Bull. Environ. Contam. Toxicol. (2000) 64:708-715

DOI: 10.1007/s001280000061

Bulletin of Environmental Contamination and Toxicology

Acute Toxicity of Arsenic to Three Species of New Zealand Chironomids: *Chironomus zealandicus, Chironomus* sp. a and *Polypedilum pavidus* (Diptera, Chironomidae)

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Received: 7 December 1999/Accepted: 10 February 2000

Acute toxicity tests are important in establishing appropriate water quality criteria and standards. It is important that the most sensitive life stage should be used in toxicity tests to properly estimate the sensitivity of the test animal and to make toxicity tests ecologically relevant (Williams et al. 1986). Many invertebrates including crustaceans, molluscs, and insects have been used as biotoxicity test organisms. However, the most widespread organisms used in toxicity tests have been genera of the family Chironomidae (Insecta: Diptera). Although different species of chironomid have frequently been used in acute toxicity tests, many of these tests have evaluated the sensitivity of only one stage of the life cycle, usually the 4th instar larvae. Data available for chironomids (Gauss et al. 1985; Williams et al. 1986) however, suggest that earlier life stages are more sensitive than later ones.

The common components of the benthos at many lakes in the central North Island of New Zealand are chironomids. The most common reprecentatives of chironomids in these lakes are *Chironomus zealandicus* (Hudson), *Chironomus* sp *a* and *Polypedilum pavidus* (Hutton). Arsenic enters the aquatic environment from a variety of natural and industrial sources and is generally regarded as highly toxic to all components of aquatic communities. However, relatively little work has been carried out on the toxicity of arsenic to freshwater macroinvertebrates. In New Zealand, dominant sources of As input into waterways are geothermal activity, herbicides and timber preservatives.

The objective of the present study was to investigate how the sensitivity of C. *zealandicus, C.* sp. a and *P. pavidus* vary with biological and non-biological facotors such as age, source system, sampling season, test temperature, and the valency state of arsenic.

MATERIALS AND METHODS

Three lake sites were chosen for the collection of wild 4th instar larvae, because each species was relatively more abundant at each of these sites.

Lake Ngaroto was chosen for C. sp. a and Sulphur Point and Hamurana Stream were chosen for *C. zealandicus* and *P. pavidus*, respectively.

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Three sets of experiments were carried out between November 1996 and June 1997, with each species to test the toxicity of arsenic as follows:

1. Each larval instar from the laboratory cultures and 4th larval instars collected from the wild were exposed to arsenic at 18°C.
2. Wild-collected 4th instar larvae of three species were tested for their sensitivity to arsenic at three different temperatures, 13°C, 18°C and 23°C.
3. The difference in sensitivity of the 4th instars of each species was compared (at 18°C) in larvae collected in two seasons, summer (November 1996) and winter (June 1997).

Test animals were carefully transferred with a paintbrush (4th instar) or a Pasteur pipette (earlier instars) into beakers containing soft synthetic water (USEPA 1991) at the test temperature and acclimatized for 24 hours before each test initiation. Beakers were gently aerated during the acclimation period.

Stock solutions of As^{3+} and As^{5+} were prepared from reagent grade sodium arsenite ($NaAsO_2$) and sodium arsenate ($Na2HAsO_4 7H_2O$), respectively. A final range of nominal concentrations for test solutions was determined by conducting preliminary range-finding tests with each instar of each species. Five concentrations of arsenic and a soft synthetic water control were run during this study. Glass beakers (150 mL) with 100 mL of test solution were used for 3rd and 4th instar larvae, and 25 mL beakers with 20 mL of test solution were used for 1st and 2nd instar larvae in each test. Each test beaker contained five larvae with three replicates per test. Each test was replicated 3 times. All replicates of a particular test were carried out at a same period. Test duration was 96 hours except tests with 1st instars which were terminated after 72 hours since these animals moult within 4 to 5 days of hatching. Test conditions were static without replacement of the test solution. Mortality was defined as lack of movement of the body, anal papillae, or mouthparts when subjected to a rapid stream of water from a Pasteur pipette or gentle probing (Gauss et al. 1985). Survival was determined at 24, 48, 72 and 96 hours, and dead larvae were removed from the test beakers.

Each set of tests was carried out in a temperature-controlled system with a photoperiod of 16:8 (L:D). Water quality parameters such as dissolved oxygen, water hardness, pH and conductivity were measured at the beginning and end of each test by standard methods. Test chambers were not aerated and no food was given during the experiments. Evapourative loss was accounted for by addition of water.

A test was only considered to be valid if control survival was greater than 80%. Four methods for estimating the Median Lethal Concentration were used as recommended by the USEPA (1991) depending on the number of partial mortalities. The graphical method was used to determine the LC_{50} when there is no partial mortality. The Spearman-Karber, trimmed Spearman-Karber or the probit method was used depending on the nature of the partial mortalities. Each replicate in these experiments was treated as an independent test. The differences between mean LC_{50} values were analysed by ANOVA followed by Tukey-Kramer pairwise comparison. Statistical differences were determined at the 95% significance level ($p < 0.05$).

RESULTS AND DISCUSSION

For all tests, dissolved oxygen: 7.9-8.5 mgO_2/L, water hardness: 43-48 mg/L as $CaCo_3$ pH: 6.7-7.2 and conductivity: 139-148 µS/cm were reported during the experiment.

Significant differences in sensitivity to arsenic were recorded between larval instars and between chironomid species. *Chironomus zealandicus* was more tolerant of both forms of arsenic than were the other two species (Table 1). *Chironomus* sp. a was the most sensitive species. In general, LC_{50} values for all three species increased with age.

Trivalent arsenic was found to be more toxic to all three species investigated. This is in agreement with the results of a previous study by McKinney (1995) who found that trivalent arsenic was more toxic than pentavalent arsenic to *C. zealandicus*. Howard et al. (1984) pointed out that the primary mode of toxicity of inorganic trivalent arsenic (As^{3+}) is through reaction with sulfhydral groups of proteins and subsequent inhibition of enzymes. Inorganic pentavalent arsenic (As^{5+}) does not react as readily with sulfhydral groups, but may uncouple oxidative phosphorylation.

One of the important factors that affects the sensitivity of a test animal to a toxicant is its age. Robinson and Scott (1995) observed that the acute toxicity of cyromazine, an insect growth regulator, to *C. zealandicus* was age dependent, and LC_{50} values increased 100 fold from 1st to 4th instars. Sensitivity to heavy metals is also age dependant. Fourth instars of C. *tentans* were 12 to 27 times more resistant to copper stress than were 1st instars (Gauss et al. 1985). Fourth instar larvae of C. *riparius* were 16 times more resistant at 10 hours and 925 times more resistant at 24 hours than 1st instar larvae to cadmium (Williams et al. 1986). This relationship is supported by findings from the present study for all three species of chironomids. Our results show that 4th instar larvae of all three species were more resistant to both forms of arsenic (Table 1). This clearly indicates the importance of using early instars if possible in toxicity tests, since one of the aims of such bioassays is to use the most sensitive life stages available.

The declining LC_{50} values with increasing test duration may have been due to progressive toxicant uptake or to starvation. Neither food nor replacement of toxicant was provided over the 96 hours of the experiment. Nutritional factors are generally not regulated and often overlooked, particularly in water-only toxicity tests. However, diet is well known to alter the toxicity of many chemicals. Feeding generally increases the amount of chemical taken up and its metabolism; therefore toxicity may be increased. However, a decrease in copper toxicity with feeding was recorded by Sosnowski et al. (1979) to a copepod, *Acartia tonsa*. Thus the effect of feeding during toxicity tests is not always known and may modify the results significantly. However, fasting is commonly recommended in short term toxicity tests (ASTM 1990), as in the protocol of the present experiment.

There were differences in sensitivity between the wild-collected and laboratory-

Table 1. 48, 72 and 96 hour LC_{50} values for As^{3+} and As^{5+} in mg/L to cultured 1st, 2nd, 3rd and 4th instars, and wild-collected 4th instars of three species of chironomid at 18°C.

		As^{3+}					As^{5+}				
		1st	2nd	3rd	4th	4th (W)	1st	2nd	3rd	4th	4th (W)
48 hours	*C. zealandicus*	36.7	79.4*	103	122	116	239	395*	1055*	2490*	4190+
		(1.6)	(6.7)	(5.0)	(8.0)	(14.0)	(15.0)	(19.0)	(27.0)	(216)	(472)
		13.1	**25.3**	**14.6**	**19.7**	**36.2**	**18.8**	**14.4**	**7.7**	**26.0**	**33.8**
	C. sp. *a*	14.7	20.4*	31.3*	39.1*	42.1	52.0	69.0	176*	1303*	2925+
		(2.4)	(1.7)	(2.7)	(3.4)	(5.3)	(6.0)	(6.0)	(10.0)	(72.0)	(304)
		49.0	**25.0**	**25.9**	**26.1**	**37.8**	**36.4**	**26.1**	**17.0**	**16.6**	**31.2**
	P. pavidus	19.0	37.4*	51.0*	84.7*	88.6	21.4	93.5*	470*	1492*	2336+
		(3.5)	(3.8)	(3.3)	(3.7)	(8.1)	(4.6)	(4.6)	(56.3)	(196)	(266)
		55.3	**30.5**	**19.4**	**13.1**	**27.4**	**64.5**	**14.8**	**35.9**	**39.4**	**34.2**
72 hours	*C. zealandicus*	29.5	24.5	81.3*	105	92.1	184	363*	944*	1707*	4028+
		(2.1)	(3.9)	(5.7)	(10.0)	(5.0)	(17.0)	(14.0)	(23.0)	(222)	(253)
		21.4	**47.8**	**21.0**	**28.6**	**16.3**	**27.7**	**11.6**	**7.3**	**39.0**	**18.8**
	C. sp. *a*	12.7	16.6	19.6*	18.0	18.3	41.7	49.2	128*	735*	1155+
		(2.3)	(0.8)	(1.0)	(2.8)	(2.3)	(1.3)	(4.0)	(5.0)	(31.0)	(162)
		54.3	**14.5**	**15.3**	**46.7**	**37.7**	**9.3**	**24.4**	**11.7**	**12.7**	**42.1**
	P. pavidus	6.2	22.2*	30.4	38.7	30.4	31.1	73.6*	267*	1127*	1191
		(0.5)	(0.8)	(4.2)	(4.7)	(3.6)	(3.5)	(4.5)	(10.0)	(133)	(176)
		24.2	**10.8**	**41.4**	**36.4**	**35.5**	**33.8**	**18.3**	**11.2**	**35.4**	**44.3**
96 hours	*C. zealandicus*		16.2	63.4*	70.4	60.0		285	919*	814	4176+
			(1.2)	(2.5)	(5.4)	(10.0)		(10.0)	(17.0)	(63.0)	(132)
			22.2	**11.8**	**23.0**	**50.0**		**10.5**	**5.5**	**23.2**	**9.5**
	C. sp. *a*		15.0	17.3*	9.8**	6.9		33.1	104*	481*	502
			(0.8)	(0.5)	(0.5)	(0.3)		(1.0)	(3.0)	(30.0)	(35.0)
			16.0	**8.7**	**15.3**	**13.0**		**9.0**	**8.6**	**18.7**	**21.0**
	P. pavidus		19.7	21.0	26.6	13.6		47.2	235*	700*	608
			(0.9)	(1.1)	(4.4)	(1.5)		(2.0)	(5.0)	(58.0)	(38.0)
			13.7	**15.7**	**49.6**	**33.1**		**12.7**	**6.4**	**24.9**	**18.8**

Each value is a mean of 9 replicates (n=5) from three tests with standard errors in parentheses and % covariance in bold face type.
Tests with 1st instar larvae were terminated after 72hours.
*- significantly greater than the previous instar; ** - significantly lower than the previous instar; + - significantly grater than cultured 4th instar larvae in 2-tailed pairwise comparison; W - Collected from the wild

cultured populations of the test species. Wild-collected 4th instars were all significantly less sensitive to As^{5+} than the cultured animals for shorter test durations

but this difference decreased with time, with the exception of C. *zealandicus.* This time-dependent alteration in senstivity was also found for As^{3+} and may reflect developing stress to laboratory conditions in the wild collected larvae. Environmental and handling stress might have affected the sensitivity of larvae, since they were collected only 48 hours prior to tests.

The differences in sensitivity between wild and cultured animals could also possibly be due to a variety of other factors such as genetic drift or differences in the amount of haemoglobin that could affect toxicant sensitivity. An increasing number of investigations on a variety of organisms have revealed that population level responses to environmental stressors may be genetically dependent (Guttman 1994; Duan et al. 1997). Although cultures of each species were originally established from the same sites from which wild animals were collected for these tests, the cultures were established approximately 30 months before the test date. There is a possibility that the genetic structure of the laboratory population could have changed during this period. Furthermore, initial high mortality (approximately 30 - 40%) in wild-collected larvae may select for animals with altered sensitivity.

The conditions of the laboratory environment may cause unanticipated alterations in the population's genetic structure. Such alterations will occur more quickly in animals with short life cycles. Woods et al. (1989) investigated genetic variability in *C. tentans* from seven scattered laboratories and a population sampled directly from the wild, and found populations differed significantly in heterozygosity, both among laboratory strains and between laboratories and the wild strain. Genetic differences among laboratory stocks, or between laboratory and natural populations, may be reflected in differential sensitivity to environmental stress. 'Since different populations will manifest somewhat different genetic profiles in response to local conditions, an exact match in toxicant sensitivity is unlikely and, even if possible, of limited utility. As Woods et al. (1989) recommended, differing responses to toxicants under conditions of altered genetic integrity must be viewed as suspect until proven otherwise.

However, the fact that this relationship between the sensitivity of wild and cultured animals is common to all three species suggests that some causative agent other than genetic drift may be responsible. As discussed earlier, arsenic is known to bind with proteins and thereby alter the metabolism of animals. If there is a variation in the amount of haemoglobin between wild-collected and laboratory-cultured populations of each species, there may be a variation in available binding sites for arsenic. This could have been one reason for the lower sensitivity of wild populations to arsenic. The laboratory-cultured chironomid larvae were observed to be pale when compared with the redder wild-collected larvae. This may be an indication of less haemoglobin in the laboratory-cultured animals. Differences in the haemoglobin content of chironomid populations may either positively or negatively affect the toxicity of arsenic. When haemoglobin binds with bioavailable arsenic, it may act as a sink for arsenic and prevent it from binding with other essential enzymes or hormones. In this case, high haemoglobin content may reduce arsenic toxicity, but this may adversely affect overall oxygen carrying capacity. However, the influence of culture

on haemoglobin levels of chironomid larvae and effect of arsenic on haemoglobin function requires further study.

Experimental conditions are also well known to alter the sensitivity of chironomids to heavy metals. The temperature effect on metal toxicity appears contradictory. Generally, temperature and toxicity are positively correlated for most chemicals (Ronald 1994). It is apparent that the temperature-mediated metabolic rate has an important role in metal toxicity. However, no consistent relationship between LC_{50} values and temperature was observed in the test designed to study the effect of temperature on arsenic toxicity (Table 2). *Chironomus zealandicus* was relatively more sensitive at 13°C and 23°C than at 18°C to both forms of arsenic. The same relationship was observed with C. sp. a and *P. pavidus* for A^{5+}. There were no significant differences in LC_{50} values for As^{3+} at 13°C and 18°C for C. sp. *a* and *P. pavidus,* but these values were significantly lower than at 23°C.

One other factor that influenced the toxicity of arsenic in this study was season. Differences were observed in 48 hour LC_{50} values for each species in different season (Table 3). The present study shows that *C. zealandicus* collected in summer (November), when the water temperature was 23°C were more resistant than specimens collected in winter (June), when water temperature was only 14°C particularly to As^{3+}. Conversely, *P. pavidus* collected in summer, when the water temperature was 16°C was less resistant than those collected in winter while water temperature was only 12°C. *Chironomus* sp. a did not exhibit such a great difference in sensitivity to arsenic with season. The water temperature at the site of collection of *C.* sp. *a* were 18°C and 14°C in summer and winter, respectively. A possible explanation for the response observed is that the optimum temperature might be species specific. *Chironomus zealandicus* appears to live in waters with elevated temperatures and reportedly as high as 34°C (Stark 1989). *Polypedilum pavidus* however seem to prefer colder water. Animals collected when temperatures were less favourable might therefore be more vulnerable to arsenic stress.

Overall, the results indicate that a number of biological (age and laboratory culture), chemical (valency state), and physical (season and temperature) factors affect the acute toxicity of arsenic to the tested chironomid species. Although the first instar larvae are the most sensitive stage to arsenic, it is the most difficult stage to deal with due to its small size and pale colour. Therefore, it may be more appropriate using second instars depending on the objective of the test. Although two of the test species in this study *(C. zealandicus* and *C* sp. *a)* are very closely related, they showed considerable differences in sensitivity to arsenic. Furthermore, the routein use of 4th instar larvae in acute toxicity tests is a practice that may seriously underestimate the sensitivity of these species since this is the least sensitive larval stage. Therefore, this practice cannot be recommended for tests that are to be used in generating data for water quality criteria and standards. This study also illustrates the importance of examining how toxicity of a substance varies with other biotic and abiotic factors. Investigation of as many life stages as possible rather than just the most easily obtainable stage is essential in order to set accurate water quality standards for the protection of the aquatic environment.

Table 2. 48, 72 and 96 hour LC_{50} values for As^{3+} and As^{5+} in mg/L to *Chironomus zealandicus, Chironomus* sp. a, and *Polypedilum pavidus* at 13°C 18°C, and 23°C

	Species	*Chironomus zealandicus*			*Chironomus* sp a			*Polypedilum pavidus*		
	Duration	**48 hr**	**72 hr**	**96 hr**	**48 hr**	**72 hr**	**96 hr**	**48 hr**	**72 hr**	**96 hr**
As^{3+}	Temperature									
	13°C	42.9	32.9	27.9	64.6	44.6*	33.7*	142	72.3*	41.9*
		(1.5)	(2.8)	(1.7)	(2.9)	(3.1)	(1.5)	(10)	(3.1)	(4.0)
	18°C	58.6*	34.5	29.2	54.8	25.1	20.4*	146	125*	76.1*
		(3.3)	(1.6)	(1.0)	(4.1)	(1.1)	(0.4)	(10)	(9)	(2.1)
	23°C	40.9	33.4	26.2	27.1*	21.7	16*	90.6	46.6*	24.8*
		(2.1)	(1.0)	(1.1)	(0.3)	(0.5)	(0)	(4.3)	(2.4)	(1.1)
As^{5+}										
	13°C	3619	2262	696	1654*	811	358	1904	906	394
		(170)	(223)	(89)	(177)	(100)	(28)	(192)	(119)	(39)
	18°C	5104*	2456	1971*	2241*	709	454	3982*	2251*	1235*
		(450)	(193)	(265)	(139)	(59)	(26)	(299)	(302)	(172)
	23°C	3519	1498*	692	1069*	639	435	1509	790	728
		(457)	(173)	(102)	(131)	(63)	(19)	(148)	(78)	(73)

Each value is a mean of 9 replicates with standard errors in parentheses.
*- significantly greater or lower than the other two temperatures in 2 tailed pairwise comparison. $p<0.05$

Table 3. 48 hour LC_{50} values of As^{3+} and As^{5+} in g/m^3 to *Chironomus zealandicus, Chironomus* sp. *a, and Polypedilum pavidus* collected from sampling sites in two seasons.

	As^{3+}		As^{5+}	
	Summer	**Winter**	**Summer**	**Winter**
Chironomus zealandicus	116[a]	58.6	4191	3619
	(14)	(3.3)	(472)	(171)
***C.* sp. A**	42.1	54.8	2925	2241
	(5.2)	(4.1)	(304)	(139)
	88.6[b]	146	2336[b]	3982
P. pavidus	(8.1)	(10)	(266)	(300)

Each value is a mean of 9 replicates with standard errors in parentheses.
[a] - significantly greater and [b] - significantly lower than the winter in 2-tailed pairwise comparison. $p<0.05$

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