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JES JOURNAL OF ENVIRONMENTAL SCIENCES

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Mutual detoxification of mercury and selenium in unicellular *Tetrahymena*

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ARTICLE INFO

Article history:
Received 28 July 2017
Revised 31 January 2018
Accepted 7 February 2018
Available online 16 February 2018

Keywords:
Mercury
Selenium
Antagonism
Mutual detoxification
Species

ABSTRACT

Selenium (Se) is commonly recognized as a protective element with an antagonistic effect against mercury (Hg) toxicity. However, the mechanisms of this Hg–Se antagonism are complex and remain controversial. To gain insight into the Hg–Se antagonism, a type of unicellular eukaryotic protozoa (*Tetrahymena malaccensis*, *T. malaccensis*) was selected and individually or jointly exposed to two Hg and three Se species. We found that Se species showed different toxic effects on the proliferation of *T. malaccensis* with the toxicity following the order: selenite (Se(IV)) > selenomethionine (SeMeth) > selenate (Se(VI)). The Hg–Se antagonism in *Tetrahymena* was observed because the joint toxicity significantly decreased under co-exposure to highly toxic dosages of Hg and Se *versus* individual toxicity. Unlike Se(IV) and Se(VI), non-toxic dosage of SeMeth significantly decreased the Hg toxicity, revealing the influence of the Se species and dosages on the Hg–Se antagonism. Unexpectedly, inorganic divalent Hg (Hg^{2+}) and monomethylmercury (MeHg) also displayed detoxification towards extremely highly toxic dosages of Se, although their detoxifying efficiency was discrepant. These results suggested mutual Hg–Se detoxification in *T. malaccensis*, which was highly dependent on the dosages and species of both elements. As compared to other species, SeMeth and MeHg promoted the Hg–Se joint effects to a higher degree. Additionally, the Hg contents decreased for all the Hg–Se co-exposed groups, revealing a sequestering effect of Se towards Hg in *T. malaccensis*.

Introduction

Mercury (Hg) is recognized as a global toxic pollutant (Jiang et al., 2006; Driscoll et al., 2013). The toxicity of Hg depends on both its concentration and species (Fitzgerald et al., 2007; Du et al., 2015; Shao et al., 2016). In aquatic systems, inorganic divalent mercury (Hg^{2+}) and monomethylmercury (MeHg) are the main species and their toxicity has been paid great attention (Fitzgerald et al., 2007; Chen et al., 2013; Peng et al., 2015). Considering the environmental risks, it is essential to explore potential pathways for mitigating the toxicity of Hg.

Selenium (Se) is commonly regarded as a protective element with an antagonistic effect against Hg. As in the case of Hg, Se occurs naturally on Earth. Unlike Hg, Se is an essential trace element for human body since it is incorporated into the activities of antioxidant selenoenzymes (Stadtman, 1991; Wyatt et al., 2016). With regard to *Tetrahymena*, the selenocysteine tRNA has been identified in *Tetrahymena thermophila* (Shrimali

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https://doi.org/10.1016/j.jes.2018.02.004
1001-0742/

et al., 2005), indicating the need for Se of this species and the potential similarity for other *Tetrahymena* species.

Tetrahymena is a type of unicellular eukaryotic protozoa located at the bottom of food chain. Owing to its rapid proliferation, unique nuclear dualism, extensive membrane structure and fast reaction upon external exposure, *Tetrahymena* has been used as a useful model organism for evaluating the toxicity and environment risks of chemicals. For example, *Tetrahymena* has been used to evaluate the toxicity of 33 organic compounds with different structures and to explore the carrier effect of TiO_2 nanoparticles on Cd bioaccumulation (Schramm et al., 2011; Yang et al., 2014). Given the wide distribution and low trophic level of *Tetrahymena* in freshwater ecosystems, the Hg uptake of *Tetrahymena* is an original and essential pathway for Hg entering food chains that can affect the transportation and transformation of Hg in the environment.

Although the existence of Hg–Se antagonism has been confirmed, the joint effects of Hg and Se are very complex and the antagonistic mechanisms are still unclear (Ganther et al., 1972; Sumino et al., 1977; Wang et al., 2016; Tang et al., 2017). The antagonistic effect has been previously proposed to involve the formation of Hg–Se complexes. These complexes are considered to decrease the bioaccumulation of Hg by reducing the uptake or promoting the removal of Hg in organisms (Sormo et al., 2011; Zhang et al., 2012; Zhao et al., 2014). Recent studies have shown that the Hg–Se antagonism in marine fish or *Caenorhabditis elegans* is highly dependent on the chemical species involved (Dang and Wang, 2011; Wyatt et al., 2016), although inconsistent results were also obtained for the joint effects of Se and MeHg. In addition, the influences of the Hg species and the dosages of Hg and Se remain uncertain. While Se has been typically regarded as a beneficial element for organisms at trace dosages, its role (*i.e.*, protective or toxic agent) under high dosages has been controversial for a long time (Hilton et al., 1980; Hodson and Hilton, 1983; Spallholz, 1994; Lemly, 2002; Hoffman, 2002; Hamilton, 2004; Branco et al., 2014; Aborode et al., 2016; Friesen et al., 2017). Thus, further detailed studies dealing with the Se toxicity and the effects of the species and dosages on the Hg–Se antagonistic mechanisms should be carried out.

This work was aimed to explore the joint effects of different species of Se and Hg at varying dosages by using a novel unicellular model organism. Thus, a type of eukaryotic protozoa, *Tetrahymena malaccensis* (*T. malaccensis*), was selected and subsequently exposed to two Hg and three Se species under various dosages. The cell numbers and total contents of Hg and Se in cell bodies were analyzed after individual or joint exposure. The effects of the different species and dosages of Se and Hg on the Hg–Se antagonism were discussed in detail. We revealed herein, for the first time, the detoxification of Hg towards highly toxic dosages of Se.

1. Materials and methods

1.1. *Tetrahymena* species and culture methods

T. malaccensis was kindly provided by Dr. Wei Miao from the Institute of Hydrobiology of the Chinese Academy of Sciences (Wuhan, China). The *T. malaccensis* used herein was grown axenically at 28°C in a medium rich in proteose peptone (Morin and Cech, 1988). The culture medium was comprised of 2% (W/V) proteose peptone (Becton, Dickinson and Company, USA), 0.2% (W/V) glucose (Sigma, USA), 0.1% (W/V) yeast extract (OXOID, Thermo Fisher Scientific, USA), and 0.003% (W/V) ferric citrate (Sigma, USA) dissolved in 1000 mL of ultrapure water (Millipore, Darmstadt, Germany) containing a 1% (V/V) penicillin–streptomycin solution (10,000 units/mL penicillin and 10,000 mg/L streptomycin, HyClone, GE Healthcare Life Sciences, USA) (Liu et al., 2017).

1.2. Exposure to gradient dosages of the three Se species

The selected three Se species, sodium selenite (Se(IV)), sodium selenate (Se(VI)), and selenomethionine (SeMeth), were all obtained from Sigma-Aldrich (USA). The exposure was carried out at the early logarithmic growth phase of *T. malaccensis* with same dosage ranges (*i.e.*, 0, 0.1, 1, 10, 100, 1000, and 10,000 μM), following a previous procedure (Wyatt et al., 2016). After exposure for 24 hr, 500 μL of the cell suspension were mixed with the same volume of a phosphate buffered saline (PBS, GE Healthcare Life Sciences, USA) solution for all groups, and the mixtures were counted by flow cytometry (Accuri C6, BD, USA). The effect of Se on *T. malaccensis* was calculated by the ratio of the cell numbers in the Se-treated groups to those in the control group. In order to observe the toxicity of Se species, *T. malaccensis* cells were photographed with a laser scanning confocal fluorescence microscope (Leica, TSC SP5, USA) after exposure to individual Se species. Three parallel experiments were carried out for each group.

1.3. Co-exposure to multiple dosage combinations of two Hg species and three Se species

Based on the results of growth inhibition induced by individual Se species, the dosages of Se species used for the co-exposure experiments were 0, 10 (low dosage, "L") and 1000 (high dosage, "H") μM. This range covered highly toxic (1000 μM Se(IV) and SeMeth) and non-toxic (the remaining) dosages. For Hg species, the following dosages were selected according to our previous work: 0; 5 μM Hg^{2+} and 4 μM MeHg (representing high dosages producing inhibitions larger than 20%, "H"); 1 μM Hg^{2+} and 0.5 μM MeHg (non-toxic low dosages, "L") (Liu et al., 2017). Either individual or combined solution was added to the medium at the early logarithmic growth phase. The individual Hg and Se solutions were used as the control, while the pure medium exclusively containing *T. malaccensis* cells (no Hg or Se addition) was used as the blank. After 24 hr exposure, the cell numbers of all groups were counted by flow cytometry. All the experiments were repeated three times.

1.4. Analysis of the total Hg and Se contents in *T. malaccensis* cells

After counting, the *T. malaccensis* cell samples were cleaned for three times (Liu et al., 2017). Then, the cell suspensions were centrifuged, collected and digested in a microwave digestion system (MASTER-40, Shanghai Sineo Microwave Chemistry Technology, China). In detail, 8 mL of concentrated HNO_3 (65%, V/V) and 2 mL of H_2O_2 (30%, V/V) were added to Teflon®

vials containing the cell samples. The mixtures were pre-digested at 50°C for 30 min in order to remove the excessive gas. The vials were subsequently subjected to microwave digestion with the following temperature programme: 130°C for 10 min, 150°C for 5 min, and 180°C for 15 min. After cooling down to room temperature, the solutions were transferred to centrifuge tubes and diluted to 50 mL with ultra-pure water (Millipore, Darmstadt, Germany) for the analysis of total Hg (THg) and total Se (TSe) contents. The concentrations of THg were determined on a MERX Automatic Total Mercury Analytical System (Brooks Rand Lab, USA) following the USEPA method 1631 (USEPA, 2002). The concentrations of TSe were determined by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher iCAP Q, USA). ^{45}Sc, ^{103}Rh, and ^{115}In were used as the internal standards to assess the stability of the instrument during the entire analytical process. In line with our previous work, the concentrations of THg and TSe in the current work were also expressed as the mean mass (ng) per 1000 cells according to the cell counting results without weighing the mass of cells.

1.5. Quality assurance/quality control (QA/QC)

To ensure the accuracy of THg and TSe analysis, analytical blanks and certified reference material (DORM-4, fish protein, National Research Council Canada, Canada) were measured in each analytical process. The determined concentrations of THg and TSe in DORM-4 (402 ± 23 for THg and 3610 ± 530 ng/g for TSe) were in good agreement with the certified values (410 ± 55 ng/g for THg and 3560 ± 340 ng/g for TSe), indicating that the methods were accurate and reliable.

1.6. Statistical analysis

Statistical analysis was performed with SPSS 19.0. The significance of the mean values among the different groups was evaluated through independent *t* test and analysis of variance (ANOVA). The statistical significance was determined at $p < 0.05$.

2. Results and discussion

2.1. Toxicity of Se species

With the aim to gain insight into the toxicity of Se species, the responses of *T. malaccensis* towards these Se species were first investigated and compared after exposure to same dosage ranges of three Se species for 24 hr. The selected ranges were relatively wide to cover non-toxic and absolute lethal dosages. As shown in Fig. 1 a, the three Se species showed different effects on the proliferation of *T. malaccensis* cells. Se(VI) showed no inhibitory effect on the growth of *T. malaccensis* at all the selected dosages (*i.e.*, the percentages of all the Se(VI) treated groups compared to the control were *ca.* 100). In contrast, Se(IV) and SeMeth displayed a significant growth-inhibitory impact on *T. malaccensis* depending on the dosages. In detail, exposure to 100 μM Se(IV) or 1000 μM SeMeth for 24 hr led to a *ca.* 95% growth-inhibitory effect. When exposed to 1000 μM Se(IV) or 10,000 μM SeMeth, no cells could survive under this working conditions. Based on these growth-inhibition degrees, the toxicity of the three mentioned Se species towards *T. malaccensis* followed the trend: Se(IV) > SeMeth > Se(VI), which was similar to some previous publications in which Se(IV) was recognized as the most toxic species (McAdam and Levander, 1987; Barceloux, 1999). However, different toxicity orders were also observed in some organisms (*i.e. Daphnia magna* and *C. elegans*): SeMeth > Se(IV) > Se(VI) (Maier et al., 1993; Wyatt et al., 2016). These differences might be associated with the different organisms used. Then, the toxicity of Se was further studied by visual observation. As shown in Fig. 1b, *T. malaccensis* cells were severely broken when exposed to 100 μM Se(IV) or 1000 μM SeMeth, whereas the cells for all the Se(VI)-treated groups remained stable and unbroken. The distinct toxicity of Se species might be related to the different uptake abilities of *T. malaccensis* to different Se species (Fig. 4). These results suggested that Se toxicity was highly dependent on its species and not all the Se compounds were safe in terms of the growth-inhibition effect on *T. malaccensis* under certain dosages. Thus, the species present in the Se compounds should be carefully considered when evaluating a potential protective effect of Se towards Hg.

2.2. Co-exposure to Hg and non-toxic dosages of Se

2.2.1. Co-exposure to non-toxic dosages of Hg and Se

We first assessed the joint effects of both non-toxic dosages of Hg and Se on *T. malaccensis* (Fig. 2). As shown in Fig. 2, cell numbers of the co-exposed groups increased to varying degrees compared to the blank, indicating the existence of a growth-promoting effect. In the case of the Hg and Se(IV) co-exposed groups, the growth-promoting effect of the L-Hg^{2+} and L–Se(IV) co-exposed group (cell count *ca.* 1.2 times of the individual Hg-treated group) was not as significant as that of the L–MeHg and L–Se(IV) co-exposed group (*ca.* 1.6 times of the individual Hg-treated group). Similarly, the cell numbers of the L–MeHg and L/H–Se(VI) co-exposed groups were significantly higher than that of the individual Hg exposed group (*ca.* 1.6 times higher for both L/H–Se(VI)). In contrast, cell numbers in non-toxic dosages of Hg^{2+}–Se(VI) treated groups remained nearly unchanged regardless the Se(VI) dosages. With regard to the L–Hg and L–SeMeth co-exposed groups, a growth-promoting effect was also observed under exposure to L–MeHg, and this effect was not observed for the L–Hg^{2+} and L–SeMeth co-exposed groups. Thus, MeHg was more active than Hg^{2+} towards the generation of joint effects of Hg and Se, which might be potentially related to its organic structure. Since the significant growth-promoting effect was observed for all the Se and MeHg co-exposed groups, the species of Hg was important in determining the joint effects of non-toxic dosages of Hg and Se. The addition of low dosages of Hg^{2+} has been reported to enhance the growth-promoting effect induced by low levels of individual Se(IV) on garlic but no discussion on the influence of Hg and other Se species was provided (Zhao et al., 2013b). Moreover, the growth-promoting effect of individual Se(IV) was not found in the current work. As previously pointed out, the growth-promoting effect can be potentially explained by stress reaction or hormesis of low-dosage icants widely observed in other organisms, whose mechanisms remain uncertain and need future study (Calabrese, 2017; Liu et al., 2017; Stebbing, 1982; Duchemin et al., 2008; Hammerschmidt et al., 2002).

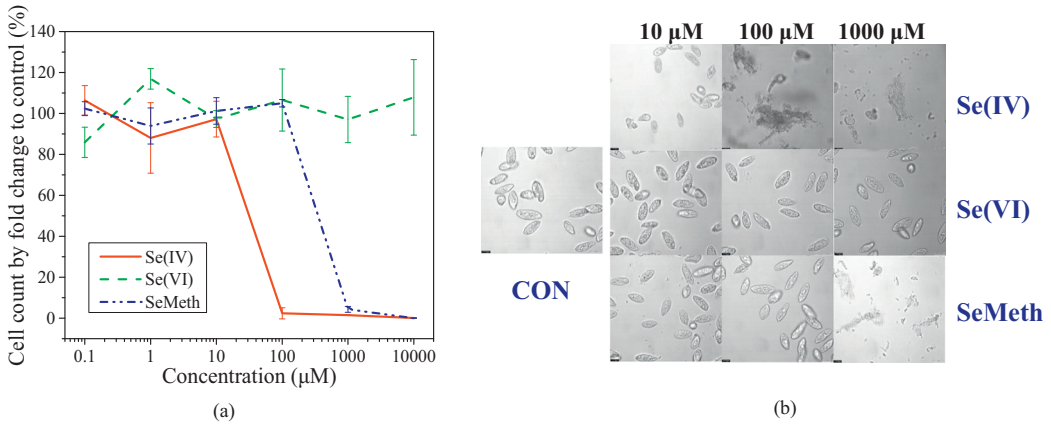


Fig. 1 – Toxicity of Se species in *T. malaccensis* after 24 hr exposure: (a) cell count of the Se-treated groups expressed by fold change to the control (%, data shown by mean ± SD, *n* = 3); (b) pictures showing the morphology of the *T. malaccensis* cells after exposure to three Se species.

Se addition has been reported to moderate the Hg toxicity in some organisms (*e.g.*, cells, daphnia, rice, garlic and fish), and this may be partially ascribed to the reduction of the Hg uptake (Maier et al., 1993; Branco et al., 2014; Bjerregaard et al., 2011; Zhao et al., 2013a, 2014). Therefore, with the aim to explore the relationship between the changes of cell numbers and the Hg uptake, the THg concentrations in cells were analyzed and expressed as the mean total Hg mass (ng Hg) per 1000 cells. As shown in Fig. 3, the THg contents in *T. malaccensis* cells for the L–Hg and L–Se(IV) co-exposed groups decreased to some extent after co-exposure. A similar decrease in THg was observed for the L–Hg and L–SeMeth co-exposed groups. In the case of the L–Hg and L/H–Se(VI) co-exposed groups, the THg contents in L–MeHg and L/H–Se(VI) co-exposed decreased remarkably, while the THg contents of the L–Hg^{2+} and Se(VI) co-exposed groups only decreased slightly. These results revealed the different abilities of Hg^{2+} and MeHg to produce joint effects with Se species again. The different growth-promoting performances suggested that the species of Hg and Se played essential roles in the Hg–Se joint effects when co-exposed to non-toxic dosages of Hg and Se. At these conditions, co-exposure to Hg and Se(VI) displayed the most significant growth-promoting effect and MeHg was more prone to generate joint effects with Se species than Hg^{2+}. Besides, various degrees of decrease in the Hg uptake of *T. malaccensis* were observed in all co-exposed groups, indicating a potential sequestering effect of Se towards Hg, similar to those in some other organisms (Bjerregaard et al., 2011; Zhao et al., 2013a, 2014). However, no definite relationship was found between the decrease in the THg contents and the increase in the cell numbers, and the identification of the potential mechanisms for this promoting effect requires additional work.

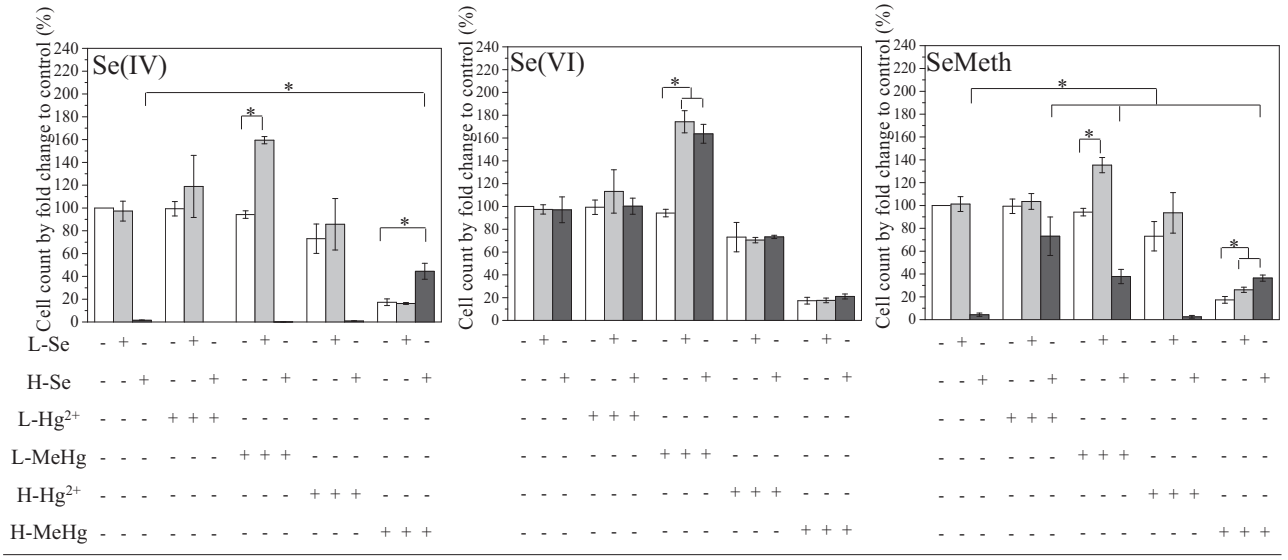


Fig. 2 – Growth effects upon individual or joint exposure to Hg and Se on *T. malaccensis*: "L" represents low dosage (10 μM for Se species, 1 μM for Hg^{2+}, 0.5 μM for MeHg); "H" represents high dosage (1000 μM for Se species, 5 μM for Hg^{2+}, 4 μM for MeHg). (data shown by mean ± SD, *n* = 3; "*" represented "*p* < 0.05")

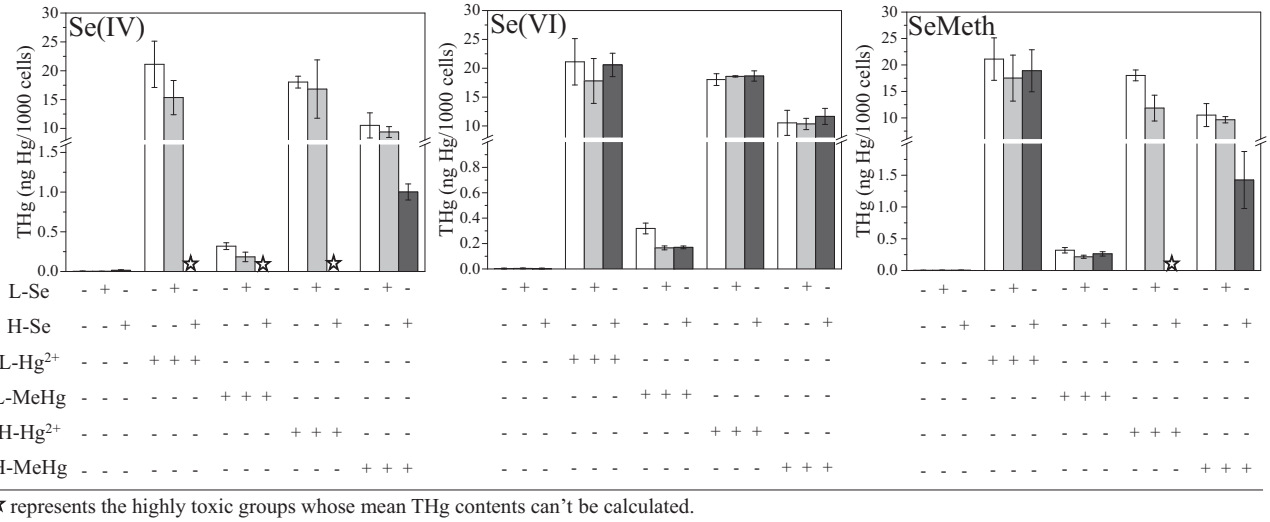


Fig. 3 – Mean total Hg (THg) contents per 1000 *T. malaccensis* cells after individual or joint exposure to Hg and Se: "L" represents low dosage (10 μM for Se species, 1 μM for Hg^{2+}, 0.5 μM for MeHg); "H" represents high dosage (1000 μM for Se species, 5 μM for Hg^{2+}, 4 μM for MeHg). (data shown by mean ± SD, *n* = 3)

2.2.2. Co-exposure to toxic dosages of Hg and non-toxic dosages of Se

The protective effects of Se against Hg^{2+} and MeHg are of our greatest concern herein. With this aim, *T. malaccensis* cells were co-exposed to non-toxic dosages of Se species along with toxic dosages of Hg^{2+} and MeHg. As shown in Fig. 2, the cell numbers of the groups co-exposed to Hg and different Se species changed to a different extent, suggesting the existence of different protective effects of Se towards Hg depending on the Se species. For the groups co-exposed to H–Hg and non-toxic L–Se(IV) and L/H–Se (VI), the cell numbers remained nearly unchanged versus the control group, and no signs of detoxification were found (Fig. 2). In contrast, a noticeable detoxifying effect was observed when co-exposed to H–Hg and L–SeMeth, as revealed by the significant increase in cell numbers after co-exposure to 4 μM MeHg and 10 μM SeMeth (a *ca.* 1.5-fold increase versus the individual Hg exposed group). Our results were not fully in agreement with previous studies. For instance, the antagonistic effect of low dosages of Se(IV) and high Hg^{2+} levels observed in HepG2 cell and garlic was negligible in *T. malaccensis* (Branco et al., 2014; Zhao et al., 2013b). Significant antagonism of Se towards Hg (*ca.* 1.2–1.4-fold increase versus the control) was observed in *C. elegans* only under high dosages of Se species, and the differences among the Se species (same species as this study) were not as significant as those observed in the current work (Wyatt et al., 2016). In general, our results revealed that not all the Se species under non-toxic dosages showed the protective effects towards Hg in *T. malaccensis*. Under the selected condition, SeMeth was more effective in detoxifying Hg as compared to Se (IV) and Se(VI). The higher efficiency of SeMeth might be ascribed to its Se^{2-} form, which is more prone to conjugate with Hg than the other two Se species. It is worth mentioning that non-toxic dosages of Se(VI) showed completely different effects when co-exposed with toxic and non-toxic dosages of Hg. These results further confirmed the impact of Hg dosages on the Se–Hg antagonism, regardless of the species involved.

As shown in Fig. 3, the mean THg contents (ng) per 1000 cells after co-exposure to H–Hg species and L–Se(IV) or L/H–Se(VI) decreased very slightly versus the control, in line with the unobserved protecting effect. In the case of the H–Hg and L–SeMeth co-exposed groups, the THg contents decreased significantly compared to the control. These results differed, to some extent, from other studies reporting a decrease in the Hg uptake of rice after Se(VI) addition and a lower efficiency of SeMeth in reducing the Hg uptake of *C. elegans* as compared to Se(IV) and Se(VI) (Tang et al., 2017; Wyatt et al., 2016). The good consistency between the decrease in THg and the increase in the cell numbers revealed that the detoxifying effect of Se might be caused by the reduction of Hg uptake at these conditions, although further work is required to confirm this inference.

2.3. Co-exposure to Hg and highly toxic dosages of Se

2.3.1. Co-exposure to toxic dosages of Hg and highly toxic dosages of Se

In order to comprehensively assess the antagonistic effects of Se against Hg, *T. malaccensis* cells were exposed to both highly toxic dosages of Hg and Se species (representing extreme conditions). The used dosages of Se species (*i.e.*, 1000 μM Se (IV) and SeMeth) were relatively high and produced a large growth-inhibition (higher than 95%) individually. 5 μM Hg^{2+} or 4 μM MeHg solutions induced growth-inhibitions higher than 20% individually. As shown in Fig. 2, the cell numbers in the H–MeHg and H–Se co-exposed groups were significantly higher (2.6 and 2.1 times for H–Se(IV) and H–SeMeth, respectively) than those of the individual MeHg-treated groups, indicating the detoxification of Se against Hg once more. Additionally, these cell numbers were 29.5 and 8.4 times higher (H–MeHg co-exposed with H–Se(IV) and H–SeMeth, respectively) than those in the individual Se-treated groups. The increases in cell numbers revealed that the joint toxicity of highly toxic dosages of Hg and Se in *T. malaccensis* cells was

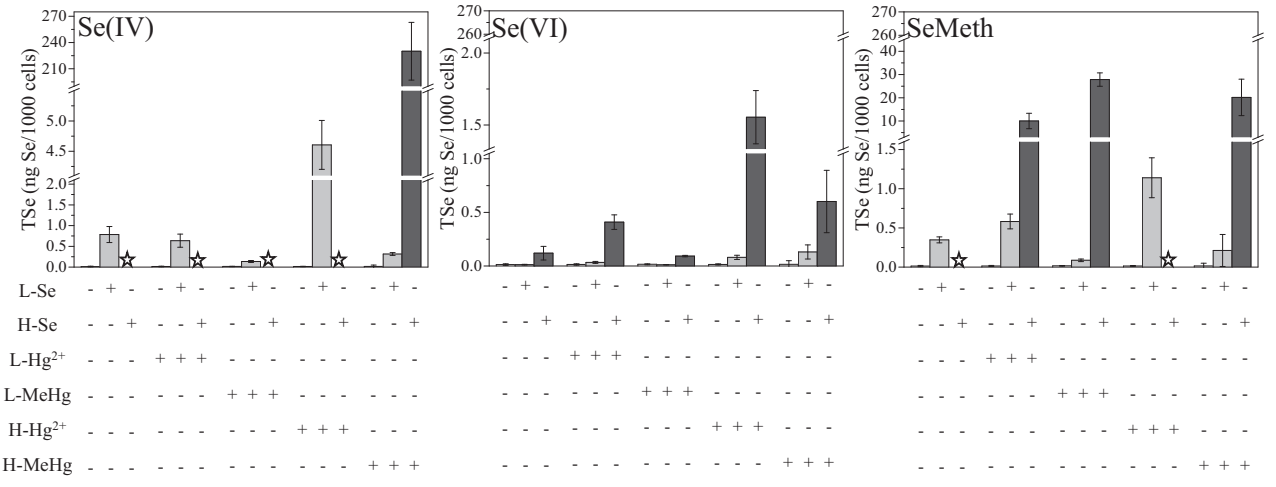
significantly lower than the individual toxicity. Therefore, the joint effects can be defined as mutual detoxification and Hg species might also have a protective effect on the toxicity of Se species at high dosages. In HepG2 cells, Se(IV) was found to cease to antagonize the Hg toxicity at high Se dosages (>2 μM) (Branco et al., 2014). However, significant antagonism was observed under co-exposure to even 1000 μM Se and 4 μM MeHg in this work. These differences were possibly related to the disparate organisms used, as revealed by the different performance of Hg–Se interaction in *T. malaccensis* compared to mammalian cells. In our opinion, the discrepancy is meaningful for wholly understanding the Hg–Se antagonism in various organisms of the environment. With regard to the few live cells, the mean THg concentrations of highly toxic groups cannot be calculated and were rejected (Fig. 3). Similar to other co-exposed assays, the mean THg mass after co-exposure decreased significantly versus the control (Fig. 3). In addition, the joint effects of Se and MeHg were more significant than those of Hg^{2+}, confirming the effect of the Hg species and the active favor of MeHg in the Hg–Se antagonism. To the best of our knowledge, the detoxifying effect of toxic dosages of Hg on extremely highly toxic dosages of Se (individually produced larger than 95% growth-inhibition) was reported for the first time. Confirmation of this mutual detoxification between Hg and Se might be useful in accurately evaluating the practical environmental risks of certain regions contaminated with both high levels of Hg and Se.

2.3.2. Co-exposure to non-toxic dosages of Hg and highly toxic dosages of Se

To further assure the detoxification effects of Hg against Se, *T. malaccensis* was co-exposed to non-toxic dosages of Hg and highly toxic dosages of Se. The results were also shown in Fig. 2. Remarkably, the detoxifying effects of both L–Hg^{2+} and L–MeHg towards highly toxic dosages of Se species were observed in *T. malaccensis*. Similar to the priority of SeMeth detoxifying Hg species, L–Hg^{2+} and L–MeHg displayed significantly detoxifying effects towards the toxicity of H–SeMeth rather than Se(IV), as revealed by the increase in cell numbers of the Hg–SeMeth co-exposed groups (16.9 and 8.7 times higher than those of the individual SeMeth groups, respectively). These results suggested that SeMeth was more prone to detoxification by non-toxic dosages of Hg species, indicating that the detoxifying effects of Hg towards Se also depended on the species of Se. In addition, L–MeHg did not decrease the toxicity of H–Se(IV) as shown in Fig. 2. The difference from the H–MeHg suggested that the protective effect of MeHg towards Se(IV) was associated with the MeHg dosage, in virtue of the higher number of cells for the H–MeHg and H–Se(IV) co-exposed group. Fig. 3 showed the THg contents per 1000 cells, revealing similar decreases in Hg uptake. In a word, our results discovered the detoxification of Hg towards Se in *T. malaccensis*, which depended on the Hg and Se species as well as their dosages. These results were indicative and should be more considered when assessing the Hg–Se antagonism.

2.4. TSe contents in *T. malaccensis* upon "L–Se" exposure

Finally, we analyzed the TSe contents of the cells and expressed the results as TSe contents (ng) per 1000 cells in Fig. 4. As in the case of Fig. 3, the mean TSe contents per 1000 cells for the highly toxic groups cannot be calculated given the little number of live cells and those results were therefore rejected. As shown in Fig. 4, the change tendencies of mean TSe contents in cells were different for the three Se species. When exposed exclusively to L–Se, the uptake ability of *T. malaccensis* towards the individual Se species differed a lot. Se(IV) and SeMeth penetrated into the cells more effectively than Se(VI), which might be used to explain their different toxicity as shown in Fig. 1. After Hg addition, the Se uptake of *T. malaccensis* varied depending on the Se species. Se(VI) hardly penetrated into the cells despite the uptake increased slightly after co-exposure to Hg. However, for L–Se (IV) and L–SeMeth, the addition of H–Hg^{2+} resulted in higher Se uptakes of *T. malaccensis*, while the addition of H–MeHg



☆ represents the highly toxic groups whose mean TSe contents can't be calculated.

Fig. 4 – Mean total seleniumT(Se) contents per 1000 *T. malaccensis* cells after individual or joint exposure to Hg and Se: "L" represents low dosage (10 μM for Se species, 1 μM for Hg^{2+}, 0.5 μM for MeHg); "H" represents high dosage (1000 μM for Se species, 5 μM for Hg^{2+}, 4 μM for MeHg). (data shown by mean ± SD, *n* = 3)

produced the opposite effect. These results further revealed the influence of the Hg species on the Hg–Se joint effects. Furthermore, the higher contents of TSe with the Hg^{2+} dosages increasing were indicative of the impact of the Hg dosage again. These results have been observed previously with various results. The higher Se levels in zebrafish's egg after MeHg and SeMeth co-feeding demonstrated the existence of a Hg–Se synergistic effect (Penglase et al., 2014). The addition of Hg^{2+} increased the Se uptake when co-exposed to low dosages of Se(IV), while the opposite effect was produced when exposed to high Se(IV) dosages in garlic (Zhao et al., 2013b). These diverse observations indicated the complex and unclear mechanisms behind the Hg–Se joint effects. As mentioned above, the formation of Hg–Se complexes has been typically invoked to explain the inhibition effect of Se towards Hg. However, the trends of the changes in Hg and Se contents obtained here did not show a clear relationship. For the H–Hg and L–Se co-exposed groups, the Hg uptake decreased while the change tendencies in Se uptake were various simultaneously (Figs. 3 and 4). These results indicated the existence of additional underlying Hg–Se antagonism mechanisms in *T. malaccensis* other than the formation of Hg–Se complexes. These mechanisms are worth studying in the future. In addition, given the different effects of Hg^{2+} and MeHg on the Se uptake, the Hg species could significantly influence the mechanisms behind the Hg–Se joint effect and should be considered in future studies.

3. Conclusions

In this work, we provided detailed exploration about the joint effects of Hg and Se in *Tetrahymena*. The Hg–Se antagonism in *T. malaccensis* resulted in mutual detoxification. This effect depended highly on the species involved and their dosages. Thus, SeMeth and MeHg promoted the Hg–Se joint effects to a higher extent as compared to the rest of species. The detoxification of Hg towards extremely highly toxic dosages of Se was described for the first time, although the identification of a plausible mechanism for this detoxification effect requires further studies. These results could help more accurately understand and evaluate the Hg–Se antagonism in the environment.

Acknowledgments

The work described here was supported by the National Natural Science Foundation of China (Nos. 41673118 and 41422306), the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDB14010400), and the National Basic Research Program of China (No. 2013CB430004).

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