Fate and behavior of five chlorinated hydrocarbons in three natural waters[1]

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Water samples removed from two rivers and from the subtidal zone of Georgia Strait in British Columbia were treated with either 0.025 ppm of DDT, lindane, α-chlordane, or γ-chlordane, or 0.1 ppm Aroclor 1260 (PCB). The samples were incubated in the laboratory for up to 12 weeks at the temperatures of these natural waters at the time of sampling. Lindane persisted in all water samples throughout the experiment, but large proportions of the other compounds were transported into the atmosphere during incubation except when the containers were sealed. No metabolic breakdown could be demonstrated. As demonstrated with ^{14}C-γ-chlordane, uneven distribution of the pesticides occurred rapidly, but was prevented, or reversed, by addition of a surfactant to the water. Total bacterial counts were generally higher in treated than in untreated water samples.

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Des échantillons d'eau prélevés de deux rivières et de la zone des basses marées dans le Georgia Strait, en Colombie Britannique, furent traités avec soit 0.025 ppm de DDT, lindane, α-chlordane, γ-chlordane, ou 0.1 ppm d'Arochlor 1260 (PCB). Les échantillons furent incubés dans le laboratoire pour des périodes allant jusqu'à 12 semaines, et aux températures des eaux naturelles au temps de l'échantillonnage. Le lindane persista dans tous les échantillons tout au cours de l'expérience, tandis que de grandes proportions des autres composés furent transportées dans l'atmosphère durant l'incubation, excepté lorsque les boîtes furent fermées hermétiquement. On n'a pu démontrer de bris métabolique. Lorsque le ^{14}C-γ-chlordane fut utilisé, on a démontré que les pesticides se distribuent inégalement et rapidement; ce phénomène est prévenu ou renversé par l'addition d'un surfactant à l'eau. Les comptes bactériens totales furent généralement plus élevés dans les échantillons traités que dans ceux non traités.

Introduction

The fate and behavior of persistent chlorinated hydrocarbons in the marine environment were recently reviewed (9). These compounds were shown to accumulate ultimately in the oceans, transported from their points of application via sewage outfalls, surface runoff, rivers, and the atmosphere. Interesting and impressive estimates were presented for the total quantities of DDT which may have been transferred to the oceans during the past. But only a few data document the actual quantities of these compounds in the open ocean environment or their impact upon the system (9).

The scarcity of relevant data necessitates making assumptions in evaluating the results of worldwide use of these compounds. The low cost and effectiveness of the chlorinated hydrocarbon insecticides are expected to cause their continued and even increased use in much of the world (12), so that increased research is needed on which to base sound judgments about the present and future impact of these compounds upon the marine environment.

Five general lines of inquiry appear to promise relevant data. (*a*) What are the actual concentrations of these compounds in the ultimate sink, i.e., the open oceans? (*b*) What is their distribution within this environment? (*c*) What are their effects on the various components of the ecosystem? (*d*) What are their rates and routes of entry into the oceans? (*e*) What are their effects on living organisms in these routes of transport? Global monitoring would furnish the most relevant data, and is the only means to answer (*a*) and (*b*) fully, but financial and technological requirements are limiting factors. Appropriately designed experiments, although second best, can give urgently needed answers to (*c*), (*d*), and (*e*) fairly quickly and economically. Furthermore, the intrinsic toxicities of the compounds are such that experimental work should be restricted to controlled laboratory investigations.

So-called hazards, based upon results of bizarre experiments, bound to produce absurd effects and bearing no resemblance to any real situation, simply add to the existing confusion. Already, the side effects of synthetic chemicals (5) and other activities of man (3) are often

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overstated. The behavior and effects of toxicants at exaggerated doses in the laboratory may not in any way be characteristic of those at low levels occurring in the environment. Experiments with high doses may be interesting and significant for toxicologists or pharmacologists, but they are likely to be useless to environmentalists, or even dangerous when the results are extrapolated. Equally dangerous pitfalls await efforts to simulate natural conditions in the laboratory. Incubation of pesticides in sealed containers (4), for instance, prevents gas exchange and is likely to change the species composition of microorganisms and the fate of the compounds studied.

The purpose of our investigation was two-fold: to test the fate and behavior of realistic amounts of some chlorinated hydrocarbons in three natural waters in the laboratory during 12 weeks, under nearly natural conditions; and to test their effects on the total number of heterotrophic bacteria under these conditions.

Materials and Methods

Water Samples

Samples were taken from the Fraser River at New Westminster in British Columbia, about 10 mi east of Vancouver; from the Nicomekl River, 2 mi above its mouth, at a point about 20 mi southeast of Vancouver; and from the shoreline of Georgia Strait, 15 mi north of Vancouver. Water temperatures were recorded at the time of sampling and the waters were immediately transported to the laboratory and put into incubation chambers set to the appropriate temperatures.

Experiment 1

Thirty-one 150-ml aliquots of each water were measured into individual 500-ml Erlenmeyer flasks. Excepting three controls with water only and three with acetone only, each flask was treated with 15 µl of an acetone solution containing 250 µg/ml of reference grades of one of the following: DDT[2], lindane (γ-BHC), α-chlordane or γ-chlordane, or 1000 µg/ml of the polychlorobiphenyl (PCB) Aroclor 1260. The concentration in each flask was thus 0.025 ppm for the insecticides and 0.1 ppm for the PCB (Tables 1, 2, and 3). The contents of each flask were swirled by hand immediately after the treatment and once every 24 h during incubation. Two samples of each treatment were analyzed after 6 and 12 weeks respectively.

Bacterial counts were determined by using nutrient agar (Difco) for the river waters and medium No. 2216 E (Difco) for Georgia Strait water. All inoculated plates were incubated at 15°C for 96 h.

[2]Throughout the paper, DDT refers to *p,p'*-DDT. No other isomers were applied or subsequently found.

Chlorinated hydrocarbons were determined by gas-liquid chromatography (g.l.c.) in the following manner: each sample was transferred into a 500-ml separatory funnel and 100 ml of a 1:1 (v:v) hexane–acetone mixture were added via the Erlenmeyer flask. The funnels were shaken vigorously by hand for several minutes. After separation, the aqueous phase was discarded and the hexane phase washed twice with 2% Na_2SO_4 in glass-distilled water. Aliquots of the hexane phase were then used for g.l.c. analysis without further cleanup. A Microtek MT 220 was used with detector, column, and conditions as described earlier (11). Silanized glass wool plugs, loosely inserted into the mouths of the flasks during incubation to prevent contamination, were each extracted with 10 ml of hexane and the hexane phase was analyzed without further treatment. The five parent compounds, added before incubation (*vide* above), were determined. In addition, the following metabolites were sought but not detected: DDD, DDE, heptachlor, heptachlor epoxide, oxychlordane, and photo-cis-chlordane.

TABLE 1

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from the Fraser River, held in the laboratory for 12 weeks at 7°C

Time, weeks	Compound	Bacteria/ml	Recovery, % Water	Glass wool
0	α-Chlordane	1.8×10^2	98.7	0*
6	α-Chlordane	1.3×10^4	59.8	9.5
6	α-Chlordane	2.3×10^4	59.7	17.7
12	α-Chlordane	4.2×10^5	41.7	7.6
12	α-Chlordane	8.8×10^4	44.1	6.3
0	γ-Chlordane	1.8×10^3	90.6	0
6	γ-Chlordane	2.2×10^4	53.0	18.6
6	γ-Chlordane	2.5×10^4	55.6	6.4
12	γ-Chlordane	2.6×10^5	37.1	9.4
12	γ-Chlordane	7.1×10^4	38.1	3.4
0	Lindane	1.8×10^2	101.0	0
6	Lindane	1.2×10^4	99.7	0
6	Lindane	1.4×10^4	101.5	0
12	Lindane	5.3×10^5	96.7	0
12	Lindane	5.3×10^4	97.2	0
0	DDT	2.0×10^2	95.6	0
6	DDT	1.8×10^4	67.4	5.6
6	DDT	2.2×10^4	71.3	3.2
12	DDT	4.8×10^4	69.7	7.2
12	DDT	3.4×10^4	68.7	3.9
0	PCB[b]	1.9×10^2	95.6	0
6	PCB[b]	5.2×10^4	60.1	1.8
6	PCB[b]	2.7×10^4	60.9	4.5
12	PCB[b]	1.1×10^5	64.1	1.2
12	PCB[b]	—	74.7	5.5
0	Acetone only	2.2×10^2	0	0
6	Acetone only	9.3×10^3	0	0
12	Acetone only	4.6×10^4	0	0
0	Control	2.3×10^2	0	0
6	Control	7.1×10^3	0	0
12	Control	4.5×10^3	0	0

*No residue detected.
[b]0.1 ppm Aroclor 1260.

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Experiment 2

Eight 150-ml aliquots of Georgia Strait water were prepared as described. Four were treated with the α- and four with the γ-chlordane solution as in experiment 1. Two flasks of each pesticide treatment were then closed with ground glass stoppers, the rest with glass wool plugs. All eight samples were incubated for 3 days at 9°C and then extracted and analyzed as described (Table 4).

Experiment 3

Samples of glass-distilled water and Fraser River water, prepared as described, were treated with γ-chlordane randomly labeled with ^{14}C, then incubated at 7°C for up to 28 days in flasks closed with glass wool plugs or glass stoppers (Table 5). An acetone solution was prepared containing 280 μg/ml of the labeled chlordane. The activity was 5.52 μCi/ml. Each 150-ml water sample was treated with 20 μl of this solution, resulting in a concentration of 0.0377 ppm (0.1104 μCi) per sample.

The fate of γ-chlordane was determined by counting (corrected for quench effects): (*a*) 1 ml of the treated water; or (*b*) 1 ml of hexane phase after extraction as described and 1 ml of the water phase after extraction; and (*c*) 1 ml of hexane with which the glass wool plugs had been extracted. For counting in the Packard model 3003, 1 ml of the water or the hexane was added to 14 ml of liquid scintillant mixture prepared according to Bray (2).

Experiment 4

Two samples of distilled water were treated with labeled γ-chlordane as in experiment 3. One hour after treatment, 1-ml aliquots were taken for counting. Then 1 ml of Triton X-100 (octylphenoxy-polyethoxy ethanol) a non-ionic surfactant, was added to each remaining water sample (149 ml); these were then swirled again by hand. One hour later, a further 1-ml aliquot was counted.

Two other 150-ml samples of distilled water were treated with 1 ml of X-100 before the addition of labeled

TABLE 2

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from the Nicomekl River, held in the laboratory for 12 weeks at 16°C

Time, weeks	Compound	Bacteria/ml	Recovery, % Water	Glass wool
0	α-Chlordane	1.5×10^4	92.0	0[a]
6	α-Chlordane	—	50.2	0.8
6	α-Chlordane	—	51.1	1.5
12	α-Chlordane	1.1×10^5	20.5	1.2
12	α-Chlordane	5.7×10^5	16.6	0.8
0	γ-Chlordane	6.4×10^3	100.8	0
6	γ-Chlordane	—	34.0	0.2
6	γ-Chlordane	—	24.7	1.3
12	γ-Chlordane	1.1×10^5	2.3	0.5
12	γ-Chlordane	5.4×10^4	13.9	1.5
0	Lindane	8.5×10^3	98.4	0
6	Lindane	—	90.5	0
6	Lindane	—	88.5	0
12	Lindane	1.2×10^4	79.0	0
12	Lindane	4.0×10^3	76.0	0
0	DDT	1.5×10^4	96.3	0
6	DDT	—	34.4	2.2
6	DDT	—	43.7	3.4
12	DDT	9.9×10^4	30.1	1.1
12	DDT	1.4×10^3	23.6	3.5
0	PCB[b]	2.4×10^4	96.2	0
6	PCB[b]	—	73.8	1.4
6	PCB[b]	—	74.5	1.6
12	PCB[b]	7.5×10^3	33.0	1.1
12	PCB[b]	4.4×10^3	56.7	1.1
0	Acetone only	1.8×10^4	0	0
6	Acetone only	—	0	0
12	Acetone only	2.6×10^4	0	0
0	Control	4.9×10^4	0	0
6	Control	—	0	0
12	Control	1.4×10^4	0	0

[a]No residue detected.
[b]0.1 ppm Aroclor 1260.

TABLE 3

The fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from Georgia Strait, held in the laboratory for 12 weeks at 9°C

Time, weeks	Compound	Bacteria/ml	Recovery, % Water	Glass wool
0	α-Chlordane	1.0×10^2	91.3	0[a]
6	α-Chlordane	1.1×10^5	41.9	0.8
6	α-Chlordane	8.9×10^4	30.3	1.0
12	α-Chlordane	3.0×10^3	50.7	0.6
12	α-Chlordane	1.3×10^5	53.2	0.6
0	γ-Chlordane	1.1×10^2	94.4	0
6	γ-Chlordane	9.3×10^4	31.9	21.5
6	γ-Chlordane	—	24.9	9.5
12	γ-Chlordane	4.5×10^4	28.4	9.6
12	γ-Chlordane	1.3×10^4	35.6	4.3
0	Lindane	8.6×10^2	99.9	0
6	Lindane	1.4×10^5	91.5	0
6	Lindane	6.0×10^4	96.0	0
12	Lindane	1.3×10^4	99.2	0
12	Lindane	1.1×10^4	90.5	0
0	DDT	8.0×10^2	90.8	0
6	DDT	5.8×10^4	65.8	3.2
6	DDT	7.2×10^4	49.3	17.2
12	DDT	1.1×10^4	53.0	6.0
12	DDT	1.9×10^3	36.6	10.7
0	PCB[b]	7.8×10^2	93.5	0
6	PCB[b]	9.8×10^4	70.7	1.3
6	PCB[b]	6.5×10^4	40.7	6.2
12	PCB[b]	3.5×10^3	37.7	2.4
12	PCB[b]	1.1×10^3	38.7	0.8
0	Acetone only	1.1×10^3	0	0
6	Acetone only	3.4×10^4	0	0
12	Acetone only	4.5×10^3	0	0
0	Control	8.0×10^2	0	0
6	Control	2.4×10^5	0	0
12	Control	5.1×10^3	0	0

[a]No residue detected.
[b]0.1 ppm Aroclor 1260.

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γ-chlordane. Then, 1 h after treatment, 1-ml aliquots were counted.

Results and Discussion

The numbers of viable bacteria within the Georgia Strait, Fraser River, and Nicomekl River waters increased upon incubation in the presence of the various pesticides (DDT, α-chlordane, γ-chlordane, lindane, all at 0.025 ppm) and the polychlorobiphenyl mixture, Aroclor 1260 (0.1 ppm). Indeed, this increase in numbers tended to be greater within these chlorinated hydrocarbon treated waters than within the untreated controls (Tables 1, 2, and 3). This may be due to a slight enhancement of bacterial growth by the added chlorinated hydrocarbons, although no metabolites were detected within the water samples. It would appear that these added chlorinated hydrocarbons, when present at low concentrations, do not adversely affect the native heterotrophic bacteria in natural waters, as determined using nutrient agar or 2216E agar plate counts.

With the consistent exception of lindane, the chlorinated hydrocarbons decreased in the samples of natural waters during incubation, even at temperatures as low as 7°C and with minimal agitation (Tables 1, 2, and 3). Nevertheless, the absence of detectable metabolites in any water sample, and the complete recovery of unaltered parent compounds from stoppered incubation flasks (Tables 4 and 5) indicated that at natural temperatures in these waters, metabolic breakdown of the compounds tested is very slow. The atmosphere is thus likely to be an important route of transport. If the chemical stability of the compounds under our conditions is indicative of their stability in river water and seawater, then atmospheric transport might account not only for their movement into the open ocean as suggested in ref. 9, but also for global redistribution, unless gradual partitioning into living organisms (13) in the freshwater and marine environments retards this process.

The persistence of lindane in our experiments differs from results obtained by Lichtenstein and Schulz (7), who reported that lindane disappeared rapidly from water, and that the vapors above the water surface were highly toxic to fruit flies. The discrepancy probably arises from the different experimental conditions. Lichtenstein and Schulz used a concentration of lindane 500 times greater than ours, and incubated at 30°C with constant shaking. Although they could not establish a direct relationship between the reported water solubilities of chlorinated hydrocarbons and their volatilization from water, in our case the water solubility of lindane might well account for the difference of results. According to Gunther *et al.* (6) the water solubility of lindane ranges from 7.3 to 14 ppm, depending on temperature and method of determination. This is much greater than the concentration we used but just around that used by Lichtenstein and Schulz (7). In addition, apparent solubilities of pesticides in water tend to increase in the presence of impurities (6), especially biological material, including cell-free enzyme preparations (10). Since we used natural waters but Lichtenstein and Schulz (7) used tap water, the presence of microorganisms may also have contributed to the retention of lindane in our samples.

Available data on the water solubility of DDT vary by as much as three orders of magnitude, namely from 0.001 to 1.0 ppm (6, 7, 8). In our experiments, DDT was diminished by between 70% and less than 30% after 6 and 12 weeks (Tables 1 to 3). Most of it was lost during the first 6 weeks. No loss occurred from Fraser River water after the sixth week. If these results are indicative of solubility in these waters, it may be assumed that the solubility of DDT in Fraser River water at 7°C is about 0.0175 ppm (70% of 0.025), and somewhat lower in the other two.

Two mechanisms may account for the loss of pesticides from water, codistillation and evaporation. At concentrations below the water solubility, losses can be expected to be low and roughly proportional to evaporation of water, which was 1 to 2% in our experiments. At concentrations exceeding water solubilities, the rate of loss will depend on the distribution of the undissolved compound, its vapor pressure, and the temperature. DDT particles appear to have an affinity for the surface of water which accounts for losses greater than those attributable to codistillation (1). This phenomenon may explain our results.

In the absence of solubility data for the two chlordane isomers and Aroclor 1260, we assume that the initial concentrations of these compounds in the waters tested were higher than

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the solubilities, and that these compounds also tend to accumulate at the water–air interface under these conditions. The higher recovery of α-chlordane after 12 than after 6 weeks from Georgia Strait water (Table 3) is difficult to explain. Possibly, an inadvertent difference in the plugs may have caused this.

TABLE 4

The fate of 0.025 ppm α- and γ-chlordane in 150-ml water samples from Georgia Strait, held in the laboratory for 3 days at 9°C in flasks closed with glass wool plugs or ground glass stoppers

Closure	Compound	Recovery (%) Water	Glass wool
Stopper	α-Chlordane	96.7	—*
Stopper	α-Chlordane	99.4	—
Plug	α-Chlordane	73.6	27.4
Plug	α-Chlordane	79.3	21.3
Stopper	γ-Chlordane	94.5	—
Stopper	γ-Chlordane	96.3	—
Plug	γ-Chlordane	65.7	36.7
Plug	γ-Chlordane	73.3	31.5

*Not applicable.

No metabolites of the compounds could be detected. Peak heights of the Aroclor 1260 chromatograms did not change in relation to one another after 6 and 12 weeks. Also, no new peaks were detected, indicating that all of the PCB isomers evaporated at similar rates from the water–air interface, and that they were not metabolized.

Loss into the atmosphere was further demonstrated using α- and γ-chlordane in a 3-day experiment (Table 4). Although 20 to 30% was lost from the water within 3 days, this could still be recovered from the glass wool plugs, and it appeared that under the experimental conditions the movement of the compounds from the water into the atmosphere was a two-step process. Initially, the compounds sublimed onto the glass wool plugs; later they evaporated into the atmosphere, as though the plugs were chromatographic columns. This curious effect was also shown by the partial recovery of Aroclor 1260 from the plugs. The various components of this PCB evaporated from the water surface at similar rates (*vide* above), but not all

TABLE 5

The fate of γ-chlordane labeled with ^{14}C in samples of distilled and Fraser River water, held in the laboratory for various periods at 7°C in flasks closed with glass wool plugs or ground glass stoppers

Time, days	Closure	μCi recovered, % Distilled water: Hexane extract	Aqueous phase	Glass wool plug	Fraser River water: Hexane extract	Aqueous phase	Glass wool plug
0	Plug	—*	79.7	0[b]	—	74.1	0
0	Plug	—	75.8	0	—	75.1	0
0	Plug	91.0(91.6)[c]	2.0[d]	0	90.9(92.0)[c]	2.3[d]	0
0	Plug	90.1(94.7)[c]	0.9[d]	0	91.6(90.1)[c]	2.5[d]	0
7	Plug	—	55.4	—	—	63.7	—
14	Plug	—	52.8	—	—	61.1	—
21	Plug	—	46.6	—	—	51.4	—
28	Plug	—	39.7	9.1	—	45.6	9.7
28	Plug	41.7	2.6[d]	5.6	56.8	3.0[d]	6.0
28	Plug	45.8	1.6[d]	7.6	51.4	2.7[d]	4.3
28	Plug	40.4	2.0[d]	11.4	48.3	3.0[d]	5.4
7	Stopper	—	75.1		—	82.2	
14	Stopper	—	75.9		—	79.0	
21	Stopper	—	76.0		—	81.2	
28	Stopper	—	74.2		—	85.8	
28	Stopper	89.3	2.0[d]		81.6	4.1[d]	
28	Stopper	91.3	2.6[d]		89.5	3.2[d]	
28	Stopper	88.9	3.5[d]		90.1	2.2[d]	

*Not determined.
[b]No residue detected.
[c]Figures in parentheses determined by gas–liquid chromatography.
[d]Counted after extraction with hexane.

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were recovered from the plugs. Apparently, others had escaped already into the atmosphere.

The data in Table 5 indicate that no water-soluble metabolites of γ-chlordane were formed under our conditions. The amount of radioactivity found after extraction was the same in distilled and in Fraser River water. More interesting was the low recovery from the water phase when it was counted directly as compared with the higher recovery after hexane extraction which was confirmed by gas–liquid chromatography. This indicated a rather rapid differential distribution within the water phase, quite likely an accumulation at the water–air interface as reported by Acree *et al.* (1).

Results of experiment 4 supported such uneven distribution. Counting 1-ml aliquots of water from two 150-ml samples 1 h after treatment with labeled γ-chlordane resulted in recovery of 75 and 80%. Subsequent addition of surfactant to these samples and swirling redistributed γ-chlordane evenly. Counting 1-ml aliquots 1 h later now gave 95.4 and 96% recovery. From two samples treated with surfactant before γ-chlordane treatment, recoveries were 97 and 98% 1 h after the treatment. These data show that surface-active pollutants in natural waters, e.g., industrial and domestic detergents, may significantly influence the behavior of highly insoluble chlorinated hydrocarbon residues.

The means by which chlorinated hydrocarbon residues are moved from points of release to the open ocean are still not completely understood, but data from this investigation suggest some possibilities: (1) when they are present in natural waters at concentrations approaching or exceeding their solubilities they appear to accumulate at the water–air interface from whence they evaporate into the atmosphere. Under these conditions, the rates of evaporation exceed those explainable by codistillation. (2) If these compounds occur at concentrations which are lower than their solubilities they are evenly distributed and their rate of transfer from the water will be lower, about proportional to the evaporation of water, i.e. they transfer by codistillation. (3) The presence of materials which promote the even distribution of chlorinated hydrocarbon residues in natural waters will decrease the transfer of residues into the atmosphere because they counteract accumulation at, and thus evaporation from, the water–air interface.

The ready escape into the atmosphere of highly insoluble and chemically stable chlorinated hydrocarbon compounds not only explains their global distribution, but also indicates that the accumulation which has resulted from their extensive use is unlikely to be reduced so long as their use is curtailed only in some parts of the world but continued or increased in others.

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