University of British Columbia/ Department of Biochemistry and Molecular Biology

Evaluating the proteome profiles in juvenile Coho gill and liver tissues exposed to the toxic tire-associated contaminant 6PPD-quinone

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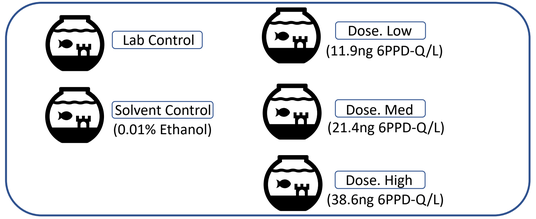
Introduction

6PPD is a commonly used antiozonant in rubber tires. Acting as a scavenger, 6PPD reacts more readily with ozone than rubber itself, extending tire life. As rubber tires wear, particles are released onto road surfaces, where 6PPD is exposed to air, reacting with ozone to create 6PPD-quinone (6PPD-Q). During rainfall events, accumulated 6PPD-Q on roadways is washed into streams and other water bodies, where it is implicated in acute mortalities of coho salmon (*Oncorhynchus kisutch*). This anadromous fish is extremely sensitive to 6PPD-Q, with very low concentrations being lethal (LC50= 41ng/L vs 500-10000ng/L for other species). We are utilizing a full range of 'omics tools to establish methods that clarify the mechanism of action and improve our understanding of coho salmon's sensitivity to 6PPD-Q. This poster specifically focuses on our findings from proteomic analyses.

Method and Study Design

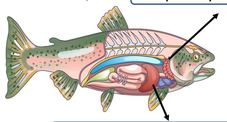
24-hour exposure to mimic a storm event, which 6PPD-Q was washed off rapidly from road.



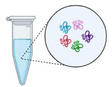
Note: Very-high and extreme dose groups were not used further due to very high mortality.

Each condition:
4 tanks, 5 individual fish/ tank

Gill: primary uptake site of dissolved chemicals



Protein extraction (TriZol) → Protein Solubilization (Urea/ Thiourea)

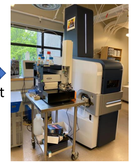


Protein Digestion (LysC & Trypsin)

Peptide Purification (STAGE-Tip)

Liver: principle detoxifying organ

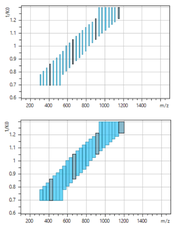
LC-MS/MS
30 min gradient



Bruker TimsTOF Pro 2

Library (top) : Gas-phase library generation using narrow window DIA-PASEF

Samples (bottom): DIA-PASEF



Computational proteomics

Mass Dynamics

Results and Discussion

1. LC50

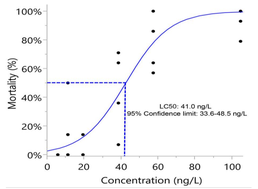


Figure 1. Concentration-response curve of 6PPD-quinone in Juvenile Coho Salmon for 24 hours
Graph obtained from Lo *et al., (2023) Environ Toxicol Chem.* The LC50 of 6PPD-quinone for coho salmon after 24 hours is 41.0 ng/L, with a confidence interval of 33.6 to 48.5 ng/L, highlighting the high toxicity of 6PPD-quinone in Juvenile Coho Salmon. Measured concentrations at test initiation were used to calculate the dose–response curve. The median lethal concentration (LC50) was calculated using a log-logistic model.

2. Liver Samples

More than 8,000 proteins were detected in the liver samples, yet no statistically significant variations were found between conditions, likely due to high variability among the samples.

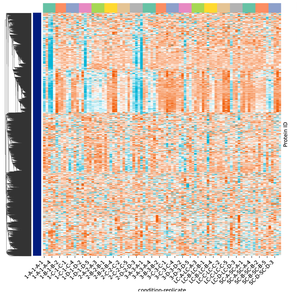
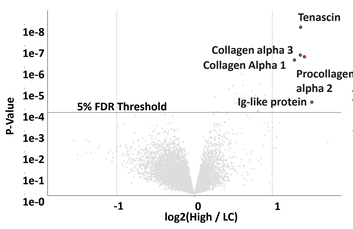


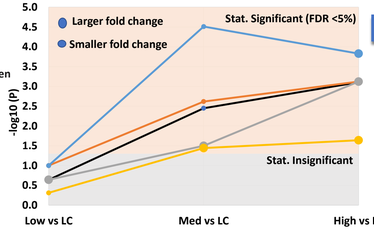
Figure 2. Intensity Heatmap of Protein Expression Profiles Across 94 Liver Samples
This heatmap represents the protein expression intensity in 94 liver tissue samples. The data exhibits considerable variability within and between groups, without any discernible patterns or trends in protein expression, varied protein expression responses among different treatment and control groups. No significant protein expression changes were seen between any experimental groups in liver after correction.

3. Gill Samples

Around 10,000 proteins were detected in the gill samples. Comparative analysis between the lab control group and the high dose group indicated a significant upregulation of several proteins in the high dose group (p-value < 0.05; refer to Figure 3(left)). Specifically, a clear pattern of escalating fold changes and significance level were observed in these proteins with increasing 6PPD-Q dosage, indicating the dose-response protein expression (refer to Figure 3 (middle & right)).

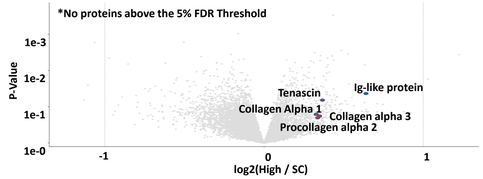
However, no statistically significant differences were detected when the solvent control was compared to any of the treatment groups or the lab control. This suggests that isolating the effects of 6PPD-Q exposure is challenging, as ethanol, used in the solvent control, may also be influencing the protein expression profiles.





Fold Change (-log$_{10}$ (P))	Low vs LC	Med vs LC	High vs LC
Collagen alpha 1	0.8 (0.6)	1 (2.4)	1.3 (3.1)
Collagen alpha 3	0.9 (1)	1 (2.6)	1.4 (3.1)
Procollagen alpha 2	1 (0.6)	0.9 (1.5)	1.5 (3.1)
Ig-like protein	1.2 (0.3)	1.4 (1.4)	1.6 (1.6)
Tenascin	1 (1)	1.3 (4.5)	1.4 (3.8)

Figure 3. (left) Volcano Plot: Differential Protein Expression in Gill Tissue Between High Dose vs Lab Control: Five proteins were significantly up-regulated (p<0.05) in the high dose group vs lab controls (p<0.05) . Three out of these proteins, namely Collagen alpha-1(IX) chain, Collagen alpha-3(IX) chain and Procollagen (type IX, alpha2) are fibril- associated collagen, which are essential for the structure of cartilage. A related study by He *et al.*, (2024) suggests that repeated injections of 6PPD-quinone in mice significantly increase collagen content, potentially leading to enhanced lung fibrosis, which highlights the pathophysiological relevance of collagen upregulation in response to 6PPD-quinone exposure. Tenascin-like proteins is a glycoprotein which plays a role in tissue repair and response to environmental stressor; **(middle) Trend Analysis of P-values for Differential Protein Expression across Dosage Levels:** The line chart demonstrates the trends in p-values for significant differential expression of key proteins across different dosage levels relative to lab control. The size of each data point on the lines corresponds to the fold change, highlighting the magnitude of expression changes: larger points indicate greater expression changes. **(right) Fold Changes in Protein Expression Across Different Dose Levels Compared to Lab Control:** The table shows a clear trend of increasing fold changes and significance with higher doses, suggesting a dose-dependent increase in the expression of these proteins.



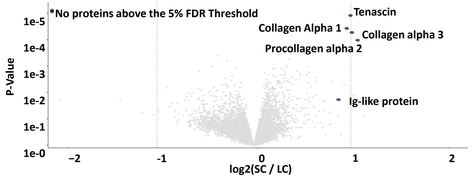


Figure 4. Volcano Plot: Differential Protein Expression in Gill Tissue. (left) High Dose vs Solvent Control: no significant differences between high dose and the solvent control were observed, suggesting that ethanol (0.01%) may also contribute to the observed protein expression changes. **(right) Solvent Control vs Lab Control:** also shows no significant differences.

Conclusion and Future Study

Sampling variability presents a significant challenge in tissue-based 'omics analyses, as evidenced by high intra-group variability observed across most proteins. To replicate the conditions of an actual storm event, coho salmon were exposed to 6PPD-Q for 24 hours; however, this short duration of exposure might not have allowed for detectable alternations in the proteome.

Reference

He, W., Chao, J., Gu, A., & Wang, D. (2024). Evaluation of 6-PPD quinone toxicity on lung of male BALB/c mice by quantitative proteomics. *Science of the Total Environment*, 171220. https://doi.org/10.1016/j.scitotenv.2024.171220
Lo BP, Marlatt VL, Liao X, Reger S, Gallilee C, Ross ARS, Brown TM. Acute Toxicity of 6PPD-Quinone to Early Life Stage Juvenile Chinook (Oncorhynchus tshawytscha) and Coho (Oncorhynchus kisutch) Salmon. Environ Toxicol Chem. 2023 Apr;42(4):815-822. doi: 10.1002/etc.5568. PMID: 36692118.
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