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Characterization of Hsp60 Response in the Rotifer, *Brachionus plicatilis*, Exposed to Multiple Environmental Contaminants

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ABSTRACT

Many bioassays examine the effects of a single stressor on organism health, but in the environment organisms are rarely, if ever, exposed to a single stressor. The development of an assay that measures overall organism health would be desirable. Stress proteins, including hsp60, are a group of highly conserved proteins that are induced in response to a variety of environmental agents and are well suited to measure the effects of multiple stressors due to their integrative nature (Sanders (1993) Critical Reviews in Toxicology ***23****, 49–75). They have been postulated to confer a protective response in the cell, shielding it from further protein damage. The goal of this investigation was to demonstrate an integrative stress response to multiple environmental contaminants using the marine rotifer,* Brachionus plicatilis, *which has a demonstrated ability to produce hsp60 (Cochrane* et al. *(1991)* Comparative Biochemistry and Physiology ***98C****, 385–390).* © *1998 Elsevier Science Ltd. All rights reserved*

Rotifers, cultured at 16°C, were exposed to either a water-accommodated fraction (WAF) of Prudhoe Bay crude oil (PBCO) or a PBCO/dispersant (Corexit 9527®) preparation of dispersed oil (DO) and ensuing hsp60 levels were measured. All exposures (WAF and DO) were conducted at concentrations where there was no significant mortality to exposed and control populations. To examine the protective nature of hsp60, rotifers were subjected to an environmental scenario similar to that of the San Francisco Bay, in which background copper (Cu^{2+}) levels are approximately 5 μg/liter at 22‰ (Flegal *et al.*, 1991) in combination with a variety of other environmental pollutants, including pesticides and petroleum hydrocarbons. Copper levels represent one-half the value of the reproductive 48-h no-observable-effect-concentration (NOEC) for Cu^{2+} in *Brachionus plicatilis* reported by Snell and Janssen (1995). Rotifers were exposed to 5 μg/liter of Cu^{2+} for 24 h to simulate the normal environment of the bay, followed by exposure to the oil/dispersant preparations for 24 h, while maintaining the Cu^{2+} concentration at 5 μg/liter. Controls of copper alone were run for both 24 and 48 h.

Exposed rotifers were homogenized and total protein content was determined using methods of Bradford (1976). Laemmli SDS–PAGE techniques (Laemmli, 1970) with western blotting (Towbin *et al.*, 1979) using hsp60-specific antibodies and chemiluminescent detection were used to isolate, identify, and measure induced hsp60 as a percentage of controls. Statistical significance was determined by two-way ANOVA. Given a significant *F*-test, a protected least significant difference (PLSD) was used to examine the significance level between each treatment and control values as well as between individual treatments. Values were expressed as percentage of control.

The WAF preparation resulted in hsp60 levels that were not significantly different from control at higher concentrations, with the 50% WAF preparation producing hsp60 levels 135% of control. There was essentially an inverse response with the highest concentration of WAF producing the lowest levels of hsp60. This could be due to a number of reasons. First, the oil proved to be so toxic that the overall cell physiology was affected such that hsp60 no longer served as a valid indicator. Another explanation is that oil levels were not toxic and subsequently did not induce stress protein synthesis. Considering that the lowest concentration of WAF did induce hsp60 at levels significantly greater than control levels, it would be expected to provoke a response at higher concentrations. Therefore, the explanation that the WAF mixture was toxic enough to inhibit stress protein induction appears to be more plausible. It is not possible to know for certain without further studies that specifically examine the level of protein synthesis in the organism, for example by measuring actual mRNA levels.

Rotifers exposed to environmental conditions with multiple stressors showed an initial induction of hsp60 in all samples after 24 h copper pre-exposure. When oil/dispersant mixtures were added, elevated hsp60 levels in the organism showed a relatively small further increase in hsp60 levels. The sum of the individual copper exposure and the individual oil/dispersant exposure was greater than the level of hsp60 produced by the combined exposure. This response suggests that the stress response observed was integrative and not additive in nature, taking into account the total stress affecting the organism.

The comparison of the response between the individual oil/dispersant exposures alone and those which had the copper pre-exposure treatment was notable. For WAF the copper pre-exposure served to elevate hsp60 levels and they remained there for the duration of the ensuing WAF exposure. All concentrations of WAF with copper pre-exposure resulted in hsp60 levels that were significantly elevated over control values in contrast to the WAF alone where hsp60 levels were not elevated at higher WAF concentrations (Fig. 1). The pre-exposure to copper increased hsp60 levels resulting from the 50% WAF preparation from 135% control without copper pre-exposure to 363% control with copper pre-exposure. This effect could be due to induced levels of hsp60 serving as a protective mechanism for subsequent stressors, rendering it less toxic to the organism. Therefore the total stress is less than the sum of the two individual stressors.

The DO mixture produced no dose–response relationship. Hsp60 levels were elevated at all exposure concentrations and there was no statistical difference between them, with an average value of $462 \pm 29\%$ control levels. The copper pre-exposures resulted in significantly increased levels of hsp60 over control populations and the further addition of DO caused an elevation in hsp60 levels, but the increase was not significant (Fig. 2), producing values that were $503 \pm 44\%$ control levels. Further investigation should address the possibility of a toxic threshold.

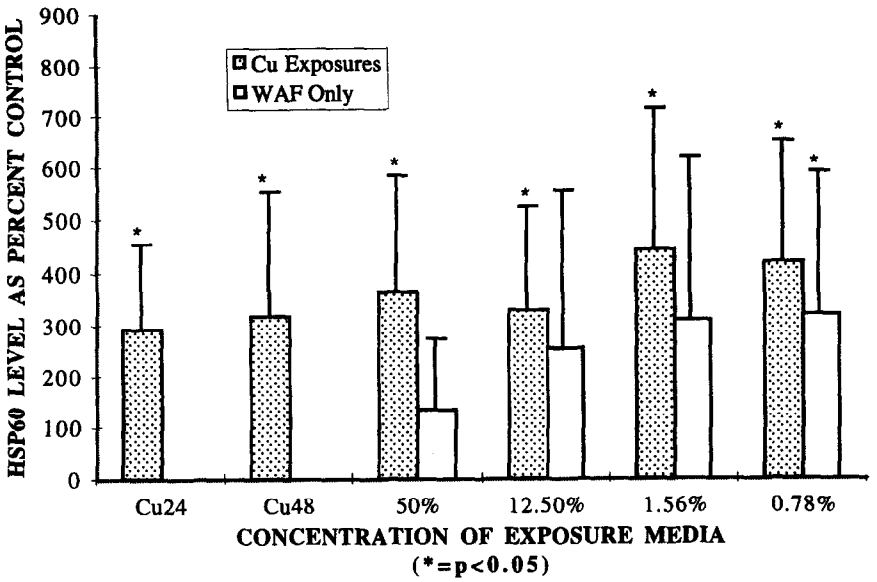

Fig. 1. Comparison of hsp60 response to the water accommodated fraction preparation (WAF) versus WAF with copper pre-exposure at 22‰. Concentrations are represented as percentage of WAF, and hsp60 response is given as percentage of control values. Standard deviations for each group are shown. C = control; Cu24 = the 24-h copper control; Cu48 = the 48-h copper control ($n = 4$).

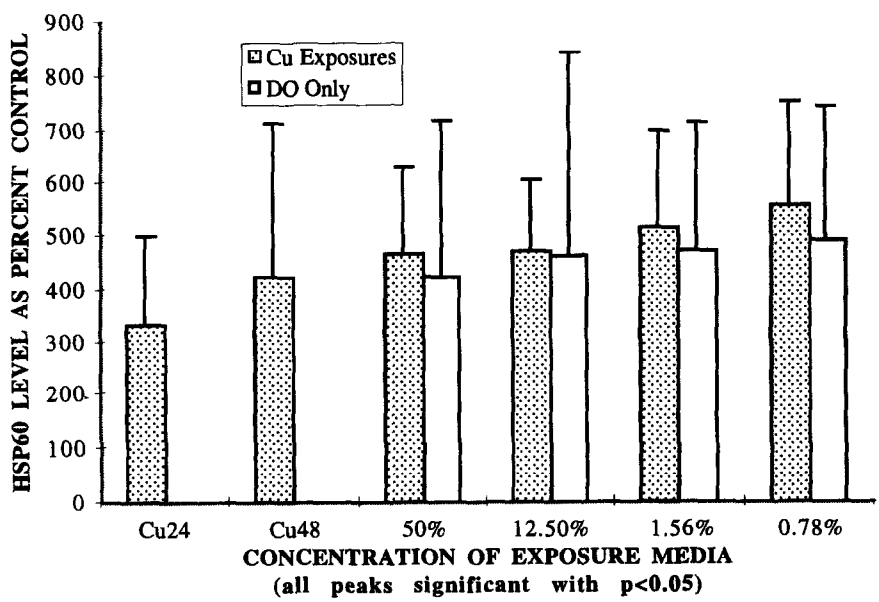

Fig. 2. Comparison of hsp60 response to the dispersed oil preparation (DO) versus DO with copper pre-exposure at 22‰. Concentrations are represented as percentage of DO, and hsp60 response is given as percentage of control values. Standard deviations for each group are shown. C = control; Cu24 = the 24-h copper control; Cu48 = the 48-h copper control ($n = 4$).

Results suggested that the induced levels of hsp60 in response to multiple environmental stressors reflected the integrative sum of the stressors affecting the organism, as demonstrated by the elevated levels of hsp60 resulting from exposure to WAF following pre-exposure to copper. Thus hsp60 was a good indicator of overall stress in this study. Its application to other environmental studies may be promising as organisms are rarely ever exposed to single stressors in the environment and it should not be assumed that the effects of toxicants are all synergetic.

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