Varied gene expression levels after HFPO-DA exposure across ten target genes involved in biological aging processes in *Caenorhabditis elegans*

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Summary

- *C. elegans* exposed to HFPO-DA
- Measured gene expression level across ten genes related to biological aging using qRT-PCR
- *hsp-70* downregulated the most and *gcs-1* upregulated the most after HFPO-DA exposure
- No statistically significant difference in gene expression levels across target genes

Abstract

GenX chemicals used in manufacturing, such as HFPO-DA, pose notable detrimental health effects in organisms. This study determines how the gene expression levels of ten different target genes are affected by exposure to HFPO-DA measured via qRT-PCR. The genes *gcs-1*, *lmd-3,* and *prx-11* were upregulated after exposure to HFPO-DA. The genes *gst-4*, *pqm-1*, *pxmp-4*, *nhr-49,* and *hsp-70* were downregulated in exposure to HFPO-DA. The genes *sir-2.1* and *daf-16* showed no changes in gene expression.

Introduction

Hypothesis

- After exposure to HFPO-DA, *prx-11*, *nhr-49, gcs-1, lmd-3,* and *gst-4* will be upregulated, while *hsp-70, sir-2.1, daf-16, pqm-1,* and *pxmp-4* will be downregulated.

GenX (HFPO-DA)

- Man-made chemical in PFAS (per- and polyfluoroalkyl substances) chemical group (EPA, 2021) [1]
- Animal studies show health effects in the kidney, blood, immune system, developing fetus, and liver, suggestive of cancer (EPA, 2021) [2]
- Used commercially in food packaging, household appliances, and firefighting foam
- Resistant to biodegradation

Model organism: *C. elegans*

- Short-lived: lifespan of around 2 weeks
- Small, adults are around 1 millimeter; can cultivate large populations in the lab (NCBI, 2021) [3]
- Transparent: can directly see effects of experiment in organs and/or fluorescence
- High conservation with humans of 83% (STRING, 2021) [4]

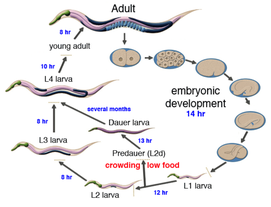


Figure 1. Life cycle of *C. elegans*. *C. elegans* hatch, mature into adulthood, and reproduce in the span of days, making them suitable model organisms.

Target Genes and Functions

- Regulation of biological aging
  - ➢ *daf-16*, *pqm-1*, *pxmp-4*: physiological development
  - ➢ *nhr-49*, *gcs-1*, *lmd-3*, *gst-4*: oxidative stress response
  - ➢ *hsp-70*, *sir-2.1*: heat shock response
  - ➢ *prx-11*: innate immune response

Significance

- This study allows us to see the effects of HFPO-DA exposure on *C. elegans* on a transcriptional level as well as indicate HFPO-DA-affected target genes involved in aging processes.

Materials and Methods

Maintenance of *C. elegans*
C. elegans N2 wild type grown using NGM in sterile environment at 23-25°C

Control: Worms were fed E. coli OP50 for 2 days

Experimental: Worms were fed E. coli OP50 incubated with 280 ng/L of HFPO-DA for 2 days

Sample Collection
1,000 worms per sample

RNA Extraction
Guanidinium-thiocyanate-phenol-chloroform liquid-liquid extraction technique used to extract RNA

qRT-PCR
Genes targeted using forward [F] and reverse [R] primers generated from NCBI primer blast in a 5' to 3' direction, and using iTaq universal SYBR.

hsp-70	F 5'-GCCGGTTGAAAAGGCACTTC-3'	R 5'-GCAGTTGAGGTCCTTCCCATT-3'
nhr-49	F 5'-TTTTGTTAGTCAACTCATTCCGTT-3'	R 5'-GCGGAAGAATCCCTTACACC-3'
pxmp-4	F 5'-TGCTGCTCTTGTCATGGGAT-3'	R 5'-TGGGAAGACTGGGAAGTTTGG-3'
pqm-1	F 5'-TTGCAGGCATAGCTCTCAGC-3'	R 5'-CGGCTGCATTAGGTTTACTGTG-3'
gst-4	F 5'-TGCTGAGCCAATCCGTATCA-3'	R 5'-AATGGGAAGCTGGCCAAATG-3'
daf-16	F 5'-GCACAAGTTTACGAATGGATGGT-3'	R 5'-GTACGCCGTGGATTCCTTCC-3'
sir-2.1	F 5'-GTGTGTCGCGATTTCTGCTC-3'	R 5'-GCAAGACGAACCACACGAAC-3'
prx-11	F 5'-TTCTGGGCCTAGCGAAACAG-3'	R 5'-GTCACTAAGCCAGGCGAGAA-3'
lmd-3	F 5'-GGGATTGAAAATGGGGCGAAG-3'	R 5'-ATGCAAATACGCCCGCATGA-3'
gcs-1	F 5'-GCAGGTGAATGCGATGCTTG-3'	R 5'-GCAAGCGATGAGACCTCCGT-3'

Data Analysis

- Target genes were normalized to the housekeeping gene tba-1
- Fold change in gene expression was calculated using delta delta Ct.
- ΔΔCt was found for the different conditions compared by taking the ΔCT difference between experiment and control groups
- Student's t-test was conducted using ΔCT values; a p-value of less than 0.05 was considered significant

Results

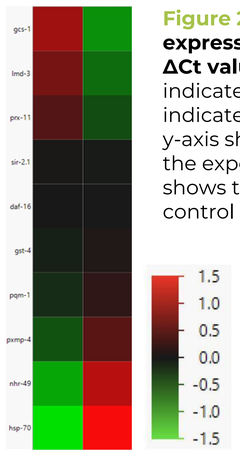
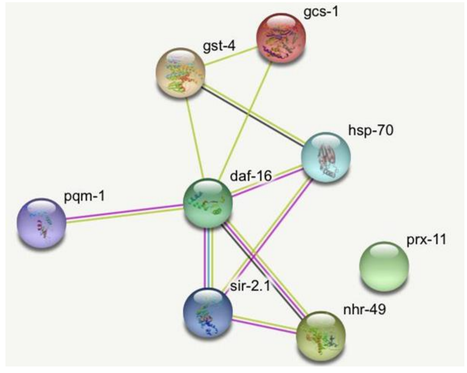


Figure 2. Heat map of gene expression in *C. elegans* based on ΔCt values from qRT-PCR. Green indicates downregulation while red indicates upregulation of a gene. The y-axis shows all the genes used in the experiment while the x-axis shows the two experimental groups: control and HFPO-DA exposure.

STRING Diagram (below). Highlighting the relationships between the target proteins. This figure shows interactions between these proteins as they respond to stress exposure.



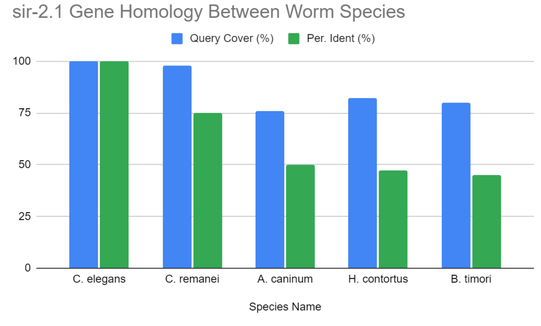


Figure 3. Gene homology bar chart showing query cover and percent identity of *sir-2.1* between *C. elegans* and different worm species using NCBI BLAST.

Discussion and Conclusion

- Downregulation of *gst-4*, *pqm-1*, *pxmp-4*, *nhr-49*, and *hsp-70* after exposure to HFPO-DA; upregulation in control condition
  - ➢ Downregulation of *gst-4* and *nhr-49* after HFPO-DA exposure suggests certain aspects of the oxidative stress response are slowed
  - ➢ Downregulation of *pqm-1* and *pxmp-4* after HFPO-DA exposure suggests certain aspects of physiological development controlled by these genes are delayed or halted
- Upregulation of *prx-11*, *lmd-3*, *gcs-1* after exposure to HFPO-DA; downregulation in control condition
  - ➢ Upregulation of *prx-11* after HFPO-DA exposure suggests activation of the innate immune response
  - ➢ Upregulation of *lmd-3* and *gcs-1* suggests increased activity in certain aspects of oxidative stress due to HFPO-DA
- No difference in *sir-2.1* and *daf-16* expression post-exposure.
  - ➢ This suggests that HFPO-DA exposure does not affect *sir-2.1* and *daf-16* in their specific functions in heat shock response and physiological development, respectively, over a period of 2 days.
- *P*-values for all comparisons, however, are larger than the 0.05 threshold set by Student's t-test. Therefore, differences observed are statistically insignificant. This is likely due to large variation between biological replicates.

Limitations and Future Inquiry

- Only one dosage of HFPO-DA used for 2 days
  - ➢ Varying doses might show different changes in gene expression
  - ➢ Changing the time C. elegans are exposed to HFPO-DA may yield a difference in gene expression
- *C. elegans* was only tested in its adult life stage
  - ➢ Observing HFPO-DA effects on earlier life stages may provide understanding on how growth and development is affected by HFPO-DA
- Only the N2 wild type strain was used in this experiment
  - ➢ Using different strains of *C. elegans* may yield different results in gene expression

Sources