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Standard Guide for Chemical Fate in Site-Specific Sediment/Water Microcosms[1]

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1. Scope

1.1 This guide provides procedures and criteria for the development and use of sediment/water microcosms for laboratory evaluations of the fate of chemical substances in the environment. It does not specify specific microcosms but it establishes minimum criteria for distinguishing acceptable microcosms from those that may be incomplete or inappropriate for site-specific extrapolation (see 5.1 and 10.1).

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 729 Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians[2]

E 1279 Test Method for Biodegradation By a Shake-Flask Die-Away Method[2]

2.2 *U.S. EPA Standard:*

Toxic Substances Control Act Test Guidelines; Proposed Rule, Site-Specific Aquatic Microcosm Test[3]

3. Terminology

3.1 *Description of Term Specific to This Standard:*

3.1.1 *microcosm*—an intact, minimally disturbed portion of an ecosystem brought into a laboratory for study under controlled experimental conditions.

4. Summary of Guide

4.1 This guide provides guidance on the development, use, and evaluation of microcosm studies used to evaluate the fate of chemical substances in specific aquatic ecosystems. It establishes minimum criteria for distinguishing acceptable site-specific fate microcosms.

5. Significance and Use

5.1 The fate of chemicals released to the environment may be evaluated in the field or in laboratory studies. This guide provides direction on the development, use, and evaluation of microcosm studies that simulate a specific aquatic ecosystem and include sediment and relevant biota. A key objective in the use of site-specific microcosms is the ability to extrapolate information obtained in the laboratory system to field situations with a reasonable degree of confidence.

5.2 Field studies can obtain important information about the fate of chemicals in a particular ecosystem but have many disadvantages. In field studies, environmental variables, in general, cannot be controlled and the study may be subject to wide fluctuations in variables such as temperature, rainfall or sunlight. Introduction of a chemical into an ecosystem may produce an unacceptable environmental risk. Furthermore, field studies often are prohibitively expensive.

5.3 Some environmental fate studies use structural or synthetic communities (not site-specific microcosms) created by placing water, soil or sediment, plants, animals and microbiota in a container according to an established protocol. Some synthetic communities have been specifically designed to examine the fate of chemical substances in aquatic environments (that is, Metcalf et al. **(1)**[4] and Isensee and Tayaputch **(2)**. These synthetic communities provide reproducible environments in which to evaluate and rank chemicals according to their fate but extrapolation to specific ecosystems is difficult. This is because they lack complex population structures and processes analogous to specific natural ecosystems. In addition, they frequently contain a biomass of organisms that is not scaled to the volume of water or sediment, thereby giving exaggerated rates of chemical metabolism.

5.4 A microcosm replicates many of the processes affecting the fate of a chemical in a complex ecosystem. A microcosm can be examined under controlled laboratory conditions in the absence of certain variables that might interfere with an understanding of a particular process. Microcosms provide an opportunity to manipulate variables and to study their effects and interactions. Microcosms also offer replication possibilities

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[2] *Annual Book of ASTM Standards*, Vol 11.05.

[3] Federal Register, Vol 52, No. 187, 1987, pp. 36352–36360.

[4] The boldface numbers given in parentheses refer to a list of references at the end of the text.

for assessing environmental variability, an advantage that is not available from field studies.

5.5 Microcosms can be used to examine the significance of various fate processes. By examining test compounds in microcosms it is possible to determine the relative effects of various fate processes (for example, biotic versus abiotic). This makes it possible to focus on critical processes and consider site-specific environmental situations where these processes predominate or are absent. Although some fate processes such as hydrolysis or partitioning to sediments may be quantified adequately in simpler studies (for example, shake-flask or aquaria tests) others such as bioturbation may require the complexity of a microcosm for adequate assessment. An important aspect of microcosm testing is determining the significance of biological processes in environmental fate. By studying test compound fate in sterilized microcosms, the role of bioturbation (that can distribute a chemical deep in sediment beds) can be assessed along with biodegradation.

5.6 The following are examples of chemical fate information that might be obtained in microcosm studies.

5.6.1 How long a chemical substance will persist in its parent form in a particular environment,

5.6.2 Whether the fate of a chemical is primarily dependent on biotic or abiotic processes,

5.6.3 The effect on the fate of a chemical by the presence of plants that may take up the chemical and store or metabolize it and that provide additional surfaces for microbial colonization,

5.6.4 The effect on the fate of a chemical by the activity of benthic organisms that move water and sediment, and

5.6.5 The effect of nutrient flux at the water sediment interface on the biodegradation of chemicals in the water column and in the sediment.

6. Preliminary Studies

6.1 A shake-flask test with site water and sediment (for example, using Test Method E 1279) is recommended to provide preliminary information about the fate of a test compound. Biotic and abiotic degradation rate constants, in the presence and absence of sediment, can be determined with this test along with an indication of potential sorption to sediments. An example of data for the pesticide fenthion generated from both shake flask and microcosm tests has been reported **(3, 4)**. The preliminary study may identify those fate processes that should receive close attention during a microcosm study and provide guidance on sampling frequency. Some test compounds, such as those that persist for a very short time period in shake flask tests, may not require further testing in a microcosm. An appropriate reference chemical such as methyl parathion **(5)** or Linear Alkylbenzene Sulfonate (LAS)[5] may be used with the shake flask and microcosm tests.

7. Design Features for Sediment/Water Microcosms

7.1 *Size*:

7.1.1 Because of their size, microcosms can model only a small part of any aquatic ecosystem. They may vary in size from a fraction of a litre to several hundred litres. Smaller sizes maximize the advantages of microcosm use, including operation within a controlled laboratory environment, replicability, containment of toxic chemicals and simplification of dosing and mixing.

7.1.2 A microcosm should be sufficiently large to permit the removal of water and sediment samples during the course of the study without significantly affecting surface to volume ratios over the course of an experiment and without significantly depleting either the water or sediment volumes. Microcosms also must be large enough to readily accommodate monitoring probes, mixing apparatus, etc.

7.1.3 The inclusion of relatively large biotic species (for example, clams and large plants) may not be appropriate in microcosms of only a few litres size. Small microcosms, however, may be the most appropriate for studies of chemical fate processes such as biodegradation and sorption, which generally are not affected significantly by this size constraint.

7.1.4 Since the size and design of a microcosm depends primarily on the issue that is being addressed **(6, 7)**, no "ideal" microcosm design can be recommended. For example, studies focusing on the interaction of a test compound with sediment, benthic macrophytes, bioturbating-macrofauna, or small fish each require specific modifications to accommodate the necessary compartments/organisms. A variety of water/sediment, site-specific microcosms have been described for studying the fate of xenobiotic compounds in the aquatic environments. These test systems vary in size from Ecocores, used by Spain, et al., containing approximately 175 mL of water **(8)**, to the 140 L test systems used by Perez et al. **(9)**, with many intermediate sizes **(3, 10, 11, 12)**.

7.2 *Water*:

7.2.1 Collect water for the microcosm from the field site above or nearly above the site of sediment core collection. Collect water by hand bucketing or non-destructive pumping. If the water column in the natural system is stratified, the microcosm water should contain samples taken from representative depths.

7.2.2 If the site water is to be the source of the test compound, sampling containers should be composed of materials such as glass or fluorocarbon plastics to minimize sorption. Take care to avoid the use of plastics (for example, plasticized polyvinyl chloride) that may leach plasticizers into the water. Transport water samples to the test facility with minimum delay and maintain field temperatures as close as possible. Effects of containerization ("wall effect") may occur soon after collection, and thus shipment over long distances may be detrimental. If, for some reason water must be held in the laboratory overnight, gently stir it using a magnetic stirrer and loosely cover the water container to prevent dissolved oxygen depletion.

7.3 *Sediment*:

7.3.1 Because of the well-documented significance of sediment in the biodegradation of many chemical substances **(13, 14, 15, 16)**, the microcosm designs covered by this guide include intact sediment cores.

[5] Linear Alkylbenzene Sulfonate (LAS) is available from Quality Assurance Research Division, Room 525, Environmental Monitoring Systems Laboratory, US EPA, Cincinnati, Ohio 45168.

7.3.2 Site-specific extrapolation of sediment-enhanced biodegradation information must take into consideration the water column volume to sediment surface area ratio. Sediment-mediated processes will be accentuated in shallow bodies of water and may be insignificant relative to processes in deep water.

7.4 *Coring*:

7.4.1 For microcosms consisting of sediment and a water column, it is important to obtain sediment cores that are as intact as possible to preserve the structural integrity of the sample, including the redox gradient and the benthic community.

7.4.2 The inner diameter of the corer may be designed such that the microcosm sediment surface area to water volume ratio equals that of the natural system.

7.4.3 For some microcosms **(17, 18)** the corer becomes the "microcosm". Insert a suitably sized glass tube (for example, the 3.5 cm diameter and 40 cm length tube used by Spain et al. **(17)**, into the sediment to a depth of 8 cm or more, sufficient to include relevant biological activity. Seal the top with a silicone stopper. Carefully remove the tube from the sediment and close the bottom of the tube with another silicone stopper. After carefully transporting the cores back to the laboratory, adjust the water volume of the microcosm to the desired water volume to core surface area ratio.

7.4.4 Alternatively, obtain an intact core in the field and extrude it into a microcosm vessel **(10, 19, 20)**. A simple and effective coring device can be made of clear acrylic tubing with serrations along the bottom for cutting through plant roots. Seal the top with an acrylic disc containing a hole that can be plugged with a stopper. Insert the corer into the sediment to a depth equal to or greater than the depth of biological habitation or activity. This could range up to 20 cm in depth **(21)**. Plug the hole on top with a stopper and carefully raise the corer and insert it into the microcosm vessel. Remove the stopper and lift the corer out of the microcosm, leaving the sediment plug intact. For large water volume to sediment surface area ratios, place the corer in a glass dish (for example, crystallization dish) with a diameter slightly larger than the corer and the entire assembly (core + corer + dish) placed into the microcosms **(19)**. Some sediments are very difficult to work with and several attempts may have to be made before an intact core can be lifted and transferred.

7.4.5 Since coring and transport from the field often suspends sediment in the overlying water column, a carboy of site water is also collected. At the laboratory, carefully siphon off the overlying water column and gently add new water to the desired volume.

7.4.6 Scuba equipment may be required to obtain undisturbed cores in relatively deep water.

7.5 *Dosing Microcosms*:

7.5.1 Maintain microcosms in either flow-through or static-renewal modes. For the latter, replace a fixed percentage of microcosm water with fresh site water. A flow-through mode is the most complex to operate but may avoid nutrient deficiencies and build-up of metabolic waste products. A one-time pulse dose of test compound may be applied in conjunction with any of these modes. For a pulse dose in a flowing system, the relationship between molecular overturn (partial replacement) time and the flow rate and volume of the microcosm chamber is characterized in a graph by Sprague **(22)**.

7.5.2 For flow-through systems employing relatively large concentrations of test compounds, as in the case of effluent testing, a pump or headbox/siphon arrangement is recommended. All parts of the pump and delivery tubing that come into contact with either test compound or diluent water should be inert and should minimize sorption of the test substance.

7.5.3 Test substance may be dissolved in a carrier (ideally, sterile diluent water) and the resulting stock solution metered into flowing diluent water. Use peristaltic pumps utilizing silicone tubing for adding diluent water but sorption of test compound to silicone tubing may be significant if it is used to deliver stock solutions. A syringe pump with glass syringes and inert fluorocarbon plungers and tubing is more desirable for introducing a test compound and carrier into flowing diluent water.

7.5.4 If the test substance is insoluble in water but soluble in a relatively nontoxic, water miscible solvent, dissolve it in the minimum volume of carrier solvent required to form a homogeneous stock solution of known concentration. Carriers other than water that are acceptable in aquatic toxicity testing (see Test Method E 729) may be used but take care that any increased organic carbon load due to the carrier does not significantly affect the contained living communities. If the test compound is added continuously, keep the stock solution and delivery lines free of microbial contamination to avoid degradation of the test compound before it reaches the microcosm.

7.5.5 The method and pattern of applying a test substance to a microcosm should reflect the release pattern expected in the natural system.

7.5.6 Use of sterile microcosms permits determination of the relative significance of biological activity on the fate of a test compound. The effects of bioturbation are indicated by distribution of the test compound to significantly greater depths in the nonsterile microcosm than in the sterile one. Differences in total quantity of test compound recovered from the nonsterile versus the sterile microcosm are a measure of biodegradation. Each sterilization process has certain advantages and disadvantages. The choice of sterilization method is up to the investigator.

7.6 *Temperature Control*—Most biotic and abiotic transformation processes are temperature dependent and temperature regulation is important. Although water has a relatively high specific heat, factors such as artificial light may have a significant effect on the temperature in aquatic microcosms. Temperature control can be obtained by placing the microcosm in a constant temperature chamber but the preferred method is to place the microcosm in a water bath or water jacket containing circulating water maintained at the temperature of the natural system.

7.7 *Light*:

7.7.1 Because of their critical roles in primary productivity, light quantity and quality are among the most important controllable parameters in microcosm studies. A variety of lighting systems has been reported. Banks of 40 W, "cool-white" fluorescent lights provide one of the least expensive

choices, and their spectra are similar to sunlight. Their intensity is very low, however, which may reduce photosynthesis. Metal halide lamps provide higher intensity and emit certain wavelengths needed by benthic macrophytes. A400-watt lamp with ballast and housing[6] has been found to be sufficient for maintaining *Thalassia testudinum* plants in the lab for short periods of up to six weeks **(23)**.

7.7.2 Adjust light intensity to the level that is equivalent to the average light intensity on the sediment surface in the natural system. Light intensity can be adjusted by covering the upper portion of the microcosm with a screen, such as a nylon net, or other spectrally neutral light filter.

7.7.3 Light photoperiod typically is controlled by simple timers and generally is fixed at some arbitrary ratio (for example, 12 h on and 12 h off) or maintained at ambient field conditions. Although it is possible to simulate seasonal changes in light to dark ratios as well as dusk to dawn transitions in intensity, it is unlikely that these factors will significantly affect fate processes over a test period.

7.7.4 In the room containing the microcosms, light sources, other than that used specifically for the microcosms, should be minimized.

7.8 *Microcosm Construction Materials*—Give careful consideration to composition of all materials in contact with microcosm water. To minimize analytical problems resulting from extraneous contamination (for example, from plasticizers leached from tubing) or sorption of test compound to surfaces, use of inert fluorocarbons and glass are recommended wherever possible. Restricting microcosm studies to these materials also will eliminate potential toxic effects. Metals and constituents of various plastics and rubber can be toxic to aquatic organisms **(24, 25)**. If stoppers must be used, those made of silicone rubber are recommended. Silicone rubber may sorb organic chemicals but appears to be less toxic to aquatic organisms than other types of rubber.

7.9 *Mixing*:

7.9.1 Mixing should be adequate to uniformly distribute the chemical in the water column but not resuspend sediment. It also may be necessary to simulate the natural movement of water at the sediment/water interface. Mixing can be accomplished with pumps, aeration, or stirrers. Use of glass or fluorocarbon stirrers attached to small motors generally is the most satisfactory approach. Aeration is often unsatisfactory because it may cause significant losses of volatile test substances and may result in uneven mixing. Aeration generally is not required for oxygenation and, if used for this purpose, may distort the natural ecosystem conditions.

7.9.2 Turbulence may be generated by glass paddles connected to drive shafts, as described by Perez et al. **(19)**. Control the turbulence level to approximate that occurring in the natural system by dissolution of gypsun slabs **(21)**. The direction of paddle rotation is frequently reversed to prevent the formation of a vortex.

7.10 *Test Compound Concentration*—Selection of test compound concentration is primarily a function of realistic or predicted environmental concentrations. Other factors may include solubility, sediment partitioning, analytical detection limits, and toxic effects on biota, including the degrader organisms. A preliminary shake-flask study (for example, using E 1279) may aid in establishing an appropriate concentration.

8. Sampling and Analysis Techniques

8.1 *Water Samples*:

8.1.1 Collect water samples in replicate from microcosms at dosing time (after an appropriate mixing period) and periodically thereafter. Design sampling regimes for both static mode systems and flow-through systems according to the expected disappearance rate of the parent compound.

8.1.2 It is recommended that water samples be taken at frequent intervals (for example, at 0, 1, 3, 6, 12, and 24 h) following the start of the test, to characterize sorption and/or volatilization. The frequency of additional sampling would depend upon loss rate constants or half-lives ($t_{½}$) determined by the preliminary shake flask test (see Section 6). Compute a preliminary rate constant from data from days 0, 1, and 2 (for microcosms, days 1, 2, and 3 may be considered to exclude non-degradative losses from sorption on day 0). If the rate constant is < −0.01/h ($t_{½}$ <3 days), take further samples on day three, four, and five. If the rate constant is −0.01 to −0.004/h ($t_{½}$ = 3 to 7 days), sampling should occur on days four, seven, and ten. If the rate constant is −0.004 to − 0.002/h ($t_{½}$ = 7 to 14 days), samples should be taken on days 7, 14, and 21. If the rate is > −0.002/h ($t_{½}$ > 14 days), sampling should take place on days 14, 21 and 28. A maximum duration for maintaining microcosms has not been determined, however site-specific microcosms have been maintained for as long as 30 days **(9)**.

8.1.3 If the test compound forms a surface film, take water samples through a “slick protector” within which the surface film has been removed by means of an absorbing membrane.

8.1.4 Take water samples near the center of the water column while it is being mixed.

8.2 *Sediment Sampling*—Obtain sediment samples periodically during the test. If the diameter of the sediment core is sufficiently large for repeated sampling without disturbance, this may be accomplished with a single microcosm for each concentration of test substance. If the nature of the sediment or the diameter of the core is such that sediment disruption is a consequence of sampling, it may be necessary to set up as many replicates as sampling times and to sacrifice an entire core at each sampling time.

8.3 *Additional Sampling*:

8.3.1 If possible, all of the test substance added to the microcosm during the study should be accounted for by mass balance. The use of radiolabeled test compounds may facilitate this.

8.3.2 A surface film formed by the test substance may be sampled prior to the collection of each water sample by sorption to a collection device, such as a filter paper.

8.3.3 Techniques have been developed to measure diffusion coefficients (for example, tritiated water **(20)** and sediment mixing by bioturbation (for example, fluorescent microspheres **(26)**).

8.3.4 Potential losses of test substance and transformation products to the atmosphere may be evaluated in several ways.

[6] The 400-watt Multi-Vapor Lamp produced by General Electric, MVR400/U, has been found satisfactory for this purpose **(23)**.

All involve trapping and sampling off-gases and some of the sampling techniques employed are described by Bourquin et al. **(27)** and in the U.S. Environmental Protection Agency Test Guidelines for a site-specific microcosm test.[3] The significance of volatilization and adsorption can also be evaluated from preliminary tests and sterile controls, and may require change of the mixing, aeration or construction materials.

9. Analytical Procedures

9.1 Analytical methods necessary to monitor the fate of test compounds are beyond the scope of this guide. Gas and liquid chromatography techniques are suitable for the quantification of many test compounds. Use of appropriately radiolabeled test substance often enhances the analytical capabilities in microcosm studies, especially when quantifying mineralization or identifying degradation products that need further characterization by conventional analysis.

9.2 Measure concentrations of the test substance, and its transformation products, if possible, for the following components of the microcosm: air, surface film (if present), water column (both particulate and dissolved fractions), various layers of benthic components, representative species of zooplankton, representative benthic organisms, and glass surfaces above and below the water surface.

10. Data Interpretation

10.1 The water/sediment microcosm, like the natural system from which it is taken, is site-specific, and care should be taken to avoid extrapolation beyond this community.

10.2 A material balance should be conducted to determine the fate, with time, of the test substance, including its transport to, from or through and appearance in all important compartments of the microcosm **(3)**, (for example, water column, various depths of sediment, and biota).

10.3 The distribution of test compound in sediment by depth can be determined by measuring residues in segments of sediment cores **(3)**.

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