Bacterial Bioconcentration of Chlorinated Hydrocarbon Insecticides from Aqueous Systems[1]

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Abstract

The prevalence of chlorinated hydrocarbon insecticide uptake by chemoorganotrophic bacteria has been investigated. Thirteen bacterial species were observed to sorb and concentrate (bioconcentrate) α-chlordane, β-chlordane, dieldrin, heptachlor epoxide, and lindane from aqueous systems. Bioconcentration, as expressed by the ratio of cellular insecticide in ng/mg (dry wt) to supernatant insecticide in ng/μl, ranged from 10 for lindane by *Enterobacter aerogenes* to a high of 55,900 for β-chlordane by *Caulobacter vibrioides* var. *limonus*. Amounts of cellular chlorinated hydrocarbon insecticides (CHI) detected and the bioconcentration ratios were observed to have the following order in magnitude: α- or β-chlordane > dieldrin > heptachlor epoxide > lindane. This decreasing order was the inverse of reported water solubilities for the CHI and the inverse relationship was mathematically defined. The CHI were not easily removed from cells by washing (desorbing) and desorption was directly proportional to insecticide water solubility. Uptake of the CHI was rapid, near-maximum amounts being sorbed within 15 min, and pH 7 appeared optimal for bioconcentration as examined over the range pH 6 to 8. Implications of this investigation are that bioconcentration of CHI by bacteria might serve as a means of introducing these toxic compounds into aquatic food chains and that the bioconcentration phenomenon might lend itself as a treatment procedure for the intentional removal of residual CHI from water supplies and wastewater.

While future use of chlorinated hydrocarbons as insecticidal agents is uncertain, current usage and seeming ubiquity of their residues in the biosphere demands more definitive evidence of their environmental effect and fate. It is now recognized that chlorinated hydrocarbon insecticides (CHI) tend to be concentrated in both aquatic and terrestrial food chains, the concentrations often

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[1] From a dissertation submitted to the Graduate Faculty of Colorado State University by D. J. G. in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Presented in part at the 71st Annual Meeting of The American Society for Microbiology in Minneapolis on May 4, 1971.

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MICROBIAL ECOLOGY, Vol. 2, 43–59 (1975)

becoming higher with each trophic level. Most investigations have studied this phenomenon at the secondary consumer level, since at this level CHI reach their highest concentration and have greatest deleterious effect. Relatively little is known about the acquisition, movement, and fate of CHI at the decomposer level of the ecosystem. Presumably, CHI can enter the food chain of a community by various routes, including *via* the decomposers, and recent evidence supports this assumption [3, 4, 13, 14, 17]. Therefore, we undertook a study to determine the prevalence, parameters, possible mechanism, and implications of chlorinated hydrocarbon insecticide uptake among chemoorganotrophic bacteria. This paper discusses the tendency of 13 bacterial species to sorb and concentrate five different CHI.

Materials and Methods

Organisms: Source, Cultivation, and Quantitation. Thirteen bacterial species representing a wide range of morphological and physiological types were selected for the study. *Chromobacterium violaceum, Escherichia coli, Pseudomonas aeruginosa,* and *Pseudomonas fluorescens* were obtained from K. L. Martin, Department of Microbiology, Colorado State University, and were isolated by him from the Cache la Poudre River. A gram-negative curved rod was isolated from Horsetooth Reservoir by D. J. Reasoner (Ph. D. Dissertation, Colorado State University, Fort Collins, 1971) and was identified by us as *Caulobacter vibrioides* var. *limonus* Poindexter [24]. *Achromobacter delicatulus* and *Micrococcus roseus* were isolated by W. O. Deason (Ph. D. Dissertation, Colorado State University, Fort Collins, 1970) from the Animas River. *Pseudomonas putida* biotype B (ATCC 25595) was isolated from soil by chlordane enrichment (D. J. Grimes, M. A. Thesis, Drake University, Des Moines, 1968), and was characterized according to Stanier *et al.* [27]. N. A. Sinclair and J. L. Stokes provided cultures of *Zoogloea ramigera* (ATCC 19623) and *Sphaerotilus natans* S15, respectively. *Bacillus megaterium, Bacillus subtilis,* and *Enterobacter aerogenes* were obtained from stock cultures being carried in our laboratory.

The medium in which cells were grown for exposure to CHI was $\frac{1}{2}$ strength m-Plate Count Broth ($\frac{1}{2}$ m-PCB, Difco). This medium, unlike all others screened, supported rapid and luxuriant growth of the 13 organisms in both stationary and agitated culture.

Cells for all experiments were obtained by inoculating 250 ml of $\frac{1}{2}$ m-PCB in a 500-ml Erlenmeyer flask with a loopful (ca. 5×10^8 colony forming units) of syneresis growth from Plate Count Agar (Difco) stock culture slants. Flasks were incubated at 28°C on a NBS model G25 gyrotory incubator-shaker (New Brunswick, N. J.) at 175 rpm until stationary growth phase was reached. The time required to reach stationary phase had been previously determined by following shake cultures of each organism turbidometrically (610 nm) with a Spectronic 20 colorimeter (Bausch and Lomb Inc., Rochester, N. Y.).

After incubation, eight 25-ml samples of the $\frac{1}{2}$ m-PCB culture were centrifuged for 10 min at 15,000 rpm in a RC-2 centrifuge with a SS-34 rotor (Ivan Sorvall, Inc., Norwalk, Conn.). Since cells were exposed to CHI in 0.067 *M* phosphate buffer, pH 7 [31], each pellet was washed once in this buffer and re-centrifuged. The 8 washed cell pellets were combined by resuspending in one 25-ml portion of buffer and used as concentrated inoculum for all studies. Dry weight measurements were performed in triplicate on each concentrated inoculum; values ranged from 11.5 to 17.3 mg/ml (mean = 15.1 mg/ml) for all experiments.

CHI, Solvents,and Glassware. Reference grade standard purity CHI were used for the study, and Table 1 lists the chemical name, purity, and source of each compound. Acetone used to rinse glassware was analytical reagent grade (Mallinckrodt Chemical Works, St. Louis, Mo.); all other solvents were of nanograde purity (Mallinckrodt).

Glassware was used to contain all insecticide solutions, and non-glass accessories (e.g., separatory funnel stopcocks and screw caps) were of Teflon or were Teflon lined. Rigid washing and drying procedures were used to ensure chemical cleanliness of the glassware and accessories [16].

Organisms Exposure to CHI. Organisms were exposed to insecticide in sterile 0.067 *M* phosphate buffer in 250-ml Erlenmeyer flasks covered with aluminum foil or stoppered with cotton plugs. With the exception of the pH studies, pH 7.0 buffer was used throughout. The hydrophobic CHI were added to the buffer as 100.0-μg/ml acetone solutions so that the final concentration of insecticide in buffer was 0.10 μg/ml and acetone in buffer 0.1% (v/v). Immediately following insecticide addition, concentrated inoculum was added to the insecticide-buffer (exposure medium) so as to dilute the cells 1:10. The concentration of cells in the exposure medium was therefore 1.15 to 1.73 mg/ml for all experiments. Cell-free controls were prepared by substituting sterile phosphate buffer for the concentrated inoculum. All flasks were incubated on the shaker (175 rpm) for 4 hr at 28°C, and 10-ml samples from each experimental and control flask were then centrifuged for 10 min (15,000 rpm) to separate treated cells from exposure medium.

Each organism was exposed to one insecticide at a time, except that both isomers of chlordane were tested together so that the exposure medium contained 0.10 μg/ml each of α- and β-chlordane. The chlordane isomers were treated in combination because they have similar physicochemical and toxicological properties, are present in approximately equal amounts in commercial technical grade chlordane formulations, and yet are separable by gas-liquid chromotography.

Initially, all 13 organisms were examined in screening experiments for bioconcentration of the five CHI at the end of 4 hr; further studies (i.e., kinetics of uptake, effect of pH, desorption) were limited to *C. vibrioides* var. *limonus, E. aerogenes,* and *Z. ramigera* and to the two insecticide systems, α- and β-chlordane and lindane.

The kinetics of bioconcentration (uptake as a function of time) was examined by sampling at times 0, 0.25, 0.5, 1, 2, and 4 hr with samples (10 ml) centrifuged as in the screening procedure. Because of centrifugation time (15 min), the cells were not actually separated from the exposure medium until 0.25, 0.5, 0.75, 1.25, 2.25, and 4.25 hr.

The persistence of α- and β-chlordane and lindane in treated cells was evaluated by placing cells into three washing or desorption media. Cells treated for 4 hr were harvested by centrifugation, and the cell pellets were resuspended in equal volume of desorption medium and washed for 2 hr at 175 rpm on the gyrotory shaker. The three insecticide-free desorption media were deionized water (autoclaved), 0.5% (v/v) acetone in deionized water (sterilized through fine sintered-glass), and pH 7.0, 0.067 *M* phosphate buffer (autoclaved). After 2 hr, 10-ml samples of each desorption system were centrifuged in the usual manner. To assess cell damage due to the extended time they were in non-nutritive media, membrane filter total plate counts were made of the cells in the exposure media after 4 hr and in the desorption media after 2 hr. The acetone loss due to sterilization and agitation was determined by a modified methyl ketone test [6].

Uptake as a function of pH was examined by phosphate buffering the exposure medium at pH 6.0, 6.5, 7.5, and 8.0. Data for pH 7.0 exposure were obtained from averages

Table 1.
Chemical Names, Purity, and Source of Experimental CHI

Compound	Chemical name[a]	% Purity[b]	Source
α-Chlordane[c]	*exo*-1, *endo*-2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan	99.8	Velsicol Chemical Corp., Chicago, Ill.
β-Chlordane[d]	*exo*-1, *exo*-2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan	99.7	Velsicol Chemical Corp., Chicago, Ill.
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo-exo*-1,4:5,8-dimethanonaphthalene	99+	Applied Science Laboratories, Inc., State College, Pa.
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan	98.4	Velsicol Chemical Corp., Chicago, Ill.
Lindane[e]	1,2,3,4,5,6-hexachlorocyclohexane	99	Applied Science Laboratories, Inc., State College, Pa.

[a] Names conform with Chemical Abstracts nomenclature.
[b] % Purities supplied by source of compounds.
[c] Also referred to as *trans-*, *endo-trans-*, or γ-chlordane.
[d] Also referred to as *cis-*, *endo-cis-*, or α-chlordane.
[e] γ Isomer of hexachlorocyclohexane, often given the misnomer γ-benzenehexachloride (BHC).

of previous experiments. The concentrated inoculum was prepared with buffer corresponding to the pH of each exposure medium. Samples (10 ml) at each pH were taken after 4 hr exposure to α- and β-chlordane or lindane and centrifuged as already described.

Extraction and Sample Preparation. Following centrifugation of the samples, supernatants (or controls) were decanted into 125-ml separatory funnels and extracted twice with 5-ml portions of 15% (v/v) ethyl ether in hexane (*n*-hexane) followed by a third extraction with 5 ml hexane. The three 5-ml extracts were pooled in 25 × 150 mm tubes with Teflon-lined screw caps and held until the extracts could be evaporated.

The method of Kates *et al.* [11] was used to extract CHI from cell pellets. Each pellet was resuspended in 2.0 ml deionized water and poured into a 25 × 150 mm tube. Methanol (5.0 ml) and then chloroform (2.5 ml) were added giving a 10:5:4 solution. Following 24 to 96 hr at room temperature, cell debris was removed by centrifugation (15 min at 15,000 rpm) and supernatants were decanted into 125-ml separatory funnels. Recovery of the CHI from supernatants was effected by readjusting the CH_3OH:$CHCl_3$:H_2O ratio to 10:10:9. This volume readjustment resulted in a separation of the system into two phases; the bottom chloroform layer containing the CHI was drawn into standard cream test (Babcock) bottles for immediate evaporation.

All extracts (cell, supernatant, and control) were evaporated to apparent dryness and reconstituted in 1.0 ml hexane for analysis. Evaporation was carried out under partial negative pressure in a water bath (65°C), and the reconstituted 1-ml samples were transferred to screw-capped 16 × 75 mm tubes and stored in a refrigerator (3°C).

Analysis. Samples were analyzed with a Varian Aerograph Series 1740-1 gas chromatograph equipped with an electron capture (tritium foil) detector (Varian Aerograph, Walnut Creek, Calif.). A 6 ft-$\frac{1}{4}$ in. OD × 2 mm ID Pyrex column (Varian Aerograph, Walnut Creek, Calif.) packed with 60/80 mesh Gas Chrom Q precoated with 5% SE-30 (Applied Science Laboratories, Inc., State College, Pa.) was used. Quantitation was accomplished by the use of external standards, and the prescribed injection technique [20] was observed. All solvents, aqueous and organic, were analyzed for contaminating and/or interfering peaks. Possible losses of CHI during extraction and evaporation were evaluated as were similar losses due to centrifugation. Losses of CHI during cell exposure could not be monitored.

Results

Preliminary Screening. Results of the preliminary screening experiments with 13 species of bacteria exposed to the five CHI are presented in Table 2. Data are presented as (A) ng insecticide per μl of supernatant, (B) ng insecticide per mg of cells (dry wt), and (C) ng insecticide detected in the cell mass separated from 1.0 μl of supernatant. Magnitudes of bioconcentration are expressed as the ratios B/A and C/A, and both ratios are ppm/ppm.

The B/A distribution ratio (Table 2) compares cellular (ng/mg) to supernatant insecticide concentration (ng/μl), and all organisms bioconcentrated the five CHI on the basis of this ratio (all B/A ratios were > 1). In all cases, the two chlordane isomers were bioconcentrated to the greatest extent. Dieldrin was generally next in a decreasing order of bioconcentration followed by heptachlor epoxide and lindane. *Caulobacter vibrioides* showed the greatest bioconcentra-

Table 2.
Insecticide Distribution (in ng) After 4 hr Between (A) 1.0 µl Supernatant and (B) 1.0 mg Cells (dry wt) or (C) the Cells Contained in 1.0 µl Exposure Medium

Organism	Insecticide	Insecticide concentration			Distribution ratio	
		Supernatant (ng/µl) (A)	Cells (ng/mg) (B)	Cells (ng/µl) (C)	B/A	C/A
Achromobacter delicatulus	α-Chlordane	0.033	IP[a]	–	–	–
	β-Chlordane	0.045	42.038	0.066	900	1.47
	Dieldrin	0.057	9.554	0.015	200	0.26
	Hep. epoxide	0.057	IP	–	–	–
	Lindane	0.063	10.191	0.016	200	0.25
Bacillus megaterium	α-Chlordane	0.008	38.235	0.052	4,800	6.50
	β-Chlordane	0.009	36.765	0.050	4,100	5.56
	Dieldrin	0.013	38.971	0.053	3,000	4.08
	Hep. epoxide	0.020	IP	–	–	–
	Lindane	0.062	4.412	0.006	70	0.10
Bacillus subtilis	α-Chlordane	0.013	29.268	0.036	2,300	2.77
	β-Chlordane	0.015	29.268	0.036	2,000	2.40
	Dieldrin	0.021	25.203	0.031	1,200	1.48
	Hep. epoxide	0.025	21.138	0.026	800	1.04
	Lindane	0.068	7.317	0.009	100	0.13
Caulobacter vibrioides	α-Chlordane	⩽0.001	50.350	0.072	⩾50,400	⩾72.00
	β-Chlordane	0.001	55.944	0.080	55,900	80.00
	Dieldrin	0.002	55.944	0.080	28,000	40.00
	Hep. epoxide	0.003	45.455	0.065	15,200	21.67
	Lindane	0.032	8.392	0.012	300	0.38
Chromobacterium violaceum	α-Chlordane	0.018	10.494	0.017	600	0.94
	β-Chlordane	0.020	10.494	0.017	500	0.85
	Dieldrin	0.037	14.815	0.024	400	0.65
	Hep. epoxide	0.046	11.728	0.019	300	0.41
	Lindane	0.064	4.321	0.007	70	0.11
Enterobacter aerogenes	α-Chlordane	0.031	7.143	0.012	200	0.39
	β-Chlordane	0.036	7.143	0.012	200	0.33
	Dieldrin	0.039	3.571	0.006	90	0.15
	Hep. epoxide	0.056	3.571	0.006	60	0.11
	Lindane	0.058	⩽0.639	⩽0.001	⩽10	⩽0.02
Escherichia coli	α-Chlordane	0.023	19.632	0.032	900	1.39
	β-Chlordane	0.025	18.405	0.030	700	1.20
	Dieldrin	0.056	11.656	0.019	200	0.34
	Hep. epoxide	0.056	3.681	0.006	70	0.11
	Lindane	0.090	1.840	0.003	20	0.03

[a]IP, interfering peak.

Continued

Table 2.
Continued

Organism	Insecticide	Insecticide concentration			Distribution ratio	
		Supernatant (ng/μl) (A)	Cells (ng/mg) (B)	Cells (ng/μl) (C)	B/A	C/A
Micrococcus roseus	α-Chlordane	0.002	29.661	0.035	14,800	17.50
	β-Chlordane	0.002	31.356	0.037	15,700	18.50
	Dieldrin	0.012	55.932	0.066	4,700	5.50
	Hep. epoxide	0.013	NA[b]	–	–	–
	Lindane	0.055	⩽0.847	⩽0.001	⩽20	⩽0.02
Pseudomonas aeruginosa	α-Chlordane	0.015	26.415	0.042	1,800	2.80
	β-Chlordane	0.014	23.899	0.038	1,700	2.71
	Dieldrin	0.020	11.321	0.018	700	0.90
	Hep. epoxide	0.025	4.403	0.007	200	0.28
	Lindane	0.035	⩽0.629	⩽0.001	⩽20	⩽0.03
Pseudomonas fluorescens	α-Chlordane	0.005	31.818	0.042	6,400	8.40
	β-Chlordane	0.005	34.848	0.046	7,000	9.20
	Dieldrin	0.015	16.667	0.022	1,100	1.47
	Hep. epoxide	0.016	7.576	0.010	500	0.63
	Lindane	0.031	⩽0.758	⩽0.001	⩽20	⩽0.03
Pseudomonas putida	α-Chlordane	0.014	21.831	0.031	1,600	2.21
	β-Chlordane	0.015	18.310	0.026	1,200	1.73
	Dieldrin	0.042	10.563	0.015	300	0.36
	Hep. epoxide	0.051	5.634	0.008	100	0.16
	Lindane	0.047	1.408	0.002	30	0.04
Sphaerotilus natans	α-Chlordane	0.001	IP	–	–	–
	β-Chlordane	⩽0.001	IP	–	–	–
	Dieldrin	0.002	32.000	0.048	16,000	24.00
	Hep. epoxide	0.010	9.333	0.014	900	1.40
	Lindane	0.030	4.000	0.006	100	0.20
Zoogloea ramigera	α-Chlordane	0.002	49.689	0.080	24,800	40.00
	β-Chlordane	0.002	45.963	0.074	23,000	37.00
	Dieldrin	0.012	18.012	0.029	1,500	2.42
	Hep. epoxide	0.015	28.571	0.046	1,900	3.07
	Lindane	0.032	10.559	0.017	300	0.53

[b]NA, not available due to leakage of $CHCl_3$ from separatory funnel.

tion (B/A = 55,900 for β-chlordane) and also exhibited the largest cellular insecticide concentrations (B) of the five compounds. *Enterobacter aerogenes* presented the other extreme; B/A distribution ratios and insecticide concentrations were the lowest. The other 11 organisms fell between these two extremes with *Z. ramigera* being second in a decreasing order of bioconcentration.

Chlorinated hydrocarbon insecticide bioconcentration from 1.0 μl of exposure medium by the cell mass contained in the 1-μl volume is expressed by the ratio C/A (Table 2). In terms of C/A ratios (Table 2): (i) *E. aerogenes* and *C. violaceum* failed to bioconcentrate the CHI (their C/A values are < 1), (ii) none of the bacteria bioconcentrated lindane, and (iii) *C. vibrioides* exhibited the largest C/A ratio, bioconcentrating β-chlordane 80 times its environment (supernatant).

Closer examination of Table 2 exhibits a trend; the degree of bioconcentration appears inversely related to water solubility of the five CHI. This apparent

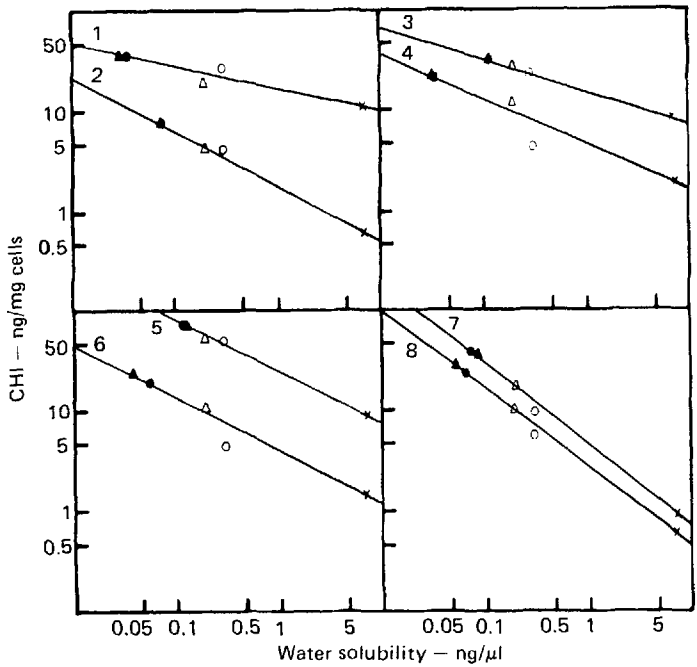

Fig. 1. Effect of water solubility (in ng/μl) on the ng of dieldrin △, heptachlor epoxide ○, and lindane X detected per mg dry-cell weight of (1) *Z. ramigera*, (2) *E. aerogenes*, (3) *B. subtilis*, (4) *E. coli*, (5) *C. vibrioides*, (6) *P. putida*, (7) *P. fluorescens*, and (8) *P. aeruginosa* after 4 hr exposure. Cellular concentrations of α-chlordane ▲ and β-chlordane ● (in ng/mg) for each organism are plotted on the slopes without regard to solubility, since the water solubilities of these isomers are unknown.

relationship was investigated by a log-log plot (Fig. 1) of water solubilities in ng/μl against cellular insecticide concentrations in ng/mg (Table 2) for 8 of the 13 organisms. For each, the concentrations of dieldrin, heptachlor epoxide, and lindane in ng/mg of cells were distributed according to their respective water solubilities in ng/μl. These solubilities are 0.186 ng/μl at 30°C for dieldrin [22], 0.350 ng/μl at 30°C for heptachlor epoxide [22], and 7.30 ng/μl at 25°C for lindane [9]. The plot points gave a line of best fit. Although the exact solubilities of α- and β-chlordane are not known (the estimated range is 0.01 to 0.10 ng/μl for both isomers; H. K. Suzuki, Velsicol Chem. Corp., personal communication), cellular insecticide concentrations for these isomers plotted on the line fell within a 0.03 to 0.14 ng/μl solubility range for all organisms (Fig. 1). The resultant slopes (Fig. 1) indicate an inverse relationship of uptake as a function of water solubility exists for, at least, dieldrin, heptachlor epoxide, and lindane. The relationship may be described with the equation $y = bx^k$, where y is the cellular insecticide concentration in ng/mg, x is the insecticide water solubility in ng/μl, b is a constant equal to y when $x = 1$ (i.e., the intercept where $\log x = 0$), and k is a constant and the slope of the straight line obtained with a log-log plot.

Kinetics of Bioconcentration. Bioconcentration of α- and β-chlordane with time is illustrated in Fig. 2. Lindane uptake is not shown, as the plots would be almost undiscernable from the x-axis over the 4-hr period (lindane values ranged from undetectable to 4 ng/mg). Bioconcentration of the chlordane isomers was rapid, reaching high levels within 15 min, and, with the exception of *C. vibrioides*, did not significantly increase with time. The two chlordane isomers were essentially taken up to the same extent and at the same rate by each organism.

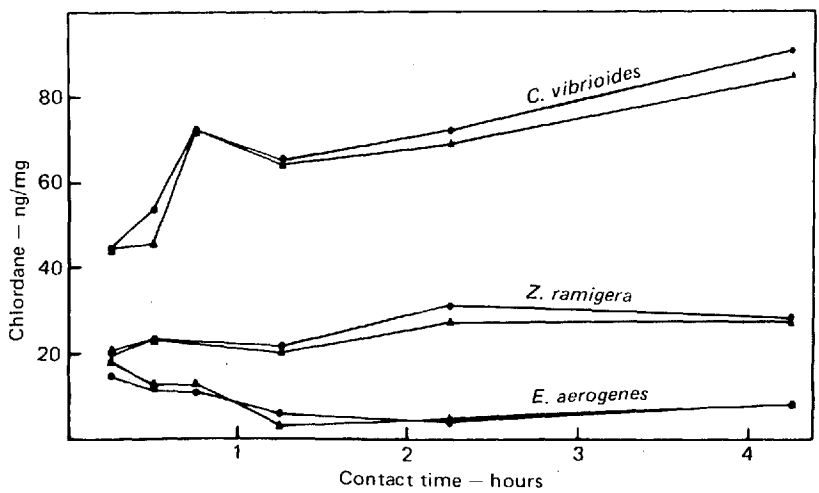

Fig. 2. Concentration of α-chlordane ▲ and β-chlordane ● in ng/mg cells (dry wt) distributed according to contact time.

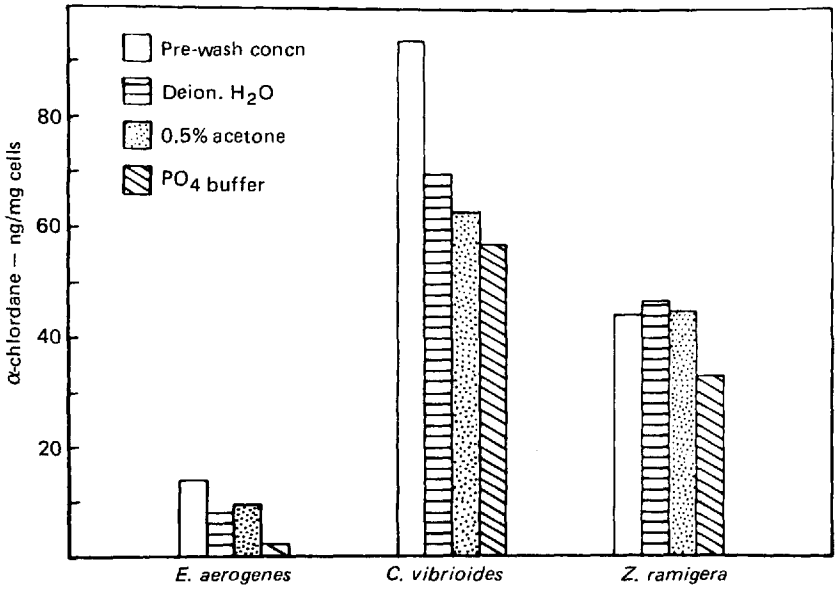

Fig. 3. Persistence of α-chlordane on treated cells washed for 2 hr with deionized water, 0.5% aqueous acetone, and pH 7, 0.067 *M* phosphate (PO_4) buffer.

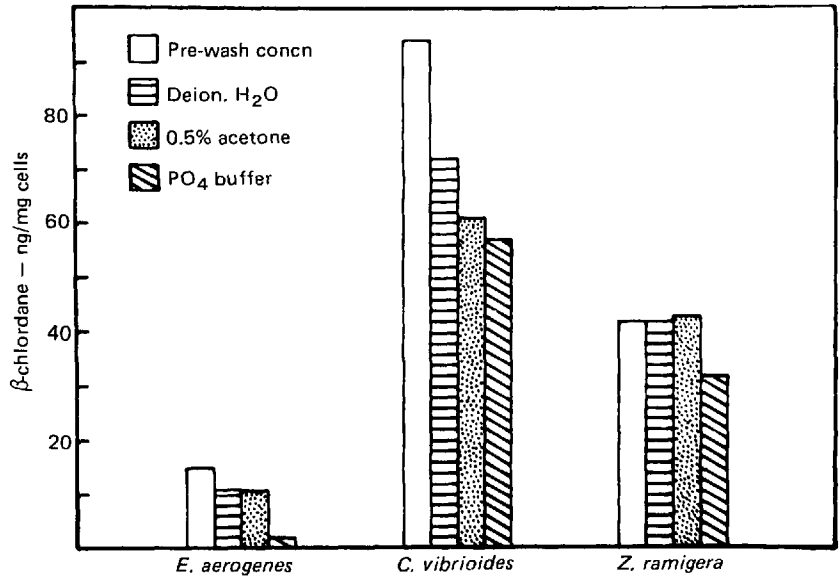

Fig. 4. Persistence of β-chlordane on treated cells washed for 2 hr with deionized water, 0.5% aqueous acetone, and pH 7, 0.067 *M* phosphate (PO_4) buffer.

Again, the magnitude of bioconcentration was *C. vibrioides* > *Z. ramigera* > *E. aerogenes.*

Desorption of Bioconcentrated CHI. These studies indicate that once sorbed, CHI are not easily desorbed from bacterial cells, and the persistence of α- and β-chlordane on the three test species is shown in Figs. 3 and 4, respectively. Cellular levels of lindane were only slightly above or at the limit of detection (0.004 ng) prior to washing and undetectable after. Phosphate buffer at pH 7.0 appeared to be a better α- and β-chlordane desorbent than water or aqueous acetone (Figs. 3 and 4).

Membrane filter total plate counts indicated there was no change in viable colony forming units during the 2-hr desorption period. Analysis of the 0.5% (v/v) aqueous acetone desorption medium showed acetone content to be 0.43% following sintered-glass filtration and 0.38% following 2 hr of agitation.

Bioconcentration as a Function of pH. Figure 5 shows that pH 7 is generally the optimum for bioconcentration of the three CHI.

Controls. GLC-EC analysis of all solvents, aqueous and organic, showed them to be completely free from detectable contaminants. The five CHI used (Table 1) approached 100% purity as evidenced by a lack of extraneous peaks on chromatograms. None of the 13 organisms contained constitutive chloroform-soluble material chromatographically similar to the five CHI.

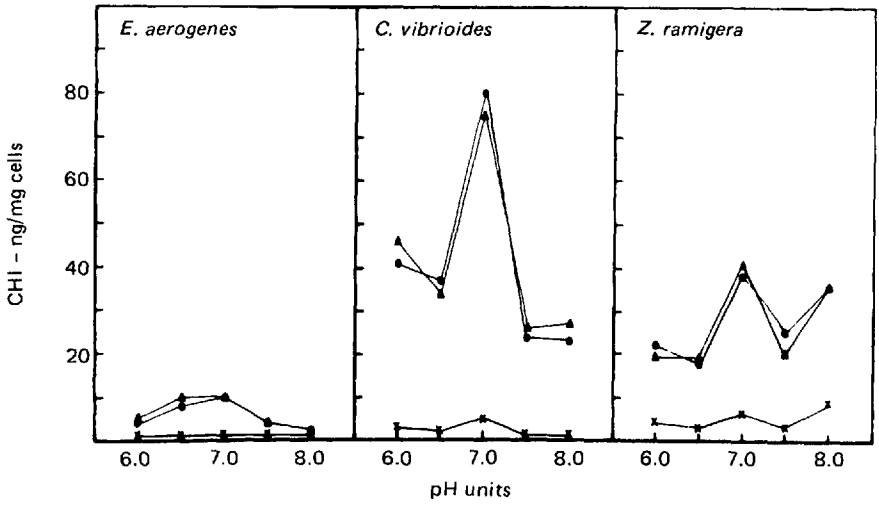

Fig. 5. Concentration of α-chlordane ▲, β-chlordane ●, and lindane X in ng/mg cells (dry wt) distributed according to pH of the exposure medium.

The efficiency of extraction and evaporation (% recovery, 3 replications) was 89.3 ± 4.6% for α-chlordane, 84.0 ± 6.9% for β-chlordane, and 101.3 ± 8.1% for lindane. Adsorptive loss of CHI to glass centrifuge tubes at 27,000 *g* was calculated to be 7.5 ± 1.4%, a loss of 0.002 ng/μl.

The mean percent insecticide recovered from all pH 7.0 control flasks (n = 83) after 4 hr agitation was 46.6 ± 18.3% of the initial 0.10 μg/ml; recovery for the experimental flasks (the sum of supernatant and cell insecticide concentations) was 58.0 ± 22.9% (n = 75).

Discussion

The low recoveries of the initial insecticide concentrations in control and experimental flasks (46.6 ± 18.3 and 58.0 ± 22.9%, respectively) were presumed largely due to the physicochemical properties of CHI. The opportunity for loss due to accumulation of CHI at, and subsequent volatilization or co-distillation with water from, air-water and glass-water interfaces was great, and this has caused similar losses in other studies [1, 3, 5, 7, 17]. In addition, other glassware and laboratory procedures probably contributed to the incomplete recoveries; biodegradation did not account for any of the observed losses.

Whether or not loss affected the total amount of insecticide bioconcentration is unknown; had the initial concentration (0.10 ng/μl) not diminished, cells may have sorbed larger amounts. This contention is supported by adsorption isotherms, particularly those described by the Langmuir and Freundlich equations which show that the amount of solute adsorbed to a particular adsorbent is directly related to the equilibrium (postadsorption) concentration of that solute in solution [12, 18, 29, 31].

Since α- and β-chlordane were used together, the amounts taken up per unit mass of cells might be either a total uptake or an uptake of each compound. While one isomer could have affected uptake of the other, bioconcentrations were considered independent of one another based on: (i) physicochemical similarities of the two isomers, (ii) experimental data indicating stability of the isomers when mixed separately or together in aqueous and nonaqueous solvents, and (iii) the findings of Bevenue and Yeo [1].

Of the different ways to express the magnitude of insecticide bioconcentration, distribution ratios were chosen for this study (Table 2). Cox [5] used distribution ratios (relative partition coefficients) to evaluate DDT uptake by three species of marine phytoplankton and obtained values comparable to many of our B/A ratios (Table 2). Similarly, when we calculated distribution ratios from the data presented by Gregory *et al.* [7], they were found to be in close agreement with B/A values (Table 2).

Cellular insecticide concentrations (ng/mg) and distribution ratios (B/A and C/A) of the five CHI in 8 of the 13 organisms (Table 2) were found to be inversely related to insecticide water solubility (Fig. 1). This same relationship has been observed by, or is apparent in the data of, others [10, 12, 29].

Distribution ratios (B/A) given in Table 2 for *C. vibrioides* were similar in magnitude to hexane : water distribution coefficients (K) for the experimental CHI. Dieldrin, heptachlor epoxide, and lindane have hexane : water K values of 36,000, 40,000, and 1,730, respectively [28]; α- and β-chlordane are estimated to have values of 60,000 - 100,000 (H. K. Suzuki, personal communication). B/A ratios calculated for the bioconcentration of α- and β-chlordane by *C. vibrioides* at the end of the 4-hr kinetics experiment (Fig. 2) were 84,800 and 90,600, respectively. Based on these similarities, and on the data showing inverse relationship between cellular uptake and water solubility of CHI, it would appear that the bacterial cell mass is acting as an organic phase to which the hydrophobic CHI molecules partition.

Pseudomonas putida biotype B (ATCC 25595) was previously shown to be capable of utilizing technical grade chlordane as its sole source of carbon and energy (D. J. Grimes, M. A. Thesis). During utilization of this material in liquid agitated culture, both floc formation and clarification of the cloudy medium occurred as incubation progressed. Results of this study (Table 2) would seem to indicate that sorption of chlordane is associated with utilization by *P. putida.* In addition, sorption is probably a prelude to utilization based on the observed rapid uptake of α- and β-chlordane (Fig. 2).

Bioconcentration kinetics indicated rapid uptake by the three bacterial species (Fig. 2) and may be the case for bacteria in general [3, 17, 18, 29, 31]. In addition to similar kinetics, Leshniowsky *et al.* [17] found that *Bacillus* sp. floc accumulated approximately 12 to 24 ng/mg (dry wt) of aldrin within 90 min, an amount similar to that observed for *B. megaterium* and *B. subtilis* in this study (Table 2).

Desorption studies (Figs. 3 and 4) revealed that cellular chlordane was not easily removed by washing with the three different aqueous solvents. Phosphate buffer was the best desorbent, removing up to 36.522 ng/mg α-chlordane from *C. vibrioides* (39% of the prewash level). Supernatant CHI in desorption media never approached solubility, the highest was 0.004 μg/ml α-chlordane in buffer supernatant from *C. vibrioides.* Supernatant concentrations did not seem to account for cell loss, indicating that some of the desorbed CHI were lost. Whatever the fate of desorbed CHI, desorption seemed to be directly related to insecticide water solubility; lindane, being more soluble in water than the chlordane isomers, was desorbed to a greater extent.

In mammalian studies, CHI are thought to localize in the bimolecular lipid layer of nerve membranes [19, 21]. Our experimental data, work of others [5, 7], and physicochemical principles permit extension of this mechanism to bacteria. Large B/A ratios (Table 2), rapid bioconcentration in agitated cell culture (Fig. 2), and resistance to desportion by aqueous systems (Figs. 3 and 4) all indicate that free energy minimization of CHI resulted from bioconcentration. Bacterial lipid would provide a hydrophobic material most capable of satisfying this minimization. Furthermore, sites of electronegativity exist on bacterial surfaces and could participate in weak London-van der Waals bonding with CHI. Such an attraction would serve as an initial sorption mechanism and could be followed by partitioning of the insecticide molecules into cellular lipid. Wedemeyer [31] has advanced a similar two-step mechanism for the uptake of 2,4-D by *Pseudomonas fluorescens.*

Insecticide bioconcentration values obtained for *C. vibrioides* were always greater than those of the other bacterial species (Table 2 and Figs. 2-5), and this too suggests an involvement of cellular lipid. *Caulobacter vibrioides* not only contains normal cytoplasmic membrane and gram-negative cell envelope lipids, but also relatively large mesosomes [24, 26] which are often continuous with the membranous material in the core of its stalk [24]. The walls of *Caulobacter* stalks are continuous with the cell envelope [24], and the particular organism used in this study (*C. vibrioides* var. *limonus*) formed a light yellow, nondiffusable pigment which was chloroform and hexane soluble and had a maximum adsorption spectrum of 400 to 450 nm. Thus, the possibility exists that pronounced uptakes for *C. vibrioides* were due to these four distinctive lipid or lipid-containing materials (mesosomes, stalk core, stalk envelope, and carotenoid-like pigment). This hypothesis is supported by Ware and Roan [30] who recently suggested that both surface area and lipid material within that surface play an important role in microbial sorption of pesticides from water and by Pate [23] and others who contend that prostheca enhance nutrient uptake.

The effect of pH on bioconcentration (Fig. 5) has not yet been explained. It may involve the initial sorption mechanism just hypothesized, since the net negative surface charge (charge density) of bacteria is affected by hydrogen ion concentration of the menstruum [15]. Another explanation, related to the observation that cellular insecticide concentrations at pH 7 were approximately twice those at pH 6 to 6.5 and 7.5 to 8 (Fig. 5), is monolayer to bilayer conversion. This explanation was ruled out however, since, by calculation, none of the organisms contained sufficient insecticide to create a monolayer.

Extrapolating the results of this study to the natural environment leads to the conclusion that *in situ* bacteria can rapidly bioconcentrate CHI from aquatic ecosystems. Bioconcentration occurs at hydrogen ion concentrations found in most freshwater lakes and streams, and bound CHI are not easily desorbed from their cellular location which was presumed to be lipid material.

Thus, CHI bound to bacterial cells would not be expected to redistribute into the aquatic environment until their bacterial sorbents died and lysed. Even then, the CHI could remain bound to the various biomolecular species responsible for sorption.

It is very probable that bacterial cells serve as a vehicle through which potentially toxic CHI enter aquatic food chains. Obviously, benthic and planktonic bacteria carrying bound chlorinated hydrocarbon insecticide molecules could serve as a direct source of these compounds for consumers, and bacteria are known to be important food sources for many primary consumers [2, 25]. In addition, bacteria could also serve as an indirect source of CHI for food chains. Previously observed uptakes of CHI by aquatic vegetation and fauna are probably due, in part, to sorption by associated *Aufwuchs* bacteria. Consumption of such surface-contaminated foodstuffs would result in a bacterial introduction of CHI into food chains.

While bacterial bioconcentration of CHI must be considered a biological hazard, there are two ways in which the phenomenon could be considered beneficial. Hill and McCarty [10] and Guenzi and Beard [8] have shown that initial degradation (e.g., reductive dechlorination) of CHI is favored under anaerobic conditions, and such conditions often exist in the bottom sediments of aquatic communities. Hence, in an anaerobic environment, benthic bacteria carrying cellular CHI would either directly metabolize (or co-metabolize) the compounds or serve as a fixed source of these materials for other potentially degradative organisms. However, ring cleavage and subsequent total mineralization of CHI would probably occur only after anaerobic metabolites had returned to aerobic areas of the aquatic environment.

The second way in which this phenomenon might prove beneficial would be the use of bacterial cells as a means of removing residual or excess CHI from water. Water or treated wastewater could most effectively be processed with multistage treatment: initial aeration to obtain maximum bacterial cell exposure to and sorption of CHI (and possibly other residual organics and inorganics), followed by an anaerobic system where initial degradation would be favored. A final aerobic treatment would then promote total mineralization. Whether or not such a treatment process would work remains to be seen; it would not seem practicable for cleaning up large bodies of water except to the extent that the process occurs naturally (e.g., sedimentation and turnover).

Acknowledgments

Velsicol Chemical Corp., Chicago, Ill. is acknowledged for suppling insecticide samples. Special thanks are extended to R. L. Zimdahl and D. L. Hyzak for allowing us access to their gas chromatograph. Laboratory assistance from K. L. Martin and G. C. Newton is also gratefully appreciated. This study was supported by the Water Quality Office of the

Environmental Protection Agency through a training grant (5T1-WP-127) awarded to S. M. Morrison and a predoctoral fellowship (1-F1-WP-26,592) awarded the senior author.

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