High Throughout Assay Development with *Raphidocelis subcapitata*

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Background

The U.S. Environmental Protection Agency (USEPA) is tasked with assessing the impacts of market chemicals on ecosystems using standardized tests across a diverse range of organisms. To support a new paradigm in toxicity testing, EPA has envisioned a tiered framework for hazard characterization that employs high throughput and high content assays as a first-tier screen, followed by successive progression to more targeted and resource intensive tests where warranted (Thomas et al. 2019). To date, assay development under this new testing paradigm has largely focused on human health, with limited or no capacity to detect hazards associated with compounds that are uniquely toxic of primary producers like plants and algae. The aim of the present study was to adapt traditional algal toxicity assays to a high throughput and high content (through the incorporation of transcriptomics-based endpoints) format could be incorporated into EPA's next generation blueprint for computational toxicology.

Objective

Develop, optimize, and evaluate a high throughput, transcriptomics-based toxicity test with the green algae Raphidocelis subcapitata (RS), currently used in a standard test guideline (OSCPP 850.4500). The present poster reports on a 96-well plate 24-hour RS assay using lab automation that yields a large amount of data that can be used to determine multiple endpoints.

Methods

- 3, 1-mL, 96-well plates were loaded with algae from a 5.0x10^6 cells/mL stock
- Plates were centrifuged to pellet algae
- Plates were placed on the Biomek i5 liquid handling automated workstation to remove media and expose to 600 uL of exposure media
  - ➢ Each plate had 2 chemical exposures, 8 concentrations of each with 5 reps
  - ➢ 30 mM and 9.5 mM CuSO4 were used as positive controls on each plate
  - ➢ Biomek programmed to avoid unbalanced treatment along edges using the following predetermined randomized layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	100.00	100.00	31.67	10.00	3.17	1.00	0.32	0.10	0.03	0.00	0.00	100.00
B	31.67	1.00	0.03	std RNA pool	10.00	0.10	0.32	3.17	10.00	0.10	1.00	31.67
C	10.00	+ Exposure B	0.10	0.00	0.00	3.17	0.32	10.00	3.17	0.00	0.03	10.00
D	3.17	100.00	31.67	100.00	0.03	3.17	31.67	+ Exposure B	10.00	0.32	3.17	3.17
E	1.00	0.32	0.10	+ Exposure B	+ Exposure A	1.00	1.00	31.67	10.00	0.03	1.00	1.00
F	0.32	100.00	31.67	0.03	0.10	10.00	std RNA pool	1.00	100.00	0.00	100.00	0.32
G	0.10	0.10	31.67	31.67	3.17	0.32	100.00	0.32	+ Exposure A	0.03	+ Exposure A	0.10
H	0.03	0.00	0.00	0.03	0.10	0.32	1.00	3.17	10.00	31.67	100.00	0.03

Chemical 1
Chemical 2
Controls
*Units in uM

- Exposed algae were resuspended and left on a shaker under constant light for 24 hours
- After 24 hours algae was pelleted as before
- One plate was frozen for RNA extraction and exposure media sampled for chemical quantification
- The 2nd plate was used for apical observations
  - ➢ Cell count, photopigments, and a fluorescence-based cytotoxicity assay all read using a fluorescence plate reader (BioTek Synergy Neo2)
- The third plate was used as a backup
- Apical observation data was run through the dose-response software BMDExpress2 to calculate an EC50 after being derandomized in Excel

Results

Growth

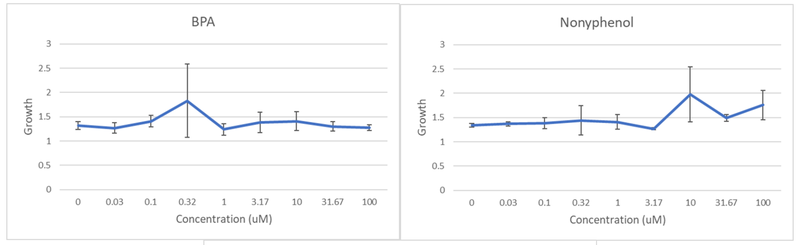
The following results are based on three example chemical exposures across three chemical classes. EC50s were calculated based on growth data and compared to those reported in the ECOTOX database.

Chemical	Exposure EC50 (mg/L)	Reported EC50 (mg/L)
BPA	> 22.83*	2.37**
Nonylphenol	> 22.04*	1.85**
Sertraline	30.93	0.09**

*Computed EC50 larger than highest concentration
**Studies were conducted 2+ days rather than 1

Resulting EC50s were higher than those reported for studies conducted over multiple days in different formats than 96 well plates.

Cell count relative to time 0 (growth):



The growth response data showed that BPA had little to no impact on growth, nonylphenol increased growth at higher concentrations, and sertraline decreased growth at higher concentrations.

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Raw data has suggested there is an impact on growth along the outside wells. Looking at wells designated as controls there are significantly higher readings on those wells on the outside compared to those more in the middle:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.256	0.245	0.276	0.366	0.365	0.288	0.626	0.311	0.292	0.3	0.293	0.269
B	0.268	0.231	0.234	0.251	0.235	0.262	0.224	0.399	0.234	0.195	0.191	0.195
C	0.247	0.225	0.238	0.215	0.209	0.206	0.223	0.199	0.195	0.204	0.212	0.222
D	0.262	0.214	0.226	0.23	0.191	0.188	0.198	0.193	0.201	0.19	0.187	0.674
E	0.243	0.221	0.218	0.213	0.197	0.205	0.192	0.214	0.205	0.188	0.196	0.241
F	0.235	0.227	0.223	0.207	0.191	0.198	0.355	0.199	0.202	0.198	0.194	0.204
G	0.25	0.214	0.211	0.209	0.195	0.191	0.2	0.202	0.193	0.186	0.182	0.211
H	0.207	0.236	0.224	0.212	0.189	0.2	0.199	0.21	0.188	0.212	0.223	0.23

Going Forward

- Chemical quantification
  - ➢ Plate used for storing exposure media will be switch to glass containment to avoid chemical absorbing into plastic. Current exposures have resulted in concentrations being much lower than nominal.
  - ➢ Efforts are ongoing to model chemical distribution to media, plate well walls, and/or algae
- Increasing initial algal cell number in exposure plates might be necessary to obtain enough RNA for downstream transcriptomics
  - ➢ Methods for RNA extraction are currently in development to obtain sufficient quantity and quality of RNA per well
- Covering the outside walls of the plates when left under the growth light to avoid edge effects
- Pigment data will be gathered using a method appropriate version of methanol extraction and calculated using reported equations (Lichtenthaler, 1987 and Haire et al., 2018)
- CellTox Green Cytotoxicity assays will be run post-exposure. This was not conducted and reported here due to resource limitations

References

Thomas, RS et al., *Toxicol Sci.* **2019**, 169(2)

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Lichtenthaler, HK, *Methods Enzymol.* **1987**, 148

Haire et al., *Front Plant Sci.* **2018**, 9(235)