

# Transcriptional profiling informs target organ phenotypic responses of agrochemicals in rats.

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The data presented here do not reflect EPA policy.

## **Benchmark dose modeling (BMD)**

#### The 4 Step Risk Assessment Process



 Traditionally, a dose that produces a predetermined change (1-10%) in response rate of an adverse effect.

• Not dependent on study design like NOAEL and LOAEL approaches.

https://www.epa.gov/risk/conducting-human-health-risk-assessment

# Lowest median pathway transcriptional BMD shown to be concordant with apical BMD.<sup>[1]</sup>



Gene response coincides or precedes adverse physical effects.

**Questions:** 

- Can BMD modeling of gene response data strengthen a 14 day in vivo experiment?
- Are phenotypic responses aligned with gene expression data?
- What is the added value?

[1] http://dx.doi.org/10.22427/NTP-RR-5.



### **Analysis Methods**



# Outline

Chemically similar compounds C1 and C2 which disrupt electron transport chain in plants.

Brief results on C1 due to compounding limitations

- 2 dose groups available due to overt toxicity (thus no BMD modeling)
- $\alpha$ -2 $\mu$  globulin nephropathy in kidney

#### C2 Results

- 1. Histopathology
- 2. Differentially expressed genes
- 3. Pathway analysis
- 4. Transcriptional BMD



# **Brief results for C1**

#### Despite overt toxicity, dose dependent increase in gene response seen in males kidney.

**C1** 

C1 eosinophilic inclusions in tubular epithelium of kidney at 14d

		Males	Females
<b>(</b> u	0	0/4	0/4
se (pp	600	4/4	0/4
Do:	2000	4/4	0/4



#### Pairwise DEG comparisons show low gene overlap in kidneys across sexes.



#### Low number of canonical pathways in common for kidneys.





#### **C1** Takeaways

- BMD modeling could not be done.
- Gene response data could be anchored to phenotypic responses.
- Added value of gene expression data
  - Reinforcement of sex differences.
  - Pathway data could help uncover mode of action.



Inhibitory dose response in females. Slight increase in DEGs response in males.



C2 observable effects at 14d								
		Males						

	iviaic5				T CITAICS			$\alpha$ - $\mu$ gioduiii			
Kidney	Increased vacuolation in S2 proximal tubules.			None.				nephropathy			
	0	200	600	2000	6000	0	200	600	2000	6000	apparent in male
	1/4	1/4	1/4	3/4	4/4	0/4	NA	NA	NA	0/4	kiuney.
Adrenals	Increased cortical vacuolation.				Increased cortical vacuolation				Adrenals had		
	0	200	600	2000	6000	0	200	600	2000	6000	similar response
	0/4	0/4	0/4	0/4	3/4	0/4	0/4	0/4	0/4	2/4	in both sexes.

# Sex differences apparent in adrenals and kidneys, but adrenals have more DEGs in common than kidneys.





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because it is proximal to phenotypic responses seen at 6000ppm.





1. BMDt values for kidney were also within 5-fold of adrenal apical BMD.

2. All BMDt values were more sensitive than lowest adrenal apical BMD, suggesting transcriptional BMDs are more health protective.

3. Female kidney DEGs also mapped to the citric acid cycle and respiratory electron transport. Pathway related to chemical intrinsic properties.

Adrenals BMD<sub>a</sub> calculated using phenotypic response data

Male mg/kg/day	69.2
Female mg/kg/day	114

Significant Median BMD<sub>t</sub> using BMDExpress 2.3 Pathway Analysis (Reactome)

Organ	Kidney	Adrenals		
Male mg/kg/day	<b>63.7</b> (Extracellular matrix organization)	<b>51.6</b> (Extracellular matrix organization)		
Female mg/kg/day	<b>43.5</b> (The citric acid (TCA) cycle and respiratory electron transport)	<b>22.8</b> (Metabolism)		

#### Conclusion

- C1
  - Transcriptional response could be anchored to observable effects.
  - Due to toxicity of higher doses, BMD modeling could not be done.
  - Added value of gene expression data
    - Reinforcement of sex differences.
    - Pathway data could help uncover mode of action.

#### • C2

- Transcriptional response aligned with observable response.
- Lowest median BMD<sub>t</sub> within tenfold of apical, potentially more health protective
- Added value of gene expression data
  - Reinforcement of sex differences.
  - More protective BMD<sub>t</sub>.
  - Lowest median pathway in female kidney supported intrinsic property of the chemical.
  - Pathway data could help uncover mode of action.

### **Future Work**

- Analyze the remainder of the chemicals
- Look into upstream regulators
- Try to develop gene response signatures for each chemical

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#### **Questions?**