INFLUENCES OF MATERNAL EXPOSURE ON THE TOLERANCE AND PHYSIOLOGICAL PERFORMANCE OF *Daphnia magna* UNDER MERCURY STRESS

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**Abstract**—We examined the tolerance development to mercury (Hg) by a population of freshwater zooplankton (*Daphnia magna*) with different pre-exposure histories to Hg. The growth and reproductive performance of the *F*$_{0}$ offspring as affected by the maternal (*F*$_{0}$) and offspring (*F*$_{1}$) exposures was quantified. The *F*$_{0}$ daphnids exposed to 2.5 and 25 nM of Hg for 4 d and followed by 4 d of depuration had elevated levels of Hg and metallothionein-like proteins (MTLPs), as well as higher tolerance to Hg toxicity than the control daphnids. The higher Hg tolerance may be attributed to the higher proportion of Hg partitioned to the MTLPs. Moreover, significant enhancement of Hg tolerance also was found in the *F*$_{1}$ offspring originating from the *F*$_{0}$ mothers exposed to 25 nM of Hg, but there was no significant induction of MTLPs in these *F*$_{1}$ offspring compared to the offspring from the control mothers. The Hg tissue concentrations in the *F*$_{1}$ neonates were approximately 25% of those in the *F*$_{0}$ adults. However, there was similar Hg tolerance in the *F*$_{1}$ offspring originating from both the control and Hg-exposed *F*$_{0}$ mothers, indicating that the Hg tolerance in the daphnids disappeared two generations after Hg contamination. Further exposure of the *F*$_{1}$ offspring to different Hg concentrations (1.5 and 15 nM for 28 d) indicated that maternal exposure history did not affect their growth and reproductive performance, which solely were influenced by the offspring exposure. Unexpectedly, the *F*$_{1}$ offspring exposed to Hg had significantly higher final wet weights and reproductive rates than the control groups, suggesting the possibility of Hg hormesis. Furthermore, the maternal exposure had no effect on the Hg accumulation and the MTLP concentrations in the *F*$_{1}$ offspring. Therefore, we concluded that the Hg tolerance might disappear quickly once the Hg contamination was removed and the maternal exposure history was not important in determining the physiological performance and Hg accumulation of the subsequent generations.

**Keywords**—*Daphnia magna* Maternal transfer Mercury tolerance Metallothionein-like proteins

**INTRODUCTION**

Mercury (Hg) is a ubiquitous environmental pollutant and can be found in increasing concentrations up the aquatic food chain (i.e., biomagnification) [1,2]. Due to its widespread occurrence, long-term exposure to Hg by aquatic organisms is a common phenomenon in nature. A recent laboratory study demonstrated that when a population of freshwater oligochaetes (*Tubifex tubifex*) was exposed to Hg in the sediment, the Hg tolerance (in terms of 96-h LC50s) was developed within single generation and inherited through several generations without sustained exposure [3].

Many trace metals (e.g., Cd, Zn) have been identified to induce tolerance in animals previously exposed to low concentrations [4–6]. This type of tolerance could be termed as phenotypic because the tolerance development occurs within a single generation. Tolerance extended to subsequent generations could be termed as a genotypic phenomenon [3]. However, not all types of metal tolerance are inherited in aquatic organisms. Regarding the effects of metal pre-exposure, bioavailability studies have shown that the pre-exposed animals could assimilate the metals (e.g., Cd and Zn) from the food more efficiently than the control animals [7,8]. Therefore, bioaccumulation potential of metals may increase in the pre-exposed (or tolerant) animals if dietary metal uptake is important for a particular metal and species. For the dissolved metal uptake, some studies found reduced uptake rates in the pre-exposed organisms [7,9], and others demonstrated higher uptake rates in the pre-exposed organisms [4]. At the biochemical level, metallothionein-like proteins (MTLPs) have been considered to play an important role to detoxify or control the internal availability of the trace metals, including both essential and nonessential ones, in many aquatic invertebrates [10,11].

However, metal tolerance may not be associated necessarily with the induction of MTLPs because some animals may use other forms of detoxification, such as the metal-rich granules, to sequester accumulated metals [7,11]. In addition, other organisms may use mechanisms such as autonomy to eliminate the toxic metals from the body by breaking down the highly concentrated body fragment physically [12]. Despite the fact that different subcellular pools (e.g., MTLP, metal-rich granules) are responsible for the detoxification for different organisms and metals, the underlying mechanisms for the inherited tolerance remain speculative. A recent study has shown that the messenger ribonucleic acid (mRNA) of MTLP in fish treated with intraperitoneal injection of Cd for 10 d could be transferred maternally to their eggs and, thus, the enhanced MTLP production can serve as a protection for the fish embryo to Cd toxicity [13]; other studies have demonstrated the maternal transfer of Cu tolerance in fish [14]. We have shown previously that a significant amount of Hg (both inorganic and methylated forms) can be transferred maternally in the freshwater zooplankton *Daphnia magna* [15]. Therefore, Hg tolerance could be markedly different from other trace metals with less potential of maternal transfer (e.g., Cd; [16]) because the transfer of the toxic metals may not be accompanied by the transfer of MTLPs during reproduction in a proportional fashion. If such a scenario occurs, the ratio of Hg to MTLPs in the offspring will be higher than that in the respective adults, possibly leading to elevated toxic stress in the subsequent
generations with the oversaturation of MTLP binding sites [10].

_Daphnia_ are an excellent model organism to study the effect of maternal exposure history, because it can be raised easily in the laboratory and mainly asexual reproduction is carried out so that the gene pool remains relatively stable throughout many generations. This study, therefore, is initiated to study the tolerance development of Hg in a population of _Daphnia magna_ and the multigenerational transfer of such tolerance (if any) and to investigate the growth and reproduction of the subsequent generations under different combinations of maternal and offspring exposures to Hg. The role of MTLPs in protecting _Daphnia_ from Hg stress and in controlling the Hg accumulation in the offspring also was considered specifically in this study.

**MATERIALS AND METHODS**

**Mercury and daphnids**

In this study, both radioactive and stable mercury (II) chloride (HgCl₂) were used together to expose the daphnids; therefore, the total Hg accumulation in the animals was traced by measuring the accumulated radioactivity. Radioisotope, ²⁰³HgCl₂ (Hg[II]) in 0.1 N HCl, was obtained from Risø National Laboratory (Roskilde, Denmark) and had a half-life of 46.6 d and a specific activity of 52.3 GBq g⁻¹. A stock of stable Hg (250 μM in 0.1 N HCl) was prepared from HgCl₂ (Sigma, St. Louis, MO, USA) and stored at 4°C in the dark. The clone of _Daphnia magna_ originated from the Chinese Academy of Sciences (Wuhan, People’s Republic of China) and was maintained in our laboratory for >4 years. Routinely, the daphnids were reared in filtered (<1.2 μm) pond water in glass beakers and fed daily with unicellular green alga, _Chlamydomonas reinhardtii_, at 10⁵ cells ml⁻¹. The water was renewed every 2 to 3 d and about two-thirds of water was replaced during each renewal. Cultures were maintained in an environmental chamber at 23.5°C with a photoperiod of 14:10-h light:dark. The culture and test water were collected from a pristine freshwater pond within the campus of the university and had a total Hg concentration of 0.75 pM, which was close to the low end of total-Hg concentrations in the uncontaminated surface water in temperate lakes and rivers [17]. Moreover, the pond water had very low levels of dissolved trace metals (e.g., 28 nM for Zn, 2.5 nM for Se, 140 pM for Cd, and <50 pM for Ag) and had dissolved organic carbon concentration of about 1 mg L⁻¹.

**Maternal exposure**

Four-day-old juveniles of _D. magna_ were collected from the stock cultures and exposed to three different Hg concentrations in the water (0 [control], 2.5, and 25 nM) continuously for a total of 4 d. The pond water was filtered through Whatman™ glass-fiber filter (GF/F; pore size: 0.7-μm; Clifton, NJ, USA) and spiked with different concentrations of stable and radioactive Hg, which was equilibrated overnight in the dark before each exposure. The Hg concentrations of radioisotopes in all exposures were ≤0.4 nM Hg. The alga, _C. reinhardtii_, was added into the exposing media at 10⁵ cells ml⁻¹ before each exposure and the exposing media and food were renewed daily. Aliquots of exposing media also were collected to determine the radioactivity as well as the total Hg concentrations. Moreover, the percentage of Hg associated with the algal cells was quantified and found to range from 1.5 to 11%. Because the contact time between Hg and the alga was relatively short (<24 h) and the inorganic Hg mainly was bound to the algal surface [18], the accumulated Hg was considered to be taken up principally from the aqueous phase. The wet weights and Hg tissue concentrations were measured after 1 and 4 d of exposure. To measure the Hg tissue concentrations, we first transferred the animals to uncontaminated water for 10 min to remove Hg from the carapace fluid as well as the Hg loosely bound to the exoskeleton and then measured the accumulated radioactivity in the animals nondestructively.

After 4 d of exposure, the animals from each treatment (i.e., control, 2.5, and 25 nM) were transferred into uncontaminated water with the same food ration provided for the animals to depurate for another 4 d. This depuration period allowed the daphnids to eliminate Hg from the quick elimination pool (<3 d) and to ensure that all the Hg in the subsequent generation (i.e., _F₁_) was delivered maternally [15], rather than through direct uptake from the exposing media via the mother’s egg sac [19]. At the end of depuration, we collected the _F₀_ adults as well as the _F₁_ neonates (<24-h-old) to quantify the MTLPs in the adults and neonates, the Hg tissue concentrations in the adults and neonates, and the subcellular distribution of Hg in the adults after exposing to Hg.

Moreover, in order to test the tolerance development of the animals to Hg after the maternal exposure, we performed a time-to-death experiment on the _F₀_ (adults) and _F₁_ (neonates) daphnids simultaneously, using an approach similar to that described in Levinton et al. [20]. Based on preliminary toxicity test, we used a nominal Hg concentration of 250 nM (stable metal only) to expose the daphnids. Thirty individuals from each of the six treatments (i.e., control–adult, control–neonate, 2.5 nM–adult, 2.5 nM–neonate, 25 nM–adult, and 25 nM–neonate) and the daphnids were incubated in 300 ml Hg-spiked solution. The survival of daphnids was monitored every 1 h for a total of 20 h. Survival curves of different generations upon Hg exposure were compared statistically by a Wilcoxon ranked-pairs nonparametric test [21] with _p_ = 0.05.

**Offspring exposure**

The _F₁_ neonates produced from the three exposure groups of _F₀_ adults (i.e., control, 2.5 nM, and 25 nM) were exposed further to different Hg concentrations for 28 d but at lower Hg concentrations than in the maternal exposure (i.e., 1.5 and 15 nM instead of 2.5 and 25 nM, spiked with trace levels of ²⁰³Hg). This was to avoid any acute toxicity in the juveniles during the early phase of offspring exposure because the neonates had significantly higher Hg uptake rates than the adults (M.T.K. Tsui, unpublished data). For the exposure of _F₁_ generation, the _F₁_ neonates from the three exposure groups of _F₀_ adults (i.e., control, 2.5 nM, and 25 nM) were exposed separately to three Hg concentrations (0, 1.5, and 15 nM). Therefore, a total of nine combinations of maternal and offspring exposures resulted. For each combination there were three replicates and 20 individuals in each replicate (i.e., 60 individuals for each combination).

Alga (_C. reinhardtii_ at 10⁵ cells ml⁻¹) and water (with or without Hg) were renewed daily. The reproduction and survival of the animals were monitored daily throughout 28 d after birth. On day 27, _F₂_ neonates <24-h-old were collected from the control adults (_F₁_) originating from the three maternally exposed groups (i.e., control, 2.5 nM, and 25 nM) and used for the second time-to-death experiment in order to test whether the Hg tolerance could be transferred from the _F₀_ to...
$F_2$ generations through the $F_1$ generation without sustained Hg exposure, using the same protocol as described above. At the end of the 28-d offspring exposure, surviving $F_1$ adults were weighed and measured for the Hg tissue concentrations and the MTLP concentrations.

Measurement of MTLPs and Hg subcellular distribution

The MTLP concentrations in the daphnids were measured using a modified Ag saturation method [8,22]. Daphnids (5–20 individuals for adults and 50–100 individuals for neonates) first were dabbed dry with a paper and transferred to preweighed 2 ml microcentrifuge tubes and their wet weights were measured immediately. The animals then were frozen at –80°C until analysis. To quantify the MTLPs, 0.5 ml of 0.25 M sucrose buffer (BDH, Poole, UK) was added to the preweighed daphnids and the soft tissues were homogenized in an ice bath using an ultrasonic homogenizer. The homogenates were centrifuged at 16,000 g for 20 min at 4°C and the supernatants were transferred into another microcentrifuge tube, to which 0.3 ml of 0.5 M glycine buffer (Sigma) and 0.5 ml of 20 μg Ag ml$^{-1}$ (Sigma) spiked with $^{110}$mAg (Riso National Laboratory) at 3.7 kBq ml$^{-1}$ were added. The mixtures were agitation and incubated for 10 to 20 min at room temperature. The MTLP binding sites were saturated with Ag in the solution within 10 min [22], and excess Ag was removed by adding 0.1 ml of rabbit red blood cell hemolysate, heating (5 min at 100°C) and centrifuging (5 min at 5,000 g) the samples for three times. For the third addition, the supernatant was spun at 16,000 g for 5 min. The final supernatant was separated and radioassayed for the amount of $^{110}$mAg, and the MTLP concentrations in the daphnids were calculated as 3.55 x the Ag concentrations measured in the samples and expressed as μg g$^{-1}$ wet weight of the daphnids.

To quantify the different subcellular components (e.g., MTLPs, enzymes, cellular debris) in binding and/or detoxifying Hg in the daphnids, the $F_0$ adults after exposure to Hg (2.5 and 25 nM) and depuration were collected and frozen at –80°C until analysis. We adopted a modified method of Wallace et al. [11] for the procedure of subcellular fractionation. To daphnid samples of 30 individuals, 0.5 ml of Tris buffer at pH 8.0 (Sigma) was added and the tissue-buffer mixture was homogenized using an ultrasonic homogenizer in ice bath. The homogenate then was centrifuged at 1,500 g for 10 min at 4°C. The supernatant from 1,500 g centrifugation was spun further at 100,000 g for 1 h at 4°C, which separated the organelles (pellet) and the cytosol (supernatant) [11]. The organelles were combined with the previous pellet to represent the insoluble fraction. The cytosol fraction was heated at 80°C for 10 min, cooled at 4°C for 1 h, and centrifuged at 30,000 g for 10 min at 4°C. The pellet after this high-speed centrifugation was defined operationally as the heat-sensitive proteins (e.g., enzymes) and the supernatant was defined as the heat-stable proteins (e.g., MTLPs) [11]. Thus, a total of three fractions were yielded from the above steps, including insoluble fraction (IF), heat-sensitive proteins (HSP), and heat-stable proteins (MTLP). Measuring the radioactivity in the samples before and after the fractionation also checked the total amount of Hg lost during these multiple steps. Approximately 100% of Hg was in the samples when combining the radioactivity of the three subcellular fractions. The amount of Hg in each fraction was determined by radioassaying and the relative distribution of Hg in the three fractions was expressed as the percentage of the total amount of Hg in the whole organism.

Chemical measurements and statistical analyses

All the water samples first were acidified with hydrochloric acid to pH < 1 and then the stable Hg concentrations in the exposing media were verified using flow injection mercury system [8,400] (Perkin-Elmer, Boston, MA, USA) and found to be 80 to 133% of the nominal concentrations with a mean of 104%. The radioactivity of Hg and Ag was determined by a Wallac 1480 NaI(T1) gamma counter (Turku, Finland). All radioactive measurements were related to appropriate standards and γ-emission of $^{203}$Hg and $^{110}$mAg was measured at 279 and 658 keV, respectively. Counting times were adjusted to yield propagated counting errors generally < 5%. Comparisons of differences between treatments were performed with one-way analysis of variance followed by Tukey’s post hoc test, where $p = 0.05$ in all cases. The statistical analyses were performed using SPSS® for Windows 11.0 (Chicago, IL, USA).

RESULTS AND DISCUSSION

Maternal exposure

Within the periods of exposure and depuration, there was no significant mortality of the daphnids. During the 4 d of exposure, the Hg tissue concentrations increased sharply in the 2.5 nM and 25 nM treatments after 1 d of exposure (Fig. 1) and then increased slightly following another 3 d of exposure. The Hg reached a steady state in the animals in which the very high influx rate of Hg into the daphnids was more or less counterbalanced by the high efflux rate from the daphnids. Following another 4 d of depuration, the Hg tissue concentrations in the two Hg treatments decreased similarly (inset of Fig. 1), and the efflux rate constants were calculated according to our previous study [15] and found to remain relatively constant (0.447 ± 0.011 d$^{-1}$ for 2.5 nM and 0.473 ± 0.032 d$^{-1}$ for 25 nM), even though the Hg tissue concentrations differed by approximately 7.5 times. The $F_1$ neonates were collected on day 8 to ensure that the Hg in the $F_0$ adults was in the slow-exchanging compartment and that the Hg in the $F_1$ neonates was derived only maternally [23]. Table 1 shows the
Table 1. Mercury tissue concentrations (nmol Hg g⁻¹ wet wt) in the \( F_0 \) adults and \( F_1 \) neonates from the two Hg treatments in the maternal exposure. The \( F_0 \) adults were the daphnids after 4 d of Hg exposure followed by 4 d of depuration, and the \( F_1 \) neonates were the <24-h-old animals reproduced by the \( F_0 \) adults after 4 d of depuration. The Hg tissue concentrations were measured simultaneously for both groups of daphnids. Data are means ± standard deviation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Maternal ( F_0 ) adults ( (n = 3) )</th>
<th>Offspring ( F_1 ) neonates ( (n = 2) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 nM</td>
<td>0.315 ± 0.016</td>
<td>0.075 ± 0.016</td>
</tr>
<tr>
<td>25 nM</td>
<td>2.37 ± 0.267</td>
<td>0.611 ± 0.059</td>
</tr>
</tbody>
</table>

Hg concentrations in the \( F_0 \) adults and the respective \( F_1 \) neonates. The Hg in the \( F_1 \) neonates were about 25% of those in the respective \( F_0 \) adults, but the Hg concentrations in the \( F_1 \) daphnids should be diluted by growth and efflux as the neonates grew without continuous Hg exposure [23]. The Hg transfer from the \( F_1 \) to \( F_2 \) daphnids was not detected because the Hg radioactivity essentially was at the background levels in the \( F_1 \) daphnids.

The MTLP concentrations in the \( F_0 \) adults and the respective \( F_1 \) neonates are shown in Figure 2. The MTLP concentrations in the \( F_0 \) adults were significantly higher after exposing to 2.5 nM (\( p = 0.009 \)) and 25 nM (\( p = 0.011 \)) of Hg than the control animals. However, the MTLP concentrations in the \( F_1 \) neonates were similar for all the treatments, implying that the MTLPs were not transferred maternally in \emph{Daphnia}. Instead of MTLP itself, Lin et al. [13] demonstrated that the mRNA of MTLP was transferred from Cd-treated fish to their larvae. Therefore, we could not rule out the possibility that there was maternal transfer of mRNA from the Hg-treated \emph{Daphnia} adults.

The subcellular distribution of Hg in the \( F_0 \) daphnids (exposed groups only) is shown in Figure 3. Generally, the relative distribution of Hg in both Hg treatments followed the order: IF > MTLP > HSP. For the 25 nM treatment, the relative distribution of Hg was not significantly different between the IF and the MTLP, but the Hg in the HSP was significantly lower. On the other hand, in the 2.5 nM treatment, the relative distribution of Hg was significantly higher in the IF than those in the HSP and the MTLP. The distribution of Hg in the MTLP fraction was significantly higher for the 25 nM than the 2.5 nM treatments (\( p < 0.05 \)), although other fractions showed no major difference between the two Hg-treatments. Therefore, MTLPs may become more important in binding the intracellular Hg if the daphnids were exposed to higher levels of ambient Hg, although the MTLP induction was similar for the daphnids from both Hg treatments (Fig. 2). It appears that the binding sites of MTLPs were not saturated fully by Hg in the daphnids from both Hg treatments. Nevertheless, a previous study observed no metal-rich granules in the gut of the daphnids after exposure to Cd, thus the MTLP presumably was the major metal-detoxifying ligand in the daphnids [4].

\emph{Tolerance of daphnids}

The time-to-death experiment was performed at a nominal Hg concentration of 250 nM and the survival of the daphnids within 20 h of exposure to Hg is shown in Figure 4. By statistical comparison between the survival curves (Table 2), it is clear that the \( F_0 \) daphnids from the two exposed groups (i.e., 2.5 nM and 25 nM) had significantly higher tolerance (\( p < 0.01 \) and \( p < 0.001 \), respectively) to Hg toxicity than the control daphnids. After proceeding to the \( F_1 \) generation, the neonates coming from the mothers treated with 25 nM of Hg had significantly higher tolerance than the individuals from the control and 2.5 nM–treated \( F_0 \) mothers (\( p < 0.05 \)). Therefore, the Hg tolerance in the \( F_1 \) generation could be transferred to the \( F_1 \) generation if the Hg contamination level was high enough (e.g., 25 nM but not 2.5 nM treatment), although there was no significant difference of MTLP measured in the \( F_1 \) daphnids among the different treatments (\( p = 0.116 \), Fig. 2). Thus, the Hg tolerance inherited in the \( F_1 \) neonates may not be due to the transfer of MTLP, but the mRNA of MTLP possibly could be transferred through reproduction [13]. Metallothionein-like protein may be synthesized as short as within 2 h in the daphnids upon exposure to trace metals such as Cd [24]. However, there was no major difference in the Hg tolerance between the different groups of the \( F_2 \) daphnids (\( p > 0.05 \), Table 2) and, thus, the Hg tolerance probably was lost during the \( F_1 \) generation. The underlying mechanism for such tolerance loss may require further examination [20].
Wilcoxon tests; Hg concentrations. The comparison was performed using generalized
in even when the animals were not exposed to Hg for several
generation (100\(\times\)10\(^{-6}\)) produced by female fathead minnows exposed to sublethal Cu
expression of the maternal detoxification mechanisms. Nevertheless, the metal tolerance of the animals may disappear either sharply (e.g., Hg in daphnids in this study) or gradually through many generations (e.g., Cd in oligochaetes) [20], but also can be retained for a long time (e.g., Hg in oligochaetes) [3].

**Offspring exposure**

In our preliminary experiments, we observed that there was no significant difference in the carapace length between the <24-h-old neonates derived from the Hg-treated and control mothers (at similar Hg concentrations as the present study, data not shown). Thus, the neonatal quality (e.g., wet wt) may be similar for both groups of offspring at the beginning and should not be a main factor in determining the physiological performance of these offspring. The results of the combined influences of maternal and offspring exposures on the Hg accumulation, reproduction, and growth of the \(F_1\) daphnids are shown in Table 3. The Hg accumulation was not influenced significantly by the different combined exposure regimes. Nevertheless, the reproduction rates were significantly higher for the \(F_1\) daphnids when exposed to 1.5 nM of Hg, regardless of the influence of the maternal exposures. Similar trends also were observed for the final wet weights of the \(F_1\) daphnids from both 1.5 nM and 15 nM offspring exposures that were not affected by the maternal exposure. Therefore, the maternal exposure history had no effect on the physiological performance of the offspring. Rather, the physiological performance of the daphnids was influenced significantly by the offspring exposure to Hg. Because the accumulated Hg could promote the reproduction and growth of the daphnids, Hg may affect certain functional metabolic pathways (i.e., hormesis). Sarabia et al. [26] found that the mean body length of the Hg-exposed *Artemia* larvae (25 nM) was higher than that of the control animals. The Hg concentration was similar to that used in our study (i.e., 1.5–15 nM), although that particular study did not provide data on Hg tissue concentrations. With increasing body size and reproductive performance, the survival time of the daphnids may be reduced accordingly. Because our study only monitored the survival of the daphnids until 28 d after birth, we did not know whether there was a trade-off between sur-

![Fig. 4. Cumulative percentage survival of the \(F_0\), \(F_1\), and \(F_2\) daphnids upon exposure to a nominal Hg concentration of 250 nM for 20 h. The differences among treatments were due to only the different maternal exposure regimes (i.e., \(F_0\) exposure). \(\bullet = F_0\) control; \(\circ = F_0\) 2.5 nM; \(\blacktriangledown = F_0\) 25 nM.](image-url)

Recently, Peake et al. [14] demonstrated that the fish larvae produced by female fathead minnows exposed to sublethal Cu concentration (100 \(\mu\)g L\(^{-1}\)) had significantly higher survivorship when they were exposed to lethal Cu concentration (800 \(\mu\)g L\(^{-1}\)) than the larvae from the control females. As an essential element, Cu should be transferred maternally in the aquatic organisms. Therefore, either the metal itself or the detoxifying agent was the cause of maternal transfer of metal tolerance. Alternatively, it may be due to the selection of the tolerant individuals and the offspring carry the tolerant genes [25]. In contrast to *Daphnia*, Hg tolerance in a freshwater oligochaete (*T. tubifex*) was developed by exposure to Hg in sediment, which then was inherited to subsequent generations even when the animals were not exposed to Hg for several
generations [3]. The underlying mechanisms for such inherited Hg tolerance in oligochaetes, however, are unknown. For *Daphnia*, we did not observe the multigenerational transfer of Hg tolerance. Although there was higher Hg tolerance in the \(F_1\) individuals from mothers treated with high Hg concentration (i.e., 25 nM), the induced Hg tolerance then disappeared when the \(F_1\) individuals were challenged with a high Hg concentration. Because only Hg was transferred maternally from the \(F_0\) to \(F_1\) daphnids, we speculate that the maternally transferred Hg, in addition to the possibility of mRNA of MTLP, may be linked to the maternally derived Hg tolerance in the \(F_1\) neonates.

Several previous studies on fish only considered the tolerance transfer between two successive generations [13,14], thus it would be interesting to know whether the metal tolerance can be lost during the second generation. Similar to Hg, the Cd resistance in *D. magna* was acquired but also lost within a single generation [4]. Consequently, the inheritance of metal tolerance may be both metal-specific (e.g., Cd vs Hg) and species-specific (e.g., daphnid vs oligochaete). Nevertheless, in an Hg-contaminated site, we should expect sustained Hg tolerance in the daphnids because the elevated Hg concentration persists and the detoxification system may be switched on continuously. However, when the site is cleaned up, the metal tolerance of the animals may disappear either sharply (e.g., Hg in daphnids in this study) or gradually through many generations (e.g., Cd in oligochaetes) [20], but also can be retained for a long time (e.g., Hg in oligochaetes) [3].

**Table 2. Statistical comparison of *Daphnia magna* tolerance to lethal Hg concentrations. The comparison was performed using generalized Wilcoxon tests; > represents significant difference \((p < 0.05)\); = represents no significant difference \((p > 0.05)\)**

<table>
<thead>
<tr>
<th>Generation</th>
<th>Comparison between different treatments due to (F_0) maternal exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F_0) adults</td>
<td>25 nM &gt; 2.5 nM &gt; control</td>
</tr>
<tr>
<td>(F_1) neonates</td>
<td>25 nM &gt; 2.5 nM = control</td>
</tr>
<tr>
<td>(F_2) neonates</td>
<td>25 nM &gt; 2.5 nM = control</td>
</tr>
</tbody>
</table>
Tolerance development in cladocerans by Hg exposure

Table 3. Mercury tissue concentrations, reproduction per female within 28 d after birth, and the final wet weights of the $F_1$ daphnids. Means for a treatment are not significantly different ($p > 0.05$) if they bear the same letter. Data are means ± standard deviation ($n = 3$). N/A = not applicable

<table>
<thead>
<tr>
<th>Maternal exposure ($F_0$)</th>
<th>Offspring exposure ($F_1$)</th>
<th>Mean value due to $F_0$ exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1.5 nM</td>
</tr>
<tr>
<td>Hg tissue concentrations (nmol g$^{-1}$ wet wt)</td>
<td>Control</td>
<td>0.93 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>2.5 nM</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>25 nM</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>Reproduction rates (neonates adult$^{-1}$)</td>
<td>Control</td>
<td>37.4 ± 3.64</td>
</tr>
<tr>
<td></td>
<td>2.5 nM</td>
<td>33.4 ± 1.11</td>
</tr>
<tr>
<td></td>
<td>25 nM</td>
<td>36.5 ± 6.7</td>
</tr>
<tr>
<td>Mean value due to $F_1$ exposure</td>
<td>35.8 ± 2.66 A</td>
<td>42.0 ± 1.55 B</td>
</tr>
<tr>
<td>Individual wet weights (mg)</td>
<td>Control</td>
<td>2.67 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>2.5 nM</td>
<td>2.69 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>25 nM</td>
<td>2.62 ± 0.08</td>
</tr>
<tr>
<td>Mean value due to $F_1$ exposure</td>
<td>2.66 ± 0.07 A</td>
<td>2.87 ± 0.08 B</td>
</tr>
</tbody>
</table>

vival time and reproductive output. If so, our results are in agreement with the life-history theory [27], which predicts that habitat disturbances leading to reduced adult survival will select for earlier maturation and increasing reproductive effort.

Similar to the physiological performance, the MTLP induction in the $F_1$ daphnids was not affected by the maternal exposure but only by the offspring exposure. Figure 5 shows that the MTLP concentrations in the $F_1$ daphnids were significantly higher for individuals exposed to 1.5 nM ($p < 0.001$) and 15 nM ($p < 0.001$) of Hg during the 28 d of offspring exposure, with the exception of the individuals exposed to 1.5 nM of Hg from the $F_0$ mothers treated with 25 nM of Hg. Bodar et al. [4] found no major difference in the effects between the multigeneration and single generation Cd acclimation on the tolerance of the daphnids to Cd toxicity, suggesting that the effect of Cd acclimation is a single generation phenomenon.

Pre-exposure to metals may increase the acute tolerance but not necessarily the chronic tolerance (i.e., reproduction). Stuhlbacher and Maltby [28] found that the Cd pre-exposure increased the Cd tolerance of a freshwater amphipod, Gammarus pulex, but had no effect on the sublethal toxicity of Cd to the amphipods. In addition, Bossuyt and Janssen [29] demonstrated that acclimation to low levels of Cu had no effect on the reproduction and intrinsic growth rate of D. magna in the laboratory. In our experiment, the maternal exposure could alter the acute Hg tolerance in the $F_1$ daphnids but not the physiological performance of the $F_1$ daphnids. Therefore, it is possible that the effect of Hg pre-exposure may influence the tolerance to the acute and chronic Hg toxicity in two different ways; the latter may involve processes such as long-term adaptation and energy redistribution.

Because MTLPs in the animals followed the metal concentrations in the tissue after either long-term natural contamination [8,30] or short-term laboratory pre-exposure [31], it has been proposed as a biomarker for metal contamination in the environment. However, with the maternal transfer of Hg from the mothers to the offspring, there were high Hg concentrations in the offspring, although the MTLP levels were similar to those of the control animals. Thus, the use of MTLP in biomonitoring program may not be appropriate for maternally transferable contaminants (e.g., Se, Zn [16]; Hg [15]), in addition to other biological and environmental influences on this biomarker [32,33].

Although our study used higher Hg concentrations than the natural Hg concentrations in the freshwater ecosystems, the Hg concentrations accumulated in the daphnids following pre-exposure (e.g., 2.5 nM in maternal exposure, ~3 nmol g$^{-1}$ dry wt) was close to those measured in the natural zooplankton samples (total Hg: ~4–6 nmol g$^{-1}$ dry wt) after high Hg contamination events such as flooding incidence in reservoirs [34]. Therefore, it is possible that the zooplankton is exposed continuously to high levels of Hg in these contaminated sites; thus the Hg tolerance in the zooplankton may be activated during their lifetime. In the natural environment, the zooplankton are exposed to both inorganic and methylated forms, although the latter, in most cases, is the dominant Hg species in the zooplankton [1,15]. Further research, therefore, is required to study the effect of methyl-Hg pre-exposure and the subsequent tolerance development to methyl-Hg, in relation to the induction of MTLPs and subcellular distribution of methyl-Hg.

CONCLUSION

In summary, when Daphnia were exposed to elevated concentrations of Hg, the MTLPs were induced to bind the Hg...
intracellularly and protected the animals from acute Hg toxicity. This Hg detoxification system was more pronounced at higher ambient Hg concentrations. The Hg tolerance was transferred maternally from the $F_0$ to $F_1$, daphnids, which was accompanied by Hg transfer but not MTLP itself. However, the Hg tolerance was not transferred further from the $F_1$ to $F_2$ generations. Furthermore, the Hg accumulation, reproduction, and growth were not influenced by the previous maternal exposure history but only by the offspring (or current) exposure. Our study implies that the removal of Hg from contaminated site could reduce quickly the Hg tolerance of this freshwater cladoceran (through 1–2 generations of time), but did not affect necessarily the subsequent Hg accumulation and the growth and reproduction of the offspring.

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