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# Chronic Toxicity of Phenanthrene to the Marine Polychaete Worm, *Nereis* (*Neanthes*) *arenaceodentata*

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Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment. While environmental concentrations are generally below acutely lethal levels (Hyland and Schneider 1976), chronic, low level exposures may result in subtle sublethal effects. PAHs accumulate in bottom sediments and may represent a hazard to the benthos. Polychaetes are important members of this community (Officer and Lynch 1989). The objective of this study is to evaluate the chronic sublethal effects of one PAH, phenanthrene (PHN), on the polychaete worm, *Nereis* (*Neanthes*) *arenaceodentata.* PHN was selected because of its high toxicity to marine invertebrates relative to other PAHs (Neff 1979).

## MATERIALS AND METHODS

Culturing methods for N. *arenaceodentata* have been described elsewhere (Dillon et al. 1993). Phenanthrene (98% purity, Lot #01128E2), obtained from Aldrich Chemical Company(Milwaukee, Wisconsin), was dissolved in reagent grade acetone to create the primary stock solution (0.1 g PHN/100 mL acetone). To estimate an appropriate chronic sublethal exposure concentration, range-finding toxicity tests were conducted with immature adult worms and 48-hr-old emergent juveniles (EJs). Immature adult worms were randomly distributed among 48 1-L beakers (5 worms/beaker) each containing 800 mL of 30% seawater [Instant Ocean@, Mentor, Ohio). There were five nominal PHN concentrations (100, 178, 316, 562, 1000 µg/L) and two controls (seawater, 1000 µg/L acetone) with four replicates per treatment. Survival was determined after 14 d.

The EJ range-finding test was initiated by randomly distributing worms among 24 250-mL beakers (10 EJs/beaker) each containing 200 mL of 30% seawater. There were five concentrations (10, 18, 32, 56, 100 µg PHN/L) and two controls (seawater, 100 µg/L acetone) with three replicates per treatment. EJ survival was determined after 4 d. Each range-finding test was run with and without culture sediment. PHN was introduced in conjunction with the

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normal worm feeding ration (a TetraMarin®-alfalfa food slurry) three times/week for the adult test and once at the beginning of the EJ test see Moore et al.(1991) for detailed description of dosing methodology.

Range-finding tests indicated 20 µg PHN/L would be an appropriate sublethal concentration. To initiate chronic exposures, approximately 2400 EJs were randomly distributed among 24 38-L aquaria (100 EJs/aquarium) each containing 30 L of seawater and 2 cm of culture sediment layered on the bottom. There were 3 treatments: 20 µg PHN/L (PHN), seawater control (SC) and the carrier control (CC), and four replicates per treatment. The CC treatment received the same volume (20 µg/L) of acetone as the PHN treatment. Worms were exposed to PHN via the thrice weekly TetraMarin®-alfalfa food slurry as in the range-finding tests. After 8-wks, all worms were removed and counted. Growth (individual dry weight) was measured in 5 randomly selected worms from each aquarium (20 worms per treatment). Animals were removed, briefly rinsed in distilled water, gently blotted dry, placed on pre-weighed aluminum pans (1 worm/pan), dried at 60°C to a constant weight ( ≈24 hr) then weighed to the nearest 0.001 mg on a Cahn (Cerritos, California) C-31 microbalance.

After 8-wk chronic exposure, effects on reproduction (fecundity and EJ production) were evaluated by establishing mated pairs from worms in each treatment. Ten males and ten females from each aquarium were used to establish pairs (40 pairs/treatment). Beakers (600 mL) containing mated pairs were randomly assigned to one of two types of observations; egg deposition or EJ production. All beakers were observed daily for the appearance of egg masses and EJs. See Moore et al.(1991) for methodological details regarding measuring reproductive endpoints.

Phenanthrene was analyzed in adult worms at the end of the 8 wk exposure period. Approximately 25 worms from each of the four treatment aquaria were pooled ( ≈2-3 g wet weight) and frozen in 50-mL glass vials. This was repeated 3 times within a treatment for a total of 4 samples per treatment. Worm tissue residue analyses were performed by NOAA/NMFS (Seattle, Washington) using analytical methods outlined by Krahn et al.(1987). Worm eggs were homogenized wet and extracted using the Bligh-Dyer (1959) method modified by the University of Maryland (Horn Point Environmental Laboratory) for analyzing total lipids in small tissue volumes. Analysis of lipids was performed using the TLC/FID Iatroscan MK-5 (RSS Inc., Costa Mesa, California).

Range-finding data were analyzed via trimmed Spearman-Karber method for estimating median lethal concentrations. Growth and reproduction data were evaluated for homogeneity of variance using Cochran's test. Growth data were log transformed and egg and EJ data were square root transformed. Carrier and PHN effects were analyzed separately via the t-test statistic using SAS. All tests for significance were analyzed at a level of $\alpha = 0.05$.

## RESULTS AND DISCUSSION

The range-finding toxicity tests indicated that: 1) EJs were an order of magnitude more sensitive to PHN than adults and 2) sediment had no effect on toxicity. EJ 96-hr LC50s (95% CL) were

identical for treatments with and without sediment; 51 µg/L (46-56) and 51 µg/L (46-57), respectively. Immature adult 14-d LC50s (95%CL) were also identical for treatments with and without sediment; 501 µg/L (427-588) and 501 µg/L (425-590), respectively.

Following the 8-wk chronic PHN exposure, survival was high and not significantly different among experimental treatments (Table 1). The acetone carrier had a significant and positive effect on growth and reproduction relative to worms in the seawater control (Table 1). For that reason, sublethal PHN effects were compared with CC via t-test. That analysis indicates that chronic exposure to PHN significantly diminished growth, fecundity and EJ production and significantly increased the time between pairing and egg deposition (Table 1). EJs produced by worm pairs from all treatments appeared normal and possessed 18 to 21 setigers. There were no significant effects on EJ dry weight (Table 1). Egg lipids doubled in the CC compared to SC and doubled again in PHN compared to CC. Because the mass of tissue available of PHN analysis was judged by NOAA to be too low, sample replicates were pooled. Worms accumulated 780 µg PHN/kg ($4.38 \times 10^{-3}$ mmol/kg) wet weight following the 8 wk exposure. PHN-exposed worms produced copious amounts of mucus and did not actively feed during the latter half of the 8 wk exposure period. When removed from aquaria they were very lethargic and did not exhibit the normal fighting behavior (Reish and Alosi 1968). Normal behavior resumed after 24 hr in clean seawater. Abnormal behavior and excess mucus production were not observed at any time in seawater control or acetone carrier treatments.

We speculate that PHN adversely affected worm growth and reproduction via reduced feeding induced by non-specific narcosis. Veith and Broderius (1990) describe non-specific narcosis as "the reversible state of arrested activity of protoplasmic structures". Our laboratory observations of reduced worm activity in the PHN treatment and return to normal behavior after a period in clean seawater ("reversible state") strongly suggest a narcotic-mediated effect. It has been observed that a variety of neutral organic chemicals, including PAHs, can induce narcosis in aquatic organisms which leads to a reduction in growth (Hermens et al. 1984). The fact that PHN reduced both growth and reproduction by similar amounts ( ≈35%) further supports a common mode of action.

Mechanisms other than narcosis, however, may also be operative. Decreased lysosomal membrane stability leading to reduced growth and reproduction is a well documented mode of action for PAH-exposed mussels (Widdows et al. 1982; 1987). Disrupted steroidogenesis may also be involved. Growth and reproduction in polychaetes are regulated via a complex neuroendocrine system (Baskin 1976). The production of neuroendocrines requires a functional cytochrome P-450 mixed-function oxygenase (MFO) system. The MFO system was probably induced in worms experiencing chronic PHN exposure. This exposure may have disrupted normal steroidogenesis leading to reduced growth and reproduction. The relationship between MFO induction, xenobiotic metabolism, and steroid metabolism in aquatic organisms is very complex (Lee 1988).

Tissue concentrations associated with acute narcosis in fish exposed to a variety of neutral organic chemicals fall within a very narrow range (2-8 mmol/kg wet weight) (McCarty and MacKay 1993). Tissue concentrations associated with chronic narcosis

Table 1 A summary of life history characteristics for N. *arenaceodentata* following 8-wk exposure to 20 µg/L phenanthrene (PHN). Mean (SE). EJ = emergent juvenile.

| | Seawater Control | Carrier Control | PHN Exposure |
|---|---|---|---|
| **Survival** | 89% (4.2) | 83% (2.3) | 90% (3.9) |
| **Growth (mg dry wt)** | 16.9 (4.2) **A** | 33.3 (12.8) | 21.3 (0.3) **A** |
| **Fecundity (eggs/brood)** | 176 (28.6) **A** | 299 (27.7) | 199 (15.4) **A** |
| **EJ Production (#/brood)** | 97 (9.5) **A** | 201 (30.8) | 128 (16.2) **A** |
| **EJ Size (µg dry wt)** | 25 (0.3) | 24 (0.8) | 23 (0.3) |
| **Time from Pairing to Egg Deposition (days)** | 27.7 (1.6) **A** | 22.3 (1.2) | 25.7 (1.9) **A** |
| **Total Egg Lipids (µg/g)** | 1.454 | 3.151 | 6.938 |
| **PHN Tissue Residues:** | | | |
| **(µg/kg wet wt)** | <dl | <dl | 780 |
| **(mmol/kg wet wt)** | <dl | <dl | $4.38 \times 10^{-3}$ |

A = significantly different from carrier control.
dl = detection limit.

would be an order of magnitude lower (McCarty and MacKay 1993). Contaminants acting via a specific mode of action have even lower critical body residues (McCarty and MacKay 1993). The PHN body burden we observed for *N. arenaceodentata* in these experiments was 2 orders of magnitude ($4.38 \times 10^{-3}$ mmol/kg; Table 1) lower than one might expect if the effects were solely mediated via chronic narcosis. Moore et al. (1987) demonstrated PHN can increased lysosomal size and membrane fragility in marine organisms. They report an association between decreased lysosomal membrane stability and PHN body burdens of 0.4 µg/g wet weight in field-collected organisms. This tissue concentration is very similar to that observed for *N. arenaceodentata* in these experiments.

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