Transcriptional profiling informs target organ phenotypic responses of agrochemicals in rats

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BACKGROUND

- In agrochemical development, data from carefully-designed short-term mammalian toxicity studies can streamline and reduce subsequent animal testing.
- Transcriptomics (TGx) data incorporated into such studies can inform potential target organ effects and provide preliminary quantitative risk assessments through benchmark dose modeling (BMD_t) of gene expression data.
- Regulatory agencies and agrochemical companies are looking to take advantage of new approach methods that utilize TGx data in their safety assessments. Currently, supporting endpoints are often required.

HYPOTHESIS

Gene expression responses following short-term (14 day) chemical treatment can quantitatively model potential biologic potency and inform histologic effects in rats for more rapid chemical risk assessments.

OBJECTIVES

- Compare transcriptional responses to histological findings via pathway analyses.
- Examine chemical, dose, and sex effects.
- Use this information to guide benchmark dose analysis and calculate a point of departure (POD) for chemical risk assessment.

EXPERIMENTAL DESIGN

Figure 1. Study design

Agrochemical 1 (C1): 0, 600, 2000, ~~6000, 12000~~ppm#, n=4/sex/group

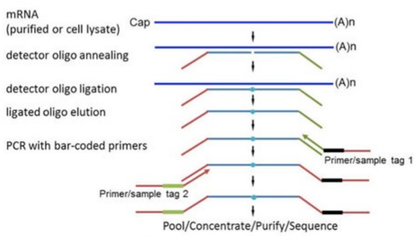
Agrochemical 2 (C2): 0, 200, 600, 2000, 6000ppm, n=4/sex/group

→ Histology report, clinical chemistry at necropsy. Target organ harvesting (adrenal gland and kidney). → RNA isolation, RNA sequencing: TempO-Seq (BioSpyder).

#2 highest doses (6000 and 12000ppm) excluded from analysis due to observed toxicity. Toxicokinetic evaluation on day 2 and 14.

SEQUENCING ANALYSIS WORKFLOW

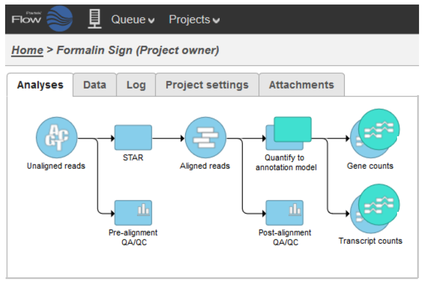
Figure 2. BioSpyder TempO-seq Assay-



(Source: https://www.biospyder.com/tempo-seq)

- TempO-Seq rat Whole Transcriptome Assay on FFPE tissue performed by BioClavis.
- Targeted RNA-seq technology
- Assay gives output on over 20,000 transcripts
- Low input (<10pg total RNA)
- Compatible with Standard PCR and Next Gen Sequencers
- Compatible with purified RNA or cell lysates

Figure 3. PartekFlow Workflow



- TempO-seq FASTQs aligned with STAR 2.4 to rn6 in PartekFlow.
- Differential gene expression with DEseq2
- Differentially expressed genes (DEGs): pval<0.05 and +/- 2 fold change cutoff
- Mapped filtered genes to pathways in IPA
- For transcriptomic BMD analysis, dose groups filtered by ANOVA.
- Responsive genes modeled in BMDExpress 2.3 and mapped to Reactome pathways
- Suspected biomarker gene modeled

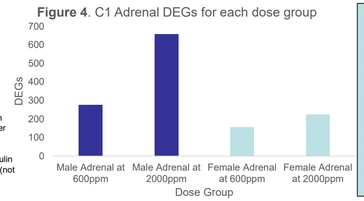
C1 PATHWAY ANALYSIS IN IPA

Table 1. Histologic findings

	C1	
	Male	**Female**
Kidney	Increased eosinophilic inclusions in the tubular epithelium.*	None.
Adrenal	None.	None.

*Found only in males at higher doses, suggestive of alpha 2u-globulin nephropathy, (not treatment-related).

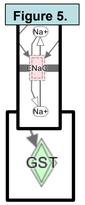
Figure 4. C1 Adrenal DEGs for each dose group



At left: DEGs dose response trend in adrenals. Males have more DEGs at each dose, which may be associated with phenotypic response in kidneys. This DEG trend not observed in male and female kidneys.

At right: Male adrenal DEGs also mapped to aldosterone signaling pathway and glutathione peroxidase inhibition at both 600ppm and 2000ppm. GST inhibition was mapped to oxidative stress pathway.

Figure 5.



Male DEGs mapped to **renal failure** pathways in both kidneys and adrenals while female DEGs mapped to more **inflammation** and **xenobiotic metabolism** pathways in both kidneys and adrenal. Findings suggest that males were more transcriptionally responsive to C1 treatment.

Due to toxic effects of C1, animals in 6000 and 12000 ppm groups were terminated early and benchmark dose modeling could not be performed for C1.

C2 PATHWAY ANALYSIS IN IPA

Table 2. Histologic findings

	Male	**Female**
Kidney	Increased vacuolation in S2 proximal tubules.*	None.
Adrenal	Increased cortical vacuolation at higher doses. n=3/4	Increased cortical vacuolation at higher doses. n=2/4

*Vacuolation seen in control (0ppm dose) and is suggestive of alpha 2u-globulin nephropathy, (not treatment-related).

At right: Male DEGs at least 1.8x female DEGs in both kidney and adrenal gland.

Figure 7. C2 DEGs comparison at 6000ppm

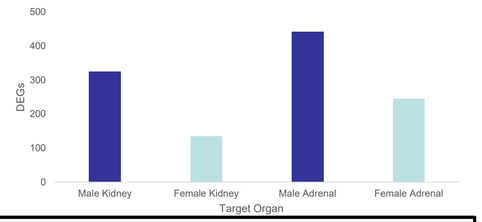
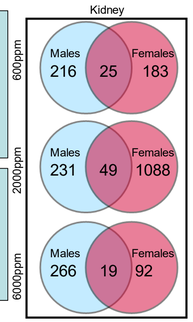


Figure 8. Common kidney DEGs in males and females within each dose group (generated in IPA).

2000ppm dose was chosen for pathway analysis because of the 49 DEGs in common.



Figures 9-10. In the male kidney, DEGs mapped to global pathways involving oxidative stress, hypoxia, and rat toxicity while female kidney DEGs mapped to renal proximal tubule toxicity biomarker. Few common canonical pathways between males and females, which agrees with the findings in the histology report which stated that only males had phenotypic responses.

Figure 9. Male kidney canonical pathways

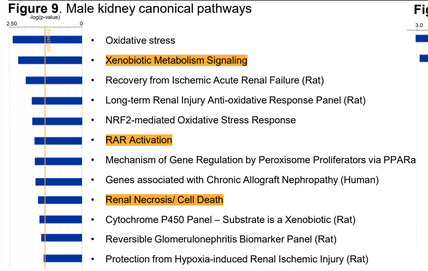


Figure 10. Female kidney canonical pathways

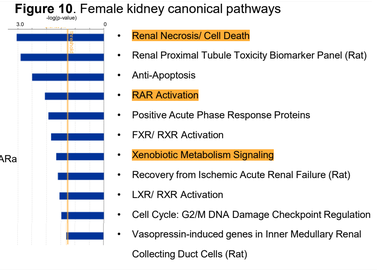
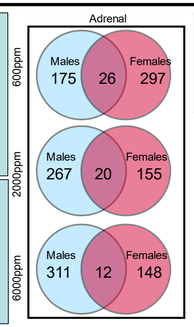


Figure 11. Common adrenal DEGs in males and females within each dose group (generated in IPA).

2000ppm dose was chosen to be predictive of phenotypic responses seen at 6000ppm dose.



Figures 12-13. Male and female adrenal DEGs mapped to similar canonical pathways. This agrees with the histology report findings that demonstrated that males and females had phenotypic responses. Male DEGs also mapped to ischemic renal failure in rat, which could be related to higher frequency of vacuolation in males.

Figure 12. Male adrenal canonical pathways

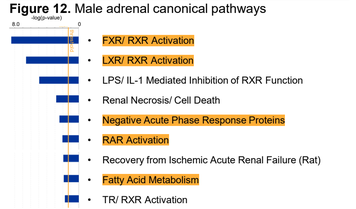
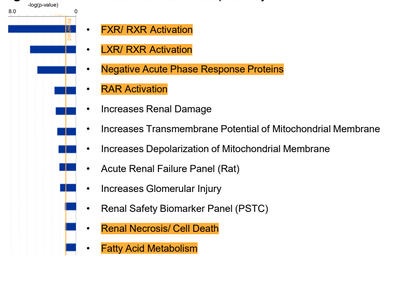


Figure 13. Female adrenal canonical pathways



C2 BENCHMARK DOSE MODELING IN BMDEXPRESS

Table 3. Median BMD_t using BMDExpress 2.3 Pathway Analysis (Reactome)

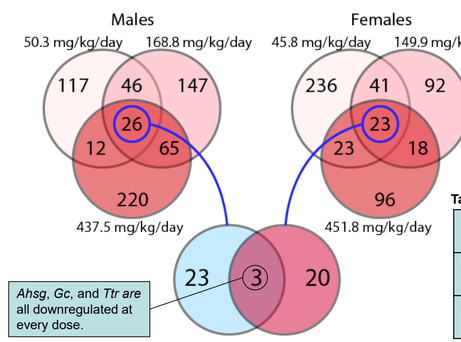
Organ	Kidney	Adrenals
Male mg/kg/day	6.17 (activation of matrix metalloproteinases)	8.05 (metabolism of polyamines)
Female mg/kg/day	15.9 (acyl chain remodeling of PS)	12.1 (nuclear receptor transcription pathway)

Table 4. BMD_a using BMDS3

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

Above: Males more transcriptionally responsive to C2. Adrenal BMD_t are within tenfold of respective apical benchmark dose (BMD_a) in Table 4. BMD_a run with only male and female adrenal because both had increased cortical vacuolation.

Figure 14. Common adrenal DEGs in male and female dose group intersection



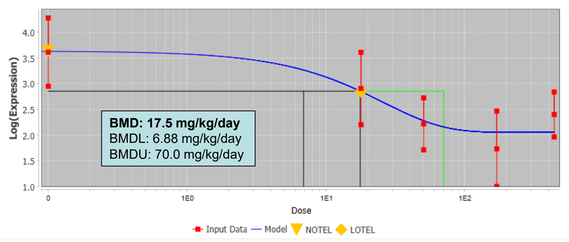
All 3 genes are involved in LXR/RXR and FXR/LXR activation. *Ahsg* is involved in negative acute phase response proteins. *Gc* is involved in increasing renal damage tox list.

Ahsg, *Gc*, and *Ttr are* all downregulated at every dose.

Table 4. Achieved doses for each group

	600 ppm	2000 ppm	6000 ppm
Male (mg/kg/day)	50.3	168.6	437.5
Female (mg/kg/day)	45.8	149.9	451.8

Figure 15. Dose-response curve (exponential model 4) of *Ttr* gene in male adrenal



At left: Using BMDExpress 2.3, One-way ANOVA identifies genes with significant dose-dependent changes. *Ttr* could only be modeled for males, and *Gc* could only be modeled for females. *Ahsg* could not be modeled for either sex. *Ttr*, however, has BMD value similar to the lowest median BMD found in BMDexpress pathway analysis.

CONCLUSIONS

C1

- In general, DEGs mapped to renal failure in males and inflammation and xenobiotic metabolism pathways in females, suggesting that females were more equipped to tolerate C1.
- Phenotypic responses seen in male kidneys could be related to high number of DEGs in male adrenals since the organs are interlinked.
- Due to insufficient number of dose groups, BMD modeling of TGx data was not possible..

C2

- Sex-dependent phenotypic responses observed only in males aligned with enriched pathways mapped by kidney DEGs.
- Similar phenotypic responses seen in adrenals strongly concurs with pathway analysis findings for both males and females.
- Benchmark dose modeling suggested that males were more transcriptionally responsive to treatment as the male BMD values were consistently lower.
- In adrenals, BMD_t was tenfold lower than BMD_a, making it health protective for assessing risk.
- These conclusions suggest that short-term TGx studies can rapidly provide added value to preliminary chemical risk assessment.

Center for Computational Toxicology and Exposure mission

- This research contributes to the USEPA/CCTE mission by using New Approach Methods (modeling early stage responses) to estimate chemical potency and more rapidly inform chemical risk assessment.