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Transfer of the Chlorinated Hydrocarbon PCB in a Laboratory Marine Food Chain

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Abstract

The transfer of chlorinated hydrocarbons (CHC) in a laboratory simulation of a three trophic level marine food chain was studied. The food chain consisted of the algal flagellate *Dunaliella* sp., the rotifer *Brachionus plicatilis*, and the larva of the northern anchovy *Engraulis mordax*. CHC were introduced into the seawater at concentrations representative of near-shore conditions off southern California without the use of dispersing agents. Each trophic level appeared to be in a steady-state at the time of first sampling, 5 days after inoculation. Apparent partition coefficients were calculated for each trophic level. The CHC contamination in the diet of the rotifers and anchovy larvae was also calculated. Unfed anchovy larvae accumulated the same amount of CHC as fed larvae and the final concentration appeared to be dependent on the CHC concentration in the seawater. The data in this report suggest that CHC accumulation is not a food-chain phenomenon but rather the result of direct partitioning of the compounds between the seawater and the test organisms.

Introduction

The presence of chlorinated hydrocarbons (CHC) in the marine environment has become a point of concern in recent years. Polychlorinated biphenyls (PCB) and pesticide residues have been detected in seawater (Cox, 1971; Harvey *et al.*, 1973; Bidleman and Olney, 1974; Pavlou *et al.*, 1974; Scura and McClure, 1975), sediments (Hom *et al.*, 1974; MacGregor, 1976), plankton (Cox, 1970; Risebrough *et al.*, 1972), fish (Zitko, 1971; MacGregor, 1974; Sims *et al.*, 1975), fish-eating birds (Moore and Tatton, 1965; Risebrough *et al.*, 1967), and marine animals (Addison *et al.*, 1973; DeLong *et al.*, 1973). CHC are accumulated by marine organisms because of the lipophilic nature of these compounds and an examination of the data show that, in general, members of the higher trophic levels contain relatively greater amounts of CHC. These observations support the conclusion that CHC are concentrated up the food chain (Woodwell *et al.*, 1967). However, information is scare concerning the mechanism of CHC transfer in marine food webs, particularly at the lower trophic levels.

Most investigators agree that the two principal means by which aquatic organisms accumulate CHC are by eating contaminated food or by a partitioning of the compounds directly from the water into the lipid of the organism until an equilibrium is established. The relative importance of these two routes of CHC accumulation has been discussed in several papers (Grzenda *et al.*, 1970; Macek and Korn, 1970; Hamelink *et al.*, 1971; Wildish and Zitko, 1971; Reinert, 1972; Södergren, 1973).

If equilibrium partitioning is an important route of CHC accumulation, then it is important that any laboratory demonstration of the transfer of CHC in an aquatic food web should have the test compound present in the water in a physical state similar to natural conditions and at concentrations known to exist in the environment. Many of the previous experiments which have been designed to demonstrate the uptake of CHC by aquatic organisms were conducted at CHC concentrations greater than the solubility of these compounds in water, and this could affect the apparent partition coefficient between the organism and the water (Butler, 1970). Most studies also used surface-active solvents such as acetone or methanol to keep the CHC in solution. If the accumulation of CHC by aquatic organisms is dependent on a partitioning of

these compounds between polar and non-polar phases, we believed it would be inappropriate to introduce even trace amounts of organic solvents which could affect the partitioning of CHC between phases.

In the research reported here we have studied the transfer of CHC in a laboratory simulation of a three trophic level marine food chain consisting of the algal flagellate *Dunaliella* sp., the rotifer *Brachionus plicatilis*, and the larva of the northern anchovy *Engraulis mordax*.

Materials and Methods

Introduction of CHC into Seawater and Media

CHC were introduced into seawater and culture media according to the method of McClure *et al.* (in preparation). This method relies on the fact that the atmosphere at La Jolla, California, USA, contains an average of 2×10^{-12} g/l CHC (predominately Aroclor® 1254). By bubbling laboratory air through 20 l glass carboys containing membrane-filtered (pore size, 0.45 µm) seawater or culture media, we introduced CHC into our experimental systems at concentrations similar to those found in samples collected near-shore off southern California (2.3 to 14.0 g x 10^{-9}/l Aroclor® 1254 parts per trillion, pptr; Scura and McClure, 1975; McClure *et al.*, in preparation).

The air was passed through a glass inlet tube (3 mm inner diameter) which extended through a cork-stopper seal to the bottom of the carboy where it was fitted with an airstone. Air was filtered through a membrane filter (pore size, 0.45 µm) before it was bubbled through the seawater at a rate of 500 to 1,000 ml/min. The air exited through a glass tube (3 mm inner diamter) which passed through the cork stopper from above the water level.

The concentration of CHC in the air was monitored by passing a measured volume of air through a mineral oil air trap as described by McClure (1976). CHC were scavenged from the air by the mineral oil and subsequently eluted with *n*-hexane (all solvents were of nanograde quality and redistilled in glass). The CHC were separated from the mineral oil on a silica gel column and analyzed by gas liquid chromatography (McClure and LaGrange, 1976).

Analysis of Seawater for CHC

Seawater and media samples were analyzed for CHC according to the method of Scura and McClure (1975). One-liter samples were pumped through a copper column (100 mm x 5 mm inner diameter) containing an adsorbent which scavenged the CHC from the seawater. The adsorbent contained by weight 5% activated carbon powder, 10% MgO, and 85% refined diatomaceous earth. The CHC were eluted from the column with 10 ml of 30% benzene in acetone (v/v). The eluate was dried with Na_2SO_4 and interfering impurities were separated from the CHC on an alumina column (McClure, 1972). Pesticides were separated from PCB on silica-gel before analysis by gas liquid chromatography (GLC) (McClure, 1972).

CHC residues were analyzed on a Hewlett-Packard Model 5700 GLC with a ^{63}Ni electron capture detector. The glass column (2 mm inner diameter x 1.83 m) was packed with a commercially available solid phase (1.5% SP2250, 1.95% SP2401 on 100/120 Supelcon AW-DMCS). The oven temperature was set at 200°C, the injection port at 250°C, and the detector at 300°C. CHC were quantified by comparing retention time and peak area with known standards (McClure *et al.*, in preparation), and CHC identities were verified by comparing the R_f values from liquid-solid chromatography on silica-gel (McClure, 1972). Pesticide standards were provided by the Environmental Protection Agency, Perrine Primate Laboratory, Perrine, Florida, USA, and the PCB standards were supplied by the Monsanto Company, St. Louis, Missouri, USA. The chromatograms of our samples had a characteristic profile of peaks which was dominated by Aroclor® 1254. However, several other peaks were frequently present, including those corresponding to dieldrin, DDT and its metabolites and other unidentified compounds. Since the predominant CHC detected in our samples was Aroclor® 1254, we decided to follow the transfer of this compound in our experimental food chain. Unless otherwise indicated, the term PCB used in the text will refer to Aroclor® 1254.

Analysis of Tissue for CHC

Algal cells, rotifers, anchovy eggs and anchovy larvae were analyzed for CHC according to a modification of the method of Stanley and LeFavoure (1965). Five to 20 mg of dried tissue were digested in a 15 ml centrifuge tube for 2 h at 60°C in 8 drops of a mixture containing equal portions of glacial acetic acid and perchloric acid (60%). After the tissue was completely digested, 0.5 ml each of hexane, acetone and distilled water were added to the sample. The sample was

thoroughly mixed and allowed to settle before the hexane-acetone fraction was removed. The tissue was extracted two more times with 0.5 ml of hexane; extracts were pooled and washed with 0.5 ml of distilled water. The solvent-CHC residue fraction was separated from the distilled water, evaporated just to dryness under vacuum at room temperature (ca. 20°C), cleaned on alumina, and the pesticides separated from PCB on silica-gel before analysis by GLC (McClure, 1972). Recovery of standard Aroclor® 1254 using this procedure ranged from 92 to 98%.

Culture Methods

The green alga *Dunaliella* sp. was isolated from San Diego Bay (by A. Dodson); it was cultured in 20 l glass carboys in a medium described by Thomas (1964). The dinoflagellate *Gymnodinium splendens* was obtained from Dr. W.H. Thomas of the Scripps Institution of Oceanography; it was also grown in 20 l glass carboys in an enriched seawater medium containing soil extract (Lasker *et al.*, 1970), kept in constant light (500 to 700 ft-c) at 23° to 25°C, and continuously aerated. The rotifer *Brachionus plicatilis* was inoculated into the *Dunaliella* sp. cultures when the algal concentration reached 10^5 to 10^6 cells/ml. The combined cultures were maintained under the same conditions as the algae (Theilacker and McMaster, 1971).

Engraulis mordax eggs were collected from a spawning school maintained at the National Marine Fisheries Service, Southwest Fisheries Center (on occasion it was necessary to use hormones to induce spawning, according to the method of Leong, 1971). The larvae, reared in membrane-filtered seawater (pore size, 0.45 µm) in 10 l containers at 17°C (methods of Lasker *et al.*, 1970), started to feed on the third day after hatching; they were fed on the dinoflagellate *Gymnodinium splendens* for 2 days because their mouths were not yet large enough to feed on rotifers (Lasker *et al.*, 1970). The average concentration of PCB in the *G. splendens* was 0.6 ppm (dry weight). After the fifth day, and for the remainder of the experiment, the anchovy larvae were fed rotifers at a concentration of approximately 50/ml.

Sampling Procedures

Algal cells were collected in 1 l aliquots and counted with a Coulter Counter (Model B). The cells were separated from the medium in a KSB-R Servall Szent-Gyorgyi and Blum continuous-flow centrifuge in which all tubing and fittings containing plasticizers were replaced with PTFE Teflon parts. At 9,000 revs/min and a flow rate of 100 ml/min, all particles with a density greater than 1.1 and a diameter greater than 1.2 µm were removed from the seawater medium. The medium was analyzed for CHC as described earlier. The cell pellet was washed in 3% ammonium formate, pipetted into 15 ml centrifuge tubes and recentrifuged at 2700 revs/min in a Servall angle centrifuge. The supernatant fluid was carefully pipetted off; the cells were resuspended in approximately 1 ml of formate solution, pipetted onto a hexane-rinsed microscope slide and dried at 55°C to a constant weight. Losses of CHC using the above procedure ranged between 3 and 30% (recovery was determined by using ^{14}C-DDT as a tracer). No corrections were made in this report for losses.

The rotifers (99 to 281 µm long and 66 to 183 µm wide) were separated from the *Dunaliella* sp. (5 to 8 µm diameter) by slow sieving through a 35 µm Nitex® screen. *Gymnodinium splendens* (53 µm average diameter) was separated from its medium in the same manner through a 20 µm Nitex® screen. The rotifers and cells were washed on the screen with 3% ammonium formate, pipetted onto a hexane-rinsed microscope slide, and dried to a constant weight at 55°C. Dried tissue samples were scraped off the slide with a solvent-cleaned razor blade, weighed on a Cahn Electrobalance®, and transferred into 15 ml centrifuge tubes for CHC analysis.

Lipid Analysis

Percent lipid per unit dry weight was determined for rotifers and anchovy larvae. Dry tissue samples were ground in an agate mortar, placed in a 5 x 100 mm glass pipette with a glass-wool plug, and dried at 55°C to a constant weight. The samples were eluted with 10 ml of a chloroform-methanol solition (2:1 v/v). The samples were again dried to a constant weight at 55°C, and percent lipid was calculated.

Continuous Introduction of CHC into a Three-Step Food Chain

Areas of rapid phytoplankton growth in the ocean must also have some degree of mixing to replenish the nutrients utilized by the algae. This mixing will

also replenish CHC as they are partitioned into the cells, resulting in a fairly constant concentration of CHC in the seawater. To duplicate these conditions in the laboratory, it is important to have a fairly constant input of CHC into the cultures.

We found that when *Dunaliella* sp. cultures were continually bubbled with air at a rate of about 500 ml/min, there was an increase in the total amount of PCB in the culture. The PCB levels in the algal cells remained relatively constant even during periods of rapid algal growth, and the amount of PCB taken up by the algae could always be accounted for by the amount of PCB lost from the medium and/or from the PCB entering the system from the bubbled air. In a typical 2-week experiment there were 3.6×10^{-9} g PCB in the air that was bubbled through a *Dunaliella* sp. culture each day (Fig. 1). The PCB concentration in the medium ranged between 2.3 and 3.1 pptr (g x 10^{-9}/l), and the algae contained from 0.17 to 0.23 ppm (parts per million). As the number of algal cells increased in the culture, PCB were partitioned onto the cells, resulting in an increase in the total amount of PCB in the culture.

Since algal cells were capable of taking up PCB in the manner described above, we used this technique to introduce CHC into a three-step food chain. *Dunaliella* sp. was cultured as described earlier while CHC were continuously introduced. Aliquots were removed every 5 days and the cells and medium were analyzed for CHC (Fig. 2). On Day 10, when the algal concentration had reached 10^5 to 10^6 cells/ml, rotifers were inoculated into the culture system. This two-step food chain (alga-rotifer) was sampled every 5 days; the rotifers were isolated from the algae and the medium. Each was analyzed for CHC. At every sampling, the volume (1 to 2 l), of the alga-rotifer culture removed was replaced with a dense suspension of *Dunaliella* sp. cells to maintain the initial algal concentration, thus insuring a healthy population of rotifers (Theilacker and McMaster, 1971). The concentration of PCB in the replacement algae was always within 0.15 ppm of the sampled algae. Starting on Day 25 of the experiment, rotifers were harvested every 1 to 5 days and fed to the anchovy larvae so that a concentration of approximately 50 rotifers/ml was maintained in the larval rearing containers. On Day 20 of the experiment, the anchovy eggs were sampled for CHC and they hatched later in the same day. The larvae were sampled again when they were 5 days old (Day 25 of the experiment) and every 5 days after that until the experiment was terminated on Day 45 when the larvae were 25 days old. Although we repeated this experiment three times, the results were not pooled because the CHC concentration in the cultures was dependent on the amount of CHC in the atmosphere which varied with time. In this report we present the results of our most complete test.

Results

The Accumulation of CHC in a Three-Step Marine Food Chain

The concentrations of CHC in the three trophic levels of our laboratory marine food chain are presented in Table 1. During the first 5 days, the number of *Dunaliella* sp. cells increased by 10-fold while the medium PCB concentration dropped from 13.1 to 2.6 pptr. Apparently, the amount of PCB introduced into the medium from the air at this time was not sufficient to keep up with the rapid uptake of PCB by the algae, so there was a decrease in the PCB concentration in the medium. However, the PCB concentration in the *Dunaliella* sp. cells remained fairly constant over the experimental period, ranging from 0.12 to 0.38 ppm (dry weight). *Brachionus plicatilis* averaged about twice as much PCB as the algae (0.29 to 0.54 ppm by dry weight). The PCB concentration in the *Engraulis mordax* larvae was also fairly constant (1.1 to 2.7 ppm by dry weight), and averaged about 5 times the level in the rotifers. DDT and DDE levels were barely detectable, but these results were included to demonstrate that other CHC were accumulated in a manner similar to PCB.

Apparently, PCB accumulation had reached a steady-state in each trophic level of this food chain by the time of first sampling on the fifth day after inoculation. If the source of PCB in the higher trophic levels was simply the result of a food-chain accumulation, the concentration of PCB in the animals should have increased with time. The fact that no such increase was observed suggests that the organisms depurated PCB directly into the water.

Dietary Sources of PCB Contamination

Information regarding the dietary sources of PCB contamination is important to an understanding of the processes involved in the transfer of PCB in a food chain. The amount of PCB in the diet of the rotifers was calculated according to the following argument. The work of Thei-

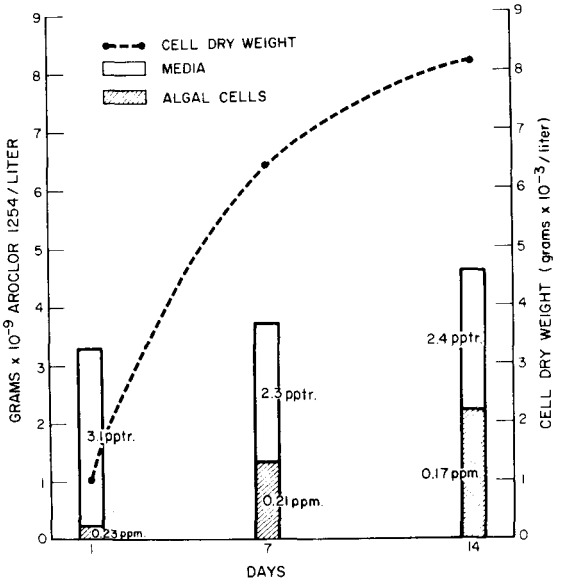

Fig. 1. Total amount of Aroclor® 1254 (PCB) in 1 l of *Dunaliella* sp. culture (includes amount in medium and cells, as indicated by height of bars) during exposure to bubbled air for 14 days. Open bars represent fraction of total PCB found in the medium; actual concentration of the medium is shown in parts per trillion (pptr). Portion of PCB associated with algal cells is depicted by cross-hatched bars and actual concentration is expressed in parts per million (ppm). Dry weight of algal cells in the culture is depicted on right ordinate

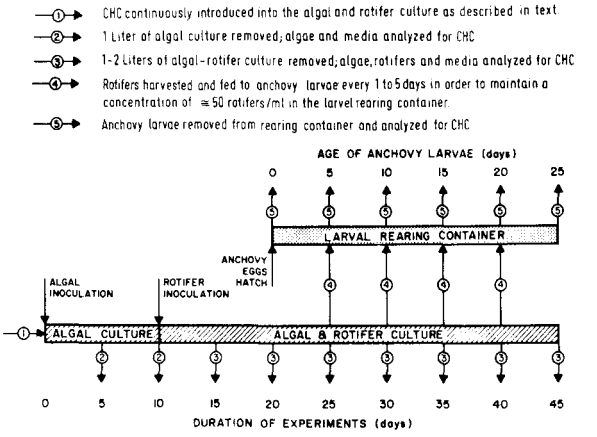

Fig. 2. Flow-diagram of three trophic level food-chain experiment, depicting inputs into system and materials removed from system

Table 1. CHC concentration in three-step marine food chain

Medium (g x 10^{-9}/l, pptr)				*Dunaliella* sp. (g x 10^{-6}/g dry tissue, ppm)			*Brachionus plicatilis* (g x 10^{-6}/g dry tissue, ppm)			Seawater in larval rearing container (g x 10^{-9}/l, pptr)			*Engraulis mordax* larvae (g x 10^{-6}/g dry tissue, ppm)		
Day	Aroclor® 1254	DDT	DDE	Aroclor® 1254	DDT	DDE	Aroclor® 1254	DDT	DDE	Aroclor® 1254	DDT	DDE	Aroclor® 1254	DDT	DDE
0	13.1	1.1	0.2	0.25[a]	0.02	ND									
5	2.6	0.6	ND	0.37	0.01	ND									
10	4.3	0.8	ND	0.25	ND	ND									
15	13.6	0.5	ND	Sample lost			0.54	0.02	ND						
20	6.6	1.0	0.3	0.28	0.01	ND	0.29	0.01	ND	2.0	ND	ND	0.35[b]		
25	8.0	0.3	ND	0.18	ND	ND	0.34	0.02	ND	*			1.93	0.18	0.29[c]
30	9.5	0.4	ND	0.12	ND	ND	0.49	0.02	ND	*			2.59	0.21	0.16
35	10.4	0.6	ND	0.16	ND	ND	0.51	ND	ND	*			1.11	Sample lost	
40	5.9	0.4	ND	0.38	ND	ND	0.42	0.02	ND	*			2.03	0.09	0.04
45							0.36	0.03	ND	*			2.67	0.09	0.01

[a] Inoculum.
[b] Anchovy eggs.
[c] 5-day old larvae were fed *Gymnadinium splendens* on Days 3 and 4.
* CHC determinations made on Day 20 only because of limited volume of seawater.
ND: Not detected.

lacker and McMaster (1971) indicates that the number of rotifers in a culture will double in 3 days at 24°C if the concentration of *Dunaliella* sp. remains at 10^5 cells/ml. The average weight of a single *Brachionus plicatilis* is 1.6×10^{-7} g (Theilacker and McMaster, 1971). Therefore, the average rotifer in a culture must assimilate at least 1.6×10^{-7} g from its food every 3 days in order to double in number. Obviously this is an overly simple explanation of events and the rotifers are not 100% efficient at converting *Dunaliella* sp. to rotifer tissue, but we have chosen this value because it results in the most conservative estimate of PCB uptake by the rotifers. In our experiments, the rotifers ate *Dunaliella* sp. which contained an average of 0.25 ppm PCB, and thereby ingested at least 1.33×10^{-14} g PCB/day. If the average rotifer assimilated all the PCB ingested it would accumulate a minimum of 0.08 ppm PCB each day or 0.42 ppm by Day 5. It seems possible, therefore, that the average rotifer could assimilate enough PCB from its diet to account for the levels detected in its tissue by the time of first sampling 5 days after inoculation. Since the level of PCB in the rotifers did not increase with time at this dietary level of PCB contamination, this indicates that a tendency toward equilibrium existed between the organism and the seawater and that PCB were also being excreted or partitioned out of the rotifer.

Similar calculations were made concerning the transfer of PCB from the rotifer to the anchovy larva. According to Hunter (1972), an *Engraulis mordax* larva between the age of 4 and 27 days makes an average of 1.28 strikes at rotifers per minute. The older the anchovy becomes, the more successful it becomes at capturing its prey. We used this information to calculate the number of rotifers eaten by an anchovy larva on Days 5, 10, 15, 20 and 25. Knowing the average concentration of PCB in the rotifers, the approximate amount of PCB eaten by a larva was determined (Table 2). To estimate the PCB accumulated at a particular age and dry weight, the amount of PCB eaten per day was ascertained by extrapolating between successive 5-day intervals and summing the daily PCB consumption.

According to these calculations, anchovy larvae could accumulate enough PCB from their diet by the age of 10 days to account for the levels detected in their tissue during the experiment, and these levels should have increased with time. However, no increase was observed; a steady-state seems to have been reached by the fifth day which was maintained throughout the remainder of the experiment.

Table 3 gives the average PCB concentration in the medium, algal cells, rotifers and anchovy larvae for the 45-day experiment. We have also calculated the amount of PCB in the lipid of these organisms, since virtually all the PCB in an organism are concentrated in the lipid. An apparent partition coefficient was calculated by dividing the PCB concentration in the lipid by the concentration in the seawater. When compared on a dry weight basis, there appears to be a biological magnification of PCB up the food chain, suggesting that higher members of the food chain accumulate PCB by eating members of lower trophic levels. This trend is not apparent when the PCB concentration is compared on a lipid basis. *Dunaliella* sp. lipid contained slightly more PCB than the rotifer lipid and, consequently, the partition coefficient between *Dunaliella* sp. lipid and seawater was greater than between rotifer lipid and seawater. What appeared to be bioamplification up the food chain when compared on a dry-weight basis was in reality only a reflection of the higher percentage of lipid in the rotifers.

However, the concentration of PCB in anchovy lipid was appreciably higher than in the lipid of the rotifers or the algae (Table 3). The partition coefficient between anchovy lipid and seawater was more than 20 times that of the algae or rotifers. Apparently, the percentage of lipid in an organism is not the only determinant of the amount of PCB accumulated by an organism. The chemical structure of the lipids of an organism might also affect partition coefficients. Grzenda *et al.* (1970) found that when goldfish (*Carassius auratus*) were exposed to 14C-DDT there was no correlation between the total lipid content of the various organ tissues and the residue concentrations. The authors interpreted this to mean that pesticides do not only accumulate in fat deposits. This finding could also be interpreted to mean that DDT has different solubilities in different types of lipids. Although it would be risky to conclude from our results that PCB are 20 times more soluble in anchovy lipid than they are in rotifer lipid, the following series of experiments do indicate that the higher concentration of PCB found in anchovy lipid was the result of equilibrium partitioning and not a food-chain phenomenon.

Table 2. *Engraulis mordax*. Calculated amount of Aroclor® 1254 (PCB) eaten by an anchovy larva

Age (days)	Average individual dry weight (g x 10^{-4})	Percent successful feeding strikes[a]	Feeding rate (organisms/min)[a]	No. of *Brachionus plicatilis* eaten per 14-h day	Dry weight of *Brachionus plicatilis* eaten per day (g x 10^{-5})	Aroclor® 1254 eaten/day (g x 10^{-11})	Aroclor® 1254 eaten/day (g x 10^{-11}; extrapolated)	Total Aroclor® 1254 eaten (g x 10^{-11})	Calculated concentration of Aroclor® 1254 in larvae (ppm)	Measured concentration of Aroclor® 1254 in larvae (ppm)
5	0.23	30	0.38	319	5.10	2.14	2.14	2.14	0.93	1.93
							2.58	4.72		
							3.02	7.74		
							3.46	11.20		
							3.90	15.10		
10	0.54	60	0.77	647	10.35	4.34	4.34	19.44	3.60	2.59
							4.62	24.06		
							4.90	28.96		
							5.18	34.14		
							5.46	39.60		
15	1.40	80	1.02	857	13.71	5.75	5.75	45.35	3.24	1.11
							5.90	51.25		
							6.05	57.30		
							6.19	63.49		
							6.34	69.83		
20	2.08	90	1.15	966	15.46	6.49	6.49	76.32	3.67	2.03
							6.64	82.96		
							6.78	89.74		
							6.93	96.67		
							7.08	103.75		
25	2.47	100	1.28	1075	17.20	7.22	7.22	110.97	4.49	2.67

[a]Data from Hunter (1972).

Table 3. Average concentration of Aroclor® 1254 (PCB) in dry tissue and lipid of algae, rotifers and anchovy larvae during 45-day food-chain experiment. Partition coefficients were calculated by dividing lipid concentration by medium concentration

Organism	Tissue concentration of Aroclor® 1254 (ppm)	Percent lipid	Lipid concentration of Aroclor® 1254 (ppm)	Medium concentration of Aroclor® 1254 (pptr)	Partition coefficient (x10^6)
Dunaliella sp.	0.25	6.4[a]	3.91	8.2	0.48
Brachionus plicatilis	0.42	15.0[b]	2.80	8.2	0.34
Engraulis mordax	2.06	7.5[b]	27.46	2.0	13.70

[a]Data from Parsons *et al.* (1961).
[b]Chloroform-methanol (2:1 v/v) extract.

Table 4. *Engraulis mordax*. Concentration of Aroclor® 1254 (PCB) in fed and unfed anchovy larvae. Partition coefficients were calculated by dividing lipid concentration by medium concentration

	Aroclor® 1254 by dry weight in eggs (ppm)	Aroclor® 1254 in seawater (pptr)	Aroclor® 1254 by dry weight in larvae (ppm)	Aroclor® 1254 in lipid of larvae (ppm)	Partition coefficient (x10^6)
Unfed 3-day old larvae	0.36	2.5	2.80	37.33	14.9
Unfed 2-day old larvae	0.33	4.5	4.70	62.67	13.9
Average of fed larvae over 25 days	0.35	2.0	2.06	27.46	13.7

Comparison of PCB Accumulation in Fed and Unfed Anchovy Larvae

In a final experiment, *Engraulis mordax* eggs of a known PCB concentration were hatched and the unfed larvae were analyzed for PCB content 2 to 3 days after hatching at the time when the yolk sac is absorbed and when they normally start feeding (Table 4). In one test, the eggs contained 0.36 ppm PCB (dry weight) and were hatched in seawater containing 2.5 pptr PCB. Three days after hatching, the unfed larvae contained 2.80 ppm PCB (dry weight). In another experiment, the initial concentration in the eggs was 0.33 ppm PCB (dry weight) and the seawater was 4.5 pptr. Two days after hatching the larvae contained 4.70 ppm (dry weight). In both tests, although the PCB concentration in the seawater and the larvae was different, the partition coefficient between the seawater and anchovy lipid was almost the same, which indicates that the PCB concentration in the larvae was dependent on the concentration in the seawater. Also, the partition coefficient of the unfed larvae was essentially the same as the partition coefficient calculated from the average PCB concentration in fed larvae from the three-step food-chain experiment. Again, this indicates that an equilibrium was established between the seawater and the larvae which was independent of the anchovy's position in the food chain.

Discussion and Conclusions

The concentration of PCB in each trophic level of a three-stop marine food chain (algae → rotifer → anchovy larvae) was monitored for 45 days. The dietary contamination to the higher trophic levels was calculated and it was determined that the PCB concentration in the food ingested was adequate to account for the residues found in the tissues of the organisms. When the PCB concentration was determined on a dry weight basis there appeared to be an amplification in a step-wise fashion up the food chain. However, when the PCB concentration was calculated on a lipid basis this trend was no longer apparent in the lower trophic levels. The algae and the rotifers contained essentially the same PCB concentration; what appeared to be a food-chain accumulation was really a reflection of the higher amount of lipid in the rotifers. This did not hold true for the higher trophic level; PCB residues in the lipid of the fish larvae were about 10 times higher than the invertebrates. Although this finding supports the theory of the bioamplification of pollutants up the food chain, we have contradictory evidence that suggests that anchovy larvae partition PCB directly from the seawater. We found that unfed anchovy larvae contained essentially the same amount of PCB as fed larvae and that the final concentration in the tissue was related to the amount of PCB in the water. Also, bioamplification suggests that the PCB concentration in an organism will increase with time if dietary inputs remain constant, but no such increase was observed in this study. Instead, all three steps of the food chain were in a steady-state by the time the first sample was taken.

The surface-area-to-volume ratio of first-feeding anchovy larvae is large enough to enable the fish to rely upon diffusion to take care of respiratory needs. Older fish maintain a large surface area of diffusive tissue in contact with the water by developing gills. Aquatic animals which rely upon diffusive interaction with the water for respiratory and metabolic needs will partition lipophilic compounds across these membranes and establish an equilibrium with the surrounding water which will depend on the concentration of the compound in the water and the solubility of the compound in the water and the lipid of the organism. It is our conclusion that this mechanism is the primary determinant of the CHC levels detected in exposed aquatic organisms.

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