Chronic Toxicity of Mercury (HgCl$_2$) to the Benthic Midge
Chironomus riparius

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ABSTRACT: In most aquatic ecosystems mercury accumulates more in the sediment than in water column. However, due to limited eco-toxicological data, it is difficult to predict the toxicity of these sediments. The present study evaluated the effects of inorganic mercury in spiked sediment on the survival, growth, and emergence of the midge *Chironomus riparius* and compared the results to mercury concentrations reported in streams and rivers in Africa. At 3.84 mg Hg/kg dry sediment, mercury significantly reduced larval survival and midges emergence success in comparison to control sediment (P<0.05). The growth of the larva was significantly inhibited (P<0.05) at 2.42 mg Hg/ kg dry weight, while emergence of *C .riparius* midges was significantly delayed at 0.93 mg/kg dry wt. These results indicate that mercury inhibits *C. riparius* characteristics at lower concentrations than those which have been measured in sediments from watersheds impacted with mercury like those found around artisanal gold mining in Africa. It is therefore possible that *Chironomus* and probably other fauna living in these watersheds are at risk.

Key words: Mercury, Artificial sediment, *Chironomus riparius*, Survival, Growth and emergence

INTRODUCTION

Mercury is a persistent, highly toxic element that bioaccumulates and biomagnifies in aquatic food webs (US EPA, 1997 and Esler, 2005). It can have adverse effects on organisms including neurological effects, reproductive effects, behavioral effects, and direct toxicity at high doses (US EPA, 1997 and Esler, 2005). Since the beginning of the industrial era, the concentrations of mercury in the global environment is estimated to have increased three fold (EU Report, 2004). In the recent past there have been numerous reports from developing countries on the contamination of rivers and natural streams by Hg released from artisanal gold mining (Kahatano *et al.*, 1997; LVEMP, 2002; Limbong *et al.*, 2003 and Ikingura *et al.*, 2006). It has been reported that in most areas Hg accumulates in the sediment (Ikingura *et al.*, 2006 and Chibunda *et al.*, 2008) where it can easily bioaccummulate and either affect the benthic organisms or even biomagnifies.

Surprisingly, the information on the distribution of Hg in the various environmental compartments exceeds by far the available information on its effects on the organisms residing in these compartments. Except for limited (eco) toxicological information on pelagic organisms such as fish, (Grippo and Heath, 2003 and Virginia and Gayle, 1982), daphnids, (Qureshi, 1980) and rotifers (Locarnini and Presley, 1996), there is very limited (eco)toxicological information on the effect of Hg to benthic organisms. The present study evaluated the effect of inorganic mercury (HgCl$_2$) in artificially constituted sediments on the survival, growth, and emergence of the nonbiting midge, *Chironomus riparius* (Diptera: Chironomidae). Chironomidae were chosen for this study as these organisms are important inhabitants of freshwater benthic habitats and have a worldwide distribution in both lentic and lotic habitats (Péry *et al.*, 2002). They feed on sediment-deposited detritus (Rasmussen, 1984) and, therefore, play a key role in organic matter cycling in aquatic ecosystems.

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Their life cycle consists of an egg stage, four larval stages, and a pupal stage (all of which are aquatic) as well as a short-lived aerial adult stage. At 20°C, *C. riparius'* life cycle is typically completed within three to four weeks which makes this species convenient for full life-cycle testing. This species is recommended as model test organism for sediment toxicity testing by different environmental regulatory bodies like the Organisation for Economic Cooperation and Development (OECD) (OECD, 2004).

MATERIALS & METHODS

The artificial sediment was prepared according to the method described in the OECD (2004) document "Sediment-water chironomid toxicity testing using spiked sediment". The composition of the sediment was as follows (on a dry weight basis): 5% sphagnum moss peat; finely ground (particle size d"1 mm); 20% kaolin clay; 75% sand (< 200 micron); deionized water was added to obtain a final moisture content in a range of 30-50%. $CaCO_3$ (obtained from Merck Darmstadt - Germany) was added to adjust the pH of the final mixture of 7.0 ± 0.5. Total Organic Carbon (TOC) was 2.5% of the dry weight and the amount of Acid Volatile Sulphide (AVS) was below the detection limit ($0.06 \mu mol/g$ dry weight).

Mercury ($HgCl_2$) stock solution was prepared by using analytical reagent grade $HgCl_2$ (Merck-Germany) in distilled and double deionized water. For each individual concentration, artificial sediment was thoroughly mixed with the appropriate amount of stock solution in acid washed plastic containers. Overlying water (distilled and double deionized) was carefully poured on top of the sediment to achieve 1:3 sediment: water ratio and then the samples were stored in the dark at 4°C for seven days, after which the overlying water was discarded. The spiked sediments were subsequently thoroughly mixed and aliquots of 100 g were dispensed into 1L test beakers and 175 mL of EPA-medium was carefully added in such a way that disruption of the sediment layer was minimal. The EPA-medium was prepared by adding 60 mg $CaSO_4.2H_2O$, 122.425 mg $MgSO_4.7H_2O$, 96 mg $NaHCO_3$ and 4 mg KCl (Merck –Germany) to 1L of deionised water, resulting in moderately hard water with a hardness of approximately 85 mg/L

as $CaCO_3$ (US-EPA, 1985). Using the same procedure control sediments (without $HgCl_2$) also were prepared. The tested Hg sediment concentrations included 0 (control), 1, 1.8, 3.2, 5.6, 10 and 18 mg Hg/ kg dry weight. The selection of the final test concentrations was based on results from our earlier range finding tests. Pore water was extracted by centrifugation of the wet sediments at g = 2054 for 30 minutes by using a Sigma – 3E-1 centrifuge.

Sediment samples were collected at the first day of the experiment for chemical analysis. Total mercury (T-Hg) analysis in sediment and pore water samples was performed by Atomic Absorption Spectrophotometer with cold vapour generation technique (ICP Ultima 2, Horiba Jobin Yvon, France). Briefly, 10 ml of filtered water ($0.45 \mu m$) was mixed with 2 mL of 1:1 H_2SO_4 to 2% potassium permanganate solution. The mixture was allowed to stand for 15 minutes; subsequently 0.5 mL of 5% potassium sulphate was added. The mixture was heated in a water-bath at 95°C for 1 hour. After cooling, the 3% hydroxylamine solution was added drop wise, until the permanganate colour discharged completely. Five ml of the digested sample was acidified, using 5 ml 6M HCl. Sediment samples were air dried in an air conditioned room set at 25°C and 65% relative humidity.

Sediments were further dried in an oven at 45°C for 48 hours and then milled using an agate planetary micro-mill (Fritsh)-Canada. The resulting fine dried powder was used for digestion. 0.5 g of sediment was mixed with 4.5 mL concentrated HCl and 1.5 mL concentrated HNO_3 in a graduated test tube and digested on a water-bath at 70°C. Thereafter, 5 mL of samples was mixed with 10 mL 6M HCl in a 50 mL test tube. After adding 1mL of KI-ascorbic acid solution, the mixture was vortexed before measuring. Acid washed glassware, analytical grade reagents and double distilled and deionised water were used in the analysis. In order to check purity of the chemicals used, one blank was run every 10 samples. There was no evidence of contamination in these blanks. Analytical quality control and assurances was ensured through the analysis of replicates of Hg standard solutions and the control reference material [BCR-580 for mercury with THg 132±3 mg/kg from European

Commission DG Joint Research Centre (IRMM)]. The recovery percentage was 84% the results were therefore not corrected for recovery.

Chironomus riparius egg ropes were obtained from a continuous culture held at the Laboratory of Animal Physiology and Toxicology at Sokoine University of Agriculture (Tanzania) and hatched in EPA-medium. The starter culture was obtained from the Laboratory of Environmental Toxicology, Ghent University (Belgium). The culture is kept at a controlled temperature of $20 \pm 2°C$ and a 12:12 h light: dark photoperiod.

This test was conducted as described in the OECD guideline 218. Eleven replicates per sediment test concentration were used: 5 of these were used to assess 14 day survival and growth, another 5 for assessment of emergence and growth at day 28 while the last replicate was used for chemical analysis at the end of the test. Tests were initiated by placing the spiked sediments into 800 ml glass vessels and adding overlying water to produce a sediment-water volume ratio of 100g sediment and 175 ml water. This was done one day prior to the introduction of the test organisms. A polystyrene plate was positioned over the sediment to minimize the disturbance of the sediment as the water was added. To each replicate, 10 larvae were introduced randomly into the overlying water below the air-water interface, using a glass pipette. Larvae were individually checked for viability (i.e. swimming motion) in the water column as they were added. Test larvae were taken from the synchronized culture and were 48h old at the start of the test.

During the exposure period the overlying water was renewed three times a week (75% renewal). Organisms were fed daily with Tetramin® (Melle, Germany) at 0.5 mg per organism per day for the first 10 days and 1 mg per organism per day for the remaining 18 days. Experiments were conducted at 20 ± 2 C° and under a light regime of 12h light and 12h dark. Temperature, pH, dissolved oxygen, hardness and ammonia were measured three times a week (i.e. before water renewal). Water samples were taken with a pipette from approximately 1 cm above the sediment surface without causing any disturbance

(to avoid contamination of the overlying water with sediment particles).

On day 14, five of the replicate vessels per concentration were sieved through a 200 µm sieve to remove the surviving larva and allow assessment of survival and growth. Recovered organisms were placed overnight in EPA medium in order to empty their digestive tracts. All organisms from each replicate were subsequently placed into pre-weighed square boats of aluminium foil, dried at 60°C for 24 hours and weighed. The average weight of individual larvae was obtained by dividing the weight of pooled larva in each replicate by the number of dried larvae.

After the removal of the 5 replicates for survival and growth assessment, the remaining vessels were covered with emergence traps and daily checked for emerged midges until day 28 of testing i.e. when the test was terminated. Newly emerged adults were collected daily, counted and recorded. This practice was continued until 100% emergence was achieved in a given replicate. Only those that had successfully broken free from the pupal skin were considered to have successfully emerged.

Statistical methods used for survival and emergence are outlined in a biometry book authored by Sokal and Rohlf, (1981). Data were arcsine square root transformed before analysis. Data for survival, growth and emergence were then tested for normality and homogeneity using the Shapiro-Wilkinson test. The differences between different treatments were determined with ANOVA (Fisher LSD test). The LC_{50} after 14 days were Bland J M, and Altman D.G (1998). calculated using the probit analysis methods according to Finney, (1971). Median emergence times (EmT50) were calculated using the Kaplain & Meier Method (Bland and Altman, 1998). Emergence times of each treatment were compared using the Mann-Whitney U test. All the statistical analysis was done with the Statistica™ 6.0 software.

RESULTS & DISCUSSION

Dry weight determination of the sediment revealed that the sediment contained 30% water. The monitoring results of the physico-chemical conditions during the 28 days period are

summarized in Table 1. Average temperature was 20°C ± 1.5°C, and the oxygen level in the water column never dropped below 4.2 mg/L. The average pH ranged between 7.4 and 7.7 and measured ammonia levels were always below 4.7 mg/L. The increase in levels of ammonia in the two highest Hg concentrations may be attributed to the accumulation and decomposition of food (Tetramin®) due to the high larva mortality which was observed in these treatments. Nevertheless, all the physico-chemical parameters were within the range recommended by OECD protocol (2004) for *Chironomus riparius* testing (OECD, 2004).

Measured Hg-concentrations in the sediment and pore water at day 0 are reported in Table 2. The measured values in the spiked sediment ranged from 52 to 75% of the nominal values. This can be expected as there is always a fraction of the spiked Hg that is not bound to the sediment, but remains in solution. Also, another fraction of Hg probably adsorbs to the mixing container walls and additional loss into the overlying water may occur during equilibration (and which was discarded after 7 days of equilibration).

The bioassay results on the survival, growth and emergence of *C. riparius* are presented in Table 2. There was no significant difference in mortality between organisms exposed to 0, 0.59 and 0.93 mg Hg /kg dry wt measured concentrations and that of the control larvae. The control and 0.59 mg/kg dry wt treatment had the same high survival (98%), while organisms exposed to 0.93 and 2.42 mg/kg dry wt Hg

exhibited a reduced survival (88% and 80%, respectively). The survival in the sediments containing 3.84 mg Hg/kg dry wt and 7.2 mg Hg/kg dry wt was significantly lower than that of the control group, i.e. 26% and 4%, respectively ($p < 0.05$). Based on these results, a 14 day LC_{50} value of 3.40 mg Hg/ kg dry wt (95% CL 2.6 - 4.2 mg Hg/ kg dry wt) was calculated while the NOEC and LOEC values (for survival) were 2.42 to 3.84 mg Hg/kg weight respectively.

No significant difference in dry body weight of larvae in the controls and those exposed for 14 days to 0.59 and 0.93 mg Hg/kg dry wt was noted ($p > 0.05$). Significant growth inhibition was observed in organisms exposed to 2.42 and 3.84 mg Hg/kg dry wt. The mean dry body weight of larvae from the 7.2 mg Hg /kg dry wt spiked sediment is not reported as the small size of the recovered larvae did not allow accurate measurement and meaningful interpretation. Based on these results, the 14 day NOEC and LOEC values based on growth were 0.93 to 2.42 mg Hg/ kg dry weight, respectively.

Emergence after 28 days of exposure in the control group (88%) was higher than the 70% validity criterion prescribed by the OECD (2004). No significant effects (compared to the control) were observed for the three lowest Hg treatments (i.e. 0.59, 0.93 and 2.42 mg/kg dry wt (Table 2). However, the emergence success of the organisms exposed to 3.84 mg Hg/kg dry wt was significantly lower than that in the control sediment ($p < 0.05$). No midges emerged from the sediment

Table 1. Mean values (+/- SD) of the different physico-chemical parameters monitored during the 28 days exposure period

Nominal Hg-conc. (mg/kg dry wt)	pH	Temperature (°C)	Ammonia (mg/L)	Hardness (mg/L CaCO$_3$)	Oxygen (mg/L)
Control	7.42±0.12	20.2 ± 0.71	0.0	252.20 ± 49.61	5.18 ± 1.30
1	7.62±0.12	19.9 ± 0.43	0.0	234.30 ± 50.87	4.90 ± 0.93
1.8	7.74±0.12	19.7 ± 0.47	0.0	178.00 ± 47.70	5.08 ± 0.79
3.2	7.64±0.23	20.4± 0.76	0.0	213.60 ± 29.25	5.17 ± 0.84
5.6	7.51±0.16	20.1 ± 0.12	1.2±1.10	240.30 ± 37.90	5.23 ± 0.79
10	7.69±0.11	20.5± 0.43	3.0±0.20	213.60 ± 46.70	5.28 ± 0.85
18	8.69±0.11	20.5± 0.43	4.2±0.20	215.63 ± 46.70	6.28 ± 0.85

Table 2. Average (± SD) for survival, growth and emergence parameters of *C. riparius* to Hg spiked sediment

Nominal conc. (mg Hg/ kg dry wt)	Measured conc. in sediment (mg Hg/ kg dry wt	Measured conc. in pore water (µg/L)	14 d- surviva l %	14.d- growth (µg/organism)	28.d- emergence (%)	median emergence time – EmT50 (days)	day of first emergence
Control	<0.02	<0.02	98 ± 2	1,357.4±47.6	88±8	17.0±1.95	15
1	0.59	<0.02	98 ± 2	1,359.75±32.9	94±4	17.0±0.45	15
1.8	0.93	85	88 ± 9.7	1,219.8±65.3	94±6.8	22.0±1.14*	17
3.2	2.42	142	80 ± 5.5	624.4±25.88 *	74±7.5	24.8±0.84*	21
5.6	3.84	316	26 ± 2.4*	304±4.0*	8±4*	27.0±0.8*	26
10	7.20	512	4 ± 2.4*	n.a	0	n.a	n.a
18	12.68	802	0	n.a	0	n.a	n.a

n.a = not applicable, * significantly different from control (p<0.05)

treated with 7.2 mg/kg dry wt. Furthermore, midges in the control and lowest Hg concentration emerged significantly earlier (p<0.05) than those exposed to the higher Hg levels (0.93, 2.42 and 3.84mg Hg/kg dry wt) as reflected by the EmT50 given in Table 2.

Information on the effects of sediment-associated mercury to life history characteristics of benthic organisms is very scarce. The current study was aimed at investigating the toxicity of inorganic mercury ($HgCl_2$) to the freshwater midge *C. riparius*. The results show that no significant effects on mortality, growth and emergence of *C riparius* occur at or below 0.93 mg Hg/kg dry weight. Median emergence time was the most sensitive of all endpoints evaluated. Our findings are consistent with results from the previous studies, which indicated that mercury is toxic to Chironomus larvae in the range of 316 to 1800 µg/L.

Previous studies performed with *C. riparius* and using water only exposures, reported 24, 48, and 96 h LC_{50}s to be in the range of 316-1800 and 400-547 µg/L, respectively (Quareshi *et al.,* 1980 and Rossaro *et al.,* 1986). Assuming that pore water was the main route of mercury exposure to *C. riparius* in the present study, the calculated

LC_{50s} is 250µg/L which is lower than ones calculated by Quareshi *et al.,* (1980) and Rossaro *et al.,* (1986). The difference between these results may be attributed to longer exposure period used in our study (14 days) compared to 24, 48 and 96 hours in the previous studies. Additionally, we used younger animals (48 hrs old) compared to 14 days old organisms used by the former authors. Young animals are mostly more sensitive to pollutants compared to older ones (Ristola *et al.,* 1999). Alternatively, it can be urged that there was an additional exposure through the solid phase of the Hg contaminated sediment as *Chironomus riparius* is a benthic detritivore, which can process large volume of contaminated sediment (Rasmussen, 1984 and Armitage *et al.,* 1995). Saouter et al. (1993) demonstrated that when mayfly larvae (benthic detritivore) were exposed to Hg via sediment, the gut contributed more Hg than the gills, which was an indication that the sediment exposure through the gut is important.

Based on dry weight, larvae growth was significantly reduced at day 14 in a concentration dependent manner from 2.42 mg Hg/ kg dry wt onwards. Growth of *Chironomus* larva has been reported to be influenced by level of feeding (Sibley

et al., 1997; Péry *et al.,* 2002; Haas De *et al,* 2006). In our study feeding was done uniformly for all tested Hg concentrations as recommended by OECD, (2004). It can therefore be argued that growth reduction of the larva in higher Hg concentrations might have been caused by mercury toxicity. Alternatively, it has been reported that Hg is capable of causing mandible deformation in C. *riparius* larva (Vermeulen *et al.,* 2000). As mandibles are important apparatus for feeding, such deformation is likely to reduce the feeding ability of the larva and result in poor growth.

The trend of midge emergence followed a similar trend as that observed for survival and growth, i.e. midges exposed to the three lowest Hg concentrations (0.59, 0.93 and 2.42 mg/kg dry wt) did not exhibit a significantly different emergence success compared to the control. There was, however, a drastic drop in the percentage of emerged midges from 74% at 2.42 mg/kg dry wt to 8% at 3.84 mg/kg dry wt and no midge emerged from 7.2 mg/kg Hg treatment. It has been suggested that for *Chironomus* midges emergence, there is a minimum weight threshold to be attained by the larvae for emergence to occur Hilsenhoff, *et al.,*(1966) and Ristola *et al.,*(1999). It is possible that the larva exposed to 3.84 and 7.2 mg/kg Hg spiked sediments did not attain the minimum weight to allow pupation and eventual emergence. For C. *tentans,* a related species to C. *riparius,* a minimum dry weight threshold for emergence was shown to be ranging from 500 to 800 μg/larvae (Schubauer-Berigan *et al,.* 1993). Based on the weight measurement obtained after 28 days in the current study, dry body weight exhibited by the larva (304 ± 4.0μg/larvae) from this treatment was much lower than the range suggested above.

Furthermore, these results indicate that emergence time (EmT50's) of the individual larvae was the most sensitive of all endpoints tested as it was the only parameter significantly affected at concentration of 0.93 mg Hg/kg dry wt (Table 2). At this concentration there was a 2 day delay in emergence compared to the control. In general, our results are in agreement with other authors who showed that for C. *riparius*, larval dry weight and emergence time are more sensitive than survival and % emergence (Kimberly *et al.*, 2004).

CONCLUSION

Results from this study have contributed to our understanding of the effects of Hg to benthic macroinvertebrates at environmental realistic sediment concentrations. Concentrations at which adverse effects were observed in this study have been reported to occur in several aquatic ecosystems; for example, in rivers and streams occurring around artisanal gold mining impacted areas in Africa. It is therefore, possible that *Chironomus* spp and other fauna inhabiting artisanal gold mining contaminated water bodies are at risk.

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