VALIDATION AND PREDICTABILITY OF LABORATORY METHODS FOR ASSESSING THE FATE AND EFFECTS OF CONTAMINANTS IN AQUATIC ECOSYSTEMS

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Foreword

The symposium on Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems was held in Grand Forks, North Dakota, 8 Aug. 1983. The symposium was sponsored by The American Institute of Biology and The Applied and Aquatic Section of the Ecological Society of America and ASTM Committee E-47 on Biological Effects and Environmental Fate. Terence P. Boyle, National Park Service, presided as symposium chairman and editor of this publication.

Related ASTM Publications

Statistics in Environmental Sciences, STP 845 (1984), 04-845000-16

Aquatic Toxicology and Hazard Assessment: Sixth Symposium, STP 802 (1983), 04-802000-16

Ecological Assessments of Effluent Impacts on Communities of Indigenous Aquatic Organisms, STP 730 (1981), 04-730000-16

Estimating the Hazard of Chemical Substances to Aquatic Life, STP 657 (1978), 04-657000-16

Bacterial Indicators/Health Hazards Associated with Water, STP 635 (1977), 04-635000-16

A Note of Appreciation to Reviewers

The quality of the papers that appear in this publication reflects not only the obvious efforts of the authors but also the unheralded, though essential, work of the reviewers. On behalf of ASTM we acknowledge with appreciation their dedication to high professional standards and their sacrifice of time and effort.

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Introduction

The assessment of the fate and effect of xenobiotic chemicals in the environment has evolved over the past several years into three categories: (1) assessment of the effects of a contaminant at the organism level in standardized acute and chronic tests; (2) assessment of contaminants at the population, community, and ecosystem level using laboratory microcosms; and (3) assessment of environmental exposure and fate of contaminants using mathematical modeling techniques. There have been increasing expressions of need among managers, decision makers, and scientists to validate and establish the limits of predictability of these assessment procedures.

The term *validate,* in the sense of this volume, means establishing the effectiveness of an assessment procedure by substantiating the degree of accuracy. This implies formulation of procedures for comparison of laboratory and field generated data. The term *predictability* implies determining the ability to forecast from laboratory results to what could be expected in a real-world situation. This involves specific advice to users of laboratory data as to the level of confidence and limits of extrapolation.

Establishing the validity and determination of the predictability of assessment procedures must depend on specific sets of hypotheses that both qualify and quantify: (1) the set of environmental variables that are critical in determining differences in exposures and response of organisms to a chemical in laboratory and field, (2) the magnitude of potential indirect effects, and (3) the relative sensitivity of organisms in the laboratory and field.

This volume presents an array of diverse research efforts toward resolving important questions on the status of determining the validity and predictability of current methods of environmental hazard assessment and research.

Terence P. Boyle
National Park Service, Water Resources Laboratory, Colorado State University, Fort Collins, CO 80523; symposium chairman and editor.

David L. Lewis,[1] *Richard B. Kellogg,*[2] *and Harvey W. Holm*[1]

Comparison of Microbial Transformation Rate Coefficients of Xenobiotic Chemicals Between Field-Collected and Laboratory Microcosm Microbiota

REFERENCE: Lewis, D. L., Kellogg, R. B., and Holm, H. W., **"Comparison of Microbial Transformation Rate Coefficients of Xenobiotic Chemicals Between Field-Collected and Laboratory Microcosm Microbiota,"** *Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems, ASTM STP 865,* T. P. Boyle, Ed., American Society for Testing and Materials, Philadelphia, 1985, pp. 3–13.

ABSTRACT: Two second-order transformation rate coefficients — k_b, based on total plate counts, and k_A, based on periphyton-colonized surface areas — were used to compare xenobiotic chemical transformation by laboratory-developed (microcosm) and by field-collected microbiota. Similarity of transformer to nontransformer community structure in blended aufwuchs was indicated by k_b values, and similarity of transformation rates per unit of periphyton-colonized surface area was indicated by k_A values. Xenobiotic chemicals used for the comparisons were methyl parathion (MP), diethyl phthalate (DEP), and 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE). Even though rate coefficients of each of the chemicals were similar among microcosm- and field-collected microbial samples, not all of the field-collected samples showed MP or DEP transformation. The MP transformation was suppressed by aqueous extracts of field-collected, algae-dominated aufwuchs. Lack of DEP transformation appeared to have resulted from an absence of DEP-transforming bacteria in field-collected aufwuchs samples.

KEY WORDS: microcosm, microbial degradation, microbial transformation, methyl parathion, diethyl phthalate, 2,4-dichlorophenoxyacetic acid butoxyethyl ester, mathematical model, aquatic ecosystems

Laboratory microcosms are used routinely to simulate environmental waters for research on the transport, fate, and effects of pollutants. We have used aquatic microcosms in the form of large, flowing laboratory ecosystems for developing

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and testing rate coefficients for microbial transformations of xenobiotic chemicals.[3,4] Our laboratory ecosystems were not intended to simulate particular rivers, lakes, or streams but were designed to provide controlled aquatic systems having a wide diversity of physical, chemical, and biological parameters. The diverse laboratory environments were used to test the applicability of rate coefficients under a wide range of conditions. Although our laboratory ecosystems were not designed to simulate any particular field sites, it is essential that they provide ranges in physical, chemical, and biological characteristics similar to those found in the natural environment. Such a comparability in ranges of characteristics between field and laboratory systems is required if the rate coefficients developed and tested with laboratory systems are to have applicability to diverse field situations.

In developing and testing microbial transformation rate coefficients, we wanted to be sure that our laboratory ecosystem microbiota were similar to microbial populations found in natural waters. To test the comparability, we used two second-order transformation rate coefficients: k_b, based on total plate counts of bacteria, and k_A, based on a ratio of colonized surface area to container volume. Because our laboratory ecosystems were dominated by aufwuchs (microbiota attached to surfaces and floating in mats), we compared transformation rate coefficients for field-collected aufwuchs and for laboratory ecosystem aufwuchs. Three xenobiotic chemicals were used in this study: methyl parathion (MP), diethyl phthalate (DEP), and 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE).

Blended aufwuchs was used for k_b determinations to provide a basis for testing the diversity of bacterial populations. We had previously shown that proportions of transformer to nontransformer bacteria varied spatially and temporally in aufwuchs samples, causing several orders of magnitude of variation in k_b for blended aufwuchs samples (see Footnotes 3 and 4). Therefore, k_b provided a basis for indicating whether laboratory ecosystems and field aufwuchs had similar ranges in ratios of transformer to nontransformer bacteria.

A second rate coefficient, k_A, based on the ratio of colonized surface area to container volume, was shown to be relatively constant under diverse laboratory ecosystem conditions (see Footnote 4). Therefore, k_A provided a basis for determining whether laboratory ecosystem periphyton had similar microbial transformation rates per unit of colonized surface area compared to field periphyton. In summary, we used second-order transformation rate coefficients to test both the diversity of transformer to nontransformer community structure and the similarity of transformation rates per unit of surface area between laboratory and field microbiota.

[3]Lewis, D. L. and Holm, H. W., *Applied Environmental Microbiology*, Vol. 42, 1981, pp. 698–703.

[4]Lewis, D. L., Kollig, H. P., and Hall, T., *Applied Environmental Microbiology*, Vol. 46, 1983, pp. 146–151.

Procedure

Experimental Approach

Our experimental approach was based on Michaelis-Menten kinetics under specific conditions of no growth and low xenobiotic chemical concentrations, whereby the transformation rates were pseudo-first-order relative to the chemical concentrations. Non-saturation conditions were ascertained by determining that the rates of chemical loss were first-order over approximately two orders of magnitude in concentration, that is, plots of the logarithms of chemical concentration versus time were linear (Fig. 1). To ensure no significant organism growth during the experiments, 10^7 to 10^9 colony forming units (CFU) of bacteria per litre were used, and transformation rates were measured during short incubation periods (usually less than 10 h). Control batch cultures that had been autoclaved and were at the same pH as test cultures were used to show that abiotic reactions were insignificant compared to biolysis rates under the conditions of the experiments. Microcosms fluctuated in pH during diurnal cycles, reaching a maximum of approximately pH 8; however, chemical hydrolysis rates were insignificant compared to biolysis rates. Autoclaved batch cultures containing several grams of aufwuchs (ash-free dry weight, AFDW) per litre were used to show that

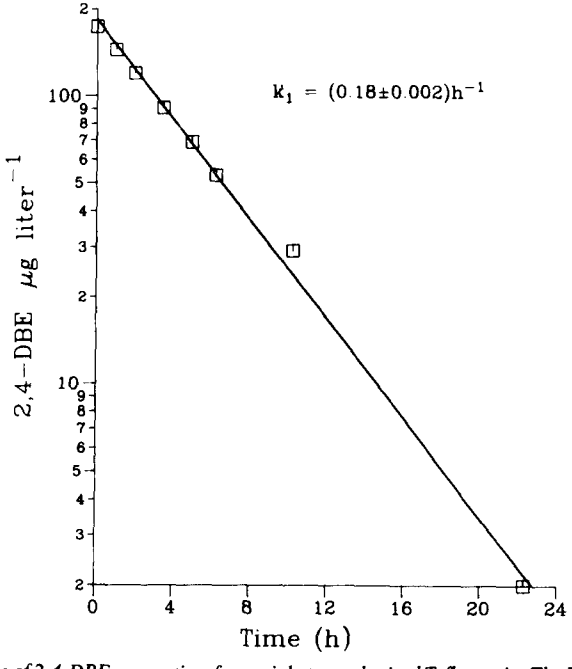

FIG. 1—*Loss of 2,4-DBE versus time for periphyton-colonized Teflon strip. The Reynolds number was 6000.*

sorption of the chemicals to biomass was not a significant process. The microcosms did not contain sediments. In our study, bacteria were shown to be the only significant transformers of MP and DEP. The 2,4-DBE was rapidly transformed by bacteria, fungi, and algae in our test systems. These conclusions were based on the use of candicidin treatments of mixed populations and by testing axenic cultures of bacteria, fungi, and algae for transformation of 2,4-DBE (see Footnotes 3 and 4). No lag in transformation rates of the chemicals occurred; that is, logarithmic plots of chemical concentration versus time were linear from the initial time of spiking (Fig. 1). Transformation rates were not mass-transport limited for suspended populations in batch cultures or microcosm systems. This conclusion was based on the observation that transformation rates did not increase for blended aufwuchs samples as turbulence increased by an order of magnitude. Mass-transport limitation was apparent for attached communities; however, transformation rate coefficients (k_1) were directly proportional to turbulence measured in Reynolds numbers for periphyton attached to Teflon strips (Fig. 2). Therefore, similar turbulences were used for comparison of transformation rate coefficients for attached communities. Transformation rate coefficients (k_1) for Teflon strips colonized with periphyton increased as colonization time increased (Figs. 2 and 3). Colonization rates were much slower in cold water (approximately 5°C) than in warm water (20°C). Strips used in these experiments were colonized at 15 to 25°C and were maturely colonized (as shown by no increase in k_1 for several additional weeks of colonization) after approximately six weeks.

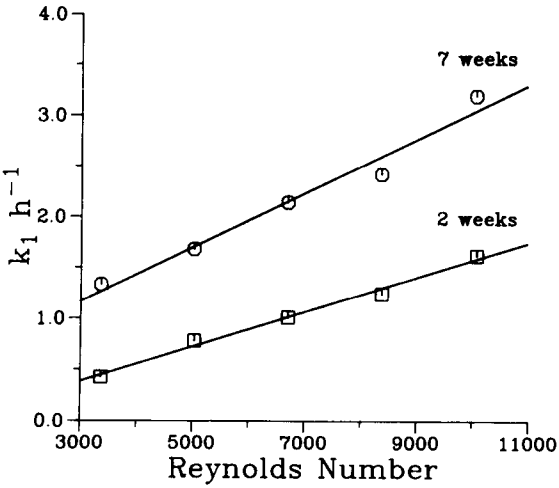

FIG. 2—*First-order transformation rate coefficients,* k_1, *of 2,4-DBE were directly proportional to turbulence measured in Reynolds numbers over the range tested. Teflon strips were colonized in a laboratory microcosm for either two or seven weeks to vary the thickness of periphyton. Biomass as AFDW was 9.0 and 71 g/m*^{2}, *respectively.*

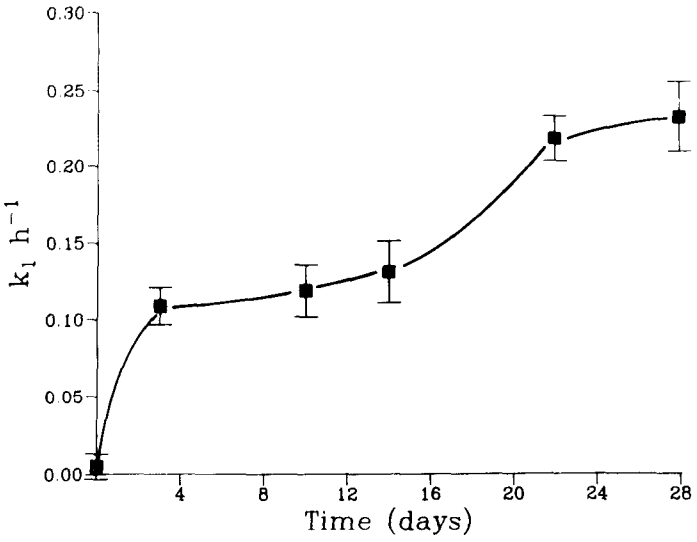

FIG. 3—*Colonization time versus first-order 2,4-DBE transformation rates. Teflon strips having equal surface areas were incubated for various lengths of time in a laboratory stream and their rate coefficients of 2,4-DBE loss,* k_1, *determined. The Reynolds number was 6000. Brackets indicate one standard deviation.*

Transformation Rate Coefficients

First-order transformation rate coefficients (k_1) were calculated using published methods (see Footnotes 3 and 4). For the study, batch cultures of blended aufwuchs or intact periphyton attached to Teflon strips were spiked to an initial concentration of approximately 150 μg/L MP, 150 μg/L 2,4-DBE, or 350 μg/L DEP. Concentrations of the chemicals were determined over time by extracting culture samples with 2,2,4-trimethyl pentane and analyzing the extracts by electron capture detection using gas-liquid chromatography. Blended aufwuchs cultures were shaken in an incubator-shaker at 20°C and intact periphyton cultures were stirred at ambient room temperature by stainless steel paddlewheels. Values of k_1 for continuous-flow microcosms were calculated from xenobiotic steady-state concentrations versus input concentrations. Second-order transformation rate coefficients (k_b) for blended aufwuchs cultures were calculated by dividing k_1 by total plate counts (see Footnotes 3 and 4). Second-order rate coefficients (k_A) for intact periphyton cultures were calculated by dividing k_1 by the ratio of colonized Teflon surface area to container volume (see Footnote 4).

Collection of Microbiota

Field-collected and microcosm microbiota were obtained by colonizing 11 by 15 cm Teflon strips for approximately ten weeks at the field sites and by

collecting aufwuchs grab samples from large laboratory microcosms and field sites. Experiments were started within 24 h after transporting the samples to the laboratory.

Laboratory Microcosms

The laboratory microcosms consisted of two independent flowing channels (designated A and B), each constituting a series of eight 250-L, continuously stirred tank reactors (CSTRs), designated 1 through 8. The water temperature was 20°C for MP and DEP and 15°C for 2,4-DBE. The channels were lighted with 21.5 klx (2000 fc) of fluorescent light on 12-h light-dark cycles for MP and DEP, and were lighted with 8.07 klx (750 fc) on 8-h light, 16-h dark cycles for 2,4-DBE. Less light was used in the 2,4-DBE experiments to discourage excessive growth of filamentous green algae. Each CSTR was tested by introducing a continuous aqueous concentrate of the xenobiotic chemical until a steady-state concentration was observed for several days. Various organic or inorganic nutrients or both were continuously added to separate CSTRs to achieve a large diversity of microbial concentrations and community structures (see Footnotes 3 and 4). The channels were inoculated weekly using water samples from local lakes and streams. Between studies for each xenobiotic chemical, the channels were cleaned and washed with sodium hypochlorite solution.

Field Sites

The field sites consisted of both lotic and lentic freshwater systems and ranged from a mountain stream to a coastal bayou. The 11 sites were the Alabama River at Highway 10 near Camden, AL; Harris Shoals Dam, a small pond in Oconee County, GA; High Shoals, a shallow shoals area in Oconee County, GA; Lassiter's Creek, a small creek in a wooded area in Oglethorpe County, GA; Oconee River in Whitehall, GA; a municipal sewage treatment plant in Athens, GA; Shope Fork Creek, a mountain stream in Coweeta, NC; the South Fork Broad River at Watson Mill State Park near Comer, GA; the St. Mary's River at the outflow of the Okefenokee Swamp at Sanderson, FL, Tom King Bayou near Gulf Breeze, FL; and Upper Three Runs Creek at the Savannah River Plant near Aiken, SC. The sites were sampled during May through August 1983.

Results

Tables 1 and 2 summarize the transformation rate coefficient data for our laboratory microcosm and field-collected microbiota. Each of the test xenobiotic chemicals was rapidly transformed in all laboratory microcosm studies. The 2,4-DBE was rapidly transformed in all field-collected microbiota samples; MP was transformed in six out of ten sites; and DEP was transformed in only two out of ten sites. Field-collected samples that did transform DEP and MP, however, did so at similar rates per unit of periphyton-colonized area and had similarly wide k_b ranges compared to laboratory microcosm data.

TABLE I — *Transformation rate coefficients for blended aufwuchs* (k_b) *and attached periphyton* (k_A) *in laboratory microcosms.*[a]

	Methyl Parathion				Diethyl Phthalate				2,4-D Butoxyethyl Ester			
Microcosm	n^b	k_b[c]	n^d	k_A[e]	n^b	k_b[c]	n^d	k_A[e]	n^b	k_b[c]	n^d	k_A[e]
A1	...	...	...	...	...	...	...	...	2	0.86 ± 0.16	3	10.0 ± 0.80
A2	...	...	...	...	...	...	...	...	1	1.5	3	9.7 ± 0.46
A3	2	0.0033 ± 0.00004	3	0.60 ± 0.16	3	0.68 ± 0.16	3	3.6 ± 0.81	2	0.76 ± 0.04	3	23.0 ± 3.4
A4	1	0.0022	3	0.93 ± 0.49	3	0.35 ± 0.10	3	4.4 ± 0.12	2	0.36 ± 0.08	3	22.0 ± 1.4
A5	2	0.0012 ± 0.00014	3	1.3 ± 0.91	3	0.03 ± 0.01	3	10.0 ± 0.17	...	...	...	...
A6	1	0.012	3	2.2 ± 0.70	3	0.11 ± 0.01	3	6.0 ± 0.55	...	...	...	...
A7	1	0.0091	3	1.9 ± 0.51	3	0.15 ± 0.05	3	4.6 ± 0.50	...	...	...	...
A8	1	0.010	3	2.6 ± 1.0	3	0.18 ± 0.03	3	5.4 ± 0.60	...	...	...	...
B1	...	...	...	...	...	...	...	...	1	0.64	3	16.0 ± 2.6
B2	...	...	...	...	...	...	...	...	...	...	3	14.0 ± 0.52
B3	1	0.0026	3	1.1 ± 0.35	1	0.77	3	4.0 ± 0.40	2	13.0 ± 1.4	3	20.0 ± 3.4
B4	1	0.0019	3	0.92 ± 0.34	3	2.2 ± 0.14	3	4.4 ± 0.12	2	0.17 ± 0.03	3	21.0 ± 2.9
B5	2	0.0064 ± 0.00014	3	1.6 ± 0.12	3	0.01 ± 0.001	3	10.0 ± 0.36	...	...	...	...
B6	1	0.0011	3	1.2 ± 0.55	3	0.04 ± 0.01	3	5.0 ± 0.21	...	...	...	...
B7	1	0.0011	3	1.5 ± 0.72	3	0.23 ± 0.05	3	3.0 ± 0.15	...	...	...	...
B8	2	0.0083 ± 0.0025	3	1.6 ± 0.12	3	0.20 ± 0.01	3	1.7 ± 0.00	...	...	...	...
k_A	Mean ± standard deviation			1.5 ± 0.57				5.2 ± 2.5				17.0 ± 5.3

[a]Microcosms were cleaned out and disinfected between studies with each chemical.
[b]Number of batch cultures.
[c]Nanolitres/cell/h based on total plate counts.
[d]Number of steady-state xenobiotic chemical concentratin determinations.
[e]Litres/m^2/h; Reynolds number was 6000.

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