

Effects of Volume on Acute Toxicity and the Transcriptome in Fathead Minnow Exposures

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Background

A core mission of the U.S. Environmental Protection Agency (USEPA) is to assess the effect of anthropogenic chemicals on freshwater ecosystems using data gathered from standardized tests across many biological taxa. One common species used for testing is the Fathead minnow (FHM), also known as *Pimephales promelas*. These tests measure chemical induced mortality and vary in observable endpoints.

- Traditionally, these tests employ long time frames and large amounts of chemical to conduct exposures.
- A modern alternative to this involves a high-throughput format of testing to cost-effectively and efficiently collect large amounts of toxicological data.

We hypothesize container volume in relation to controls in multi-well plates should not significantly affect transcriptomic response nor LC50 values in this type of screening assay compared to traditional methods of testing and thus, facilitate a high-throughput approach to future chemical risk assessment.

Objective

To evaluate the effect that differences in exposure volume and associated loading density could have on both survival and the transcriptome-level response in a high-throughput format

Exposures

- 24-hour static exposures to FHM larvae were conducted with 12 concentrations of CuSO₄, NiSO₄, and ZnSO₄
- Three exposure volumes were tested, specifically: 96-well plates (96WP), 24-well plates (24WP), and single 15mL cups (CUP)
 - 96WP: 1 FHM/well; 700uL exposure media/well; 8 replicates
 - 24WP: 1 FHM/well; 2.5mL exposure media/well; 4 replicates
 - CUP: 1 FHM/cup; 15mL exposure media/cup; 3 replicates



96-well plate
(96WP)



24-well plate
(24WP)



15 mL cup
(CUP)

BMDEExpress2

BMDEExpress2, <https://www.sciome.com/bmdexpress/>, is a desktop application that enables analysis of dose/concentration-response data produced in differential gene expression experiments

- Transcriptomic response using RNA-Seq data was assessed in pairwise comparisons between container volumes using control data only^{1,2,3}
- Points of Departure (POD) were calculated from Differentially Expressed Genes (DEGs) in each volume independently^{1,2}
- Volume-specific LC50 values were calculated with BMDEExpress2 and were compared with published LC50s from ECOTOX knowledgebase, <https://cfpub.epa.gov/ecotox/index.cfm>⁴

Survival

LC50s, with 95% confidence interval, were assessed with 96WP exposures in full concentration-response with 12 concentrations of half-log dilutions corresponding to each chemical starting at high concentrations of 2, 15.8, & 4 mg/L of CuSO₄, NiSO₄, & ZnSO₄, respectively

	BMDEExpress2	Published Literature	
	96WP (24h)	Traditional (24h)	Traditional (96h)
Chemical	LC50	LC50	LC50
CuSO ₄ (mg/L)	0.57 (0.47 - 0.69)	0.74	0.25
NiSO ₄ (mg/L)	11.3 (9.2 - 13.7)	N/A	6.2
ZnSO ₄ (mg/L)	3.8 (3.3 - 3.9)	2.46	2.19

RNA Seq Data

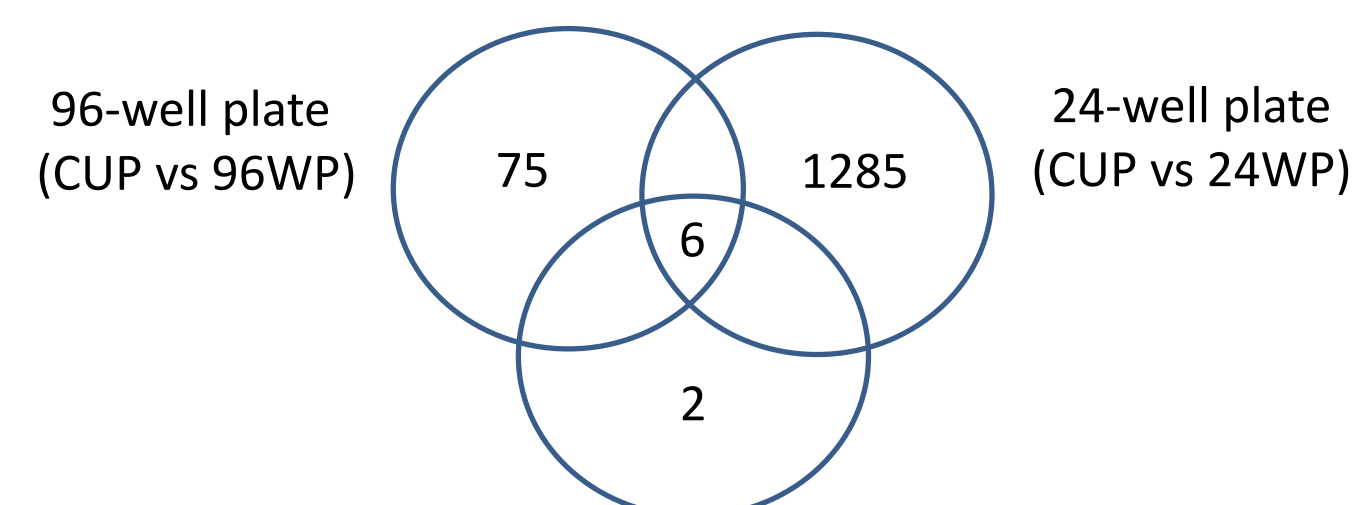
RNA-Seq raw reads were assembled into transcript models, aligned with annotations, counted, normalized, and log₂ transformed for each transcript

- Low count feature filtering: any given feature had to have a count of 10 or more in a minimum of 4 samples or that feature was filtered out

RNA-seq data was collected from all 12 concentrations of the 96WP and only 3 concentrations of CuSO₄ from 24WP and CUP formats^{2,3}

- The impact of exposure volume on the transcriptome of controls was explored by comparing lists of DEGs from pairwise comparisons
- Transcriptomic-derived PODs from each volume were compared to determine the impact exposure volume may have on the analysis

of Differentially Expressed Genes (DEGs) in Controls Across Volumes³



15 mL vessel (24WP vs 96WP)

POD Calculation for CuSO₄ in each Volume

BMDEExpress2 Results	Volume Format		
	CUP ²	24WP ²	96WP
#DEGs via ANOVA	53/32996	11/32996	4139/32548
#DEGs via Williams Trend Test + 2-Fold Change	369	159	208
#DEGs passing NTP filters ¹	128/369	52/159	108/208
Median POD (mg/L)	0.0445	0.045201	0.025
Median POD lower limit (mg/L)	0.028027	0.028289	0.017
Median POD upper limit (mg/L)	0.101045	0.106327	0.046

Highlights

- Survival across the different exposure formats was similar in all three chemicals, and LC50 values calculated from these were similar to published 24-hour LC50's. For instance, the LC50 for CuSO₄ from exposure in a 96-well plate was 0.57 mg/L, comparable to 0.74 mg/L, the median value from several published tests documented in the EPA's ECOTOX knowledgebase. Comparable results were also observed for NiSO₄ and ZnSO₄
- Results of pairwise comparisons between controls across volumes suggests negligible biological response related to volume format
- PODs calculated for CuSO₄ from each volume were similar indicating final POD calculations were not impacted by volume

Future Recommendations

- Analyze full concentration-response data for 24WP and CUP formats for a complete comparison to 96WP format
- Compare water quality parameters (ex. pH, DO, etc) across volumes
- Expand volume comparisons to other chemical modes of action

¹ Due to COVID restrictions, data for NiSO₄ and ZnSO₄ is pending

² A full 12 concentration-response was calculated for the CuSO₄ 96WPs, however only 3 concentrations were analyzed for the 24WP and CUP formats

³ DEGs calculated by pairwise comparisons using One-Way Analysis of Variance (ANOVA)

⁴ Follows National Toxicology Program (NTP) Approach to Genomic Dose-Response Modeling, https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr05_508.pdf?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=rr05