Effects of methyl and inorganic mercury exposure on genome homeostasis and mitochondrial function in *Caenorhabditis elegans*

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A B S T R A C T

Mercury toxicity mechanisms have the potential to induce DNA damage and disrupt cellular processes, like mitochondrial function. Proper mitochondrial function is important for cellular bioenergetics and immune signaling and function. Reported impacts of mercury on the nuclear genome (nDNA) are conflicting and inconclusive, and mitochondrial DNA (mtDNA) impacts are relatively unknown. In this study, we assessed genotoxic (mtDNA and nDNA), metabolic, and innate immune impacts of inorganic and organic mercury exposure in *Caenorhabditis elegans*. Genotoxic outcomes measured included DNA damage, DNA damage repair (nucleotide excision repair, NER; base excision repair, BER), and genomic copy number following MeHg and $HgCl_2$ exposure alone and in combination with known DNA damage-inducing agents ultraviolet C radiation (UVC) and hydrogen peroxide (H_2O_2), which cause bulky DNA lesions and oxidative DNA damage, respectively. Following exposure to both MeHg and $HgCl_2$, low-level DNA damage (∼0.25 lesions/10 kb mtDNA and nDNA) was observed. Unexpectedly, a higher MeHg concentration reduced damage in both genomes compared to controls. However, this observation was likely the result of developmental delay. In co-exposure treatments, both mercury compounds increased initial DNA damage (mtDNA and nDNA) in combination with H_2O_2 exposure, but had no impact in combination with UVC exposure. Mercury exposure both increased and decreased DNA damage removal *via* BER. DNA repair after H_2O_2 exposure in mercury-exposed nematodes resulted in damage levels lower than measured in controls. Impacts to NER were not detected. mtDNA copy number was significantly decreased in the MeHg-UVC and MeHg-H_2O_2 co-exposure treatments. Mercury exposure had metabolic impacts (steady-state ATP levels) that differed between the compounds; $HgCl_2$ exposure decreased these levels, while MeHg slightly increased levels or had no impact. Both mercury species reduced mRNA levels for immune signaling-related genes, but had mild or no effects on survival on pathogenic bacteria. Overall, mercury exposure disrupted mitochondrial endpoints in a mercury-compound dependent fashion.

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1. Introduction

Mercury is a heavy metal of great interest because of its pervasive presence in the environment and recognized adverse impacts on multiple systems, including most prominently the nervous system. Nervous system and other impacts, including cell injury and death, are likely derived from multiple mechanisms that could also impact DNA homeostasis and alter mitochondrial functions. Proposed genotoxic mechanisms are mostly indirect and include: altering the balance of antioxidant enzymes in favor of a pro-oxidant environment [1,2]; impairing DNA repair enzymes [3]; reactive oxygen species (ROS) generation as a result of the inhibition of electron transport chain (ETC) proteins [4,5]; and altered calcium homeostasis which has been associated with apoptosis and necrosis [6].

Protein impairment from both inorganic and organic exposure is considered an important mechanism in mercury toxicity. Mercury has a strong affinity for thiol and selenol groups, and by binding to these groups mercury can impair protein function [1]. Reductions in antioxidant enzyme activity occur following chronic exposure due to inhibition of enzymes such as glutathione reductase, glutathione peroxidase, and superoxide dismutase [7,8], and

in one report persisted beyond mercury elimination after developmental exposures in rodents *via* an unidentified mechanism [9]. Declines in antioxidant function can promote oxidative stress as a result of diminished ability to properly cope with endogenous as well as exogenous oxidants [10,9]. The promotion of oxidative stress may also be related to mitochondrial impacts. Mercury accumulates in the mitochondria, in addition to the nucleus and lysosomes [11–13]. In addition, ROS including superoxide anion and hydrogen peroxide are produced at higher than normal levels following methylmercury exposure due to the inhibition of ETC proteins and reduced superoxide dismutase scavenging activity [5]. Other reported mitochondrial impairments include increased oxygen consumption and increased membrane permeability from augmented calcium influx [14,5,15]. The combination of reduced antioxidant capabilities and increased ROS may promote damage to macromolecules, including DNA.

DNA damage following mercury exposure has been observed and is thought to occur through either direct oxidation of DNA [16,17] and/or reduced repair capacity due to impaired DNA repair enzymes [18–21]. Laboratory, field, and epidemiological studies have reported increased strand breaks, chromosome aberrations, 8-hydroxy-2′-deoxyguanosine (8-OHdG), micronuclei, and reduced DNA repair [22,19,21]. However, there are discrepancies among these studies. In some studies, mercury has a strong dose-response relationship below cytotoxic levels, but in others, increased DNA damage was not observed at exposures below cytotoxic levels or when not in combination with another exposure (radiation, H_2O_2) [23,18,20]. Epidemiological studies indicate that DNA damage is possible in fish-eating populations, in children, and most importantly at exposure levels lower than those known to adversely impact the nervous system [22,24,18,25,26]. The focus of previous studies has primarily been on impacts to the nuclear genome, with only one study to date reporting mtDNA impacts [27]. Depending on the kind of damage, increased mtDNA damage and damage accumulation in the mitochondrial genome are possible as there are fewer DNA repair pathways in this organelle [28–30]. Damage to DNA, mitochondria, and other cellular components can lead to broader impacts.

In addition to neurological impacts, multiple effects of mercury exposure on immune responses have been reported, including both increased and decreased antibody and cytokine concentrations [31–33]. Immune system impacts may be in part related to impaired mitochondrial function as mitochondria have an important role in immune system function, including ROS signaling and generation of mitochondrial specific damage-associated molecular patterns that activate pattern-recognition receptors [34,35]. *Caenorhabditis elegans* is a useful model to study innate immunity because it lacks an adaptive immune response, simplifying interpretation of outcomes, and has an innate immune system that shares similarities at the molecular level with that of higher eukaryotes. *C. elegans* mounts bacterial immune responses through well studied signaling pathways including p38 mitogen-activated protein kinase (MAPK) pathway, an insulin/insulin-like growth factor receptor (IGF) pathway, and transforming growth factor (TGF)-beta pathway [36–43]. The p38 MAPK pathway can be affected by ROS [44–47]. Additionally, human pathogens including gram-negative *Pseudomonas aeruginosa* can kill *C. elegans* using virulence factors required for pathogenicity in mammalian systems [37,48,49]. In this paper, we test potential impacts of mercury on one innate signaling pathway, through a mitogen-activated protein kinase (MAPK), in *C. elegans*. The impact of one environmental stressor, heat-shock, has been observed to impact innate immunity in *C. elegans* [50,51], but the effect of other environmental exposures, such as heavy metal exposures, has not been assessed to date in this system.

The aim of this study was to assess genotoxic, mitochondrial, and immunotoxic endpoints following inorganic and organic mercury exposure using the model organism *C. elegans*, which has increasingly been used as a model for assessing DNA damage and mitochondrial toxicity [52–54] caused by environmental stressors [55–59]. Our objectives were to assess the impact of mercury exposure on: 1) DNA damage and repair, 2) mitochondrial parameters (DNA copy number and steady-state ATP levels), and 3) innate immunity.

2. Materials and methods

2.1. C. elegans *and bacterial strains*

Nematode populations were maintained at 20 °C on K agar plates seeded with OP50 strain *Escherichia coli* unless otherwise stated [60]. N2 (wild-type) and JK1107 *glp-1(q224)* were obtained from the Caenorhabditis Genetics Center (CGC, University of Minnesota). PE255 *glp-4 (bn2)* strain was generously provided by Dr. Christina Lagido (University of Aberdeen, UK). KU25 *pmk-1(km25)* were obtained from CGC. *glp* strains were maintained at 15 °C, until time of experiment, and then shifted to the restrictive temperature of 25 °C to limit germ cell production. Experiments involving DNA damage utilized the germline-deficient mutant (*glp-1*) to minimize the potential confounding of DNA damage dilution from dividing germ cells, as no cell division occurs in adult the adult life-stage [61]. Steady-state ATP levels were determined using the transgenic, firefly luciferase-expressing nematode strain PE255 (*glp-4*), as previously described [62,63]. N2 and *pmk-1* were utilized to assess the impact of mercury on both immunocompetent and immune compromised nematodes. PMK-1 is a p38 mitogen-activated protein kinase that has a protective role in infection and is required for immune induction [40,64]. The pathogenic *Pseudomonas aeruginosa* strain PA14 was also used for the immune function experiments. For all experiments, synchronized L1 larvae were obtained by treating gravid adults with a 5% sodium hypochlorite solution and hatching eggs in the absence of food (K medium plus $MgSO_4$, $CaSO_4$, and cholesterol) [60]. A general schematic of the experimental design is presented in Fig. 1.

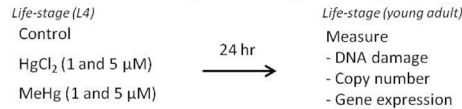### 2.2. Experiment 1: DNA damage and genome copy number following mercury exposure

Nematodes (*glp-1*) were grown to young adult stage (36 h at 25 °C) and then transferred to 24-well plates (100–230 nematodes per well) and exposed to control conditions, $HgCl_2$ (1 and 5 μM), or MeHgCl (1 and 5 μM) for 24 h and then sampled to assess DNA damage. Liquid exposures were performed in EPA reconstituted moderately hard water (hereafter described as "EPA water") plus UVC-killed *E. coli* (UVRA strain), to eliminate the potentially confounding effect of bacterial metabolism on exposures, as previously described [65,66]. The experiment was repeated three times separated in time (n = 9–27).

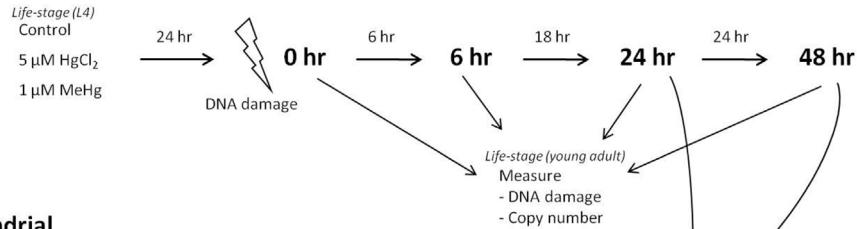### 2.3. Experiment 2: DNA damage repair and removal following mercury exposure

Young adult *glp-1* nematodes were exposed to mercury and then two prototypical DNA damage-inducing agents, ultraviolet C radiation (UVC) and hydrogen peroxide (H_2O_2), to test if mercury would inhibit repair or removal of DNA damage. $HgCl_2$ (5 μM) and MeHgCl (1 μM) concentrations were chosen based on the exposures that caused similar increases in DNA damage in Experiment 1 but did not result in significant growth reductions. We excluded growth-inhibiting exposure levels because delayed development is associated with increased DNA repair gene expression [67], and, in

Genotoxicity

Experiment 1: DNA damage following mercury exposure

Life-stage (L4)
Control

HgCl$_2$ (1 and 5 μM) $\xrightarrow{\text{24 hr}}$

MeHg (1 and 5 μM)

Life-stage (young adult)
Measure
- DNA damage
- Copy number
- Gene expression

Experiment 2: DNA damage repair and removal following mercury exposure

Life-stage (L4)
Control

5 μM HgCl$_2$ $\xrightarrow{\text{24 hr}}$ **0 hr** $\xrightarrow{\text{6 hr}}$ **6 hr** $\xrightarrow{\text{18 hr}}$ **24 hr** $\xrightarrow{\text{24 hr}}$ **48 hr**

1 μM MeHg

DNA damage

Life-stage (young adult)
Measure
- DNA damage
- Copy number

Mitochondrial

Experiments 1 and 2: Genome copy number following mercury exposure

Experiment 3: ATP determination following mercury exposure and DNA damage

Innate Immunity

Experiment 4: Innate immunity in wild type and immune deficient nematodes, survival assay
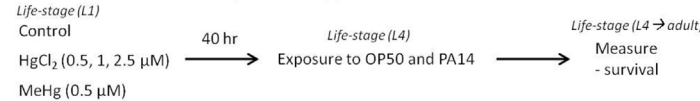
Life-stage (L1)
Control

HgCl$_2$ (0.5, 1, 2.5 μM) $\xrightarrow{\text{40 hr}}$

MeHg (0.5 μM)

Life-stage (L4)
Exposure to OP50 and PA14 $\longrightarrow$

Life-stage (L4 → adult)
Measure
- survival

Fig. 1. General experimental schematic.

this study, with lower basal levels of DNA damage (Fig. 1). These differences would confound our ability to isolate the effect of mercury exposure. Nematodes were then washed and exposed to UVC or H$_2$O$_2$. UVC exposure was chosen to assess nucleotide excision repair (NER) which removes bulky lesions including photodimers in the nucleus, and mitophagy which removes the same types of damage from mtDNA. H$_2$O$_2$ exposure was chosen to evaluate base excision repair (BER) which is responsible for removal of most oxidative damage in both genomes.

Nematodes (*glp-1*) were raised to young adult stage in the same manner as Experiment 1. Following 36 h, nematodes were transferred to 24-well plates (100–220 nematodes per well) containing control, 5 μM HgCl$_2$, or 1 μM MeHgCl in EPA water plus UVC-killed *E. coli* for 24 h. For UVC exposures, nematodes were transferred to K agar plates without OP50 and exposed to 50 J/m^2 UVC using an ultraviolet lamp (UVLMS-38 EL Series 3UV Lamp, UVP, Upland, CA, USA) with peak emission at 254 nm. UVC doses were quantified using a UVX digital radiometer. For H$_2$O$_2$ exposures, nematodes were transferred to 24-well plates and exposed to 5 mM H$_2$O$_2$ for 1 h without food. Immediately following the DNA damage event nematodes were sampled (0 h) and then returned to their original medium (control or mercury condition). DNA damage removal was assessed by sampling at 6, 24, and 48 h following the DNA damage event. The experiment was repeated three times separated in time (n = 9–21 for each time-point).

2.4. Experiment 3: ATP determination following mercury exposure and DNA damage

Utilizing a similar experimental design as Experiment 2, steady-state ATP levels were determined at 24 and 48 h following the DNA damage event, essentially as described [62,63]. Differences included using a transgenic, luciferase-expressing nematode strain (PE255 *glp-4*). For each time point 100 nematodes (in 100 μL K-medium) were loaded into each well of a white 96-well plate, such that each treatment was loaded into four separate wells (i.e. four technical replicates). GFP fluorescence, which is used to normalize luminescence readings to GFP-luciferase fusion protein expression levels, was measured using a FLUOstar OPTIMA BMG Labtech plate reader (Ortenberg, Germany; excitation filter: 485 nm; emissions filter: 512 nm). An automated dispenser was then used to deliver 50 μl of luminescence buffer (citrate phosphate buffer pH 6.5, 0.1 mM D-luciferin, 1% DMSO, 0.05% Triton X-100) to each well, and luminescence was measured in the visible spectral range of 300–600 nm. All luminescence values were normalized to their corresponding GFP fluorescence value and ATP values are reported as percent of control for each time-point. The experiment was repeated four times (n = 4 for each time-point).

2.5. Experiment 4: Innate immunity in wild type and immune deficient nematodes, survival assay

The impact of larval mercury exposure (HgCl$_2$ and MeHgCl) on innate immunity against pathogenic bacteria was assessed by exposing N2 and *pmk-1* nematodes to *P. aeruginosa* PA14. L1 nematodes were transferred to 24-well plates (800–2500 worms per well) and exposed to control, HgCl$_2$: 0.5, 1, 2.5 μM, or MeHgCl: 0.5 μM for 40 h at 20 °C. Developmental stage is an important factor that needs to be considered when utilizing this survival assay, because survival time on PA14 is significantly shorter in younger worms [49]. To avoid potential confounding, mercury concentrations that did not significantly impair nematode growth were used in this experiment. Note that these experiments involved exposures from the first larval stage, which is more sensitive than the later stages used in experiments reported in Figs. 2–7 . Of these developmental exposures, only 2.5 μM HgCl$_2$ modestly reduced nematode growth.

Following larval exposure, nematodes were washed and then transferred to survival plates containing *E. coli* OP50 or *P. aeruginosa* PA14. The bacterial lawns used for *C. elegans* killing assays were prepared by placing a 20 μL drop of an overnight culture of the bacterial strains on modified NGM agar on Plates 3.5 cm in diameter.

Full lawn plates used for *C. elegans* killing assays were prepared by spreading a 25 μL drop of an overnight culture grown at 37 °C of *P. aeruginosa* on the complete surface of modified NGM agar in 3.5 cm diameter Petri plates. Plates were incubated at 37 °C for 12–16 h. Plates were cooled to room temperature for at least one hour before seeding with synchronized young adult animals. The killing assays were performed at 25 °C and live animals were transferred daily to fresh plates. Animals were scored at the times indicated and were considered dead when they failed to respond to touch. The experiment was repeated 3–4 times separated in time (n = 97–341).

2.6. DNA damage and genome copy number analysis

For damage and genome copy number assays, six nematodes were pooled and treated as a biological replicate. nDNA and mtDNA damage were assessed using a QPCR-based method as previously described [68]. This assay measures lesion frequency based on decreases in amplification efficiency relative to controls, which are assumed to be undamaged [69]. Two nuclear genome targets (*unc-2* and small nuclear; 9.3 and 0.2 kb) and two mitochondrial genome targets (*nd-1* and small mitochondrial; 10.9 and 0.2 kb) were amplified. The amount of amplified long PCR product provides a measurement of lesion frequency, while the amount of short PCR product provides normalization to DNA concentration and genome copy number [70].

2.7. Gene expression analysis

Expression of autophagy, mitophagy, biogenesis, BER-related DNA repair, and p38 MAPK-related genes were measured using the experimental design for Experiment 1. Nematodes were reared to the young adult stage and exposed to control conditions, HgCl₂ (1 and 5 μM), or MeHgCl (1 and 5 μM) for 24 h. Total RNA from *glp-1* nematodes was extracted using a RNeasy kit (Qiagen, Valencia, CA, USA), quantified with a NanoDrop Fluorospectrometer (NanoDrop Technologies, Wilmington, DE, USA), and converted to cDNA using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) using methods previously described [71]. Average fold change of each target gene was calculated by comparing the CT (cycle threshold) of the target gene to two housekeeping genes (*tba-1* and *pmp-3*). Real-time PCR conditions were optimized for previously published and designed primers; the primer sequences and conditions are listed in Table A9.

2.8. Assessment of autophagic foci

Autophagy was assessed using the QU1 izEx1[Plgg-1::gfp::lgg-1+rol-6] (LGG-1:GFP) reporter strain, where increased or impaired autophagic flux is represented by a change in the number of LGG-1:GFP foci [72], using the experimental design for Experiment 1. Nematodes were reared to the young adult stage and exposed to control conditions, HgCl₂ (1 and 5 μM), or MeHgCl (1 and 5 μM) for 24 h. Following exposure, nematodes were picked onto 10% agar pads with 10 ml of 150 mM sodium azide (Sigma Aldrich) [73]. Single-plane images of seam cells were taken using a Zeiss 780 confocal microscope at 63× magnification and LGG-1:GFP foci in each seam cell were counted manually. The experiment was repeated two times separated in time. In total, 21–48 seam cells from 8 to 18 nematodes were analyzed per treatment.

2.9. Statistical analysis

Experiment 1 data were analyzed using a 2-way ANOVA with mercurial type (HgCl₂, MeHg) and mercury concentration as factors. Significant interactions were followed up with Tukey's post-hoc test for pairwise comparisons. Data from experiment 2 was analyzed with a 3-way ANOVA. For DNA damage data, significant 3-way ANOVAs were followed up with 2-way ANOVAs (mercury compound × time) for UVC/H₂O₂ exposed and non-UVC/H₂O₂ exposed nematodes. For copy number data, significant 3-way ANOVAs were followed up with 2-way ANOVAs (mercury compound × UVC/H₂O₂ exposure) for each time-point. Significant 2-way interactions for DNA damage data were followed up with Tukey's post-hoc test for pairwise comparisons. ANOVA tables for Experiment 2 are presented in the Appendix A. Experiment 3 data were analyzed with a 2-way ANOVA and significant interactions were followed up with Tukey's post-hoc test for pairwise comparisons. Gene expression and LGG1:GFP data was analyzed using a 2-way ANOVA, similar to Experiment 1. Statistics for all experiments except survival were conducted using R (Version 3.2.2, Vienna, Austria). Nematode survival in Experiment 4 was plotted as a non-linear regression curve using the PRISM (version 4.00) computer program. Prism uses the product limit or Kaplan-Meier method to calculate survival fractions and the Mantel-Cox log-rank test to compare survival curves. Significance for all experiments was accepted at a level of p < 0.05.

3. Results

3.1. Experiment 1: DNA damage and genome copy number following mercury exposure

To test the impact of mercury on mtDNA and nDNA damage and copy number, young adult nematodes were exposed to two concentrations of HgCl₂ and MeHg for 24 h. DNA damage profiles were similar between the mitochondrial and nuclear genomes. For both mtDNA and nDNA the direction of the change in damage level from low to high concentration was different for the two mercury compounds (interaction between mercury compound and concentration, p < 0.001). Increasing lesions were observed with increasing HgCl₂ exposure, but for MeHg exposures there was increased damage at 1 μM and decreased lesions at 5 μM (Fig. 2A). Genome copy number following mercury exposure differed between the two genomes. In the mitochondrial genome, there was a significant interaction between mercury compound and concentration (p < 0.001), as the 5 μM MeHg treatment resulted in reduced mtDNA copy number, but other treatments did not. In the nuclear genome, mercury compound was a significant factor influencing copy number, with MeHg reducing significantly reducing copy number (main effect, p = 0.03) by about 4% (Fig. 2B).

To better understand the surprising decrease in mtDNA and nDNA damage at the high (5 μM) MeHg concentration, we carried out additional experiments to test possible hypotheses for this observation. First, we tested whether autophagy was increased, because autophagy can remove damaged mtDNA [73] and may also reduce nDNA damage [74]. Increased autophagy would also be consistent with the reduced mtDNA copy number that we also observed (Fig. 2). Consistent with this hypothesis, we identified increased formation of seam cell autophagic foci (Fig. A1). However, foci number was also increased at 1 μM MeHgCl, where we saw increased rather than decreased DNA damage. Importantly, increased foci may also result from inhibition of the autophagic process (i.e., inability to resolve autophagic foci [75], so we also measured mRNA levels of inducible autophagy related genes [76]. The high MeHg concentration (5 μM) significantly reduced *atg-18*, *dct-1*, and *hmg-5* and reduced (p = 0.08) *bec-1* expression, suggestive of decreased, rather than increased, autophagy and mitophagy (Fig. A2). We also tested for a transcriptional signal for induction of biogenesis (Fig. A2) that might offset autophagic removal of mitochondria, but did not find support for this possibility. We next considered whether transcripts for key genes involved in BER,

which repairs oxidative damage likely to be caused by mercury exposure, would be increased. While not generally considered a highly inducible process, there is evidence for upregulation of some BER genes after exposures [77]. Instead, BER genes were expressed at similar or lower levels after both HgCl$_2$ and MeHg (Fig. A3). We near considered the possibility that BER activity might have
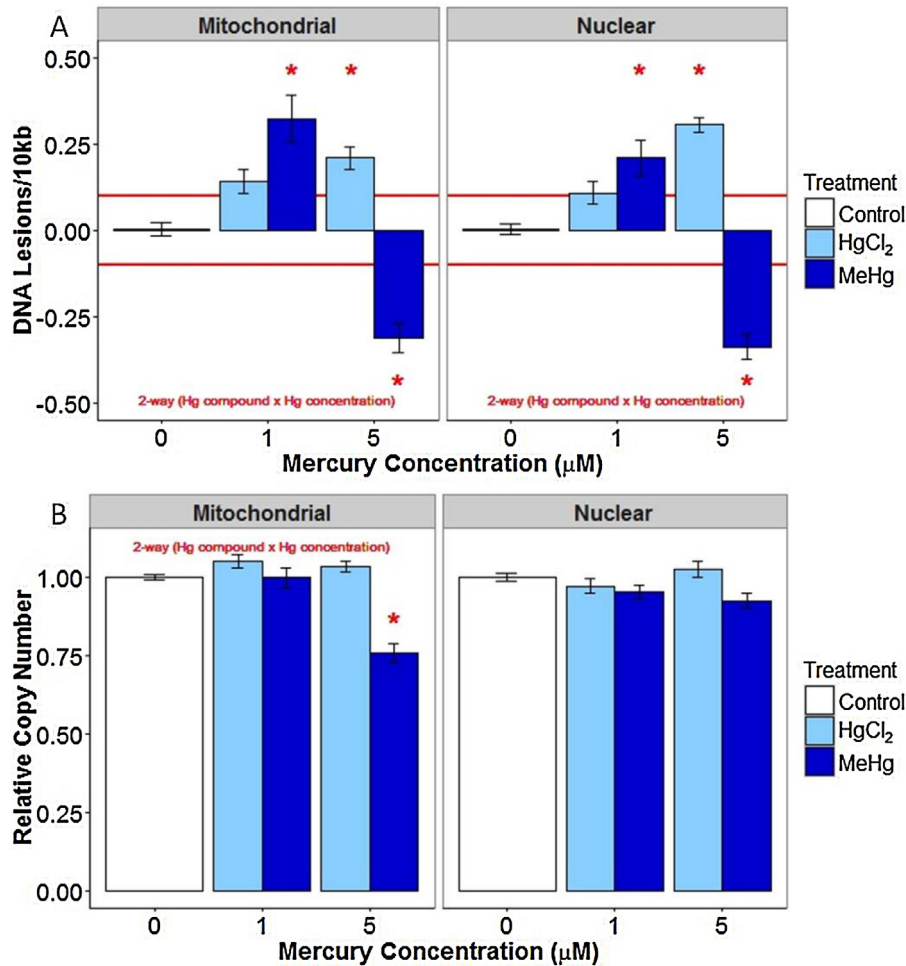
Fig. 2. Mitochondrial and nuclear DNA damage (average lesions/10 kb ±SE) (A) and relative copy number (average% of control ±SE) (B) in young adult nematodes following 24 h HgCl$_2$ and MeHg exposure. Red lines indicate the limit of detection for DNA lesions (0.1 lesions/10 kb). * indicates significantly different (p < 0.05) from control.
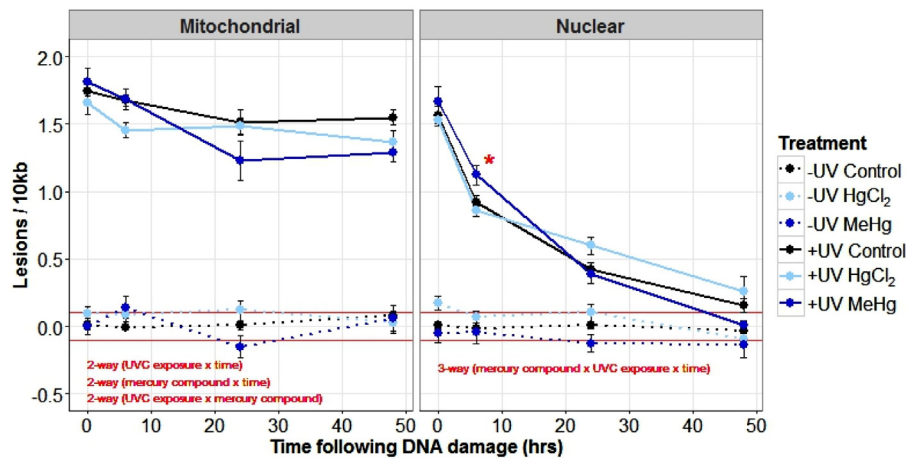
Fig. 3. Mitochondrial and nuclear DNA damage (average lesions/10 kb ±SE) in control (black), 5 μM HgCl$_2$ treated (light blue), and 1 μM MeHg treated young adult nematodes (darkblue) that were either exposed (solid lines) or not exposed (dotted lines) to UVC (50 J/m^2). DNA damage was measured at four time-points following UVC exposure. Red lines indicate the approximate limit of detection for DNA lesions (0.1 lesions/10 kb). In the mitochondrial genome, all three 2-way ANOVAs were significant. In the nuclear genome the 3-way interaction was significant. * indicates a significantly different from the control and HgCl$_2$ treatments at 6 h. See Results Section for further statistical explanation.

been induced, leading to above-normal repair of endogenous DNA damage (i.e., negative lesions). Our data related to this hypothesis is presented below (Section 3.2.3), but overall did not explain the observation of below-baseline damage only in the high MeHg exposure group. Finally, we considered the possibility that the developmental delay observed at the high concentration of MeHg (Fig. A4) could explain these results. Minimal growth retardation (<5% growth reduction) was observed following $HgCl_2$ (1 and 5 μM) and 1 μM MeHgCl exposures, while 5 μM MeHgCl reduced growth by ~10% (Fig. A4). While there haven't been previous reports of baseline changes in DNA damage with development, both nDNA and, more dramatically, mtDNA copy number increase during these lifestages [53], including in *glp-1*(q244) nematodes [78], and the decreased mtDNA copy numbers that we observed could well be explained by developmental delay. We measured mtDNA and nDNA damage levels in unexposed, wildtype and *glp-1* nematodes at multiple timepoints after age-synchronization at the first larval stage (as previously described: [73]). We found that DNA damage in both genomes and strains increased with developmental stage (Fig. A5), supporting the hypothesis that the decreased DNA damage observed at 5 μM MeHg could be explained by the developmental delay that was also observed.

3.2. Experiment 2: DNA damage repair and removal following mercury exposure

Next, we carried out experiments designed to test the influence of prior mercury exposure on the response in both genomes to DNA damage induced by exposure to UVC or H_2O_2.

3.2.1. Repair and removal of UVC-induced DNA damage

The impact of mercury on removal of UVC induced mtDNA and nDNA damage, assessed using a 3-way ANOVA (mercury compound × UVC exposure × time), was mild. In the mitochondrial genome, the 3-way interaction was not significant (p = 0.56); however, mercury altered lesion removal over time (mercury compound × time interaction, p = 0.01), although the difference was small (Fig. 3).

For nDNA lesions the 3-way interaction was significant (p = 0.02), and so the data was further analyzed as a function of UVC exposure. In UVC exposed nematodes, there was a difference in nDNA lesion removal over time between the three treatments (mercury compound × time interaction, p = 0.004), but significant

differences in levels of damage between treatments were only observed at one time-point (6 h), and the degree of change was relatively small. In non-UVC exposed nematodes, the MeHg exposure resulted in significantly lower nDNA lesions (main effect, p = 0.001) than control or $HgCl_2$ treatments. However, again, the effect size was small ($\leq$0.1 lesions) (Fig. 3).

3.2.2. Relative copy number following UVC exposure

The most dramatic impact was that MeHg in combination with UVC led to a decrease in mtDNA copy number. For relative mitochondrial copy number, the 3-way (mercury compound × UVC exposure × time) ANOVA interaction was significant (p < 0.001) and the data was further analyzed by time-point. At 0 h, copy number was significantly decreased by the MeHg treatment compared to both the control (p < 0.001) and $HgCl_2$ (p = 0.003) treatments and also by the UV treatment (p = 0.03). At 6 h, copy number was significantly reduced by both MeHg (p < 0.001) and $HgCl_2$ (p = 0.03) treatments compared to controls and by the UV treatment (p < 0.001). At 24 h, copy number was significantly lower in MeHg (main effect, p < 0.001) and UV (main effect, p < 0.001) treatments and further reduced in the MeHg-UV co-exposed treatment (interaction, p < 0.001). At 48 h, copy number was significantly lower in MeHg (p < 0.001), $HgCl_2$ (p < 0.001), and UV (p < 0.001) treatments and further reduced in the MeHg-UV co-exposed treatment (interaction, p < 0.001) (Fig. 4).

We did not observe large effects on nuclear copy number. The 3-way interaction was not significant (p = 0.05). Though some main effects were significant, the resulting change in nuclear copy number was small, less than 5% (Fig. 4, Table A4).

3.2.3. Repair of H_2O_2-induced DNA damage

Mercury exposure significantly increased the DNA damage caused by H_2O_2, and also affected damage removal in complex fashions. The impact of mercury and H_2O_2 exposure on nDNA and mtDNA damage was assessed using a 3-way ANOVA (mercury compound × H_2O_2 exposure × time). For mtDNA lesions, the 3-way interaction was not significant (p = 0.08). mtDNA damage depended on both the mercury compound and H_2O_2 exposure (mercury compound × H_2O_2 exposure interaction, p = 0.007). Across timepoints MeHg exposed nematodes had significantly higher damage compared to the $HgCl_2$ treatment after H_2O_2 exposure (p = 0.006). MeHg-exposed nematodes had significantly higher damage compared to controls in non-H_2O_2 exposed nematodes (p = 0.01). We
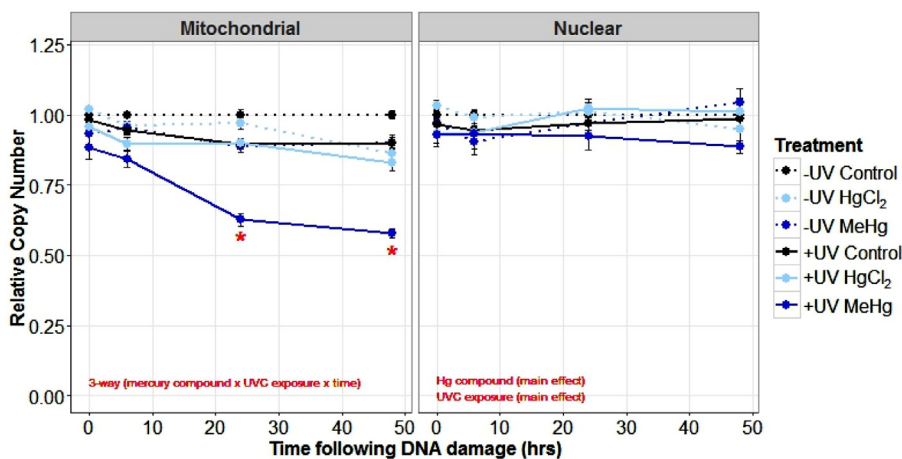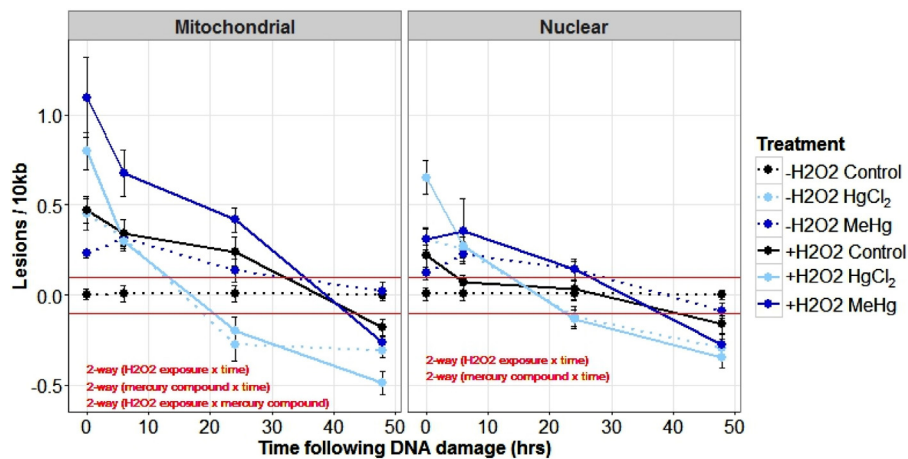

Fig. 4. Mitochondrial and nuclear relative copy number (average % of control ±SE) in control (black), 5 μM $HgCl_2$ treated (light blue), and 1 μM MeHg treated young adult nematodes (dark blue) that were either exposed (solid lines) or not exposed (dotted lines) to UVC (50 J/m^2). Relative copy number was measured at four time-points following UVC exposure. For mitochondrial copy number the 3-way interaction was significant. * indicates significant 2-way interaction with the MeHg +UV treatment further reducing mitochondrial copy number. See Results Section for further statistical explanation.

could not formally test whether exposure to either mercury compound resulted in a greater number of lesions immediately after H_2O_2 exposure because of the lack of a significant 3-way interaction.

mtDNA damage also changed over time following H_2O_2 exposure (time $\times$ H_2O_2 exposure interaction, p < 0.001), where nematodes exposed to H_2O_2 had significantly more DNA damage than non- H_2O_2 exposed nematodes at all time-points except at 48 h where there was less damage. Additionally, mtDNA damage changed over time depending on the mercury compound (time $\times$ mercury compound interaction, p < 0.001), where at 0 h both MeHg and $HgCl_2$ treatments had higher DNA damage compared to controls, at 6 h the MeHg treatment had significantly more damage compared to controls, and at 24 and 48 h $HgCl_2$ treatments had significantly less (in fact, "negative" lesions—i.e., below control baseline) damage compared to control and MeHg treatments (Fig. 5).

The 3-way interaction for nDNA lesions was also not significant (p = 0.76). The change in nDNA damage over time differed between H_2O_2 exposures (H_2O_2 exposure $\times$ time interaction, p < 0.001), with significantly more damage in H_2O_2 treatments at 0 h (p < 0.001) and

significantly less damage at 48 h (p = 0.006). Mercury compound also impacted nDNA lesions at different time-points (mercury compound $\times$ time interaction, p < 0.001). At 0 h $HgCl_2$ treated nematodes had significantly more nDNA lesions than control and MeHg treatments, at 6 h both MeHg and $HgCl_2$ nematodes had significantly more damage compared to controls, at 24 h $HgCl_2$ nematodes had significantly less damage compared to the control and MeHg treatments, at 24 h the MeHg treatment also had also had significantly more damage than $HgCl_2$ treatment, and at 48 h $HgCl_2$ treated nematodes had significantly less damage compared to the control and MeHg treatments (Fig. 5). Below-baseline DNA damage (mitochondrial and nuclear) observed at the last time point (48 h) in mercury exposed animals suggested that BER was induced by mercury exposure. To test for transcriptional induction of BER, we measured gene expression of BER related genes in young adult animals. However, we did not find evidence for induction; rather, we measured decreased gene expression for some BER related genes. The greatest MeHg and HgCl2 concentrations (5 μM) significantly reduced *nth-1*, *exo-1*, and *lig-1* expression, while the 5 μM HgCl2 exposure also reduced (p = 0.10) *rnr-2* expression (Fig. A4).

Fig. 5. Mitochondrial and nuclear DNA damage (average lesions/10 kb ±SE) in control (black), 5 μM $HgCl_2$ treated (light blue), and 1 μM MeHg treated young adult nematodes (dark blue) that were either exposed (solid lines) or not exposed (dotted lines) to H_2O_2 (5 mM). DNA damage was measured at four time-points following UVC exposure. Red lines indicate the approximate limit of detection for DNA lesions (0.1 lesions/10 kb). See Results Section for further statistical explanation.
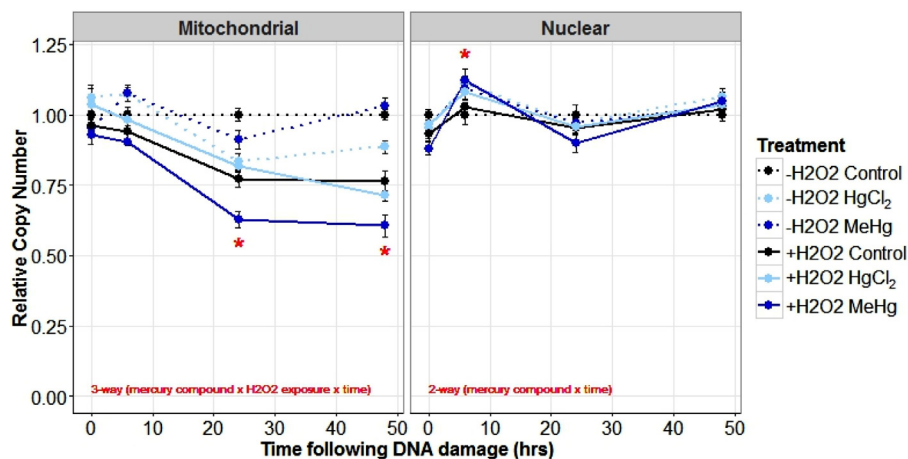
Fig. 6. Mitochondrial and nuclear relative copy number (average % of control ±SE) in control (black), 5 μM $HgCl_2$ treated (light blue), and 1 μM MeHg treated young adult nematodes (darkblue) that were either exposed (solid lines) or not exposed (dotted lines) to H_2O_2 (5 mM). Relative copy number was measured at four time-points following UVC exposure. Exposure groups are dodged for better visual representation. See Results Section for further statistical explanation.
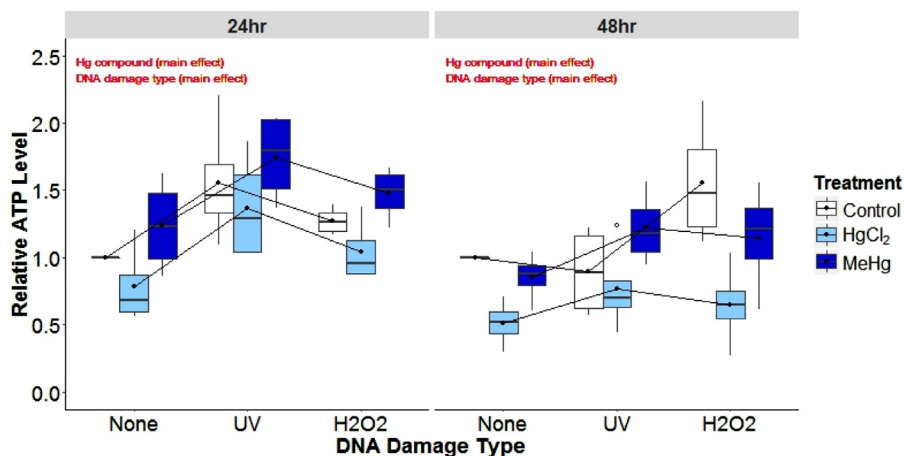
Fig. 7. Interaction plots of steady-state ATP levels (% of control) of young adult nematodes exposed to control, mercury (5 μM HgCl$_2$, 1 μM MeHg), or DNA damage (UVC, H$_2$O$_2$) conditions. Interaction lines and box-plots are dodged for better visual representation.

3.2.4. Relative copy number following H$_2$O$_2$ exposure

The major effects that we observed were a decrease in mtDNA copy number following H$_2$O$_2$ exposure that was exacerbated by prior exposure to MeHg. Again, the impact of mercury and H$_2$O$_2$ exposure on relative copy number was assessed using a 3-way ANOVA (mercury compound $\times$ H$_2$O$_2$ exposure $\times$ time). For mtDNA copy number, the 3-way interaction was significant (p = 0.004), so the data was further considered by time-point. At 0 h, copy number was significantly lower in the MeHg treatment compared to the HgCl$_2$ treatment (p = 0.01). At 6 h, copy number was significantly reduced in the H$_2$O$_2$ exposure (p < 0.001). At 24 h, copy number was reduced in the MeHg (p < 0.001) and H$_2$O$_2$ (p < 0.001) treatments and further reduced in the MeHg-H$_2$O$_2$ co-exposure treatment (interaction, p < 0.001). At 48 h, copy number was reduced in the HgCl$_2$ (p = 0.01) and H$_2$O$_2$ (p < 0.001) treatments and further reduced in the MeHg-H$_2$O$_2$ co-exposure treatment (interaction, p < 0.001) (Fig. 6).

The 3-way interaction for nuclear copy number was insignificant (p = 0.37). Mercury affected copy number over time (mercury compound $\times$ time interaction, p = 0.01), but differences in nuclear copy number between treatments were only observed at one time-point (6 h). At 6 h, MeHg and HgCl$_2$ treatments had significantly higher nuclear copy number compared to the control treatment. No other significant differences were observed at the other time-points (Fig. 6).

3.3. Experiment 3: in vivo ATP levels following exposure to mercury, UVC, and H$_2$O$_2$

To assess the impacts of mercury compound, type of DNA damage, and their interaction on ATP levels, we carried out an experiment similar to that in which we analyzed impacts on DNA. A 2-way ANOVA was performed for each time-point. At the 24 h time-point, mercury compound (main effect, p = 0.007) and DNA damage type (main effect, p < 0.001) affected ATP levels. ATP levels in the MeHg treatment were significantly higher than the HgCl$_2$ treatment (p = 0.005). Additionally, ATP levels after UVC were significantly higher compared to the control treatment (p < 0.001) and higher than the H$_2$O$_2$ treatment (p = 0.05) (Fig. 7).

At the 48 h time-point, mercury compound (main effect, p = 0.04) and DNA damage type (main effect, p < 0.001) affected ATP levels. ATP levels in the HgCl$_2$ treatment were significantly lower compared to the control (p = 0.001) and MeHg (p = 0.004) treat-

ments. H$_2$O$_2$-exposed nematodes had significantly increased ATP levels compared to controls (p = 0.03) (Fig. 7).

### 3.4. Experiment 4: Innate immunity in wild type and immune deficient nematodes

Mitochondria are important immune response signaling organelles and mercury-induced alterations to ROS concentrations and mitochondrial status and function (ATP, copy number) could have downstream impacts on immune response. We hypothesized that mercury-induced changes to these processes could alter p38 MAPK, an innate immune signaling pathway that can be activated by redox changes [44,45,46,47]. Impacts on this pathway were first assessed by measuring mRNA levels of p38 MAPK pathway genes. *sek-1* expression was significantly reduced following 5 μM MeHg exposure and reduced (p = 0.07) following 5 μM HgCl$_2$ exposure. The 5 μM concentration of both MeHg and HgCl$_2$ significantly reduced *pmk-1* expression (Fig. 8).

As we observed that both mercury species impacted this pathway we then assessed the influence of early larval mercury exposure on animals with mutations in the p38/PMK-1 MAPK pathway by measuring survival time of N2 and *pmk-1* nematodes on *E. coli* OP50 or *P. aeruginosa* PA14. HgCl$_2$ and MeHg treatments were analyzed separately as there was considerable variation in control survival between these trials. There were no survival differences following HgCl$_2$ exposure for either nematode strain on OP50 or PA14 (Fig. A6). MeHg exposure significantly reduced survival time for N2 nematodes on OP50 (median survival difference 21 h), while no survival differences were observed on PA14. Survival was also not different for the *pmk-1* strain on either strain of bacteria (Fig. A7).

4. Discussion

Mercury is well known for having adverse impacts on multiple systems, though the specific mechanisms are not well understood. Proposed mechanisms for injury include increased ROS [3,5]; reduced cell membrane integrity [14,15]; altered cell signaling [79,80]; mitochondrial impacts [81,82,5]; altered DNA repair [83,18,84,19,20,21]; and immunomodulatory impacts [85–88]. Here we report the outcomes of HgCl$_2$ and MeHg exposure on DNA damage, DNA repair, mitochondrial endpoints (copy number and ATP), and PMK-1 (p38 mitogen-activated protein kinase) mediated innate immune impacts in *C. elegans*. Of the outcomes we investigated, mitochondrial impacts were particularly notable.
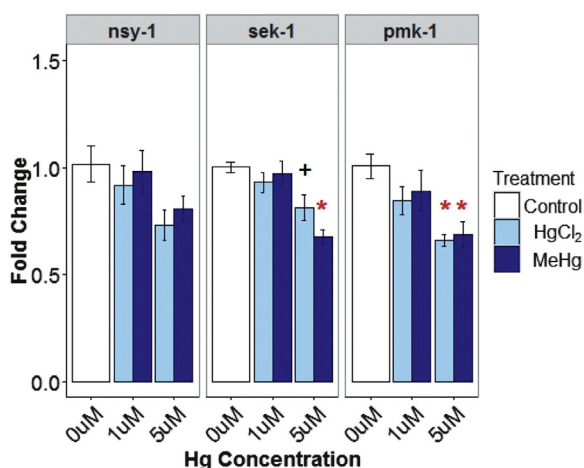
Fig. 8. Relative expression (fold change ±SE) of p38 MAPK related genes (*nsy-1*, *sek-1*, *pmk-1*) in young adult nematodes. * indicates significantly different (p < 0.05) from control and + indicates 0.05 ≤ p < 0.1.

4.1. DNA damage and damage repair

We found that following exposure to both inorganic and organic mercury species, increased DNA damage and altered DNA repair occurred in both genomes. We predicted DNA damage to both genomes because mercury accumulates in both organelles, but we hypothesized that mitochondria may be more susceptible to damage as mitochondria lack some of the pathways that repair damage to nuclear DNA [11–13,89]. Until recently studies had primarily focused on nuclear genome impacts following mercury exposure, because the methods used in many studies (*e.g.* the comet assay) predominantly evaluated nDNA [90,18,16,21]. mtDNA impacts have been addressed in only a few studies. In an *in vitro* study, plausibility for mtDNA damage was established through microscopy images that depicted increased ROS in a qualitative manner and DNA damage co-occurring in cellular space with mitochondria [91]. In a field study, some evidence for increased mtDNA damage was obtained in bats from areas with high potential for Hg exposure, but individual-level correlations with Hg levels were poor [27]. Here we report the first quantified impacts of mercury on mtDNA in controlled experiments in an *in vivo* model.

DNA damage following mercury exposure alone was dependent on life-stage and concentration. Low-level damage in both genomes was only detected at the earliest time-point examined (Fig. 2), which suggests that there is increased susceptibility to damage earlier in life. Surprisingly, the high- concentration MeHg resulted in less damage compared to controls. It is important to note that our DNA damage assay defines "control" samples as having no damage, although cells have a normal steady-state level of damage. Negative damage levels are thus interpreted as improved repair or clearance of damaged genomes, relative to standard control conditions. With this assay, younger life-stages appear to have less nDNA and mtDNA damage compared to older life-stages. Our results suggest that the small decrease in lesion number (0.3 lesions/10 kb) in the highest MeHg exposure is related to a slight developmental delay, rather than changes damage removal processes (Figs. 2 and A6). Developmental delay in this treatment is also supported by our observation of reduced copy number as both nDNA and, more dramatically, mtDNA copy number increase during these lifestages [78,53]. The increase in DNA damage with developmental stage that we observed is interesting in its own right and consistent with our previous observation of developmental stage decreases in DNA repair genes [67]. It also suggests that any exposures that result in developmental rate and also cause DNA damage could lead to

DNA damage levels being underestimated, since the developmental delay by itself would result in a lower level of DNA damage.

Our data support findings from previous studies, that nonlethal mercury exposure alone does not induce substantial DNA damage in either genome [90,18]. nDNA damage has been observed in some studies, but the weight of the evidence so far seems to support the hypothesis that compromising genetic damage may only occur at cytotoxic exposures [92,20]. However, although mercury exposure does not appear to directly damage DNA, there is evidence that in combination with other exposures, considerably increased damage can occur.

Elevated DNA damage was observed in both genomes after co-exposure to H_2O_2 in this study, and has also been observed *in vitro* with nDNA [20]. We did not observe increased damage in conjunction with UVC exposure, confirming a previous report [18], though elevated damage has been noted with UVA and also X-ray co-exposures [90,92]. Collectively, these findings are consistent with one of mercury's major proposed mechanisms of action, creating an environment sensitive to oxidative stress due to reduced antioxidant capabilities: H_2O_2, UVA, and X-ray exposures induce ROS generation, but UVC-induced ROS is very minor [93–96]. Another proposed mechanism for induced DNA damage is the impairment of DNA repair enzymes.

Damage removal is similar between *C. elegans* and mammals, with NER acting in the nucleus, photodimer removal from mitochondria occurring *via* mitophagy, and BER acting in both genomes [71,97–100]. Our data indicates that not all types of repair and removal are impacted by mercury exposure, as there were no differences in lesion removal following UVC exposure in either genome. This is the first assessment of photodimer removal in mitochondria after MeHg exposure. Our observation that inorganic mercury exposure does not impact NER is consistent with other studies [18,84]. Although mercury has not been observed to inhibit proteins involved in NER (ex. mammalian XPA protein [83]), there is evidence for alterations to nonhomologous end joining, homologous recombination, and BER.

Inorganic mercury has been reported in multiple studies to impair BER. Reduced repair of double strand breaks through either nonhomologous end joining and/or homologous recombination repair has been consistently observed with inorganic mercury exposure and gamma radiation [21] and X-ray exposure [92,18,84]. *In vitro* studies indicate that BER impairment occurs from reduced glycosylase expression (ex. OGG1) following inorganic mercury exposure [19] and reduced repair enzyme recruitment from decreases in the poly(ADP-ribosyl)ation signaling reaction that is induced by strand breaks [20]. Our gene expression data are consistent with previous studies in that we observed reduced expression of BER associated-genes, including a glycosylase, an apurinic/apyrimidinic endonuclease, and DNA ligase following exposure to the highest concentrations of $HgCl_2$ and MeHg. Further, these data suggest that at least some of the impairment of BER may be transcriptionally-mediated. However, our *in vivo* repair kinetics data were not as clear-cut. Impairments the repair rate were not readily evident, as might be expected from decreased gene expression; rather, in some cases, repair appeared to be increased. Of course, *in vivo* repair integrates transcriptional regulation, protein-level changes (*e.g.*, inhibition by Hg), and other physiological conditions. It is possible that in some cases, elevated ATP levels permitted increased kinetics of DNA repair, despite decreased transcription of repair genes, although we did not test this. In the mitochondria, nematodes co-exposed to H_2O_2 and $HgCl_2$ or MeHg began repair with a similar level of mtDNA damage and both had similar repair rates. nDNA repair was slower at early time-points in nematodes co-exposed to MeHg and faster in $HgCl_2$ co-exposures. However, a limitation of this study is that actual repair kinetics could not be calculated due to the low number of

time-points and the fact that DNA repair was assumed to be non-linear, as previously observed in *C. elegans* for repair of photodimers [99].

Despite these limitations, ours is the first report of increased BER following Hg exposure. We observed an apparent overcompensation of repair, evidenced by DNA damage levels in both genomes falling below the damage levels in controls at late time-points after exposure to both mercury and a DNA damaging agent. Overcompensation occurred primarily with $HgCl_2$ in mtDNA and with both $HgCl_2$ and MeHg in nDNA; we speculate that the levels of mercury utilized in this study stimulated a regulatory mechanism that activated DNA repair. For example, methylmercury in particular activates poly(ADP-ribose)polymerase (PARP), well known for the role it plays in DNA damage response signaling [101]. An overcompensation in repair has also been noticed following moderate oxidative damage in *in vitro* studies [102,103].

4.2. Mitochondrial impacts

Mitochondria are one target of mercury toxicity and impacts to mitochondria are important to understand because of the important role this organelle has in cellular maintenance [57]. Mitochondrial impacts observed in this study included the mtDNA damage and altered repair described in the previous section and altered copy number and steady-state ATP levels. Changes in copy number and ATP content were dependent on mercury compound exposure, supporting the notion that inorganic and organic mercury toxicities can occur through different mechanisms [104–106]. In *C. elegans*, MeHg and $HgCl_2$ behave differently in terms of uptake and interactions with other co-exposures (*e.g.* selenium compounds) [107].

In this study, both MeHg and $HgCl_2$ reduced mitochondrial copy number; however, the greatest copy number reductions ($\geq$25%) occurred with MeHg exposures. In Experiment 1, acute exposure to the high MeHg concentration alone decreased mitochondrial copy number, while in Experiment 2, chronic exposure to a lower concentration diminished copy number only in nematodes that were co-exposed to UVC or H_2O_2. Following UVC or H_2O_2 exposure in MeHg-treated nematodes, mitochondrial copy number decreased over time ($\sim$40% reduction). These reductions coincide with DNA damage removal which is consistent with mitochondrial dynamics (fission, fusion) and autophagy playing an important role in mtDNA damage removal, and with the removal processes differing between MeHg and $HgCl_2$ treatments [73]. "Slow" mtDNA removal following H_2O_2 exposure has also been observed in HeLa cells [108].

Declines in mtDNA copy number could result in altered physiology and cell homeostasis. However, the degree to which mitochondrial copy number reductions impact cellular health is not well understood, because many cells have high mtDNA redundancy, copy number varies between tissues, and there is a wide range of apparently normal mtDNA copy number in humans [109]. We recently found that small decreases in mtDNA copy number have only mild impacts on ATP levels in *C. elegans* [110]. Nonetheless, mtDNA depletions around 65% can cause disease in humans [111], and a certain level of mtDNA is required to pass some developmental milestones in *C. elegans* [53]. Therefore, we also investigated the impacts of Hg on energy metabolism.

In our experiments, ATP levels were increased by MeHg exposure while they were decreased by $HgCl_2$ exposure. *In vitro* data supports our $HgCl_2$ related findings as respiration was suppressed in fish liver cells following exposure [112]. The finding of increased (or in one case unchanged) ATP levels across treatments following MeHg exposure was a surprise, especially given that MeHg exposures resulted in decreased copy number, particularly in combination with UVC and H_2O_2 exposures. This increase in ATP levels could be explained by increased production or decreased utiliza-

tion, possibly in combination with a shift in metabolism; additional work will be required to elucidate the cause.

Again to our surprise, we also observed increased ATP levels after both UVC and H_2O_2, potentially reflecting a regulated response that devotes energy resources to DNA damage removal. Another explanation is that cellular respiration and resulting ATP levels are correlated to the ratio of functional and dysfunctional mitochondria. Reduced mitochondrial copy number and increased ATP levels have been observed with lithium exposure in *C. elegans*. The authors of that study observed that the metal positively influenced the lifespan of *C. elegans* and made the argument that mitophagy could benefit mitochondrial energetics by selectively removing damaged mitochondria, thus altering cellular respiration [113]. Though we observed reduced expression for some mitophagy and autophagy genes, these processes could be upregulated post-translationally.

4.3. Innate immune impacts

Although we observed altered mitochondrial parameters and reduced gene expression of a p38 MAPK (*pmk-1*) and MAPKK (*sek-1*) following mercury exposure, effects on innate immunity were mild. Larval mercury exposure to MeHg reduced survival of wildtype N2 nematodes on OP50, which is suggestive of a general impairment of innate immune response. OP50 is generally considered a control food source, but it does have some level of pathogenicity, as animals incubated on this bacteria have a higher expression of an antimicrobial gene, suggesting innate immunity activation [114]. Though the reduction in survival time was small (<24 h), MeHg does not appear to reduce lifespan from a separate experiment using heat killed OP50 and antibiotics (data not shown). The absence of an observed difference on PA14, a more pathogenic bacteria, seems surprising but may be related to the difficultly in discerning differences where there is a rapid reduction in survival. MeHg exposure appears to affect the PMK-1-mediated immune response, since MeHg had an effect on survival of N2 but not *pmk-1* animals. No difference in survival in *pmk-1* animals was observed, which also could be related to the difficulty to perceive survival differences when the overall survival time is short. Data from this study supports previous observations of inorganic mercury related innate immune modulation [115,116,33] and suggests that organic mercury may also have an impact on innate immune signaling.

5. Conclusions

In summary, our data provides evidence that genotoxic, metabolic, and innate immune impacts can result following mercury exposure. Mercury impacts on DNA damage outcomes were similar between genomes, resulting in low-level damage following individual mercury exposures, synergistically increased damage with oxidative co-exposure, and altered DNA damage repair through BER. A major finding was that mercury compounds affect mitochondrial outcomes differently, including genome copy regulation and ATP levels, supporting the hypothesis that MeHg and $HgCl_2$ toxicities operate through different mechanisms. Mitochondrial impacts should be further examined because of the mitochondria's importance to cellular functions. Additionally, innate immune impacts, though moderate, differed between mercury compounds.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

Appendix A.

See Tables A1–A9 and Figs. A1–A7 .

Table A1
mtDNA damage, 3-way ANOVA (mercury compound × UVC exposure × time).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.213	0.107	1.7931	0.168226
UV_yn	1	188.326	188.326	3169.292	<2.2e-16
Timepoint	3	1.322	0.441	7.4159	8.30E-05
Hg_type:UV_yn	2	0.545	0.273	4.5877	0.010901
Hg_type:Timepoint	6	1.132	0.189	3.1762	0.004898
UV_yn:Timepoint	3	1.248	0.416	6.9984	0.000145
Hg_type:UV_yn:Timepoint	6	0.286	0.048	0.8031	0.568108
Residuals	300	17.827	0.059		

Table A2
nDNA damage, 3-way ANOVA (mercury compound × UVC exposure × time).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.48	0.24	5.3429	0.005248
UV_yn	1	53.186	53.186	1183.051	<2.2e-16
Timepoint	3	26.258	8.753	194.6894	<2.2e-16
Hg_type:UV_yn	2	0.131	0.066	1.4584	0.234261
Hg_type:Timepoint	6	0.619	0.103	2.295	0.035055
UV_yn:Timepoint	3	20.351	6.784	150.8947	<2.2e-16
Hg_type:UV_yn:Timepoint	6	0.643	0.107	2.3845	0.028878
Residuals	300	13.487	0.045		
+UVC	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Hg_type	2	0.1	0.05	0.9392	0.393235
Timepoint	3	46.362	15.4539	289.9922	<2.2e-16
Hg_type:Timepoint	6	1.041	0.1734	3.2547	0.004898
Residuals	150	7.994	0.0533		
-UVC	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Hg_type	2	0.5114	0.255716	6.9823	0.001261
Timepoint	3	0.2477	0.082566	2.2544	0.084406
Hg_type:Timepoint	6	0.2216	0.036929	1.0084	0.422097
Residuals	150	5.4935	0.036623		

Table A3
Mitochondrial copy number, 3-way ANOVA (mercury compound × UVC exposure × time).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.98592	0.49296	96.088	<2.2e-16
UV_yn	1	0.68226	0.68226	132.9868	<2.2e-16
Timepoint	3	0.43753	0.14584	28.4276	3.31E-16
Hg_type:UV_yn	2	0.20756	0.10378	20.229	5.74E-09
Hg_type:Timepoint	6	0.25561	0.0426	8.3039	2.47E-08
UV_yn:Timepoint	3	0.13169	0.0439	8.5565	1.81E-05
Hg_type:UV_yn:Timepoint	6	0.13842	0.02307	4.4968	0.000222
Residuals	300	1.5391	0.00513		
0 h	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Hg_type	2	0.09549	0.047745	8.2053	0.000583
UV_yn	1	0.02814	0.028142	4.8364	0.030827
Hg_type:UV_yn	2	0.00799	0.003993	0.6862	0.506499
Residuals	78	0.45387	0.005819		
6 h	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Hg_type	2	0.075866	0.037933	9.4618	0.000209
UV_yn	1	0.106285	0.106285	26.5112	1.91E-06
Hg_type:UV_yn	2	0.010047	0.005024	1.253	0.291319
Residuals	78	0.312707	0.004009		

Table A3 (*Continued*)

24 h	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.49831	0.24915	48.066	2.50E-14
UV_yn	1	0.34159	0.34159	65.897	5.57E-12
Hg_type:UV_yn	2	0.1049	0.05245	10.118	0.000124
Residuals	78	0.40432	0.00518		

48 h	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.54443	0.27221	48.795	9.80E-14
UV_yn	1	0.34525	0.34525	61.887	4.59E-11
Hg_type:UV_yn	2	0.21574	0.10787	19.336	2.46E-07
Residuals	66	0.3682	0.00558		

Table A4
Nuclear copy number, 3-way ANOVA (mercury compound × UVC exposure × time).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.0824	0.041222	3.3721	0.035624
UV_yn	1	0.1046	0.104558	8.5531	0.003712
Timepoint	3	0.0404	0.013482	1.1029	0.348196
Hg_type:UV_yn	2	0.0082	0.0041	0.3354	0.715342
Hg_type:Timepoint	6	0.0259	0.004317	0.3531	0.907819
UV_yn:Timepoint	3	0.0142	0.004744	0.3881	0.761665
Hg_type:UV_yn:Timepoint	6	0.156	0.025998	2.1267	0.050227
Residuals	300	3.6674	0.012225		

Table A5
mtDNA damage, 3-way ANOVA (mercury compound × H_2O_2 exposure × time).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	2.7149	1.3575	24.1444	4.43E-10
H_2O_2_yn	1	1.9902	1.9902	35.3987	1.25E-08
Timepoint	3	15.8078	5.2693	93.7218	<2.2e-16
Hg_type:H_2O_2_yn	2	0.5632	0.2816	5.0086	0.007579
Hg_type:Timepoint	6	3.7738	0.629	11.1872	1.07E-10
H_2O_2_yn:Timepoint	3	4.1309	1.377	24.4915	1.83E-13
Hg_type:H_2O_2_yn:Timepoint	6	0.6278	0.1046	1.861	0.089453
Residuals	192	10.7947	0.0562		

Table A6
nDNA damage, 3-way ANOVA (mercury compound × H_2O_2 exposure × time).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.3199	0.15996	4.4674	0.01269
H_2O_2_yn	1	0.1168	0.11676	3.2609	0.07252
Timepoint	3	6.9804	2.32681	64.9858	<2.2e-16
Hg_type:H_2O_2_yn	2	0.0212	0.0106	0.296	0.74415
Hg_type:Timepoint	6	2.8791	0.47986	13.402	1.13E-12
H_2O_2_yn:Timepoint	3	1.028	0.34268	9.5707	6.40E-06
Hg_type:H_2O_2_yn:Timepoint	6	0.12	0.02	0.5587	0.76281
Residuals	192	6.8745	0.0358		

Table A7
Mitochondrial copy number, 3-way ANOVA (mercury compound × H_2O_2 exposure × time).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.10879	0.0544	6.748	0.001472
H_2O_2_yn	1	1.17798	1.17798	146.1282	<2.2e-16
Timepoint	3	1.43665	0.47888	59.4053	<2.2e-16
Hg_type:H_2O_2_yn	2	0.20676	0.10338	12.8244	5.92E-06
Hg_type:Timepoint	6	0.21496	0.03583	4.4444	0.00031
H_2O_2_yn:Timepoint	3	0.45123	0.15041	18.6584	1.16E-10
Hg_type:H_2O_2_yn:Timepoint	6	0.16015	0.02669	3.311	0.004
Residuals	192	1.54777	0.00806		

0 h	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.10618	0.053088	4.1621	0.02153
H_2O_2_yn	1	0.01128	0.011277	0.8841	0.3518
Hg_type:H_2O_2_yn	2	0.00047	0.000234	0.0183	0.98182
Residuals	48	0.61225	0.012755		

Table A7 (*Continued*)

6 h	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.030473	0.015236	2.739	0.07475
H_2O_2_yn	1	0.156475	0.156475	28.1288	2.85E-06
Hg_type:H_2O_2_yn	2	0.031038	0.015519	2.7898	0.07142
Residuals	48	0.267014	0.005563		

24 h	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.11907	0.05953	8.8583	0.000532
H_2O_2_yn	1	0.42122	0.42122	62.6739	2.94E-10
Hg_type:H_2O_2_yn	2	0.18299	0.09149	13.6135	2.07E-05
Residuals	48	0.3226	0.00672		

48 h	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.06804	0.03402	4.7209	0.013437
H_2O_2_yn	1	1.04024	1.04024	144.3521	4.47E-16
Hg_type:H_2O_2_yn	2	0.15242	0.07621	10.5753	0.000157
Residuals	48	0.3459	0.00721		

Table A8
Nuclear copy number, 3-way ANOVA (mercury compound × H_2O_2 exposure × time).

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Hg_type	2	0.02318	0.011588	1.5732	0.21003
H_2O_2_yn	1	0.01852	0.018517	2.5139	0.11449
Timepoint	3	0.56815	0.189382	25.7104	5.01E-14
Hg_type:H_2O_2_yn	2	0.00167	0.000833	0.1131	0.89315
Hg_type:Timepoint	6	0.12291	0.020485	2.781	0.01289
H_2O_2_yn:Timepoint	3	0.03457	0.011523	1.5643	0.19942
Hg_type:H_2O_2_yn:Timepoint	6	0.04762	0.007937	1.0775	0.37737
Residuals	192	1.41427	0.007366		

Table A9
Primer sequences and RT-PCR conditions for gene expression analysis.

Gene target	Primer sequence	Temp. (°C)
tba-1	R - CCGACCTTGAATCCAGTTGG F - TGATCTCTGCTGACAAGGCTT	60
pmp-3	R - ACACCGTCGAGAAGCTGTAGA F - GTTCCCGTGTTCATCACTCAT	60
dct-1	R - GGACAGTCTTTGGAGGTGTATT F - ATCGCACAATCTCCTCACGT	58
polg-1	R - TTGGAGCCGTCCGGATT F - CTGCCTAATACCGTTGCCTTCTT	60
hmg-5	R - GCTTCTTCGCTTCGTCTGTG F - TGTCTGGAGCTGGAATGGAA	60
bec-1	R - TTCGATCCTTGAGCTCTTTCA F - ATGGCATCGACATGGAAACG	60
lgg-1	R - CCTCGTGATGGTCCTGGTAG F - GCACCAAAGTCAAAGCTCCA	60
atg-18	R - CCAAGATGTGTAAGATTTTCGCC F - TGGGGCACAAAGATGGCTA	58
nth-1	R -GGCATTGCATCTGACCGAAT F - AATCGACTCGGCTGGATCAA	60
exo-1	R - CGAGGGTTTTGGTGTAGTTCTTA F - AGCTGGAAGTTTGTGTGCTG	60
apn-1	R - GCCACCCACTTCACCGAAAT F - ATTTAAGTTCTCGAGACAATGGC	60
lig-1	R - TGAGGAGTGCACTAATGGCA F - ATTCGCTGATCAAGGCTGTT	60
rnr-1	R - AGTCTTTCAGCGAACGAAGC F - TGAGAACTTGTGCGAACGAT	60
nsy-1	R - TCTTGAGCATGAAGTAGGAGAGA F - TGTTCCCCGTCGCCTTGATA	60
sek-1	R - AATTATCCCGCTCTGCCTGT F - ATGGAGCGAAAAGGACGTGA	60
pmk-1	R -TCGATGTGATCAGATCCAGGG F - TGGATTGGCACGTCAAACTG	58
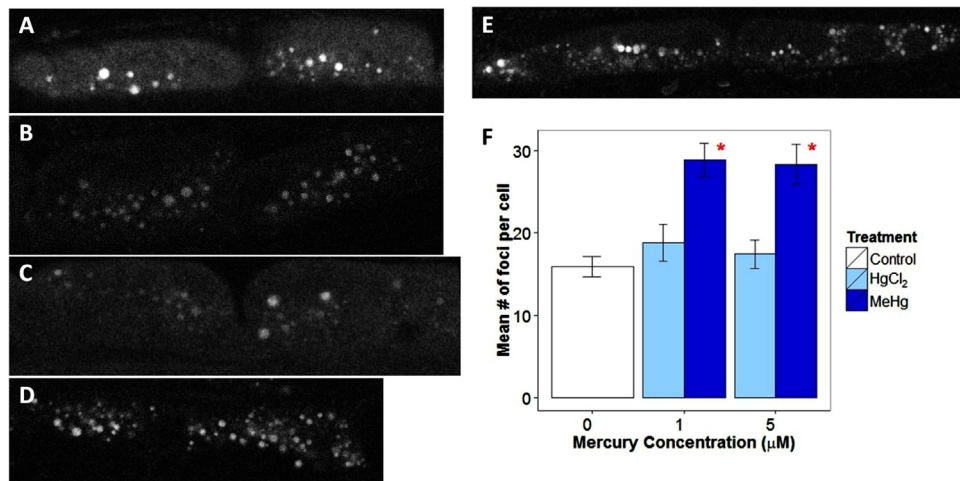
Fig. A1. Average number of LGG-1:GFP foci per seam cell under the following experimental conditions A) control, B) 1 μM $HgCl_2$, C) 5 μM $HgCl_2$, D) 1 μM MeHg, and E) 5 μM MeHg.
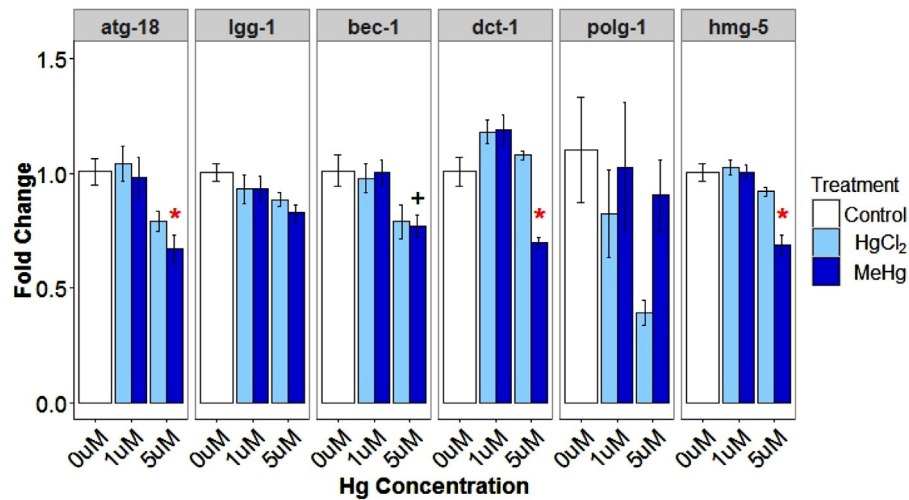
Fig. A2. Relative expression (fold change ±SE) of mitophagy (*dct-1*), autophagy (*bec-1*, *lgg-1*, *atg-18*), and biogenesis (*polg-1*, *hmg-5*) related genes in young adults. * indicates significantly different ($p < 0.05$) from control and + indicates $0.05 \leq p < 0.1$.
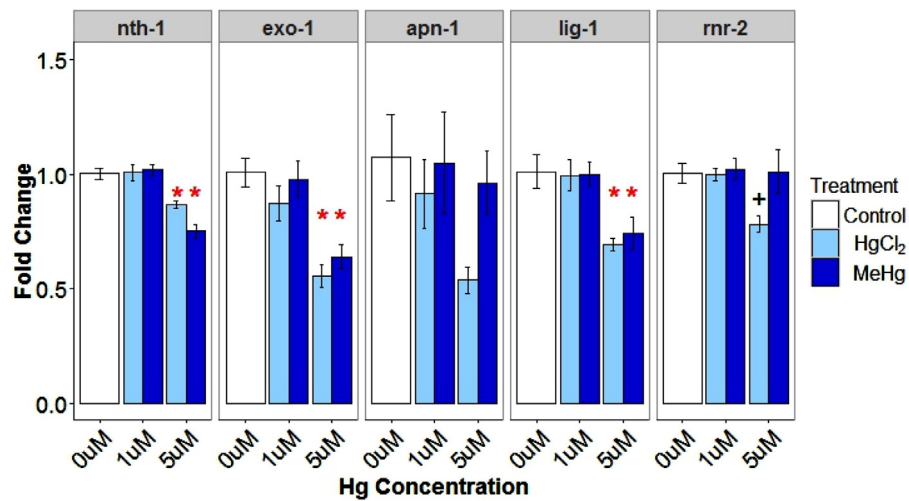
Fig. A3. Relative expression (fold change ±SE) of BER related genes (*nth-1*, *exo-1*, *apn-1*, *lig-1*, *rnr-2*) in young adults. * indicates significantly different ($p < 0.05$) from control and + indicates $0.05 \leq p < 0.1$.
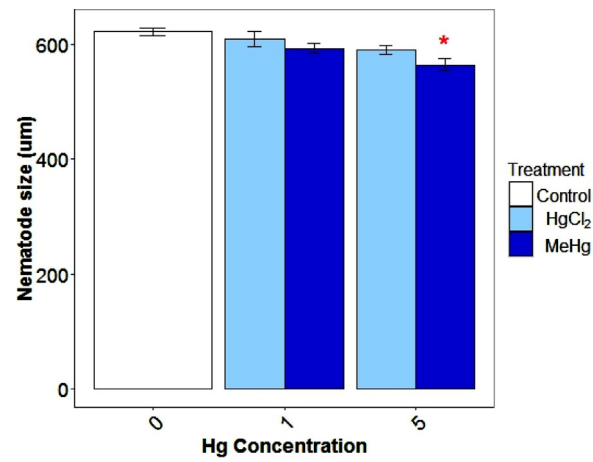
Fig. A4. *glp-1* nematode size (μM) following treatment with either control, HgCl₂, or MeHg conditions at the young adult life-stage.
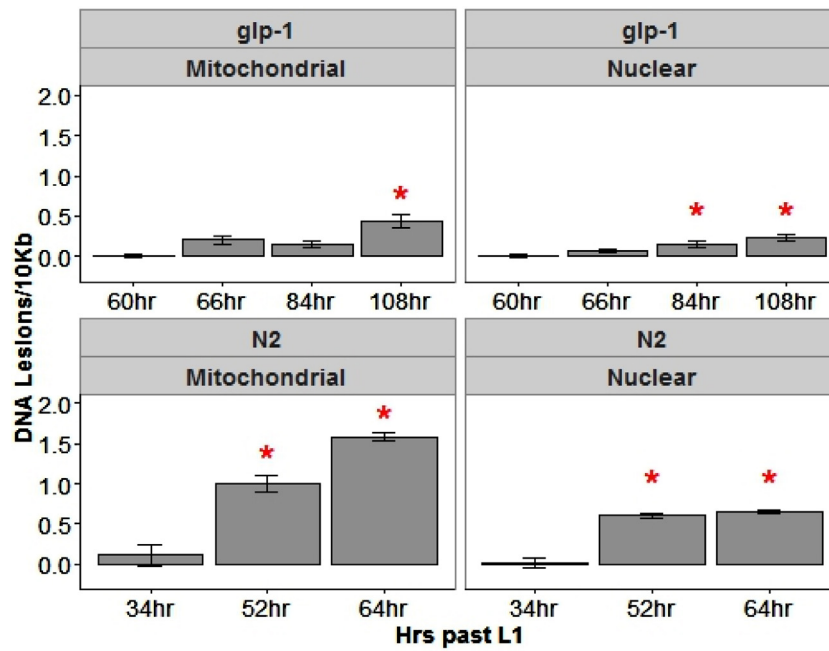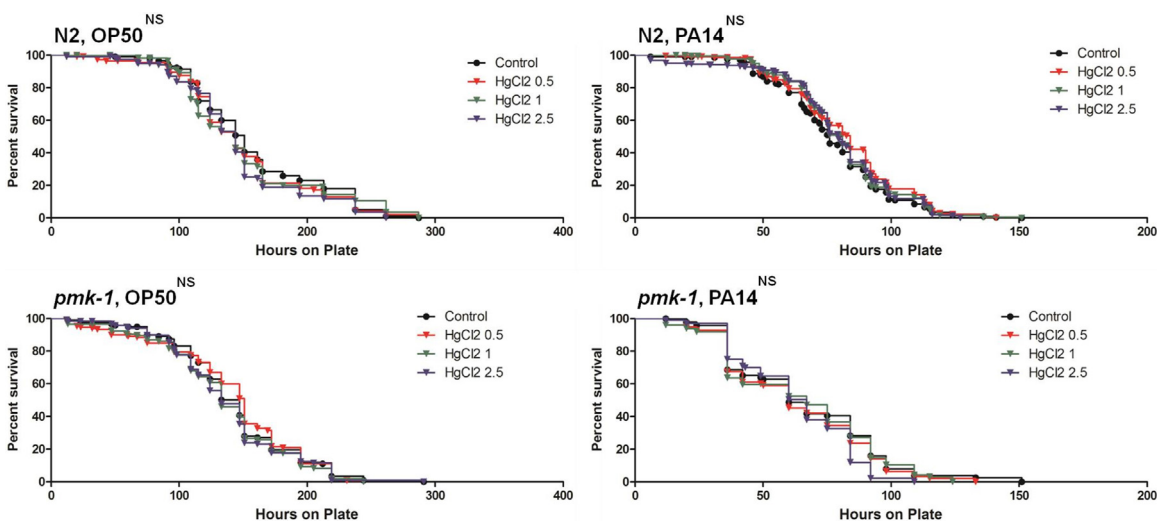

Fig. A5. mtDNA and nDNA damage in *glp-1* and N2 nematodes using the earliest time-point as the reference for the comparison. Nematodes were cultured on plates under control conditions at 25 °C and sampled at 60, 66, 84, and 108 h post placement on food after overnight hatch for synchronization for *glp-1* nematodes and at 34, 52, and 64 h for N2 nematodes.

Fig. A6. Survival curves for N2 and *pmk-1* nematodes exposed from the L1 stage to control (black), 0.5 (red), 1 (green), and 2.5 μM (blue) HgCl₂ on OP50 and PA14 survival plates. More than 40 animals were used for each curve.
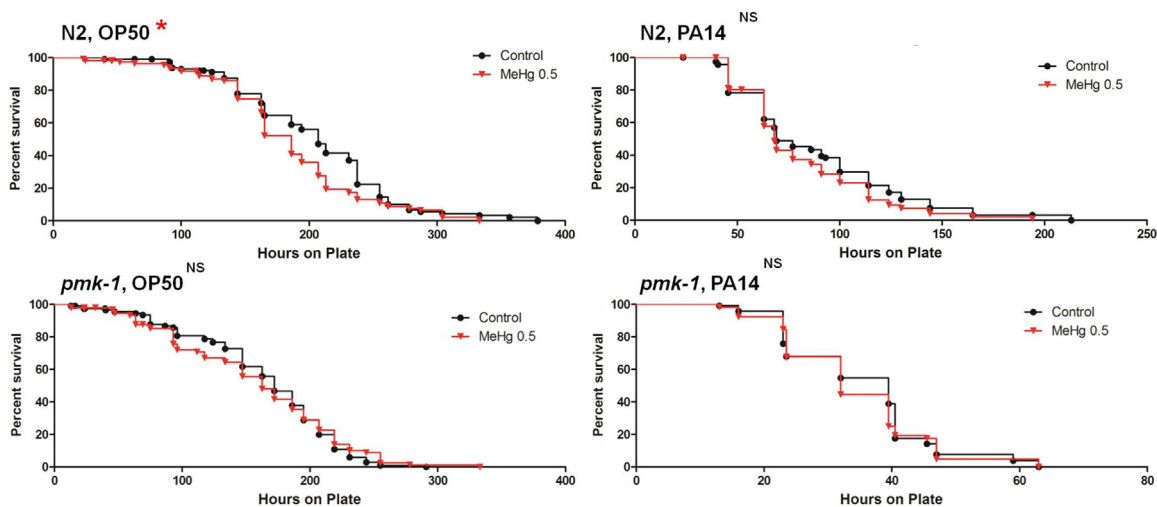
Fig. A7. Survival curves for N2 and *pmk-1* nematodes exposed from the L1 stage to control (black), 0.5 μM (red) MeHg on OP50 and PA14 survival plates. More than 40 animals were used for each curve. MeHg significantly reduced survival of N2 nematodes on OP50 (p < 0.05, Mantel-Cox log-rank test).

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