NTP
National Toxicology Program

Application of Genomic Benchmark Dose Analysis to the Elk River Chemical Spill

Scott S. Auerbach PhD DABT

SOT Workshop on
Bioactivity-Based Margin of Exposure Safety Assessment: The Next Stop along the Road to 21st Century Safety Assessments

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Disclaimer

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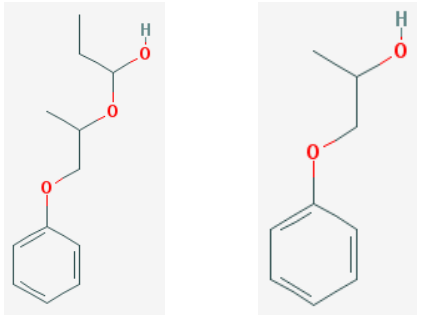
Background

- On January 9, 2014 approximately 10,000 gallons of liquid (crude MCHM and stripped PPH) was leaked from a tank into the Elk River
- The leak occurred 1.5 miles upstream of the water intake facility serving 300,000 people across 9 counties in the Charleston, WV area
- The main chemical from the spill (4-methylcyclohexanemethanol; MCHM) made it into the water supply and was detectable by residents (licorice smell)
- CDC issued a Drinking Water Advisory Level (DWAL) of 1 ppm for MCHM and 1.2 ppm for PPH (propylene glycol phenyl ether) which limited exposure to the chemicals
- Despite the efforts of CDC, along with state and local authorities, a number residents manifest symptoms chemical exposure including rash, skin irritation, diarrhea, nausea, and respiratory illness
- Exposure continued at low levels for a couple months after spill

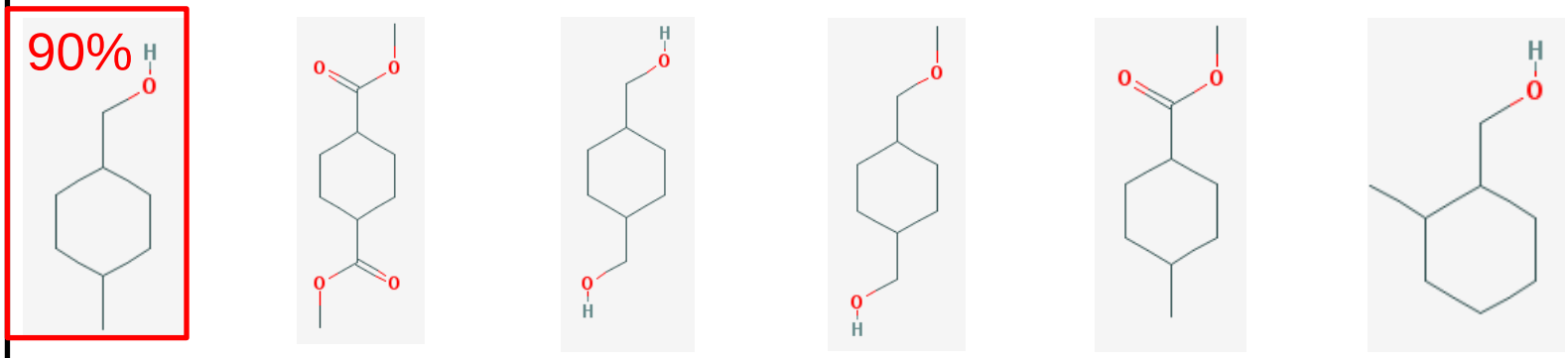
Spilled chemicals

Stripped PPH

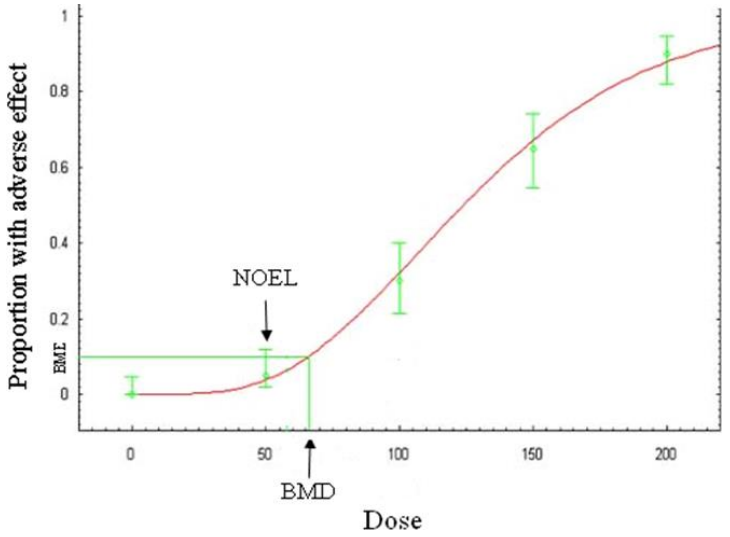


Crude MCHM

90%



July 2014



NCEH/ATSDR (CDC) request the NTP undertake research to address lingering uncertainties in the toxicology dossier for a number the spilled chemicals

"A research effort aimed at providing meaningful information to public health decision-makers ***over the coming year*** would be most useful."

-CDC Nomination letter to NTP

Issues Addressed by NTP Studies

- Reduce uncertainty around the **point of departure** and **safety factors** used to develop the drinking water advisory levels
  - NOEL/NOAEL
    - MCHM: 100 mg/kg/day – kidney and liver effects
    - PPH: 40 mg/kg/day – maternal toxicity
  - Drinking Water Advisory Level
    - MCHM: 1 ppm, which equals 0.1 mg/kg/day for a child
    - PPH: 1.2 ppm, which equals 0.04 mg/kg/day for a pregnant woman

Remember these numbers

- Determine if there are **life-stage specific hazards**
- Screen minor components of the mixture to determine if there are significant **deviations in potency or toxicological properties**

NTP Studies on Elk River Chemicals

Test Article [Abbreviation, CAS Number]	Studies							
	Rat Prenatal Toxicity	Mouse Dermal Irritation and Hypersensitivity	5-Day Rat Toxicogenomic	Bacterial Mutagenicity	Zebrafish Developmental	Nematode Toxicity	High Throughput Screening	Structure Activity Relationship (SAR) Analysis
4-Methylcyclohexanemethanol [MCHM, 34885-03-5]	X	X	X	X	X	X	X	X
Dipropylene glycol phenyl ether [DiPPH, 51730-94-0]				X	X	X		X
Propylene glycol phenyl ether [PPH, 770-35-4]			X	X	X	X	X	X
1,4-Cyclohexanedimethanol [CHDM; 105-08-8]				X	X	X	X	X
2-Methylcyclohexanemethanol [2MCHM, 2105-40-0]				X	X	X		X
4-(Methoxymethyl)cyclohexanemethanol [MMCHM, 98955-27-2]				X	X	X		X
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC, 94-60-0]				X	X	X	X	X
Methyl 4-methylcyclohexanecarboxylate [MMCHC, 51181-40-9]				X	X	X		X
Technical product ["crude MCHM"]		X	X	X	X	X		

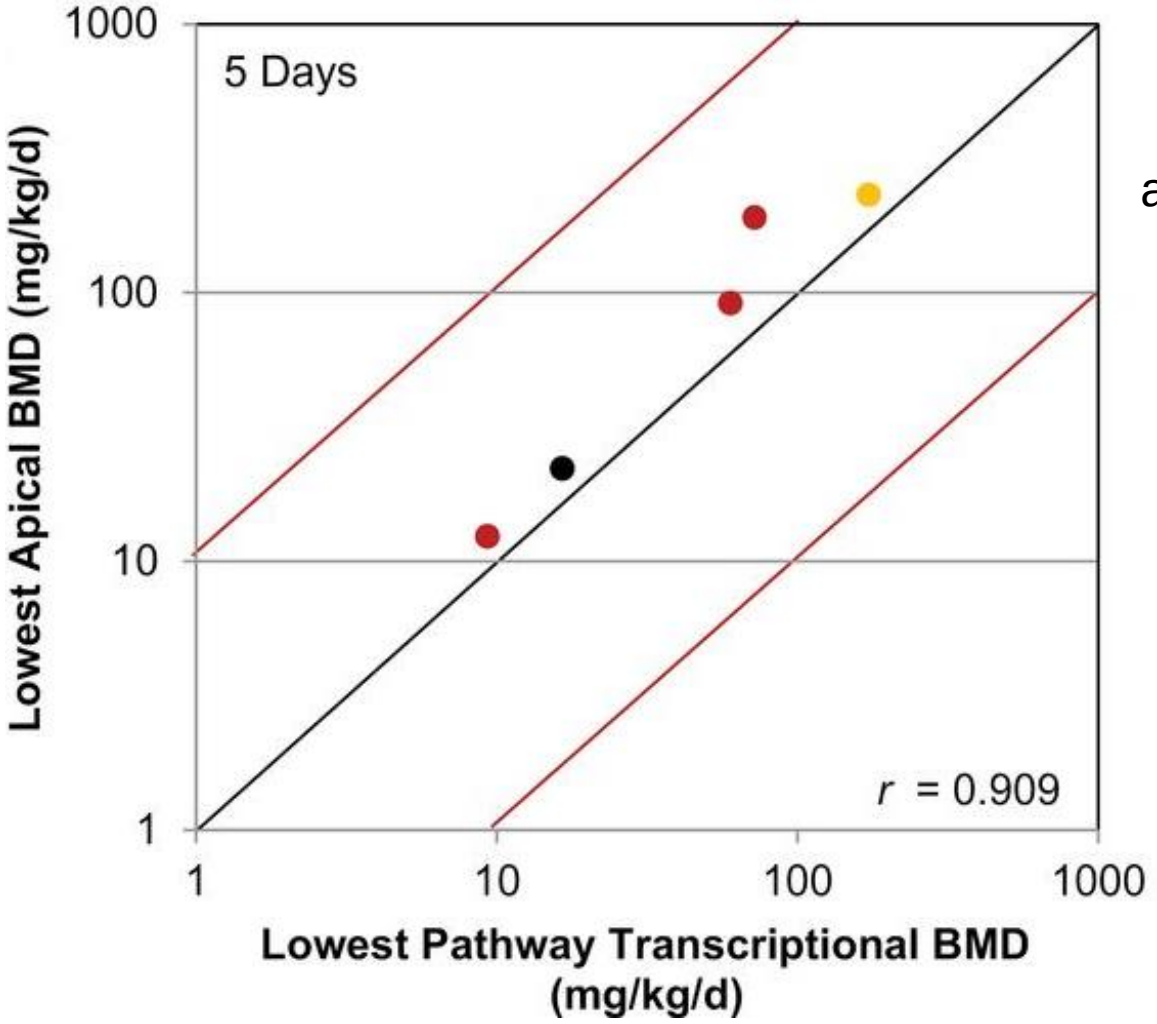
Guideline studies
Non-guideline studies

http://ntp.niehs.nih.gov/results/areas/wvspill/studies/index.html

Poster 2864: Mouse Dermal Irritation and Hypersensitivity Studies (Wed morning)

Why 5-Day Toxicogenomic Studies?

Genomic Pathway Level Benchmark Dose



Quickly **query a wide swath of biological space** to identify a **biological point of departure** that will be as **sensitive** or more sensitive than traditional toxicological endpoints

Identify a plausible **lower bound** for most toxicological effects and reduce POD uncertainty

Thomas et. al., Tox Sci, 2013

Study Design

- **Model**: Harlan Sprague Dawley Rat (male)
- **Route**: Oral (corn oil gavage)
- **Dose range**:
  - 0.1 to 500 mg/kg/day (MCHM and Crude MCHM)
  - 1 to 2000 mg/kg/day (PPH)
  - 6 dose levels plus control group
- **Dosing regiment**: 5 repeated doses, euthanize 24 hrs. after last dose
- **Organs for transcriptomics**: Liver and Kidney
- **Other endpoints**: Clinical observations, body and organ weights, clinical pathology, micronuclei

Non-genomic Effects in the 5-Day Studies

- MCHM: All effects were marginal and occurred at 300 and or 500 mg/kg/day
  - Increased **liver weight** (trend); increased **triglycerides**; decreased serum **glucose** and **eosinophils**; No effect on micronuclei
- Crude MCHM: All effects were marginal and occurred at 300 and or 500 mg/kg/day
  - Increased **liver weight**; decreased **thymus weight**; increased **triglycerides, creatinine, total protein, albumin** and **mean cell volume**; decreased serum **glucose** and **eosinophils**; No effect on micronuclei
- PPH: All effects were limited to the 500, 1000 and/or the 2000 mg/kg/day dose groups
  - **Mortality** and **clinical signs** at the 1000 and 2000 mg/kg/day groups; increase **ALT** and decreased **monocytes**

Genomic BMD Analysis (Published Standard)

- Black et. al, Tox. Sci, 2014**;** Thomas et. al., 2013
- Identifies genomic BMDs that approximate apical BMDs
- **All probe sets** are fit to 4 different models (power, linear, poly2 and poly3)
- "Best fit model" for each probe set is selected and BMD and BMDL are reported
- Probe sets considered to have acceptable fits (fit p-value threshold) in the "best fit models" are passed into the *gene*, pathway, biological process analysis
- Pathways are populated by the genes and a mean or median BMD/BMDL is determined for pathways that contain 5 or more genes

Genomic BMD Results (Published Approach)

Chemical	Organ	ANO (FDR< (n=31,
MCHM	Liver	18
	Kidney	**0**
cMCHM	Liver	38
	Kidney	**0**
PPH	Liver	20
	Kidney	31

Modeling the noise

- Identified ~100 microarrays from vehicle treated rat liver (TG-Gates, 7 day) with no batch effect
  - http://toxico.nibio.go.jp/english/index.html
- Randomly sample arrays to create 5 null data sets of 30 microarrays
  - Dose levels 0, 0.1, 1, 10, 100, 1000
  - 5 samples per dose group
- Ran null sets through BMDExpress using published approach (i.e., all probe sets fit to models)

Null Data Sets (Published Standard)

Null Set	ANOVA (FDR<0.05) (31,000)	Individual Genes (n=14073)	KEGG (n=206)	GO Biological Processes (n=12355)	MSigDB Pathways (n=4725)
Set 1		2672	101	3108	2881 (3, 6)
Set 2					3805 (8, 5)
Set 3	0	4670 (5, 4)	134 (19, 10)	4031 (14, 7)	3691 (13, 7)
Set 4	0	3635 (5, 4)	106 (18, 7)	3647 (12, 7)	3401 (12, 6)
Set 5	0	5116 (5, 3)	149 (13, 6)	4451 (10, 5)	3910 (12, 6)

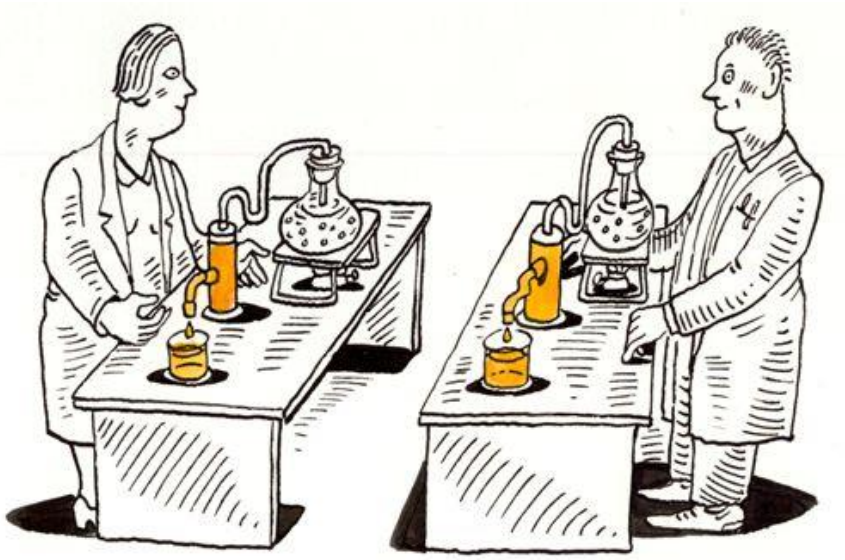
Overly Permissive

Active count
(Lowest BMD, BMD_L)

Issues

Eliminating the noise

Reproducibility



Eliminating the Noise

Modeling the noise

- 5 null data sets
- 12 different gene filters with a complete BMD Analysis
  - Statistical Threshold
  - Multiple testing correction
  - Fold change
- Ranked the filtering methods based on lowest number of “active” genes and pathways with BMDs
- If the multiple methods reported “0” genes or pathways with BMDs than we ranked the more permissive method higher

Eliminating the Noise (Null Data Sets)

Genes

ANOVA (p-value)	Multiple Testing Correction	Fold Change	Individual Genes (n=14073) # Active Genes (Permissivity Rank)	Rank
0.1	Yes	None	0 (10)	1
0.01	No	2	0 (11)	2
0.05	Yes	None	0 (12)	3
0.001	No	None	1(9)	4
0.01	No	1.7	2 (7)	5
0.05	No	2	3 (8)	6
0.01	No	1.5	3 (6)	7
0.05	No	1.7	8 (5)	8
0.005	No	None	10 (4)	9
0.05	No	1.5	13 (3)	10
0.01	No	1.2	20 (2)	11
0.05	No	1.2	70 (1)	12

Eliminating the Noise (Null Data Sets)

Pathways

ANOVA (p-value)	Multiple Testing Correction	Fold Change	MSigDB Pathways (n=4725) # Active Pathways (Permissivity Rank)	Rank
0.01	No	1.2	0 (2)	1
0.005	No	None	0 (4)	2
0.05	No	1.7	0 (5)	3
0.01	No	1.5	0 (6)	4
0.01	No	1.7	0 (7)	5
0.05	No	2	0 (8)	6
0.001	No	None	0 (9)	7
0.1	Yes	None	0 (10)	8
0.01	No	2	0 (11)	9
0.05	Yes	None	0 (12)	10
0.05	No	1.5	4 (3)	11
0.05	No	1.2	12 (1)	12

Reproducibility

Paired chemical studies

- 3, 7, 14 and 28 day liver studies from TG-Gates
  - 3 dose levels and control
- Chemical pairs
  - Gemfibrozil and Clofibrate
  - WY-14,643 and Fenofibrate
  - Naproxen and Ibuprofen
- 12 different gene filters with a complete BMD Analysis
  - Statistical Threshold; Multiple testing correction; Fold change
- Reproducibility Metric
  - Percent of overlapping genes/pathways with a BMD

Pair Chemical Reproducibility

Genes

ANOVA (p-value)	Multiple Testing Correction	Fold Change	Individual Genes (n=14073) % Overlapping	Rank
0.05	No	1.2	15.1	1
0.05	No	1.5	14.5	2
0.05	No	1.7	14.3	3
0.05	No	2	12.2	4
0.01	No	1.5	12.6	5
0.01	No	1.7	12.6	6
0.01	No	2	12.2	7
0.01	No	1.2	12	8
0.1	Yes	None	10.3	9
0.001	No	None	8	10
0.05	Yes	None	8	11
0.005	No	None	5.8	12

Pair Chemical Reproducibility

Pathways

ANOVA	Multiple Testing Correction	Fold Change	MSigDB Pathways (n=4725) % Overlapping	Rank
0.05	No	1.2	37.9	1
0.1	Yes	None	18.7	2
0.01	No	1.2	18.4	3
0.05	No	1.5	16.3	4
0.05	No	1.7	12.6	5
0.05	No	2	11.1	6
0.01	No	1.5	10.8	7
0.05	Yes	None	10	8
0.01	No	1.7	9.1	9
0.01	No	2	8.4	10
0.001	No	None	5.9	11
0.005	No	None	4.7	12

Selection of Optimal Modeling Approach

Genes

ANOVA (p-value)	Multiple Testing Correction	Fold Change	Noise Elimination Rank	Reproducibility Rank	Overall Rank
0.01	No	1.7	5	5	1

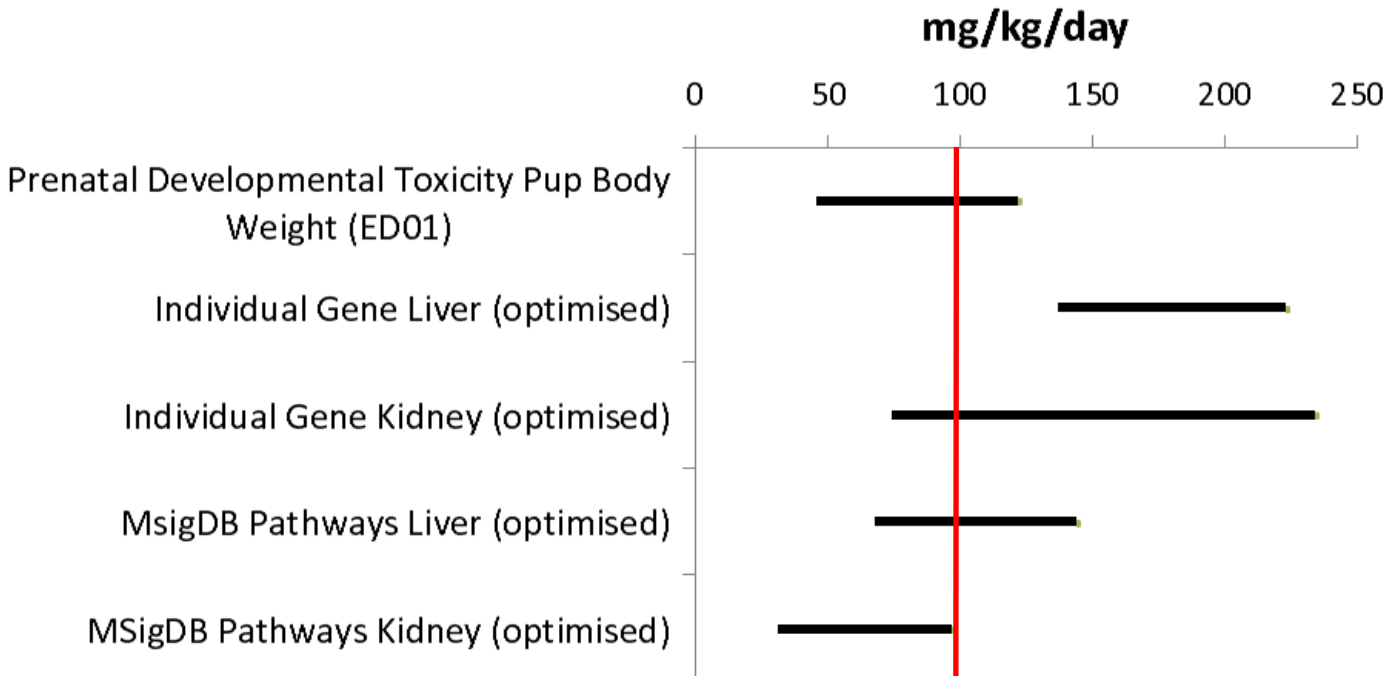
Pathways

ANOVA (p-value)	Multiple Testing Correction	Fold Change	Noise Elimination Rank	Reproducibility Rank	Overall Rank
0.01	No	1.2	1	3	1

Elk River with Optimized Modeling Approach

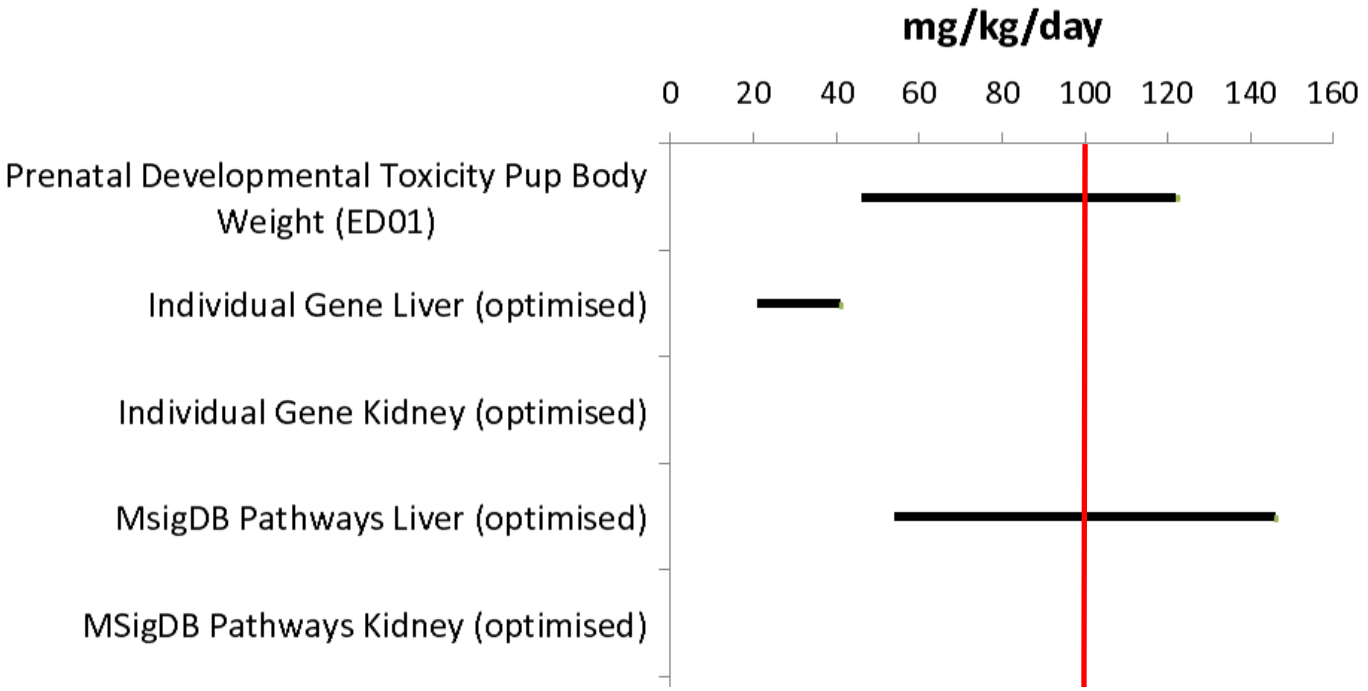
Chemical	Organ	Individual Genes (n=14073) Active Count Method: 0.01 No MTC, 1.7 FC	MSigDB Pathways (n=4725) Active Count Method: 0.01 No MTC, 1.2 FC
MCHM	Liver	14	28
	Kidney	6	44
cMCHM	Liver	18	27
	Kidney	0	0
PPH	Liver	24	32
	Kidney	33	156

MCHM: Apical vs. Genomic



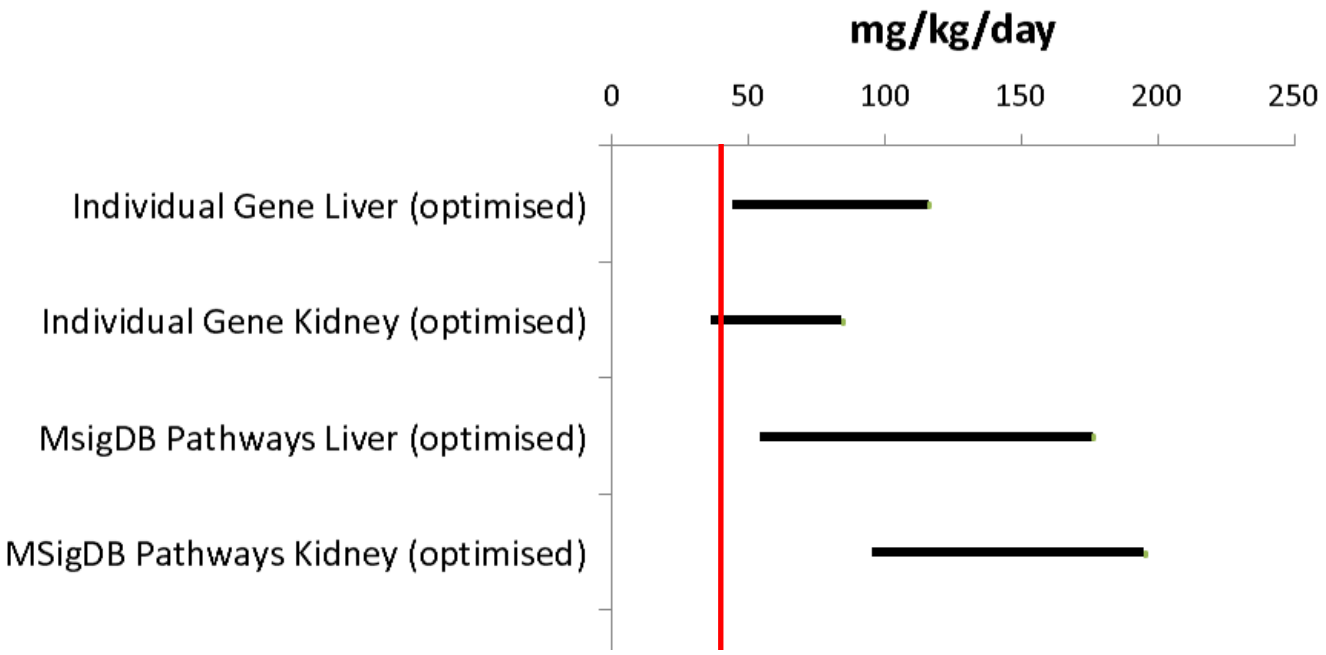
Rat 28-Day Study of MCHM (NOAEL)

Crude MCHM: Apical vs. Genomic



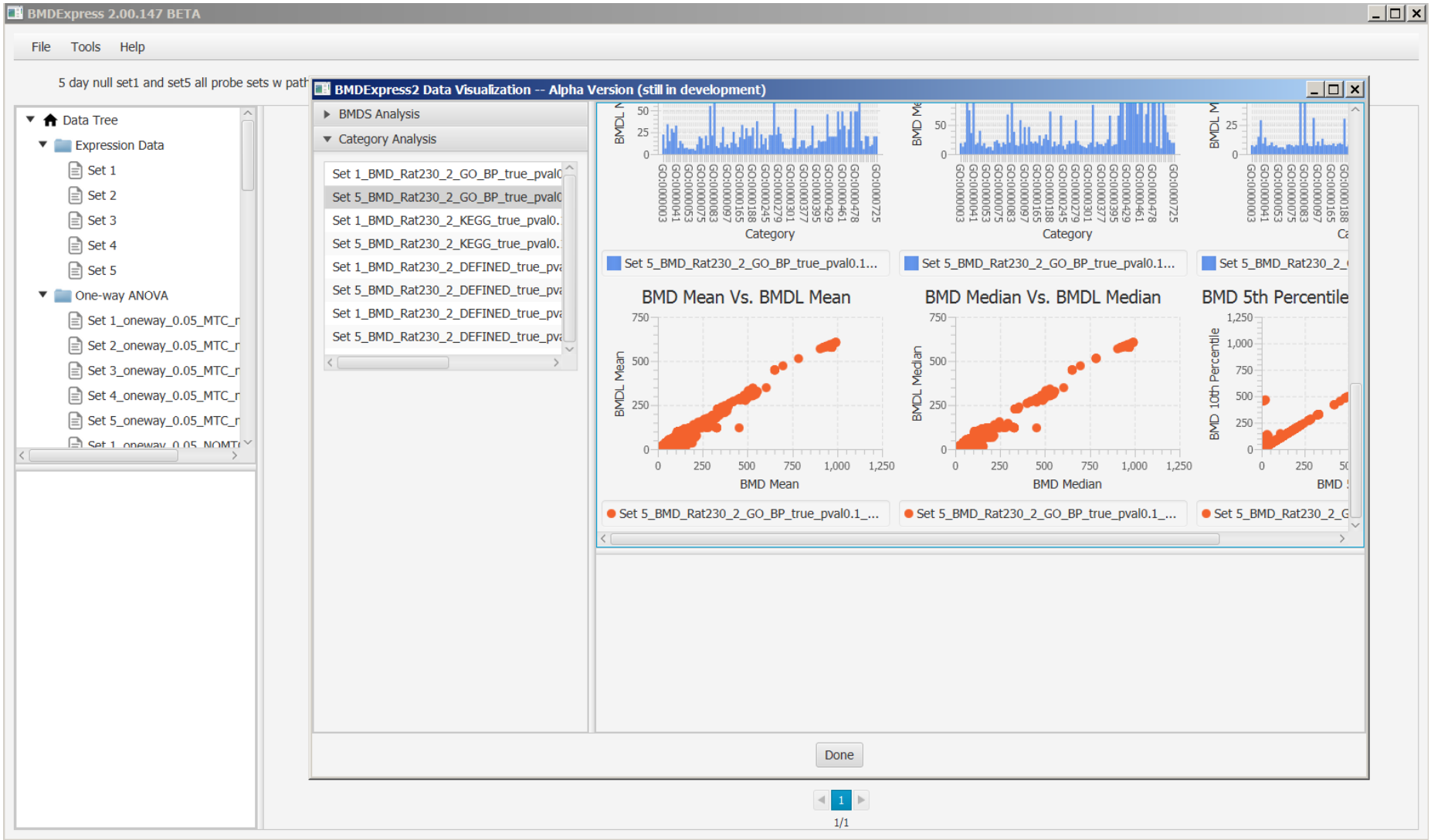
Rat 28-Day Study of MCHM (NOAEL)

PPH: Apical vs. Genomic



Maternal Toxicity in Rat Teratology (NOAEL)

BMDExpress 2.0



Conclusions

- A best practices in genomic benchmark dose modeling needs to be established
- Published approach used here is likely not appropriate for weak signal chemicals
  - >99% of the genes reported were noise
- Optimized methods like the ones described here will help in balancing signal/noise and increase the reproducibility of genomic BMD results
- Both gene level and pathway level BMD/BMD_L performed well in estimating the most sensitive apical NOAEL or BMD/BMD_L therefore reducing the uncertainty around the PODs used for the develop the DWAL

Acknowledgements

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Acknowledgements

auerbachs@niehs.nih.gov

Extra slides

Most Sensitive Genes

Chemical	Organ	Gene	Full Gene Name
MCHM	Liver	Ces2c	carboxylesterase 2C
	Kidney	Pxmp4	peroxisomal membrane protein 4
cMCHM	Liver	Dusp6	dual specificity phosphatase 6
	Kidney		
PPH	Liver	Gpt	glutamic-pyruvate transaminase
	Kidney	Ccnb1	cyclin B1

Most Sensitive Pathways

Chemical	Organ	Pathway/Gene Set Name
MCHM	Liver	REACTOME_METAL_ION_SLC_TRANSPORTERS
	Kidney	WEST_ADRENOCORTICAL_TUMOR_MARKERS_UP
cMCHM	Liver	KEGG_PYRUVATE_METABOLISM
	Kidney	
PPH	Liver	MOOTHA_GLUCONEOGENESIS
	Kidney	KUMAMOTO_RESPONSE_TO_NUTLIN_3A_DN