

Upregulation of oxidative stress-response gene *lmd-3* by 2.4-fold in *Caenorhabditis elegans* after GenX exposure

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Summary

- GenX is the common name for a chemical used to replace perfluorooctanoic acid (PFOA).
- We investigated GenX effects on *lmd-3*, a stress response protein in *Caenorhabditis elegans* (*C. elegans*).
- Expression of *lmd-3* was upregulated in GenX-exposed *C. elegans*.

Abstract

GenX is a new chemical used in a variety of different manufacturing processes. To determine if GenX is a possible health concern, its effects on *C. elegans* gene expression were investigated. Synchronized *C. elegans* were split into two groups: control and GenX-exposed. qRT-PCR was used to analyze expression of genes such as *lmd-3*, which is responsible for senescence and stress response. We found that the gene is upregulated in the GenX group, indicating that GenX activates stress response pathways.

Introduction

GenX

- Hexafluoropropylene oxide-dimer acid (HFPO-DA) is commonly known as GenX.¹
- Widely applied in commercial products like non-stick coating on pans.¹
- How GenX is harmful to living organisms is largely unknown.

C. elegans

- Roundworms that usually live in temperate soil, and a model organism.²
- Average lifespan of 2-3 weeks.
- Life cycle starts from eggs, then go through 4 larval stages before maturing into adults.



Figure 1: Larval stage 2 (L2) *C. elegans*. This photo was taken using an inverted microscope at 10X magnification.

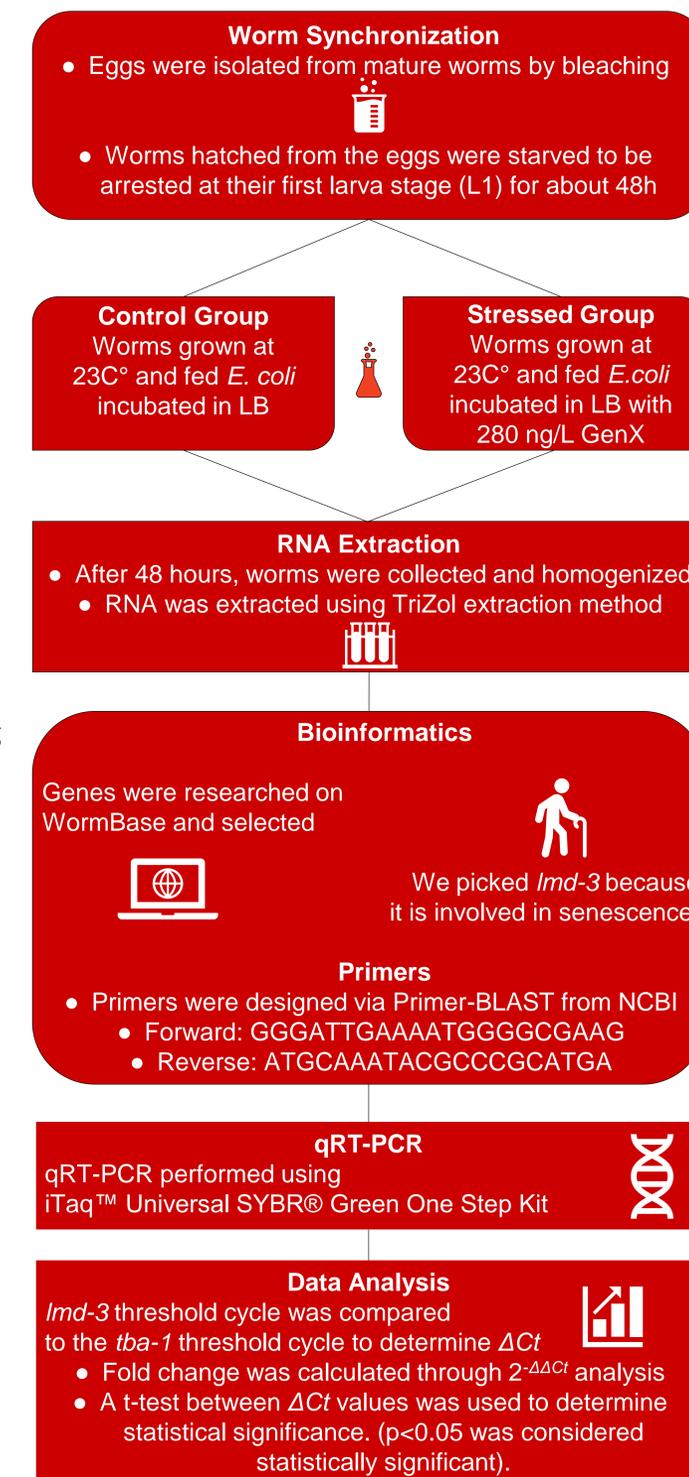
Target Gene: *lmd-3*

- Involved in heat stress and oxidative stress responses.³
- An ortholog of human *NCOA7* (nuclear receptor coactivator) and *OXR1* (oxidation resistance 1). *OXR1* is essential for oxidative stress response.³

Treatment

- *C. elegans* were grown on a diet of *E. coli*, which were incubated in LB with 280 ng/L GenX (EPA health advisory limit).^{4,5}

Methodology



Results

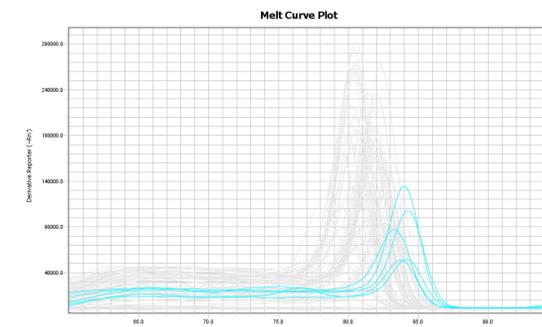


Figure 2. qRT-PCR melt-curve plot for *lmd-3*. The similar placement of peaks on the x-axis indicates similarity amongst amplification products. The curve was generated using QuantStudio™ Design & Analysis Software.

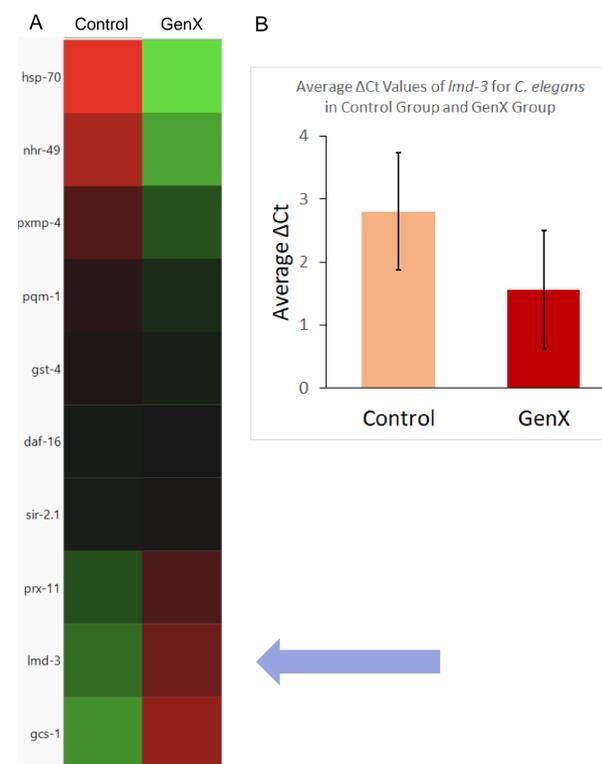


Figure 3. Heatmap (A) and bar graph (B) showing differential expression of *lmd-3*. *lmd-3* is indicated by the arrow in the heatmap. Green indicates relatively low expression level, and red indicates relatively high expression level. The heatmap was generated using JMP Pro 14. Standard deviations of the ΔCt values are represented by error bars in the bar graph. The difference of expression level is not statistically significant ($p=0.28$).

Conclusion

- The proximity of the melt curve peaks to each other indicates RNA transcripts of *lmd-3* for each technical replicate were similar, and specific.
- GenX changes the expression level of *lmd-3* in *C. elegans* through *E. coli* at 280 ng/L, calculated from the EPA draft reference dose of chronic GenX exposure for humans.
- *lmd-3* codes for proteins affecting senescence. These proteins are also involved in heat and oxidation responses.
- Due to *lmd-3* being an ortholog for the human genes *NCOA7* and *OXR1*, this study shows GenX may activate human oxidative stress response, heat stress, and affect aging through these genes.

Study Limitations

- *C. elegans* were indirectly exposed to GenX by ingesting *E. coli* exposed to GenX.
- 280 ng/L was the only concentration tested.
- *C. elegans* were exposed only from L1 stage to early adult.

Future Directions

- Repeat experiment with direct exposure methods.
- Repeat experiment with multiple concentrations to generate a dose-response relationship.
- Expose the worms to GenX for a longer period to study chronic exposure effects.
- Measure the worms' lifespan and other factors to determine phenotypic GenX effects.
- Study expression responses to GenX in genes coding for chaperone proteins, another key component of oxidative stress and heat stress response.

References

