APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1979, p. 878-885 Vol. 37, No. 5
0099-2240/79/05-0878/08$02.00/0

Comparative Effects of Aroclor 1254 (Polychlorinated Biphenyls) and Phenanthrene on Glucose Uptake by Freshwater Microbial Populations

G. S. SAYLER,* L. C. LUND, M. P. SHIARIS, T. W. SHERRILL, AND R. E. PERKINS

Department of Microbiology and The Graduate Program in Ecology, The University of Tennessee, Knoxville, Tennessee 37916

Received for publication 22 February 1979

The effects of polychlorinated biphenyl (PCB) and phenanthrene stress on glucose uptake by natural microbial populations were examined by the heterotrophic potential technique. Temporal and spatial distributions in glucose uptake velocities were examined for natural samples as well as PCB- and phenanthrene-stressed samples. Statistical analysis indicated significant variability among the various samples. It was demonstrated that the environmental variables contributed significantly to the variability in uptake kinetics. Although general trends indicated a PCB-induced stimulation in uptake velocities, these trends were in part masked by sample variability. Data analysis indicated no statistically significant PCB or phenanthrene effect on either total glucose uptake velocities or the proportion of $^{14}CO_2$ evolved, as compared to natural unstressed samples.

Numerous investigators have demonstrated the role and abilities of microorganisms in the aquatic decomposition of environmental pollutants. However, relatively little information is available concerning the effects of environmental contaminants on aquatic microbial communities.

Toxicant perturbations of aquatic ecosystems may adversely effect the overall microbial community or specific microbial populations within the community. Either type of effect may cause serious disruptions in productivity, nutrient and element cycling, and decompositional activity. The consequences of such perturbations may pose a threat to the stability of an aquatic ecosystem as serious as the failure of microorganisms to degrade a specific toxicant.

This study was undertaken to elucidate the effects of Aroclor 1254, a group of (commercial preparation) polychlorinated biphenyls (PCB), and a model polycyclic aromatic hydrocarbon, phenanthrene (PHE), upon glucose uptake by an aquatic microbial community. Both PCBs and polycyclic aromatic hydrocarbons are recognized as persistent aquatic contaminants of global distribution (2, 8, 14-16, 28). In addition, their potential for continued contamination of aquatic systems seems assured due to their past heavy usage, in the case of PCBs, and their relationship to fossil fuel technology, in respect to the polycyclic aromatic hydrocarbons. Both groups of contaminants share similar physical and chemical characteristics, including low water solubility: ca. 56 μg liter^{-1} (13) and 1.6 mg liter^{-1} (34), respectively, for Aroclor 1254 and PHE. In addition, both PCB and PHE have been shown to be subjected to biodegradation (3, 9, 10, 19, 21, 27, 33).

Glucose uptake velocities were chosen as a parameter for measuring the effects of PCB and PHE upon heterotrophic microbial activity. The "heterotrophic potential" assay has been suggested as a sensitive method for the assessment of eutrophication (1). It has also been applied, in a comparative fashion, for industrial and domestic wastewater analysis (18). Although variable in its information content, the assay provides an index for gross heterotrophic metabolism if respiratory corrections (17) are included. Numerous investigators have employed a variety of techniques to assess the relative heterotrophic potential of aquatic habitats. It was felt that these techniques would provide a framework for elucidating toxicant effects on heterotrophic metabolism. In addition, valuable information would be obtained on the consequences of heterotrophic potential assessment in habitats with an unrecognized source of contamination.

MATERIALS AND METHODS

Sampling. Samples were collected from five sample sites located on Center Hill Reservoir in central Tennessee (Fig. 1). Center Hill Reservoir is a U.S. Army Corps of Engineers impoundment of the Caney Fork River. The reservoir lies within the eastern highland rim of Tennessee at an elevation of 648 feet (ca.

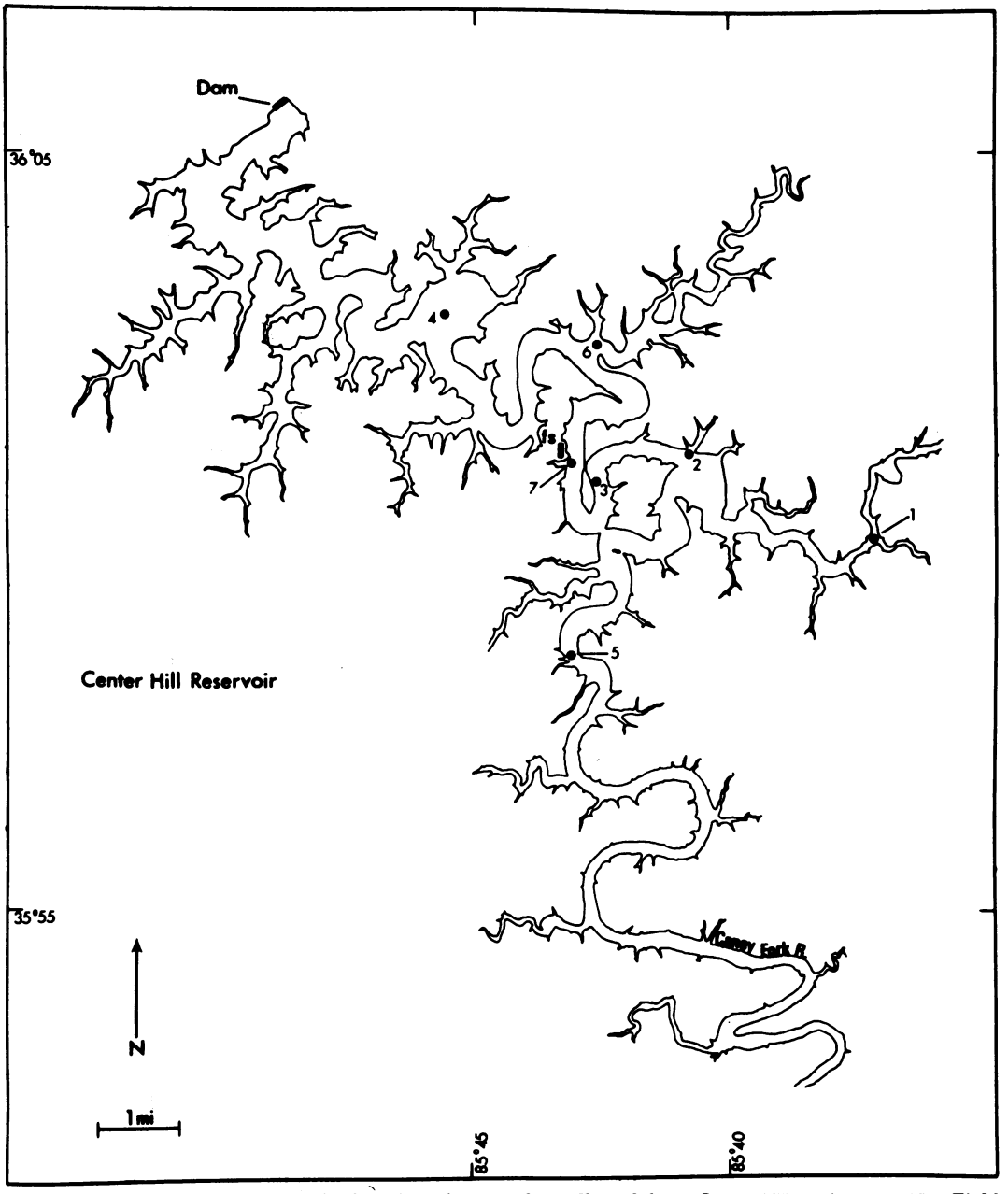

FIG. 1. *Center Hill Reservoir site locations for samples collected from June 1977 to August 1978. Field station (fs) facilities are located adjacent to site 7.*

197.5 m) above sea level. Maximum reservoir depths approach 250 feet (ca. 76.2 m). The reservoir has a restricted, relatively undeveloped shoreline exceeding 400 miles in length and encompasses a drainage of 2,195 square miles. The reservoir is surrounded by a second-growth deciduous forest. The reservoir is essentially free of industrial and domestic wastes and is considered relatively oligotrophic.

Samples were collected during June, August, September, and December of 1977 and August of 1978. All samples were taken from a depth of 1 m with the aid of a Niskin sterile bag sampler (General Oceanics, Miami, Fla.). The samples were returned to shore-based laboratory facilities located at the Tech Aqua biology field station (Fig. 1). Sample processing was initiated within 1 h of sample collection. In the interim,

the samples were maintained at ambient water temperature. A total of 10 samples were examined during the course of this investigation. All samples were processed in triplicate.

Field sampling variables assessed during the course of this investigation included: dissolved oxygen (YSI DO meter, model 51B; Yellow Springs Instrument Co., Yellow Springs, Ohio); water temperature and conductivity (YSI conductivity meter, model 33); transparency (Secchi disk; Wildlife Supply Co., Saginaw, Mich.); pH (Orion model 407A pH meter; Orion Research Inc., Cambridge, Mass.); and air temperature, mercury-filled glass thermometer.

Assessment of bacterial populations. Total viable heterotrophic bacterial counts (TVC) were determined by spread-plating each sample (after appropriate dilution in autoclaved lakewater) onto yeast extract peptone glucose (YEPG) agar. YEPG agar is formulated with (per liter of distilled water): dextrose, 1.0 g; polypeptone, 2.0 g; yeast extract, 0.2 g; NH_4NO_3, 0.2 g; and purified agar (Fisher), 18.0 g. The spread plates were incubated at room temperature and examined for bacterial growth at both 2 and 4 weeks. All observable colonies were recorded; however, only those plates having statistically valid numbers of colonies, 30 to 300 colonies per plate, were used for data analysis.

Enumerations of PCB- and polycyclic aromatic hydrocarbon-resistant microorganisms were performed by spread plate inoculations of PCB agar and PHE agar, respectively. PCB agar and PHE agar were prepared with the same basal salts mixture, as follows (per liter of distilled water): $NaNO_3$, 4.0 g; KH_2PO_4, 1.5 g; Na_2HPO_4, 0.5 g; $FeSO_4$, 0.0007 g; $MgSO_4 \cdot 1H_2O$, 0.2 g and $CaCl_2 \cdot 2H_2O$, 0.01 g. Yeast extract (BBL Microbiology Systems), 0.001 g liter^{-1}, and purified agar (Fisher), 20.0 g liter^{-1}, were added to the basal salts mixture prior to sterilization. Aroclor 1254 (a commercial PCB mixture, 54% chlorine by weight; Monsanto Chemical Co., St. Louis, Mo.) or certified PHE (Eastman Organic Chemicals, Rochester, N.Y.) was added to the basic medium formulation at a concentration of 1.0 g liter^{-1}. The media were autoclaved at 15 lb/in^2 for 15 min and then aseptically sonicated for 15 min to dispense the Aroclor 1254 or PHE as an emulsion in the medium. PCB agar is opaque, and colony growth was viewed under reflected light. PHE agar is translucent, and colonies may be viewed under transmitted light. Sample dilution, plate inoculation and incubation, and the recording of results were handled as described for TVC analysis.

Heterotrophic uptake. Heterotrophic uptake analysis of [U-^{14}C]glucose was performed according to the methods of Parsons and Strickland (23) as modified by Wright and Hobbie (35). Reaction vessels used for heterotrophic uptake studies were 60-ml serum bottles fitted with serum stoppers with suspended plastic center wells (Kontes).

For each sample collected, three heterotrophic uptake studies were performed in triplicate, including: a natural control study with unamended lakewater and 10 μl of acetone; lakewater dosed with 100 μg of Aroclor 1254 per liter, delivered in 10 μl of acetone; and lakewater dosed with 100 μg of phenanthrene per liter in 10 μl of acetone. Appropriate sterile controls, using autoclaved lakewater or lakewater dosed with 1.0 mg of $HgCl_2$ per ml, were included for all experiments.

The procedures for performing the heterotrophic uptake studies maintained a well-standardized format that was consistent for every sample collected. A 10-ml lakewater sample was aseptically pipetted into sterilized reaction vessels. Natural control samples received no toxicant supplements, and the toxicant-dosed samples received supplements of either Aroclor 1254 or certified PHE as previously described. The samples in the reaction vials were allowed to acclimate for approximately 1 hr. At this time [U-^{14}C]glucose (New England Nuclear or Amersham), specific activities ranging from 5.4×10^4 to 4.0×16^6 dpm μg^{-1}, was added at varied concentrations to triplicate groups of reaction vessels. The reaction vessels were again sealed and incubated at temperatures approximating field conditions at the time of sampling. For the majority of experiments, incubation times were held constant at 1 h. After incubation, 1.0 ml of 2 N H_2SO_4 was added to the reaction mixture (final pH, 3.5) via a syringe needle through the serum stopper, to terminate heterotrophic uptake and drive $^{14}CO_2$ out of solution. Liberated $^{14}CO_2$ was collected by β-phenethylamine absorption by injecting 0.4 ml of the absorber through the serum stopper into the center wells. The reaction vessels were allowed to stand undisturbed for a minimum of 1 h to allow complete absorption of liberated $^{14}CO_2$. After absorption of CO_2, the serum stoppers and center wells were removed from the reaction vessels, and the center wells were placed directly into liquid scintillation vials containing a liquid scintillation cocktail, 0.4% Omnifluor (New England Nuclear), in scintillation-grade toluene (Fisher).

Cell-bound [^{14}C]glucose was determined by withdrawing a 3.0-ml subsample, via syringe, from the reaction vessel. The subsample was then filtered through a 25-mm-diameter, 0.45-μm-pore size filtration membrane (Amicon) inserted in a Swinnex adapter attached to the syringe. Each membrane was rinsed with 3.0 ml of distilled water. The filtration membranes were removed from the adapters and placed in scintillation vials containing 0.8% Omnifluor in scintillation-grade dioxane (Burdick and Jackson Laboratories). The processed samples were returned to the laboratory for scintillation counting, which was completed within 24 to 48 h after termination of the heterotrophic uptake studies. Liquid scintillation counting employed a Packard Tricarb liquid scintillation counter. Counting efficiency (75 to 94%) was determined by the use of internal quenched and unquenched standards.

Statistical analysis. Raw sampling data, plate count data, and heterotrophic uptake velocity data were subjected to computer analysis using a DEC 10, IBM 360/5-370/148 computer system. Statistical analysis subprograms for analysis of variance, multiple correlation and linear regression, polynomial regression, and multiple regression were derived from *Statistical Package for the Social Sciences* (22). Significance limits were set at the 95% level.

RESULTS

Sampling site characteristics. Physical, chemical, and microbiological parameters mon-

itored for each site during the investigation are given in Table 1. Site depth was highly variable from site to site, ranging from 5 m at site 2 to greater than 30 m at sites 4 and 5. Temperature, dissolved oxygen, transparency, conductivity, and pH were variable on a temporal basis, but were similar from site to site. TVC ranged from 2.54 to 4.68 $\log_{10}$ ml^{-1} while PCB- and PHE-resistant organisms ranged from 1.56 to 3.74 $\log_{10}$ ml^{-1} and 1.0 to 3.37 $\log_{10}$ ml^{-1}, respectively. PCB-resistant organisms represented their highest proportion of the TVC in August 1977 and August 1978. PHE-resistant organisms maintained a nearly uniform population density throughout the study except for samples collected at site 7 in September 1977 and site 7 in August 1978.

Natural glucose turnover. Glucose turnover times for each of the eight samples examined are presented in Table 2. Natural, undosed uptake velocities approximated saturation kinetics in only one instance, site 4, December 1977. With minor exceptions all other uptake velocities appeared linear with respect to increasing glucose substrate levels from 5 to 120 μg liter^{-1}. Consequently, turnover times were calculated on the basis of linear uptake kinetics as described by Wright and Hobbie (35). Overall, no distinct patterns in glucose turnover were observed. The fastest rate of glucose turnover was observed at site 7, August (245 h), and the slowest rate was observed at site 2, June (2,500 h). In general, there were no temporal or spatial trends indicated by either turnover times or raw uptake velocities.

Effects of PCB and PHE on glucose turnover. PCB and PHE stress resulted in both stimulation and inhibition of natural glucose turnover (Table 2). PCB and PHE stresses were observed to stimulate rates of glucose turnover in four and two samples, respectively. In two samples each, inhibition of glucose turnover was observed for both PCB and PHE. However, as indicated by Table 2, these effects were not necessarily concurrently demonstrated by both PCB and PHE in the same sample. Comparatively, PCB and PHE concurrently stimulated glucose turnover in samples from sites 2 and 4, June, and site 7, August, while they concurrently inhibited glucose turnover at site 5, June. PCB stress effects ranged from a 10-fold stimulation to a 3-fold inhibition in glucose turnover. Similarly, PHE stress effects ranged from approximately a fourfold stimulation to a twofold inhi-

TABLE 2. *Comparative effects of PCB and PHE on natural glucose turnover among temporally and spatially isolated samples*

Date	Site[a]	Turnover time[b] (h)		
		Natural	PCB stress	PHE stress
6-77	2	2,500	355*	575*
	4	270	675*	310
	5	260	645*	1,430*
8-77	1	1,940	620*	2,550
	7	245	75*	110*
9-77	4	1,430	120*	1365
	7	1,295	1,230	2,940
12-77	4	690	550	630

[a] See Fig. 1.

[b] Turnover times were estimated from linear glucose uptake kinetics over a glucose concentration range of 5, 30, 60, and 120 μg liter^{-1}. * Indicates those values significantly different from unstressed natural samples ($\alpha = 0.05$).

TABLE 1. *Site characteristics for samples collected from Center Hill Reservoir*[a]

Date	Site[b]	Depth[c] (m)	Temp (°C)	DO (mg liter^{-1})	SECC (m)	COND (μmho)	pH	TVC	PCB	PHE
6-77	2	5	27.0	—	1.8	132	9.4	4.35	2.60	2.30
	4	>30	25.5	8.0	2.0	112	8.2	3.30	2.30	2.30
	5	>30	28.0	—	1.0	160	9.0	4.68	2.27	2.30
8-77	1	21	29.0	7.5	1.4	159	7.5	4.00	3.90	2.30
	7	8	29.0	7.6	2.5	151	8.4	3.38	2.38	2.30
9-77	4	>30	28.5	7.8	4.0	280	6.2	3.47	1.56	2.30
	7	8	28.5	7.4	2.0	280	6.5	2.54	1.90	1.00
12-77	4	>30	13.0	10.0	1.0	117	8.0	3.25	2.27	2.27
8-78	7	8	32.0	7.8	1.5	150	—	4.05	3.74	2.37

[a] Abbreviations: DO, dissolved oxygen; SECC, transparency; COND, conductivity; TVC, PCB, PHE, $\log_{10}$ of the total viable count and PCB- and PHE-resistant microorganisms, respectively.

[b] See Fig. 1.

[c] Sample site depth; all samples were collected 1 m below the surface.

bition in glucose turnover.

In December 1977 (site 4) and August 1978 (site 7), examination of the PCB and PHE stress effects on glucose turnover, at elevated glucose concentrations, was undertaken. Samples collected in December exhibited glucose turnover rates an order of magnitude higher than any samples previously examined (Table 3). There was an insignificant increase in glucose turnover for those December samples stressed with either PCB or PHE. A higher significant PCB and PHE inhibition of glucose turnover was observed for those samples collected in August 1978 (Table 3). This inhibition in glucose turnover was the result of the virtual cessation of increased rates of glucose uptake beyond a glucose substrate concentration of 265 μg liter^{-1}.

Effect on glucose mineralization. PCB and PHE stress effects on substrate mineralization were examined by partitioning $^{14}CO_2$ evolution from total [^{14}C]glucose uptake (Table 4). An average of 61.9% of the total glucose uptake was respired by natural samples. In comparison, 63.1 and 66.4% of the total glucose uptake was respired by PCB- and PHE-stressed samples, respectively. These averages included all ranges of glucose substrate concentration. Least-significant range analysis indicated that there were no significant differences (α = 0.05) between the mean proportions of $^{14}CO_2$ evolved for the three sample types.

Comparative effects of toxicants and environmental variables on glucose uptake. In an effort to explain the nature of the variation in glucose uptake and turnover during stressed and unstressed conditions, an analysis of the effects of environmental variables was undertaken. A mixed-model, three-way analysis of variance examining the effects of sample type, sample treatments, and glucose substrate concentration was performed (Table 5). Statistically significant sample type, treatment, and substrate concentration effects were observed. In addition, there was also a significant interaction between sample types and treatments as well as sample types and substrate concentration. These results indicated significant variation in terms of glucose uptake, in response to both treatment and glucose concentration effects. These effects are readily discernible in Table 2, as previously described. However, it is also apparent that uncontrolled variables characteristic of each sample type also significantly affected glucose uptake velocities and were a potential source of variation in terms of the interaction between sample types or glucose additions. Preliminary correlation analysis indicated no apparent correlation between bacterial cell densities (TVC, PCB, PHE) and glucose uptake velocities, although uptake velocities themselves were significantly intercorrelated.

Regression analyses were performed to describe accurately the variations in glucose uptake velocities as a function of the independent variables. Initially, uptake velocities were re-

TABLE 3. *Comparative effects of PCB and PHE on natural glucose turnover at elevated glucose levels*

Date	Site[a]	Turnover time[b] (h)		
		Natural	PCB stress	PHE stress
12-77	4	13	8	9
8-78	7	130	36,720*	18,300*

[a] See Fig. 1.

[b] Turnover times were estimated from linear glucose uptake kinetics over a glucose concentration range of 50 to 1,000 μg liter^{-1} (increment of approximately 100 μg liter^{-1}). * Indicates those values significantly different from unstressed natural samples (α = 0.05).

TABLE 4. *Comparative glucose mineralization[a] for natural Center Hill samples and those stressed with PCB or PHE*

Treatment	No. of observations	% CO_2 evolved	Standard deviation
Natural	33	61.9	24.0
PCB dosed	36	63.1	26.4
PHE dosed	28	66.4	23.4

[a] Proportion of the total glucose uptake evolved as $^{14}CO_2$.

TABLE 5. *Three-way analysis of variance of glucose uptake velocities of the Center Hill Reservoir microbial community*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F[a]
Main effects				
Sample[b]	7	1.25×10^8	1.78×10^7	9.294*
Treatment[c]	2	2.09×10^7	1.05×10^7	5.448*
Substrate concn[d]	3	1.04×10^8	3.48×10^7	18.150*
Interactions				
Sample × treatment	14	5.43×10^7	3.88×10^6	2.02*
Sample × substrate concn	21	1.29×10^8	1.18×10^6	3.28*
Treatment × substrate concn	6	1.37×10^7	2.29×10^6	1.194
Explained	53	4.48×10^8	8.46×10^6	
Residual	42	8.07×10^7	1.92×10^6	
Total	95	5.29×10^8		

[a] *, Significant F statistic, α = 0.05.

[b] Sample site and data.

[c] Natural or stressed with PCB or PHE.

[d] [*U*-^{14}C]glucose concentration range.

gressed solely on glucose substrate concentrations in model I linear regression. Figure 2 represents the resulting linear regression plots of glucose uptake velocity versus the added glucose concentration for natural samples and PCB- and PHE-dosed samples. Statistically significant linear regression relationships were found for each of three uptake velocity responses examined. However, as indicated by the r^2 values, only 28, 27 and 16% of the variation of the independent variable, glucose uptake velocities for TVC, PCB, and PHE (VTVC, VPCB, and VPHE), respectively, could be attributed to the linear regression of glucose uptake velocity on the glucose substrate concentration. Second- and third-order curvilinear models were also examined, and, although significant regressions were observed, there was no greater explained variation attributed to the regression model. These results indicated that variations in glucose uptake were attributable, in part, to other independent variables in addition to glucose substrate concentration.

To elucidate these effects more fully, a multiple regression analysis between glucose uptake velocities, substrate concentration, and field sampling variables was performed. For the purpose of this analysis the following variables were examined: glucose uptake velocities for untreated samples (VTVC), PCB-stressed samples (VPCB), and PHE-stressed samples (VPHE); glucose substrate concentrations; logarithmic transformation of TVC, PCB-degrading or -resistant bacterial counts, and PHE-degrading or -resistant bacterial counts; water temperature; dissolved oxygen; Secchi disk transparency; conductivity; and pH. The results of this analysis are presented in Table 6. As expected, the major contributing factor to variation in uptake velocity was the glucose substrate concentration. However, the added variance components explained 46, 45, and 39%, comparatively, for VTVC, VPCB, and VPHE, of the total variation attributed to the multiple regression. Aside from the substrate concentration, the two greatest sources of variation were conductivity and pH. Increases in the multiple r^2 value due to conductivity, relative to increased variation in uptake velocity, were 0.08, 0.08, and 0.20 for VTVC, VPCB, and VPHE, respectively. Variation, attributable to pH, increased r^2 values by 0.2, 0.0, and 0.1, respectively, for VTVC, VPCB, and VPHE. Although a significant portion of the total variation of glucose uptake velocity was accounted for by the individual regression equations, the overlapping 95% velocity confidence limits at the mean glucose substrate concentration (57 μg liter^{-1}) indicated no significant difference between the natural uptake and the uptake of those samples dosed with PCB or PHE.

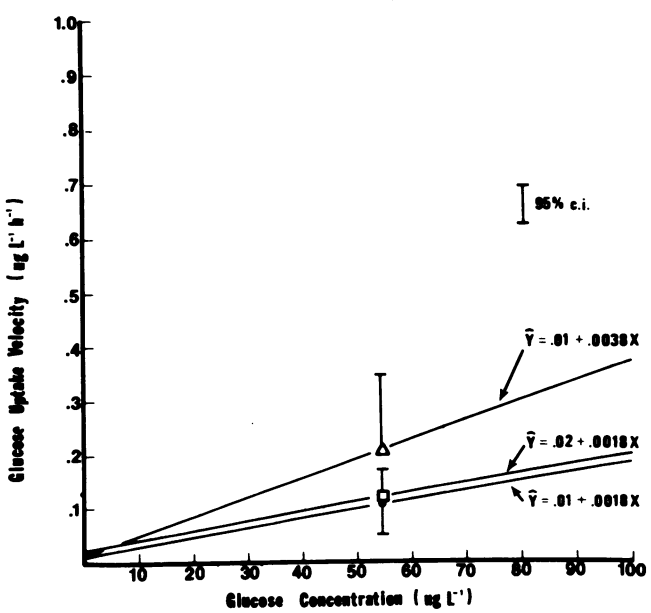

FIG. 2. *Comparative regressions of glucose uptake velocity on glucose concentration for pooled samples examined in Table 2.* (●) *Natural uptake velocities;* (△) *PCB-stressed samples;* (□) *PHE-stressed samples. 95% c.i., Confidence interval.* r^2 *values for natural and PCB- and PHE-stressed samples were 0.28, 0.27, and 0.16, respectively.*

TABLE 6. *Comparison of multiple regression coefficients of glucose uptake velocities for natural samples, PCB-stressed samples, and PHE-stressed samples*

Independent variable[a]	Regression coefficient[b] (*b*)			Multiple coefficient of determination (r^2)		
	VTVC	VPCB	VPHE	VTVC	VPCB	VPHE
SUBCON (X)	0.18	0.36	0.18	0.28	0.26	0.15
TEMP (X_2)	0.12	0.36	0.53	0.28	0.28	0.16
DO (X_3)	−0.24	0.33	0.41	0.30	0.30	0.16
SECC (X_4)	0.15	0.15	0.13	0.30	0.33	0.17
COND (X_5)	−0.26	−0.14	−0.19	0.38	0.45	0.37
PH (X_6)	−0.11	0.75	−0.27	0.46	0.45	0.38
TVC (X_7)	0.14	0.47	0.20	0.46	0.45	0.39
Y intercept (*C*)	0.01	−0.03	−0.04			
Mean velocity ($\bar{Y}$)[c]	0.11	0.22	0.12			
95% confidence limits of $\bar{Y}$	±0.04	±0.09	±0.09			

[a] SUBCON, Glucose substrate concentrations; TEMP, water temperature; DO, dissolved oxygen; SECC, Secchi disk transparency; COND, conductivity; PH, pH.

[b] $b \times 10^2$.

[c] Micrograms per hour per liter.

DISCUSSION

A variety of effects could contribute to the lack of saturation kinetics and the highly variable turnover and stress effects observed in the samples examined in this study. Specifically, microbial diversity and composition of the samples, species-specific saturation coefficients (K_s), and methods of assay termination can all influence kinetic expression of glucose uptake (12, 24, 25, 30–32).

The effects of environmental variables on glucose uptake cannot be over emphasized. Much of the variation in glucose uptake between samples can be attributed to subtle differences in the physical-chemical environment. In addition, endogenous organic carbon and mineral nutrients play an important role in heterotrophic activity (1, 26).

Specific environmental variables such as pH and conductivity may relate directly to phytoplankton metabolism. Consequently, interactions of glucose uptake with pH and conductivity could be attributed in part to phytoplankton densities or specific phytoplankton populations. It is highly probable that increased glucose turnover at glucose concentrations from 100 to 1,000 μg liter^{-1}, observed at site 7, August 1978 (Table 3), were due in part to algal uptake. Furthermore, the high significant PCB suppression of glucose uptake at elevated glucose concentrations (August 1978) may have been due to PCB-phytoplankton stress interactions. Other investigators have previously demonstrated phytoplankton sensitivity to PCB stress (4, 11, 30), although the effect on organic solute uptake has not been demonstrated.

The available information relating to PCB and PHE stress on heterotrophic bacterial population indicates variable, but minor, stress effects. In examining the effects of PCB on pure cultures of environmental isolates, PCB has demonstrated both inhibitory and stimulatory effects (6, 7, 20, 27). In a sense, this investigation supports both types of observations. In the short term, individual samples exhibited both PCB-induced inhibitions and stimulations in heterotrophic glucose uptake.

The results of these investigations have demonstrated that on a temporal basis there is no statistically significant effect of either Aroclor 1254 or PHE on glucose uptake by naturally occurring microbial populations. A trend did exist that indicated a stimulated uptake of low concentrations of glucose by Aroclor 1254. However, natural variability of glucose uptake velocities masked this trend and its true importance. On an individual sampling-site basis, significant variation, greater than the control samples, could be attributed to the stress effects of Arclor 1254 and PHE. In the majority of samples, the PHE-induced stress was less pronounced than the PCB stress. These results were further supported by regression analysis, which demonstrated an identical uptake velocity regression coefficient (*b*) for both the natural samples and PHE-dosed samples. It is important to point out that these short-term acute dosing studies with PHE and PCB need supportive information on the long-term multifaceted effects of chronic PCB and PHE stress before it can be conclusively demonstrated that these pollutants do not significantly perturb the heterotrophic microbial community.

ACKNOWLEDGMENTS

This investigation was supported by the National Institutes of Health (Public Health Service grant ES01521-02) and by

Union Carbide, Oak Ridge National Laboratories, subcontract no. 7182.

LITERATURE CITED

1. **Albright, L. J., and J. W. Wentworth.** 1973. Use of the heterotrophic activity technique as a measure of eutrophication. Environ. Pollut. **5**:59–72.
2. **Andelman, J. B., and J. E. Snodgrass.** 1974. Incidence and significance of polynuclear aromatic hydrocarbons in the water environment. C. R. Environ. Contam. **4**: 69–83.
3. **Ballschmiter, K., C. Unglert, and H. J. Neu.** 1977. Decomposition of chlorinated aromatics. III. Microbial decomposition of polychlorinated biphenyls (PCB). Chemosphere **7**:51–56.
4. **Biggs, D. C., R. G. Rowland, H. B. O'Connors, Jr., C. D. Powers, and C. F. Wurster.** 1978. A comparison of the effects of chlordane and PCB on the growth, photosynthesis, and cell size of estuarine phytoplankton. Environ. Pollut. **15**:253–263.
5. **Blakemore, R. P.** 1978. Effects of polychlorinated biphenyls on macromolecular synthesis by a heterotrophic marine bacterium. Appl. Environ. Microbiol. **35**:329–336.
6. **Blakemore, R. P., and A. E. Carey.** 1978. Effects of polychlorinated biphenyls on growth and respiration of heterotrophic marine bacteria. Appl. Environ. Microbiol. **35**:323–328.
7. **Bourquin, A. W., and S. Cassidy.** 1975. Effect of polychlorinated biphenyl formulations on the growth of estuarine bacteria. Appl. Microbiol. **29**:125–127.
8. **Crump-Wiesner, H. J., H. R. Feltz, and M. L. Yates.** 1974. A study of the distribution of polychlorinated biphenyls in the aquatic environment. Pest. Monit. J. **8**:157–161.
9. **Dagley, S.** 1971. Catabolism of aromatic compounds by microorganisms. Adv. Microbial Physiol. **6**:1–46.
10. **Evans, W. C., H. M. Fernley, and E. Griffiths.** 1965. Oxidative metabolism of phenanthrene and antracene by soil pseudomonads. Biochem. J. **95**:819–831.
11. **Fisher, N. S.** 1975. Chlorinated hydrocarbon pollutants and photosynthesis of marine phytoplankton: a reassessment. Science **189**:463–464.
12. **Hamilton, R. D., and J. E. Preslan.** 1970. Observations on heterotrophic activity in the eastern tropical Pacific. Limnol. Oceanogr. **15**:395–401.
13. **Haque, R., D. W. Schmedding, and V. H. Freed.** 1974. Aqueous solubility, adsorption and vapor behavior of polychlorinated biphenyl Aroclor 1254. Environ. Sci. Technol. **8**:139–142.
14. **Hardy, R., P. R. Mackie, and K. J. Whittle.** 1977. Hydrocarbons and petroleum in the marine ecosystem—a review. Rapp. P. V. Reun. Cons. Int. Explor. Mer. **171**:17–26.
15. **Harrison, R. M., R. Perry, and R. A. Wellings.** 1975. Polynuclear aromatic hydrocarbons in raw, potable and waste waters. Water Res. **9**:331–346.
16. **Harvey, G. H., and W. G. Steinhauer.** 1976. Transport pathways of polychlorinated biphenyls in Atlantic water. J. Mar. Res. **34**:561–575.
17. **Hobbie, J. E., and C. C. Crawford.** 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnol. Oceanogr. **14**: 528–532.
18. **Hobbie, J. E., and C. C. Crawford,** 1969. Bacterial uptake of organic substrate. New methods of study and application to eutrophication. Vert. Int. Verein Theor. Angew. Limnol. **17**:725–730.
19. **Kaiser, K. L. E., and P. T. S. Wong.** 1974. Bacterial degradation of polychlorinated biphenyls. I. Identification of some metabolic products from Aroclor 1242. Bull. Environ. Contam. Toxicol. **11**:291–296.
20. **Keil, J. E., S. H. Sandifer, C. D. Graber, and L. E. Priester.** 1972. DDT and polychlorinated biphenyl (Aroclor 1242) effects of uptake on *E. coli* growth. Water Res. **6**:837–841.
21. **Kiyohara, H., and K. Nago.** 1978. The catabolism of phenanthrene and naphthalene by bacteria. J. Gen. Microbiol. **5**:69–75.
22. **Nie, N. H., C. H. Hull, J. G. Jenkins, K. Stenbrenner, and D. H. Bent (ed.).** 1970. Statistical package for the social sciences, p. 280–286, 320–360, 411–421. McGraw-Hill, New York.
23. **Parsons, T. R., and J. D. H. Strickland.** 1961. On the production of particulate organic carbon by heterotropic processes in sea water. Deep-Sea Res. **8**:211–222.
24. **Ramsay, A. J.** 1976. The effect of acidification on measurements of microbial uptake of radioactive glucose in fresh water. Limnol. Oceanogr. **21**:922–926.
25. **Robinson, G. G. C., L. Hendzel, and D. C. Gillespie.** 1973. A relationship between heterotrophic utilization of organic acids and bacterial populations in West Blue Lake, Manitoba. Limnol. Oceanogr. **18**:264–269.
26. **Sayler, G. S., and C. M. Gilmour.** 1978. Heterotrophic utilization of organic carbon in aquatic environments. J. Environ. Qual. **7**:385–391.
27. **Sayler, G. S., M. Shon, and R. R. Colwell.** 1977. Growth of an estuarine *Pseudomonas* sp. on polychlorinated biphenyl. Microb. Ecol. **3**:241–255.
28. **Sayler, G. S., R. Thomas, and R. R. Colwell.** 1978. Polychlorinated biphenyl (PCB) degrading bacteria and PCB in estuarine and marine environments. Estuar. Coast. Mar. Sci. **6**:553–567.
29. **Sinclair, T., S. Garland, T. Arnason, P. Hope, and M. Granville.** 1977. Polychlorinated biphenyls and their effects on photosynthesis and respiration. Can. J. Bot. **55**:2679–2684.
30. **Vaccaro, R. F.** 1969. The response of natural microbial populations in seawater to organic enrichment. Limnol. Oceanogr. **14**:726–735.
31. **Vaccaro, R. F., and H. W. Jannasch.** 1967. Variations in uptake kinetics for glucose by natural populations in seawater. Limnol. Oceanogr. **12**:540–542.
32. **Williams, P. T. LeB.** 1973. The validity of the application of simple kinetic analysis to heterogenous microbial populations. Limnol. Oceanogr. **18**:159–165.
33. **Wodzinski, R. S., and J. E. Coyle.** 1974. Physical state of phenanthrene for utilization by bacteria. Appl. Microbiol. **27**:1081–1084.
34. **Wodzinski, R. S., and M. J. Johnson.** 1968. Yields of bacterial cells from hydrocarbons. Appl. Microbiol. **16**: 1886–1891.
35. **Wright, R. T., and J. Hobbie.** 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology **47**:447–464.