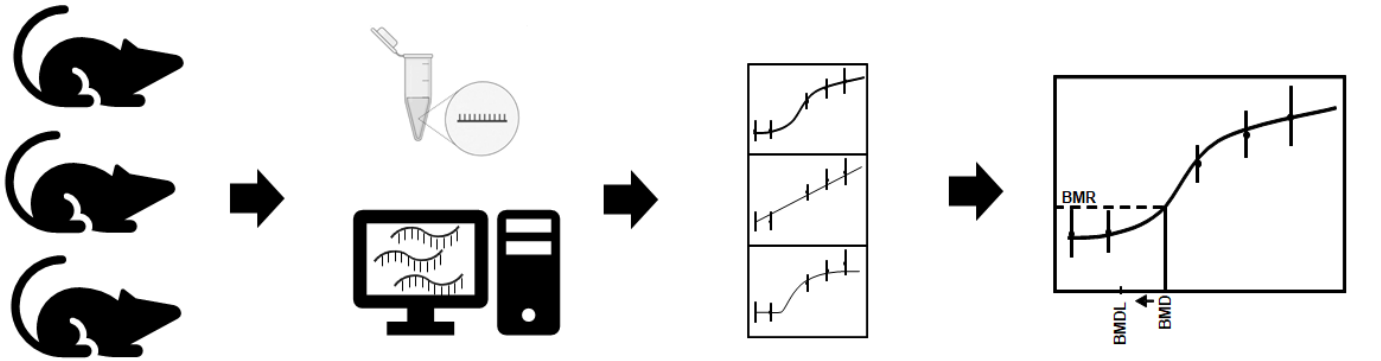
Thomas et al., *Toxicol Sci,* 2007

- Thomas et al. applied benchmark dose (BMD) modeling methods that are commonly used in regulatory risk assessment to transcriptomic data
- Demonstrated alignment of gene set-based transcriptomic and apical effect BMD values from chronic toxicity study
- A growing number of studies began using and adapting the approach to compare transcriptomic BMD values from short-term studies with apical responses in traditional toxicity studies



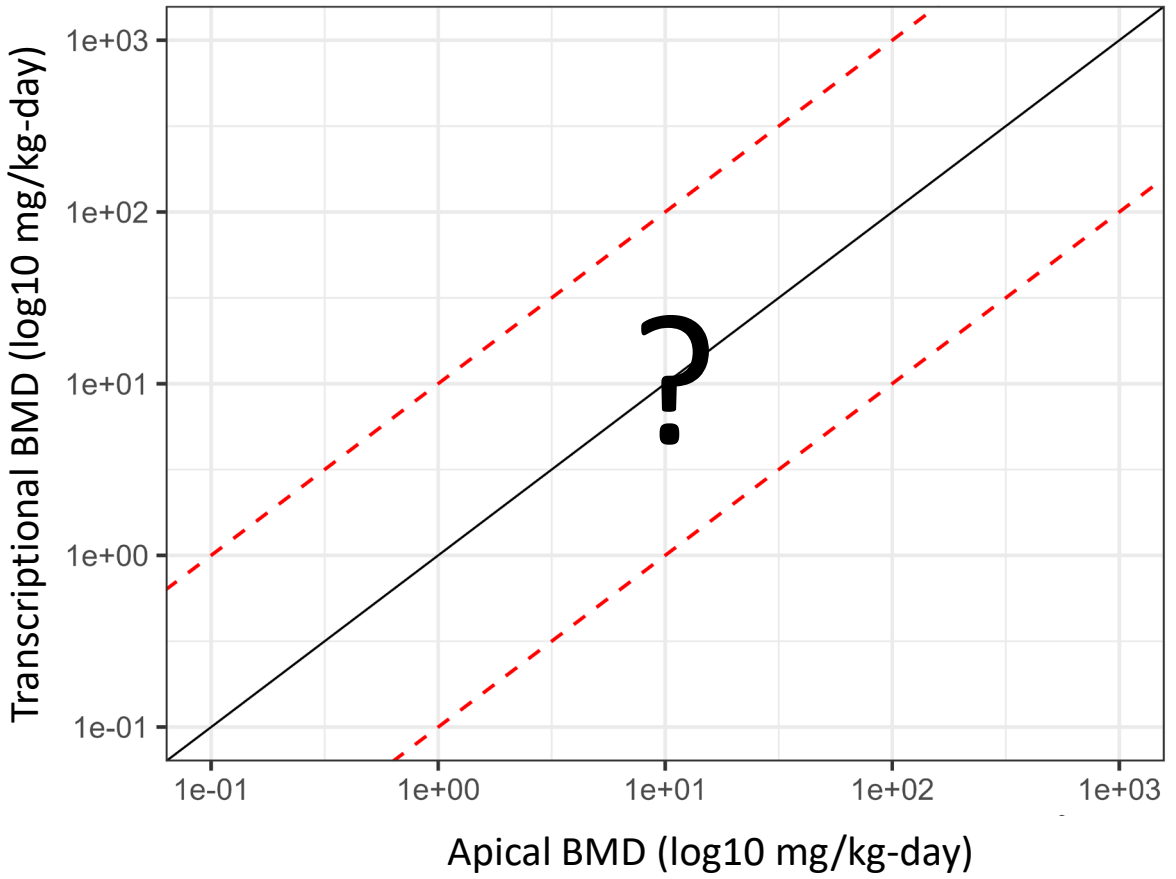

Short-term animal study

Measure gene expression

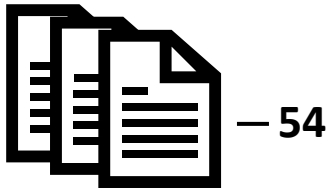
Fit gene transcripts to statistical models using BMD (and map to gene sets)

Identify point of departure typically based on BMD from gene set

# Initiated literature review to evaluate broader evidence base

# Used a set of expertly curated papers to identify studies with BMD modeling of gene expression data



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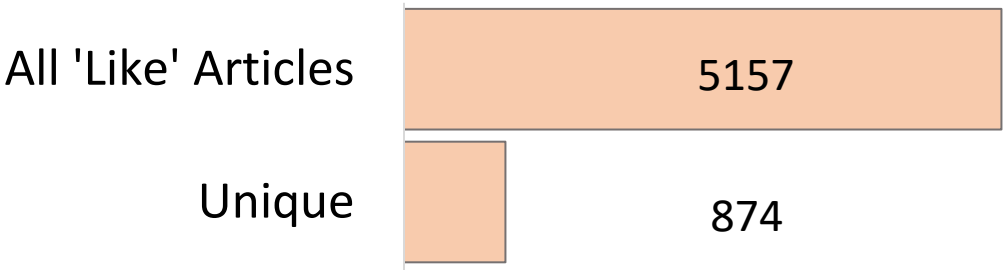


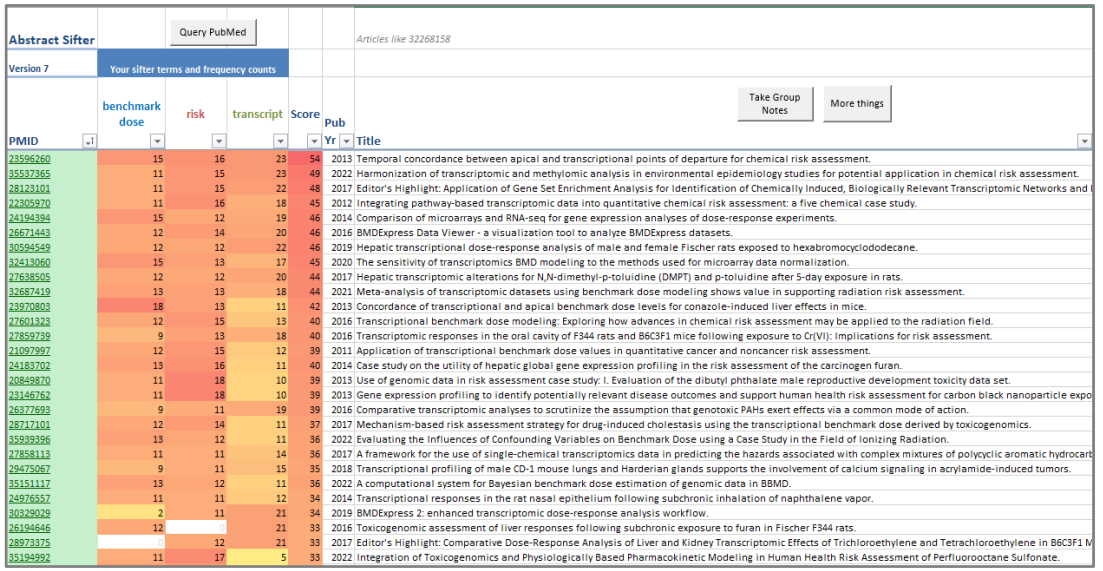

Abstract Sifter | Query PubMed | Articles like 32268158

Version 7 | Your sifter terms and frequency counts | Take Group Notes | More things

| PMID | benchmark dose | risk | transcript | Score | Pub Yr | Title |
|---|---|---|---|---|---|---|
| 23596260 | 15 | 16 | 23 | 54 | 2013 | Temporal concordance between apical and transcriptional points of departure for chemical risk assessment. |
| 35537365 | 11 | 15 | 23 | 49 | 2022 | Harmonization of transcriptomic and methylomic analysis in environmental epidemiology studies for potential application in chemical risk assessment. |
| 28123101 | 11 | 15 | 22 | 48 | 2017 | Editor's Highlight: Application of Gene Set Enrichment Analysis for Identification of Chemically Induced, Biologically Relevant Transcriptomic Networks and |
| 22305970 | 11 | 16 | 18 | 45 | 2012 | Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: a five chemical case study. |
| 24194394 | 15 | 12 | 19 | 46 | 2014 | Comparison of microarrays and RNA-seq for gene expression analyses of dose-response experiments. |
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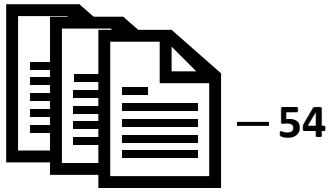
An initial set of 54 expertly curated papers was assembled focused on transcriptomic dose response analysis with application of benchmark dose methods.

https://pubmed.ncbi.nlm.nih.gov/help/#computation-of-similar-articles

# 214 articles identified as maybe relevant



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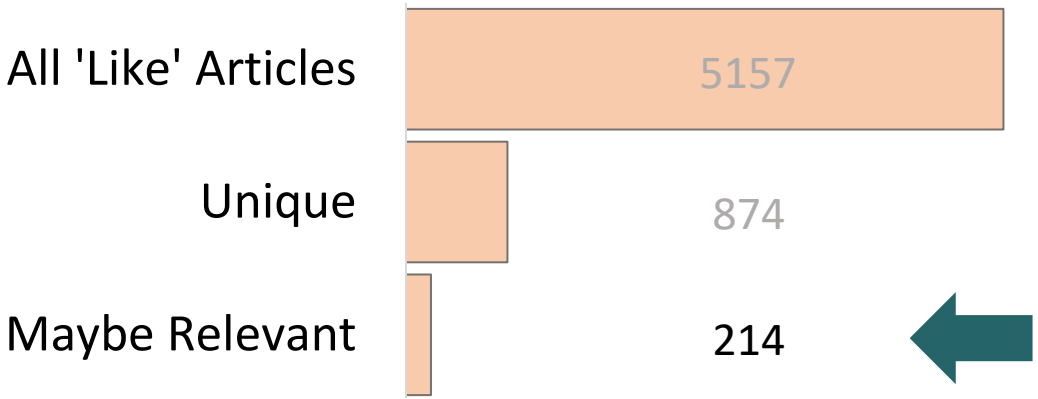


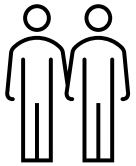

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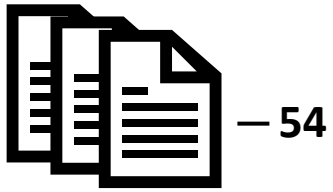
# 85 articles identified as likely relevant



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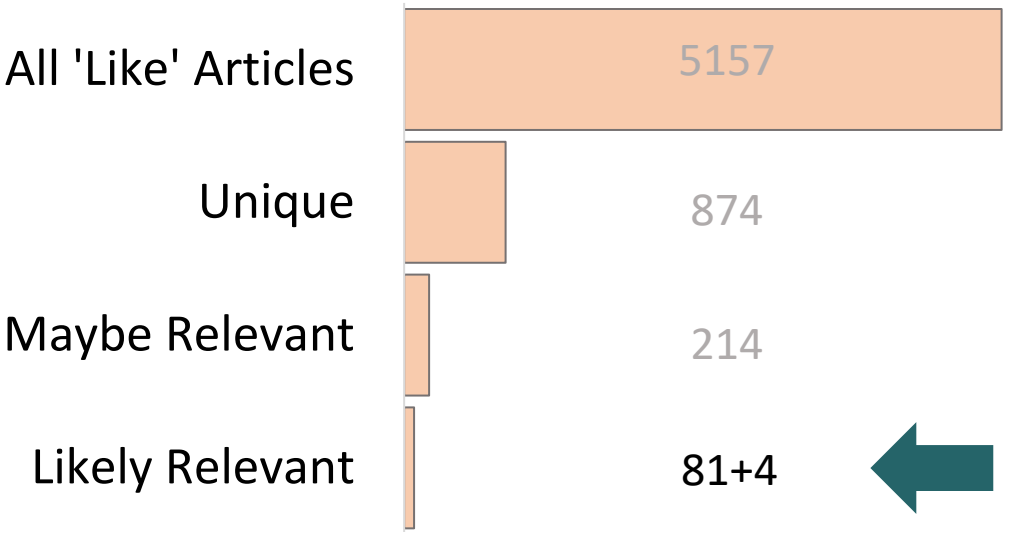
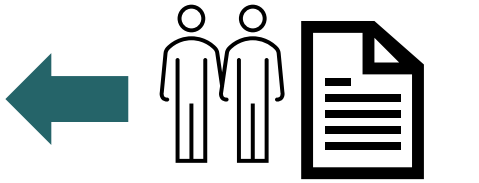


**NTP reports**

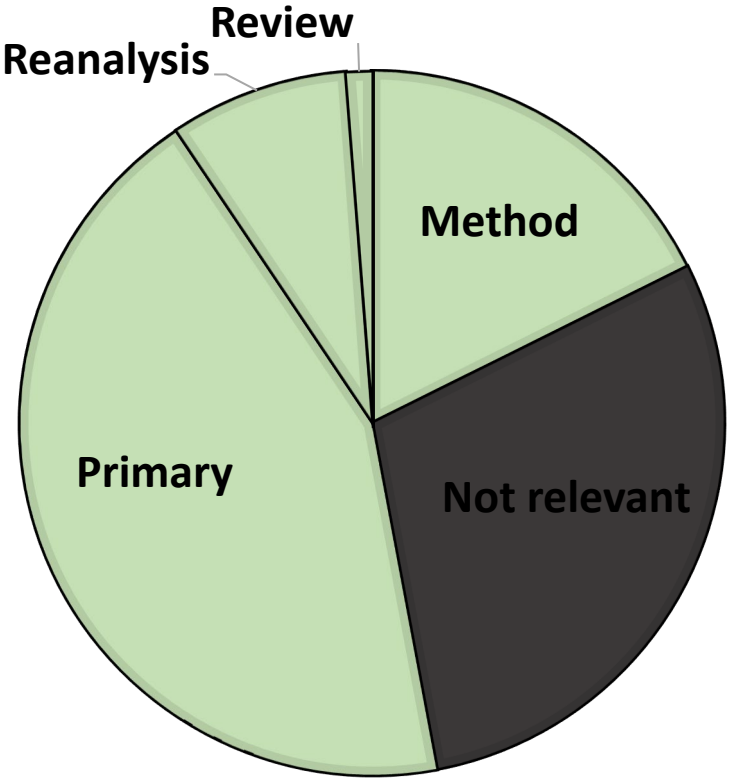
## Inclusion Criteria

- *In vivo* studies
- Mouse or rat
- Chemical exposures (non-particulate)
- At least 3 dose levels
- BMD on gene expression data
- Apical endpoint data (BMD or NOAEL/LOAEL)

# Almost half of relevant articles were primary research

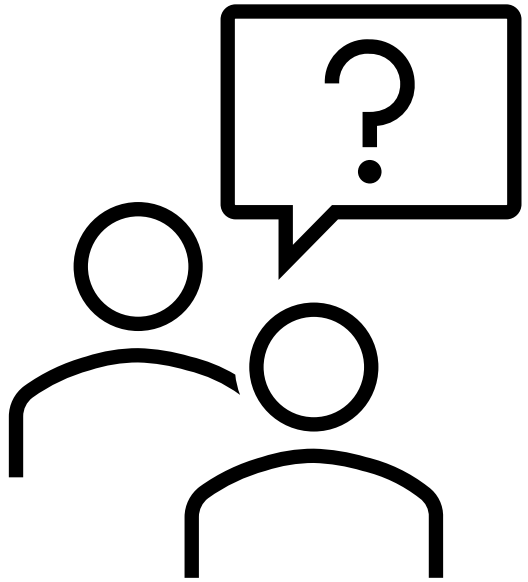
**32 primary studies**

- 140 chemicals
- Both genders
- Mix of mouse and rat
- Multiple durations
- Differing modes of action
- Oral and inhalation routes of exposure
- Target and sentinel tissues
- Three gene expression platforms

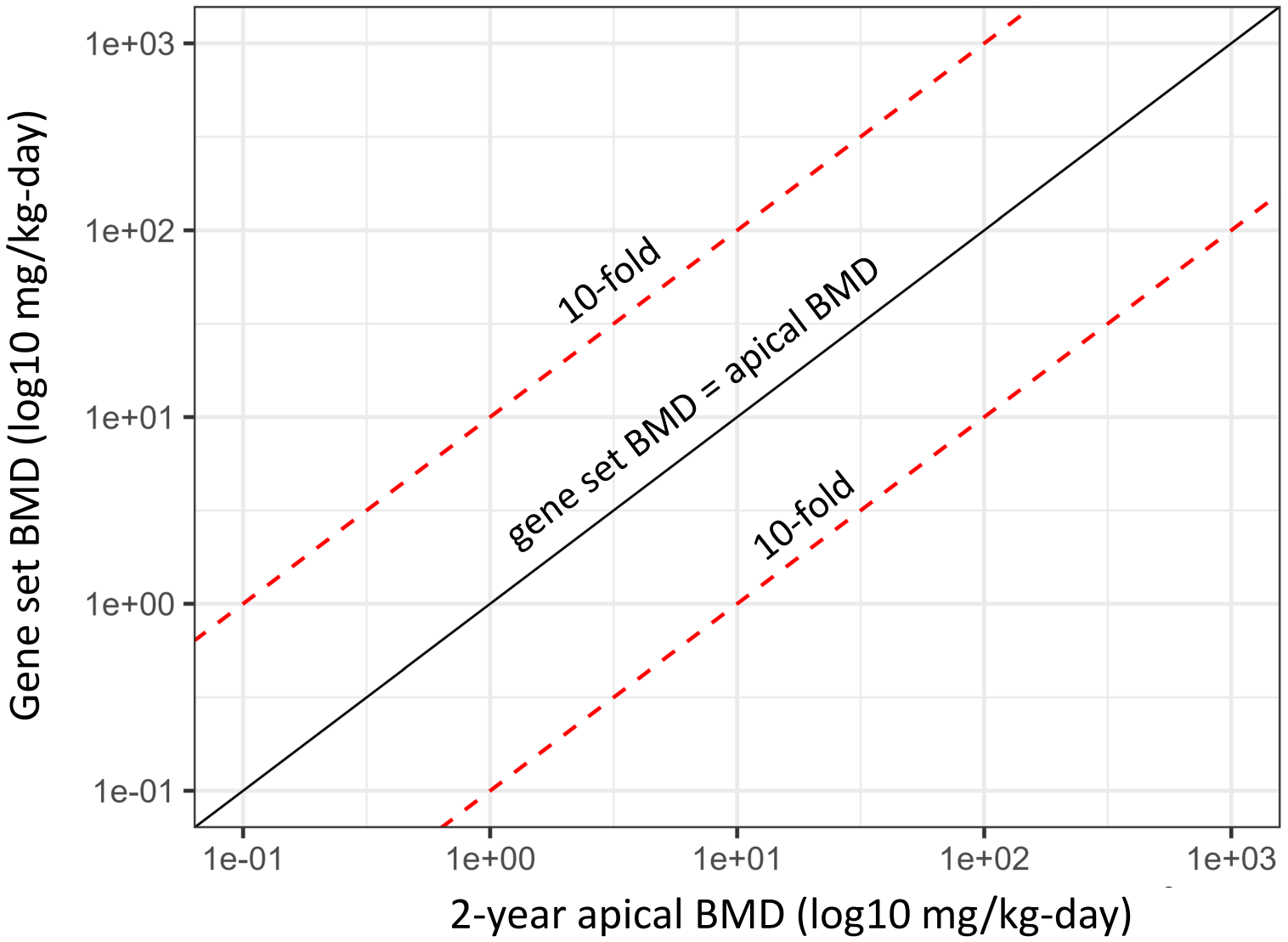


# Analysis of literature studies to evaluate important considerations for application of dose response assessment to transcriptomics

- Study duration
- Chemical modes of action
- Chemical properties
- Route of exposure
- Tissue selection
- Gene expression platform

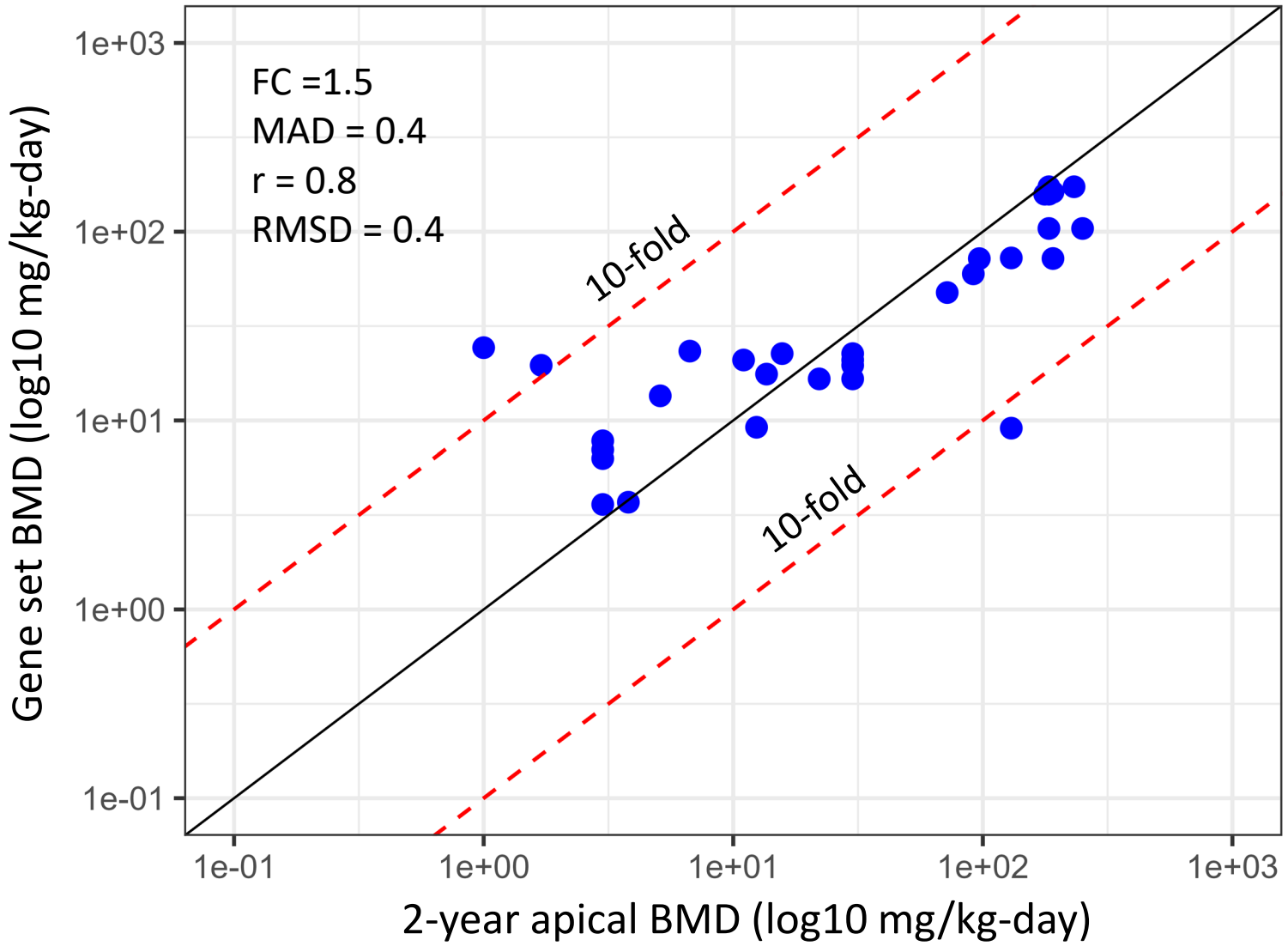


# Scatter plots used to compare gene set BMD to apical BMD




On solid line: Gene set BMD = Apical
Shift right: Gene set BMD < Apical
Shift left: Gene set BMD > Apical

# Example scatter plots used to compare gene set BMD to apical BMD



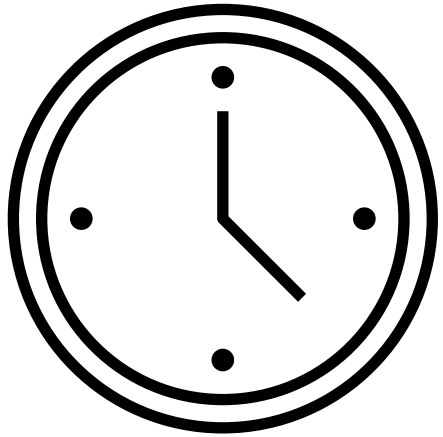

On solid line: Gene set BMD = Apical
Shift right: Gene set BMD < Apical
Shift left: Gene set BMD > Apical

- **FC** - median absolute fold-change
- **MAD**- median absolute deviation of FC
- **r** – Pearson correlation coefficient
- **RMSD** - root mean squared difference

**Data on graph are not real and provided for demonstration purposes.*

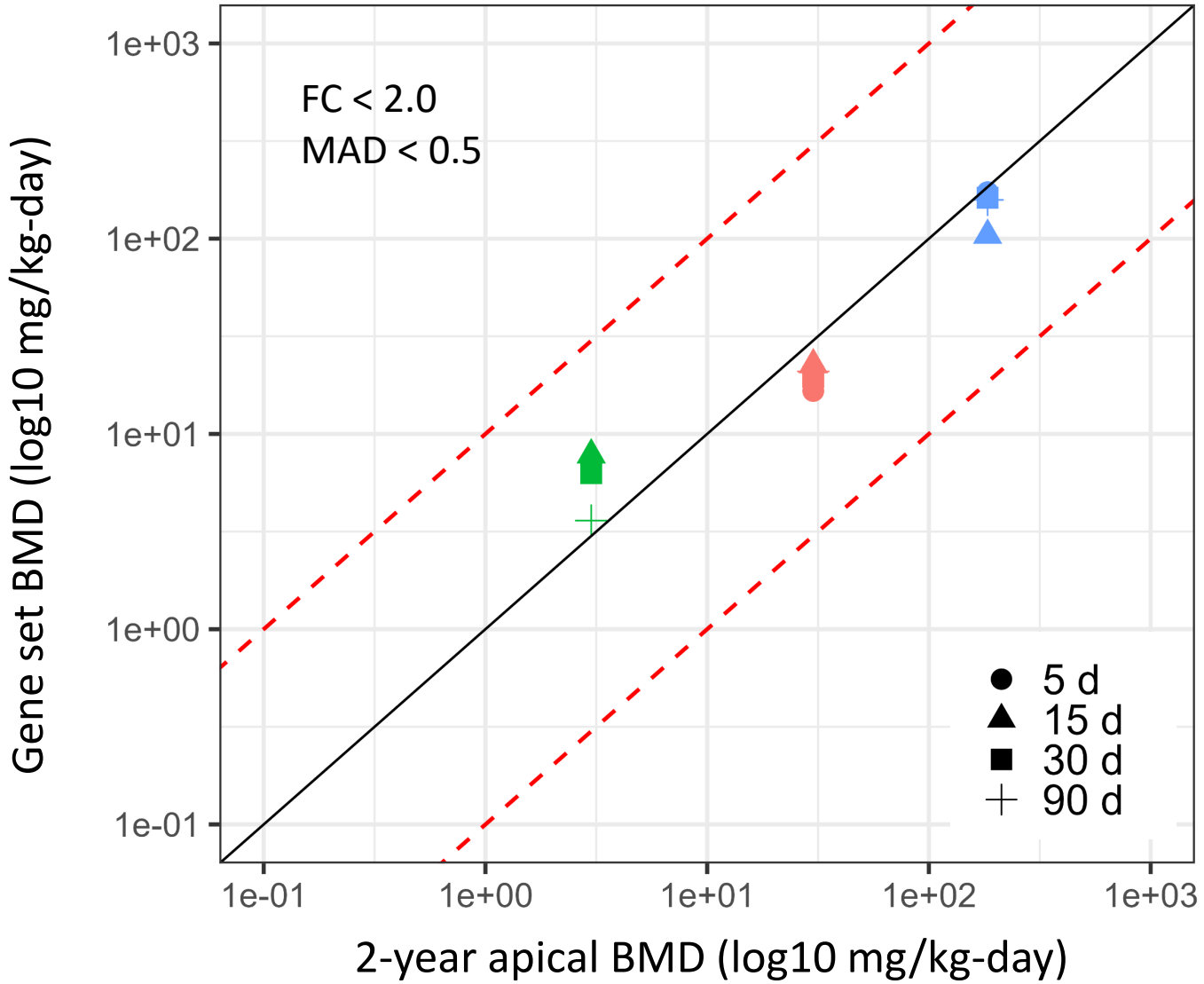
# Wide range in study durations identified

- Gene-set BMD(L)
  - 1 to 90 days
- Apical comparisons
  - Concurrent or later
  - 1 to 720 days
  - LOAEL/NOAEL
  - BMD(L)
- Few systematic investigations of duration



**Identify study duration sufficient to indicate chronic adverse effects**

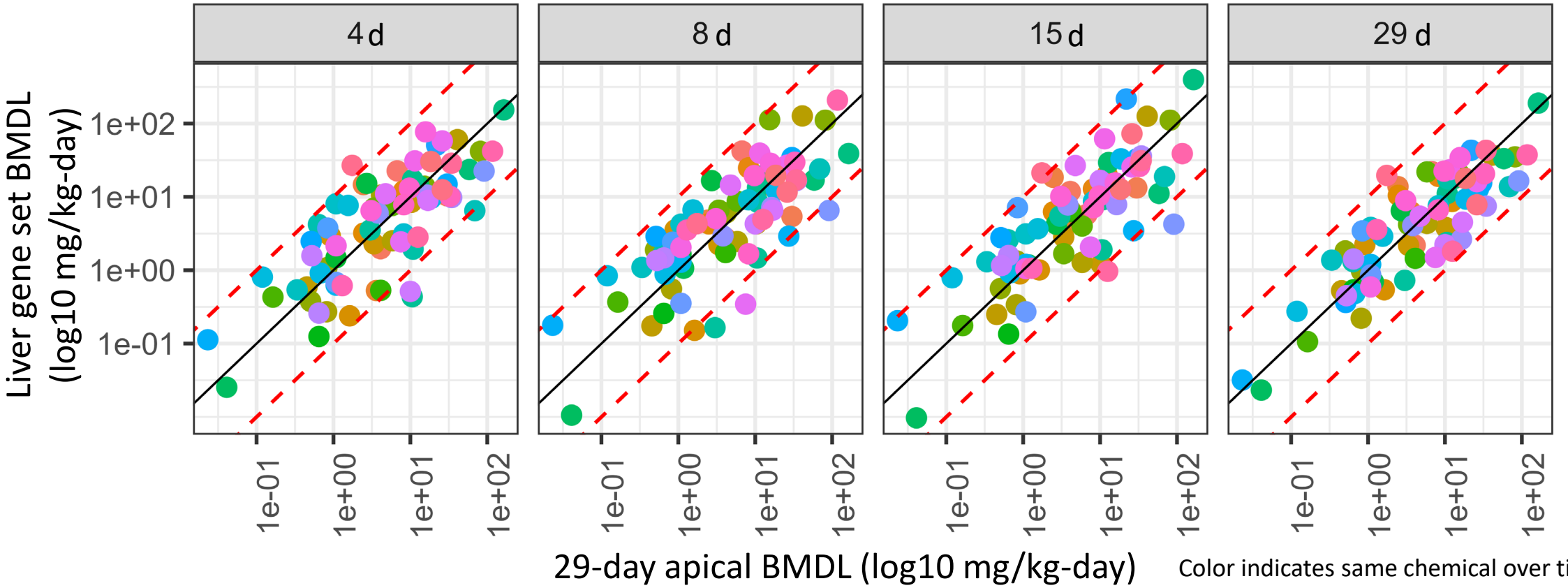
# Gene set BMD consistent with 2-year apical BMD across time for 3 industrial chemicals




4,4-methylenebis (n,n-dimethyl) benzenamine
hydrazobenzene
n-nitrosodiphenylamine

Thomas et al. 2013

# Gene set BMDL consistent with 29-day apical BMDL across time for 79 chemicals

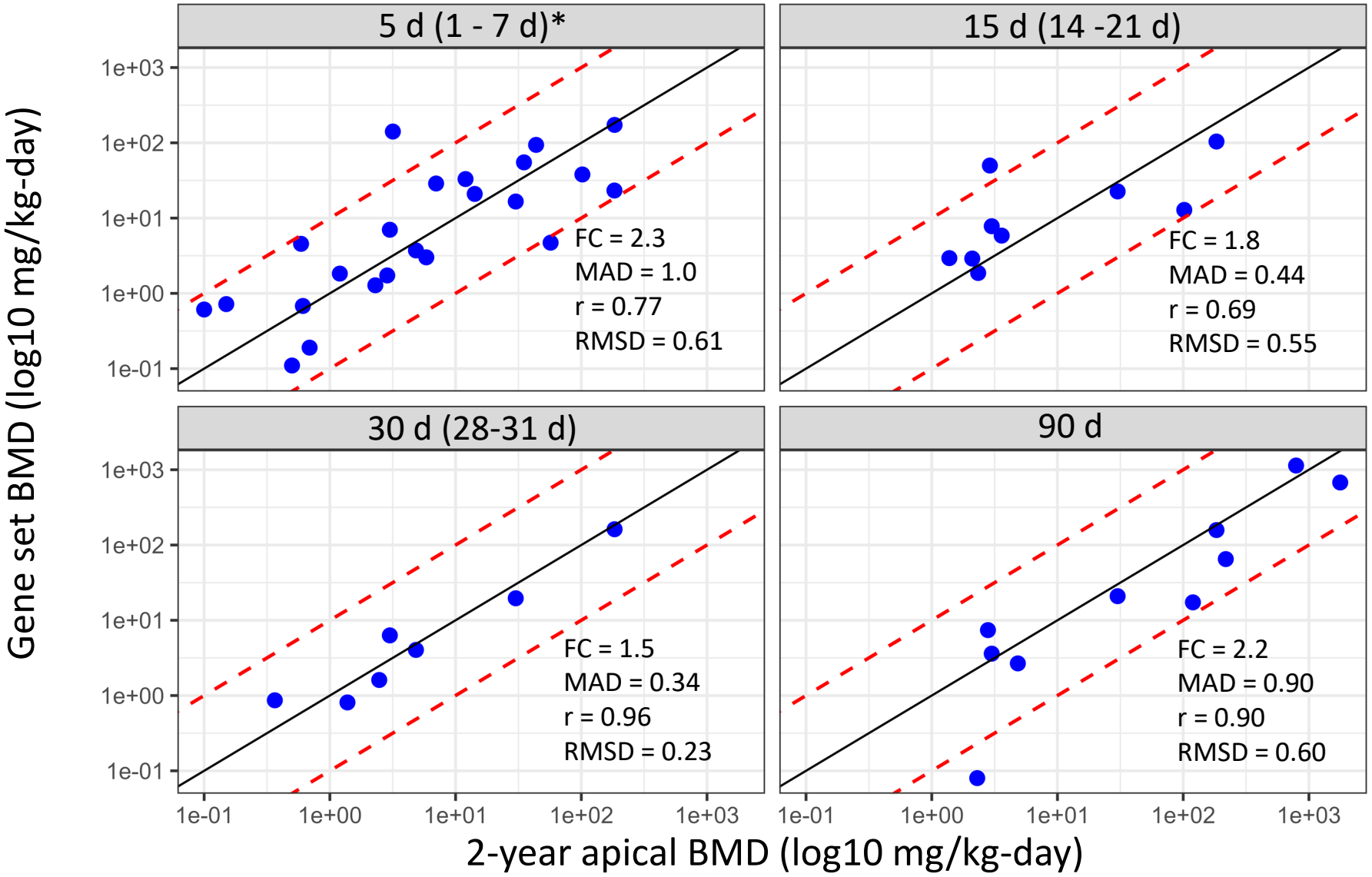



Color indicates same chemical over time

| Day | 4 | 8 | 15 | 29 |
|---|---|---|---|---|
| FC | 2.27 | 2.37 | 2.00 | 1.97 |
| MAD | 0.91 | 0.87 | 0.89 | 0.74 |
| r | 0.77 | 0.78 | 0.80 | 0.86 |
| RMSD | 0.52 | 0.51 | 0.49 | 0.41 |

Johnson et al 2020

# Gene set BMD consistent with 2-year apical BMD across time



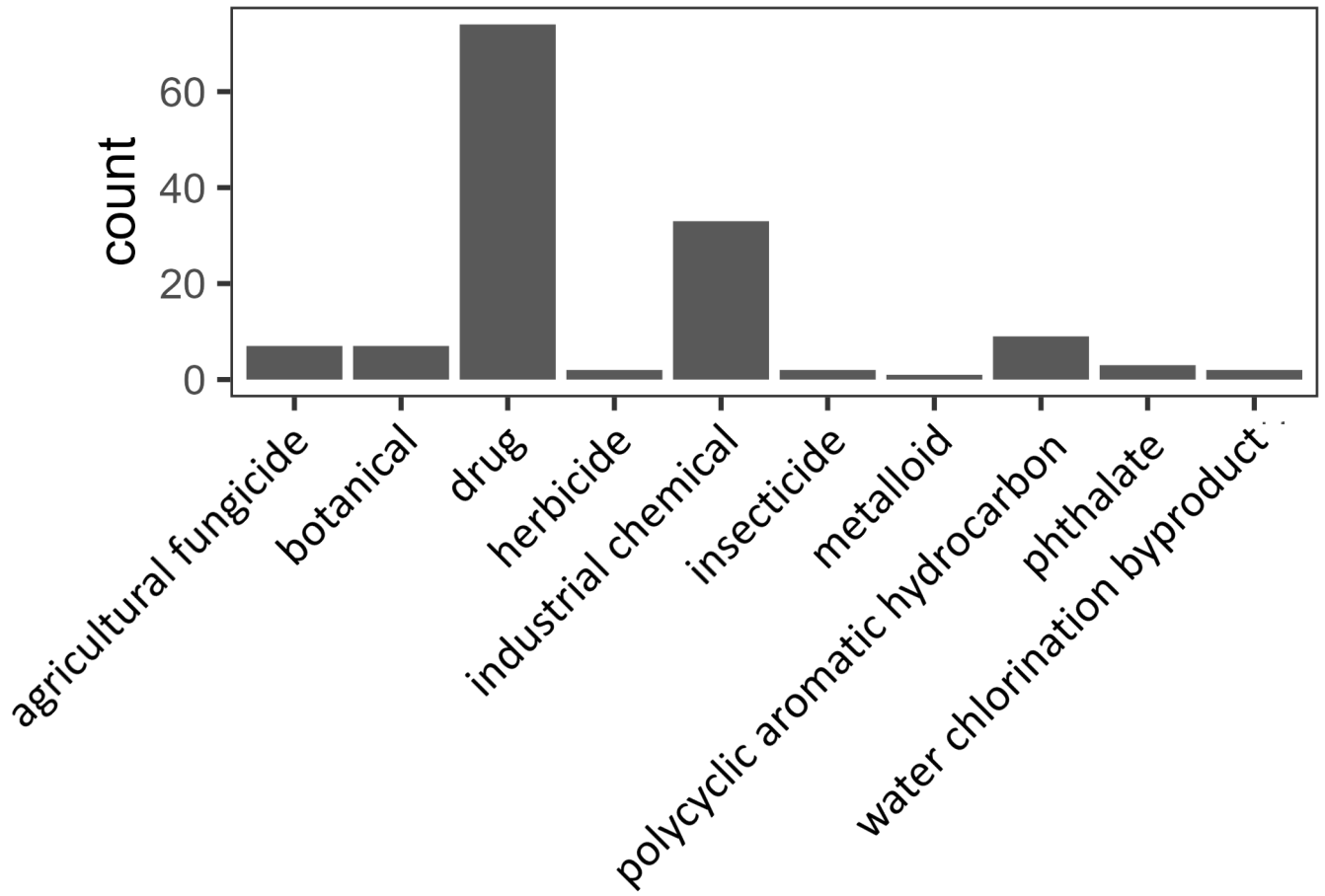

1-90-day BMD vs. 2-year BMD

- 38 chemicals
- 14 studies
- r = 0.82
- FC = 1.9
- MAD = 0.68
- RMSD = 0.56

***5 d (1-7 d) gene set BMD mainly derived from surrogate tissue***

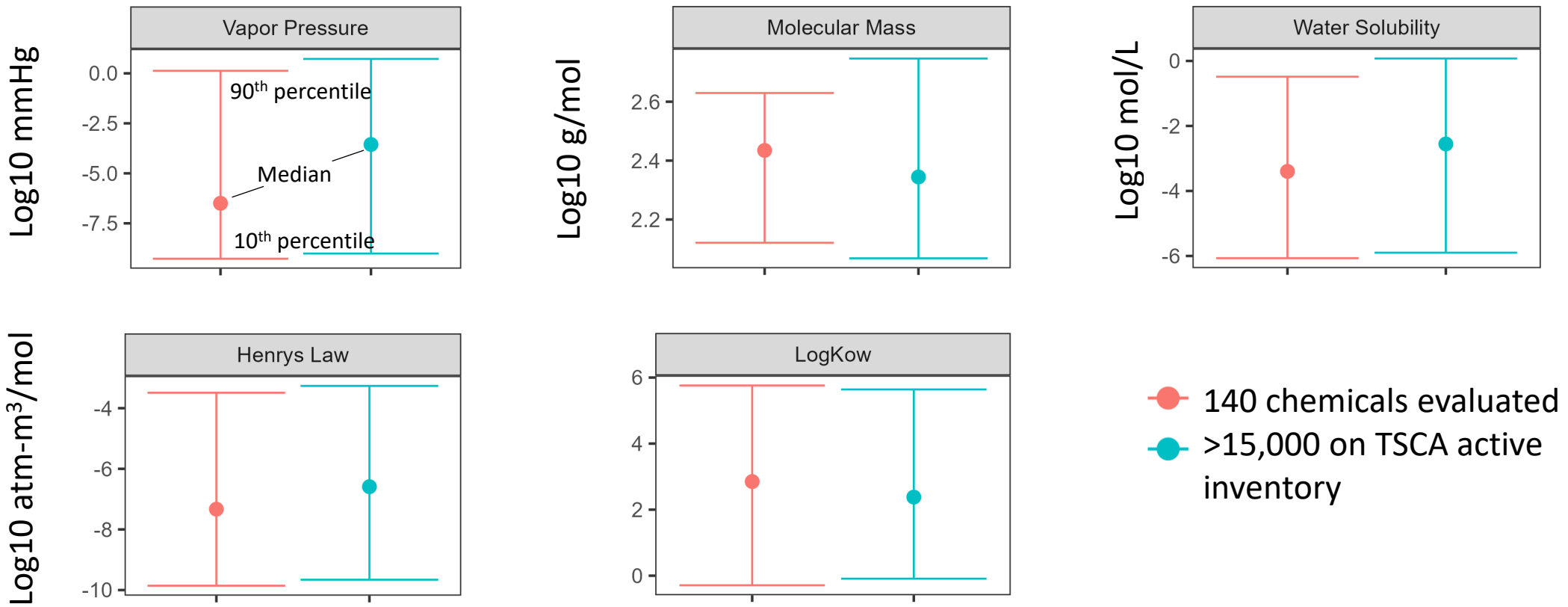
Andersen et al. 2010; Bercu et al. 2010; Bhat et al. 2013; Bianchi et al. 2021; Cannizzo et al. 2022; Chepelev et al. 2017, 2018; Dong et al. 2016; Gwinn et al. 2020; Jackson et al. 2014; LaRocca et al. 2020; Thomas et al. 2011; Thomas et al. 2013a; Thomas et al. 2013b

# Evaluated chemicals span a range of toxicity domains, types, and modes of action



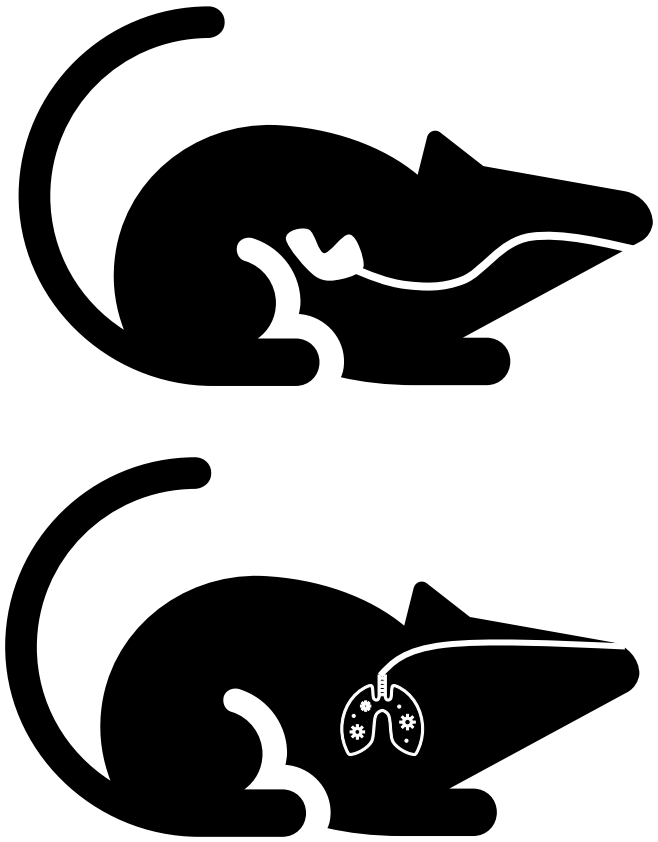

- Neuroactive chemicals
- Anticancer agents
- Endocrine active chemicals
- Receptor mediated effects
- Genotoxic and carcinogenic chemicals

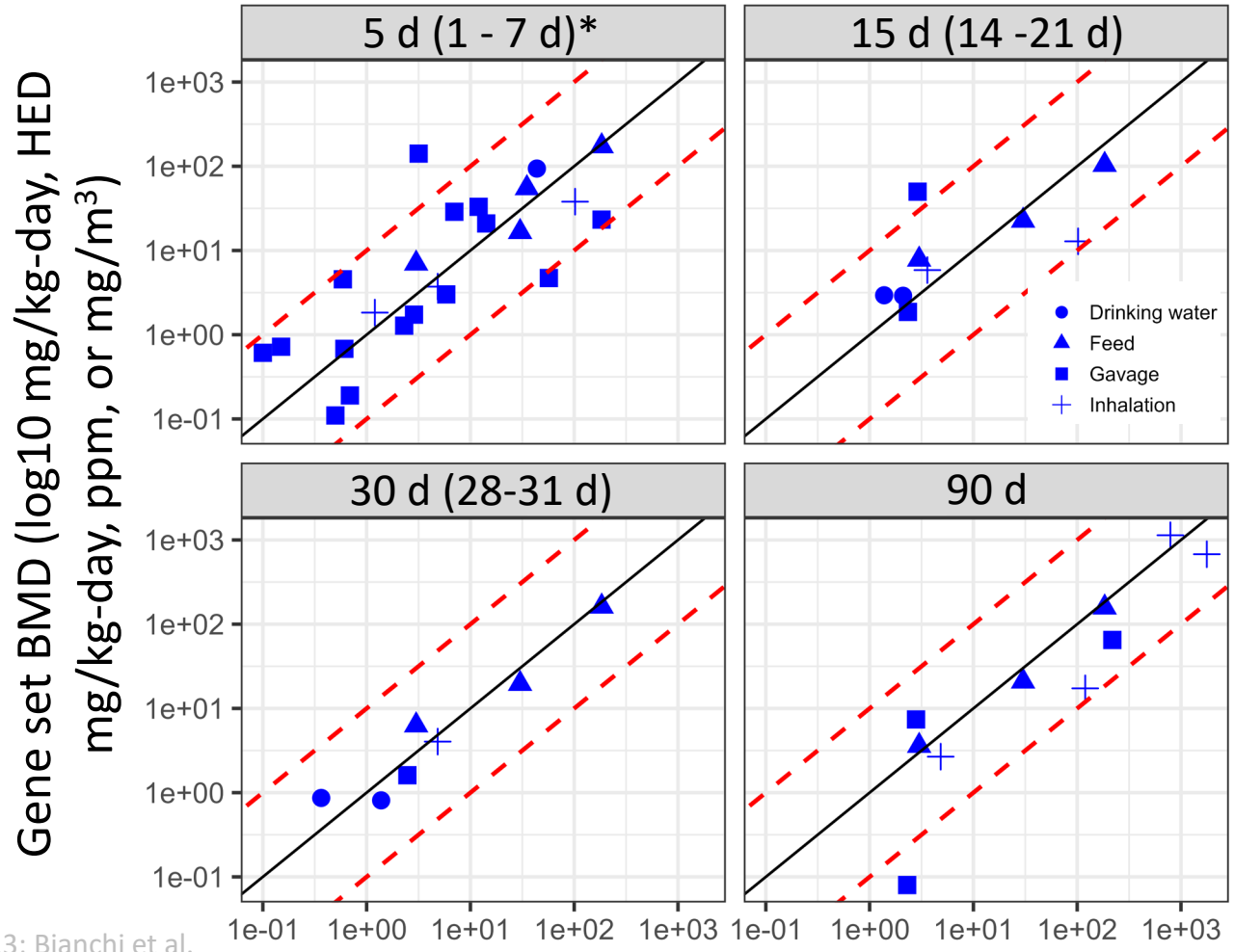
# Physicochemical properties of tested chemicals consistent with TSCA active inventory

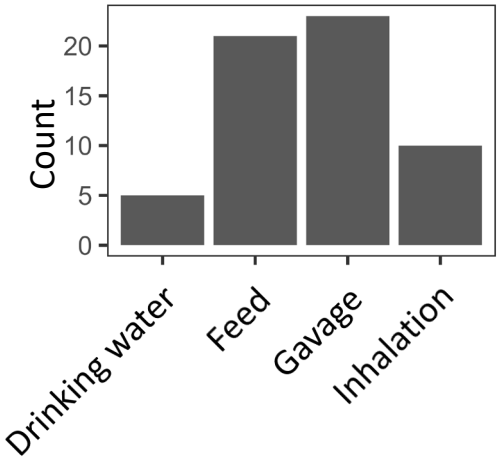



Predicted physicochemical properties were obtained from the EPA CompTox Chemicals Dashboard using the OPERA model. Physicochemical properties were not able to be predicted for all chemicals.

# Route of exposure did not greatly impact 1 to 90-day gene set BMD concordance





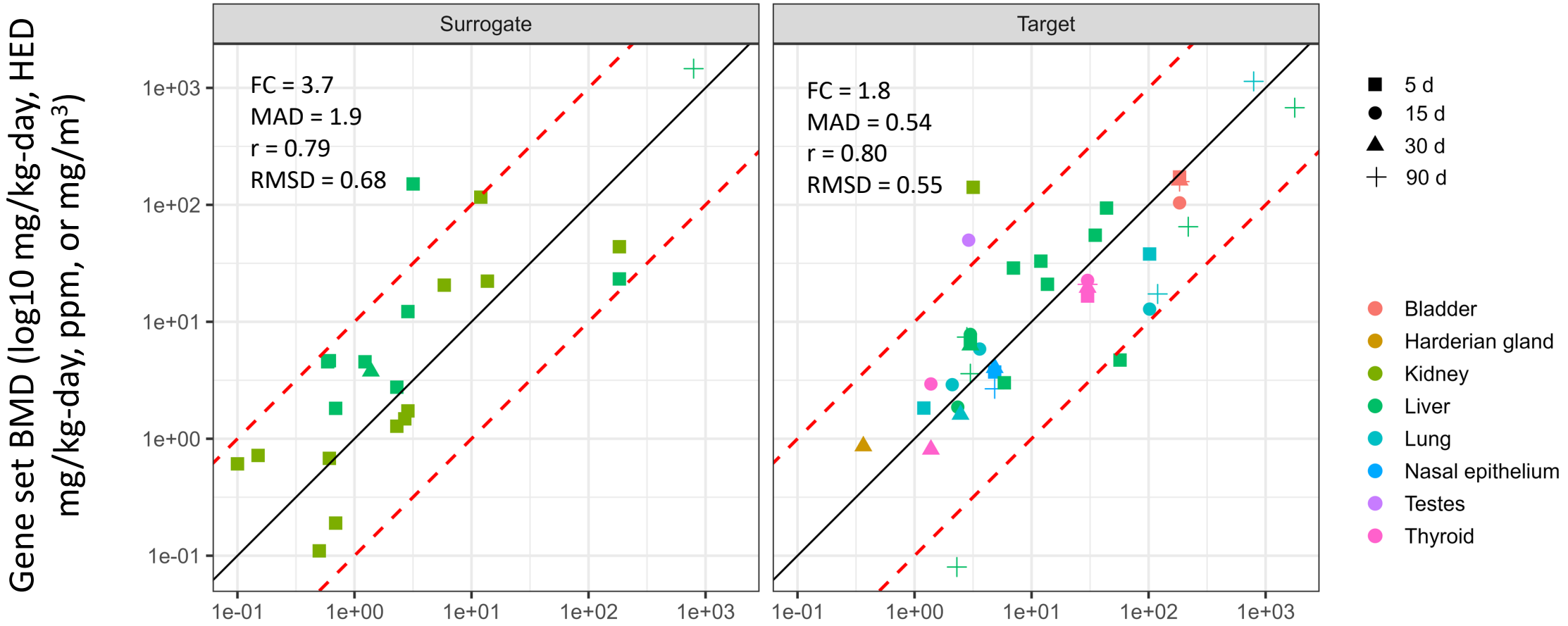

Oral

- r = 0.82
- FC = 2.1
- MAD = 0.72
- RMSD = 0.59

***5 d (1-7 d) gene set BMD mainly derived from surrogate tissue***

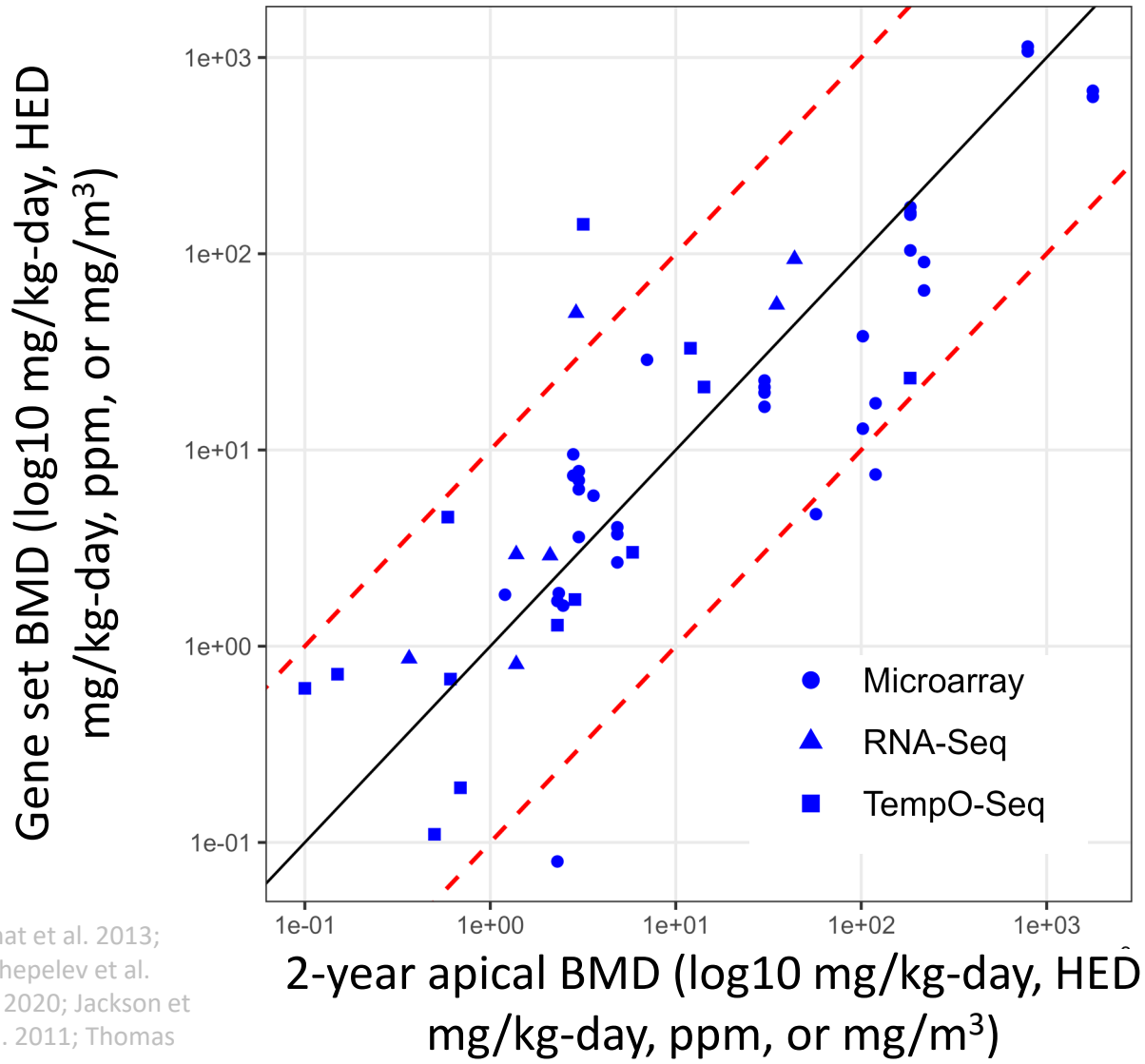
Andersen et al. 2010; Bercu et al. 2010; Bhat et al. 2013; Bianchi et al. 2021; Cannizzo et al. 2022; Chepelev et al. 2017, 2018; Dong et al. 2016; Gwinn et al. 2020; Jackson et al. 2014; LaRocca et al. 2020; Thomas et al. 2011; Thomas et al. 2013a; Thomas et al. 2013b

# Target tissue generally performed better for 1 to 90-day gene set BMD concordance




Andersen et al. 2010; Bercu et al. 2010; Bhat et al. 2013; Bianchi et al. 2021; Cannizzo et al. 2022; Chepelev et al. 2017, 2018; Dong et al. 2016; Gwinn et al. 2020; Jackson et al. 2014; LaRocca et al. 2020; Thomas et al. 2011; Thomas et al. 2013a; Thomas et al. 2013b

# Gene set BMD concordance consistent across platforms




Chemicals and experimental designs differed across platforms

Andersen et al. 2010; Bercu et al. 2010; Bhat et al. 2013; Bianchi et al. 2021; Cannizzo et al. 2022; Chepelev et al. 2017, 2018; Dong et al. 2016; Gwinn et al. 2020; Jackson et al. 2014; LaRocca et al. 2020; Thomas et al. 2011; Thomas et al. 2013a; Thomas et al. 2013b

# Summary

- The combined set of 1-90-day gene set BMDs are generally concordant with 2-year apical BMDs
  - Pearson correlation r = 0.83
  - RMSD = 0.56; similar interstudy variability of traditional toxicity studies
  - FC = 1.9; MAD = 0.7
- Gene set BMDs following 5-day exposure showed similar concordance with 2-year apical BMDs as other time points
- Concordance of gene set BMDs with 2-year apical BMDs was robust across route of exposure, physicochemical properties, mode-of-action, and measurement platform
- Gene set BMDs from target tissues were more concordant with 2-year apical BMDs than surrogate/sentinel tissues supporting the collection and analysis of multiple tissues in an ETAP