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**STUDY TITLE:** Development and Application of a Sublethal Toxicity Test to PAH Using Marine Harpacticoid Copepods

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**BACKGROUND:** Polycyclic aromatic hydrocarbons (PAH) are a highly toxic group of compounds present in crude oil and many petroleum products. Estuarine salt marshes are critical marine habitat because of their high productivity and importance as nursery grounds for many commercially important species. One impediment to understanding the response of estuarine organisms to contaminants is the lack of bioassay protocols with benthic organisms. Harpacticoid copepods in many ways are almost ideal organisms for toxicity tests. They have short life histories, all life history stages are benthic and selected species are easy to culture. No sublethal bioassays have been conducted with a meiofaunal taxon using PAH as a contaminant.

**OBJECTIVES:** The intent was to first investigate the effects of sediment-bound polynuclear aromatic hydrocarbons on survivorship and reproduction of harpacticoid copepods through the development of a sub-lethal toxicity-testing system. Due to their

ecological importance and their suitability as experimental organisms, we wished to refine methods to use harpacticoids in toxicity tests to study effects relevant to the goals of MMS. The objective was to develop an experimental protocol to determine the effects of PAH on estuarine harpacticoids and to use this technique to apply it to specific problems associated with contaminant effects.

**DESCRIPTION:** Survivorship and sublethal reproductive tests were conducted in 96-h toxicity tests. Test units (28 x 45 mm carrier glass vials) were filled with one ml of sediment treatment dispensed with minimal disturbance using a pipette creating a 2-3 mm sediment layer. Four replicates were used per sediment treatment (consisting of phenanthrene at 0, 0 S, 126, 261, 514, 1030 µg/g; fluoranthene at 0, 0 S, 137, 249, 451, 990, 2170 µg/g; and diesel fuel at 45, 93, 185, 370 µg/g). Vials were placed inside moisture chambers. They were kept in the dark at 25 °C in an incubator with no illumination. *Schizopera knabeni* or *Nitocra lacustris* were introduced to each experimental unit and vials were returned to the incubator. After 96 h, the contents of all vials were sieved through a 45-µm mesh and retained copepods were enumerated as live or dead. Additional bioassays for grazing and avoidance tests were conducted and effects on life stages were examined using the bioassay protocols developed.

**SIGNIFICANT CONCLUSIONS:** A successful bioassay was developed. Test protocols yielded repeatable results and statistically sound data. Adult harpacticoids were found to be relatively tolerant to PAHs in 10-day exposures. Species-specific differences in sensitivity were detected. Early life history stages were much more sensitive than adults in one species but not in the other. Low concentrations of PAHs decreased copepod offspring production, egg hatching success, and embryonic and early-stage development, demonstrating the high sensitivity of life history related endpoints. In addition, grazing on microalgae was significantly impaired at low concentrations after short exposures (< 30 h). Finally it was demonstrated that harpacticoids can actively avoid contamination. These protocols are proving useful to address other questions regarding contaminant effects on benthic organisms.

**STUDY RESULTS:** The results of the life history studies suggest that, for *S. knabeni*, offspring production is the most sensitive life-cycle variable, followed probably by age at first reproduction and egg hatching success. For *N. lacustris*, results suggest that survivorship at the copepodite stages is the most sensitive variable, followed by offspring production, and egg hatching.

**STUDY PRODUCTS:** Fleeger, J.W. and G.R. Lotufo. Development and application of a sublethal toxicity test to PAH using marine harpacticoid copepods: Final report. OCS Study MMS Pub. No. MMS 99-0001. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, Louisiana, 51 pp.

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