Low-concentration GenX exposure did not significantly affect the expression of *pqm-1* & *daf-16*, two lifespan-regulating genes, in *Caenorhabditis elegans*

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Summary

- GenX is a replacement for perfluorooctanoic acid (PFOA), a chemical shown to be detrimental to human health. However GenX toxicity is largely untested.
- *pqm-1* and *daf-16* both code for proteins involved in *C. elegans* lifespan determination.
- In GenX-treated *C. elegans*, fold changes for *pqm-1* and *daf-16* were 0.81 and 1.01, respectively.
- GenX exposure alters gene expression but does not appear to alter lifespan determination specifically.

Abstract

GenX is a chemical used in the production of everyday goods such as food wrappers and nonstick coatings, but its toxicity is largely unknown. Examining how GenX affects *C. elegans* may help us understand how these chemicals affect us as well. Focusing on genes involved in lifespan determination, *pqm-1* and *daf-16*, we observed that low-concentration GenX treatment did not alter the expression of these genes. We conclude that at the tested concentration, GenX is not affecting *C. elegans* lifespan determination by changing the expression of *pqm-1* and *daf-16*.

Introduction

Hypothesis:

- Expression of pqm-1 and daf-16 is significantly altered in C. elegans with their food source *Escherichia coli* (*E. coli*) exposed to 280 ng/L of GenX.

Model organism:

- *C. elegans* share many similar biological characteristics with humans such as skin, muscles, neurons, and gut.[1]
- The genome of *C. elegans* has been fully sequenced.

Figure 1. Microscope image of a *C. elegans* at larval stage 1 (L1). The photo was taken at 10X magnification.

GenX:

- GenX is a replacement for PFOA, a chemical shown to be detrimental to human health.[2]
- GenX is a chemical that can be found in food packaging, non-stick products, paints, and firefighting foam.[2]
- In preliminary studies, GenX has been shown to cause liver damage.[3]
- EPA's draft reference dose for GenX is four times of that for PFOA, so the experimental concentration (280 ng/L) was determined to be four times of the health advisory level for PFOA (70 ng/L).[4,5]

Target genes:

pqm-1: predicted to have nucleic acid binding activity; mutants have been linked to an increased lifespan in *C. elegans*.[6,7]

daf-16: involved in DNA-binding transcription factor activity and enzyme binding activity; regulates lifespan.[6,8]

Methodology

Preparing the *E. coli* and *C. elegans*

- LB medium was used to culture *E. coli* OP50 in a sterile environment at 37°C.
- *C. elegans* were cultured on NGM plates inoculated with *E. coli*.
- Bleaching method was used to synchronize the control group and the experimental group to the L1 stage.

Control Condition

E. coli cultured in regular LB were added to NGM plates with L1 worms. The plates were kept at 23°C for 2 days.

Stressed Condition

E. coli were cultured in LB with 280 ng/L GenX for 2 days, then added to NGM plates with L1 worms. The plates were kept at 23°C for 2 days.

RNA Extraction

Worms were homogenized with a bead mill. RNA samples were extracted using the TriZol RNA extraction method.

Figure 2. An artistic representation of RNA extraction by Kalia Reece.

qRT-PCR

Primers

pqm-1	TTGCAGGCATAGCTCTCAGC	(FWD)
	CGGCTGCATTAGGTTTACTGTG	(REV)
daf-16	GCACAAGTTTACGAATGGATGGT	(FWD)
	GTACGCCGTGGATTCCTTCC	(REV)

qRT-PCR was performed using iTaq Universal SYBR® Green kit with 20 µl reaction volume.

Data Analysis

Ct number normalized with *tba-1* (housekeeping gene) → *ΔCt* → used to generate the heatmap. *ΔΔCt* determined by calculating the difference between the *ΔCt* values of the control group and GenX-exposed group. t-test was performed for the *ΔCt* values of the technical replicates, and a p value of less than 0.05 was considered statistically significant.

Results

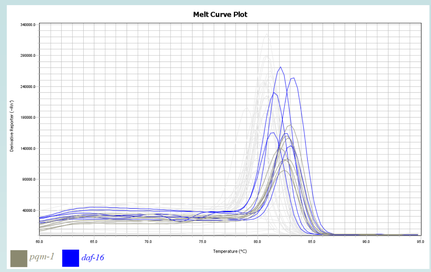

Figure 2. qRT-PCR melt curve plot for *pqm-1* and *daf-16*. Proximity among peaks indicate similarity among amplification products. The plot was generated by QuantStudio™ Design & Analysis Software v1.5.1.

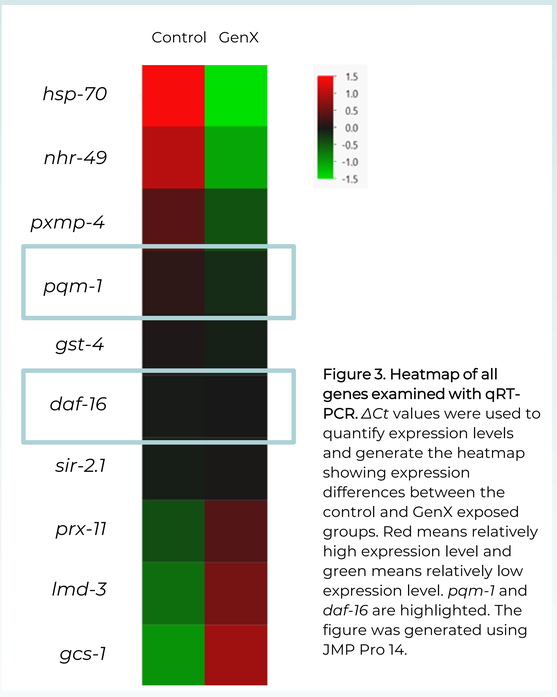

Figure 3. Heatmap of all genes examined with qRT-PCR. *ΔCt* values were used to quantify expression levels and generate the heatmap showing expression differences between the control and GenX exposed groups. Red means relatively high expression level and green means relatively low expression level. *pqm-1* and *daf-16* are highlighted. The figure was generated using JMP Pro 14.

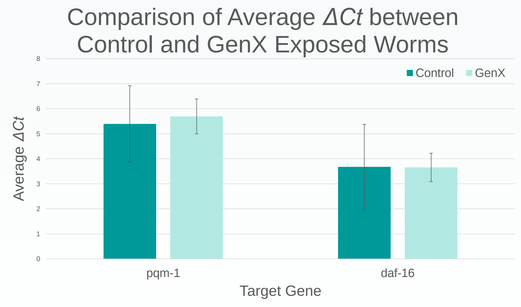

Figure 4. Average change in threshold cycle between control and GenX treated samples. Gene expression was quantified through qRT-PCR and normalized to *tba-1*. Between the *ΔCt* values of the control group and the GenX-exposed group, there is no statistically significant differences for *daf-16* (p=0.988) and *pqm-1* (p=0.770). Error bars represent standard deviations.

Discussion

- The peaks in the melt curve plot are close to each other, indicating that the amplified RNA transcripts were similar both in the case of *pqm-1* and *daf-16* (Fig.2).
- In *C. elegans* fed with *E. coli* exposed to GenX, *pqm-1* and *daf-16* gene expression was not significantly different from the control (Fig. 4).
- GenX does not affect *C. elegans*' lifespan through *pqm-1* and *daf-16* at the tested concentration.
- *daf-16* is an ortholog of human FOXO genes,[8] meaning that low level of GenX might not affect human via FOXO.
- Other processes in C. elegans were affected by GenX exposure (Fig. 3).
- The concentration of GenX tested was calculated from the EPA draft reference dose for human, and we can see that even this level changes gene expression in many cases.

Study Limitations

- *C. elegans* were not exposed to GenX during their adulthood. Exposures only spanned from L1 stage to early adult life.
- *C. elegans* were not directly exposed to GenX.
- Only one concentration of GenX was used.
- *C. elegans* were only an experimental organism and we do not have proof and evidence to conclude that the same effects will occur in humans.
- RNA was extracted from a pooled sample of *C. elegans*; individual variation was not considered.

Future Directions

- Exposing adult *C. elegans* to GenX to infer how adult animals are affected by GenX.
- Culturing *C. elegans* in liquid medium with GenX.
- Testing more concentrations of GenX to develop a dose-response relationship, as different concentrations of GenX may have different effects.
- Exposing other model organisms to GenX using similar methods.
- Testing more genes would be useful to see if GenX affects different *C. elegans* systems that were not observed.
- Looking for directly observable phenotypic changes and biomarkers of response other than transcriptional changes.

Bibliography