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Project Summary

Cycling of Xenobiotics Through Marine and Estuarine Species

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Cycling of xenobiotics was studied using time-lapse photography to evaluate effects of Kepone and sodium pentachlorophenate on feeding activity of the lugworm, *Arenicola cristata.* The fate and effects of methyl parathion in microcosms inhabited by lugworms were determined. Uptake and depuration of chrysene by lugworms were evaluated.

A toxic sediment bioassay system was developed to test effect of dredged material. The system included mysid shrimp, *Mysidopsis bahia,* oysters; *Crassostrea virginica;* and lugworms, *Arenicola cristata.* Criteria of effects were survival of mysids, shell deposition and bioaccumulation by oysters, substrate reworking and bioaccumulation by lugworms, and settlement of zooplankton. Kepone-sorbed sediment and dredge spoil from James River and Houston Ship Channel were tested. Long-term tests were used to evaluate effects of a specific drilling mud from an active exploratory platform.

Predator-prey tests of sublethal effects of xenobiotics demonstrated effects in one-prey and two-prey systems. The effects of methyl parathion on predator-prey relationships between grass shrimp, *Palaemonetes pugio;* juvenile sheepshead minnows, *Cryprinodon variegatus;* and gulf killifish, *Fundulus grandis,* were demonstrated.

Evaluation of sublethal effects, such as avoidance of pollution gradients, was studied in a trough-type avoidance-response system. The system was tested with pinfish to demonstrate that they will avoid chlorine-produced oxidants.

The assessment of the potential impact of environmental contaminants depends on the accurate measurement of the fate and effects of these pollutants on both field and laboratory. This project was directed toward developing methods to provide more sensitive evaluators, other than acute and chronic toxicity tests for a xenobiotic's fate and effect in estuarine and marine ecosystems. The goals of the project were to:

(1) evaluate cycling of selected xenobiotics or uptake and effect of selected energy related compounds in experimental systems that include the lugworm, *Arenicola cristata;*

(2) develop a toxic sediment assay system involving the lugworm and other species;

(3) develop tests involving estuarine and marine crustaceans and fishes designed to evaluate how exposure to xenobiotics can alter predator-prey relationships;

(4) develop and test behavioral assays that provide reliable means to evaluate sublethal effects.

This Project Summary was developed by EPA's Environmental Research Laboratory, Gulf Breeze, FL, to announce key findings of the research project that is documented in a separate report of the same title (see Project Report ordering information at back).

Cycling of Xenobiotics by the Lugworm, *Arenicola Cristata*

The impetus to design an assay system involving a lugworm resulted from development of culture methods for that species and recognition that toxicity tests employed by EPA for estuarine and marine species do not include an infaunal organism.

Benthic Photo-Bioassay System

The lugworm, *Arenicola cristata* is ideal for this type of test because it is widely distributed in littoral habitats and has major ecological impact due to its ability to recycle sediment and transport xenobiotics into the substrate. The photobioassay system constructed was based on the lugworm's habit of creating feeding funnels on the surface of sediment it occupies. Patterns on the surface of the substrate which indicate activity of the worm, were monitored with time-lapse photographs taken at 12-hour intervals for 72 hours. Areas of feeding funnels in exposed and control aquaria were calculated and compared.

Results indicated that *A. cristata* was sensitive to Kepone at all concentrations tested. The highest concentration was acutely toxic. Lugworms appeared to be more sensitive to Kepone than many other species normally used in toxicity tests. It appeared that in Kepone-contaminated habitats the ability of lugworms to rework sediment would be markedly decreased.

Cycling of Methyl Parathion by Lugworms

A second evaluation of cycling of xenobiotics by lugworms was directed toward determining compartmentation and degradation dynamics of methyl parathion in a small-scale microcosm occupied by only the worm and microorganisms associated with the organic material on which they feed. Ninety percent of radio-labelled methyl parathion disappeared from the water column in aquaria after 14 days. Movement into the sediment proved to be the major compartmentation phenomenon, with over half of the total radioactivity residing in the sediment after two weeks. The lugworm enhanced movement of radioactivity into the sediment and caused dispersion throughout the sediment. Although volatilization losses were negligible, steadily decreasing mass balance of radioactivity in the system suggested accumulation of unextractable residues in the sediment. Analysis of extractable radioactivity in the sediment and water compartments by thin-layer chromatography and autoradiography demonstrated rapid degradation of methyl parathion into a number of more polar products, including *p*-nitrophenol and amino-methyl parathion. Although *A. cristata* was shown to metabolize methyl parathion readily to *P*-nitrophenol, microbial activity accounted for the majority of biological degradation in the system.

Effect of Sodium Pentachlorophenate on Lugworm Activity

The third analysis of effect of xenobiotics on activities of the lugworm was the evaluation of effect of sodium pentachlorophenate (Na-PCP) on feeding activity. Na-PCP was used because it is an energy-related compound (oil-well drilling fluids) and because it enters estuarine and marine systems occupied by lugworms from numerous non-point sources. The photo-bioassay methods described previously were used. Stock solutions of Na-PCP prepared from a commercial bactericide were introduced into experimental aquaria at 45, 80, 156, and 276 μg/l. Comparisons were made between the areas of feeding funnels in exposed and control aquaria. Na-PCP had no marked effect on feeding activity at the lowest concentration tested; however, at the other concentrations, there was significant decrease in activity. Some death occurred at the higher concentration.

Uptake and Depuration of Chrysene by Lugworms

The final analysis of cycling of xenobiotics by lugworms involved uptake and depuration of chrysene, another energy-related compound. Worms were exposed to chrysene at measured concentrations of 0.07, 0.69, and 2.76 μg/l large wooden tanks in an open system that simulated ambient conditions and the natural habitat. After 14 days, exposed worms were moved to uncontaminated systems and allowed to depurate for 14 days. From lowest to highest exposure, lugworms accumulated 65, 516, and 682 μg/l in 14 days. There was a continued increase in accumulation during that period, so it is probable that had exposure time been increased, higher concentrations of chrysene would have been encountered before equilibrium was reached. Little depuration was observed. This suggested that lugworms are unable to degrade chrysene; thus their potential to introduce chrysene into various food chains utilized by man is high.

Toxic Sediment Bioassay System

Many xenobiotics in marine environments have a high affinity for particulate material (especially organics) and thus become sequestered in bottom sediments. Due to increased dredging and maintenance of navigable water, there is a greater need to evaluate impact of toxic sediments on the biota. For that reason, grant activities were directed toward developing a flow-through toxicity test that could be used to determine biological effects of contaminated sediments on representative estuarine organisms and to evaluate resiliency of benthic communities exposed to contaminated sediments. The test developed incorporated several established toxicity tests that were modified to examine acute and sublethal effects of dredged sediments on the biota. It was designed to serve as a screening tool to detect potential hazards of dredge spoils prior to disposal in the marine environment.

Kepone-Sorbed Sediment

Small scale estuarine microcosms were assembled using 10-gallon aquaria that received flowing, unfiltered seawater. Artificially prepared sediments containing Kepone at 0.1, 1.0, and 10.0 μg/l were used. Three aquaria received different concentrations of test sediments, while three others remained unperturbed and served as controls. Comparisons were made after 28 days. Organisms included in the test were representative of three environmental compartments affected by dredging activities. Included were mysid shrimp, *Mysidopsis bahia;* oysters, *Crassostrea virginica;* and lugworms, *Arenicola cristata.* Test criteria used to identify effect were: (1) survival of mysids; (2) shell deposition and bioaccumulation of known contaminants by oysters; (3) substrate reworking and bioaccumulation by lugworms; and (4) resiliency of the benthic community in terms of numbers and variety of macrofaunal organisms that settled onto test sediments as planktonic larvae within 28 days.

Effect of Kepone-sorbed sediment on mysid survival was time- and dose-

dependent. Oyster shell growth was significantly inhibited. Lugworms had an increasing dose-dependent relationship in concentration of Kepone. Whole-body residues were 0.043, 0.46 and 1.1 μg/l. Nineteen macrofaunal species from four major taxa were identified. Using test criteria, only polychaetes were affected at the highest exposure.

Dredge Material

Tests with actual dredged material from the James River and Houston Ship Channel were conducted. James River sediment did not affect mysids significantly although there was some effect on oysters. Lugworms substrate reworking was reduced in experimental aquaria. Oysters and lugworms concentrated Kepone. Little difference was seen in survival of recruited larvae, perhaps because few larvae entered the system during the winter when it was in operation. Houston Ship Channel sediment did not significantly affect mysid survival or oyster shell deposition; nor did lugworm activity or macrofaunal composition vary significantly between control and experimental units.

Drilling Muds

A long-term (100-day) toxicity test was conducted using the toxic sediment assay system to determine effects of a specific drilling mud. Drilling muds were obtained weekly from an active exploratory platform and tested within one week of collection. Three dilutions were tested: 10, 30, and 100 ml/l. These concentrations represented those expected to occur at intervals of from several meters to several hundred meters from a point source. Mud was added to test aquaria to simulate periodic discharge. The same species previously employed were included in this test but mysids were exposed for only 10 days.

Mysids exposed in the system were not affected acutely. Oyster shell growth was inhibited significantly at concentrations of 30 and 100 ml/l but there were no deaths. Lugworms were severely affected by exposure to the mud. Mortalities observed were 75% at 100 ml/l, 64% at 30 ml/l, and 33% at 10 ml/l. Twenty recruited species were present after 100 days. There was no significant difference between populations in the aquaria. Ba, Cr, and Pb were found to have accumulated significantly in oyster tissue.

The results indicate that physical as well as chemical properties must be considered before environmental impact of drilling fluids can adequately be assessed. It was also recognized that composition of drilling muds is highly variable; thus, impact should be considered on a case-by-case basis.

Predator-Prey Tests

Sublethal concentrations of xenobiotics, especially pesticides, may be expected to affect various aspects of behavior. If pesticides have different effects on species in a multiprey system, predators consume a higher than normal proportion of affected species. The result would be more rapid accumulation of a xenobiotic.

Palaemonetes pugio and juvenile sheepshead minnow, *Cyprinodon variegatus,* were exposed to methyl parathion for 24 hours before introduction of *Fundulus grandis,* the predator. The killifish consumed a greater proportion of grass shrimp relative to sheepshead minnows. Increasing the concentration resulted in increased consumption of grass shrimp relative to fish prey, an example of how a pesticide can alter relative proportions of prey in a predator's diet.

Evaluation of Sublethal Effects in Special Test Systems

Avoidance of Pollution Gradients

It has often been observed that fish and invertebrates avoid pollution gradients. Most apparatus designed to detect avoidance of pollutants by aquatic organisms require visual observation of the test organisms in steep pollution gradients. The Aquatic Gradient Avoidance Response System (AGARS) was developed to eliminate these limitations. This system allows animals to choose among one uncontaminated zone and three increasingly toxic zones in a gradient trough that is monitored for extended periods by infrared light sources, sensors, and a microprocessor. Initial tests in AGARS indicated that pinfish, *Lagodon rhomboides,* avoid chlorine-produced oxidants at concentrations of 0.02-0.04 mg/l.

Toxicant Induced Changes in Cyclic Burrowing Patterns

The pink shrimp, *Penaeus duorarum,* is a species that is very sensitive to xenobiotics. Since no life-cycle toxicity test exists for penaeid shrimp, the only criterion of effect that has been used for hazard assessment is death. Pink shrimp normally remain buried in substrate during the day and emerge at night. Stress from both lethal and sublethal pesticide exposures disrupt this pattern and may result in the shrimp's continuous presence above the substrate. Such activity would increase predation and cycling of xenobiotics. To evaluate the effect of toxicant-induced disruptions in the cyclic burrowing pattern, an apparatus was constructed from a modified AGARS system.

The results indicated variability in absolute activity level of a particular shrimp on different days as well as between shrimp. On a daily basis, there was significant difference in activity between days when toxicant was added and days when it was not.

Conclusions

The toxic sediment bioassay system developed under this project shows promise of becoming a useful method in determining biological effects of potentially toxic sediments on representative estuarine organisms and benthic communities. It provides several distinct advantages over existing dredged material tests. Thus, it may be a suitable methodology for future inclusion in the testing manuals used to generate data in support of permitting programs such as S. 103 of The Marine Protection, Research and Santuaries Act of 1972.

The behavorial tests investigated demonstrate that measurable biological responses occur at contaminant levels below those acutely toxic. Further research is required, however, to achieve full understanding and interpretability of the information generated by these tests.

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The complete report, entitled "Cycling of Xenobiotics Through Marine and Estuarine Species," (Order No. PB 82-239 252; Cost: $9.00, subject to change) will be available only from:
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