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Effects of early life exposure to methylmercury in *Daphnia pulex* on standard and reduced food ration

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Abstract

As a well-known eco-toxicological model organism, *Daphnia pulex* may also offer advantages in human health research for assessing long-term effects of early life exposures to coupled stressors. Here, we examine consequences of early life exposure to methylmercury (MeHg) under standard and reduced food ration. We exposed *Daphnia* for 24 h in early life to varying concentrations of methylmercury(II) chloride (0, 200, 400, 800 and 1600 ng/L) and thereafter kept *Daphnia* on either a standard or a reduced food ration. The data suggests an additive effect of MeHg concentration and food ration on decreasing lifespan, although MeHg concentration does not affect survival linearly. Food ration and MeHg concentration were predictive of reduced reproduction, and there is some evidence of an interaction ($p = 0.048$). Multi-stressor work in alternative model systems may be useful for prioritizing research, taking into account potential antagonistic, additive or synergistic effects that nutritional status may have on chemical toxicity.

Keywords

Methylmercury; *Daphnia pulex*; Early life; Nutrition; Reproduction; Survivorship

1. Introduction

The need to better evaluate the risk of methylmercury (MeHg) toxicity in humans lies in the importance of fish in the diet of many different world cultures. Marked differences in nutritional status exist across regions with Sub-Saharan Africa and southern Asia having the highest prevalence of undernourishment [1]. One in eight people between 2011 and 2013 in the world are estimated to be suffering from chronic hunger [1]. While human populations are exposed simultaneously to chemical stressors and other factors including differing

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Conflict of interest

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nutrition, safe limits of exposure are based primarily on single chemicals tested in highly controlled laboratory settings. Consequently, the effects of MeHg within optimal nutritional parameters in test animals are relatively well documented [2].

In a review of nutrition and MeHg toxicity, Chapman and Chan [3] observe that more studies designed specifically to address the role of nutrition in MeHg toxicity are needed. In deriving the reference dose (RfD) for MeHg, three epidemiological studies were considered by the United States Environmental Protection Agency (USEPA): The Seychelles Child Development Study (SCDS) [4], the Faroe Island study [5] and the New Zealand Children study [6]. With comparative levels of maternal MeHg, the SCDS yielded no impairment related to MeHg exposure but the other two studies found a dose related adverse effect on neurobehavioral endpoints. Some have suggested that nutritional status likely played a role in the differing results across these epidemiologic studies [3]. Seafood, a primary source of MeHg exposure, is also a source of protein, beneficial lipids, and micronutrients thought to be important for the developing brain [7]. Specifically, the selenium and dietary antioxidant content of species consumed are thought to alter the toxic responses elicited by MeHg and could in part explain the differences in response across the three populations [8–10]. For example, selenium has been shown to reduce MeHg target organ dose, preserve antioxidant activity, and prevent neurotoxicity of MeHg in rodent models [11–16].

Current developmental toxicity testing protocols [17,18] rarely require examination of effects throughout the lifespan; yet, studies have suggested subtle effects that are only unmasked later in life are possible [19]. Studies have shown that effects of MeHg have a latent period between exposure and visible effects [20–22]. For example in the Iraq grain incidence of MeHg poisoning, ill effects of MeHg was seen weeks and in some cases months after the exposure stopped [21]. In the case of the Minamata poisoning, low chronic doses of MeHg may not have produced observable behavioral effects for periods of time measured in years [20–23]. In addition, several studies suggest multigenerational effects are possible from early life exposure to chemicals [24,25], with diethylstilbestrol (DES) being a well-known example where granddaughters of women who were exposed to DES had a higher frequency of hip dysplasia, irregular periods, older age at menarche, and potentially an increased risk of infertility [26,27]. A more recent study also shows that nicotine may induce trans-generational asthma in rats [28].

The high cost and time required to test interacting stressors (e.g. chemical and nutrition) across the lifespan in traditional animal models makes the development of novel model systems critically important. Invertebrate models have been used extensively in biomedical research to elucidate mechanisms of aging, and studies have identified conserved pathways associated with increased lifespan upon caloric restriction [29,30]. Alternative animal models have been used previously to evaluate MeHg toxicity. A dose-dependent inhibition of embryonic development and perturbation of highly conserved pathways in neural development is seen in *Drosophila* embryos after exposure to MeHg [31]. Despite these findings, the interaction between diet and low level MeHg exposure in invertebrate model systems is unknown.

Daphnia are a standardized USEPA [32] and Organization for Economic Co-operation and Development (OECD) ecotoxicology model system [33] and are also a recent addition to the NIH list of model organisms for biomedical research [34]. The acute 48-h and subchronic 21 day *Daphnia* toxicity tests are common protocols currently being used to develop water quality criteria for stressors in aquatic environments [32–34]. Some unique characteristics of *D. pulex* make it an ideal organism for complimenting other alternative models systems in biomedical research, particularly for evaluating multiple stressor hypotheses and long-term effects of early life exposures. *Daphnia* reproduce by cyclic parthenogenesis (switching between asexual and sexual reproduction) thereby allowing for indefinite culture of clonal populations to either limit or evaluate the contribution of genetic variation in response to environmental exposures. A relatively short lifespan (60 days), transparency through adulthood, release of live broods upon molting, worldwide distribution, well characterized ecology, and low maintenance cost are also important features of this whole animal model system for efficiently evaluating early life environmental mediators of toxicity [35–37].

Here, we evaluate the effects of an early life exposure to varying doses of MeHg in a standard and low food ration regime on time to first reproduction, total number of offspring, average brood size, and lifespan. We hypothesized that a low food regime would increase MeHg toxicity as measured by development, reproduction and lifespan endpoints in *D. pulex*.

2. Materials and methods

2.1. *Daphnia pulex* culture system

The *D. pulex* culture was established from a clonal population from Dr. Joseph Shaw's laboratory (Indiana University) received in June of 2011 and maintained in our laboratory using previously established culture protocols [38,39]. Prior to sending to us, it had been in culture for at least 8 years and has high hybridization efficiency to the *D. pulex* TCO microarray (pers comm.). *Daphnia* were maintained in COMBO media. COMBO is a reconstituted water recipe and consists of ultrapure deionized water (Elga:Purelab Ultra MK2, TOC 3-10X) with added Magnesium Sulfate Heptahydrous, Sodium Bicarbonate, Sodium Metasilicate Nihydrous, Boric Acid, Potassium Chloride, and Calcium Chloride Dihydrous with Selenium and Animate stocks [35,38,39] and is recommended as a softer medium similar to natural water [40,41]. *Daphnia* were kept in an environmental chamber (Percival Intellus Ultra Controller Model I36NLXC8) and maintained at 22.5 °C (± 0.5 °C) with a 12 h light–12 h dark cycle (12:12 L:D) program for lighting.

Daphnia were fed the green algae *Ankistrodesmus falcatus*, cultured in our laboratory using Woods Hole MBA macro and micro nutrient stocks for media preparation. This culturing protocol has been described previously as a food source for *Daphnia* [38]. The original algae cells were purchased from Carolina Biologicals (15-1955 *Ankistrodesmus* Alga-Gro freshwater). The alga is cultured in a 20 liter clear carboy, continually aerated and exposed to 12:12 L:D cycle. Algae were centrifuged down and rinsed 3 times with COMBO media after a 14 day growth period. After harvesting, at least 2 dilutions are made and light absorbance obtained at 680 nm wavelength is measured using a spectrophotometer (DU 800 Series). The concentration of cells is estimated by a standard curve developed relating the

light absorbance measure to manual cell number counts using a hemocytometer. Algae was refrigerated at 4 °C and used within a month or stored in a –80 °C freezer. The *Daphnia* are fed at 80,000 cells/ml every other day (during media change) unless otherwise stated. This concentration has been shown to ensure that *Daphnia* have enough access to algae to ensure adequate nutrition [38].

2.2. MeHg exposures

A stock solution of 11.4 mg/L MeHg (methylmercury(II) chloride of analytical standard Sigma–Aldrich Prod. # 33368) was made by first dissolving MeHg in 1.5 mL of methanol, then adding it to 1 L deionized ultrapure water. Based on previous studies evaluating the effects of MeHg on *Daphnia* [42,43], we evaluated the following doses: 0, 200, 400, 800 and 1600 ng/L of methylmercury(II) chloride in the current study (Table 1). To eliminate the sorption of MeHg by algae and therefore a potential confounder, *Daphnia* were not fed during exposure as per the OECD Guidelines for testing of chemicals [33]. Vehicle controls were exposed to the same methanol concentration (0.2 µL/L) as in the 1600 ng/L MeHg dose group. Mercury concentration in stock solution and in *D. pulex* tissue following the 24 h. exposure to the 1600 ng/L was measured via dual Purge and Trap Cold Vapor Atomic Fluorescence Spectrometry (P/T-CVAFS) by EPA 1631e [44] at Southern Research Institute (Birmingham, AL). The mercury concentration in prepared stock solution was measured at 9.98 ppm as Hg, confirming minimal loss of Hg in the preparation and use of stock solution. For the *D. pulex* sample, the sample vial was rinsed with several aliquots of an L-cysteine and nitric acid solution, dried slowly at 50 °C, and then weighed to determine mass recovered from the original vial. The sample was then reconstituted with BrCl and heated at 60 °C for ~4 h. The sample was then diluted in matrix and introduced to the instrument.

2.3. Experimental design

Neonates (born between 0 and 48 h) from the third or later clutches were collected from cultures of 20 mature *D. pulex* that were kept individually [45]. These neonates, born to mothers in a standard feed environment, were exposed to MeHg-containing or control media for 24 h in closed flasks, after which *Daphnia* were rinsed and transferred to clean COMBO media in 50 ml tubes with either a standard feed ration (80,000 cells/ml) or half feed ration (40,000 cells/ml) diet ($N = 20$ per group). The day of exposure to MeHg, vehicle or no exposure was considered Day 1 of the life table analysis. Experiments were started on the same day for each dose group and experiments for all treatment groups were started within a three week time frame. Between 55 and 135 *Daphnia* were introduced into exposure or control solutions (Table 1). Out of those recovered from the initial exposure, we separated out the *Daphnia* into individual tubes until we had at most 20 per exposure group. Death and number of live offspring were recorded for each animal every other day.

2.4. Statistical analysis

A chi-square test was used to evaluate differences in acute survival immediately following 24 h exposures. Kaplan–Meier survival curves were constructed and a Gehan–Breslow–Wilcoxon test was used to determine differences amongst the treatments. *Daphnia* that died prior to Day 12 or before first reproduction were not included in the survival analysis, as per

previous studies [46]. Cox proportional hazards models were built to determine whether early life exposure to MeHg interacts with food ration to affect survival. Three model fits were compared:

- A. Lifespan ~ Food ration
- B. Lifespan ~ Food ration + MeHg concentration (additive or ‘main’ effects only, no interaction)
- C. Lifespan ~ Food ration + MeHg concentration + Food ration * MeHg concentration (interaction model)

The effect of MeHg concentration and food ration on time to first reproduction was evaluated using negative binomial regression and likelihood ratio tests. Average brood size and total reproductive output were modeled using linear regression with a food ration * MeHg concentration interaction term included. Differences were evaluated using F tests. To evaluate reproductive output over time, Poisson regression models were used where reproduction rate may vary additively by food ration/concentration group (GROUP) as a function of time independent of GROUP (A) or with additional terms allowing reproduction rate to vary as a function of time dependent on GROUP (B):

- A. Reproductive rate ~ GROUP + time + time²
- B. Reproductive rate ~ GROUP + time + time² + GROUP * time + GROUP * time²

Models were compared using a likelihood ratio test. Significance levels for all tests was set at an alpha = 0.05. All reported *p*-values are nominal (unadjusted for multiple comparisons). Statistical analyses were performed in *R*. Survival curves and bar graphs were generated in GraphPad Prism version 5.00 for Windows.

3. Results

3.1. Life history effects of early life exposure to MeHg

Based on a previous study, a 10 day MeHg exposure in neonates suggested an Effective Dose 50 (ED₅₀) of 707 ng/L for reduction in reproduction in *D. pulex* [43] (Table 1). Since we were particularly interested in determining potential subtle long-term effects when exposure was confined to an early developmental stage, we exposed *Daphnia* (0–48 h old) to 1600 ng/L (FN 1600), 800 ng/L (FN 800), 400 ng/L (FN400), and 200 ng/L (FN200) for 24 h. These doses are below what is expected to cause lethality in *Daphnia* [42,43]. Whole animal tissue concentrations following the highest dose tested (1600 ng/L) were measured at 1.9 ppm (ng/mg) as Hg.

Mortality in the various treatments after a 24 h exposure was assessed. Between 9% and 52% of *Daphnia* initially introduced into the exposure solutions were lost after the 24 h. exposure period across MeHg and control groups (Table 2). A chi-square test suggests that the six groups do not have the same underlying probability of survival after exposure ($p = 1.9 * 10^{-7}$), although a dose-response relationship is not evident. Animals in the 1600 ng/L experienced higher mortality compared to the 800 ng/L, 200 ng/L, control, and vehicle

control groups and the 400 ng/L group had heightened mortality compared to the vehicle control.

Survivorship in *D. pulex* exposed to MeHg in early life and observed for lifespan effects are shown in Fig. 1. Although not significantly different across the six groups ($p = 0.23$), we do note a trend such that higher MeHg exposed groups (400 ng/L, 800 ng/L, and 1600 ng/L) have higher mortality, particularly in mid-life (20–40 days), when compared to low dose (200 ng/L) and control groups.

For reproductive endpoints, we examined time to first reproduction, reproductive output per day lived and average brood size of the groups that were exposed to MeHg (Fig. 2).

Outcomes were analyzed using regression models. MeHg concentration was not a significant predictor of time to first reproduction, which marks maturation of the *Daphnia* ($p = 0.13$) (Fig. 2A). MeHg concentration was a significant predictor of reduced reproductive output over the lifespan ($p = 0.0013$) (Fig. 2B). Average brood size (Fig. 2C) was weakly affected by treatment ($p = 0.026$) (Fig. 3C).

3.2. Effects of early life exposure to MeHg in a low food regime

To elucidate the effects of a reduced food ration on MeHg toxicity, *Daphnia* exposed to MeHg were either fed at a standard food ration (80,000 cells/ml) or at a low food ration (40,000 cells/ml *A. falcatus*) throughout life. Survival curves across MeHg doses and fed low food ration are shown in Fig. 3. Cox proportional hazards models were developed to determine the effect of food ration and MeHg exposure on lifespan across the 12 dose/diet groups. Both diet and MeHg dose were significant negative predictors of lifespan, however the addition of a food ration * MeHg interaction term did not improve the model fit ($p = 0.31$), suggesting diet and MeHg are likely acting independently on survival in this model system.

Reproductive outcomes across the low food ration MeHg dose groups are shown in Fig. 4. Regression models suggest days to first reproduction is not affected by low food ration ($p = 0.13$), but average brood size ($p < 10^{-15}$) and reproductive output ($p < 10^{-15}$) are strongly reduced by low food ration. There is weak evidence of an interaction between MeHg concentration and food ration for reproductive output ($p = 0.048$) and no evidence of an interaction for average brood size ($p = 0.092$).

Data on reproduction collected every other day suggests reproduction peaks between days 22 and 30 in full ration (FN) control and low MeHg (200 ng/L) exposure groups, but peaks later in life in the full ration (FN) higher MeHg (400, 800, and 1600 ng/L) exposed groups (Fig. 5). Examining the reproductive output from *Daphnia* fed half ration (HN) suggest reproduction later in life may be reduced by early life MeHg exposure (Fig. 5). Comparing Poisson regression models to fit the data suggests significant effects on reproduction rate over time as a function of early life MeHg exposure and food ration (p -value $< 10^{-16}$).

4. Discussion

This study evaluated lifespan and reproduction in *D. pulex* exposed to MeHg early in life at 5 different MeHg concentrations ranging from 0 to 1600 ng/L. The purpose was to evaluate the long-term effects of a short exposure confined to early life. Tsui and Wang (2006) estimated the 24-h median lethal concentration in *Daphnia magna* to be between 12,000 ng/L and 55,000 ng/L (Table 1). The LC₅₀ reported for *D. pulex* was 31,205 ng/L in a 24 hr period and 5700 ng/L over a 48 h exposure in neonates of *D. pulex* [43] (Table 1). Based on these studies, the lower concentrations in this present research were not expected to be acutely lethal to *D. pulex*, although we did find acute mortality was increased in our 1600 and 400 ng/L dose groups.

The concentrations of MeHg evaluated in the current study are generally higher than those found in natural waters. For example, a study in Canada measured surface water levels between 0.02 and 4 ng/L [47] while a study in California wetlands reported MeHg between 0.1 and 37 ng/L [48]. Invertebrates harvested from these waters had MeHg concentrations approximately 10 to 100 times that of surface waters, ranging from 0.2 to 394 ng/g [49]. With reference to human health, the U.S. EPA has determined bioconcentration factors (BCFs) to estimate a water concentration that would result in a fish concentration of concern [47]. This modeling involves numerous points of uncertainty and assumptions, but allows us to estimate whether the doses we are currently testing are relevant to human exposures. The median EPA BCF estimate is 33,000 µg of MeHg/kg in fish to 1 µg of MeHg/L in water. Therefore, the dose of 200 ng/L through to 1600 ng/L would translate into that which yields a concentration of 6.6 mg/kg through to 52.8 mg/kg of MeHg in fish tissue if exposed continuously. The U.S. EPA RfD is based on a cord blood level of 58 ppb calculated as the benchmark dose level (BMDL) of mercury in cord blood from the Boston Naming Test results of Faroese children. A study in patients that died from the Minamata disease had brain Hg levels of 2.6–24.8 µg/g and kidney levels of 21.2–140 µg/g [23].

Our data did not show any statistically significant differences in lifespan between individual MeHg dose groups however Cox proportional hazards models do suggest MeHg concentration is a significant predictor of reduced lifespan when added to a model with only food ration as a predictor. Examining the survival curves presented suggest the largest difference in survival in higher MeHg dose groups compared to lower and control groups occurs between day 20 and day 45 (Figs. 1 and 3). Tsui and Wang (2004) showed that over 70% of a 33.3 ± 1.91 µg/g body burden resulting after a 5 day dietary exposure of MeHg was eliminated over a 20 day period in *Daphnia magna*. The biological retention half-life was 9.07 ± 0.12 d for MeHg in that study [50]. Assuming a similar half-life in *D. pulex*, our results suggest that early life exposure may affect survivorship even after the body burden of MeHg is significantly reduced, especially considering the low tissue concentrations measured in the present study (1.9 ppm (ng/mg) as Hg) after the highest dose tested (1600 ng/L).

This initial analysis of long-term effects of food ration and an early life MeHg exposure suggests future areas of research. In the present study, low MeHg exposure group (FN 200) and controls tended to have higher total reproductive output than those in the higher MeHg

exposed groups (FN 400, FN 800 and FN 1600). This result is consistent with the findings by Tsui and Wang (2004) which showed that a high body burden of MeHg resulted in undeveloped eggs [51]. MeHg may influence the production of eggs or cause arrested or aberrant development, therefore in future studies it will be important to track eggs in the brood sac as well as live offspring. In addition further evaluation of food rations at varying levels above and below the standard food regime may help tease out interactive effects. The amount of cells consumed should be accounted for within the MeHg exposed daphnia to determine whether MeHg exposure affects the amount of food consumed. Potential trade-offs in energy allocation in different food ration * MeHg concentration environments would need to consider egg size, neonate growth and subsequent fitness of offspring.

We set out to determine if a low dose exposure to MeHg confined to early life has an effect on lifespan and also if food ration influences the effect of MeHg exposure. In summary, the results suggest that both low food ration and MeHg concentration additively affect survivorship and reproduction, with some evidence of a food ration * MeHg concentration interaction on reproduction that requires further evaluation.

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References

1. Food and Agriculture Organization of the United Nations. The State of Food Insecurity in the World 2013: the multiple dimensions of food security. 2013.
2. Rice DC, Gilbert SG. Effects of developmental methylmercury exposure or lifetime lead exposure on vibration sensitivity function in monkeys. *Toxicol Appl Pharmacol*. 1995; 134(1):161–9. [PubMed: 7676451]
3. Chapman L, Chan HM. The influence of nutrition on methyl mercury intoxication. *Environ Health Perspect*. 2000:29–56. [PubMed: 10698722]
4. Myers GJ, Marsh DO, Davidson PW, Cox C, Shamlaye CF, Tanner M, et al. Main neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet: outcome at six months. *Neurotoxicology*. 1995; 16(4):653–64. [PubMed: 8714870]
5. Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol*. 1997; 19(6):417–28. [PubMed: 9392777]
6. Kjellstrom, T.; Kennedy, P.; Wallis, S.; Mantell, C. Physical and mental development of children with prenatal exposure to mercury from fish. Stage 1: Preliminary tests at age 4. Report 3080. Solna, Sweden: National Swedish Environmental Protection Board; 1986.
7. WHO. Joint FAO/WHO Expert Committee on Food additives. Seventy-third meeting; 2010. <http://www.who.int/foodsafety/publications/chem/summary73.pdf>. JECFA/73/SC
8. Goyer RA. Toxic and essential metal interactions. *Annu Rev Nutr*. 1997; 17(1):37–50. [PubMed: 9240918]
9. Watanabe C. Modification of mercury toxicity by selenium: practical importance. *Tohoku J Exp Med*. 2002; 80:71–7. [PubMed: 12498318]

10. Mergler D1, Anderson HA, Chan LH, Mahaffey KR, Murray M, Sakamoto M, et al. Methylmercury exposure and health effects in humans: a worldwide concern. *Ambio*. 2007; 36(1): 3–11. [PubMed: 17408186]
11. Ganther HE. Modification of methylmercury toxicity and metabolism by selenium and vitamin E: possible mechanisms. *Environ Health Perspect*. 1978; 25:71. [PubMed: 720304]
12. Ralston NV, Ralston CR, Blackwell JL 3rd, Raymond LJ. Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology*. 2008; 29(5):802–11. [PubMed: 18761370]
13. Khan MA, Wang F. Mercury-selenium compounds and their toxicological significance: toward a molecular understanding of the mercury-selenium antagonism. *Environ Toxicol Chem*. 2009; 28(8):1567–77. [PubMed: 19374471]
14. Rayman MP. The importance of selenium to human health. *Lancet*. 2000; 356(9225):233–41. [PubMed: 10963212]
15. Watanabe C, Yoshida K, Kasanuma Y, Kun Y, Satoh H. *In utero* methylmercury exposure differentially affects the activities of selenoenzymes in the fetal mouse brain. *Environ Res*. 1999; 80(3):208–14. [PubMed: 10092441]
16. Nielsen JB, Andersen O. The toxicokinetics of mercury in mice offspring after maternal exposure to methylmercury—effect of selenomethionine. *Toxicology*. 1992; 74(2):233–41. [PubMed: 1519245]
17. USEPA. Guidelines for developmental toxicity risk assessment. *Fed Regist*. 1991; 56(234):63798–826. <http://www.epa.gov/raf/publications/pdfs/DEVTOX.PDF>.
18. OECD. Test No. 414: Prenatal Development Toxicity Study. OECD Publishing; 2014.
19. Fox DA, Grandjean P, de Groot D, Paule MG. Developmental origins of adult diseases and neurotoxicity: epidemiological and experimental studies. *Neurotoxicology*. 2012; 33(4):810–6. [PubMed: 22245043]
20. Weiss B, Clarkson TW, Simon W. Silent latency periods in methylmercury poisoning and in neurodegenerative disease. *Environ Health Perspect*. 2002; 110(Suppl 5):851. [PubMed: 12426145]
21. Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, et al. Methylmercury poisoning in Iraq. *Science*. 1973; 181(4096):230–41. [PubMed: 4719063]
22. Trasande L, Landrigan PJ, Schechter C. Public health and economic consequences of methylmercury toxicity to the developing brain. *Environ Health Perspect*. 2005; 113(5):590. [PubMed: 15866768]
23. Harada M. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *CRC Crit Rev Toxicol*. 1995; 25(1):1–24.
24. Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect*. 1993; 101(5):378. [PubMed: 8080506]
25. Mably TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A, Peterson RE. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: 3. Effects on spermatogenesis and reproductive capability. *Toxicol Appl Pharmacol*. 1992; 114(1):118–26. [PubMed: 1585364]
26. Giusti RM, Iwamoto K, Hatch EE. Diethylstilbestrol revisited: a review of the long-term health effects. *Ann Intern Med*. 1995; 122(10):778–88. [PubMed: 7717601]
27. Titus-Ernstoff L, Hatch EE, Hoover RN, Palmer J, Greenberg ER, Ricker W, et al. Long-term cancer risk in women given diethylstilbestrol (DES) during pregnancy. *Br J Cancer*. 2001; 84(1): 126. [PubMed: 11139327]
28. Rehan VK, Liu J, Naeem E, Tian J, Sakurai R, Kwong K, et al. Perinatal nicotine exposure induces asthma in second generation offspring. *BMC Med*. 2012; 10(1):129. [PubMed: 23106849]
29. Austad SN. Is there a role for new invertebrate models for aging research? *J Gerontol A Biol Sci Med Sci*. 2009; 64(2):192–4. [PubMed: 19181713]
30. Masoro EJ. Overview of caloric restriction and ageing. *Mech Ageing Dev*. 2005; 126(9):913–22. [PubMed: 15885745]
31. Bal-Price AK, Coecke S, Costa L, Crofton KM, Fritsche E, Goldberg A, et al. Conference Report Advancing the Science of Developmental Neurotoxicity (DNT) Testing for Better Safety Evaluation. ALTEX Society ALTEX Edition, Kuesnacht, Switzerland. 2012; 29(2):202–15.

32. 2000, U., Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136). EPA 821-B-00-004 July 2000. http://water.epa.gov/scitech/methods/cwa/wet/upload/2007_07_10_methods_wet_wetguide.pdf
33. OECD. *Daphnia* sp., Acute Immobilisation Test and Reproduction Test No: 202 1948249.pdf. www.oecd.org/dataoecd/17/21/1948249.pdf [retrieved on 2012]
34. NIH. Model organisms for Biomedical Research. Available at: <http://www.nih.gov/science/models/>
35. Colbourne JK, et al. The ecoresponsive genome of *Daphnia pulex*. Science. 2011; 331(6017):555–61. [PubMed: 21292972]
36. Colbourne JK, Pfrender ME, Gilbert D, Thomas WK, Tucker A, Oakley TH, et al. *Daphnia* as an emerging model for toxicological genomics. Adv Exp Biol. 2008; 2:165–328.
37. LeBlanc G. Acute toxicity of priority pollutants to water flea (*Daphnia magna*). Bull Environ Contam Toxicol. 1980; 24(1):684–91. [PubMed: 7459457]
38. Shaw JR, Colbourne JK, Davey JC, Glaholt SP, Hampton TH, Chen CY, et al. Gene response profiles for *Daphnia pulex* exposed to the environmental stressor cadmium reveals novel crustacean metallothioneins. BMC Genomics. 2007; 8
39. Asselman J, Shaw JR, Glaholt SP, Colbourne JK, De Schamphelaere KA. Transcription patterns of genes encoding four metallothionein homologs in *Daphnia pulex* exposed to copper and cadmium are time- and homolog-dependent. Aquat Toxicol. 2013; 142:422–30. [PubMed: 24113165]
40. Samel A, Ziegenfuss M, Goulden CE, Banks S, Baer KN. Culturing and bioassay testing of *Daphnia magna* using Elendt M4; Elendt M7, and COMBO media. Ecotoxicol Environ Saf. 1999; 43(1):103–10. [PubMed: 10330328]
41. Kilham SS, Kreeger DA, Lynn SG, Goulden CE, Herrera L, et al. COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia. 1998; 377(1–3):147–59.
42. Tsui MT, Wang WX. Acute toxicity of mercury to *Daphnia magna* under different conditions. Environ Sci Technol. 2006; 40(12):4025–30. [PubMed: 16830577]
43. Tian-yi C, McNaught D. Toxicity of methylmercury to *Daphnia pulex*. Bull Environ Contam Toxicol. 1992; 49(4):606–12. [PubMed: 1421856]
44. EPA U. Method 1631, Revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. Washington, DC: US Environmental Protection Agency; 2002.
45. Adema D. *Daphnia magna* as a test animal in acute and chronic toxicity tests. Hydrobiologia. 1978; 59(2):125–34.
46. Kim E, Ansell CM, Dudycha JL. Resveratrol and food effects on lifespan and reproduction in the model crustacean *Daphnia*. J Exp Zool A Ecol Genet Physiol. 2014; 321(1):48–56. [PubMed: 24133070]
47. Hall BD, Baron LA, Somers CM. Mercury concentrations in surface water and harvested waterfowl from the prairie pothole region of Saskatchewan. Environ Sci Technol. 2009; 43(23):8759–66. [PubMed: 19943643]
48. Alpers CN, Fleck JA, Marvin-Di Pasquale M, Stricker CA, Stephenson M, Taylor HE. Mercury cycling in agricultural and managed wetlands, Yolo Bypass, California: spatial and seasonal variations in water quality. Sci Total Environ. 2014; 484(15):276–87. [PubMed: 24332791]
49. Bates LM, Hall BD. Concentrations of methylmercury in invertebrates from wetlands of the Prairie Pothole Region of North America. Environ Pollut. 2012; 160:153–60. [PubMed: 22035939]
50. Tsui MT, Wang WX. Maternal transfer efficiency and transgenerational toxicity of methylmercury in *Daphnia magna*. Environ Toxicol Chem. 2004; 23(6):1504–11. [PubMed: 15376536]
51. Tsui MTK, Wang WX. Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*. Environ Sci Technol. 2004; 38(3):808–16. [PubMed: 14968868]

Fig. 1. Survivorship of *D. Pulex* after early life exposure to MeHg. FN 1600 ($n = 20$), FN 800 ($n = 18$), FN 400 ($n = 13$), FN 200 ($n = 20$) represents *Daphnia* fed on a standard feed regime and exposed to 1600 ng/L, 800 ng/L, 400 ng/L, 200 ng/L of MeHg. CFN ($n = 20$) and MFN ($n = 20$) represent control and vehicle control (MeOH 0.2 μ g/L) *Daphnia*, respectively.

Fig. 2. Reproduction in MeHg exposed groups. (A) Time to first reproduction. (B) Reproduction per lifespan. (C) Average brood size of the *Daphnia*. Error bars represent SEs. FN 1600 ($n = 17$), FN 800 ($n = 17$), FN 400 ($n = 13$), FN 200 ($n = 17$) represents *Daphnia* fed on a standard feed regime and exposures to 1600 ng/L, 800 ng/L, 400 ng/L, 200 ng/L of MeHg, respectively. CFN and MFN represent *Daphnia* with no exposure and vehicle controls (MeOH 0.2 $\mu\text{g/L}$), respectively.

Fig. 3.

Survivorship in *D. pulex* on low food ration and exposed to MeHg FN0 *D. pulex* fed a standard diet in the vehicle control and no exposure control groups ($n = 40$). HN 1600 ($n = 20$), HN 800 ($n = 20$), HN 400 ($n = 17$), HN 200 ($n = 19$) are animals fed at a reduced food ration and exposed to MeHg at 1600 ng/L, 800 ng/L, 400 ng/L and 200 ng/L, respectively. CHN ($n = 20$) and MHN ($n = 20$) are *Daphnia* fed a low food ration and exposed to no MeHg and MeOH (0.2 μ l/L), respectively.

Fig. 4. Reproductive effects in *Daphnia pulex* exposed to MeHg and on low food ration. (A) Time to first reproduction. (B) Average reproductive output per day (C) Average brood size. FN0 are *D. pulex* fed a standard diet in the vehicle control and control groups ($n = 39$). HN 1600 ($n = 18$), HN 800 ($n = 18$), HN 400 ($n = 14$), HN 200 ($n = 16$) are animals fed a low food ration and exposed to MeHg at 1600 ng/L, 800 ng/L, 400 ng/L and 200 ng/L, respectively. CHN ($n = 18$) and MHN ($n = 16$) are *Daphnia* fed at a low food ration and exposed to no MeHg and MeOH (0.2 μ L/L), respectively. Error bars represent SEs.

Fig. 5.

Reproduction over lifespan in *D. pulex* exposed to MeHg early in life and kept on either full or half food ration. *Y* axis represents average number of offspring counted every 2 days.

Lowess smoother has been used to generate curves.

Table 1

Previous MeHg and mercury toxicity studies in *Daphnia* sp.

Citation	Test species	Chem	Doses range (ng/L)	Duration of dose	Timing of dose	Endpoints	(ng/L)
Tian-yi and McNaught [43]	<i>D. pulex</i>	MeHg	0–1000	10 days	Neonate	Reproduction	707 (EC ₅₀)
Tian-yi and McNaught [43]	<i>D. pulex</i>	MeHg	0.1–10,000	24 h	Neonate	Lethality	31,205 (LD ₅₀)
Tian-yi and McNaught [43]	<i>D. pulex</i>	MeHg	0.1–10,000	48 h	Neonate	Lethality	5700 (LD ₅₀)
Tsui and Wang [42,50]	<i>D. magna</i>	Hg	0–80,000	24 h	Neonate	Lethality	12,400 (LD ₅₀)

Table 2Acute mortality of *D. pulex* after MeHg exposure.

Exposure	# at initiation of exposure	# recovered after 24 h exposure	% recovered
1600 ng/L	135	65	48.15
800 ng/L	60	42	70.00
400 ng/L	59	32	54.24
200 ng/L	60	39	65.00
Vehicle control	55	50	90.91
Control	60	46	76.67