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Effects of Four Aromatic Organic Pollutants on Microbial Glucose Metabolism and Thymidine Incorporation in Marine Sediments†

JAMES E. BAUER AND DOUGLAS G. CAPONE*

Marine Sciences Research Center, State University of New York, Stony Brook, New York 11794

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The metabolism of D-[*U*-^{14}C]glucose and the incorporation of [*methyl*-^{3}H]thymidine by aerobic and anaerobic marine sediment microbes exposed to 1 to 1,000 ppm anthracene, naphthalene, *p,p'*-dichlorodiphenyltrichloroethane, and pentachlorophenol were examined. Cell-specific rates of [^{14}C]glucose metabolism averaged 1.7×10^{-21} and 0.5×10^{-21} mol/min per cell for aerobic and anaerobic sediment slurries, respectively; [^{3}H]thymidine incorporation rates averaged 43×10^{-24} and 9×10^{-24} mol/min per cell for aerobic and anaerobic slurries, respectively. Aerobic sediments exposed to three of the organic pollutants for 2 to 7 days showed recovery of both activities. Anaerobic sediments showed little recovery after 2 days of pre-exposure to the pollutants. We conclude that (i) anaerobic sediments are more sensitive than aerobic sediments to pollutant additions; (ii) [^{3}H]thymidine incorporation is more sensitive to pollutant additions than is [^{14}C]glucose metabolism; and (iii) the toxicity of the pollutants increased in the following order: anthracene, *p,p'*-dichlorodiphenyltrichloroethane, naphthalene, and pentachlorophenol.

Coastal marine sediments, which are sites of intensive and important microbial activities (8), accumulate organic pollutants (2, 4, 16). The effects of organic pollutants on the sediment biota, although possibly substantial, generally have not been considered. Several studies have examined the acute and chronic effects of complex organic mixtures such as fuel oils (10, 11, 14) on organic carbon metabolism by sedimentary microorganisms. A few studies have considered the alteration of activities in marine sediments as a result of individual pollutants (5, 18; J. Slater and D. G. Capone, *in* I. Duedall, ed., *Wastes in the Ocean, vol. 9: Proceedings of the Fourth Ocean Disposal Symposium*, in press).

In the present investigation, we assessed the effects of four aromatic organic pollutants, anthracene, naphthalene, *p,p'*-dichlorodiphenyltrichloroethane (DDT), and pentachlorophenol (PCP), on two microbial activities common to both oxic and anoxic coastal marine sediments. Anthracene and naphthalene are polycyclic aromatic hydrocarbons, whereas DDT and PCP are representative chlorinated aromatic pollutants. The activities chosen for study were D-[*U*-^{14}C]glucose metabolism and [*methyl*-^{3}H]thymidine ([^{3}H]TdR) incorporation. The study allows comparison of the sensitivity of [^{3}H]TdR incorporation relative to [^{14}C]glucose metabolism in toxicity studies involving sedimentary systems, comparison of the sensitivity of aerobic and anaerobic marine sediments to pollutants, and assessment of the relative toxicity of the four pollutant compounds tested.

MATERIALS AND METHODS

Sampling and sample preparation. Sediments for all laboratory studies were obtained from an intertidal mudflat of Flax Pond Saltmarsh, Old Field, N.Y. The mud consisted of a fine organic-mineral mixture (3 to 7% loss on ignition) at the sediment-water interface which became more mineral-rich at depths of 5 to 10 cm. The depth of the oxidized surface layer varied seasonally from approximately 1 to 3 mm in summer to as much as 3 cm in winter.

Sediments for aerobic studies were obtained by scraping the top 0 to 2 mm of sediment with a sterile spatula and placing the sample in a sterile glass beaker. Sediment for anaerobic studies was collected from depths of 5 to 10 cm and maintained anoxically until return to the Marine Sciences Research Center, within 1 h from the time of collection. Flax Pond seawater, with a salinity of about 28 parts per thousand, was obtained from Flax Pond laboratory where it had undergone coarse (10 μm) filtration through nylon cartridge filters.

Sediment subsamples were diluted 1:100 (volume sediment:volume seawater) in filtered seawater (0.45-μm, type GA-6; Gelman Sciences, Inc., Ann Arbor, Mich.) and kept well mixed on a magnetic stirrer while slurry was dispensed. For anoxic sediments, all diluent seawater was purged of oxygen by bubbling with nitrogen gas for at least 30 min before dilution. All vessels containing anoxic slurries were immediately stoppered (butyl rubber stoppers; Thomas Scientific Co., Philadelphia, Pa.) after they were dispensed, and the headspace was purged with nitrogen gas for 1 min. All experimental slurries were maintained at 25°C in the dark in temperature-controlled water baths with rotary shaking to ensure rapid equilibration between the gas and liquid phases and dispersal of pollutants.

Pollutant effects studies. The effects of anthracene, naphthalene (Eastman Kodak Co., Rochester, N.Y.), DDT (R.F.R. Corp., Hope, R.I.), and PCP (Aldrich Chemical Co., Milwaukee, Wis.) on D-[*U*-^{14}C]glucose metabolism and [^{3}H]TdR incorporation were examined in short-term acute and longer term experiments. All comparisons of rates of activities among treatments were analyzed by single classification analysis of variance (23). Significance levels were set at $P < 0.05$ for all analyses.

In acute pollutant effects studies, 5 ml of diluted sediment slurry was transferred from batch preparations to 20 ml of acid-washed borosilicate scintillation vials (Wheaton Industries, Millville, N. J.). Controls received 5 μl of pure acetone carrier. It was found in preliminary experiments that this volume of acetone did not alter significantly either [^{3}H]TdR incorporation or [^{14}C]glucose metabolism compared with samples that received no acetone. Pollutants were added to

* Corresponding author.
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treatment slurries in 5-μl acetone solutions; final concentrations ranged from 1 to 1,000 ppm (micrograms of pollutant per milliliter of slurry). Samples were shaken continuously and incubated for 1 to 2 h before activity assays were performed. In addition, subsamples were taken for the estimation of total bacterial numbers (20) and determination of cell-specific activity rates.

The effects of longer term exposures of the four pollutants on oxic [^{14}C]glucose assimilation were assessed by preparing large batch slurries (ca. 600 ml) of diluted sediment and dispensing 100 ml into 125- or 250-ml Erlenmeyer flasks. Individual flasks then received 100 μl of acetone or organic stock solutions containing one of the four pollutants (100 ppm final concentration). Triplicate subsamples were taken from each flask and assayed for [^{14}C]glucose metabolism in scintillation vials at selected intervals over a 7-day period. Single flasks were deemed suitable for each experimental level on the basis of preliminary results, from which no significant differences among subsamples from replicate flasks (receiving no treatment) with respect to total [^{14}C]glucose metabolism or percent respired to $^{14}CO_2$ or [^{3}H]TdR incorporation were found.

To investigate possible acclimation of microbial populations to pollutants through previous exposure, as well as the effects of additional inputs on activities, 100-ml batch slurries were prepared as described above and dosed with 1 to 1,000 ppm of the pollutants or acetone. After 2 days, triplicate 5-ml subsamples of both control and pretreated batches were dispensed into scintillation vials. Initial controls then received either 5 μl of acetone (control) or organic pollutant stock to the same level as the parallel pretreated samples (dosed, no pretreatment). Pretreated batch subsamples received either 5 μl of acetone (pretreatment control) or an additional quantity of organic pollutant identical to the pretreatment concentration (pretreatment plus retreatment).

[^{3}H]TdR incorporation. The incorporation of [^{3}H]TdR to trichloroacetic acid (TCA)-insoluble macromolecules was investigated by a modification of previously described methods (7, 9, 21). An autoclaved seawater solution (20 μl) of 20 μCi of [^{3}H]TdR per ml (25 Ci mmol^{-1}; Amersham Corp., Arlington Heights, Ill.) was added to 5-ml subsamples of sediment slurry through the stoppers with a microliter syringe. The final concentration of added thymidine in the solution was approximately 3 nM. After the addition of substrate, all samples were returned to the rotary shaker. Duplicate Formalin-killed controls were assayed at each time point to correct for [^{3}H]TdR adsorption. Abiotic adsorption of [^{3}H]TdR generally amounted to between 5 and 10% of the microbial incorporation of the substrate, and total incorporation was corrected by this factor. Abiotic adsorption accounted for less than 0.5% of the total added tritium label.

At the completion of incubation, samples were placed in an ice-chilled water bath (0 to 4°C). Stoppers were removed, and 5 ml of a chilled solution of 2 mM unlabeled TdR in 10% TCA was injected into the sample vial (final concentrations: TCA, 5%; TdR, 1 mM). Extraction of TCA-insoluble macromolecules was allowed to proceed for 20 to 40 min. On completion of extraction, samples were thoroughly mixed in their incubation vials and filtered through 0.45-μm-pore-size membrane filters (type HA; Millipore Corp., Bedford, Mass.). Incubation vials were rinsed with 1 ml of chilled 5% TCA, and this fraction was added to the filter unit. Filtered samples were then rinsed three successive times with 1-ml portions of chilled 5% TCA. On completion of filtration, filters with their light coating of collected sediment were removed and placed in 20-ml scintillation vials. Ethyl acetate (1 ml) was added to dissolve the filters, after which Protosol tissue digester (1 ml; New England Nuclear Corp., Boston, Mass.) was also added. Vials were then capped, and digestion proceeded overnight at 25 to 30°C. The next day, 10 ml of Econofluor scintillation cocktail (New England Nuclear) was added to each vial. Samples were thoroughly shaken, and the light floc was allowed to settle.

The radioactivity in each sample was quantified by counting in a Packard Tri-Carb scintillation counter. Quench curves were constructed by the external standards method. Counting efficiency was relatively low (8 to 10%) in these samples because of color quenching which could not be removed by 10% benzoyl peroxide oxidation.

To ensure that all single time point measurements of [^{3}H]TdR incorporation were representative of linear uptake kinetics, periodic time series of incorporation were performed. Both oxic and anoxic incorporation were linear for at least 60 min (data not shown). Generally, oxic experiments were conducted for 30 to 45 min, whereas anoxic experiments never exceeded 60 min.

[^{14}C]glucose metabolism assays. The format for assaying the metabolism of [^{14}C]glucose was similar to that described above for the [^{3}H]TdR assays. An autoclaved filtered seawater solution (10 μl) of 5 μCi of (18.0 mCi mmol^{-1}) D-[*U*-^{14}C]glucose (New England Nuclear) per ml was added to each 5-ml sediment slurry subsample through the stopper with a microliter syringe. The final amount of glucose to be added was determined by a preliminary study which assessed the kinetics of uptake, as suggested by Griffiths et al. (13). Glucose uptake appeared to be saturated at glucose concentrations of about 100 μg/liter of slurry, and this concentration was used in all subsequent incubations. In preliminary experiments, uptake of [^{14}C]glucose (100 μg/liter of slurry) by oxic sediment slurries (5 ml) remained linear for at least 60 min. For this reason, all experimental incubations were kept to 45 min or less.

[^{14}C]glucose incubations were terminated by injecting 0.2 ml of 2 N H_2SO_4 through the stoppers (15). Shaking was continued for 1 h to aid in removing $^{14}CO_2$ from solution. It is possible to underestimate the uptake of [^{14}C]glucose by acidification (12). These underestimates were assessed in preliminary studies by comparison of acidification with Formalin and filtration termination methods. Results (data not shown) indicate that the amounts of cellularly incorporated ^{14}C-label may have been underestimated by up to 23% through acidification. This lost material may have been in cell-associated labeled pools that had not yet been incorporated into macromolecules (12). Consequently, percentages of ^{14}C-label respired to $^{14}CO_2$ may have been overestimated.

$^{14}CO_2$ was collected from incubation vial headspaces by placing incurrent and excurrent 22-gauge needles (Becton-Dickinson & Co., Baltimore, Md.) through the stoppers and purging with compressed air for 10 min. Effluent gas containing $^{14}CO_2$ was passed through 5 ml of a premixed phenethylamine-containing scintillation cocktail (^{14}C-Oxosol; National Diagnostics Inc., Somerville, N.J.). The efficiency of this trapping system was determined to be 83 ± 1.3%. Radioactivity was directly determined in these trapped samples. $^{14}CO_2$ was found to be released to the incubation vial headspace for up to 1 h after acidification and was trapped only after that period.

After collection of respired $^{14}CO_2$, samples were filtered and vials were rinsed with 1 ml of filtered seawater. Filters and their sediments were then extracted in 1 ml of ethyl

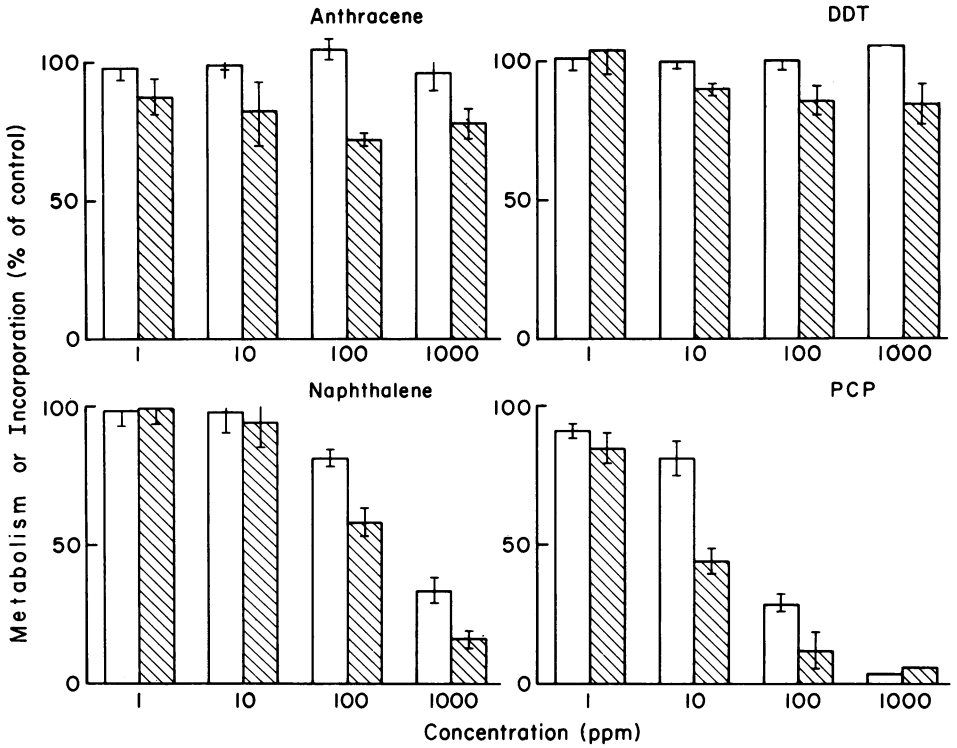

FIG. 1. Rates of [^{14}C]glucose (□) metabolism and [^{3}H]TdR (▨) incorporation by oxic salt-marsh sediments under exposure to 1 to 1,000 ppm anthracene, DDT, naphthalene, and PCP. All values are expressed as a percentage of the control (5 μl of acetone) rates. All values are the mean ± standard deviation (n = 3 replicates).

acetate overnight in scintillation vials. Scintiverse scintillation cocktail (10 ml; Fisher Scientific Co., Pittsburgh, Pa.) was then added to each vial, and the radioactivity was quantified. The counting efficiency for these samples was consistently 85 to 86%. Abiotically adsorbed [^{14}C]glucose accounted for 1.5 to 3% of the cellularly associated ^{14}C-label and was no more than 0.1% of the total added [^{14}C]glucose. For all ^{14}C-labeled samples, quenching was determined by the external standards method.

RESULTS

Acute effects. The effects of 1 to 1,000 ppm of anthracene, DDT, naphthalene, and PCP on [^{14}C]glucose metabolism and [^{3}H]TdR incorporation by oxic sediment slurries are shown in Fig. 1. [^{14}C]glucose metabolism was not significantly ($P < 0.05$) altered in oxic sediment slurries by 1 to 1,000 ppm anthracene or DDT. In contrast, and except for 1 ppm DDT, [^{3}H]TdR incorporation was increasingly and significantly inhibited by greater concentrations of both of these compounds. The response of oxic sediments to naphthalene and PCP was more pronounced. [^{14}C]glucose metabolism and [^{3}H]TdR incorporation were significantly ($P < 0.05$) inhibited at all concentrations of PCP and at 100 and 1,000 ppm naphthalene. In most cases, [^{3}H]TdR showed greater decreases in the rate of incorporation relative to controls than did [^{14}C]glucose metabolism.

Control values of both activities were fairly consistent among experiments. [^{14}C]glucose metabolism ranged from 530×10^{-12} to 700×10^{-12} mol min^{-1} 5-ml slurry^{-1} (1.5×10^{-21} to 1.9×10^{-21} mol min^{-1} cell^{-1}), whereas [^{3}H]TdR incorporation rates showed a greater range among experiments at 14×10^{-12} to 20×10^{-12} mol min^{-1} 5-ml slurry^{-1} (38×10^{-24} to 52×10^{-24} mol min^{-1} cell^{-1}).

Total microbial metabolism of [^{14}C]glucose is the sum of the cellularly incorporated and respired ^{14}C-label. It is of additional interest to compare the ratios of the two transformations of ^{14}C-label. The ratio most commonly encountered is the percentage of total assimilated ^{14}C-label respired to $^{14}CO_2$, expressed as: [dpm in $^{14}CO_2$/(dpm in $^{14}CO_2$ + dpm in ^{14}C-labeled cellular material)] × 100 (dpm is disintegrations per minute).

Percentages of [^{14}C]glucose respired by oxic controls averaged 30 ± 0.5% (standard error; n = 4 experiments). Anthracene and DDT had no significant effect on the percent of the [^{14}C]glucose label respired, although there was an apparent reduction at the highest level of anthracene (Table 1). However, the addition of naphthalene to oxic sediment slurries resulted in an apparent dose-dependent increase in the percentage of ^{14}C-label respired at all concentrations. Linear regression of the difference in percentage of ^{14}C-label respired from controls against the logarithm of pollutant concentration ($y = 11x + 15$; $r^2 = 0.941$) indicates an 11% increase in the amount of ^{14}C-label respired to $^{14}CO_2$ relative to controls for each 10-fold increase in naphthalene concentration. The addition of PCP to oxic sediment slurries caused highly significant increases in the percentage of ^{14}C-label respired at all levels (Table 1). By using the 1-, 10-, and 100-ppm values for which a consistent trend exists, the regression line ($y = 26x + 4.9$; $r^2 = 0.996$) indicates a 26% increase in ^{14}C-label respired (relative to controls) for each 10-fold increase in PCP concentration.

Figure 2 presents the effects of these same compounds on anoxic sediment slurries. Neither anthracene nor DDT

TABLE 1. Change in percentage of metabolized [^{14}C]glucose respired relative to controls by oxic and anoxic sediments under acute pollutant additions

Expt	Dose (ppm)	Difference from controls (percent respired) in the following sediments[a]:	
		Oxic	Anoxic
Anthracene	1	2.0 (0.03)	−6.3 (0.4)
	10	1.4 (0.02)	−5.3 (1.0)
	100	−1.0 (0.03)	2.6 (0.1)
	1,000	−9.2 (0.07)	1.3 (0.3)
DDT	1	3.3 (0.08)	−1.0 (0.04)
	10	6.6 (0.08)	0.6 (0.1)
	100	3.6 (0.07)	1.0 (0.1)
	1,000	3.6 (0.03)	−3.9 (0.4)
Naphthalene	1	6.6 (0.04)	−11.2 (1.6)
	10	9.4 (0.09)	−6.3 (0.4)
	100	25.2 (0.84)[b]	−0.7 (0.2)
	1,000	38.1 (0.68)[c]	28.4 (3.4)[b]
PCP	1	5.9 (0.04)[c]	4.8 (0.2)
	10	29.1 (1.1)[d]	−1.3 (0.2)
	100	58.2 (1.8)[d]	24.5 (1.9)[b]
	1,000	33.3 (2.0)[c]	0

[a] Mean ± standard deviation (n = 3 replicates).
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

caused significant alteration of [^{14}C]glucose metabolism except at 100 and 1,000 ppm DDT. Higher anthracene and DDT concentrations resulted in greater inhibition of [^{3}H]TdR incorporation, although these differences were only significant ($P < 0.05$) at 100 and 1,000 ppm anthracene and 100 ppm DDT.

Naphthalene and PCP additions to anoxic sediment slurries caused greater alterations in activities than did anthracene and DDT. Significant ($P < 0.05$) reduction in [^{14}C]glucose metabolism and [^{3}H]TdR incorporation occurred at 100 and 1,000 ppm naphthalene and 100 and 1,000 ppm PCP, as well as at 10 ppm PCP for glucose metabolism. High replicate variance resulted in nonsignificant inhibition in several cases in which apparent reduction in activity occurred. With the exceptions of 1 ppm anthracene, DDT, and naphthalene and 1,000 ppm naphthalene, the relative responses in [^{3}H]TdR incorporation rates were always greater than those of [^{14}C]glucose metabolism.

Coefficients of variation (data not shown) indicate a greater replicate variance in anoxic incubations, in [^{3}H]TdR incorporation assays, and in dosed samples compared with oxic, [^{14}C]glucose metabolism, or undosed incubations, respectively. We believe this may be partly a result of the greater number of manipulations involved in the former experiments.

Control rates of [^{3}H]TdR incorporation in all anoxic experiments were up to 10-fold lower than in oxic sediment slurries. [^{3}H]TdR incorporation in anoxic slurries ranged from 1.7×10^{-12} to 2.9×10^{-12} mol min^{-1} 5-ml slurry^{-1} (6×10^{-24} to 12×10^{-24} mol min^{-1} cell^{-1}). Rates of anoxic [^{14}C]glucose metabolism were up to fivefold lower than oxic metabolism, ranging from 61×10^{-12} to 224×10^{-12} mol min^{-1} 5-ml slurry^{-1} (0.3×10^{-21} to 1.1×10^{-21} mol min^{-1} cell^{-1}). When rates of [^{14}C]glucose metabolism, [^{3}H]TdR incorporation, and bacterial cell numbers were grouped (for all oxic and anoxic experiments individually) and subjected to correlation analysis, it was found that [^{14}C]glucose metabolism was positively correlated with [^{3}H]TdR incorporation ($P < 0.01$ in oxic slurries; $P < 0.05$ in anoxic slurries). Neither activity was found to correlate with cell counts.

The effects of each compound on the percentages of ^{14}C-labeled glucose respired to $^{14}CO_2$ for all anoxic experiments are also shown in Table 1. The percentage of label respired by controls was similar to that noted in oxic experiments (ca. 30%) except for the PCP experiment, in which controls were anomalously high (ca. 48%). In contrast to the oxic slurries, as many anoxic samples treated with organic compounds showed decreased as showed increased percentages of ^{14}C-label respired.

For all acute pollutant effects experiments, the percentages of responses relative to controls of both activities as a function of the $\log_{10}$ pollutant concentration for 1 to 1,000 ppm organic compounds were subjected to linear regression analysis for purposes of regression coefficient (slope) comparisons. All data from both oxic and anoxic acute effects experiments are summarized in Table 2. Although many of the slopes were not statistically significant (i.e., H_o:b = 0), several comparisons are possible. First, regression coefficients increased (negatively) in all cases in the order anthracene < DDT < naphthalene < PCP for both activities in oxic and anoxic slurries (with the exception of oxic [^{14}C]glucose metabolism under DDT treatment). Second, regression coefficients were more negative for [^{3}H]TdR incorporation than for [^{14}C]glucose metabolism in all cases except for PCP treatment. Finally, coefficients of both activities for any given compound were greater under anoxic conditions when compared with parallel oxic assays, with the exception of those for naphthalene treatments.

Long-term exposure. The results of experiments examining the effects of longer term (7 days) exposures of the four compounds on oxic [^{14}C]glucose metabolism are shown in Fig. 3. Although naphthalene and PCP initially inhibited [^{14}C]glucose metabolism (day 0, t = 3 h), by day 7 anthracene, DDT, and naphthalene had greatly stimulated activity. Slurries treated with PCP maintained a lower activity than controls throughout the experiment. Respiration of [^{14}C]glucose was slightly lower than that of controls in all treatments except PCP which, by day 7, resulted in [^{14}C]glucose respiration that was 120 to 160% that of the controls (data not shown). An additional 100 ppm dosing of all compounds on day 7 (Fig. 3) had no immediate effect on glucose metabolism.

Slight but consistent patterns in activities were observed in oxic and anoxic sediments which were treated with 1 and 100 ppm naphthalene or PCP or 10 and 1,000 ppm anthracene or DDT and held for 2 days before activity assays were done (Table 3). All oxic slurries (except PCP) had a slight positive slope, indicating stimulation, whereas all anoxic slurries showed negative regression coefficients (increasing inhibition with concentration) under the same treatments. [^{3}H]TdR activity was more severely affected, relative to [^{14}C]glucose metabolism, in all cases under anoxic conditions and for PCP assayed under oxic conditions. A 2-day naphthalene preincubation significantly ($P < 0.001$) stimulated [^{3}H]TdR incorporation.

When oxic pretreated sediments were dosed with additional 1 or 100 ppm PCP or 1,000 ppm DDT, activities underwent smaller decreases relative to their respective controls than samples which received no pretreatment; a similar response of lower magnitude was exhibited by anoxic pretreated sediment slurries (Fig. 4). Oxic and anoxic sediment slurries which were pre-exposed for 2 days to 1 to

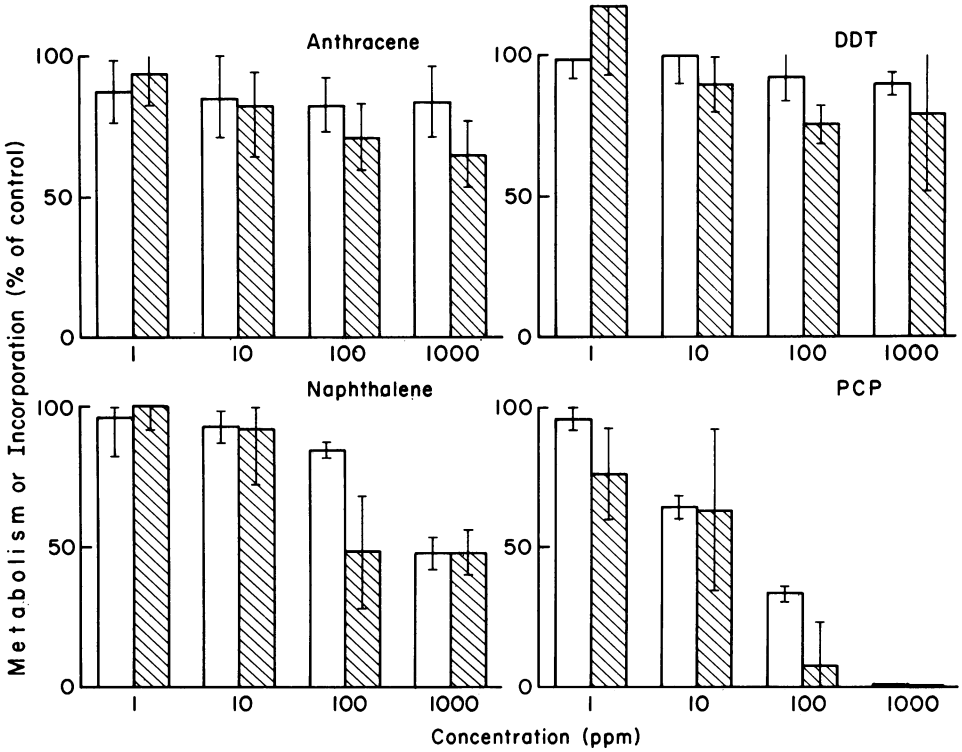

FIG. 2. Rates of [^{14}C]glucose metabolism (□) and [^{3}H]TdR (▨) incorporation by anoxic salt-marsh sediments under exposure to 1 to 1,000 ppm anthracene, DDT, naphthalene, and PCP. All values are expressed relative to the control (5 μl of acetone) rates. All values are the mean ± standard deviation (n = 3 replicates).

1,000 ppm anthracene or naphthalene, retreated with an additional amount of the compounds, and assayed for glucose metabolism and thymidine incorporation showed little discernible difference in the percentage of responses from controls (data not shown).

DISCUSSION

In this study, we emphasized more general estimates of microbial activity (i.e., glucose metabolism and TdR incorporation) rather than more physiologically restricted measures (e.g., sulfate reduction, methanogenesis, nitrogen fixation or denitrification [5, 18; Slater and Capone, in press]). This approach affords a broader evaluation of pollutant effects on the microbiota by using measurements of an activity that is common to a greater proportion of the microbes present. Such an approach is useful in identifying pollutants which have broad spectrum effects. Conversely, if particular physiological groups are more sensitive to particular toxicants, these differences might not be elucidated through the use of general measures of metabolism.

Our examination of the alteration of sediment microbial activities by the acute addition of organic pollutants found differences in compound toxicity, the relative sensitivity of each activity to compound addition, and the relative susceptibility of oxic and anoxic sediments to pollutant addition. In few instances did acute pollutant addition result in the stimulation of either activity relative to controls.

The apparent order of compound toxicity was as follows (in increasing order): anthracene < DDT < naphthalene < PCP. Although this is the first study which assesses the effects of these four organic pollutants on [^{14}C]glucose metabolism and [^{3}H]TdR incorporation, other studies have examined their effects on anaerobic activities. The four compounds tested in this study have also been found to exhibit some degree of toxicity to N_2 fixation, denitrification (Slater and Capone, in press), sulfate reduction, and methanogenesis (18).

In general, [^{3}H]TdR incorporation was found to be affected to a greater extent by acute pollutant addition than was [^{14}C]glucose metabolism. In all but 7 cases (3 oxic, 4 anoxic of 32 total; Table 1) acute pollutant addition resulted in [^{3}H]TdR incorporation rates being inhibited more than

TABLE 2. Slopes of linear regressions of percent response (relative to controls) of [^{14}C]glucose metabolism and [^{3}H]TdR incorporation versus $\log_{10}$ (pollutant) for all oxic and anoxic acute pollutant addition experiments

Expt and pollutants	[^{14}C]glucose metabolism		[^{3}H]TdR incorporation	
	r^2	Slope	r^2	Slope
Oxic				
Anthracene	0.0030	−0.2 (2.3)[a]	0.5813	−3.9 (2.3)[a]
DDT	0.4719	1.7 (1.3)	0.7972	−6.4 (2.9)
Naphthalene	0.7902	−21.3 (7.8)	0.9138	−29.1 (6.3)[b]
PCP	0.9444	−31.1 (5.5)[b]	0.9298	−27.4 (5.3)[b]
Anoxic				
Anthracene	0.8346	−1.9 (0.6)	0.9800	−10.0 (1.0)[b]
DDT	0.8636	−3.3 (0.9)	0.7725	−13.0 (4.7)
Naphthalene	0.7965	−15.5 (5.5)	0.8526	−20.0 (5.8)
PCP	0.9999	−31.9 (0.3)[b]	0.9072	−28.3 (6.4)[b]

[a] Standard error of slope.
[b] $P < 0.05$.

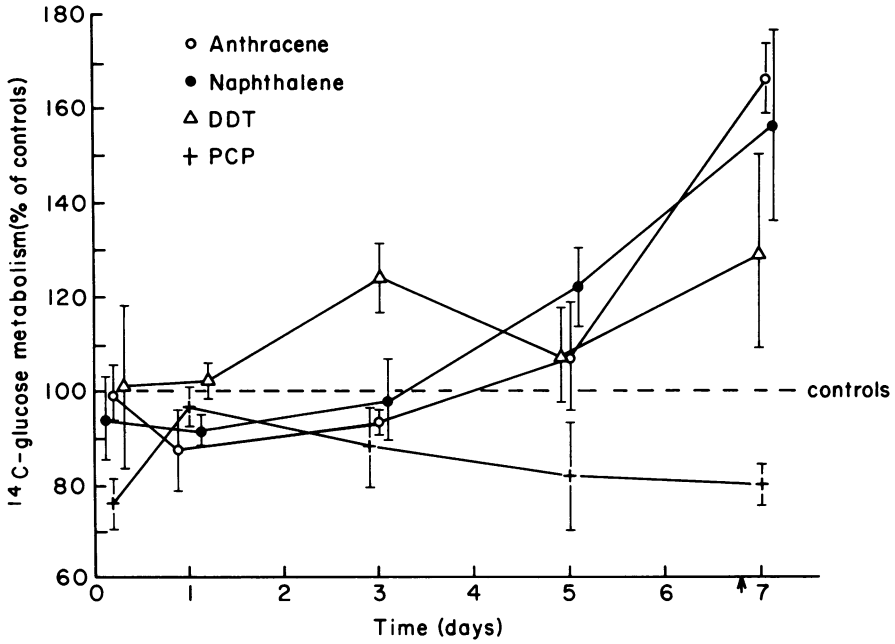

FIG. 3. Week-long time series of total [^{14}C]glucose metabolism by oxic batch sediment slurries (100 ml) exposed to 100 ppm anthracene, DDT, naphthalene, or PCP. Metabolism is expressed relative to the control (5 μl of acetone) values. All values are the mean ± standard deviation (n = 3 replicates). Arrow indicates when an additional 100 ppm of each organic pollutant was added before assays done on day 7.

[^{14}C]glucose metabolism. Regression analyses of dose-response curves (Tables 2 and 3) also support the greater sensitivity of [^{3}H]TdR incorporation. Recent evidence presented by Jonas et al. (17) has indicated that [^{3}H]TdR incorporation is affected to a greater extent than [^{14}C]glutamate uptake by Chesapeake Bay bacterioplankton exposed to several organometallic compounds. Blakemore (3) has also shown the selective action of polychlorobiphenyls on the growth and nucleic acid synthesis of a marine pseudomonad but not on protein synthesis. The fact that [^{3}H]TdR incorporation to cellular macromolecules is altered to a greater extent than assimilation of carbon and energy substrates suggests that these organic pollutants may selectively act on the mechanisms responsible for either nucleoside transport across cell membranes or their incorporation into DNA.

TABLE 3. Linear regression slopes of percent response (relative to controls) of [^{14}C]glucose metabolism and [^{3}H]TdR incorporation versus $\log_{10}$ ([pollutant] + 1)[a] for all oxic and anoxic samples pretreated with 1 to 1,000 ppm organic pollutants and held for 2 days before activity assays

Expt and pollutants	[^{14}C]glucose metabolism		[^{3}H]TdR incorporation	
	r^2	Slope	r^2	Slope
Oxic				
Anthracene[b]	0.0073	1.2 (3.9)[c]	0.7200	1.9 (2.0)[a]
DDT[b]	0.5278	2.5 (3.3)	0.2330	1.6 (4.6)
Naphthalene[d]	0.5880	0.8 (1.6)	0.8546	3.1 (0.02)[e]
PCP[d]	0.6940	−10.5 (2.2)	0.7346	−23.3 (3.7)
Anoxic				
Anthracene[b]	0.0488	−0.2 (4.3)	0.3903	−1.7 (3.2)
DDT[b]	0.3573	−2.0 (3.9)	0	
Naphthalene[d]	0.3882	−2.9 (9.8)	0.8929	−4.2 (0.2)[f]
PCP[d]	0.9615	−20.6 (3.9)	0.8143	−42.0 (2.6)[f]

[a] $\log_{10}$ (pollutant +1) transformation utilized to allow inclusion of 0 concentration (23).
[b] Samples received 0, 10, and 1,000 ppm organic compounds.
[c] Standard error of slope.
[d] Samples received 0, 1, and 100 ppm organic compounds.
[e] $P < 0.001$.
[f] $P < 0.05$.

In the present study, glucose metabolism was defined as the total ^{14}C-label that was both respired and incorporated into cellular material. Even when any increases in the percentage of respiration are taken into account in oxic experiments (Table 1), the amount of ^{14}C-label incorporated exclusively to cellular biomass was, in most cases, inhibited to only a slightly greater extent than was total [^{14}C]glucose metabolism. For instance, slopes of regressions of the percentage of ^{14}C-label incorporated into cellular material relative to controls under oxic and anoxic naphthalene and PCP treatment were, respectively, −20.1, −30.8, −18.6, and −32.6 (cf. Table 2). This illustrates that the ^{14}C-label incorporated into cellular biomass alone is also not as sensitive an indicator of pollutant stress as [^{3}H]TdR incorporation. However, this additional decrease in cellularly incorporated ^{14}C-label is additional evidence of decreased microbial growth and cellular carbon production.

The acute effects of organic pollutants on sediment microbial carbon cycling and potential growth and production were also found to vary based on the sediment redox state. Regression analysis of dose-response curves found greater negative regression coefficients for three of four compounds for both activities under anoxic conditions (except for naphthalene).

Evidence that oxic and anoxic sediments respond differently to pollutant addition over the longer term was provided by both week-long batch enrichments and 2-day acclimation studies. The addition of anthracene, DDT, and naphthalene stimulated oxic glucose metabolism in both experiments either through their use as carbon sources or alteration of

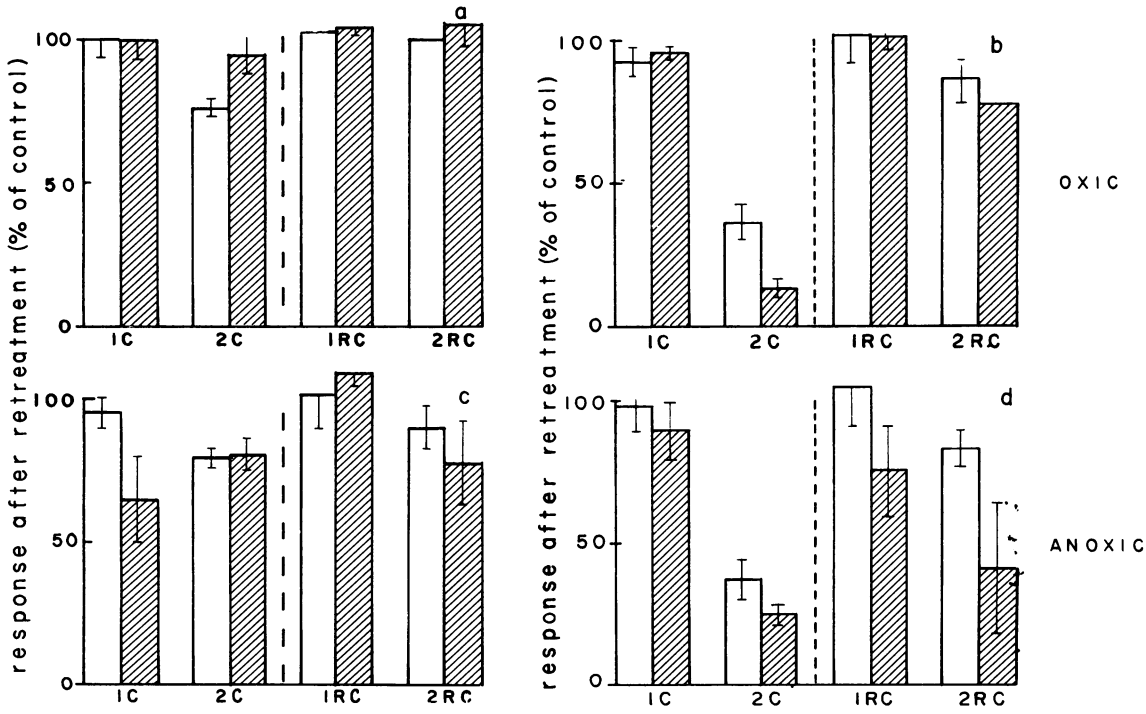

FIG. 4. Metabolism of [^{14}C]glucose (□) and incorporation of [^{3}H]TdR (▨) by batch sediment slurries exposed to DDT or PCP for 2 days before second dosing (retreatment) and activity assays. Treatment or retreatment (1) received 1 ppm (micrograms per milliliter of sediment slurry) PCP or 10 ppm DDT. Treatment or retreatment (2) received 100 ppm PCP or 1,000 ppm DDT. (1C) and (2C) were dosed after 2-day incubation immediately before assay at the level indicated; (1RC) and (2RC) were treated at both zero time and 2 days at the indicated level and compared with controls which were dosed at the same respective level at zero time. (a) and (c) Oxic and anoxic DDT, respectively. (b) and (d) Oxic and anoxic PCP, respectively. All values are the mean ± standard deviation (n = 3 replicates).

biochemical pathways (Fig. 3 and Table 3). [^{3}H]TdR incorporation in oxic experiments was also stimulated after 2 days by all compounds except PCP. This indicates that oxic sediment activities may recover and be stimulated by compounds which were initially toxic. In all anoxic cases, regression coefficients were negative after 2-day dosings, suggesting a lack of recovery of anoxic sediments after treatment and a possible role of oxygen in ameliorating the toxic effects of the compounds.

These results agree to some extent with those of other studies. Alexander and Schwarz (1) have found no inhibition of oxic sediment glucose utilization by crude oil exposures of up to 1 day. In a comprehensive study, Griffiths et al. (11) have observed sediment glucose uptake, methanogenesis, nitrogen fixation, and denitrification to be affected by exposure to crude oil for 8 months. The degree of inhibition or stimulation of these activities depends on the organic content of the sediments.

When oxic sediments that were pre-exposed for 2 days were redosed with an additional 100 ppm of DDT or PCP immediately before activity assays, at least some resistance to further pollutant input was noted (Fig. 4). Although actual adaptation to a particular compound or concentration is difficult to prove, in both cases those samples that had been exposed to the compounds for 2 days were inhibited less than those which had not undergone pre-exposure. Although some resistance to additional pollutant input was suggested in anoxic sediments, a precise interpretation of the results is difficult because redosed samples may have had up to two times the concentration of their controls. In several cases with anoxic sediments, one activity exhibited some degree of recovery, whereas the other did not. This contrasts with the oxic sediments case, in which parallel recovery occurred for both activities.

The concentrations of organic compounds used in our studies were generally higher than those used by other investigators and were higher than would be found in sediments not subjected to heavy pollutant loading. However, concentrations of polycyclic aromatic hydrocarbons in the New York Bight area have been found to reach levels of 1 to 120 ppm (19). Polychlorobiphenyls in regions of Buzzards Bay and Fall River Estuary, Mass., have been detected at levels of 50 to 100,000 ppm (22). Therefore, the levels utilized here would represent those of moderately to severely polluted areas. However, these concentrations were often necessary to elicit any toxic response (cf. 18; Slater and Capone, in press). It is possible that higher concentrations of organic pollutants may be tolerated by sediment microbes than by those of the water column because of complexation of pollutants with naturally occurring sediment organics (10).

Studies of the degradation and mineralization of the pollutants under investigation may shed additional light on the effects of these compounds on sediment microbial activities. For example, the absence of degradation of many aromatic compounds under anoxic conditions (6; Bauer and Capone, in review) may be responsible for the prolonged toxicity observed in these sediments. Anthracene and naphthalene are readily degraded and would be expected to be rapidly removed from oxic sediments (J. E. Bauer and D. G. Capone, submitted for publication) and exhibit short-term toxicity only. Therefore, it can be hypothesized that certain

organic pollutants accumulate in anoxic sediments and result in long-term exposure of sediment microbes; those in oxic sediments would be degraded and might represent both a carbon and energy source for aerobic microbes (accounting for the stimulation in activities after 2 days).

The temporal and spatial variability in the oxidation state of coastal marine sediments likely would result in intermediate degrees of compound toxicity and degradation. Anoxic sediments that are periodically oxidized (through macrofaunal bioturbation or physical mixing) may eliminate polycyclic aromatic hydrocarbons more efficiently than those which are strictly anoxic, but less effectively than fully oxidized surface sediments.

It is concluded that anoxic marine sediment microbiota may be more severely affected and affected for longer periods of time by particular organic contaminants than those of oxidized sediments because of both the apparently greater toxicity to the anoxic sediment microbiota and the potentially greater persistence of these compounds in anoxic environments.

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