Effects of Suspended Material on Measurement of DDT in Estuarine Water

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Accumulation of pesticides by suspended material in sea water has been well documented. COX (1971) noted that 90% of DDT residues recoverable from whole sea water was not bound to particulate material greater than one to two microns in diameter. RICE and SIKKA (1973) showed that approximately 90% of the maximum uptake by six species of marine algae occurred within 2 hours after exposure to ^{14}C-DDT. PIERCE et al. (1974) reported the humic acid fraction of sea water to have a greater adsorption capacity than clay and sediment. OLOFFS et al. (1973) showed that all detectable DDT residues moved into sediments after 6 weeks of incubation in the laboratory. Other studies have shown that phytoplankton and suspended particulate material have relatively large capacities for sorption of pesticides (COX, 1970; GREGORY et al., 1969; HILL and MCCARTY, 1967; KIEL and PRIESTER, 1969; POIRRIER et al., 1972; SODERGREN, 1968, 1973; SUFFET, 1973b; VANCE and DRUMMOND, 1969; WARE et al., 1968; WHEELER, 1970). Recent monitoring studies show residues of DDT in plankton from the open ocean (GIAM et al., 1973; HARVEY et al., 1974; WILLIAMS and HOLDEN, 1973).

The present study was undertaken to investigate the effect of suspended material on measurement of DDT in estuarine water. Preliminary studies (WILSON et al., 1970) reported a decrease in extractable DDT from estuarine water with time. These results were observed in laboratory studies in which DDT was added to natural estuarine water and incubated in sealed glass containers. The results were interpreted by many workers as indication of DDT breakdown. The objective of the studies described herein was to learn if suspended material accumulated DDT under the conditions of this experiment and prevented its complete recovery. No attempt was made to evaluate the separate effects of biotic and abiotic suspended material.

There are various methods for measuring pesticides in water. Due to their simplicity, many analysts employ liquid-liquid extraction (LLE) methods of the batch or serial type. This method consists of extracting up to about four liters of water with an organic solvent (KALLMAN et al., 1962). Other LLE methods are the continuous multichamber systems such as that described by KAHN and WAYMAN (1964). Carbon has been used to adsorb pesticides (MIDDLETON and LICHTENBERG 1960). Recently, other adsorptive techniques have been described. HARVEY (1972) described a

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synthetic resin for the analyses of pesticides in sea water. AHLING and JENSEN (1970) used reversed liquid-liquid partition methods for extraction of chlorinated pesticides from water. Once a method of extraction has been selected, it is usually evaluated by fortification of a water sample with the compound and determining if the method produces acceptable accuracy and precision (MCFARREN et al., 1970). Isotope tracers are also used in determining acceptability of a method.

The following experiments were designed to determine the efficiency of serial LLE of DDT fortified estuarine water and evaluate the recovery rate from fortified samples by extracting suspended material and water separately.

RECOVERY STUDIES OF LLE OF DDT FORTIFIED ESTUARINE WATER

Methods. Duplicate 4 liter clear glass bottles, containing 3.5 l of estuarine water or distilled water, were fortified with 10.5 µg of p,p' -DDT in 350 µl of acetone to yield a concentration of 3.0 ppb (parts per billion). Five hundred ml samples were taken from each bottle and extracted by shaking vigorously for one minute in a separatory funnel as follows: three times with 50 ml of petroleum ether, two times with 50 ml of 15% ethyl ether in hexane followed by 50 ml hexane, or three times with 50 ml of methlyene chloride. All solvents were dried with sodium sulfate, concentrated to an appropriate volume and analyzed by electron capture gas chromatographs equipped with 2.0% OV-101 and 0.75% OV-17: 0.97% OV-210 in glass columns. Methylene chloride extracts were transferred to petroleum ether by concentrating the methylene chloride to about 10 ml, adding 50 ml of petroleum ether and reconcentrating to about 10 ml. This removed the methylene chloride which cannot be used in electron capture gas chromatography. Just prior to extraction, all samples were fortified with o,p' -DDE as an internal standard. The recovery rate of o,p' -DDE in all tests was greater than 89%, indicating no significant loss during analyses. The estuarine water was collected in Santa Rosa Sound, Florida. The salinity ranged from 16 to 24 ppt.

After initial sampling, the bottles were sealed and incubated at 20 C under controlled light conditions (5000 lux; 12 hours light, 12 hours dark). Duplicate samples of 500 ml were extracted at various time intervals.

Results. Table 1 and 2 show the average percentage recovery of p,p' -DDT extracted from duplicate estuarine water or distilled water samples up to 14 days after initiation of the experiment. p,p' -DDE was the only metabolite measured, but since it never exceeded 2% of the parent compound it is not included in the percent recoveries.

Table 1 shows that immediately after the estuarine water (21 ppt salinity) was fortified with 3.0 ppb of DDT, all solvent systems removed 93% of the DDT. After six days of incubation, less was recovered with all solvents. However, methylene chloride was the most efficient.

TABLE 1

PERCENTAGE RECOVERY OF P,P' -DDT FROM ESTUARINE WATER BY DIFFERENT EXTRACTION SOLVENTS

Day	Extraction Solvent		
	Petroleum Ether	15% Ethyl Ether In Hexane	Methylene Chloride
0	93	93	93
6	67	66	76

An experiment was performed with estuarine water (21 ppt salinity) and distilled water using petroleum ether and methylene chloride. Table 2 shows that immediately after fortification, recoveries were 90% or greater. After 14 days, similar recoveries were obtained only from distilled water. In estuarine water however, there were 49% and 28% reduction in recovery from zero day with petroleum ether and methylene chloride respectively. Since distilled water is devoid of particulate matter, this suggests that DDT may be absorbed or adsorbed to particulate matter found in estuarine water and the DDT sorbed onto this matter was not removed, resulting in low recoveries. This explains the initially high extraction efficiency of DDT followed by decline in recovery as DDT became associated with the particulate phase. Since methylene chloride was the most polar solvent used, it had the greatest affinity for removing sorbed DDT.

TABLE 2

PERCENTAGE RECOVERY OF P,P' -DDT FROM ESTUARINE WATER AND DISTILLED WATER BY PETROLEUM ETHER AND METHYLENE CHLORIDE

	Estuarine Water		Distilled Water	
Day	Petroleum Ether	Methylene Chloride	Petroleum Ether	Methylene Chloride
0	90	94	90	91
7	58	78	90	91
14	46	68	94	92

The above data are typical of results obtained from several tests with estuarine water of varying salinity (16 - 24 ppt). These experiments suggest that LLE methods are not efficient for the extraction of DDT from suspended material. To test this hypothesis, experiments were done in which the suspended material was separated from the water and both constituents analysed separately.

RECOVERY OF DDT FROM WHOLE SEA WATER AND SUSPENDED MATERIAL

Methods. Duplicate 4 liter bottles of estuarine water was fortified and incubated under controlled temperature and lighting conditions as described above. Samples were removed at various time intervals and analysed as follows: 500 ml samples were taken from each bottle and extracted in a separatory funnel three times with 50 ml of methylene chloride. In addition, 500 ml samples from each bottle were filtered through 47 mm, 0.4 micron porosity Nucleopore filters. The filter was placed in a 200 mm x 25 mm (O.D.) screw top test tube containing 10 ml acetonitrile. The test tube was then placed in a Varian Ultrasonic Cleaner and sonicated for 30 minutes at 45 C. Twenty five ml of 2.0% aqueous sodium sulfate and 5 ml of hexane were added. The test tube was sealed with a teflon-lined cap and shaken for one minute. After the solvent phases separated, the upper hexane layer was transferred with a dropping pipet to a clean 25 ml graduated cylinder. This was repeated three times with 5 ml of hexane. The combined extracts were analysed by electron capture gas chromatography.

The filtrate was extracted as follows: the filtering apparatus and the graduated cylinder used to measure the sample were rinsed with acetonitrile and the rinses added to the 500 ml filtrate. The combined filtrate and acetonitrile rinses were extracted in a separatory funnel three times with 50 ml of petroleum ether. The extracts were dried with sodium sulfate and concentrated to an appropriate volume for analyses by electron capture gas chromatography. Before extraction, the filter and filtrate were fortified with o,p'-DDE as an internal standard.

Estuarine water for these tests was collected from Santa Rosa Sound, Florida (salinity range 22 - 28 ppt). However, in one test, artificial estuarine water was used. This was prepared by dissolving 210 grams of Rila salts in 7 liters of distilled water. The resulting solution (23 ppt salinity) was filtered through a glass filter. A mixed algal culture consisting of Chlorella sp., Dunaliella tertiolecta and Chlamydomonas sp. was added to the artificial estuarine water.

Results. Table 3 compares the percentage recovery of DDT from methylene chloride extractions of the entire water sample with percentage recovery after extraction of the suspended material and water separately (filter + filtrate). In all tests there was a significant increase in the recovery of DDT when the suspended material was analysed separately. The greatest increase was in fortified artificial estuarine water containing the algal culture.

TABLE 3

PERCENTAGE RECOVERY OF DDT FROM EXTRACTION OF WHOLE WATER AND SEPARATE EXTRACTION OF SUSPENDED MATERIAL AND FILTRATE

Day	Percentage Recovery (Std dev)		Percentage Increase
	Whole water	Suspended Material + Filtrate	
Exp. 1 (Estuarine water)			
0	85 (1.8)	89 (4.4)	4.7
4	75 (1.8)	101 (6.2)	35
9	77 (2.6)	90 (12)	17
Exp. 2 (Estuarine water)			
0	84 (4.4)	91 (3.5)	8.3
4	84 (0)	100 (.89)	19
Exp. 3 (Estuarine water)			
0	72 (8.6)	89 (1.5)	25
6	76 (3.6)	99 (1.8)	30
Exp. 4 (Estuarine water)			
0	91 (0)	---	---
7	56 (.89)	83 (5.6)	48
8	---	88 (8.2)	---
Exp. 5 (Artificial estuarine water + algae)			
0	85 (0)	84 (5.4)	0
5	54 (5.9)	85 (2.7)	57

DISCUSSION

These experiments show the pitfalls of sample fortification. Liquid-liquid extraction of estuarine water immediately after fortification yielded acceptable recovery levels with all solvent systems tested. However, analyses several days later gave only partial recovery. Field residues may be subject to similar physical and chemical transformations and therefore complete recovery of DDT may not be possible by these methods. Similar studies reported by OLOFFS et al. (1972) showed up to 60% decrease in recovery of DDT from estuarine water fortified with 25 ppb DDT and incubated for 12 weeks in flasks stoppered with glass wool. The mechanism suggested for this loss was evaporation and co-distillation. SUFFET (1973a) suggested that the recovery from fortified laboratory water samples approach actual recovery from field samples if a pesticide is completely dissolved and not associated with suspended matter and the water properties are similar to natural water. However, estuarine water has a pH above 7.0, is of high ionic strength, and contains suspended material.

A more exhaustive extraction was required to remove the sorbed DDT from the suspended material (Table 3). Experiment 5 shows that phytoplankton will accumulate DDT and LLE methods will not remove all the sorbed DDT.

Different results may be observed if experiments are conducted below the solubility of DDT (1.2 ppb in distilled water; reported by BOWMAN et al., 1960). EICHELBERGER and LICHTENBERG (1971) fortified natural river water with 1.0 ppb DDT and incubated replicate samples in sealed glass containers for eight weeks. No loss of DDT was observed by LLE methods. However, COX (1970) studied ^{14}C-DDT uptake in three species of marine phytoplankton and noted concentration factors ranging from 2.5 to 8.0 x 10^4 at 0.8 to 3.0 parts per trillion. RICE and SIKKA (1973) also observed rapid uptake of DDT by marine algae at 1.0 ppb.

Interaction of pesticides between water and particulate matter is complex. Evaluating LLE methods of herbicides from river water, SUFFET (1973b) observed that the isopropyl ester of 2,4-D was adsorbed to particulate material in river water and that the amount changed by alteration of the pH of the water. HUANG and LIAO (1970) found that adsorption of DDT to clays was rapid but the amount differed with the type of clay. A mixed culture of algae consisting mainly of Vauchenia had a greater adsorption for DDT than bentonite according to HILL and MCCARTY (1967). The experiments reported in this paper support the work of other investigators in that DDT is extremely hydrophobic and can easily be adsorbed or abosrbed by suspended matter in liquid solutions. These studies indicate the observed loss of DDT by this author's earlier studies (WILSON et al., 1970) was due to sorption of DDT to suspended material which prevented complete recovery of DDT by the methods used in the experiment.

It is difficult to relate laboratory findings directly to that of the estuary or open ocean. However, the laboratory data described here illustrate clearly some problems that could be encountered in monitoring estuarine water for pesticide pollution. Data obtained by analysis of water by liquid-liquid extraction methods or other methods which do not efficiently extract sorbed pollutants from suspended material may be misleading.

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