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Mortality and Abnormalities Observed After Experimental Hg Exposure in the Polychaete *Eurythoe complanata* (Pallas) from Mazatlan, Mexico

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Abstract Lethal effects of Hg on *Eurythoe complanata* held under laboratory conditions were evaluated (LC_{50} and LT_{50}). Worms were exposed to 0–900 µg/L of Hg for 10 days. Mortality occurred in all the treatments, being faster at 200–900 µg/L, which was confirmed by a Friedman ANOVA non-parametric test. The 4-day $LC_{50} = 197.15$ µg/L (200 µg/L $LT_{50} = 3.4$ days) was similar to that reported for other Hg tolerant annelids. Abnormalities were observed in worms exposed to all the treatments, becoming more severe as Hg concentrations increased: body darkening, rough, white and opaque skin, everted and swollen proboscis and gut evisceration.

Keywords Fireworm · Hg · Metal · LC_{50} · LT_{50}

Mercury is one of the most toxic metals for polychaetes (Reish et al. 1976; Reish 1988). Field concentrations reported for coastal areas can reach up to 0.06 µg Hg/L (Roth and Hornung 1977); however, levels recorded from factory effluents from several chloralkali plants in Europe, which eventually discharge into the coastal systems, can range from 1,600 to 7,600 µg/L (von Canstein et al. 1999). Some polychaete species have been considered as good candidates for ecotoxicological studies in the laboratory (Reish 1980). Effects of Hg on cultured annelids have been poorly studied on polychaete mortality (Reish et al. 1976; Reish and Carr 1978; Reish 1988; Reish and LeMay 1991) and oligochaete morphological abnormalities (Vidal and Horne 2003).

The fireworm *Eurythoe complanata* (Pallas 1766) is widely distributed in tropical and sub-tropical regions especially on both sides of the American continent, in intertidal and sublittoral rocky shores (Fauchald 1977). It has been used as a test organism for toxicological studies in the laboratory (Reish et al. 1989; Marcano et al. 1996; Nusetti et al. 1998), but none of these bioassays have been performed with Hg. Recently, we studied bioaccumulation and elimination of Hg on this species kept in laboratory conditions (Vázquez-Núñez et al. 2007). Thus, the aim of this study was to analyze the effect of Hg on mortality of *E. complanata* after exposure to solutions with different concentrations under laboratory conditions. Moreover, abnormalities produced by this metal, which have not been reported previously, are described here.

Materials and Methods

Worms were collected under rocks from the mesolittoral zone of Cerritos Beach, Mazatlan (23°18′30″N 106°29′22″W) during August 2003. About 300 organisms (13–15 cm length) were maintained in aquarium tanks containing 20 L of seawater (34 PSU), a 5 cm layer of sand, and rocks with attached algae from the same collection site, to simulate natural conditions. Stock cultures were maintained at room temperature (26–28°C) in semi-dark conditions for 17 days prior to the experiments. The rocks were replaced every 8 days to allow animals to feed on the algae. In addition, 1 g of fish food (Sera Vipan) was added weekly as a complementary food source. Water exchange was performed every 4 days.

In order to select the Hg concentrations, data from the literature were taken into account. Reish et al. (1976) and

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Reish and Carr (1978) indicated 96 h-LC_{50} values ranging from 22 μg/L (*Neanthes arenaceodentata*) to 1,000 μg/L (*Capitella capitata*). Recently, Vidal and Horne (2003) exposed the oligochaete *Sparganophilus pearsei* to Hg solutions from 10 to 2,400 μg/L. Based on this information and on a preliminary screening test with a wide range of Hg concentrations (1 to 1,000 μg/L on logarithmic scale) performed in our laboratory, the experimental concentrations were (0, 100, 200, 300, 400, 500, 600, 700, 800, and 900 μg/L). A stock solution (10^6 μg Hg/L) was prepared using mercuric chloride ($HgCl_2$) diluted in filtered (<10 μm) and UV-purified seawater (INSTA PURA filter) with a salinity of 34 PSU. To prepare the experimental solutions with initial concentrations of 100, 200, 300, 400, 500, 600, 700, 800, and 900 μg/L, the stock solution was diluted using filtered (<10 μm) and UV-purified seawater. The Hg concentrations of these solutions were determined previous to the experiment by cold vapor atomic adsorption spectrophotometry (CV-AAS), using $SnCl_2$ as a reducing agent (Loring and Rantala 1992), with a Hg analyzer (Buck Scientific).

Ten individuals per treatment (two replicates) were examined and the duration of this bioassay was 10 days. Worms were placed in 3 L (base: 12 × 20 cm; high: 12 cm) plastic aquarium tanks containing 1 L of aerated Hg solution or filtered (<10 μm) and UV-purified seawater (INSTA PURA filter), with a salinity of 34 PSU for the controls. They were maintained at 26–28°C in the dark. Daily observations were performed in order to observe mortality and possible abnormalities produced by Hg exposure. When dead animals were observed, they were removed from the aquaria.

Since data were not normally distributed, the effect of Hg concentrations on *E. complanata* mortality was tested by the Friedman ANOVA non-parametric test (Zar 1996) for mean differences (paired test) over the experimental time (n = 11 observations, considering days 0–10). The mean daily mortality percentages for each treatment were compared using the Statistica ver. 5.5, 2000 program. The LC_{50} and LT_{50} were determined for different periods of time and concentrations, respectively by the probit method (Finney 1971), which consists of a straight-line graphical interpolation (Probit, ver. 1.0, 1990 program).

Results and Discussion

Total mortality was observed in all the tested Hg concentrations. Worms exposed to 100 μg/L began to die at day 6, but did not survive at the end of the bioassay, while 100% mortality occurred faster in the 200–900 μg/L treatments (Fig. 1). The effect of Hg concentrations on *E. complanata* mortality was confirmed by the Friedman ANOVA non-parametric test, since significant differences were found between the control groups, and all the Hg treatments (Table 1). Moreover, significant differences were observed among the lower Hg treatments (100–300 μg/L). The lack of significant differences in the highest concentrations (400–900 μg/L) was due to the high mortality at the beginning of the experiment (Fig. 1).

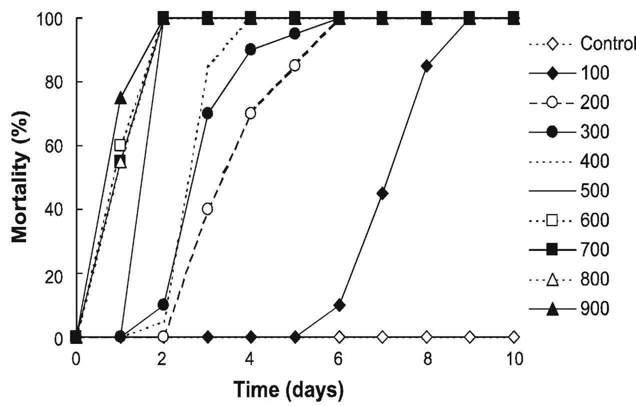


Fig. 1 Mortality of *Eurythoe complanata* exposed to the different Hg concentrations (μg/L)

Table 1 Friedman ANOVA paired test for effects of Hg concentrations on *E. complanata* mortality (n = 11; df = 1) (* $p < 0.05$)

	Controls		100 μg/L		200 μg/L		300 μg/L		400 μg/L		500 μg/L		600 μg/L		700 μg/L		800 μg/L	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
100 μg/L	5	0.025*																
200 μg/L	8	0.005*	6	0.014*														
300 μg/L	9	0.003*	7	0.008*	4	0.046*												
400 μg/L	9	0.003*	7	0.008*	4	0.046*	1	0.317										
500 μg/L	9	0.003*	7	0.008*	4	0.046*	4	0.046*	2	0.157								
600 μg/L	10	0.002*	8	0.005*	5	0.025*	5	0.025*	3	0.083	1	0.317						
700 μg/L	10	0.002*	8	0.005*	5	0.025*	5	0.025*	3	0.083	1	0.317	1	0.317				
800 μg/L	10	0.002*	8	0.005*	5	0.025*	5	0.025*	3	0.083	1	0.317	1	0.317	–	–		
900 μg/L	10	0.002*	8	0.005*	5	0.025*	5	0.025*	3	0.083	1	0.317	1	0.317	1	0.317	1	0.317

Table 2 LC_{50} and LT_{50} values with 95% confidence limits

Days	LC_{50} (μg/L)	Lower limit (μg/L)	Upper limit (μg/L)	Hg (μg/L)	LT_{50} (days)	Lower limit (days)	Upper limit (days)
2	443.6	–	–	100	7.2	6.8	7.5
3	329.3	122.8	462.2	200	3.4	2.8	3.8
4	197.2	0.8	308.2				
5	161.2	–	–				
6	139.5	25.0	222.6				
7	84.4	38.4	123.6				

Results of LC_{50} and LT_{50} testing were reported only for the cases when data fitted significantly the probit lines (Table 2). The LC_{50} value obtained here at day 4 is in the same order of magnitude as those reported for the polychaetes *N. arenaceodentata* (96-h LC_{50} = 150 μg/L) and *Ophryotrocha labronica* (96-h LC_{50} = 160 μg/L), studied by Reish and LeMay (1991). According to these authors, these species were more tolerant to Hg than *C. capitata* (96-h LC_{50} = 47 μg/L), *Nereis grubei* (96-h LC_{50} = 90 μg/L) and *Pectinaria californiensis* (96-h LC_{50} = 60 μg/L) tested in simultaneous experiments. Similar results were obtained at pH = 7 and 10°C for the oligochaetes *Limnodrilus hoffmeisteri* (96-h LC_{50} = 150 μg/L), *Stylodrilus heringianus* (96-h LC_{50} = 140 and 180 μg/L) and *Tubifex tubifex* (96-h LC_{50} = 140 μg/L) (Chapman et al. 1982). Comparisons with these tolerant polychaete and oligochaete species suggest that *E. complanata* could be considered as a candidate to be a biomonitor species for Hg contaminated zones. To strengthen this suggestion, several toxicological studies should be performed using this species.

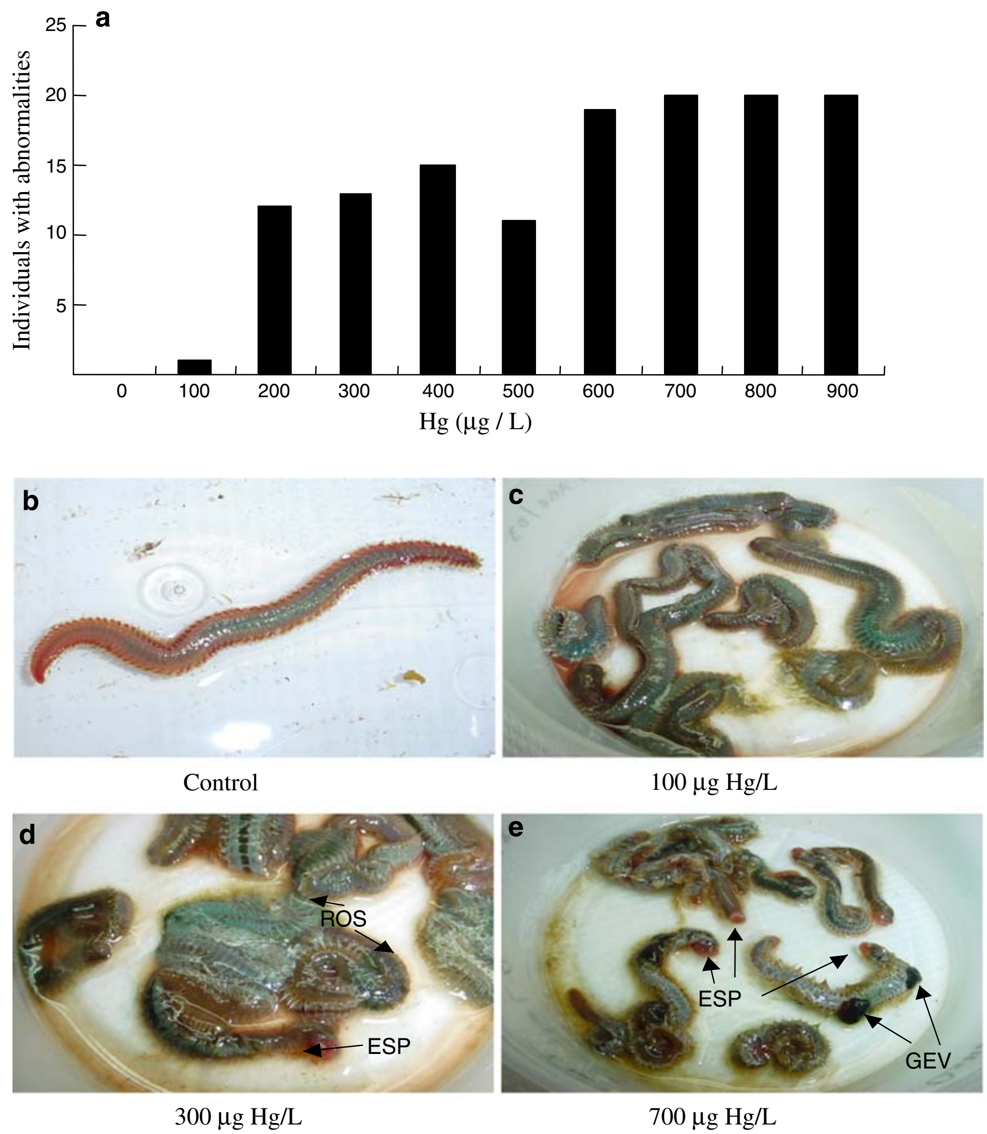
Comparisons of LC_{50} and LT_{50} data should be interpreted with caution since differences in experimental periods as well as variations in experimental conditions can produce completely different results (Reish and Carr 1978). For instance, Hg 96-h LC_{50} results obtained by Reish et al. (1976) and Reish and LeMay (1991), differed during several experiments with same analysed polychaete species like *C. capitata* (<100 vs. 47 μg/L, respectively) and *N. arenaceodentata* (22 vs. 150 μg/L, respectively). According to these authors, it is unknown if differences are due to genetic adaptation or differences in the experimental protocols. Moreover, Sturmbauer et al. (1999) demonstrated that LC_{50} values were highly variable among different natural populations of the oligochaete *T. tubifex* exposed to several Cd concentrations and concluded that different degrees in resistance could be due, in part, to differences in genotype compositions.

Several abnormalities were observed in worms exposed to all the treatments, and became more severe as Hg concentrations increased (Fig. 2). These observations were recorded once the worms died. Individuals from the 100 μg/L treatment experienced a change in color from brownish-orange to gray, which became darker at 200 μg/L Hg. Under 300 μg/L, the skin of worms became rough and opaque, turning white in color. Moreover, the proboscis looked everted and swollen. These abnormalities were also observed in worms exposed to 400–600 μg/L. At 700 μg/L, worms had the swollen proboscis and their skin began to break provoking a gut evisceration. These abnormalities were also observed in the 800 and 900 μg/L treatments. The number of individuals with abnormalities increased with increasing Hg concentrations, as shown in Fig. 2. None of these abnormalities was observed in worms that died in natural conditions (i.e., from the stock culture and from the controls after the experiments), which indicates that they were produced by Hg exposure.

Most of the toxicological studies in laboratory performed with annelids are focused on testing, quantitatively, dose-response relationships at ecological, physiological and biochemical levels, but external abnormalities produced by toxicants are rarely described. For instance, Lucan-Bouché et al. (1999) found that Cu and Pb exposure produced abnormal caudal regions (completely missing or regenerating) on the oligochaete *T. tubifex*. Geracitano et al. (2004) observed histological and morphological abnormalities in the polychaete *Laeonereis acuta* caused by Cu exposure. Previous studies performed with *E. complanata* have reported injuries at an immunological level. Marcano et al. (1997) and Nusetti et al. (1998) demonstrated the immunosuppressor action of sublethal doses of Cu, which provoked a decrease in phagocytosis as well as in the development of eritrocitic and secretory structures, but external abnormalities were not mentioned. The autotomy experiments performed by Vidal and Horne (2003) with the oligochaete *S. pearsei* exposed to Hg (10–2,000 μg/L) showed that, in most cases, the metamere caudal region formed sphere-like shapes with a “rosary bead” appearance. The strong macroscopic abnormalities provoked by Hg in *E. complanata* described in this study had not been previously reported for polychaetes.

To date there are no protocols at physiological or ecological levels to study the effects of Hg on *E. complanata*. Vázquez-Núñez et al. (2007) proposed this species as a possible ecotoxicological test organism based on

Fig. 2 **a** Number of individuals of *Eurythoe complanata* with abnormalities assessed at the time of death under different Hg concentrations. Abnormalities: **b** none, **c** dark body, **d** rough and opaque skin (ROS) and everted and swollen proboscis (ESP), **e** ESP and gut evisceration (GEV)



bioaccumulation and elimination experiences. In this study, information about the effect of Hg on polychaete mortality (Reish et al. 1976; Reish and Carr 1978; Reish 1988) was considered to design our experiment. Although the experimental Hg concentrations were relatively high, the sensitivity to Hg from 200 to 900 µg/L exhibited by *E. complanata*, as well as its size (i.e., 13–15 cm length), and its relatively easy collection and culture suggest that this species could be a good candidate to be a test species after the performance of physiological, ecological and biochemical studies. In addition, histological studies to closely observe abnormalities produced by this metal should be done.

This study constitutes the first report of LC_{50} and LT_{50} data for *E. complanata* exposed to Hg in solution under laboratory conditions. The mortality recorded from 200 µg/L and the macroscopic abnormalities observed in all the treatments could have important ecological implications for natural populations, since ecosystems with concentrations as high as 100 µg/L, like those reported for sporadic discharges of industrial wastewater by von Canstein et al. (1999) could produce serious damage to polychaetes or other benthic communities.

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References

Chapman PM, Farrel MA, Brinkhurst RO (1982) Effects of species interactions on the survival and respiration of *Limnodrilus hoffmeisteri* and *Tubifex tubifex* (Oligochaeta, Tubificidae) exposed to various pollutants and environmental factors. Water Res 16:1405–1408

Fauchald K (1977) Polychaetes from intertidal areas in Panama with a review of previous shallow-water records. Smithsonian Contr Zool 221:1–81

Finney DJ (1971) Probit analysis. Cambridge University Press, Cambridge

Geracitano LA, Luquet C, Monserrat JM, Bianchini A (2004) Histological and morphological alterations induced by copper exposure in *Laeonereis acuta* (Polychaeta, Nereididae). Mar Environ Res 58:263–267

Loring OH, Rantala RTT (1992) Manual for the geochemical analysis of marine sediments and suspended particulated matter. Reference methods of marine pollution studies No 63

Lucan-Bouché ML, Biagianti-Risbourg S, Arsac F, Vernet G (1999) An original decontamination process developed by the aquatic oligochaete *Tubifex tubifex* exposed to copper and lead. Aquat Toxicol 45:9–17

Marcano L, Nusetti O, Rodríguez-Grau R, Vilas J (1996) Uptake and depuration of copper and zinc in relation to metal-binding protein in the polychaete *Eurythoe complanata*. Comp Biochem Physiol Part C Pharmacol Toxicol Endoc 114:179–184

Marcano L, Nusetti O, Rodríguez-Grau R, Briceño J, Vilas J (1997) Coelomic fluid lysozym activity induction in the polychaete *Eurythoe complanata* as a biomarker of heavy metal toxicity. Bull Environ Contam Toxicol 59:22–28

Nusetti O, Salazar-Lugo R, Rodríguez-Grau J, Vilas J (1998) Immune and biochemical responses of the polychaete *Eurythoe complanata* exposed to sublethal concentration of copper. Comp Biochem Physiol Part C Pharmacol Toxicol Endoc 119:177–183

Pallas PS (1766) Miscellanea zoologica quibus novae imprimis atque obscurae Animalium species describuntur et observationibus iconibusque illustrantur. Hague Comitum, pp 1–224

Reish DJ (1980) Use of Polychaetous Annelids as test organisms for marine bioassay experiments. In: Buikema AL Jr, Cairns J Jr (eds) Aquatic invertebrate bioassays. ASTM STP 715. American Society for Testing and Materials, Philadelphia, pp 140–154

Reish DJ (1988) The use of toxicity testing in marine environmental research. In: Soule DF, Kleppel GS (eds) Marine organisms as indicators. Springer-Verlag, New York, pp 231–245

Reish DJ, Carr RS (1978) The effect of heavy metals on the survival, reproduction, development and life cycles of two species of polychaetous annelids. Mar Pollut Bull 9:24–27

Reish DJ, LeMay JA (1991) Toxicity and bioconcentration of metals and organic compounds by Polychaeta. Ophelia Suppl 5:653–660

Reish DJ, Martin JM, Plitz FM, Word JQ (1976) The effect of heavy metals on laboratory populations of two polychaetes with comparison to the water quality conditions and standards in Southern California marine waters. Water Res 10:299–302

Reish DJ, Asato SL, LeMay JA (1989) The effect of cadmium and DDT on the survival and regeneration in the amphinomid polychaete *Eurythoe complanata.* VII Simposio Internacional de Biología Marina, 1–5 June 1989. La Paz, México

Roth I, Hornung H (1977) Heavy metal concentrations in water, sediments and fish from Mediterranean coastal area, Israel. Environ Sci Technol 11:265–269

Sturmbauer C, Opadiya BGB, Niederstätter H, Riedmann A, Dallinger R (1999) Mitochondrial DNA reveals cryptic oligochaete species differing in cadmium resistance. Mol Biol Evol 16:967–974

Vázquez-Núñez R, Méndez N, Green-Ruíz C (2007) Bioaccumulation and elimination of Hg in the fireworm *Eurythoe complanata* (Annelida: Polychaeta) from Mazatlan, Mexico. Arch Environ Contam Toxicol 52:541–548

Vidal DE, Horne AJ (2003) Mercury toxicity in the aquatic oligochaete *Sparganophilus pearsei* II: autotomy as a novel form of protection. Arch Environ Contam Toxicol 45:462–467

von Canstein H, Li Y, Timmis KN, Deckwer WD, Wagner-Döbler I (1999) Removal of mercury from chloralkali electrolysis wastewater by a mercury-resistant *Pseudomonas putida* strain. Appl Environ Microbiol 65:5279–5284

Zar JH (1996) Biostatistical analysis. Prentice Hall, New Jersey