Metabolic genes *nhr-49* and *pxmp-4* downregulated by 4.3 fold and 1.8 fold in GenX-stressed *Caenorhabditis elegans (C. elegans)*

Gaxiola S.[1], Mesri L.[2], Whall D.[2], Villarreal C.[3,4], Engelhart D.[3,4], Feng Z.[3]

[1]Academy of Our Lady of Peace, [2]La Jolla High School, [3]Boz Life Science Research and Teaching Institute, [4]UC San Diego Extension

Summary

- Exposed *C. elegans* to the potentially toxic chemical, GenX.
- Investigated changes in gene expression of *nhr-49* and *pxmp-4* using qRT-PCR.
- *nhr-49* and *pxmp-4* were downregulated 4.3 fold and 1.8 fold following GenX exposure.

Abstract

GenX is a chemical used to manufacture nonstick cookware, waterproof coatings, and more, but its effects on organisms are largely unknown. To better understand them, we exposed *C. elegans* to GenX and analyzed expression of genes such as *nhr-49* and *pxmp-4*, which are involved in metabolism. qRT-PCR reveals that GenX affects gene expression in *C. elegans*, decreasing expression of these genes.

Introduction

Hypothesis:
Exposure to GenX through *Escherichia coli* (*E. coli*) causes an increase in nhr-49 and pxmp-4 gene expression in *C. elegans*.

C. elegans
- Common model organism
  - Chosen for its short lifespan, size, transparency, ability to easily reproduce, and simple maintenance.[1]

GenX:
- Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals that include PFOA, PFOS, and GenX.
- GenX is used in the production of "high-performance polymers" (coating textiles, paper products, cookware, etc.).
- GenX is a PFOA replacement with largely unknown toxicity and an environmental pollutant.[2]

Genes
- Nuclear Hormone Receptor-49 (*nhr-49*) - regulator of fat usage, controls energy metabolism, and helps to determine adult lifespan; ortholog of HNF4A and HNF4G in human.[3]
- Peroxisomal Membrane Protein-4 (*pxmp-4*) - encodes peroxisomal membrane protein. Peroxisomes are involved in lipid metabolism. It is an ortholog of PXMP4 in human.[4]

Treatment
- *C. elegans* were fed with *E. coli* incubated in 280 ng/L GenX (EPA health advisory limit).[5]

Methodology

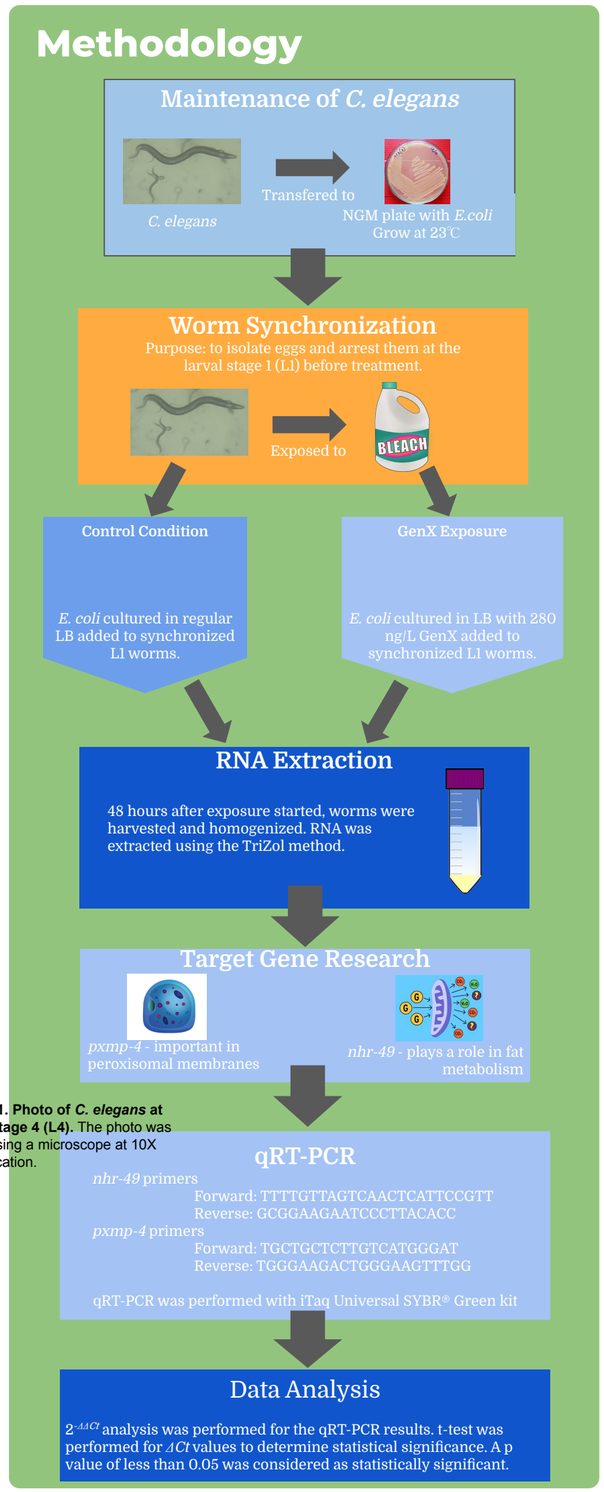

Figure 1. Photo of *C. elegans* at larval stage 4 (L4). The photo was taken using a microscope at 10X magnification.

Results

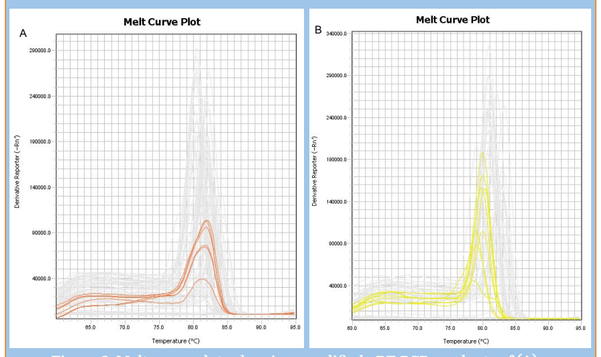

Figure 2. Melt curve plots showing amplified qRT-PCR products of (A) *nhr-49* and (B) *pxmp-4* primers. The plots were created by QuantStudio™ Design & Analysis Software v1.5.1.

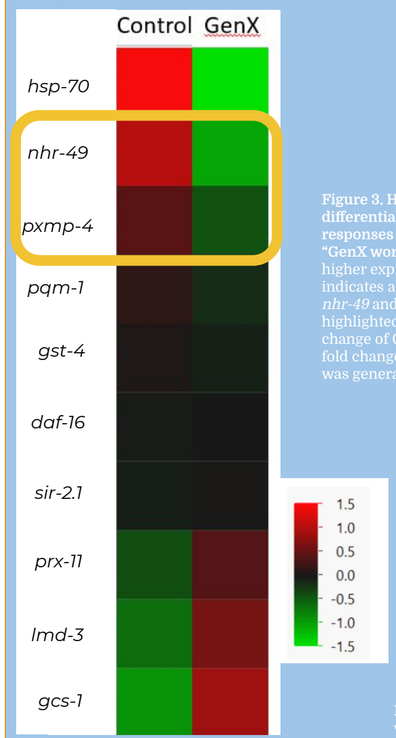

Figure 3. Heatmap showing differential gene expression responses in "control worms" and "GenX worms". Red indicates a higher expression level and green indicates a lower expression level. *nhr-49* and *pxmp-4* are highlighted. *nhr-49* had a fold change of 0.234 and *pxmp-4* had a fold change of 0.551. This figure was generated using JMP Pro 14.

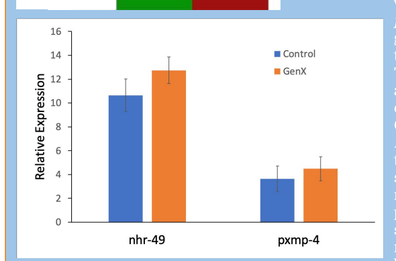

Figure 4. Average ΔCt values of *nhr-49* and *pxmp-4*. The data from this graph was obtained through the QuantStudio™ 3 System. The graph compares average ΔCt values in control worms and GenX-exposed worms for *nhr-49* and *pxmp-4*, and there is no statistically significant differences for nhr-49 (p=0.07) and pxmp-4 (p=0.30). The standard deviations are represented by the error bars.

Discussions & Conclusions

- GenX affects *C. elegans* through *pxmp-4* and *nhr-49* at the tested concentration (Fig. 3).
- *nhr-49*
  - Decreased expression indicates that GenX negatively impacted the organism's energy metabolism, fat usage.
  - Adult lifespan could be affected if the exposure did not stop at L4. [3]
- *pxmp-4*
  - Slightly decreased expression suggests decreased demand for peroxisome membrane proteins, and potentially impacted on lipid metabolism.[4]
- Potentially similar gene expression effects in humans, due to *pxmp-4* and *nhr-49* being also present in humans.[6]
- Melt curve analysis shows there is a secondary amplification product for one technical replicate of *pxmp-4*, but taking out that data point will not affect our conclusion .

Study Limitations & Future Directions

- Worms were only exposed to one concentration of GenX. Experiments should be conducted at more concentrations of GenX.
- GenX exposures were conducted only from L1 to early adult stages in worms. Next steps should include exposing worms into adulthood.
- Experiments used pooled worms, and cannot account for individual variations.
- *C.elegans* are distant from humans, and in the future, more experiments on other model organisms should be performed.

Sources

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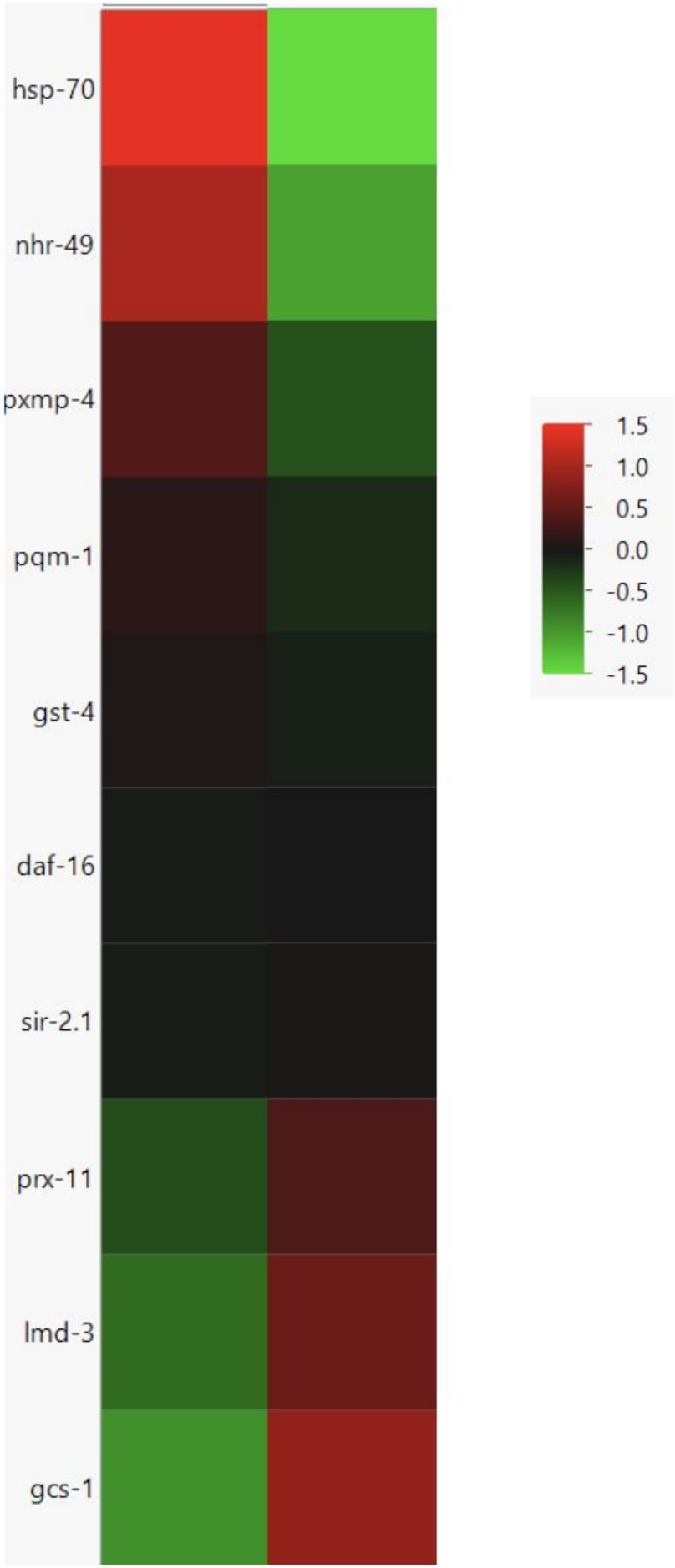
Link for QR code:



FWD PXMP	TGCTGCTCTTGTCATGGGAT
REV PXMP	TGGGAAGACTGGGAAGTTTGG

GenX is a chemical used to manufacture nonstick cookware, waterproof coatings, and more, but its dangers are largely unknown. To better understand them, we treated *C. elegans* with GenX and analyzed expression of genes such as *nhr-49* and *pxmp-4,* which are involved in metabolism. qRT-PCR reveals that GenX affects gene expression in *C. elegans*, decreasing expression of these genes making it potentially harmful to humans since GenX is an environmental pollutant.

FWD NHR-49	TTTTGTTAGTCAACTCATTCCGTT
REV NHR-49	GCGGAAGAATCCCTTACACC

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Abstract

GenX is a chemical used to manufacture nonstick cookware, waterproof coatings, and more, but its effects on organisms are largely unknown. To better understand the potential adverse effects, we exposed *C. elegans* to GenX and analyzed expression of genes such as *nhr-49* and *pxmp-4*, which are involved in metabolism. qRT-PCR reveals that GenX affects gene expression in *C. elegans*, decreasing expression of these genes.

Introduction

Hypothesis:
Exposure to GenX through *Escherichia coli* (*E. coli*) downregulates nhr-49 and pxmp-4 gene expression in *C. elegans* due to similarities between GenX and PFOA.

C. elegans
- Common model organism
  - Chosen for its fully sequenced genome, short lifespan, small size, transparency, and simple maintenance.

Figure 1. Photo of *C. elegans* at larval stage 4 (L4). The photo was taken using a microscope at 10X magnification.

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- GenX is used in the production of "high-performance polymers" (coating textiles, paper products, cookware, etc.).
- GenX is a PFOA replacement with largely unknown toxicity and an environmental pollutant.
- PFOA is an agonist of peroxisome proliferator-activated receptors (PPARs).

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- Nuclear Hormone Receptor-49 (*nhr-49*) - regulator of fat usage, controls energy metabolism, and helps to determine adult lifespan; ortholog of HNF4A and HNF4G in human. Functionally similar to mammalian PPAR-α.
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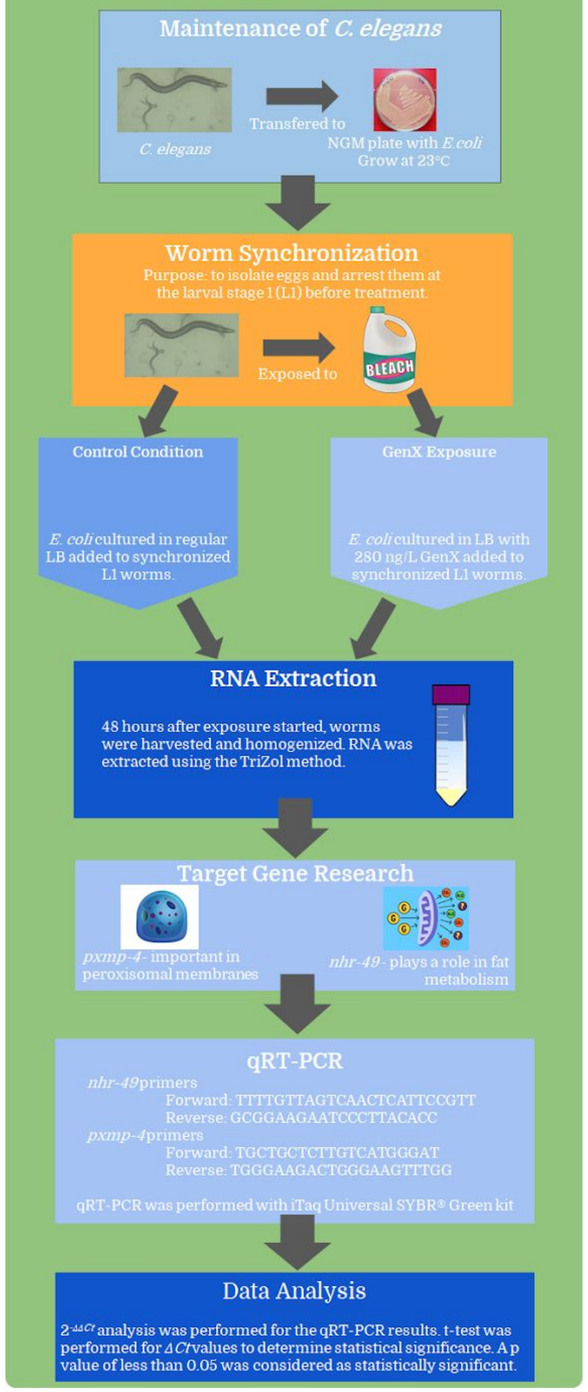

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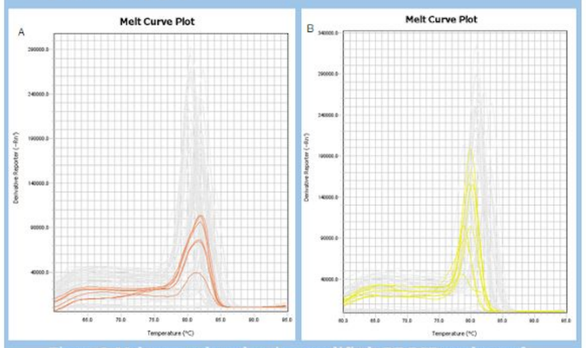

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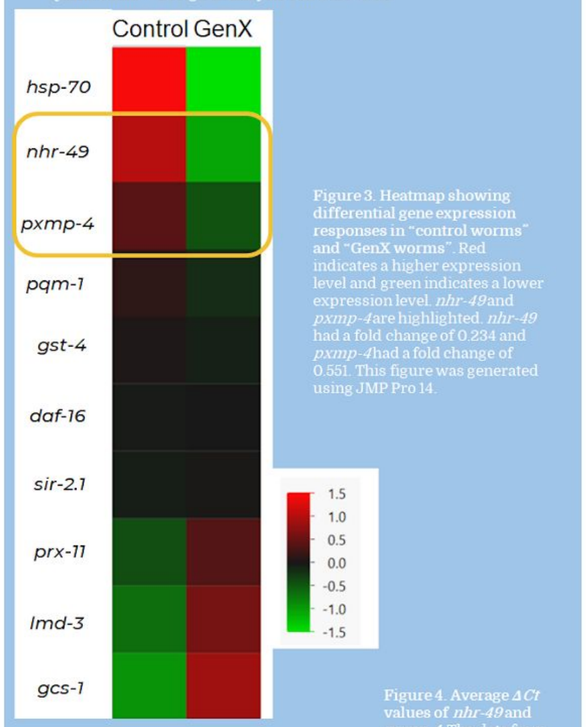

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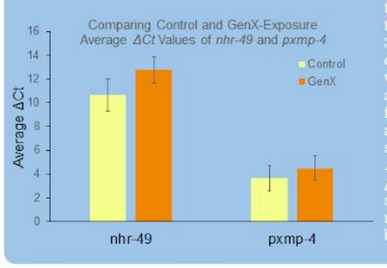

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Discussions & Conclusions

- As expected, GenX affects *C. elegans* through *pxmp-4* and *nhr-49* at the tested concentration (Fig. 3).
- A possible reason for the downregulation is GenX acted as an agonist of PPAR.
- *nhr-49*
  - Decreased expression indicates that GenX could have negatively impacted the organism's energy metabolism, fat usage.
  - Adult lifespan could be affected if the exposure did not stop at L4.
- *pxmp-4*
  - Slightly decreased expression suggests decreased demand for peroxisome membrane proteins, and potentially impacted on lipid metabolism.
- GenX could potentially induce similar gene expression effects in humans, due to *pxmp-4* and *nhr-49* having orthologs in humans.
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