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Comparison of Methods to Measure Acute Metal and Organometal Toxicity to Natural Aquatic Microbial Communities†

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Microbial communities in water from Baltimore Harbor and from the mainstem of Chesapeake Bay were examined for sensitivity to mercuric chloride, monomethyl mercury, stannic chloride, and tributyltin chloride. Acute toxicity was determined by measuring the effects of [^{3}H]thymidine incorporation, [^{14}C]glutamate incorporation and respiration, and viability as compared with those of controls. Minimum inhibitory concentrations were low for all metals (monomethyl mercury, <0.05 μg liter^{-1}; mercuric chloride, <1 μg liter^{-1}; tributyltin chloride, <5 μg liter^{-1}) except stannic chloride (5 mg liter^{-1}). In some cases, mercuric chloride and monomethyl mercury were equally toxic at comparable concentrations. The Chesapeake Bay community appeared to be slightly more sensitive to metal stress than the Baltimore Harbor community, but this was not true for all treatments or assays. For culturable bacteria the opposite result was found. Thymidine incorporation and glutamate metabolism were much more sensitive indicators of metal toxicity than was viability. To our knowledge, this is the first use of the thymidine incorporation method for ecotoxicology studies. We found it the easiest and fastest of the three methods; it is at least equal in sensitivity to metabolic measurements, and it likely measures the effects on the greater portion of the natural community.

Highly toxic heavy metals and organometals are common contaminants of natural waters (4, 11, 13). Sources of these substances include industrial and domestic wastewater, atmospheric deposition, erosion, and even direct application, e.g., algicides and antifouling coatings. Research efforts have been directed toward determining "safe" levels of these substances which would not adversely affect the biota of aquatic environments.

One experimental approach has been to measure concentrations of toxic metals in a certain aquatic environment and then, by comparison with results of toxicity studies conducted with standard laboratory organisms, to predict toxic impact on the natural communities within that environment. However, it is uncertain that standard laboratory organisms will respond to toxic metals in the same way as species belonging to the natural community. In another approach, indigenous organisms are removed from their natural habitat and tested under laboratory conditions. The latter experimental design has been used to investigate responses of microorganisms to heavy metals (3, 24). A serious problem with this method, which requires isolation and maintenance of the microorganisms on artificial media usually containing high nutrient concentrations, is that the toxicity tests are conducted under conditions bearing little relationship to the habitat from which the organisms were originally isolated.

A better approach is to investigate toxic effects of metals on intact microbial communities. However, if these communities are exposed to heavy metals in nutrient medium (10), (i) the medium composition would be expected to alter the physiochemical equilibrium of the metal species compared to the natural water, (ii) only lethal effects can normally be observed, and (iii) the selectivity of the nutrient medium limits observation to only that small part of the microbial community that can be cultured under the chosen nutrient conditions.

From an ecological viewpoint, toxicity testing with microorganisms should be conducted under conditions as similar as possible to those existing in the natural environment. For example, the effective concentration of the toxicant may be a function of the community composition, organic carbon concentration, salinity, pH, and other variables. Only a few investigators have incorporated this rationale into their experimental designs. Albright et al. (1) and Albright and Wilson (2) used the heterotrophic potential technique (16, 26) to evaluate the impact of a variety of metallic salts on [^{14}C]glucose assimilation and respiration by an intact heterotrophic microbial community from a freshwater pond. They attempted to mimic the pond conditions as closely as possible by adding the metal salts solutions directly to freshly collected water samples, which were subsequently incubated at in situ temperature. Vaccaro et al. (23) also used the kinetic approach (16, 26) to evaluate copper toxicity to microbial communities in Sannich Inlet water enclosed in a large-volume controlled ecosystem pollution experiment (CEPEX) mesocosm. Copper was added directly to the enclosures, and both acute and long-term responses were determined by measuring [^{14}C]glucose metabolism in small samples at various time intervals. In some instances replicate samples from the mesocosms were amended with additional copper to determine possible changes in community sensitivity to the metal. A problem with the kinetic approach to measurement of heterotrophic potential is that only potential metabolic rates at higher than natural substrate concentrations are measured, although calculations derived from these measurements often give accurate determinations of turnover time in eutrophic systems.

Values approaching natural metabolic rates can be determined directly by using a radiolabeled substrate at true

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tracer concentrations (7, 25). The tracer method has been used by Sunda and Gillespie (20) to evaluate the metabolic response of a marine bacterium to cupric ion stress and by Jonas (Ph.D. thesis, University of North Carolina, Chapel Hill, 1981) to assess the effect of cupric ion on salt marsh-estuarine microbial communities.

In the tracer technique, the specific substrate is normally chosen on the basis of its probable utilization by the largest possible portion of the natural microbial community. Nevertheless, even with experimental evidence that the above is true, it is unlikely that all members of the natural community are capable of transporting or metabolizing the specific substrate. Furthermore, there is no reason to assume that utilization of any carbon and energy source is necessarily the most sensitive indicator of toxicity. Therefore, we have investigated the effect of heavy metals and organometals on the incorporation of the radiolabeled nucleic acid precursor, [*methyl*-^{3}H]thymidine. We hypothesized that changes in thymidine incorporation should be a better indicator of acute metal toxicity than other measurements because thymidine is more universally utilized than carbon and energy source metabolites (6) and thus could reflect toxic effects exerted upon systems other than macromolecular synthesis, e.g., poisoning of any cell function could conceivably inhibit cell growth as estimated by methyl thymidine incorporation. To test this hypothesis, we simultaneously tested the influence of heavy metals and organometals on [*methyl*-^{3}H]thymidine incorporation and on glutamic acid metabolism by the microbial community as well as on viability of the culturable portion thereof.

MATERIALS AND METHODS

Sampling. Water samples (10 liters each) were collected ca. 10 cm below the surface with a 10% (vol/vol) HCl-rinsed polyethylene bucket or polypropylene bottles. Five separate samples were collected sequentially at each of two sites in Chesapeake Bay. A relatively pristine site was located along the mainstem of the Chesapeake Bay approximately 200 meters east of the mouth of the Patuxent River (Patuxent buoy no. 1, 38°19′ N, 76°22′ W). Samples were collected at this site on 1 December 1982 when the surface water temperature was 10°C and the salinity 17‰. The second site was offshore from Hawkins Point in Baltimore Harbor, 39°18′ N, 76°35′ W. Samples were collected from an ore dock on 20 December 1982 at a water temperature of 6°C and 2‰ salinity.

Samples were returned to the laboratory in an insulated chest as rapidly as possible (<2 h). To provide a uniform sample representative of each site (R. B. Jonas, Ph.D. thesis), 750-ml portions of each of the five samples were filtered through a 100-μm Nitex screen and mixed in a sterile, HCl-rinsed, 4-liter polypropylene beaker from which 10-ml subsamples were removed to appropriate polypropylene incubation vessels for metal treatments. In situ water temperatures were maintained throughout.

Metal treatments. The following metals or organometals were used over a range of added concentrations: mercuric mercury (Hg) was added as $HgCl_2$ (1 to 100 μg of Hg liter^{-1}); monomethylmercury (MeHg) as CH_3HgCl (0.05 to 5.0 μg of Hg liter^{-1}); stannic tin (Sn) as $SnCl_4$ (5 to 100 mg of Sn liter^{-1}); and tributyltin (TBT) as $[CH_3(CH_2)_3]_3SnCl$ (5 to 500 μg liter^{-1}). Sn and TBT were obtained from Alfa Products (Danvers, Mass.), MeHg was from ICN Pharmaceuticals Inc. (Plainview, N.Y.), and Hg was from Fisher Scientific Co. (Fairlawn, N.J.). The metals were diluted in sterile distilled water (Hg and Sn) or absolute ethanol (MeHg and TBT) so that the addition of 100 μl of metal solution to a water sample provided the desired final concentration. Treatments were initiated by metal addition followed immediately by radiotracer addition where appropriate. Solvent controls consisted of water amended only with 100 μl of distilled water or with 100 μl of absolute ethanol.

[*methyl*-^{3}H]thymidine incorporation. The rate of [*methyl*-^{3}H]thymidine incorporation was determined by a method similar to that of Fuhrman and Azam (5, 6). For each metal treatment and solvent control, approximately 0.5 to 1.0 μCi of [*methyl*-^{3}H]thymidine (ICN) was added to each of four replicate 10-ml water samples contained in 50-ml polypropylene screw-cap tubes. One of the four, treated with formaldehyde (2% [vol/vol] final concentration), served as an abiotic control. After [*methyl*-^{3}H]thymidine addition, the samples were incubated for exactly 59 min at in situ temperature with rotary shaking at 120 rpm. The incubation tubes were then chilled in an ice bath for 1 min followed by the addition of 10 ml of cold (4°C) 10% (wt/vol) trichloroacetic acid to terminate the reaction and precipitate macromolecules. The 1-h incubation period was chosen on the basis of preliminary experiments, which gave linear rates of thymidine incorporation with untreated water samples over periods up to 2 h. After trichloroacetic acid addition, the samples were mixed well and held for a minimum of 15 min in an ice bath, and the particulate radioactive material was collected on 0.2-μm porosity Nuclepore filters (Nuclepore Corp., Pleasanton, Calif.). The filters were washed twice with 2-ml portions of cold 5% trichloroacetic acid, and radioactivity was assayed by liquid scintillation counting (Packard Instrument Co., Inc., Rockville, Md.; model 3330) in Aquasol II (New England Nuclear Corp., Boston, Mass.). Quench correction was by the channels ratio method. Mean disintegrations per minute for the three replicates were computed, and values for abiotic controls were subtracted before comparing treatment effects. [*methyl*-^{3}H]thymidine incorporation in metal treated samples was compared with that of the appropriate solvent controls; organometals were compared with ethanol solvent controls, and metals were compared with distilled water solvent controls.

Glutamic acid metabolism. The effect of metals on incorporation and respiration of glutamic acid was determined in a manner analagous to that described above by a modification of the tracer level technique of Williams and Askew (25). Approximately 0.1 μCi of L-[*U*-^{14}C]sodium glutamate (ICN) was added to each 10-ml sample contained in a 20-ml screw-capped polypropylene vial fitted with a polypropylene cup suspended from the interior surface of the cap. The cup contained a filter paper wick and 0.2 ml of hyamine hydroxide (New England Nuclear) to trap $^{14}CO_2$ (12). After incubation for 1 h at in situ temperatures, 0.3 ml of 0.4 N H_2SO_4 was injected through a silicone rubber-sealed port in the vial cap to stop activity and to liberate $^{14}CO_2$ from the water. The samples were then shaken on a rotary shaker at 120 rpm for 1 h at room temperatrure to permit $^{14}CO_2$ absorption. $^{14}CO_2$ trapping efficiency was 86% by this procedure. The filter paper wicks were removed to scintillation vials for counting, and the cups were rinsed with several volumes of Aquasol. Particle-associated radioactivity was collected from the acidified water and assayed as described above, except that the filters were washed with 5 ml of Nelson salt solution (14) of the appropriate salinity.

Preliminary experiments with untreated Chesapeake Bay water indicated that rates of glutamate incorporation and respiration were linear over a minimum of 1.5 h of incubation. Acid treatment of the water to remove $^{14}CO_2$ resulted

in a 37% decrease in particle-associated radioactivity compared with samples filtered immediately after incubation. This loss has been attributed to the release of unmodified radiolabeled substrate pools (8). Therefore, particle-associated radioactivity measured in these experiments likely represents true incorporation rather than accumulation of glutamate into intracellular pools (22).

Viability testing. The bactericidal effects of the metals and organometals on the culturable fraction of the microbial community were assessed as in the radiotracer experiments. Metal solutions or appropriate solvents were added to single 10-ml samples contained in polypropylene tubes. After 1 h of incubation at the in situ temperature, portions of each sample were diluted in bicarbonate buffered (pH 7.7) Nelson salts of the appropriate salinity. Appropriately diluted samples were then spread onto half-strength Nelson agar plates at pH 7.7 and in situ salinity. Colonies were enumerated after incubation for 10 days at 22 ± 2°C. The data for these experiments are expressed as CFU.

RESULTS

Thymidine incorporation, glutamate metabolism, and CFU. Preliminary experiments indicated that thymidine incorporation, glutamate metabolism, and CFU were the same in raw water as in raw water with 100 μl of distilled water. Likewise, there were no significant differences between values for samples amended only with distilled water or ethanol at either sampling site (Table 1). Control values for all parameters remained the same when measured before or after the 1-h time period required for the initiation of all the different metal treatments. Therefore, comparisons made between metal treatments and their respective controls are valid.

Thymidine incorporation, glutamate metabolism, and numbers of culturable microorganisms were uniformly higher in Baltimore Harbor water than in mid-Bay water (Table 1). Turnover time of thymidine was threefold faster in Baltimore Harbor than in the mid-Bay and compared well with the threefold greater number of culturable microorganisms in the Baltimore Harbor water. On the other hand, glutamate metabolism was considerably higher in Baltimore Harbor than would be predicted from comparison of thymidine turnover and CFU between the two sites. The major difference was in the rate of glutamate incorporation. Glutamate incorporation and respiration by the mid-Bay microbial community represented approximately equal portions of glutamate metabolism (velocity of metabolism/velocity of incorporation, 2.1), whereas in Baltimore Harbor water, incorporation significantly exceeded respiration (velocity of metabolism/velocity of incorporation, 1.7). These results suggest differences not only in the composition of the two communities but also in their physiological state.

Toxicity of Hg and MeHg. The sensitivity of the microbial communities to added Hg at the two sites was approximately the same whether determined by thymidine incorporation or glutamate incorporation and respiration (Fig. 1A and B). Thymidine incorporation was the most sensitive indicator of Hg toxicity at both sites. Although the degree of inhibition was the same at most Hg concentrations, the data suggest that the community at the mid-Bay site was somewhat more sensitive to Hg (~90% inhibition at 1 μg of Hg liter^{-1}) than that at Baltimore Harbor (~60% inhibition at 1 μg of Hg liter^{-1}). Glutamate incorporation and respiration were inhibited equally as the Hg concentration was increased.

Although thymidine incorporation and glutamate metabolism detect either bactericidal or bacteriostatic effects, CFU responses clearly indicated that Hg was bactericidal (Fig. 1A

TABLE 1. Thymidine incorporation, glutamate metabolism, and CFU in solvent controls containing 100 μl of distilled water or ethanol

Site	Solvent	[^{3}H]thymidine incorporation[a]		[^{14}C]glutamate[b]						CFU ($\times 10^2$) ml^{-1}
				Incorporation		Respiration		Metabolism[c]		
		V	T_t	V	T_t	V	T_t	V	T_t	
Mid-Bay	Distilled water	4.0 ± 2.0[d]	254.6 ± 129.1	149.7 ± 65.0	216.2 ± 93.8	164.1 ± 60.3	197.2 ± 72.4	313.8 ± 125.3	103.3 ± 41.2	185 ± 32
	Ethanol	3.9 ± 1.5	259.2 ± 102.1	146.6 ± 51.4	220.8 ± 77.5	180.0 ± 50.8	179.8 ± 50.7	326.6 ± 102.2	99.2 ± 31.0	208 ± 21
Mid-Bay Baltimore Harbor	Distilled water	35.6 ± 2.8	74.6 ± 5.8	1,336.3 ± 177.1	25.3 ± 3.4	973.6 ± 360.7	34.7 ± 12.9	2,309.9 ± 537.8	14.6 ± 3.4	594 ± 280
Baltimore Harbor	Ethanol	29.9 ± 5.6	88.6 ± 16.7	1,241.0 ± 359.2	27.2 ± 7.9	831.0 ± 331.4	40.7 ± 16.2	2,072.0 ± 690.6	16.3 ± 5.4	619 ± 235

[a] The amounts of [*methyl*-^{3}H]thymidine added were 0.44 μCi per 10 ml (1.02 nmol liter^{-1}) and 1.14 μCi per 10 ml (2.65 nmol liter^{-1}) for Chesapeake Bay water and Baltimore Harbor water, respectively. A maximum of 1.3% added thymidine was incorporated. V, Apparent velocity of incorporation, respiration, or metabolism in units of picomoles per liter per hour; T_t, turnover time in hours.

[b] The amounts of [^{14}C]glutamate added were 0.089 μCi per 10 ml (32.4 nmol liter^{-1}) and 0.093 μCi per 10 ml (33.8 nmol liter^{-1}) for Bay water and Baltimore Harbor water, respectively. A maximum of 6.8% added glutamate was metabolized.

[c] Metabolism is defined as the sum of incorporation and respiration.

[d] One standard deviation, n = 9 for [*methyl*-^{3}H]thymidine incorporation and [^{14}C]glutamate metabolism; n = 3 for viable bacterial counts.

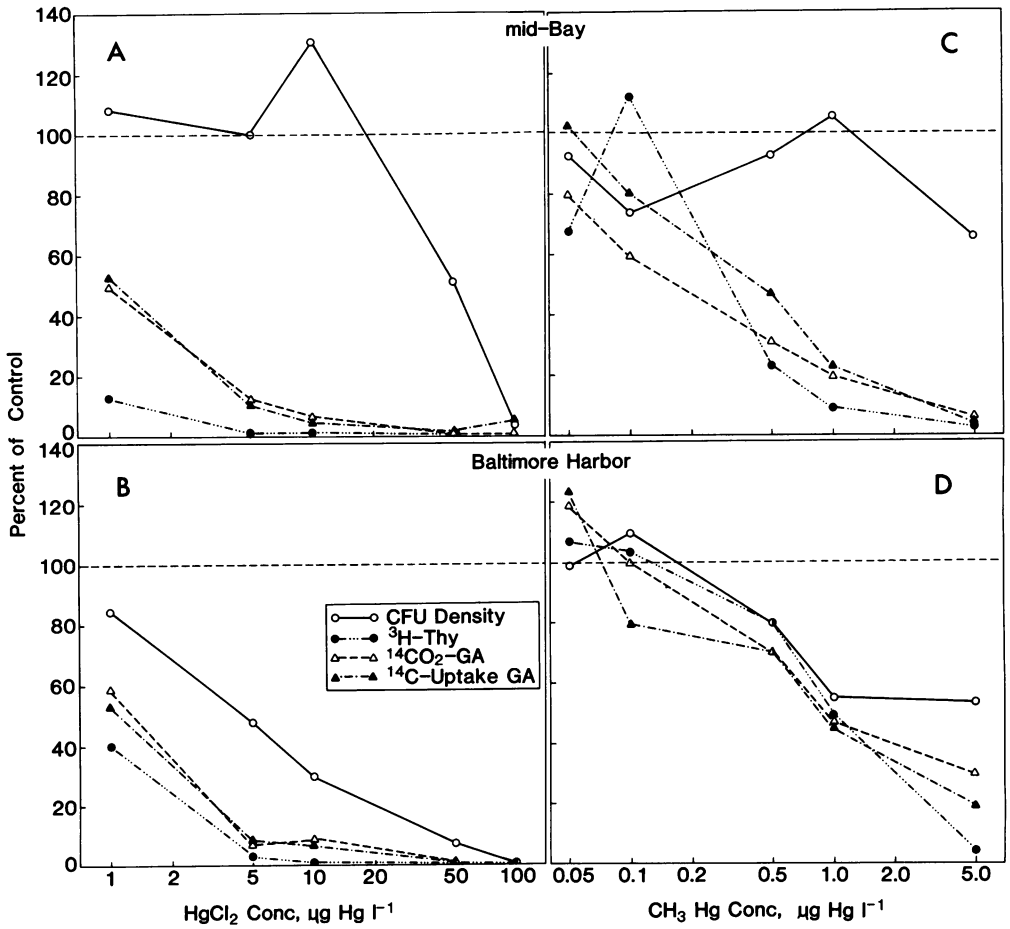

FIG. 1. Impact of increasing Hg (A and B) and MeHg (C and D) concentrations on thymidine incorporation (●), glutamate incorporation (▲), glutamate respiration (△), and CFU density (○) in Chesapeake Bay water (A and C) and Baltimore Harbor water (B and D).

and B). However, at the mid-Bay site, the Hg concentration required to kill the bacteria was more than 1 order of magnitude greater than that required to inhibit thymidine incorporation and glutamate metabolism. The addition of 50 µg of Hg liter^{-1} reduced CFU by 50%. By comparison, in Baltimore Harbor 50% reduction in CFU occurred at 5 µg of Hg liter^{-1}, indicating that the culturable portion of the community in Baltimore Harbor was much more sensitive to Hg than was the mid-Bay community.

The mid-Bay community was more sensitive to MeHg than was the Baltimore Harbor community with respect to thymidine incorporation and glutamate metabolism (Fig. 1C and D). For example, 1 µg of Hg liter^{-1} as MeHg caused approximately a 50% inhibition of the Baltimore Harbor community, but an 80 to 90% inhibition of the mid-Bay community. At each site, thymidine incorporation and glutamate metabolism were nearly equally inhibited by MeHg.

Again, the CFU data indicated that the culturable portion of the Baltimore Harbor community was more sensitive to toxicant, in this case MeHg, than was the mid-Bay community (Fig. 1C and D). In contrast to Hg treatments (Fig. 1B), the CFU parameter was nearly as sensitive an indicator of MeHg toxicity as were the radiotracer methods in Baltimore Harbor water. Although some differences in the toxicity of Hg and MeHg as measured by thymidine incorporation and glutamate metabolism were noted at the two sites, the magnitude of those differences was relatively small.

Toxicity of Sn and TBT. Inorganic Sn was by far the least toxic of the metals and organometals tested; 5 mg of Sn liter^{-1} or more was required to significantly inhibit the microbial communities at either site (Fig. 2A and B). This concentration greatly exceeds tin concentrations measured in Chesapeake Bay waters (10). In agreement with Hg and MeHg data, glutamate incorporation and respiration were affected similarly by increasing Sn concentrations. In contrast to all other data from both sites, thymidine incorporation in Baltimore Harbor water was a slightly less sensitive indicator of toxicity than was glutamate metabolism (Fig. 2B). The 50% inhibition level for Sn in Baltimore Harbor water was about 2.5 times greater for thymidine incorporation than for glutamate metabolism.

In agreement with data for both mercury compounds, the culturable microbial community in Baltimore Harbor was more sensitive to Sn toxicity than was the culturable community in the mid-Bay. In the latter community CFU was a less sensitive indicator of toxicity than either thymidine incorporation or glutamate metabolism.

As in the case of MeHg (Fig. 1C and D), the mid-Bay community was somewhat more sensitive to TBT inhibition of thymidine incorporation and glutamate metabolism than was the Baltimore Harbor community (Fig. 2C and D). A concentration of 5 µg of TBT tin liter^{-1} caused a 50 to 60% decrease in thymidine incorporation and glutamate metabolism in mid-Bay water, but only a 20 to 30% decrease in

Baltimore Harbor water. At both sites thymidine incorporation and glutamate incorporation were inhibited about equally by TBT. However, glutamate respiration was slightly less inhibited in Baltimore Harbor water than either thymidine or glutamate incorporation at all concentrations tested (Fig. 2D).

TBT was clearly bactericidal in Baltimore Harbor water: 100 μg of TBT tin liter^{-1} killed about 50% of the culturable microorganisms (Fig. 2D). However, even the highest TBT concentration tested, 500 μg liter^{-1}, did not reduce CFU numbers in mid-Bay water. Although the greater metal tolerance of the mid-Bay culturable community was most striking in the TBT tests, this pattern was consistent for all four compounds.

MIC. Table 2 lists the minimum inhibitory concentrations (MIC) for the four metals and organometals tested. Owing to the high sensitivity of the thymidine incorporation and glutamate metabolism measurements and the need to compare all the methods over the same concentration range, MIC values are often not defined precisely.

The differences in MIC between mid-Bay and Baltimore Harbor water were relatively small except for CFU measurements. Apart from Sn, differences in MIC determined from thymidine incorporation and glutamate metabolism data indicated that the Baltimore Harbor community was slightly more tolerant of the metals under the treatment conditions used. This trend was reversed for Sn effects on glutamate metabolism. In terms of MIC, CFU measurements suggested that the mid-Bay community was more tolerant to the metals than was the Baltimore Harbor community.

DISCUSSION

Plate count techniques and heterotrophic potential measurements have been used previously to assay metal toxicity to natural microbial communities (1, 10, 23). To our knowledge this is the first report of the use of [*methyl*-^{3}H]thymidine incorporation for this purpose. As an indicator of toxicity, thymidine incorporation was as sensitive as glutamate metabolism and was far more sensitive than CFU measurements. Not only is [^{3}H]thymidine less costly than ^{14}C-radiolabeled substrates, but also the assay is more rapid and may include a larger portion of the exposed community than does the metabolic assay, which is dependent on the utilization of a single carbon and energy source.

In attempting to assay whole community responses to toxicant stress, a technique which measures the activity of the largest portion of the community should be employed. In the two estuarine pelagic environments studied, the community response curves obtained for glutamate metabolism and thymidine incorporation were similar. Thus, either the microorganisms that incorporated thymidine also utilized glutamate, leading to the observed similar responses, or the glutamate-metabolizing portion of the community mimicked the response of the entire community to metal toxicity.

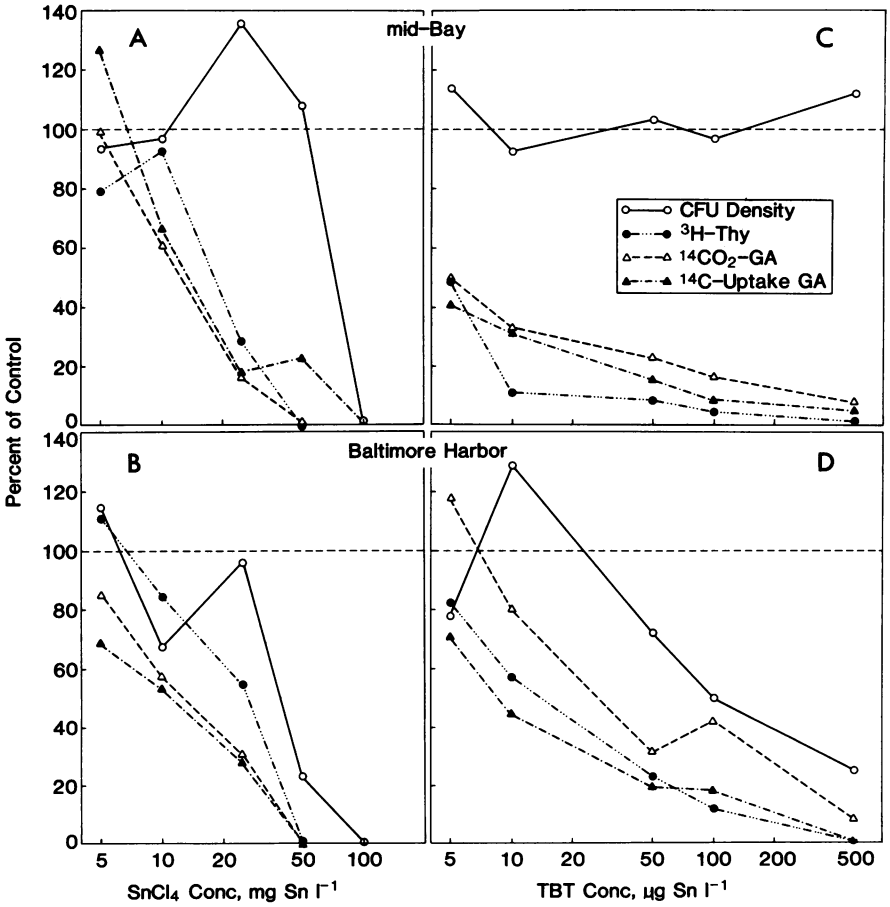

FIG. 2. Impact of increasing Sn (A and B) and TBT (C and D) concentrations on thymidine incorporation (●), glutamate incorporation (▲), glutamate respiration (△), and CFU density (○) in Chesapeake Bay water (A and C) and Baltimore Harbor water (B and D).

TABLE 2. MIC of Hg, MeHg, Sn, and TBT

Treatment	Sample location	MIC (μg liter^{-1}) by the following method:			CFU
		[^{3}H]thymidine incorporation	[^{14}C]glutamate		
			Incorporation	Respiration	
Hg	Mid-Bay	<1	<1	<1	10–50
Hg	Baltimore Harbor	<1	<1	<1	<1
MeHg	Mid-Bay	0.1–0.5	0.05–0.1	0.05	1–5
MeHg	Baltimore Harbor	0.1–0.5	0.05–0.1	0.1–0.5	0.1–0.5
Sn	Mid-Bay	5×10^3	5×10^3–10×10^3	5×10^3–10×10^3	50×10^3–100×10^3
Sn	Baltimore Harbor	5×10^3–10×10^3	$<5 \times 10^3$	$<5 \times 10^3$	25×10^3–50×10^3
TBT	Mid-Bay	<5	<5	<5	>500
TBT	Baltimore Harbor	<5	<5	5–10	50–100

Despite the similarity of results with the thymidine and glutamate methods in this study, thymidine incorporation, a measure of cell growth, integrates many metabolic and biosynthetic pathways and thus could generally be used to detect a wider range of cell damage than metabolism of any single carbon and energy source.

Except in the case of MeHg treatment of Baltimore Harbor water, the CFU technique was a significantly less sensitive indicator of metal toxicity. Therefore, it would be unwise to rely on this method alone to determine potential toxicity of heavy metals and organometals to natural microbial communities. Nevertheless, inhibition of thymidine incorporation or glutamate metabolism does not indicate whether the mode of action of these compounds was bacteriostatic or bactericidal. It is clear from the CFU data that all four compounds were bactericidal, at least to the Baltimore Harbor community (Fig. 1 and 2). The sensitivity of the CFU test might be increased by lengthening metal exposure times, but previous data for copper toxicity indicates that 1 h is sufficient for development of the full bactericidal effect on CFU (Jonas, Ph.D. thesis).

The mid-Bay culturable microbial community was consistently less sensitive to the bactericidal effects of the metals and organometals tested than was the Baltimore Harbor community. This result contrasts with thymidine incorporation and glutamate metabolism determinations, which indicated that the microbial communities at both sites exhibited roughly similar sensitivities to the test compounds. The comparative insensitivity of the culturable portion of the mid-Bay community to the two organometals is striking. The explanation for these observations may lie simply in the fact that the culturing technique selects only a portion of the total metabolically active community and the metal sensitivity of the culturable community differs from that of the whole. For example, it is possible that a large part of the Baltimore Harbor culturable microbial community consisted of zymogenous bacteria derived from upland sources. Alternatively, the Baltimore Harbor community may have been stressed in its natural state by metals or other toxic compounds, and that small additional stress, i.e., metal treatment, may have caused a relatively greater inhibitory effect than in the less-contaminated mid-Bay. The data do not provide a choice between these alternatives, but the approximately 1:1 ratio of assimilation and respiration of glutamate at both sites (Table 1) suggests that neither community was severely stressed. Rather it seems likely that the culturable communities at the two sites differed significantly in their respective relationship to the entire community.

The data reported here indicate that some of the compounds tested are considerably more toxic to microorganisms than previously reported. Hallas and Cooney (9), using a technique in which metals are incorporated into solidified media, reported that the most sensitive bacterial isolates from Chesapeake Bay were inhibited by less than 1 mg of TBT tin liter^{-1} and by about 12 mg of $SnCl_4$ tin liter^{-1}. Our data indicate that the MIC in the intact community was less than 5 μg liter^{-1} for TBT and less than 5 mg liter^{-1} for Sn. Singh and Bragg (17) reported that 2 mg of TBT liter^{-1} inhibited amino acid metabolism in *Escherichia coli* by 50%. In water disinfection applications, approximately 500 to 1,000 μg of TBT liter^{-1} was needed to inhibit *Leginonella pneumophila* (19).

As little as 1 μg of Hg liter^{-1} reduced metabolic activity of the Cheaspeake Bay and Baltimore Harbor communities by 50 to 90%, and 100 ppb was completely bacteriostatic (Fig. 1). In a freshwater pond 100 ppb of added Hg had no effect on CFU density and reduced glucose metabolism by only about 85% (1). Similarly, Singleton and Guthrie (18) found that in mixed freshwater and brackish water ecosystems 40 μg of Hg liter^{-1} had no effect on culturable bacteria. The data presented here indicate that intact microbial communities in Chesapeake Bay are extremely sensitive to Hg. The actual MIC may be considerably lower than 1 μg liter^{-1}, the lowest concentration tested (Fig. 1).

Because the exposure environment can markedly influence metal speciation and toxicity (20, 21), the quantitative relationships between metal concentration and community inhibition are somewhat uncertain. However, our data are conservative in the sense that the active form of an inorganic metal (usually the free ion activity) is less than the total concentration of that metal. Organometals are probably also toxic as the covalently bound free cation form and may exist in aquatic systems primarily in that state. Therefore, the MIC for organometals are also likely to be conservatively high.

At comparable concentrations (Fig. 1) MeHg was no more toxic than Hg. This suggests that methylation of Hg to the more volatile MeHg may be an effective means of protecting microorganisms from the toxicant. This suggestion is supported by results of Pan-Hou and Imura (15), which show that mercury methylation by *Clostridium cochleanium* T-2C is a primary mechanism of protection against mercuric mercury in this anaerobe.

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