Bull. Environ. Contam. Toxicol. (2004) 73:167–173

DOI: 10.1007/s00128-004-0409-x

Bulletin of Environmental Contamination and Toxicology

Comparison of Cadmium Toxicity to *Asellus aquaticus* (L.) Populations Following 17 Years Isolation in Pond and Laboratory Cultures

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Received: 5 January 2004/Accepted: 22 April 2004

There is evidence that invertebrate populations can develop increased tolerance following long term exposure to toxicants (Posthuma & Van Straalen, 1993) and some indication that this tolerance may be genetically based (Klerks and Weis 1987; Spurgeon and Hopkin 2000). Although this occurs in the natural environment with populations which are genetically diverse (Hoffman & Fisher, 1994), a practical demonstration of the development of genetically inherited resistance would normally involve controlled culture of the test organism, over several generations, in an environment containing the toxicant (Klerks & Levinton, 1993). However, in a toxicant-free culture, with the absence of such a strong selective pressure, genetic diversity would be expected to decrease, particularly when inbreeding, due to isolation of a relatively small population, occurs. This is one of the main arguments presented to support the use of laboratory cultures for routine toxicity testing i.e. it is a means of standardising the conditions and possibly the responses recorded, in a test. Consequently, it may seem unlikely that pollutant tolerance would develop in a toxicant-free culture. However, it has been suggested (Baird, 1992) that the genetic bottlenecking of laboratory populations could actually result in an increased tolerance to toxicants compared to genetically diverse field populations. This could occur because laboratory cultures are based upon the selection of animals which are tolerant to the culture conditions and perhaps also to other stressors. This would, of course, have implications for the relevance of laboratory toxicity tests with cultured animals. The current study was designed to compare the toxicity of cadmium to several populations of the isopod crustacean *Asellus aquaticus* (L.) originating from the same source, but with some having been maintained in culture, without added toxicant, for several years.

Cadmium was selected for this investigation as it is a common pollutant entering the aquatic environment from a variety of natural and industrial sources and is generally regarded as being highly toxic to all components of freshwater ecosystems. *A. aquaticus* is an important component of freshwater ecosystems providing a source of food for many fish species (McCormack and Le Cren 1971). It is commonly found in organically enriched waters and is a key organism in many biotic indices used to monitor pollutant effects in rivers. Toxicity investigations including the effects of cadmium (Ham et al. 1995; Williams et al.

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1985; van Hattum et al. 1989), platinum (Rauch and Morrison 1999), aluminium (Elangovan et al. 1999), copper (Brown 1977; de Nicola Giudici et al. 1987), iron (Maltby et al. 1987), phenol (Green et al. 1988; McCahon et al. 1990) and lindane (Blockwell et al. 1998) have been carried out with this species

MATERIALS AND METHODS

A. aquaticus were collected in 1985 by netting from beds of *Elodea canadensis* in the Brecon and Monmouthshire canal, South Wales, at a location with no known pollution history. They were then maintained in laboratory culture in dechlorinated water (approximate water quality conditions: temperature 13°C, conductivity 292 $\mu S.cm^{-1}$, pH 7.8, cadmium <1.0 $\mu g\ l^{-1}$ and hardness 176 $mg\ l^{-1}$ as $CaCO_3$) until required. The toxicity of cadmium to different life-history stages of these specimens was determined and reported by Green et al. (1986). Animals not used in the study were divided into two groups. One group was maintained as before in laboratory culture and a second group was transferred to a small, isolated outdoor pond (approximate water quality conditions: conductivity 156 $\mu S.cm^{-1}$, pH 7.1, cadmium 2.7 $\mu g\ l^{-1}$ and hardness 61 $mg\ l^{-1}$ as $CaCO_3$) containing vegetation and subject to natural variation in environmental conditions. Although water quality conditions in the laboratory and pond obviously varied over the long culture period there were no significant changes from the representative values given above. No additional animals were added to the laboratory culture and pond after the initial introduction.

In 2002 cadmium toxicity tests were carried out again to compare the responses of three *Asellus* populations i.e. those maintained in the laboratory for 17 years (laboratory population), those maintained in the outdoor pond for 17 years (pond population) and a population freshly collected in 2002 (canal population) from the same original site on the Brecon and Monmouthshire canal (conductivity 134 $\mu S.cm^{-1}$, pH 7.4, cadmium 1.8 $\mu g\ l^{-1}$ and hardness 51 $mg\ l^{-1}$ as $CaCO_3$). Toxicity tests were carried out using standard static-with-renewal procedures in six tanks (32x22x20 cm) each containing 12 L of cadmium solution (17.5 $\mu g\ Cd\ l^{-1}$, 50 $\mu g\ Cd\ l^{-1}$, 175 $\mu g\ Cd\ l^{-1}$, 500 $\mu g\ Cd\ l^{-1}$, 1750 $\mu g\ Cd\ l^{-1}$) or dechlorinated water as a control. Cadmium solutions were prepared by dilution of a stock solution of cadmium chloride with dechlorinated water. Three plastic baskets (10x10x6 cm) with 500 µm nylon mesh bases were suspended within each tank. The tanks and baskets were pre-soaked for 24 h and the test solutions were then replaced with fresh solution. Twenty *A. aquaticus* (3-6 mm body length) from each population were transferred to a basket within each of the six tanks thus ensuring that each population was exposed to the same test solutions throughout the study. Animals were initially provided with 5 conditioned (Bird and Kaushik, 1985) alder, *Alnus glutinosa* (L.) leaf discs (7 mm diameter) as food and additional discs were supplied as required. The test continued for 30 days with daily observations for mortality and any dead animals (indicated by cessation of pleopod beat) were removed and recorded.

Tests were carried out at 13 °C ± 2°C with a photoperiod of 16 h light and 8 h dark.

Tanks were aerated with a centrally positioned air stone and 4 litres of test solution were replaced twice weekly. Conductivity and pH of the control water were recorded with portable meters before and after each solution change and at the same times samples were acid fixed (to 1% with Aristar® nitric acid) for hardness (calcium and magnesium) and cadmium measurement by atomic absorption spectrophotometry.

Mortality data were analysed by Litchfield's (1949) time-response methods, which enabled median lethal times (LT50) and slope functions of the probit response lines for the three *Asellus* populations to be compared. Median lethal concentrations (LC50) and slope functions were also determined and compared at different exposure times by Litchfield and Wilcoxon's (1949) concentration-response methods.

RESULTS AND DISCUSSION

There is ample evidence that invertebrate populations can diverge as a result of genetic adaptation to heavy metals following both natural and experimental exposure (Posthuma & Van Straalen, 1993). However, this study sought to examine whether population divergence, through isolation, in the absence of toxicant stress would result in changed sensitivity to cadmium compared with the original population.

Water quality parameters (mean ± SE) recorded for the control dilution water were: conductivity (281.2 ± 3.8 $\mu S.cm^{-1}$), pH (8.1 ± .04), cadmium <0.5 $\mu g\ l^{-1}$ and hardness (176 ± 8.8 mg l^{-1}as $CaCO_3$). Measured cadmium concentrations shown in parentheses as the mean ± SE were close to nominal and so the latter values were used in all toxicity data analyses: 17.5 μg Cd l^{-1} (20.9 ± 0.57); 50 μg Cd l^{-1} (55.0 ± 1.55); 175 μg Cd l^{-1} (179.5 ± 3.01); 500 μg Cd l^{-1} (464.7 ± 8.3); 1750 μg Cd l^{-1} (1695 ± 9.6).

Time-response data are shown in Fig.1. Less than 50% of the animals died at the lowest test concentration (17.5 μg Cd l^{-1}) so these are not represented graphically. However, it is clear that the pattern of mortality was similar for all three populations and statistical comparisons confirmed that the LT50's and slope functions at the same cadmium concentrations for the different populations were not significantly different ($p > 0.05$). The relationship between LC50 and exposure time is shown in Fig. 2 and it is again evident from statistical comparisons of LC50s and slope functions, at the same exposure times, that there was no significant difference ($p > 0.05$) between the laboratory maintained, pond maintained and newly collected canal populations in their responses to cadmium. Although not statistically compared, an examination of these 2002 data with those from 1986 also suggested that there were no significant differences (Table 1) between the populations.

In a study carried out to test Baird's (1992) hypothesis that laboratory populations could exhibit increased tolerance to toxicants Barata et al. (2000) examined the

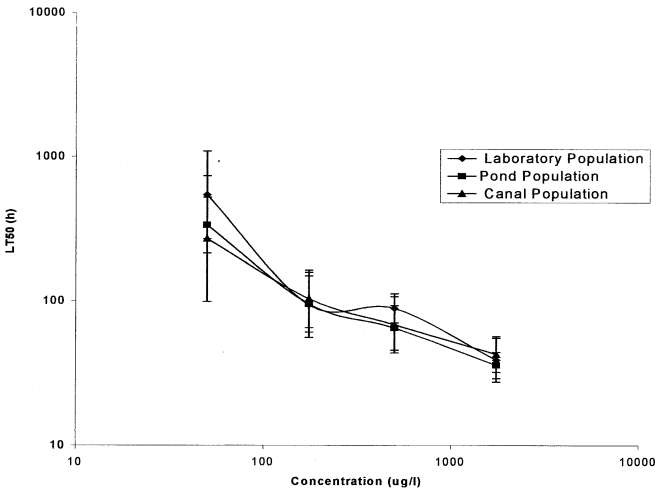

Figure 1. Median Lethal Time (LT50) with cadmium concentration for three populations of *A. aquaticus*

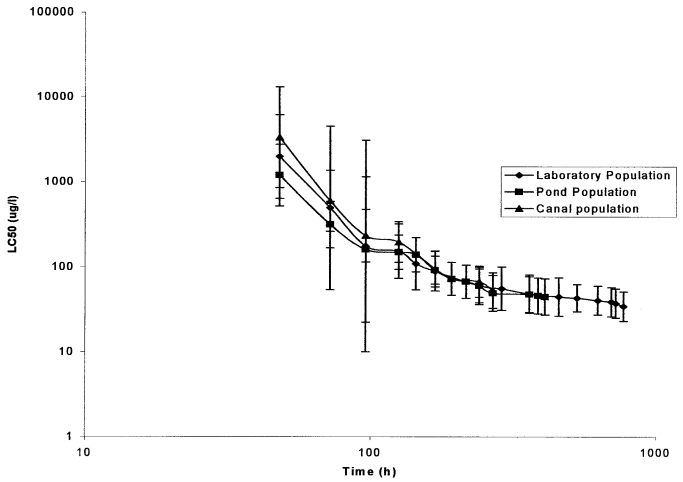

Figure 2. Median Lethal Concentration (LC50) with time for three populations of *A. aquaticus*

response of two laboratory (clonal lines) and one field population of *Daphnia magna* to cadmium. They also found that the field population had a similar mean tolerance to cadmium as the laboratory populations. Barata et al. (2002) suggested that this might have been because the laboratory populations were derived from geographically distinct populations. However, in the current study with *A. aquaticus* the laboratory and pond populations were originally derived from the same field population which was employed in the toxicity study.

Table 1. A comparison of median lethal times (LT50) and median lethal concentrations (LC50) determined in 2002 with values obtained by Green et al., (1986).

	LT50 with 95% confidence intervals at 1750 μg Cd l^{-1} hours	96 h LC50 with 95% confidence intervals μg Cd l^{-1}
1986 study	44 (37-52)	230 (105-510)
2002 laboratory population	39 (27-55)	76 (10-3093)
2002 pond population	36 (29-44)	160 (22-1141)
2002 canal population	43 (32-57)	233 (114-473)

Hoffman & Fisher (1994) investigated the laboratory toxicity of several insecticides to a field population of *Chironomus riparius* and a laboratory strain established approximately 16 years earlier. Their results suggested that the two populations had diverged significantly, with the field population showing greater resistance to three classes of insecticides than the laboratory population. LC50 values ranged from 15-230 times greater than those for the laboratory chironomids. The authors were able to correlate the enhanced resistance with greater activity of metabolising enzymes compared to the laboratory animals and speculated that this had occurred as a result of site specific selection pressures which were not a factor for laboratory maintained animals. The selective pressures acting on each population were not, however actually measured in their work. In the current study, neither the laboratory and pond populations nor the natural field populations were exposed to added cadmium and we are not aware of any pollution events affecting the field population. Consequently selective pressure to develop enhanced cadmium detoxication processes was absent. The responses of these different populations to cadmium in a controlled toxicity test were subsequently found to be similar and although lethality is a relatively crude indicator of toxicity, the results confirm that, in the absence of a specific selection pressure, long established cultures of *A. aquaticus* were neither more nor less tolerant to cadmium than the original population from which they were derived.

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