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# Toxicity of Cadmium and Mercury to Horseshoe Crab *(Limulus polyphemus)* Embryos and Larvae

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This paper is part of a series of investigations into the lethal and sublethal effects of heavy metals on developmental stages of the American horseshoe crab, *Limulus polyphemus* (Botton et al. 1998a, b; Itow et al. 1998a, b). These studies have been prompted by concerns about the apparent decline in horseshoe crabs from the middle Atlantic coast of the US, leading to the recent development of an interstate fisheries management plan for the species (Schrading et al. 1998). Horseshoe crabs are an important commercial fishery in the region; an estimated 500,000 to 1,000,000 adult crabs have been collected from Delaware Bay and coastal NJ, DE, and MD in each of the last five years for use as bait in the eel and whelk *(Busycon* sp.) fisheries (Schrading et al. 1998). Uneasiness over the status of horseshoe crab populations is linked to the fact that *Limulus* eggs are the most important food source for hundreds of thousands of migrant shorebirds during their northward (Spring) migration through Delaware Bay (Botton et al. 1994).

Horseshoe crabs spawn on intertidal estuarine beaches; eggs develop within beach sediments for a period of three to four weeks before they hatch out as swimming "trilobite" (first instar) larvae. This life history strategy exposes the developing embryos to potential contaminants in the sediments and pore water. Exposure of eggs to pollutants could be toxic or impair development; furthermore, shorebirds ingesting contaminated eggs might in turn experience adverse effects (Burger 1997). The Japanese horseshoe crab, *Tachypleus tridentatus*, has been severely impacted by coastal pollution, especially in some of the heavily industrialized areas within the Sea of Japan (Itow 1997, Itow et al. 1998a).

The purpose of this paper is to evaluate the toxicity of mercury and cadmium to horseshoe crab embryos and larvae in short-term (24, 48, and 72 hr) and continuous exposure. Kennish (1992) ranked these as the two most toxic of 11 heavy metals in the estuarine environment. I compare the toxicities of Hg and Cd, which are not metabolically essential, to Cu and Zn which have

biochemical uses (hemocyanin biosynthesis, enzyme cofactors) (Viarengo 1985).

## MATERIALS AND METHODS

Freshly laid clutches of horseshoe crab eggs were collected in Spring, 1996 from High's Beach in lower Delaware Bay, NJ. Horseshoe crab eggs were cultured in 20‰ Instant Ocean artificial seawater (ASW), made up using distilled water, at ambient room temperature. Water was changed daily, except on weekends. Developing embryos were examined under a dissecting microscope and staged according to the criteria in Sekiguchi (1988). Stage 20-1 embryos were used in all bioassays; these embryos are enclosed by a clear membrane, enabling observation of gill and leg movements. About one week later, embryos hatch out as first instar or "trilobite" larvae, approximately 3.3 mm in carapace width (Sekiguchi 1988).

Stock solutions of 1 g/L $CdCl_2$ (Sigma Chemical Co.) and $Hg(C_2H_3O_2)_2$ (Mallinkrodt) were made in 20‰ ASW. Nominal metal concentrations of 0.01, 0.1, 1, 10, 25, 50, 75, 100, 250 and 500 mg/L were made by dilution into ASW. All heavy metal treatments and controls consisted of 60 animals reared in groups of 20 in three replicate 100 mm diameter plastic Petri dishes, with approximately 20 cc of test solution per dish. To minimize microbial growth and to eliminate the possibility of metal adsorption onto particulate material, all animals were raised in the absence of sediments. No food was added, as neither the embryos nor trilobite larvae are known to feed.

In the short-term bioassays, embryos and trilobite larvae were exposed to mercury or cadmium for 24, 48, or 72 hr. Test solutions were replaced daily. Following treatments, animals were rinsed twice with ASW and then reared in 20‰ ASW for the duration of the experiment. In the long-term (continuous) experiments, the animals remained in the test solution for the entire experiment, with solutions renewed every 24 hr, except over weekends. I defined the endpoints of the study as either molting (embryos into trilobites, or trilobites into second instars) or death (when animals showed no gill and leg movements, and were unresponsive when touched with a probe). Most animals that molted did so within 14-21 days after the trial began; a few remained in moribund condition for up to 1 month prior to death. Mortality in ASW control dishes was 0.4% for embryos and 3.9% for trilobites.

Median lethal concentrations ($LC_{50}$) and 95% confidence intervals were estimated using probit analysis (Lieberman 1983). The estimates of $LC_{50}$ are conservative since I assessed mortality on the basis of the number of animals which ultimately completed molting, not simply the number which remained alive at the end of 24, 48, or 72 hr.

**Table 1.** Median lethal concentrations for horseshoe crab embryos exposed to mercury and cadmium.

| Metal | Stage | Exposure time (hr) | $LC_{50}$ (mg/L) | 95% Confidence Interval | |
|---|---|---|---|---|---|
| Cd | Embryo | 24 | > 1000 | - | - |
| Cd | Embryo | 48 | 503.3 | 678.1 | 410.0 |
| Cd | Embryo | 72 | 171.9 | 208.0 | 145.6 |
| Cd | Embryo | Continuous | 39.5 | 43.4 | 35.5 |
| Cd | Trilobite | 24 | 304.0 | 472.2 | 221.6 |
| Cd | Trilobite | 48 | 139.9 | 180.6 | 112.8 |
| Cd | Trilobite | 72 | 167.7 | 250.0 | 121.3 |
| Cd | Trilobite | Continuous | 71.7 | 105.8 | 50.3 |
| Hg | Embryo | 24 | 12.8 | 15.2 | 12.3 |
| Hg | Embryo | 48 | 5.1 | 6.6 | 3.8 |
| Hg | Embryo | 72 | 3.1 | 4.6 | 1.9 |
| Hg | Embryo | Continuous | 3.2 | 4.5 | 2.1 |
| Hg | Trilobite | 24 | 56.0 | 64.6 | 48.4 |
| Hg | Trilobite | 48 | 19.6 | 25.6 | 14.6 |
| Hg | Trilobite | 72 | 7.6 | 10.7 | 5.2 |
| Hg | Trilobite | Continuous | 0.7 | 1.1 | -2.0 |

(-) indicates that 95% confidence interval was not calculated because median lethal concentration was outside the range of the test concentrations (0.01 - 1,000 mg/L).

## RESULTS AND DISCUSSION

Both stage 20 embryos and trilobite larvae were far more tolerant of cadmium than mercury (Table 1). Mortality generally increased with increasing exposure period for both heavy metals. The highest mortality was observed among embryos and larvae that were continuously exposed to metals, except for the embryo treatments with mercury where there were no significant differences among the 48 hr, 72 hr, and continuous treatments. Larvae were more tolerant of mercury than embryos except during continuous exposure. In contrast, embryos were more tolerant of cadmium than larvae during short-term exposure but the opposite trend was seen in the continuous exposure experiment.

**Table 2.** Toxicities of other heavy metals to horseshoe crab Stage 20 embryos and trilobite larvae.

| Stage | Metal | $LC_{50}$ Values 24 hr | 48 hr | 72 hr | Continuous |
|---|---|---|---|---|---|
| Embryo | Copper | 151 | 180 | 171 | 185 |
| | Zinc | > 1000 | > 1000 | 715 | 170 |
| | Tributyltin | 44 | 20 | 14 | |
| Trilobite | Copper | > 1000 | 855 | 637 | 136 |
| | Zinc | > 1000 | > 1000 | > 1000 | 87 |
| | Tributyltin | > 1000 | 742 | 594 | 42 |

All experimental animals were obtained from Delaware Bay, NJ except copper and zinc experiments which used animals from Sandy Hook Bay, NJ. $LC_{50}$ values are in mg/L except TBT which are in µg/L. Modified from Botton et al. (1998 a,b).

In comparison to the early developmental stages of other marine arthropods, horseshoe crab embryos and trilobite larvae showed a high tolerance to mercury, and especially to cadmium. For example, green crab *(Carcinus maenas),* shrimp *(Crangon crangon),* and lobster *(Homarus americanus)* larvae exposed to $HgCl_2$ had 48 hr $LC_{50}$'s of 0.014, 0.01, and <0.001 mg/L, respectively (Connor 1972). Grass shrimp *(Palaemonetes pugio)* larvae had <28% survival over 21 days when exposed to 0.01 mg/L $HgCl_2$ (Kraus et al. 1988). Mance (1987) reported $LC_{50}$'s of 0.1 mg/L or lower for in which marine crustacean adults and larvae were exposed to mercury. For cadmium, the differences between horseshoe crabs and other marine arthropods are even more striking. Larvae of the grapsid crab, *Paragrapsus quadridentatus,* had a 96 hr $LC_{50}$ of 1.23 mg/L for Cd (Ahsanullah and Arnott 1978). Larvae of the portunid crabs, *Portunus sanguinolentus, P. pelagicus,* and *Charybdis feriatus,* had 48 hr $LC_{50}$'s of 0.38 mg/L for Cd (Greenwood and Fielder 1983). Larval mud crabs, *Scylla serattu,* had a 48 hr $LC_{50}$ of 0.078 mg/L (Ramachadran et al. 1997), and larvae of Dungeness crab, *Cancer magister,* had 96 hr $LC_{50}$'s of 8.2 µg/L to Hg and 247 µg/L to Cd (Martin et al. 1981). Blue crab, *Callinectes sapidus,* embryos had 50% hatching failure at 0.25 µg/L Cd (Lee et al. 1996). In fact, *Limulus* embryos and larvae can tolerate Cd levels that are well above the $LC_{50}$ for most adult Crustacea. For example, amphipods *(Corophium volutator)* of similar size (4-7 mm length) to *Limulus* trilobite larvae had a 96 hr $LC_{50}$ of 9.03 mg/L in seawater and 12.50 mg/L in sediment (Bat et al. 1998). Hong and Reish (1987) reported 96 hr $LC_{50}$'s

ranging from 0.24 to 1.27 for five marine amphipods and 0.41 to 7.12 mg/L for two isopods. Hickey and Roper (1992) found that the amphipods *Paracorophium excavatum* reared in Cd-contaminated sediments had a 10 day $LC_{50}$ of 18.3 mg/kg. Phillips (1980) summarized a number of bioassays in which adult crustaceans were exposed to cadmium; 48 hr's $LC_{50}$'s ranged from 0.5 to 28 mg/L, and 96 hr $LC_{50}$'s ranged from 0.3 to 61.5 mg/L.
This study agrees with previous investigations showing that *Limulus* embryos and larvae are highly tolerant of exposure to copper and zinc (Botton et al. 1998a), organotin (Botton et al. 1998b), and other pollutants (Laughlin and Neff 1977, Neff and Giam 1977, Strobe1 and Brenowitz 1981, Weis and Ma 1987). It is clear, however, that *Limulus* is much more susceptible to mercury than cadmium, zinc, or copper (Table 2). The chemical interactions between mercury and aquatic systems are complex (Boudou and Ribeyre 1997), and further experiments would be required to determine whether mercury toxicity in these bioassays was related to inorganic mercury ions (HgI, HgII) or organomercurial compounds (methylmercury, dimethylmercury). Itow et al. (1998a) found that mercury, and to a lesser extent, cadmium, induced developmental anomalies in horseshoe crab embryos, including missing or fused body segments and appendages, and poorly differentiated anterior regions. Exposure of trilobites to 1 mg/L Hg or Cd inhibited limb regeneration (Itow et al. 1998b).

The decline of the Japanese horseshoe crab, *Tachypleus tridentatus,* is partially attributed to the poor survival of eggs which develop in polluted areas (Itow 1997, Itow et al. 1998a). Although there are no data on pore water metal concentrations from Delaware Bay beaches, fewer than 1% of the *Limulus* embryos from this estuary exhibited developmental anomalies (Itow et al. 1998a). Most horseshoe crab eggs develop on beaches in the lower reaches of Delaware Bay at salinities above 15‰ (Botton et al. 1994). Church et al. (1988) showed that most trace metal concentrations decreased as a function of increasing salinity along the gradient from the heavily industrialized upper estuary to the mouth of Delaware Bay. Engel and Fowler (1979) showed that Cd toxicity was inversely proportional to salinity, due to the complexation of Cd ions with chloride. Based on the high tolerance of *Limulus* embryos and larvae to heavy metals, and the fact that most of the important spawning sites occur in the comparatively unpolluted lower bay, it seems unlikely that heavy metal pollution is a major factor responsible for the declining horseshoe crab population in Delaware Bay. However, the possible uptake of contaminants by horseshoe crab eggs during vitellogenesis (Kannan et al. 1995) or subsequently may have potentially important consequences for shorebirds and gulls feeding in Delaware Bay and other coastal areas (Burger 1997).

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