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Comparative Toxicology of Mercurials in *Caenorhabditis elegans*

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Abstract

Mercury is a toxic metal that can exist in multiple chemical species. Humans are commonly exposed to methylmercury and mercury vapor, which is converted to mercuric mercury in the body. Despite years of research, there is a paucity of information on the similarity and differences in the mechanisms of mercury toxicity. The relative toxicity of mercuric chloride (HgCl_2) and methylmercury chloride (MeHgCl) in *C. elegans* was determined using assays that measured growth, feeding, reproduction, and locomotion. The effect of HgCl_2 and MeHgCl on the expression of several archetypal stress-response genes was also determined. There was no significant difference between the EC_{50} s of the two mercurials on *C. elegans* growth. However, MeHgCl was more toxic to *C. elegans* than HgCl_2 when assessing feeding, movement and reproduction, all of which require proper neuromuscular activity. Methylmercury chloride exposure resulted in increased steady-state levels of the stress response genes at lower concentrations than HgCl_2 . In general, MeHgCl was more toxic to *C. elegans* than HgCl_2 , particularly when assaying behaviors that require neuromuscular function.

Keywords

C. elegans; Mercury; Methylmercury; Stress-response; Metal toxicity

Introduction

Methylmercury and inorganic mercury are two mercurial species of great concern. Approximately one third of global mercury emissions are natural in origin, while anthropogenic sources account for the remaining two thirds [1]. Anthropogenic emissions in 2050 are projected to as much as double 2006 emission levels [2]. Mercury undergoes complex environmental cycling and can exist in multiple chemical forms. Atmospheric mercury can be oxidized, then dissolve in water and return to the earth's surface in rain [3]. In the aquatic environment, microbes can methylate divalent mercury [4]. The methylated mercury can undergo biomagnification, so that top predators (e.g., tuna) have methylmercury levels that are several orders of magnitude higher than those found in the environment [5]. Humans are exposed to methylmercury through the consumption of fish, where approximately 95% of the mercurial can be absorbed in the gastrointestinal tract [6]. Humans are also exposed to various forms of inorganic mercury from dental amalgams, chlor-alkali plants, fluorescent lamp factories, and artisanal gold mining [7, 8]. Mercury vapor may not be toxic to cells, but it can be rapidly oxidized to divalent mercury, which is believed to be the proximate toxic form [9]. Humans are also exposed to inorganic mercury

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through their diet, although less than 10% of ingested inorganic mercury is absorbed in the gastrointestinal tract [10].

The toxicity of methylmercury became evident after the mass poisonings in the region surrounding Minamata Bay, Japan in the 1950s and 1960s, and in Iraq in the early 1970s [9]. Affected adults presented various neurological deficits. In addition, the sensitivity in developing humans was demonstrated by the severe cognitive and physical congenital defects observed in children whose mothers were asymptomatic [11]. Inorganic mercury is a well-known renal toxicant, and occupational exposure to this metal has been associated with neurological and renal defects [7, 12, 13].

Despite years of research, only a fragmented understanding of the mechanisms of mercurial toxicity exists. In addition, there is a paucity of information regarding the molecular mechanisms of toxicity for inorganic and methylmercury. The mechanistic data that are available are primarily based on in vitro studies using cultured cells. In cultured cells, exposure to either mercurial results in oxidative stress, microtubule disruption, and alterations in intracellular calcium levels [14–19]. Little is known about the molecular mechanisms of mercurial toxicity in vivo.

There is a need for a whole organism model that can be used to investigate the mechanisms of toxicity of different mercurials. *Caenorhabditis elegans* (*C. elegans*) has many attributes that make it a good toxicological animal model [20]. The short life cycle (~70 h), small size (~1 mm) and transparency of *C. elegans* facilitate their use in medium and high-throughput toxicity assays. Using these assays, it is possible to assess the effects of toxicants on large numbers of animals using various endpoints [21]. While the evolutionary distance between *C. elegans* and humans is considerable, a high level of conservation in regulatory signal transduction pathways and a high degree of genetic homology exist between the two species. Homologs to 60–80% of human genes have been identified in *C. elegans* [22]. An impressive array of genetic tools coupled with the ability to study whole organism effects makes *C. elegans* a useful model organism in which to study the toxicity of mercurials.

In the present study, the toxicity of mercuric chloride (HgCl_2) and methylmercury chloride (MeHgCl) in *C. elegans* were compared. *C. elegans* growth, reproduction, feeding, movement, and expression of stress-response genes were monitored as toxicological endpoints. In all of the assays, MeHgCl was more toxic to *C. elegans* than HgCl_2 . The relative toxicity between the mercurials however, differed by endpoint. These results provide insight into the mechanisms of mercurial toxicity and establish a framework in which the molecular toxicity of mercurials can be further examined.

Materials AND Methods

Maintenance of *C. elegans*

The Bristol N2 strain of *C. elegans* was used in all experiments with the *Escherichia coli* OP50 strain as a food source. *C. elegans* were maintained on K-agar plates at 20°C unless otherwise noted [23]. *C. elegans* were age synchronized by treating gravid adult nematodes with sodium hypochlorite/sodium hydroxide and allowing the embryos to hatch in the absence of food [24].

Growth assay

The effect of HgCl_2 and MeHgCl on *C. elegans* growth was determined as previously described [24]. Briefly, 50 age-synchronized L1 larvae were dispensed into 96-well plates containing a range of concentrations of either HgCl_2 or MeHgCl in complete K medium plus OP50 [25]. Nematodes were incubated at 20°C for 48 h. Under these conditions, untreated

L1 larvae will develop to the L4 larval stage. At the end of 48 h, the size of each nematode, as determined by its optical density (EXT), was assessed using the COPAS Biosort (Union Biometrica). Twelve conditions (eleven concentrations plus untreated control) were assayed for both mercurials. The concentrations examined for HgCl₂ were: 1.5, 2.1, 3, 4.3, 6.1, 8.7, 12, 18, 25, 35, and 50 µM and those for MeHgCl were: 1, 1.3, 1.8, 2.5, 3.3, 4.5, 6, 8.1, 11, 15 and 20 µM. Each condition was assayed in six wells per 96-well plate (~300 nematodes/condition/plate), and three replicates were performed. Growth was calculated by subtracting the mean EXT of the starting L1 larvae from the EXT measurement of each nematode at the end of the 48 h exposure. The 50% effective concentration (EC₅₀) was estimated by fitting growth data to the Hill function [26].

Reproduction assay

A reproduction assay that uses the COPAS Biosort to directly count the number of offspring was used to examine the effect of mercurials as previously described [27]. In this assay, five age-synchronized L4 larvae were dispensed into each well of a 96-well plate containing varying concentrations of either HgCl₂ or MeHgCl in K-medium with OP50. Six wells were used for each concentration (total of 30 L4s for each concentration). Twelve conditions (eleven concentrations plus untreated control) were tested for HgCl₂, while seventeen conditions (sixteen concentrations plus untreated control) were tested for MeHgCl. The concentrations assayed for HgCl₂ were: 0.3, 0.48, 0.75, 1.2, 1.9, 3, 4.8, 7.5, 12, 19, and 30 µM and those for MeHgCl were: 0.03, 0.048, 0.075, 0.12, 0.19, 0.3, 0.48, 0.75, 1.2, 1.9, 3, 4.8, 7.5, 12, 19, and 30 µM. After 48 h incubation at 20°C, the number of L1 *C. elegans* was determined. Each concentration had three to eight replicates. Fifty percent effective concentrations were estimated by fitting the data to the Hill function.

Feeding assay

The effect of mercurial exposure on feeding in *C. elegans* was determined as previously described [28]. In brief, 25 three-day-old age-synchronized adult nematodes were dispensed into each well of a 96-well plate containing various concentrations of HgCl₂ or MeHgCl in K-medium with OP50. Six wells (total of 150 adult nematodes) were used for each concentration (eleven concentrations plus untreated control). The concentrations for HgCl₂ were: 2, 3.2, 5, 8, 13, 20, 32, 50, 80, 130, and 200 µM and for MeHgCl: 0.5, 0.8, 1.3, 2, 3.2, 5, 8, 13, 20, 32, and 50 µM. Nematodes were incubated for 5 h at 20°C. At the end of the incubation, red fluorescent microspheres (0.5 µm diameter) were added to each well, and nematodes were allowed to feed for 15 min. Nematodes were then anesthetized with sodium azide, and the COPAS Biosort was used to measure the amount of red fluorescence (RFP) in each nematode. Feeding was determined by subtracting the RFP value post-feeding from the mean background RFP of nematodes at the beginning of the experiment. Three replicates were performed and EC₅₀s were estimated by fitting to the Hill function.

Motion tracking

The effects of mercurial exposure on locomotion was determined as previously described [21]. This assay utilized a *myo-2::GFP* strain of *C. elegans* (CB5584), which expressed high levels of green fluorescent protein in pharyngeal muscles [29]. Motion tracking was performed using an inverted fluorescence microscope (Leica) equipped with a camera (Hamamatsu), motorized stage, and temperature/humidity controlled incubator (Okolab). Approximately 40 age-synchronized L4 larval *C. elegans* were exposed five concentrations of HgCl₂ or MeHgCl in K-medium for 4 h. The HgCl₂ concentrations used were: 1, 3, 10, 30, and 100 µM and for MeHgCl were: 0.3, 1, 3, 10, and 30 µM. Exposed nematodes were then transferred to agar pads (1% agar, 51mM NaCl) and nematode movement was then monitored at low magnification for ~35 sec (3.5 frames/sec). The motion tracking software (Hamamatsu) generated Computer Assisted Sperm Analysis-like statistics for each animal.

Three parameters were reported: curvilinear distance, which is the total distance traveled by the nematode; curvilinear velocity, which is the speed of the nematode; and mean amplitude of the head (ALH mean), which is the average amplitude of the sinusoidal movement of the nematode. The Kruskal-Wallis test was used to find significant differences between treated and untreated samples. Three replicates were performed and the criterion for statistical significance was set at $p < 0.01$.

Quantitative reverse transcription-real time PCR

Quantitative reverse transcription-real time-PCR (qRT-PCR) was used to measure the effect of the mercurials on steady-state mRNA levels of the *C. elegans* genes: *gcs-1* (γ -glutamylcysteine synthetase), *gst-38* (glutathione S-transferase), *hsp-16.2*, *hsp-70* (heat shock protein). Mixed stage populations of *C. elegans* in complete S-medium liquid culture [30] were then exposed to HgCl_2 (1, 2.5, 4, 8, 10, 16 or 25 μM) or MeHgCl (0.1, 0.25, 0.6, 1.6, 4, 10 or 16 μM) for 24 h at 20°C with constant agitation.

Mixed stage populations were used for qRT-PCR due to the higher concentrations used in this study. These concentrations may delay nematode development; therefore, mixed-stage populations were used to help ensure that differences in gene expression were due to mercurial exposure, and not differences in the developmental stages of different treatment groups.

After exposure, *C. elegans* were collected and stored at -80°C until RNA purification, as previously described [31]. Total RNA was isolated using RNeasy Mini kit (Qiagen) according to manufacturer's instructions. cDNAs were generated using the SuperScript® First-Strand Synthesis System for RT-PCR from 2 μg total RNA according to manufacturer's instructions (Invitrogen). cDNAs were subsequently used in qRT-PCR using Power SYBR Green RT-PCR kits according to manufacturer's instructions (Applied Biosystems). Quantitative PCR was performed in an ABI 7900 HT Fast Real-Time System and fold-changes in mRNA levels were calculated using the $\Delta\Delta\text{Ct}$ method [32] using *mlc-2* (myosin light chain) as reference mRNA. Primers (Table 1) were designed using the open source Primer3 program [33]. Results were presented as the mean $\pm$ standard error of the mean. The significance of mean differences was determined by 1-way ANOVA of log-transformed data followed by Dunnett's multiple comparison test to find differences in treated and control samples. Three replicates were performed and the criterion for statistical significance was set at $p < 0.05$.

Results

Effect of mercurials on *C. elegans* growth

C. elegans increase in size as they develop from the L1 larval stage to reproductive adult [34]. Thus, while the growth assay measures the change in nematode size, a deficit in growth indicates delayed or arrested development. The effect of HgCl_2 and MeHgCl on the growth of *C. elegans*, relative to untreated nematodes, was determined following a 48 h exposure to eleven concentrations of each mercurial (Fig. 1). The EC50s, the concentration of a mercurial that inhibits growth by 50%, were not significantly different between the two mercurials. The EC50 for HgCl_2 was 11.8 μM , while the EC50 for MeHgCl was 11.3 μM (Table 2). However, differences in lethality, defined as nematodes that were immobile and smaller than L1s, were observed between the mercurials at higher concentrations. Nematodes exposed to HgCl_2 at concentrations greater than 25 μM appeared to arrest in the L1 stage, while the same MeHgCl concentrations resulted in nematode death. Exposure to MeHgCl concentrations higher than 20 μM was 100% lethal (data not shown), while nematodes tolerated HgCl_2 concentrations as high as 50 μM (Fig. 1).

Effect of mercurials on *C. elegans* feeding

Feeding in *C. elegans* is accomplished by the coordinated pumping of three sections of the pharynx, which simultaneously push bacteria into the intestine and expel excess liquid out of the mouth. The neuromuscular activity of feeding is regulated by a self-contained nervous system [35]. The effect of mercurials on feeding was assessed by exposing adult nematodes to eleven concentrations of HgCl₂ and MeHgCl for 5 h (Fig. 2). The feeding EC₅₀ for HgCl₂ was 34.8 μM and for MeHgCl it was 11.3 μM (Table 2). Feeding was completely inhibited after exposure to 32 μM MeHgCl. In contrast, exposure to 200 μM HgCl₂ did not completely inhibit feeding (Fig. 2). The difference between the EC₅₀s for the two mercurials suggested that MeHgCl was more neurotoxic than HgCl₂.

Effect of mercurials on *C. elegans* reproduction

Reproduction in *C. elegans* involves the integration of several processes: gamete formation, embryonic development and egg laying, which is a neuromuscular activity. This makes reproduction a sensitive endpoint in measuring effects of toxicants. The effect of mercurials on *C. elegans* reproduction was assessed by determining the number of progeny produced after 48 h exposure to different concentrations of either HgCl₂ or MeHgCl (Fig. 3). There was a 14-fold difference between the effective concentrations of HgCl₂ and MeHgCl: EC₅₀ for HgCl₂ was 11.3 μM, while the EC₅₀ for MeHgCl was 0.81 μM (Table 2). The difference between the HgCl₂ and MeHgCl on the reproduction EC₅₀s was the greatest of the endpoints examined. In addition, the EC₅₀ for MeHgCl reproduction was 14-fold lower than that calculated for the growth assay. As both the growth and reproduction endpoints assess the effects of a 48 h exposure, these results indicate that *C. elegans* reproduction is particularly sensitive to MeHgCl exposure.

Effect of mercurials on *C. elegans* locomotion

C. elegans move forward or backward in a sinusoidal motion across solid substrates. To determine the effect of mercurials on locomotion, *C. elegans* movement was measured following a 4 h exposure to HgCl₂ or MeHgCl. The distance (curvilinear distance), speed (curvilinear velocity) and amplitude of the nematodes sinusoidal motion (ALH mean) all decreased in a exposure-dependent manner in response to exposure to either mercurial (Fig. 4). Exposure to 1 μM MeHgCl significantly affected *C. elegans* by all three motion measurements. Conversely, exposure to 3 μM HgCl₂ was required to significantly decrease curvilinear velocity and exposure to 10 μM HgCl₂ was required to significantly decrease curvilinear distance and ALH mean. Supplemental Figure 1 shows representative computer-generated tracks for untreated nematodes and those exposed to 10 μM of HgCl₂ and MeHgCl. The effect of MeHgCl on the length and amplitude of the tracks of nematodes is particularly evident.

Effect of mercurials on stress-response gene expression

To determine the effect of mercurials on *C. elegans* at a molecular level, the exposure-response of HgCl₂ and MeHgCl on steady-state mRNA levels of four stress-responsive genes: *gcs-1*, *gst-38*, *hsp-16.2*, and *hsp-70*; were determined. γ-glutamyl-cysteine synthetase (*gcs-1*) catalyzes the rate-limiting first step in the synthesis of glutathione and is involved in the oxidative stress pathway [36] Glutathione S-transferase-38 is up-regulated in response to multiple stressors [37, 38]. The genes that encode the *C. elegans* heat shock proteins, *hsp-16.2* and *hsp-70* are up-regulated by heat shock and other environmental stressors [39, 40]. Steady-state mRNA levels of these genes were measured following a 24 h exposure of a mixed-stage population of *C. elegans* to HgCl₂ or MeHgCl (Fig. 5).

There were exposure-dependent increases in mRNA levels after exposure to either mercurial. There was a significant increase in mRNA levels of *gcs-1* and *gst-38* in nematodes exposed to 1.6 μM MeHgCl. Conversely, exposure to 16 μM HgCl₂ was required to significantly increase *gcs-1* levels, and exposure to 48 μM HgCl₂ was required to significantly increase *gst-38* mRNA levels. A lower concentration of MeHgCl was also required for induction of heat shock protein mRNAs. Exposure to 10 μM MeHgCl significantly increased mRNA levels of both *hsp-16.2* and *hsp-70*, while a 25 μM HgCl₂ exposure was required to significantly increase mRNA levels of the two heat shock proteins.

Discussion

Mercury is an environmental toxicant of great public health concern. In the present study, the toxicity of an inorganic (HgCl₂) and organic (MeHgCl) mercury in *C. elegans* was compared. When examining the effect of mercurials on growth, there was not a significant difference between the EC₅₀ values (Table 2). MeHgCl concentrations greater than 20 μM , however, were lethal to L1 larvae. In contrast, exposure to HgCl₂ at concentrations greater than 20 μM resulted in viable, but growth arrested L1 larvae. For feeding, the HgCl₂ EC₅₀ was approximately three-fold higher than that for MeHgCl. In addition, exposure to 32 μM MeHgCl completely inhibited feeding, while *C. elegans* exposed to HgCl₂ concentrations as high as 200 μM did not completely abrogate feeding. For the three measurements of locomotion, MeHgCl had significant inhibitory effects at concentrations lower than those observed following HgCl₂ exposure. The largest difference in toxicity between HgCl₂ and MeHgCl was observed in reproduction. The HgCl₂ EC₅₀ was ~14-fold higher than that for MeHgCl. Finally, MeHgCl exposure induced significant increases in stress-response gene expression at lower concentrations than HgCl₂. While the relative toxicity between HgCl₂ and MeHgCl differed by end-point, MeHgCl was consistently more toxic to nematodes than HgCl₂ (Table 2).

Previous studies have used different methods to investigate the toxicity of HgCl₂ or MeHgCl in *C. elegans*. The 24 h LC₅₀ of HgCl₂ in adult *C. elegans* exposed on agar plates or liquid culture were 500 μM and 90 μM , respectively [23, 41]. The difference in LC₅₀s may be due to a greater bioavailability of HgCl₂ in liquid. In agreement with the data presented here, a study that examined the effect of continuous MeHgCl exposure on *C. elegans* brood size found that *C. elegans* cultured on agar containing 10 μM MeHgCl had a 90% decrease in brood size and a 40% increase in time to adulthood [42]. However, a study of the effects of a 15 h MeHgCl exposure (100 μM – 400 μM) in *C. elegans* found no effect on life span or brood size, a temporary deficit in feeding and delayed growth [43]. It is therefore possible that nematodes can recover from MeHgCl toxicity after cessation of MeHgCl exposure. The present study is the first to directly compare the toxicity of HgCl₂ and MeHgCl in *C. elegans*.

Methylmercury was generally more toxic than HgCl₂ in *C. elegans*. This observation is consistent with experiments using cultured cells. Among three human neural cell lines, SH-SY5Y (neuroblastoma), U373MG (glioblastoma) and D407 (retinal pigment epithelial), the effects of 24 h and 48 h exposures to HgCl₂ and MeHgCl on mitochondrial enzyme activity were compared [44]. As was observed in the present study, MeHgCl exposure was more toxic than HgCl₂, though the relative toxicity of the mercurials varied by cell line and time of exposure. For example, the 24 h EC₅₀s of HgCl₂ and MeHgCl in D407 cells were not significantly different. In contrast, the 24 h HgCl₂ EC₅₀ was eight-fold greater than the MeHgCl EC₅₀ in SH-SY5Y cells. Similarly, the 24 h HgCl₂ EC₅₀ was five-fold greater than that for MeHgCl in differentiated rat pheochromocytoma cells (PC12) [45].

Human exposure to mercury vapor can produce tremors and loss of coordination, while exposure to methylmercury can result in ataxia [11, 46]. Similarly, a disruption of neuromuscular activity was observed in *C. elegans* after mercurial exposure. Neuromuscular function was more sensitive to MeHgCl than HgCl₂. For feeding, the MeHgCl EC₅₀ was 3-fold lower than that for HgCl₂. Methylmercury exposure also inhibited locomotion at lower concentrations than HgCl₂. There was not a significant difference between the mercurial on *C. elegans* growth, which is not a sensitive measure of neurotoxicity. This suggests that MeHgCl is a more potent neurotoxicant than HgCl₂ in *C. elegans*.

In utero methylmercury exposure results in congenital cognitive and anatomical defects in humans in the absence of overt maternal toxicity [47]. Methylmercury also causes a dose-dependent decrease in mouse litter size [48]. Little is known about *in utero* inorganic mercury exposures, but the progeny of pregnant Long-Evans rats exposed to mercury vapor exhibited developmental toxicity. This only occurred, however, at concentrations that caused significant maternal toxicity [49]. *C. elegans* early life stages were highly susceptible to MeHgCl, but not HgCl₂, toxicity. For reproduction, the MeHgCl EC₅₀ was 14-fold lower than the HgCl₂ EC₅₀ (Table 2). Exposure to 0.81 μ M was not toxic to post-embryonic nematodes. In contrast, the HgCl₂ EC₅₀s for reproduction and growth were not significantly different. These data suggest that the phenomenon of exquisite susceptibility of early life stages to methylmercury, but not inorganic mercury, is conserved across species.

Both HgCl₂ and MeHgCl exposure resulted in an increase in the expression of stress response genes, though the induction of these genes varied by mercurial and target gene. Induction of heat shock proteins required higher concentrations of either HgCl₂ or MeHgCl than did *gcs-1* or *gst-38*. The induction of glutathione-S-transferase and γ -glutamylcysteine synthetase transcription is used as a marker of toxicant induced intracellular oxidative stress [50]. Glutathione is critical in the oxidative stress response, as supplementation with a glutathione precursor protects against both MeHgCl and HgCl₂ toxicity [51, 52]. Thus both mercurials induce oxidative stress in *C. elegans*. In addition, MeHgCl induces oxidative stress at lower concentrations than HgCl₂. Methylmercury exposure induced the glutathione-response genes (*gcs-1* and *gst-38*) at 5–10-fold lower concentrations than HgCl₂ exposure. In contrast, MeHgCl exposure induced expression of heat shock genes at concentrations only two to three-fold lower than HgCl₂. The heat shock response may be a secondary response following mercurial- or reactive oxygen species-mediated protein damage, which occurs when the oxidative stress defense mechanisms are depleted. In all genes tested, lower concentrations of MeHgCl were required to induce significant increases in expression relative to HgCl₂. This suggests that MeHgCl is a particularly potent inducer of intracellular damage and this damage may be a consequence of mercurial-induced oxidative stress.

Inorganic and methylmercury remain toxicants of great concern to human health. The extent to which inorganic and methylmercury have similar or divergent mechanisms of toxicity is a question that requires further inquiry. In the present study, a detailed comparison of the toxicity of inorganic and methylmercury in *C. elegans* was performed. The *C. elegans* model has many traits that make it an appealing model in which to study molecular mechanisms of toxicity. The present study establishes the framework in which comparisons in the molecular mechanisms of mercurial toxicity can be studied in *C. elegans*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure. 1.

Effect of mercurials on *C. elegans* growth. Nematode size is expressed as the log of the absorbance (EXT) of individual nematodes at the end of 48 h exposure to HgCl_2 or MeHgCl minus the mean absorbance of all nematodes at the beginning of exposure. Lines in box plots indicate mean, boxes include 25th–75th percentile, whiskers include 10th–90th percentile. Data represent combined results of three independent experiments. Each box plot represents 500 to 900 nematodes.

Figure. 2.

Effect of mercurials on *C. elegans* feeding. The level of feeding (LogRFP) was measured in individual nematodes following a 5 h exposure to HgCl₂ or MeHgCl followed by a 15 min incubation with fluorescent beads. LogRFP values are the measured fluorescence values following exposure minus mean background fluorescence of all nematodes at the beginning of mercurial exposure [28]. Lines in box plots indicate mean, boxes include 25th to 75th percentile, whiskers include 10th to 90th percentile. Data represent combined results of 3 independent experiments. Each box plot represents 200 to 400 nematodes.

Figure. 3. Effect of mercurials on *C. elegans* reproduction. Nematodes in final larval stage were exposed to HgCl₂ (●) or MeHgCl (■) for 48 h. The number of offspring from treated nematodes was then measured [27]. Data presented are mean $\pm$ standard mean error of 3 to 8 independent experiments.

Figure. 4.

Effect of mercurials on *C. elegans* locomotion. Movement of L4 larvae was tracked after a 4 h exposure to various concentrations of HgCl_2 or MeHgCl . Three motion parameters were then calculated: curvilinear distance (*upper panel*), curvilinear velocity (*middle panel*), and mean amplitude of the head (ALH mean) (*lower panel*). Data presented are mean $\pm$ standard mean error of three independent experiments. Significant differences relative to untreated are indicated with asterisks.

Figure. 5.

Effect of mercurials on stress-response gene expression. Relative mRNA levels of four *C. elegans* stress-responsive genes, *gcs-1*, *gst-38*, *hsp-16.2* and *hsp-70*, were measured from mixed-stage *C. elegans* populations following a 24 h exposure to HgCl₂ or MeHgCl. Data represent mean $\pm$ standard mean error of 3 to 4 independent experiments. Significant differences relative to untreated are indicated with asterisks. Values for the fold change in stress-response gene expression can be found in Supplemental Table 1

Table 1

Primers used in qRT-PCR reactions

Gene	Forward (5'→3')	Reverse (5'→3')
<i>mlc-2</i>	TTGACAGGAACTGACCCAGAGG	ATAGCCTTGACCTCATCCTCG
<i>gcs-1</i>	CCACCAGATGCTCCAGAAAT	TGCATTTTCAAAGTCGGTCA
<i>gst-38</i>	AAGATAACAGACTTACCGATGAGGA	GAAGCTGGTTGTATGGGGTTT
<i>hsp-16.2</i>	TGCAGAATCTCTCCATCTGAGT	TGGTTTAAACTGTGAGACGTTGA
<i>hsp-70</i>	CGGTATTATCAAAATGGAAAGGTT	TACGAGCGGCTTGATCTTTT

Table 2Levels of mercurial toxicity in *C. elegans*

Assay	Mercury chloride		Methylmercury chloride	
	EC50 (μM)	95% Confidence Interval (μM)	EC50 (μM)	95% Confidence Interval (μM)
Growth	11.8	11.2 – 12.4	11.3	10.4 – 12.2
Feeding	34.8	29.9 – 39.7	11.3	10.8 – 11.9
Reproduction	11.3	7.1 – 15.5	0.81	0.68 – 0.95

EC50, 50% effective concentration