

HFPO-DA exposure has a statistically significant effect on gene expression in C-elegans roundworms.

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Summary - Pranav

- Null Hypothesis: There is no statistically significant evidence that gene expression levels for C-Elegans are inhibited from exposure to HFPO-DA.
- The gene expression for the gene was assessed using qRT-PCR.
- Heat map and delta-delta ct calculations reveal major correlation between HPFO-DA exposure and tba-1 gene expression in *c.elegans*.

Abstract - Pranav

The project was conducted within the confinement of our homes over a period of four weeks. During those four weeks, fellow group members used various simulations and data analysis software available through the internet to simulate a "lab experience" due to the limits brought on by COVID-19. Through the use of qrt-PCR, delta delta CT calculations, as well as simulations on the National Center for Biotechnology Information, we were able to conclude that there was a drastic effect of the HPFO-DA chemical on gene expression, as shown by our findings.

Introduction - Juwairia

Hypothesis

- There is a significant difference in expression levels of tba-1 between the control group and the group of *C. elegans* incubated with HPFO-DA
- The target genes are expressed differently after exposure to HFPO-DA.

HPFO-DA

- HPFO-DA is an industrial replacement for PFAS and PFOS¹. Similar to PFAS and PFOS, HPFO-DA is resistant to biotic and abiotic degradation¹.
- Due to the widespread use of HPFO-DA in manufacturing, this chemical compound tends to accumulate in the environment².
- HPFO-DA is found in various products such as food-packaging, non-stick coatings, paint, and fire-fighting foam².
- Previous studies show that exposure to per- and polyfluoroalkyl substances can result in chronic toxicity in other species like the Sprague-Dawley rat³.

Genes

- Target Genes: Hsp70, nhr-49, prx-11, lmd-3, and *gst-4*
- Housekeeping Genes: Genes sir-2.1 and daf-16 will be used as a control group

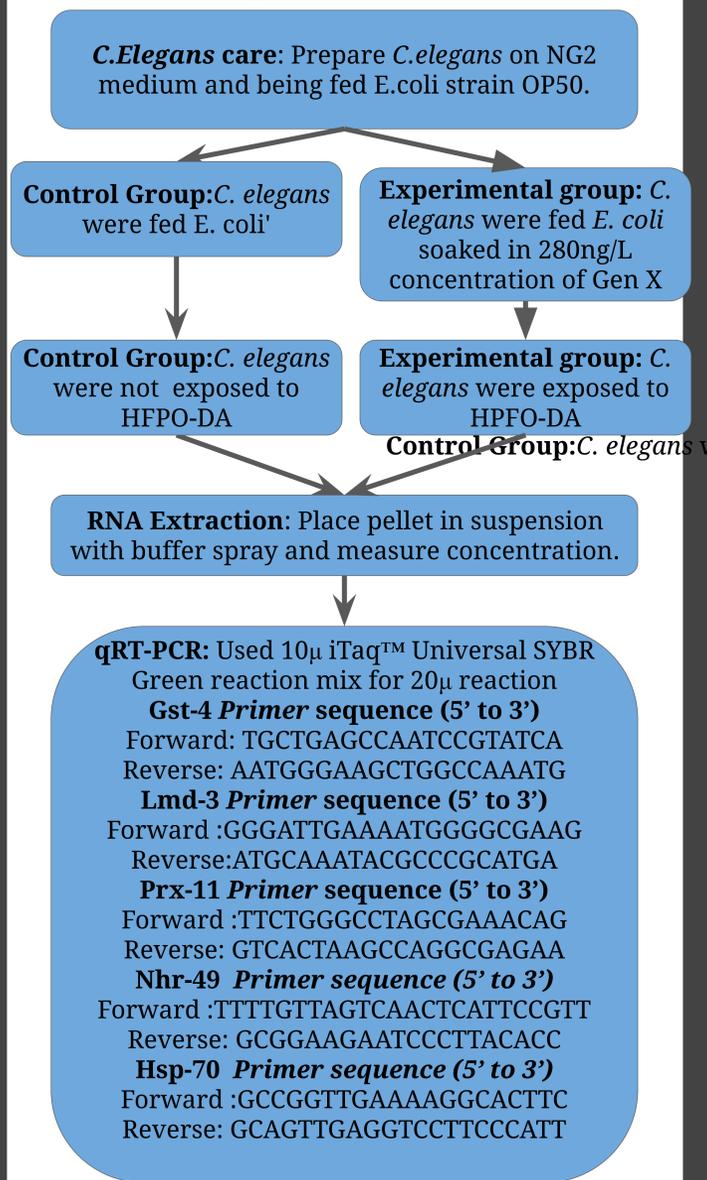
Why *C. elegans*?

- About 35% of *C. elegans* genes have human homologs⁴
- C. elegans* have short lifespans and live about 12-18 days under stable conditions⁵
- Fully sequenced genome
- Reproduce quickly and in large amounts
- Previous studies show that *C. elegans* genes have significantly different expression patterns between groups when incubated with HPFO-DA⁶.

Significance

- Understanding the effects of HFPO-DA exposure levels on gene-expression related to stress and biological aging under controlled conditions will set up baseline information for future stress experiments, and eventually, give direction to research relevant to the effects of toxicants like HPFO-DA as an environmental stressor

Methodology - Pranav



Data Analysis: Used delta delta Ct to calculate fold change

- The housekeeping gene sir 2.1 had the forward primer sequence: GTGTGTCGCGATTTCTGCTC and reverse sequence: GCAAGACGAACCACACGAAC.
- Housekeeping gene daf-16 also had the primer sequence: GCACAAGTTTACGAATGGATGGT; Reverse: GTACCCGTGGATTCTTCC
- The quality of QRC-PCR results were analyzed using melt curves.

Results - Atharv

- Our results were statistically significant.
- Chose TBA-1 gene as housekeeping gene
- significant fold-change difference between treatment (exposure to HFPO-DA) and control.
- Hsp-70, nhr-49, prx-11, lmd-3 and *gst-4* were chosen as target genes since their treatment and control fold-change difference is significantly different.
- Calculated fold change using the Delta Delta Ct technique.
- Figure 1: Target and Housekeeping Genes

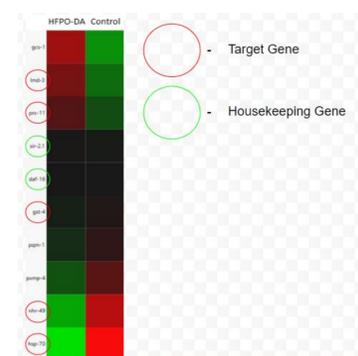


Figure 2: P-Values for each housekeeping gene

Gene of Interest	Fold Change Treated	Fold Change Control	Fold Change Control - Treated	Standard Deviation	Mean	P Value	Final P-Value
DAF-16							
hsp-70	0.171	2.284	2.113	10.36860122	13.3614	0.033189027	0.024434946
nhr-49	1.817	15.454	13.637				
prx-11	1.857	23.357	21.5				
lmd-3	1.435	23.357	21.922				
gst-4	2.3	2.355	0.055				
SIR-2.1							
hsp-70	0.105	1.925	1.82	3.209761409	3.107	0.239804775	
nhr-49	7.654	3.737	-3.917				
prx-11	0.49	4.276	3.786				
lmd-3	0.38	4.275	3.895				
gst-4	0.953	1.321	0.368				

- Performed a 2 sample 1 tailed t-test for each housekeeping gene between their "Fold Change Treated" and Fold Change Control".
- Assumed a Critical Value of 0.05, as I did not want to be too conservative (with a critical value of 0.01) since the sample size was relatively small (<30), yielding a higher standard deviation.
- Point of interest was in our overall P-Value, calculated using a T-Test between all fold change results for control, and all fold change results for treatment in both housekeeping genes.
- Yielded a P-Value of 0.024, which is less than my critical value. Therefore, we can confidently reject the null hypothesis.

Discussion/Conclusion - Atharv

Since our P-Value was less than 0.05, it proves that our results were statistically significant. In other words, HFPO-DA does have a significant negative effect on gene expression in *C-Elegans* roundworms.

Study Limitations - Juwairia

- Since the commercial development of HPFO-DA only began in 2009², there is little prior research done on this and several long-term effects on the environment and the human body are still relatively unknown

Future Directions - Juwairia

In this preliminary experiment, we studied the gene expression levels of *C. elegans* under regular conditions to establish a baseline. Our goal is to investigate the correlation between stress response and exposure to HFPO-DA. Through this, we want to gain a better understanding of what genes this chemical targets and how it changes their expressions. We strive to quantify the effects of this toxicant as a stressor and help better understand the long-term effects of HFPO-DA on the environment.

References

- R.A. Mullin E.J., Spink D.C., Legacy and emerging per-and polyfluoroalkyl substances: Analytical techniques, environmental fate, and health effects, International Journal of Molecular Sciences, Volume 22, 2021
- Mullin L, Katz D, Riddell N, Plumb R, Burgess JA, Yeung LWY, Jogsten IE. Analysis of hexafluoropropylene oxide-dimer acid (HFPO-DA) by Liquid Chromatography-Mass Spectrometry (LC-MS): Review of Current Approaches and Environmental Levels. Trends Analyt Chem. 2019;118:828-839. doi: 10.1016/j.trac.2019.05.015. PMID: 31501636; PMCID: PMC6733277.
- Caverly Rae JM, Craig L, Slone TW, Frame SR, Buxton LW, Kennedy GL. Evaluation of chronic toxicity and carcinogenicity of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate in Sprague-Dawley rats. Toxicol Rep. 2015 Jun 30;2:939-949. doi: 10.1016/j.toxrep.2015.06.001. PMID: 28962433; PMCID: PMC5598527.
- Lai, C. H., Chou, C. Y., Ch'ang, L. Y., Liu, C. S., & Lin, W. (2000). Identification of novel human genes evolutionarily conserved in *Caenorhabditis elegans* by comparative proteomics. *Genome research*, 10(5), 703-713. <https://doi.org/10.1101/gr.10.5.703>
- Riddle DL, Blumenthal T, Meyer BJ, et al., editors. *C. elegans* II. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997. Section III. Aging in *C. elegans*. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK20213/>
- Stylianou M, Björnsdóttir MK, Olsson PE, Ericson Jogsten I, Jass J. Distinct transcriptional response of *Caenorhabditis elegans* to different exposure routes of perfluorooctane sulfonic acid. *Environ Res*. 2019 Jan;168:406-413. doi: 10.1016/j.envres.2018.10.019. Epub 2018 Oct 18. PMID: 30388497.

HFPO-DA Chemical Proven to Inhibit Gene Expression in *C. elegans* Roundworm

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Hypothesis - Atharv

HPFO-DA Background - Atharv & Pranav

Genes - Juwairia

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Significance - Juwairia