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Project Summary

Experimental Marine Microcosm Test Protocol and Support Document

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The purpose of the experimental ecosystem (microcosm) test protocol is to determine the fates and ecological effects of various "photostable" chemicals within a site-specific marine or estuarine ecosystem. The protocol, microcosm facility and associated experimental procedures correct some of the limitations imposed by single species and single variable or process test systems by simulating most of the relevant dimensions of the natural system in both the water column and the benthos. The data resulting from comparisons of experimental controls with the field are far more applicable to predictive environmental assessments than those derived from simpler tests. Furthermore, since the information generated via microcosm experiments is integrated at the system level of organization, quantitative relationships can be determined for factors such as chemical exposure concentrations and transport rates to measured body burdens, ecological effects, input concentrations and loadings.

There are, however, two constraints upon the use of the system: 1) Because of the size of the microcosm tank, macrofauna cannot be tested; thus, data on some economically important species cannot be derived. In addition, the exclusion of macrofauna could affect significant variables in the system, but whether or not this would be the case, particularly within the experimental time frame of 30 days, has not been established; 2) The microcosm test system was developed from a temperate Northeast (U.S.) estuarine ecosystem where intertidal and reef subsystems are either small or lacking. The applicability of this protocol to other ecosystems where either shallow conditions or major reef subsystems exist has yet to be determined.

A support document, incorporated as Part (c) of the full report, provides a rationale for the use of the test system.

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

Introduction

Chemical companies are required, under the Toxic Substances Control Act (TSCA), to submit information about new and existing chemicals. This information is then used by the U.S. Environmental Protection Agency (EPA) to develop an environmental or risk assessment for each chemical. In the marine environment, the assessment of risk from a specific chemical includes the effects of that chemical on the receiving body or ecosystem and the potential for indirect human exposure through the consumption of marine organisms. Such information is usually derived from simple test systems. For example, components of the ecosystem (single species) or processes (e.g., water solubility or biological breakdown) are isolated and either exposed to or measured in the presence of the chemical of interest. These data are then used to develop the environmental assessment. However, such assessments have been subject to ques-

tion on three major grounds. First, the data base is usually confined to the measurement of only a few variables. Second, the validity of these data is tenuous, since the physical, chemical, and biological complexities of the natural ecosystem are not simulated. Third, the use of these data to generate a meaningful and accurate prediction of the fate, transport, and ecological effects of the chemical in a natural ecosystem is highly questionable.

Test Protocol

The Microcosm Test Protocol described in the full report is designed to overcome many of the concerns associated with environmental assessments based upon existing simple (single species and processes) test systems. Specifically, undisturbed, natural pelagic, and benthic communities are coupled within a single system, the physical and chemical conditions of which are equated to those in the natural system being simulated.

For purposes of the protocol, the natural system undergoing assessment is defined in terms of its physical and temporal boundaries, its light and temperature regime, its water composition, turbulence and turnover rate, the ratio of benthic surface area to sea water volume, the sediment characteristics, and the water flow rate over the sediment surface.

The biota of the water column are characterized by the number and species composition of phytoplankton, zooplankton, and transient larval forms found at the designated collection site at mid-tide. The benthic subsystem is characterized by the sediment type and the structural composition of the benthic community. If the natural system has more than one distinct benthic community, then those organisms directly linked to human consumption or important to the economics and the aesthetics of the area should be considered for experimental use. If some benthic communities contain species which are known to be more sensitive to environmental contaminants than others, these communities should also be considered.

Microcosm Facility

The microcosm facility (Figure 1) is composed of two basic parts: (1) the support equipment (room, waterbath, light and turbulence fixtures, test compartments, and an air evacuation system), and (2) the experimental microcosm (tanks, paddles, benthic cylinders, pumps and pump air supply).

The microcosm test system is placed in operation as follows: (1) The test water is collected from the natural system at mid-tide by hand bucketing or non-destructive pumping, (with a diaphragm pump) and transported to the test facility in glass tanks. The test water is then distributed equally among the microcosm tanks until the prescribed volume is reached. (3) Benthic cylinders are used by divers to collect undisturbed sediment cores from the prescribed portion of the natural system. The bottoms of the cylinders (cores) are sealed, prior to installation in the microcosm tanks, by placing them in slightly larger diameter crystallization dishes. The benthic cylinders and crystallization dishes containing the sediment cores are then placed in the tanks, and any disturbed sediment is allowed to settle (for approximately 30 minutes). (4) The benthic pumps are placed adjacent and connected to the benthic cores. Air lines supplying low pressure air are attached to create the desired water flow rates over the sediment surfaces. (5) Paddles are installed, and the speed of rotation is established to generate a water column turbulence level equivalent to that in the natural system. (6) Light intensity to the water in the tanks is controlled by adjusting the shading of fluorescent lights to appropriate levels, and the photoperiod is set equal to ambient conditions. (7) Water flow in the bath is adjusted to provide temperature tracking within 1°C of the natural system. Flow rates must also be sufficient to maintain all experimental tanks within 1°C of each other. (8) After the disturbed sediment in the benthic cylinders settles, the low pressure air to the benthic pumps is turned on, and water flow to the benthic cylinders is begun at an average rate equal to the average tidal flow over the benthic substrate in the natural system. (9) Any resuspended sediment that settles on the bottom of the microcosm tanks at any time during the experiment should be collected with a tubing

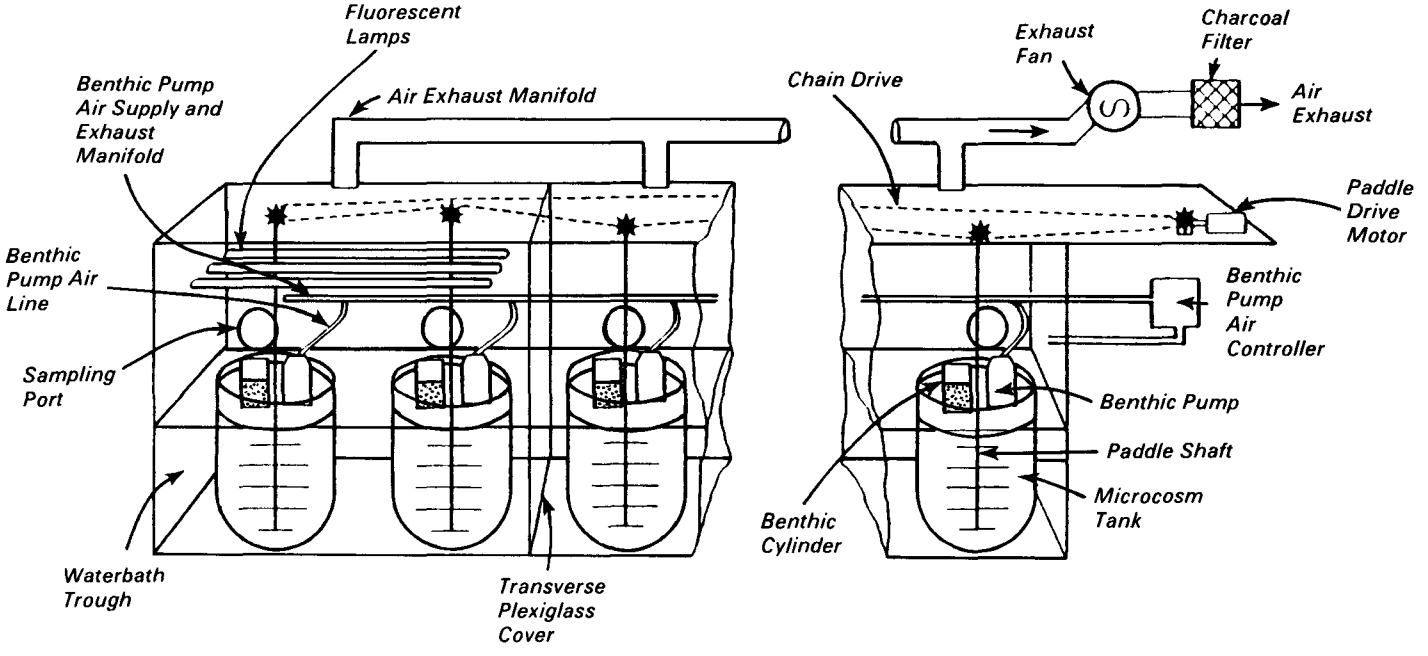

Figure 1. Experimental microcosm facility.

pump and replaced into the bentnic cylinders. (10) Water turnover volume and frequency are established to match the exchange rate of the natural system. Water exchange in the microcosm tanks should be scheduled at least three times a week and should coincide with the biological and chemical sample collections. (11) If a surface film containing the test substance is formed, a portion of that film must be removed during each water exchange to simulate natural transport. The area of surface film to be removed by wicking with filter pads or other suitable absorbent material is defined by:

$$\text{Area (cm}^2\text{)} = \frac{\text{water turnover volume/turnover day}}{\text{total microcosm water volume.}} \text{ X benthic surface area}$$

Entry of Test Substance

If the mode (i.e., atmospheric and/or aqueous) and form (i.e., gas, liquid, or solid) of entry of the test substance into the marine environment is known, as with existing chemicals, or predictable, as with "new" chemicals under TSCA, then similar mechanisms of entry will be simulated in the experimental microcosms. If the only mode of entry is the atmosphere, then this protocol is not applicable at this time. However, if the mode of entry is partially or completely aqueous, then the test substance, in its realistic form, will be added to achieve nominal water concentrations (i.e., those based upon projected or measured field exposures and varying by at least two orders of magnitude). If the mode and form of entry of the test substance into the marine environment is unknown, then the mode of entry into the microcosms will be aqueous. The form of entry will depend upon the solubility of the test substance in sea water. If the test substance is insoluble in sea water, then an appropriate carrier will have to be used. An appropriate carrier would completely dissolve the test substance and result in a uniform water column distribution of the test substance at the start of the experiment.

Ecological and Chemical Effects Sampling

Simultaneous measurements of the test substance and ecological effects are prescribed for a thirty-day period. This sampling period enables the investigator to establish either the time for the test substance to reach quasi-equilibrium or the degree to which equilibrium is reached within the various physical and biotic compartments of the experimental microcosm.

Those measurements required to describe ecological effects (i.e., phytoplankton, zooplankton, and benthic organism abundance, and ammonia levels and flux rates) are dependent on the characteristics of both the contained biotic communities and the test substance. Ideally, a structural and functional measure should be made from each trophic level. It is recommended that additional ecological measures be made in those compartments exhibiting significant accumulation of or exposure to the test substance.

The test substance is measured within the microcosms to establish (1) material budget (2) total transport, and (3) bioaccumulation and potential trophic transfer.

It is necessary to develop a material budget of the test substance and resultant breakdown products throughout the course of the experiment. This indicates the accuracy of measured concentrations and rates of removal within selected microcosm compartments. The compartments identified as essential to a material budget are the overlying gas phase, surface film (if produced), water column, sediment, glass surfaces and benthic macrofauna not included in the sediment sub-cores. Water analysis should include determination of test substance concentrations in terms of the total, dissolved, and particulate fractions.

Bioaccumulation of the test substance can result in subsequent ecological effects and may also be a potential source for human exposure via direct consumption or upward trophic transfer. It is essential to be able to define quantitatively the relationship between the amount of test material added (i.e., the input function) and subsequent bioaccumulation levels, trophic transfer, and ecological effects.

Analysis of sediment sub-cores is required to determine the vertical profile of the test substance and, thus, exposure concentrations for some of the benthic organisms.

The amount of test substance found in the surface film and subsequently lost through gas transport or decomposition can, depending on the characteristics of the substance, form a significant fraction of the total budget. As a result, the design and performance of any chemical sampling program must take the contribution of the surface film into consideration.

The temporal dynamics and quantities of the test substance and breakdown products within selected microcosm compartments enable the investigator to determine: (a) the exposure concentrations and the relationships to the body burdens of associated biota, (b) the concentration of the test substance at which ecological effects are observed, and (c) the total transport of the test substance and breakdown products. All of these measures can then be related to the three different quantities of the test substance added to the microcosms (season and the interaction of season and test substance, if included as a variable in the microcosm).

Cleaning

The combination of benthic biological activity and water flow through the benthic chamber will result in significant but realistic quantities of suspended sediment in the microcosm tanks. Under natural conditions, this resuspended sediment settles back to the sediment surface. However, in the microcosms, the resuspended sediment settles to the bottom of the larger microcosm tanks. All such settled sediment is collected at the time of water turnover and returned to the benthic box in order to compensate for and minimize the effects of this artifact.

Data Analysis

One of the major features of the recommended experimental design and statistical analysis (see Section 6.1 of the full report) is its ability to establish independently the quantitative effect of (1) the solvent carrier (if used) and, (2) the test substance for all the variables measured.

Furthermore, the degree of divergent biotic behavior between the control microcosms and the natural system provides a measure of the validity of the microcosm responses to the test substance.

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The complete report, entitled "Experimental Marine Microcosm Test Protocol and Support Document: Measurement of the Ecological Effects, Fate and Transport of Chemicals in a Site-Specific Marine Ecosystem," (Order No. PB 83-230 854; Cost: $10.00, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

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