Deriving Points of Departure using Toxicogenomics for Chemical Risk Assessment

Andrew Williams
Environmental Health Science and Research Bureau
Health Canada
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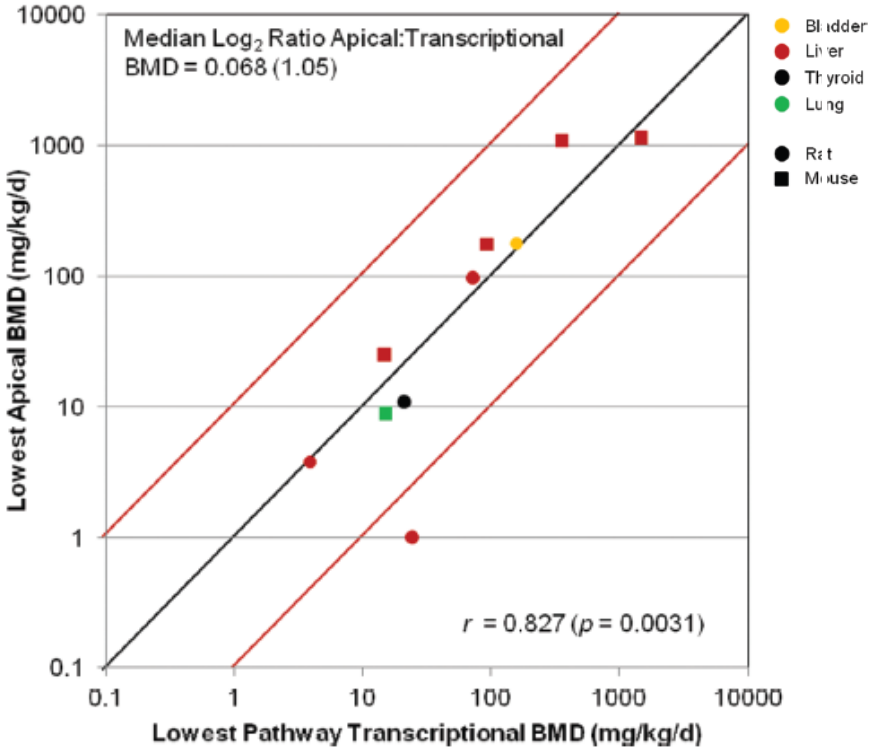
Temporal Concordance Between Apical and Transcriptional Points of Departure for Chemical Risk Assessment

Russell S. Thomas,*,1 Scott C. Wesselkamper,† Nina Ching Y. Wang,† Q. Jay Zhao,† Dan D. Petersen,† Jason C. Lambert,† Ila Cote,‡ Longlong Yang,* Eric Healy,* Michael B. Black,* Harvey J. Clewell III,* Bruce C. Allen,§ and Melvin E. Andersen*



Data at 13 weeks of exposure when combined with data from a previous study (Thomas et al., 2012).

Gene Expression Omnibus

GSE45892

- Affymetrix Arrays
- Time Points (5 days and 2, 4 and 13 weeks)
- 5 Doses per Chemical
- RMA Normalization with a log2 transformation
- BMDExpress (Version 1.41)
  - Linear
  - 2° & 3° Polynomial
  - Power
- Benchmark response (BMR)
  - 1.349 multiplied by the SD in the controls
- Model Selection
  - LRT and AIC
- Filtering
  - BMDs > Highest Dose
  - Goodness-of-fit p value < 0.1
- Gene Sets
  - GeneGo Metacore database (744 Gene Sets)
  - Gene Sets with fewer than 5 genes with BMD values were removed
- Median BMD and BMDL values were used to summarize each pathway.

Comparison of toxicogenomics and traditional approaches to inform mode of action and points of departure in human health risk assessment of benzo[*a*]pyrene in drinking water

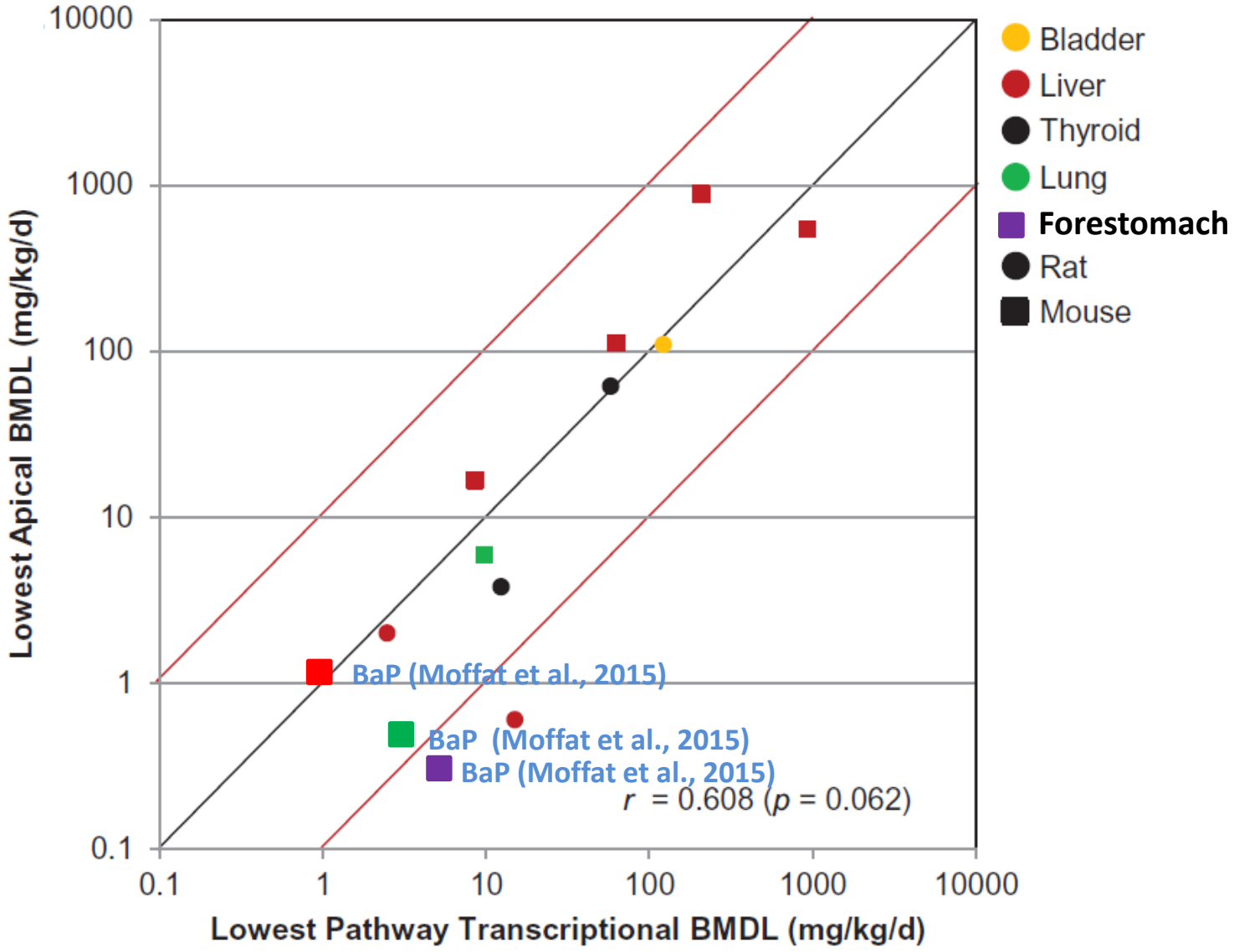
Ivy Moffat[#1,2], Nikolai Chepelev[#2], Sarah Labib[2], Julie Bourdon-Lacombe[1,2], Byron Kuo[2], Julie K. Buick[2], France Lemieux[1], Andrew Williams[2], Sabina Halappanavar[2], Amal Malik[2], Mirjam Luijten[3], Jiri Aubrecht[4], Daniel R. Hyduke[5], Albert J. Fornace Jr.[6], Carol D. Swartz[7], Leslie Recio[7], and Carole L. Yauk[2]

Crit Rev Toxicol. 2015 January ; 45(1): 1–43.

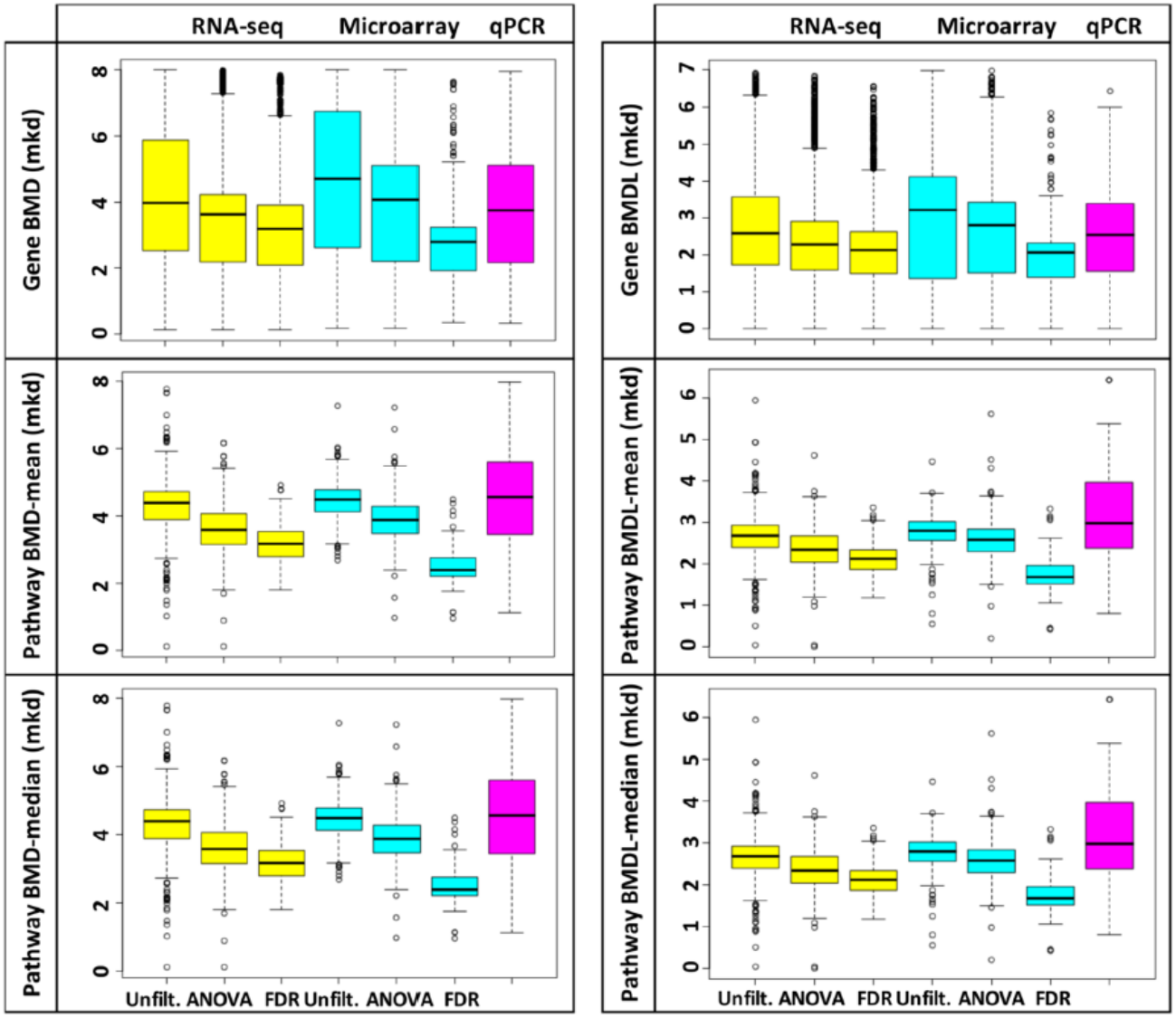
Approach	Liver		Lung		Forestomach	
	BMD/BMDL	Response	BMD/BMDL	Response	BMD/BMDL	Response
Traditional	1.8/1.2	tumors	0.8*	tumors	0.8/0.5	tumors
Mutations	7.2/4.8	mutations	2.2/1.4	mutations	0.5/0.3	mutations
Toxicogenomics						
Key Event preceding the committed step	8.1/1	DNA damage	14.8/3.7	DNA damage	11.4/7.4	p53 signaling
Lowest MOA associated pathway	8.1/1	DNA damage	14.8/3.7	DNA damage	11.4/7.4	p53 signaling
10th Percentile BMD Pathway	0.3/0.2	notch signaling	15.7/2.1	cellular effects of sildenafil	16.1/4.5	phenylalanine degradation IV

"PODs for traditional and transcriptional approaches were similar (liver 1.2 vs. 1.0 mg/kg-bw/day; lung 0.8 vs. 3.7 mg/kg-bw/day; forestomach 0.5 vs. 7.4 mg/kg-bw/day)"

Temporal Concordance of Apical and Transcriptional PODs



Impact of Filtering Genes and/or Pathways following Furan Exposure



Webster et al. (2015). Impact of Genomics Platform and Statistical Filtering on Transcriptional Benchmark Doses (BMD) and Multiple Approaches for Selection of Chemical Point of Departure (PoD). PLoS One. 2015 Aug 27;10(8)

Arch Toxicol (2017) 91:2045–2065
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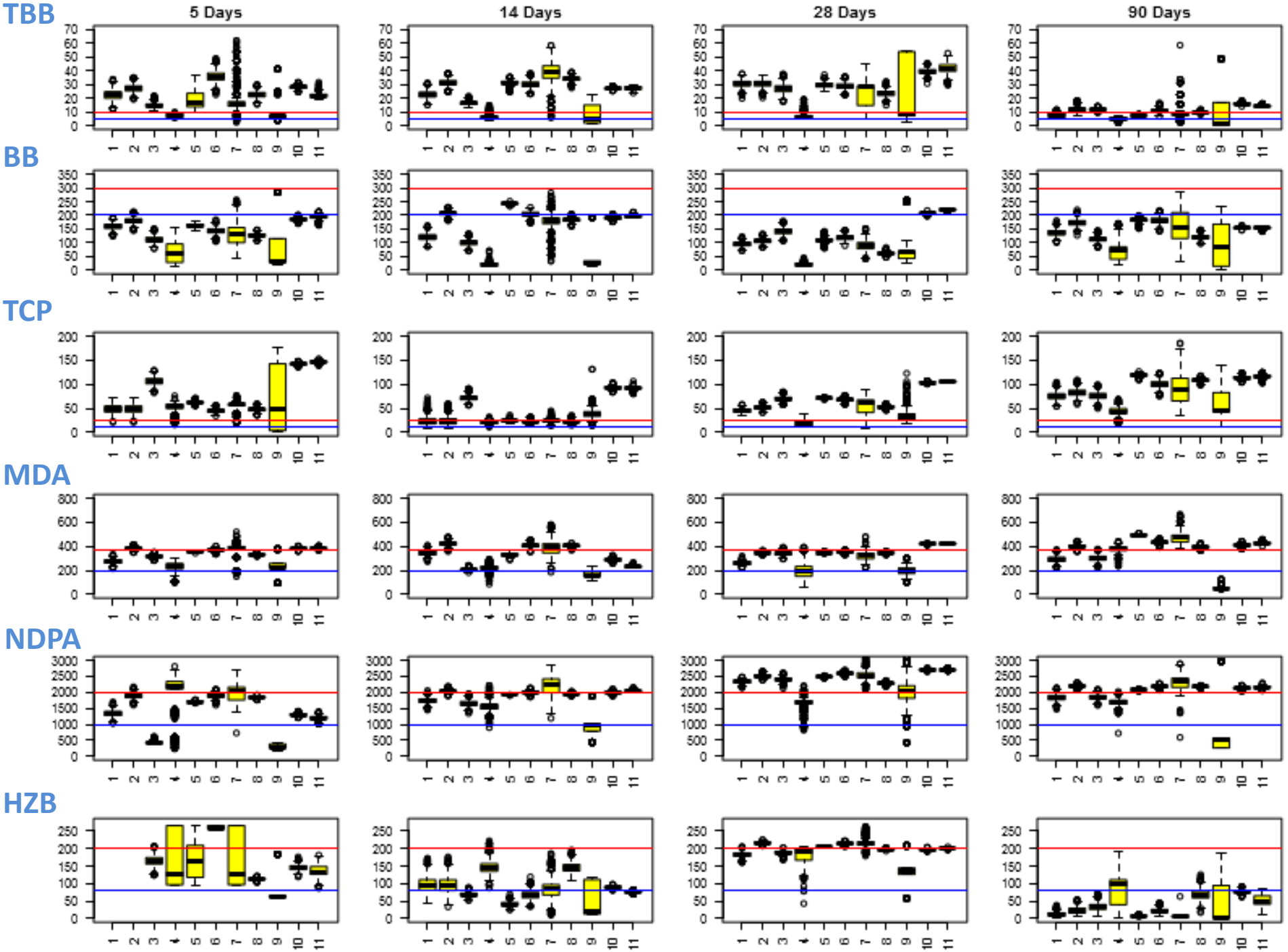
REGULATORY TOXICOLOGY

Recommended approaches in the application of toxicogenomics to derive points of departure for chemical risk assessment

Reza Farmahin[1] · Andrew Williams[1] · Byron Kuo[1] · Nikolai L. Chepelev[1] · Russell S. Thomas[2] · Tara S. Barton-Maclaren[3] · Ivan H. Curran[4] · Andy Nong[1] · Michael G. Wade[1] · Carole L. Yauk[1]

1. The 20 significantly enriched pathways with the lowest BMDs.
2. The 20 most statistically significantly enriched pathways.
3. The 20 lowest pathway BMDs.
4. The 20 genes with the largest fold changes relative to controls.
5. Genes with BMDs within the 25th–75th percentile.
6. The 20 pathways with the greatest number of shared genes.
7. The 20 genes that contribute to the greatest number of enriched pathways.
8. The BMDs of genes that are regulated by the 20 most significant upstream regulators.
9. The significantly enriched pathway with the lowest BMD (i.e., most sensitive pathway).
10. The mean of gene BMDs across all pathways.
11. The median gene BMD across all pathways.


TBB
5 Days
14 Days
28 Days
90 Days
BB
TCP
MDA
NDPA
HZB

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]

Arch Toxicol (2017) 91:1685–1696

Experimental Design

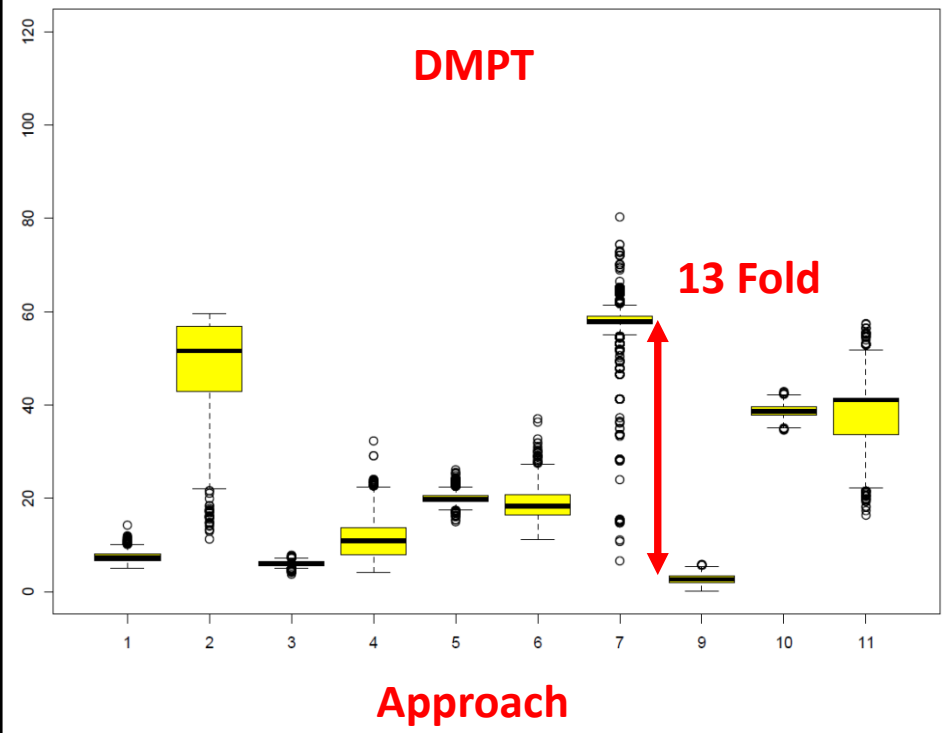
- Tissue: Liver
- Platform: Affymetrix Arrays
- Exposure: Orally for 5 Days
- Doses: 6 Doses per Chemical

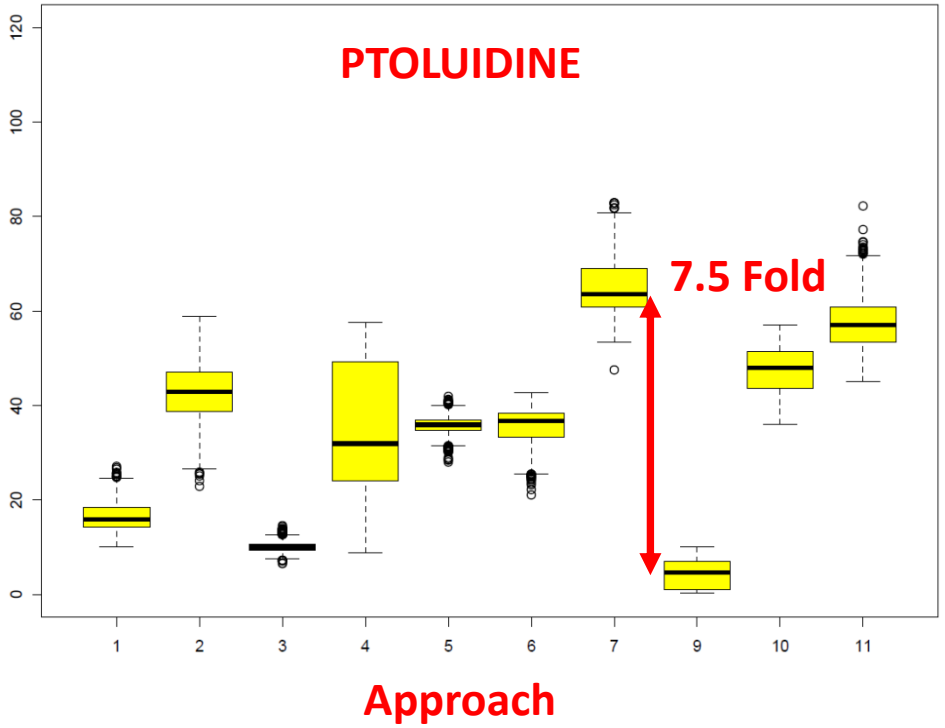
Microarray Results

- DEGs
  - DMPT: 2, 28, 176, 125 and 454
  - p-toluidine: 2, 11, 41, 81 and 305

BMDExpress

- GO Biological Process Category Pathways (2882)
- Filtering: ANOVA FDR p-value < 0.05,
- BMDLs
  - Regulation of Fatty Acid Transport
    - DMPT: 2 mg/kg/day
    - p-toluidine: 7 mg/kg/day
  - Prostanoid or Prostaglandin metabolic process
    - DMPT: 0.5 mg/kg.





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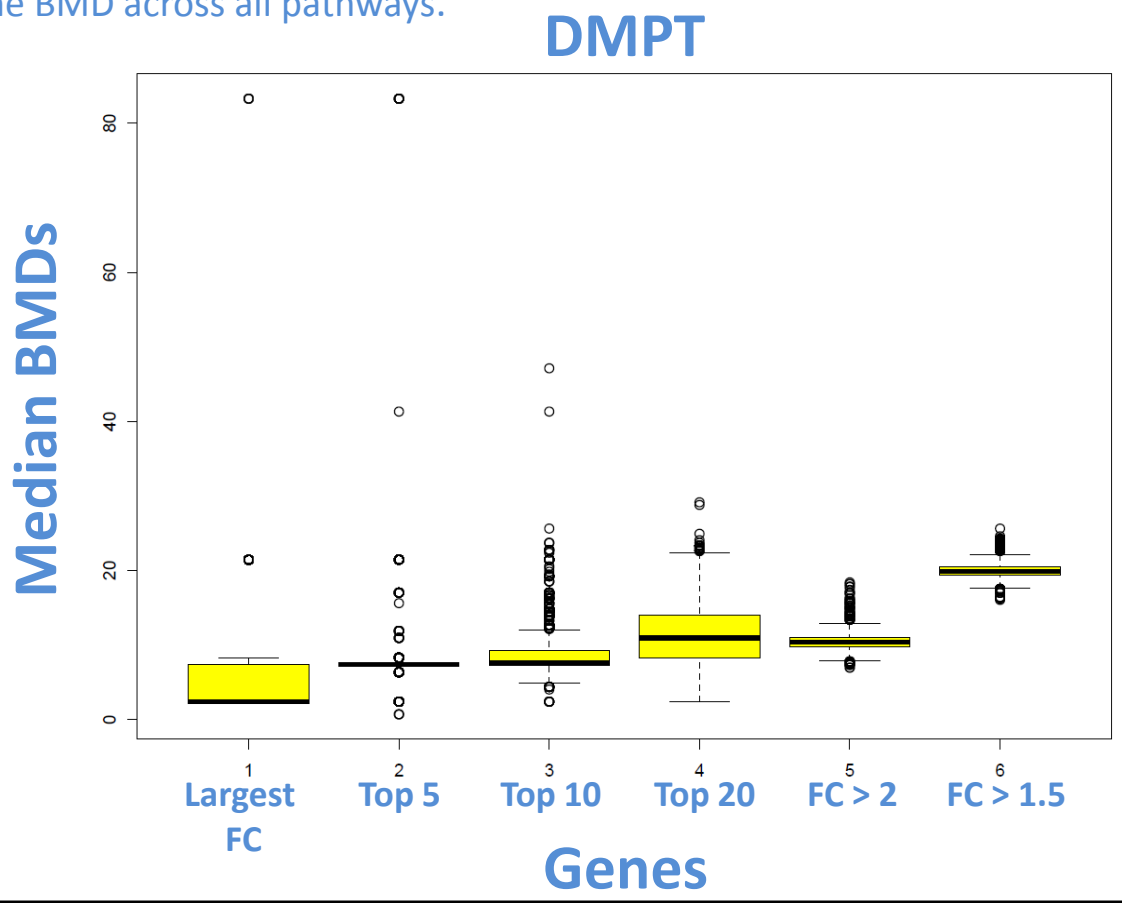

Pathway Approaches

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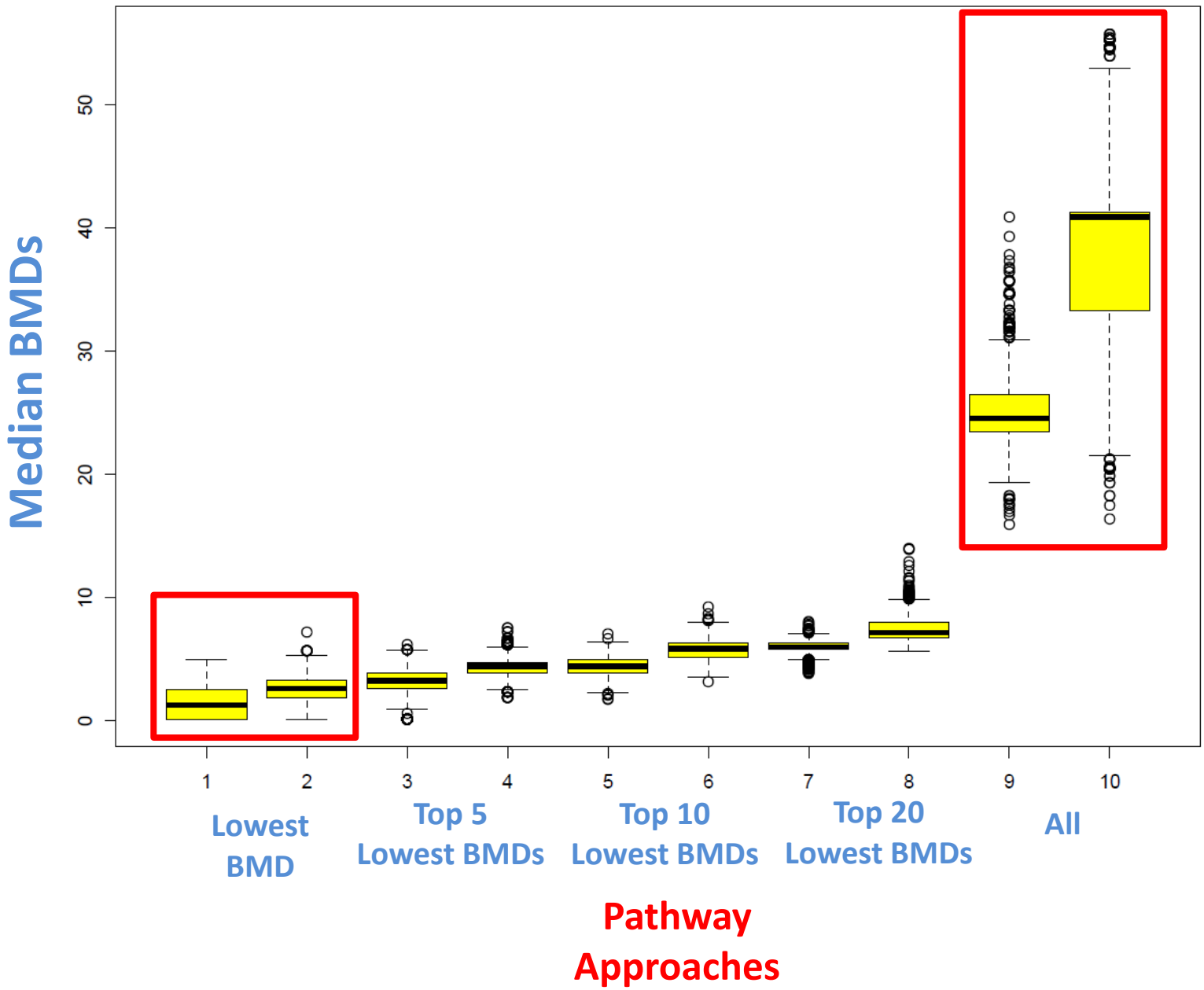
DMPT


Median BMDs
0
10
20
30
40
50
1
2
3
4
5
6
7
8
9
10
Lowest BMD
Top 5 Lowest BMDs
Top 10 Lowest BMDs
Top 20 Lowest BMDs
All
Pathway Approaches

Application of Gene Set Enrichment Analysis for Identification of Chemically Induced, Biologically Relevant Transcriptomic Networks and Potential Utilization in Human Health Risk Assessment

Jeffry L. Dean,*,[1] Q. Jay Zhao,* Jason C. Lambert,* Belinda S. Hawkins,* Russell S. Thomas,† and Scott C. Wesselkamper*

TOXICOLOGICAL SCIENCES, 157(1), 2017, 85–99

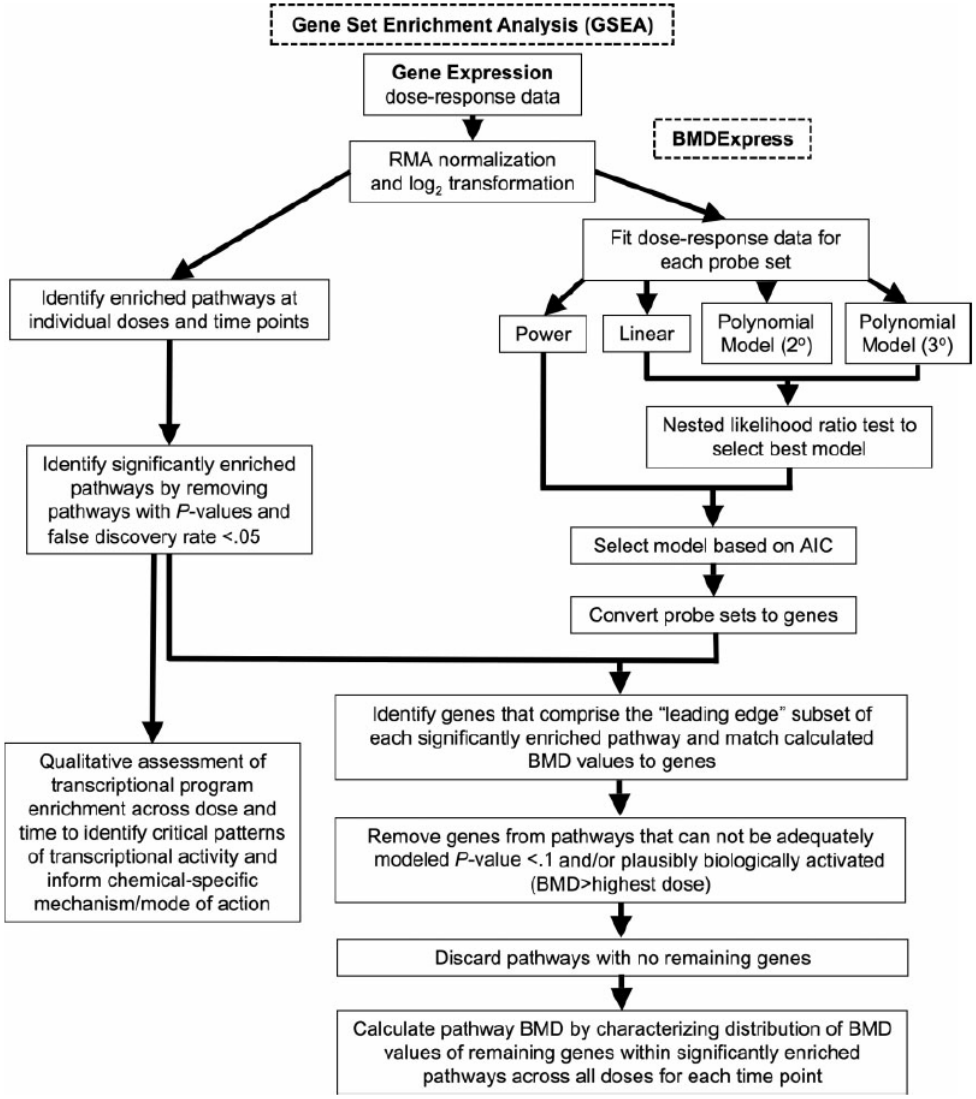
"BMD values from GSEA identified genes and most sensitive biologically enriched pathways were shown to be good predictors of the most sensitive apical response BMD values."

Gene Sets: MSigDB version 5.1

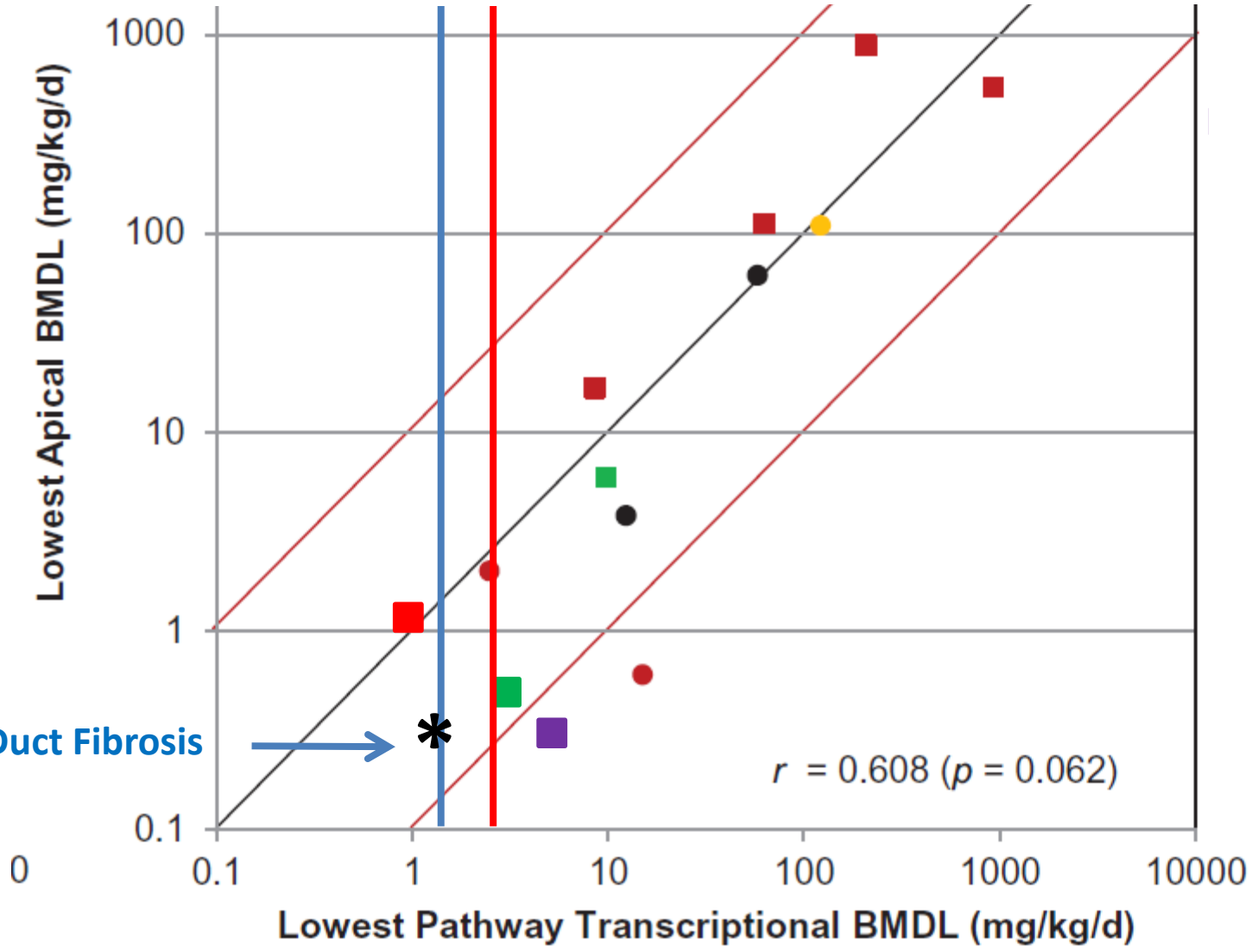
- **Hallmark Gene Set Definitions**

GSEA

- **Unfiltered Gene Lists**
- **Normalized Enrichment Score (Subramanian et al., 2005)**
- **p-values were FDR corrected**



DMPT
BMDL: 2.18 mg/kg/d
Pathway: G1 PHASE
GSEA FDR p-value = 0.0003



Bile Duct Fibrosis

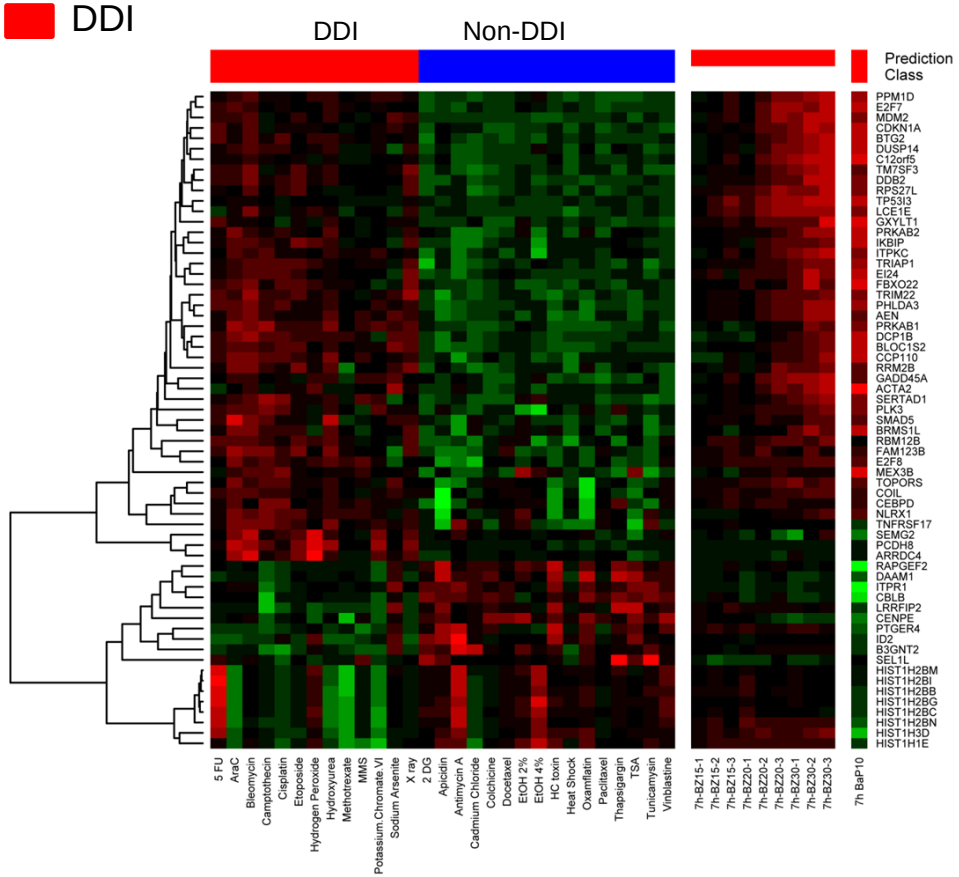
Integration of the TGx-DDI genomic biomarker with the flow cytometry micronucleus test to assess the genotoxicity of disperse orange and 1,2,4-benzenetriol in human TK6 cells

Julie K. Buick[a], Andrew Williams[a], Byron Kuo[a], John W. Wills[a], Carol D. Swartz[b], Leslie Recio[b], Heng-Hong Li[c], Albert J. Fornace Jr.[c], Jiri Aubrecht[d], Carole L. Yauk[a,*]

Mutat Res Fund Mol Mech Mutagen 806 (2017) 51–62

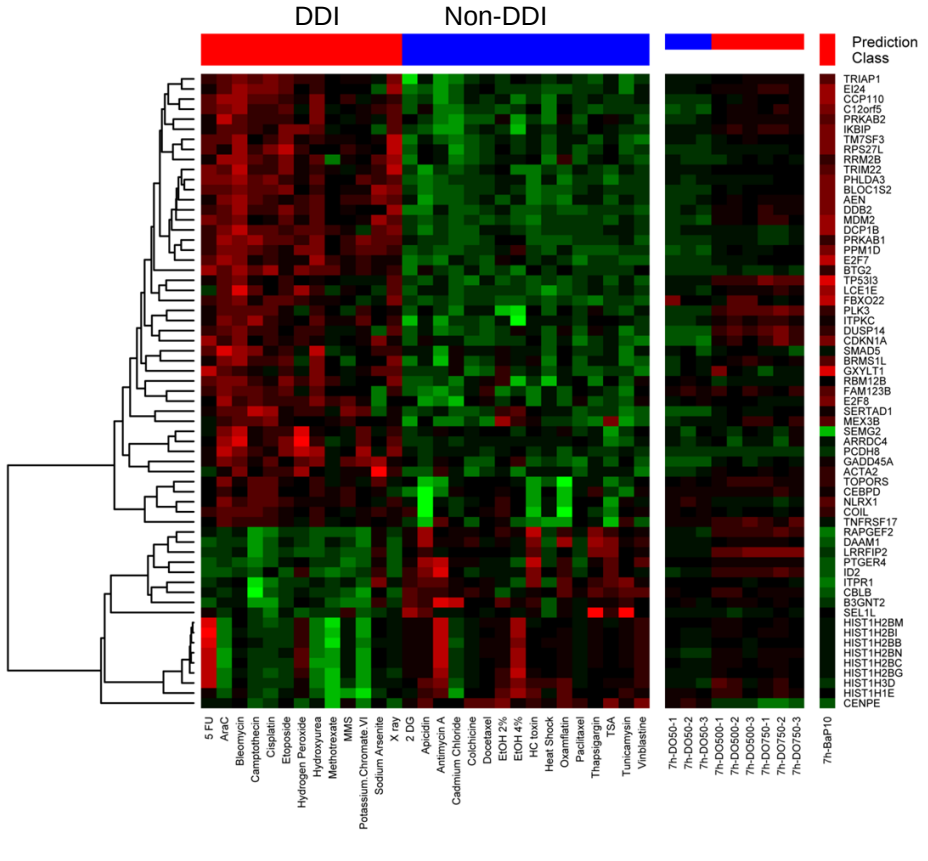
Non-DDI
DDI

Benzenetriol



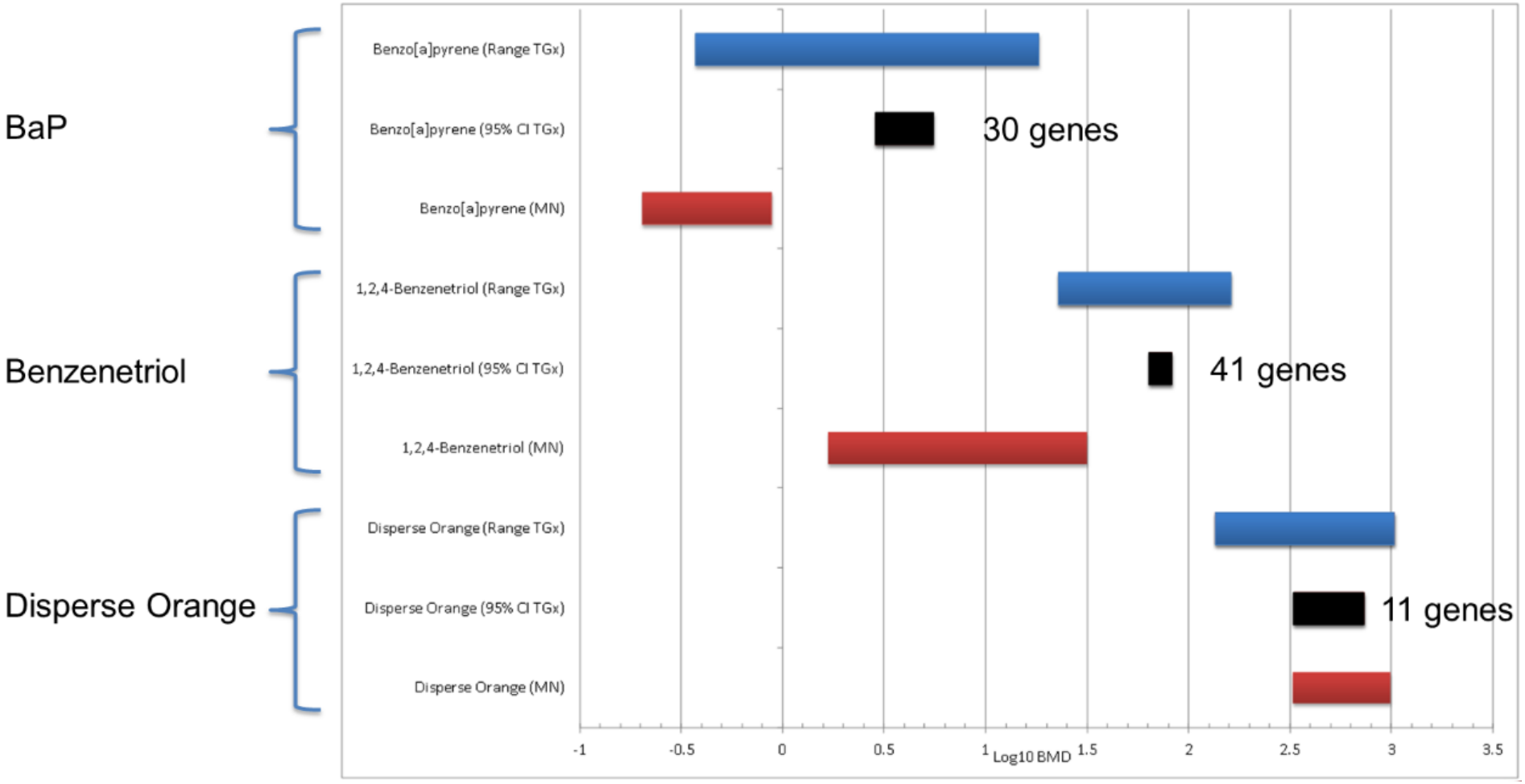
Probability Analysis indicates that all the concentrations are DDI

Orange Azo Dye

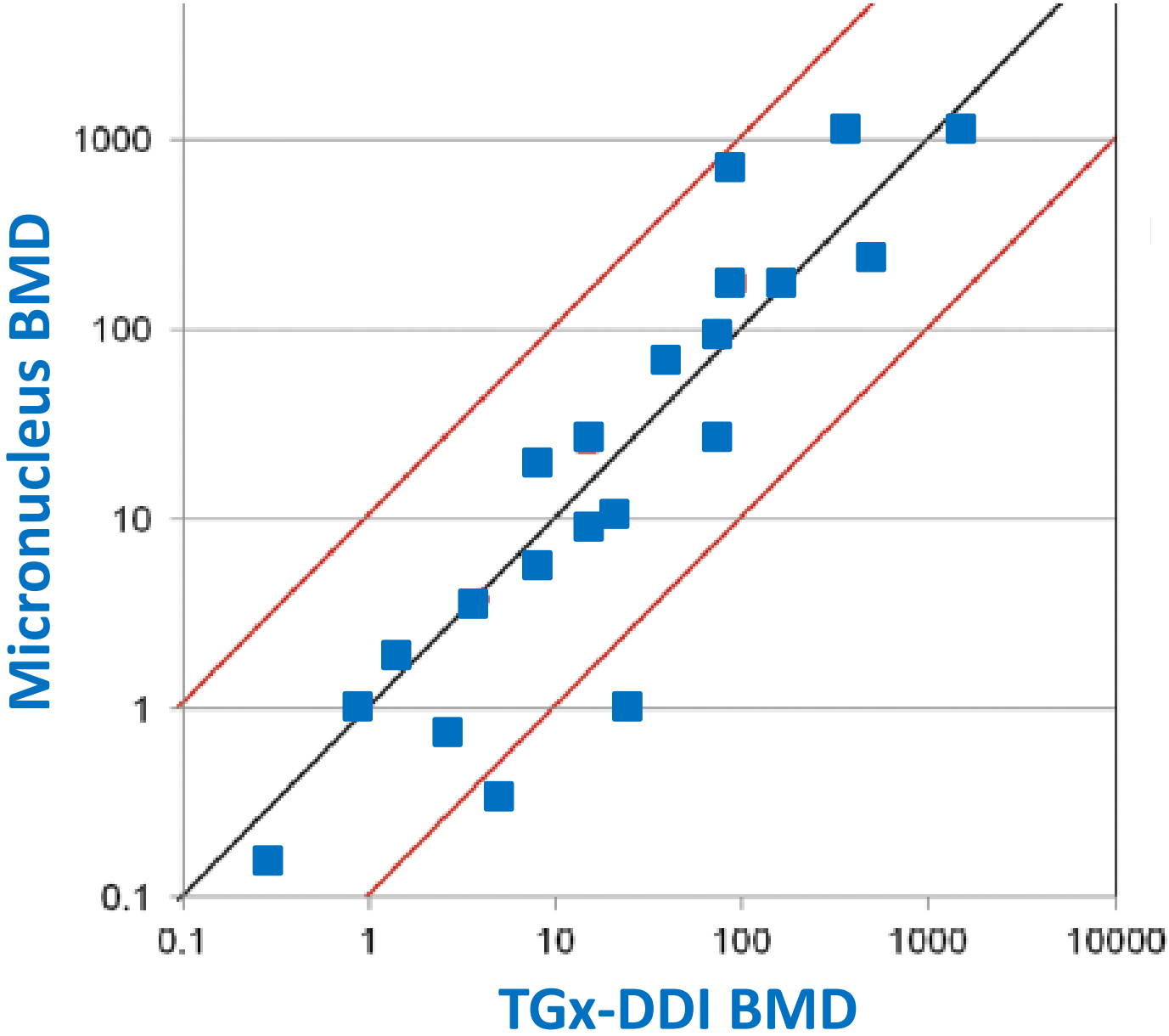


Probability Analysis indicates that the top two concentrations are DDI

BMD potency comparison for all endpoints are consistent: BaP > Benzenetriol > Disperse Orange



Micronucleus vs TGx-DDI BMDs
We need to Populate the Plot



Recommendations:

We like the NTP Approach to Genomic Dose-Response Modeling

1. "Genes don't act alone…"
   Significant Gene Sets, Pathways and/or Signatures over individual genes
2. Modelling Composite Scores
   - Modelling the GSEA Score
   - First Principle Component
   - Cumulative Expression Differences (Parfett et al., 2013; Moser, 1991; Coffee et al., 2007)
3. Filtering
   - Significant Gene Sets, Pathways and/or Signatures
   - MAQC (unadjusted p-value < 0.05 & 1.5 fold change cut-off)

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Dr. Francina Webster

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Anna Stamatogiannakis