Antagonistic Growth Effects of Mercury and Selenium in *Caenorhabditis elegans* Are Chemical-Species-Dependent and Do Not Depend on Internal Hg/Se Ratios

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S Supporting Information

ABSTRACT: The relationship between mercury (Hg) and selenium (Se) toxicity is complex, with coexposure reported to reduce, increase, and have no effect on toxicity. Different interactions may be related to chemical compound, but this has not been systematically examined. Our goal was to assess the interactive effects between the two elements on growth in the nematode *Caenorhabditis elegans*, focusing on inorganic and organic Hg (HgCl₂ and MeHgCl) and Se (selenomethionine, sodium selenite, and sodium selenate) compounds. We utilized aqueous Hg/Se dosing molar ratios that were either above, below, or equal to 1 and measured the internal nematode total Hg and Se concentrations for the highest concentrations of each Se compound. Observed interactions were complicated, differed between Se and Hg compounds, and included greater-than-additive, additive, and less-than-additive growth impacts. Biologically significant interactions were only observed when the dosing Se solution concentration was 100–25 000 times greater than the dosing Hg concentration. Mitigation of growth impacts was not predictable on the basis of internal Hg/Se molar ratio; improved growth was observed at some internal Hg/Se molar ratios both above and below 1. These findings suggest that future assessments of the Hg and Se relationship should incorporate chemical compound into the evaluation.

INTRODUCTION

Mercury (Hg) is an environmental contaminant of great concern owing to its persistence in the environment; human and wildlife exposures are common, with recognized toxic impacts, making it important to understand factors that can mediate Hg toxicity. Hg exists naturally in the environment but also has considerable anthropogenic mobilization, with industrial inputs expected to increase in the future.¹ In the environment, Hg exists in three main chemical forms: elemental Hg(0), inorganic divalent Hg(II), and organic forms such as monomethylmercury (MeHg).² The majority of human exposures are to inorganic and organic Hg through occupational and dietary routes, respectively. Chemical speciation is important to toxicity; for example, harmful impacts to the nervous and reproductive systems are associated with MeHg exposure, and negative renal system impacts are associated with inorganic Hg exposure.^{3–6} Hg toxicity can also be altered by a variety of other factors, including selenium coexposure.

Selenium (Se), like Hg, is a naturally occurring element in the environment, but unlike Hg, Se is a necessary micronutrient. It is necessary for the proper function of selenoenzymes, which have important roles including anti-

oxidant functions. Due to its biological importance, organic Se (e.g., selenomethionine) and inorganic Se (e.g., selenate, selenite) have commonly been used in human and animal supplements. However, although Se is required at low levels, it has a very narrow therapeutic index and is toxic at higher concentrations. The interaction between Hg and Se is complex and has commonly been described as antagonistic, with Se coexposures having the ability to mitigate Hg toxicity.^{7,8} Conversely, synergistic effects have also been observed.^{9–11} The difference between antagonistic and synergistic impacts may result from the chemical species and dosing concentrations used and may depend on the organism and biological outcome examined. However, the mechanisms that underlie either interaction are not well-understood.

Proposed mechanisms for antagonism include: (1) reductions in bioavailable Hg due to Hg–Se complex formation (i.e., reduced uptake); (2) decreased distribution to target tissues or increased excretion of Hg due to Hg–Se complex formation;

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and (3) improved antioxidant function, as some antioxidants are Se-dependent enzymes (ex. glutathione peroxidase, GPX; thioredoxin reductase, TrxR), and supplemental Se could reduce the Hg-induced depletion of selenoenzymes.^{12,13} Related to the first two hypotheses, it has been specifically postulated that the protective effects of Se occur when the molar Se/Hg molar ratio is ≥ 1 because Se may reduce the biological availability of Hg through physical sequestration due to the high affinity between Hg and Se.^{14–17} Potential mechanisms for synergism are less clear, but may include altering antioxidant capabilities, thereby promoting a pro-oxidative environment.^{8,9,18}

We sought further knowledge of Hg and Se interactions by systematically varying multiple chemical species and concentrations in the model organism *Caenorhabditis elegans*. *C. elegans* growth was used as the toxic end-point because it represents an integrated measure of multiple developmental processes. Growth impairments from exposure to some species of Hg or Se individually have also been previously described in *C. elegans*, making it a logical model to investigate coexposures.^{19–21} Additionally, many antioxidants in *C. elegans* act similarly to mammalian homologues in terms of reducing oxidative stress (GPX, TrxR, and superoxide dismutase SOD).^{22–25} We note that *C. elegans* may be a particularly useful model for addressing the antagonism theories described in the preceding paragraph because the likelihood of the third hypothesis is especially low in *C. elegans*. *C. elegans* has only one selenoenzyme (TrxR-1) compared to the 25 in humans,^{26–28} deletion of this gene has not been observed to alter Se toxicity in *C. elegans*,²⁹ and low levels of Se stimulate nematode growth^{21,30} and are protective against oxidative stress in *C. elegans*.^{31–34} Our objective was to measure growth impacts from multiple combinations of inorganic and organic forms of Hg and Se and, in doing so, examine the importance of (1) Se compound and concentration, (2) Se/Hg molar ratio of the exposure medium, and (3) internal Se/Hg molar ratio to toxicity recovery.

MATERIALS AND METHODS

***C. elegans* Maintenance.** N2 (Bristol) nematodes were maintained at 20 °C on K agar plates seeded with *Escherichia coli* (OP50 strain). 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 MgSO₄, CaSO₄, and cholesterol).³⁵

Individual Dose–Response Curves for Selecting Hg and Se Concentrations for the Coexposure Growth Assay. L1 nematodes were distributed into 24 well plates (100–300 nematodes per well in 1 mL of dosing solution) containing a range of concentrations of Hg or Se compounds in EPA reconstituted moderately hard water plus UVC-killed *E. coli* (UVRA strain) to eliminate the potentially confounding effect of bacterial metabolism on exposures, as previously described.³⁶ Dose–response curves were obtained for HgCl₂ (0–10 μ M), MeHgCl (0–10 μ M), selenomethionine (0–1 mM, SeMeth), sodium selenite (0–5 mM, Se(IV)), and sodium selenate (0–50 mM, Se(VI)). Nematodes were exposed at 20 °C for 48 h, with additional UVC-killed *E. coli* added after 24 h (5% of solution volume). Following 48 h, nematode size was measured using a COPAS Biosort (Union Biometrica, Holliston MA), using extinction, the optical density of a nematode, as a growth end-point (72–1479 nematodes measured per exposure).³⁷

Compound concentrations for the coexposure growth assay were chosen on the basis of growth reductions. We selected concentrations of 2 and 5 μ M MeHgCl and HgCl₂ because of their similar impacts on growth (both species caused ~15 and ~35% reduction at these doses) and because these exposure levels alter neuromuscular function (locomotion) in *C. elegans*.²⁰ We chose Se concentrations that included a high concentration that reduced growth 10–20% (500 μ M SeMeth, 1500 μ M Se(IV), and 50 mM Se(VI)) along with lower concentrations to assess the impacts of dosing solutions with molar ratios of Hg/Se that were either above, below, or equal to 1. Although we did not observe a growth response at the lower Se concentrations (0.25, 2, and 5 μ M), which could suggest that Se may not be entering the nematodes, these doses were considered appropriate for the coexposure growth assay, as toxicities to other end-points have been observed in this concentration range in other invertebrate studies. We note that although some Se concentrations are high compared to those employed in some other studies, they are sublethal and consistent with previous reports of the same chemicals in studies with *C. elegans*.^{19,29,38,39}

Hg and Se Coexposure Growth Assay. L1 nematodes were exposed to HgCl₂, MeHgCl, SeMeth, Se(IV), or Se(VI) using the same methods that were used to obtain the individual dose–response curves. The following conditions were assayed individually and in Hg–Se combinations: control; 2 and 5 μ M HgCl₂; 2 and 5 μ M MeHgCl; 0.25, 2, 5, and 500 μ M SeMeth; 0.25, 2, 5, and 1500 μ M Se(IV); and 0.25, 2, 5, 1000, 10000, and 50000 μ M Se(VI). Following the 48 h exposure, nematode size was determined (163–409 nematodes per exposure).

Internal Hg and Se Exposure Determination. L1 nematodes were distributed into 250 mL tissue culture flasks (49 000–85 000 nematodes per flask in 30 mL of dosing solution) and exposed to a range of concentrations of HgCl₂, MeHgCl, SeMeth, Se(IV), or Se(VI). Internal concentrations were only determined for the highest Se concentrations because interactive impacts on growth in combination with Hg exposure were most clearly present at these exposure levels. Following the 48 h of exposure, nematodes were washed three times with K medium and briefly damaged with a Bullet Blender (Next Advance, Averill Park, NY; four repetitions of 15 s pulses) to break the cuticle. The solution containing damaged nematodes was then centrifuged (3 min at 300g) to remove the cuticle from the solution. The remaining solution was considered to be representative of the internal nematode environment.

Total Hg concentrations from the internal worm solution were measured using direct thermal decomposition, amalgamation, and atomic absorption spectrometry (Milestone-DMA-80).⁴⁰ Analysis of standard reference material (DORM-4 Fish Protein) resulted in Hg measurements that were $95 \pm 4.2\%$ of the certified value. The limit of detection (LOD) for Hg by this method was 0.001 mg/kg. For Se determination, 0.5 mL of sample was microwave digested in 2.5 mL of 90% nitric acid solution (CEM Discover SP-D Microwave Digestion System). Total Se was determined by inductively coupled plasma–mass spectrometry (ICP–MS; Agilent 7700X equipped with Octopole Reaction System). Analysis of a standard reference material (DORM-4 fish protein) resulted in recoveries of $101 \pm 2.1\%$ ($n = 7$) of the certified Se value. The LOD for Se by this method was 3.8 μ g/L. For internal nematode Hg and Se determination, the LOD value was used for samples with concentrations below the LOD for each method. Hg and Se content were normalized to total protein content to account for

Figure 1. Nematode size (logEXT) following combination mercury (HgCl_2 and MeHg) and selenium (sodium selenate, sodium selenite, and selenomethionine) exposures. Coexposures include: (A) selenate and HgCl_2 , (B) selenate and MeHg, (C) selenite and HgCl_2 , (D) selenite and MeHg, (E) selenomethionine and HgCl_2 , and (F) selenomethionine and MeHg. Selenium exposure is represented on the x -axis against nematode growth (logEXT), with mercury exposure represented by the grouped lines and box-plots. White box-plots indicate $0 \mu\text{M}$ Hg exposure, light gray box-plots indicate $2 \mu\text{M}$ Hg exposure, and dark gray box-plots indicate $5 \mu\text{M}$ Hg exposure. Data for each group represents three to four biological experiments separated in time with between 163 and 409 individual nematodes. Interaction lines and box-plots are dodged for better visual representation. Lines between box-plots connect the means for each treatment. For each selenium–mercury pairing, mercury altered nematode growth compared to selenium-only exposures ($p < 0.05$). The dashed red line indicates the average nematode size before dosing (L1).

nematode size differences between treatments. Protein content was determined following manufacturer instructions in the bicinchoninic acid (BCA) protein assay kit (Thermo Scientific Pierce BCA Protein Assay Kit). Average protein concentration per sample was approximately $80 \mu\text{g/mL}$, ranging from 39 to $133 \mu\text{g/mL}$.

Statistical Analysis. Individual dose–response curves were analyzed using ANOVA, followed by Dunnett’s post-hoc test for pairwise comparisons to the control. Interactions between Hg and Se were assessed by using contrast matrices to measure differences in nematode growth from the coexposure assay. Using these matrices, null hypotheses were defined as additive, with equal weight given to both Hg and Se individual exposures. Significant deviations from the null would indicate interactions that were either greater or less than additive, with less than additive responses signifying an antagonistic interaction. Matrices were constructed, for each Hg and Se compound pair, in a stepwise manner, and significance was determined using an F-statistic (see [Supporting Information](#)). For cases in which the expected nematode size was smaller than

the size before dosing, the coexposure treatment was compared to the size before dosing so that the calculated change in growth would not be overinflated. Changes in expected growth were expressed as percentage relative to control nematode growth. A multiplicative model was also assessed, and instances in which multiplicative interactions are significant and differ from additive are described in the text. Additive representations are presented graphically because they are more straightforward to interpret. Additive and multiplicative models were compared using an F-test, Akaike information criterion (AIC), and Bayesian information criterion (BIC) scores. An amelioration of growth inhibition (i.e., toxicity reduction, sometimes described as “rescue” in the pharmacological or genetic literature), is referred to as a reduction or mitigation of toxicity in this manuscript. Internal Hg and Se analyses were performed twice, separated in time. The impact of the highest Se exposures on the internal Hg and Se concentrations and the Hg/Se molar ratio was determined using a two-way ANOVA. Statistics were calculated using R version 3.2.2 (Vienna, Austria), and significance was accepted at a level of $p < 0.05$.

Figure 2. Nematode growth difference (average % of control $\pm$ SE) from expected growth under additive conditions for all selenium and mercury coexposures. Coexposures include: (A) selenate and HgCl₂, (B) selenate and MeHg, (C) selenite and HgCl₂, (D) selenite and MeHg, (E) selenomethionine and HgCl₂, and (F) selenomethionine and MeHg. The x-axis has categorically increasing selenium exposures (μ M). HgCl₂ and MeHg exposures are plotted separately and are indicated by open and closed circles, respectively. Exposure concentration is represented by a solid line for 2 μ M exposures and a dashed line for 5 μ M exposures. Positive percentages indicate an antagonistic interaction (more growth than expected) and negative percentages greater than additive interactions (less growth than expected). Asterisks indicate significance compared to the expected additive relationship ($p < 0.05$).

Equilibrium Speciation Calculations. The speciation of Hg(II) and MeHg in the Hg–Se(IV) and Hg–Se(VI) mixtures was calculated using Visual MINTEQ (v. 3.1). Input parameters included the recipe for the EPA moderately hard water matrix (96 mg of NaHCO₃, 60 mg of MgSO₄, 60 mg of CaSO₄, and 4 mg of KCl in 1 L of H₂O), pH 7.5, and 2 μ M total Hg (as Hg(II) or MeHg). The concentration of total Se as Se(VI) or Se(IV) was varied using the same range as the coexposure experiments (from 0.1 μ M to 50 000 μ M). The calculations utilized the thermodynamic stability constants in the Visual MINTEQ database, including constants for Hg²⁺ complexes with OH[−], Cl[−], and SeO₃^{2−}. Constants for CH₃Hg⁺ complexes with OH[−], Cl[−], SeO₃^{2−}, and SeO₄^{2−} were obtained from the reference literature (NIST) and manually entered into the program database (Table SI-2).⁴¹ The calculations assumed that other components of the exposure matrix (e.g., UV-killed bacteria, *C. elegans*) did not alter the solution-phase speciation of Hg and Se.

RESULTS

Individual Dose–Response Curves for Selecting Hg and Se Concentrations for the Coexposure Growth Assay. Nematodes were exposed to a range of individual Hg and Se compound concentrations to evaluate exposure concentrations that impact growth and could be used in the coexposure assay. Data for the individual dose–response curves represents one to nine biological experiments separated in time. All tested Hg and Se compounds exhibited dose–responses

with respect to growth with the following observed toxicity order: MeHg $\approx$ HgCl₂ > SeMeth > Se(IV) > Se(VI) (Figure SI-1 top panel). For individual compounds, the lowest concentrations at which consistent and significant growth reductions were observed were: 1 μ M MeHg, 2 μ M HgCl₂, 200 μ M SeMeth, 700 μ M Se(IV), and 50 mM Se(VI) (Figure SI-1 bottom panel).

Hg and Se Coexposure Growth Assay. Next, to assess the interaction between Hg and Se, F-statistics were computed to determine if there were significant interactions and whether these interactions were less than additive (antagonistic) or greater than additive. Nematode size for each treatment is displayed in Figure 1. Data for coexposure experiments represents three to four biological experiments separated in time. Initial matrices for all Hg–Se comparisons rejected the null hypotheses that Hg exposure in general (H_{Aa}) and at each dosing concentration (H_{Ab1} and H_{Ab2}) had no impact on nematode growth compared to that in Se-only exposures. Additive interaction directionality (antagonistic or more than additive) for each Hg–Se pairing is indicated in Figure 2. Multiplicative interactions were significant and different from the additive model at all instances in which growth differed from additive conditions, with the exception of no significant multiplicative interaction at 0.25 μ M Se(VI) in combination with 2 μ M HgCl₂. In most instances, differences between additive and multiplicative models was modest (AIC and BIC score difference <20). However, for coexposures that resulted in more than 15% growth differences additive and multi-

Figure 3. Interaction plots for internal mercury and selenium concentrations (average ng of compound/ μ g of protein $\pm$ SE) and internal Hg/Se ratios (average $\pm$ SE). The figure is grouped into three main sections with internal mercury concentrations are represented in the left panels (A–D), internal selenium concentrations are represented in the middle panels (E–H), and internal Hg/Se ratios in the right panels (I–L). Each of these sections contains faceted plots to represent exposure to no selenium compounds (none; A, E, I), selenate (50 mM; B, F, J), selenite (1500 μ M; C, G, K), or selenomethionine (500 μ M; D, H, L). The x-axis has categorically increasing mercury exposures (μ M) to either HgCl₂ (open circle, solid line) or MeHg (closed circle, dotted line). In the internal Hg/Se ratio section, note that the y-axis scale is different for each plot, and the red dashed line indicates a 1:1 Hg/Se ratio. In the internal mercury and selenium sections, points with at least one value above or below detection limits are indicated by OD and LOD, respectively; for a full list of sample values, see Table SI-1. For the internal mercury plots, main effects for mercury compound and concentration and their interaction were significant ($p < 0.05$) for each selenium exposure. For the internal selenium plots, the main effect of mercury compound was significant ($p < 0.05$) in the selenate plot but not ($p = 0.08$) in the selenite or selenomethionine plots.

plicative models differed greatly (AIC and BIC score difference >70), with the multiplicative models having the lower AIC and BIC scores. The better model fit with the multiplicative models in these instances is likely due to the defined multiplicative interaction being a more intermediate response compared to the additive model.

Following Se(VI) exposures, antagonistic interactions were mainly observed at the highest Se(VI) dosing concentration (50 000 μ M). Smaller ($<15\%$) antagonistic and greater than additive interactions also occurred at lower Se(VI) doses (Figure 2). Following Se(IV) exposures, antagonistic interactions only occurred at the highest Se(IV) dosing concentration (1500 μ M), with significant growth improvements ($>20\%$) observed in combination with both MeHg concentrations. Greater-than-additive interactions with Se(IV) were also observed at one coexposure with HgCl₂ and multiple coexposures with 2 μ M MeHg (Figure 2). Following SeMeth exposures, significant antagonistic interactions ($>20\%$) were observed at the highest Se dosing concentrations for all Hg compounds and doses. Smaller antagonistic interactions were also observed at lower SeMeth doses in combination with both low and high concentrations of HgCl₂ and MeHg (Figure 2). Antagonistic relationships identified for some very high (5 μ M)

Hg exposures (Figure 2, Table SI-3) should be taken with caution because growth was highly impaired at this Hg concentration. There were seven cases in which the expected nematode size was smaller than the size before dosing. These cases are indicated in Table SI-3.

Internal Hg and Se Exposure Determination. We next determined if the interactions observed at the highest Se dosing concentrations, which were uniformly antagonistic, were related to the intraorganismal Se/Hg molar ratio. We measured internal Hg and Se concentrations for individual Hg exposures and for the coexposures that occurred at the highest Se dosing concentration for each Se compound. As expected, Hg concentrations increased with increasing exposure concentration, but unexpectedly, higher Hg concentrations were detected in HgCl₂-exposed rather than MeHg-exposed nematodes ($p < 0.05$ for Hg compound effect). In Se(IV) and Se(VI) coexposed nematodes, internal HgCl₂ concentrations were reduced but not significantly ($p = 0.074$). In SeMeth and MeHg coexposed nematodes, the internal Hg concentration was similar to the internal concentration of the treatment exposed only to MeHg, and the internal Hg concentration was significantly reduced in Se(IV) and Se(VI) coexposures ($p < 0.05$). Nematodes exposed to Se(IV) had

significantly reduced internal Hg compared to the Se(VI) treatment (Figure 3).

Internal Se concentrations were not statistically significantly impacted by either Hg compound in SeMeth and Se(IV) coexposed nematodes (Figure 3). In nematodes exposed to Se(VI), the internal Se(VI) concentration was higher in MeHg coexposed nematodes (Figure 3).

Finally, we assessed the effects of coexposures on Hg/Se molar ratios (Figure 3), and the relationship between those ratios and growth effects (Figure 4). We observed significant

Figure 4. Hg/Se ratio (average $\pm$ SE) plotted against nematode growth difference (average % of control $\pm$ SE) from expected growth under additive conditions for selenium–mercury coexposures at the highest selenium exposure. Faceted plots represent exposure to (A) selenate (50 mM), (B) selenite (1500 μ M), or (C) selenomethionine (500 μ M). HgCl₂ and MeHg exposures are indicated by open and closed circles, respectively. Red dashed line indicates a 1:1 Hg/Se ratio.

main effects for Hg concentration and compound and their interaction on Hg/Se molar ratios for Se(VI) and SeMeth (Figure 3). For Se(IV), the main effect of Hg compound was significant, but the main effect for Hg concentration ($p = 0.053$) and the interaction between compound and concentration ($p = 0.055$) were not. As Se concentrations were held constant at the highest exposure level, increasing the Hg exposures increased the Hg/Se molar ratio, and the Hg/Se molar ratio was higher in HgCl₂ coexposed than in MeHg coexposed nematodes. Antagonistic interactions resulting in greater than expected growth were observed at Hg/Se molar ratios both above and below 1 for all Se compounds at the highest Se exposure levels (Figure 4). We also observed one instance (Se(VI) treatment) in which a Se/Hg molar ratio of >1 did not result in the reduction of growth inhibition (Figure 4).

DISCUSSION

We observed growth impairments from single exposures to either Hg or Se that were consistent with other *C. elegans* studies. Reduced growth was comparable for MeHg and HgCl₂ and occurred in the low- μ M range, as observed by McElwee and Freedman.²⁰ At first glance, this finding is unexpected because MeHg is frequently identified as the more toxic compound for most end points, but in both of our studies, growth was reduced similarly on the basis of external dosing solution concentrations. However, our measurements of internal Hg demonstrated that MeHg was more toxic than HgCl₂ because internal nematode MeHg concentrations were lower than HgCl₂ concentrations for identical external exposure concentrations.

The tested Se compounds were at least 2 orders of magnitude less toxic than the Hg compounds. Biological impairments resulting from Se(IV) occurred in the same range (1–5 mM) reported by Li et al.¹⁹ for growth impairment and by Morgan et al.³⁴ for impaired motility. Growth sensitivity to Se varied greatly between compounds, with toxicity observed in following order: SeMeth $>$ Se(IV) $>$ Se(VI); this order has also been observed in other invertebrates.^{29,38,39} Coexposure outcomes were not as straightforward.

In our experiments, the instances in which Hg and Se did interact were complex and depended greatly on the chemical compound and the concentration administered. For reference, most dosing Se concentrations that we utilized were in the range of total Se concentrations found in child (2–32 μ M) and adult (3–12 000 μ M) blood and plasma in populations exposed to Hg through diet.^{42–47} We observed growth impacts from coexposures that ranged from antagonistic to greater-than-additive. Antagonism has been the most frequently reported Hg and Se interaction and was observed in this study primarily with Se(IV) and SeMeth exposures. Growth improvements, compared to the individual Hg exposure, were observed with Se(IV) and organic Hg, while growth improvements were noted for SeMeth in combination with both inorganic and organic Hg (Figure 2). Some antagonistic interactions occurred at some lower Se exposures and resulted in small growth improvements ($\leq 13\%$), and biologically meaningful antagonism, which we defined here as that which altered growth by 20% or more, only occurred when the Se exposure was much greater than the Hg exposure (100–25 000 times). Toxicity reduction at the highest Se exposures also varied between compounds, with SeMeth causing the greatest reduction in combination with both Hg compounds (34–47%), followed by Se(IV) with MeHg (30–42%). Se(IV) in combination with HgCl₂ and Se(VI) with both Hg compounds provided 9–28% toxicity reduction. Antagonism between Hg and Se has been observed previously, with other end-points with many different selenocompounds in a number of ameliorated or partially ameliorated end points including enzyme activity, enzyme expression, and DNA damage.^{8,18,48}

In addition to the antagonistic interactions described above, we observed additive and greater than additive interactions. Additive impacts were most common, especially at the lower Se exposures (Figure 2). Various interactions between the two elements have also been noted in other studies. Impacts ranging from little impact of Se supplementation to reduction of toxicity were also highlighted in a rodent dietary study in which Se delayed and reduced MeHg's impact for some end points but not others.⁴⁹ Though only observed to a small degree in

this study, greater than additive toxicity has been noted in other aquatic toxicology studies, resulting in reduced survival and development in aquatic insect larvae,⁵⁰ lower hatching success and survival in ducklings,⁵¹ and reduced reproduction in fish.¹⁰ This synergism in younger life-stages appears to have been noted most frequently in studies that have assessed end-points at young and adult life-stages. However, the same group that reported synergistic effects of reduced reproduction also observed partial mitigation of embryonic selenoprotein gene mRNA levels, GPX activity, and larval locomotion.¹⁸ The mechanisms that underlie greater than additive toxicity are not well-understood.

One theory that seeks to explain some of these interactions is that antagonism and toxicity mitigation occur when the Se/Hg molar ratio is ≥ 1 . This hypothesis is based on the premise that when the two elements are present at equimolar ratios, Se binds to and sequesters Hg.^{14,17} However, the majority of the studies addressing Se/Hg molar ratios have been ecological in nature, focusing on natural ratios present in fish.^{52–54} In laboratory studies with rodents, both beneficial and nonadvantageous health outcomes of Se coexposure have been noted.^{14,55} Furthermore, although Se has a beneficial impact on some health outcomes, there has not been strong evidence for interactions between Hg and Se in epidemiological studies.^{42,43,46,47} The reasons for which different interactions have been observed are not entirely clear but could be attributable to study design differences including: different studies employing different species of Hg and Se, typically a relatively small number of concentrations, different species of test organism, and different toxic end-points. The results of our study, in which we directly tested outcomes in experiments in which Se/Hg molar ratio were deliberately manipulated over a wide range of ratios do not support the Se/Hg molar ratio hypothesis. Antagonism was not consistently observed when either the external or internal Se/Hg molar ratio was ≥ 1 , suggesting that the simple model of Se-mediated sequestration of Hg is an insufficient explanation for antagonism. Another possibility, less dependent on the molar ratio per se, is that Se supplementation replenishes Se sequestered by Hg, thereby rescuing toxicity by providing ample Se for selenoprotein production and activity.^{14,17} This hypothesis, however, presumes that all Hg toxicity can be explained by selenoprotein depletion or inhibition, which seems unlikely given that Hg also has a well-documented interaction with sulfhydryl groups in proteins. Of note, this possibility is especially unlikely in our model organism because *C. elegans* has only one selenoprotein. However, it is important to note that this study did not assess tissue-specific impacts or effects beyond growth. While this means that we capture many potentially important biological targets, it also means that we could not distinguish specific impacts at the cellular or tissue level. To address the potential interactions between the elements in the dosing solution, we calculated that changes in Se and Hg speciation and the binding of the two in solution are unlikely to explain the biological impacts observed.

The speciation of Se and Hg in exposure experiments was likely dependent on the Se/Hg molar ratio and the type of Se and Hg tested. For example, the calculated equilibrium speciation for the exposure media in our study suggested that Se(VI) was not binding substantial amounts of MeHg⁺ in the experimental solutions (Figure SI-2), even though we observed antagonism at the highest Se(IV)/MeHg molar ratio. Se(IV) also was not influencing inorganic Hg²⁺ and MeHg⁺ speciation

at most Se/Hg molar ratios. The exception was the mixture with the highest Se(IV)/MeHg molar ratio (1500 μ M Se(IV) and 2 μ M MeHg), where the CH₃HgSeO₃[−] concentration was predicted to be 1.3 μ M (or 67% of the total MeHg). We also note that at this highest Se(IV)/MeHg molar ratio, antagonistic effects were observed for the growth of the nematodes (Figure 2). Stability constants for Hg²⁺ complexes with Se(VI) and Hg²⁺ as well as MeHg⁺ complexes with SeMeth were not found in the literature, so speciation calculations were not performed for these mixtures. Therefore, the effects of these ligands, particularly SeMeth, on Hg speciation in the exposure mixtures could not be determined. Overall, the changes in Hg speciation in the exposure medium coincided with antagonisms in one case (Se(IV)–MeHg mixtures) but not in other cases (Se(VI)–MeHg and Se(IV)–Hg mixtures).

Understanding factors that may mitigate Hg toxicity is important to environmental and human health. Our experiments focused on Se and utilized multiple Hg and Se compounds and concentrations to assess interaction impacts on toxicity. Our results indicate that even in a simple system, the relationship between the Hg and Se is complex and that straight antagonism is not the only interaction. Our most noteworthy findings emphasize the importance of including Se compound as a part of the analysis when assessing Hg and Se interaction and de-emphasizing the weight given to the Se/Hg molar ratio.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b06044.

Table SI-1: internal mercury and selenium concentrations (average ng of compound/ μ g of protein) for nematodes exposed to selenium (50 mM selenite, 1500 μ M selenite, and 500 μ M selenomethionine) and mercury (HgCl₂, MeHg). Table SI-2: stability constants for the mercury–selenium species considered in the equilibrium calculations. Table SI-3: expected growth (logEXT) following coexposure assuming an additive interaction between mercury and selenium compounds. Figure SI-1: nematode size (logEXT) dose–responses for individual mercury and selenium compounds. Figure SI-2: the calculated equilibrium speciation of MeHg species and selenite species for the MeHg+selenite test mixtures. Figure SI-3: equilibrium speciation of mercury in the Hg(II)-selenite mixtures and the MeHg-selenite mixtures. (PDF)

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Notes

The authors declare no competing financial interest.

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