Bull. Environ. Contam. Toxicol. (1986) 37:402–406

Bulletin of Environmental Contamination and Toxicology

Accumulation of Mercury in Larvae and Adults, *Chironomus riparius* (Meigen)

B. Rossaro,[1] G. F. Gaggino,[2] and R. Marchetti[1,2]

[1]Department of Biology, University of Milan, Via Celoria 26, 20133 Milano and
[2]Water Research Institute (I.R.S.A.), Via Occhiate, 20100 Brugherio, Italy

Among benthic aquatic insects there are taxa that grow abundant in sediments polluted with organic matter. Some of them also tolerate high levels of heavy metals. For example, Winner et al. (1980) observed that there are only Tubificid worms and Chironomids (among aquatic insects) in the more heavily polluted ections of Shayler Run stream. On the other hand, Yasuno et al. (1983) found that Chironomids do not survive in very polluted rivers, with high heavy metal concentrations, but these aquatic insects can tolerate moderate metal pollution.

In this research short exposure and partial life cycle tests (Anderson 1980) were carried out to evaluate the accumulation of mercury in Chironomus riparius Meigen larvae, pupal exuviae and adults from water enriched with $HgCl_2$. Their abundance in heavily polluted waters and the fact that it is easy to rear them (Credland 1973) suggested the use of this species for the toxicity tests considered in our present research.

MATERIALS AND METHODS

Short exposure tests were carried out to evaluate the LC_{50} of $HgCl_2$ for the 4th instar larva of C. riparius Meigen. Stock solutions were prepared by dissolving $HgCl_2$ in slightly acidified tap water with hardness of 50 mg/l $CaCO_3$ and a pH of 6.8. Concentrations from 50 ppb to 30 ppm of Hg were used.

The tests were conducted with an intermittent-flow exposure system. The test chambers were plexiglass. The stock solutions were stored in glass Mariotte bottles with a capacity of about 50 l. A 6-channel toxicant injection system was used to deliver 5 toxicant concentrations and a control. To avoid cannibalism among larvae, single larvae were tested, with a total of 12 larvae at each Hg concentration. Batch experiments in glass dishes containing 200 ml of test solution were also carried out with the test solution changed every 24 hours.

A partial life cycle test was carried out to evaluate the accumulation of Hg by larvae exposed to sublethal concentrations and the transfer of metal into pupal exuviae and adults. 5 plexiglass chambers (square base 900 cm^2, height 40 cm), each containing about 100 larvae, were made. The concentrations of Hg in the water ranged from 2 to 9 ppb in the treated samples and from 0 to 0.52 ppb in the control. A continuous flow of the toxic solution at 0.6 ml/min per liter was maintained for 30 days. Samples of larvae, pupal exuviae and adults were collected at intervals of about 48 hours and their mercury contents analyzed. Addition of food (pellets) was necessary at the beginning of the experiment for pupation to occur and to avoid cannibalism among the larvae. There was no further addition of food during the accumulation test.

Both the short term and the partial life cycle tests were carried out at room temperature, about 20 °C.

The mercury concentration in the water (ppb) was analyzed by flameless atomic absorption, using the volatile hydride technique, with $NaBH_4$ in alkaline medium. The samples were dissolved in concentrated nitric acid (Julshamn et al. 1975), as modified by Gaggino (1982). Test specimens were rinsed with tap water before the analysis. The concentrations in the Chironomids are reported as ppm Hg for fresh body weight

RESULTS AND DISCUSSION

The results of the short-term toxicity tests are summarized in Table 1. The LC_{50} was higher in the static tests than in the intermittent flow tests. The bioaccumulation tests were carried out with an intermittent flow of tap water containing a mean value of 5.5 ppb Hg, with the concentration for the controls 0.20 ppb. The concentration of 5.5 ppb apparently has no effect on the emergence of adults: about 70% of the larvae reached the adult stage in both control and treated samples. In the larvae, we always observed mean values of less than 8.0 ppm in untreated samples, while the concentration in the treated larvae increased from 10.7 ppm after 24 hours to 107.6 ppm after 29 days (fig. 1). Increasing concentrations of Hg during treatment are also observed in the emerging adults, but the levels are lower than in the larvae, always less than 40 ppm. Pupal exuviae have higher concentrations than the adults and lower than the larvae. The pupal exuviae concentration of Hg rose from 27 ppm after 6 days to 88 ppm after 27 days. The concentrations that cause 50% mortality of the larvae of C. riparius are rather high, but not much higher than

Table 1. Short term toxicity test results: a and b are the intercept and the slope of the regression lines with probits and log10 concentrations as dependent and independent variable respectively, calculated at 3 different exposure time

Exposure time hours	LC_{50} ppb Hg	Fiducial limits		a	b	DF
		Intermittent flow tests				
24	1074	760	1520	4.94	1.83	17
48	316	230	440	6.24	2.48	17
96	100	50	180	7.01	2.01	17
		Static tests				
24	1028	880	1200	4.90	8.39	21
48	750	660	850	5.83	6.65	21
96	547	480	630	6.66	6.34	20

that found for rainbow trout (Salmo gairdneri), which is 500 ppb after 76 hours at 18 °C (Boudou et al. 1980). We found LC_{50} values lower than those for Carassius carassius found by the same author, 500 ppb after more than 10 days.
The accumulation of mercury in the different trophic levels of the food chain is a well known phenomenon (Boudou et al. 1981), but there is no information at present about the transfer of the metal from contaminated sediments to aquatic insect larvae and from them to the emerging adults. Nuorteva et al.(1982) analyzed the fate of mercury in sarcosaprophagous insects (Calliphoridae) and in their predators, and have shown that higher levels along the trophic chain concentrate the metal, especially in the methylated form, but that adults have mechanisms to rid themselves of a part of the toxic element and conclude that the larvae of sarcosaprophagous insects, which might eat contaminated flesh, are adapted to tolerate high mercury levels. The situation is similar for C. riparius Mg, which has an aquatic larva that is tolerant to organic pollution and appears to be able to accumulate mercury in the larval body. Passive adsorption of mercury on to the larval surface accounts for a small part of the total mercury present in the larva only: 100 larvae kept for only 1 minute in water containing 5 ppb of Hg and dried on filter paper had a total concentration of 9.32 ppm in the body. The bioaccumulation factor is calculated from the data in Table 2 to be 12600 for larvae and 12470 for pupal exuviae with respect to the water content. On the other hand, in the adults there is some means for getting rid of a portion of the toxic element. The concentration in the adults is only 29% of that in the larvae. Because larval density is expected to be very high in sediments with high organic matter contents (1000-10000 specimens per square meter and above), assuming a mean fresh weight of about 20 mg per larva, we would expect, from our results, to find that in a water polluted with 5 ppb of mercury, about 70 ppm would be present in the larvae and somewhat less in the adults.
Biomagnification of toxic metals in insectivorous birds would become possible at this point, but this problem has not yet been studied.

Table 2. Mean mercury content in the water (ppb) and in different developmental stages of Chironomus riparius (ppm/fresh weight)

	Water (ppb)	larvae	pupal exuviae (ppm/fresh weight)	adults
Control				
Mean	0.23	4.20	19.54	7.76
St. dev.	0.22	1.90	9.36	13.28
Treated				
Mean	5.50	69.35	57.61	19.93
St. dev.	2.85	42.00	24.48	11.44

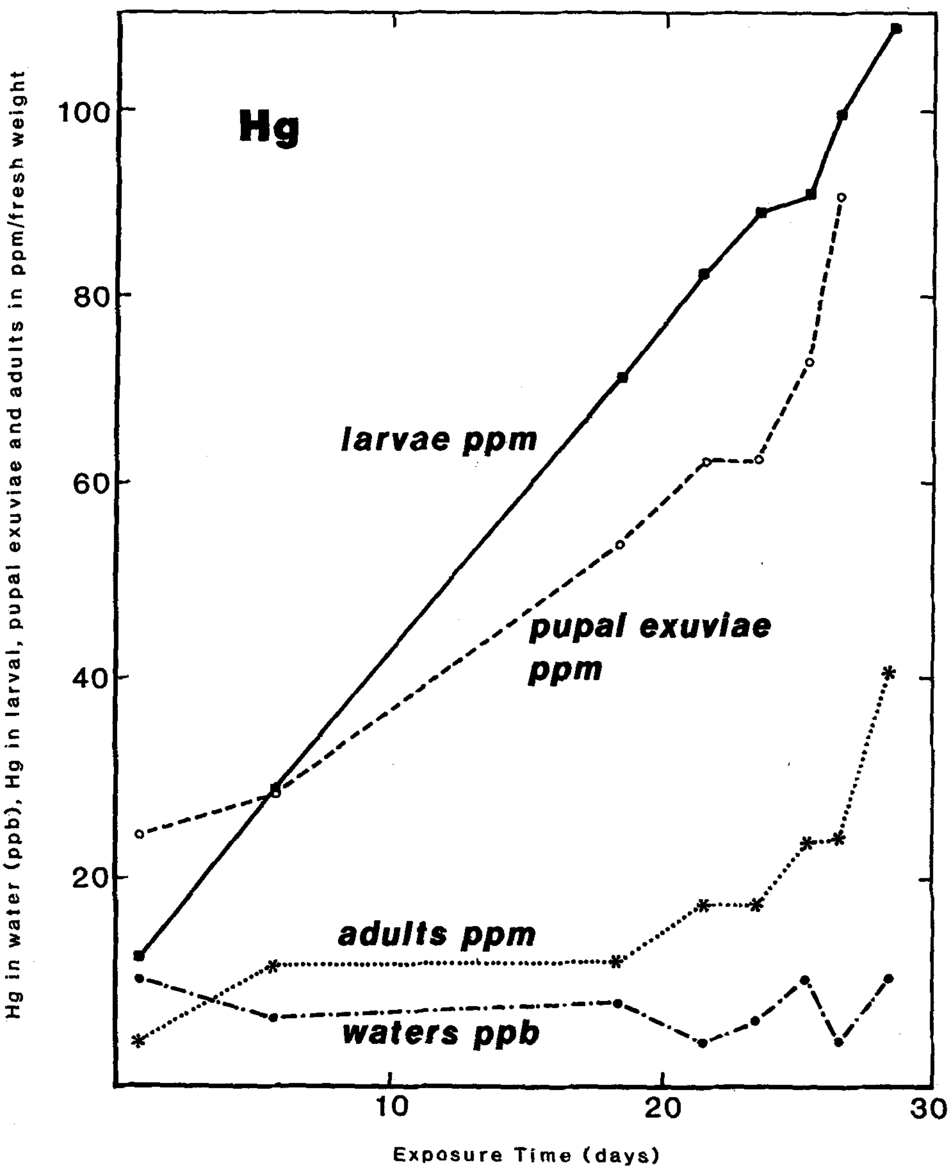

Figure 1. Partial life cycle test of bioaccumulation.

REFERENCES

Anderson R L (1980) Chironomidae toxicity tests-biological background and procedures. Aquatic invertebrate bioassays. In: Buikema A L Jr & Cairns J Jr (ed) ASTM-STP 715, American Society for Testing and Materials. Philadelphia, p 70-80

Boudou A, F Ribeyre, A Delarche & R Marty (1980) Bioaccumu= lation et bioamplification des derivs du mercure par un consommateur de troiseme ordre: Salmo gairdneri- Incidences du facteur temperature. Wat Res 14: 61-65

Boudou A & Ribeyre F (1981) Comparative study of the trophic transfer of two mercury compound $HgCl_2$ and CH_3HgCl between Chlorella vulgaris and Daphnia magna. Influence of temperature. Bull Environ Contam Toxicol 27:624-629

Credland P F (1973) A new method for establishing a permanent laboratory culture of Chironomus riparius Meigen. Freshwater Biology 3: 45-51

Gaggino G F (1982) Contenuti di alcuni metalli pesanti nella fauna ittica del fiume Po. Inquinamento 6:25-28

Julshamn K & Braekkan O R (1975) Determination of trace elements in fish tissues by the standard addition method. Atom Abs Newsl 14: 49-52

Nuorteva P, Nuorteva S L (1982) The fate of Mercury in Sarcosaprophagous Flies and in Insects Eating Them. Ambio,2(1): 34-37

Yasuno M, Hatakeyama H & Sugaya Y (1983) Characteristic distributions of Chironomids in the rivers polluted with heavy metals. XXII Congr. S.I.L. Lyon, 21-28/8/83:376

Winner R W, Boesel W M & Farrell M P(1980) Insect Community Structure as an Index of Heavy Metal Pollution in Lotic Ecosystems. Can J Fish Aquat Sci 37:647-655

Received September 28,1985; accepted January 20, 1986