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William T. Lammers

UNION CARBIDE CORPORATION
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OAK RIDGE GASEOUS DIFFUSION PLANT

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THE ACCUMULATION OF DDT BY PARTICULATE AND DISSOLVED FRACTIONS OF NATURAL WATER

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January 24, 1969

To: Holders of Union Carbide Corporation, Nuclear Division,
Report Number K-1755

Report Number K-1755, Page 15, Table I

Data presented in the subject report have been reviewed and corrected. Specifically, the value of the Student's "t" parameter has been recalculated and corrected where necessary.

Please remove and replace the old table with the enclosed corrected table.

Yours sincerely,

J.W. Amburgey, Jr.

J. W. Amburgey, Jr.
Biophysical Limnology Laboratory
Separation Systems Division

JWA:egc

Enclosure: Corrected Table I

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ABSTRACT

The study of DDT distribution in natural water is essential to a better understanding of its true biohazards contributions in the aquatic environment. In order to determine this distribution and biohazards effect, the suspended materials must be isolated from natural water samples and studied. During the past two and one-half years such studies have been conducted in the Biophysical Limnology Laboratory at Oak Ridge whereby centrifugal techniques were used to isolate the particulate fractions contained in samples of DDT contaminated water. The isolated fractions were then studied analytically to determine the amount of DDT associated with each fraction.

The reasons for making these studies were: one, to establish the feasibility of using centrifugal techniques for concentrating and isolating suspended materials from DDT contaminated water; two, to determine the pattern of DDT distribution within the major particulate fractions; and three, to check the effects of seasonal changes on the DDT distribution patterns.

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THE ACCUMULATION OF DDT BY PARTICULATE AND DISSOLVED FRACTIONS OF NATURAL WATER

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INTRODUCTION

The water fractionation program was initiated in the fall of 1965 for the purpose of investigating the applicability of ultracentrifuge techniques to separating waterborne particulate materials and conducting studies relating to water pollution. One of the first pollution-type studies to be considered included an attempt to determine the DDT distribution among the various suspended fractions contained in a natural body of water that drains an area of moderate agricultural activity. This particular study was one of several pollutant related investigations to be conducted under one of the U. S. Atomic Energy Commission's Management of Facilities Capabilities projects.

The acknowledged presence of DDT over our entire planet presents serious problems in assessing its effect on non-target organisms, including man (1). Knowledge of the simple physical concentration levels of DDT in water and soil are not enough because DDT, by design, is biologically and surface active. As a result, low initial levels can be concentrated in cycling through the food chain so that a real hazard is presented to the non-target organisms at or near the top of the chain (2, 3). The increasing use of pesticides and the reuse of water add emphasis to the need for information about DDT distribution within a given volume of water or soil (4).

The information that is needed about DDT in water is not simply the absolute amount in a given volume of water, but more important, the distribution of that given amount among the various fractions suspended in that water mass. Knowledge of the nature of this differential distribution of the DDT will allow meaningful assessments of the potential biohazard.

Natural water is a heterogeneous mixture of numerous types of particles and solutes suspended or dissolved in the solvent water (5). Some of these fractions are surface active and others are biologically active. These active fractions especially can be expected to accumulate a significant proportion of the DDT. Any portion of the DDT sorbed by a fraction of the water mass which plays no part in the food chain has a low potential as a biohazard. On the other hand, any DDT accumulated by an active fraction of the food chain will have an increased potential as a biohazard.

As reported in previous papers (6, 7) the particles in natural water can be rapidly concentrated and isolated into a number of relatively homogeneous fractions by centrifugal techniques. A major advantage of this method is particles and contaminants existing in low quantities per unit volume may be recovered from large volume samples (20 to 40 liters) so the total quantities isolated lie within the range of sensitivity of analytical methods. This method of rapid centrifugal concentration and isolation also causes a minimum of distortion and injury to particles and a minimum loss of accumulated DDT. Patchy distribution of particles and seasonal variations in a natural water mass may be circumvented by multiple sampling on a randomized year round schedule.

In the investigation reported here, the goal was to establish the feasibility of centrifugal concentration and isolation of particles from DDT contaminated water and to investigate the pattern of DDT distribution within the major fractions of water both in a single sample and with seasonal changes. This investigation does not use a statistically designed sampling schedule, but does demonstrate the potential of the method for an extended study.

SUMMARY

The distribution of the DDT among the isolated fractions may depend on its mode of entry into the water. If the DDT were derived from a terrestrial application that later washed into the stream, one would expect that the DDT still would be associated with the particles which had sorbed it before its entry into the stream. In this case most of the DDT would be expected to be found associated on suspended or colloidal inorganic particles, and DDT association with this fraction should be highest after a rainy period and especially following periods of crop dusting. There does seem to be a seasonal variation in the observed DDT distribution that can be roughly related to expected periods of crop dusting, growing seasons, and rain distribution.

The seasonal distribution of DDT in the organic fraction also appears to roughly follow the expected planktonic population variations with an obvious early spring increase.

The results indicate that detectable DDT is present in all fractions of all samples. A 20-liter sample is of sufficient volume for accurate analysis, and the centrifugal method of fractionating natural water is applicable to this problem. The results, presented as a "balance sheet" of distribution, are in a useful form for assessing the pattern and possible consequences of DDT in the aquatic sphere.

EXPERIMENTAL PLAN AND TECHNIQUE

The Tennessee Valley Authority has kept long-term records of DDT use and concentration levels in the Valley reservoirs. In addition, the TVA environmental scientists have made thorough long-term investigations of various biological, physical, and chemical phenomena in the Valley reservoirs. Because of this background of interest in high quality water, TVA readily cooperated in supplying water samples. The TVA water intake on Wilson Lake at Muscle Shoals, Alabama, was chosen as the sampling site. Water from Wilson Lake was known to have DDT in it, the refrigerated samples could be shipped easily and quickly to the Biophysical Limnology Laboratory by air express, and the existing long-term investigations at that site could provide backup information about the results. In this study the 20-liter samples were collected on a regular monthly timetable, packed in a refrigerated, insulated crate, and flown to the laboratory at

Oak Ridge. The fractionation was started the morning after collection and was complete that day, so only about 30 hours elapsed from sample collection until complete fractionation. The sample was kept below 10° C at all times.

The fractionation scheme used in this investigation is shown in figure 1. The particles were isolated from the suspending water by continuous flow centrifugation using a centrifugal impulse (force x time) sufficient to sediment suspended organic and inorganic particles (> 20,000 S) in the first centrifugation (56,000 g x min) and organic and inorganic colloids (> 150 S) in the second (1.44×10^7 g x min). The > 20,000 S particles were removed from the rotor and thoroughly resuspended. Linear density gradients of physiologically inert 3,5-DP (methyl glucamine salt of 3,5-diiodo-4-pyridone N acetic acid) in a pH 7.2 phosphate buffer were made with a gradient preforming device (8, 9). The density range of the gradients was from a minimum of 1 to a maximum of 1.5 g/cc and sector-shaped centrifuge tubes were used to prevent wall effects during centrifugation (10). The suspended 20,000 S particles were carefully layered over the density gradient and then separated into an organic and an inorganic fraction on the basis of density differences by density gradient centrifugation using the same impulse as in the initial centrifugation.

Following centrifugation the tube contents were pumped through the optical flow cell of a densiometer and the optical density of the gradient recorded at 530 mμ. The organic particles, suspended in the gradient, were recovered in a fraction collector and dialyzed against a pH 7.2 phosphate buffer. The area of the absorption peak on the chart recording of the gradient optical density was measured with a polar planimeter and this value was taken as one measure of the amount of organic material. The amount of organic material present was also estimated by a direct count of an aliquot using phase contrast microscopy and a Petrof Hausser counting chamber and method.

The inorganic suspended particles, forming a pellet at the centrifugal side of the centrifuge tube, were recovered by washing with the phosphate buffer. The number of particles in the inorganic fraction was estimated by a direct microscope count of an aliquot using the same method as for the organic particles.

The colloidal fraction (< 20,000 S, > 150 S) was treated as a single entity and the centrifugal pellet concentrated to a 10 ml volume by differential centrifugation at a centrifugal impulse of 1.44×10^7 g x min.

The supernatant from the second continuous flow centrifugation (< 150 S), was concentrated to a 100 ml volume by vacuum evaporation in a two-liter rotary spray evaporator at 35° C vapor temperature in the flask. The flask contents were removed with washes of 1N HCl and 1N NH_4OH.

RESULTS

Codistillation of dissolved DDT is possible, but if one assumes that nearly all free DDT is quickly sorbed by a particle, then little should

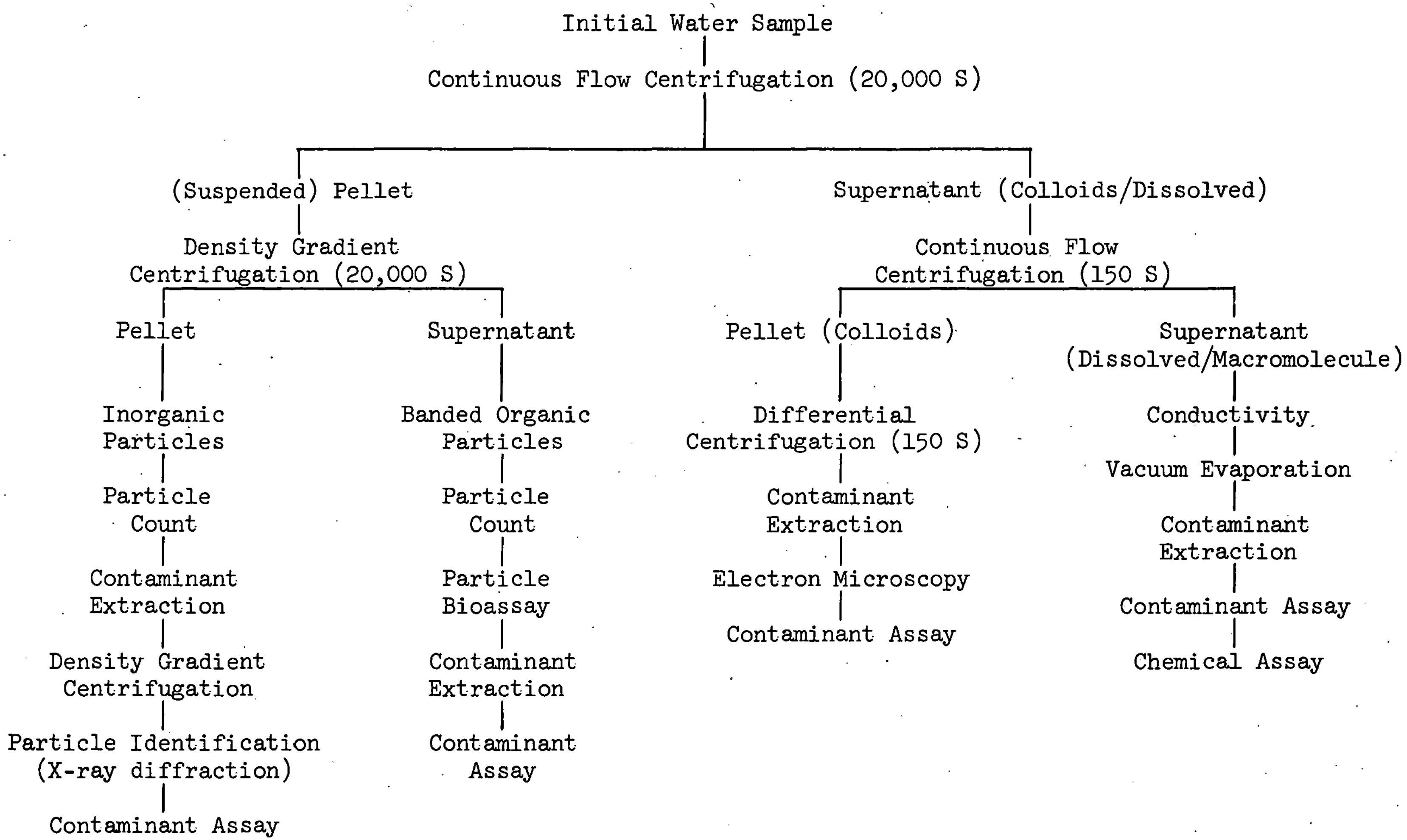

FIGURE 1

SCHEME OF FRACTIONATION OF A WATER SAMPLE be lost in evaporation. The amount of loss was investigated in one experiment by collecting and redistilling the 18 ℓ of vapor. In this case, 0.08 μg of DDT was recovered in the second evaporation. In a second experiment a glass filter was packed with activated carbon and incorporated into the vapor line from the evaporator. After 18 ℓ had passed through the filter, the carbon was hexane extracted and 0.09 μg of DDT were recovered. While this amount of DDT may be significant with very low levels, in the experiments reported here, it seems to be a small portion of the total DDT and probably represents some of the DDT free in solution.

The concentrated, isolated fractions were extracted with chromatographic grade hexane for 24 hours. It was usually necessary to clean-up the concentrated hexane extract by thin layer chromatography. The hexane extract was spotted on Eastman 6062 "Chromagram" sheet and developed by ascending chromatography with hexane. The isolated sample was removed from the plate by scraping off the adsorbent and re-extracted with hexane. The DDT was then identified and determined by electron capture gas chromatography using a Barber-Colman Model 5120 cell (11). The results were recorded as μg of para para DDT with a precision of ± 10%.

Figure 2 shows the population numbers of organic and inorganic suspended particles (> 20,000 S) and of colloidal particles (< 20,000 S and > 150 S) in each sample. The amount of < 150 S material (macromolecules and dissolved particles) is represented by the conductivity (μmho/cm at 25° C) of the supernatant from the second continuous flow centrifugation (< 150 S in supernatant).

The amount of DDT accumulated by each fraction is shown in figure 3. Some observations can be made from these data. The solubility of DDT in water at 25°C is reported to be 15 μg/ℓ for 1000 Å particles (12). This is 300 μg for a 20-liter sample. Since no sample showed this amount of DDT, the water does not appear to be saturated and the particles and water are probably not in exchange equilibrium. On the basis of the amount of DDT on the suspended inorganic particles, a considerable amount of total DDT should be found sorbed on the inorganic particles in the bottom sediment. This portion of the DDT may be available to "blooms" of bacteria or algae. The bottom sediment probably will become increasingly saturated due to settling of contaminated inorganic particles. If this were so, the bottom sediment would remain a source of DDT contamination of the water long after the use of this particular pesticide ceased.

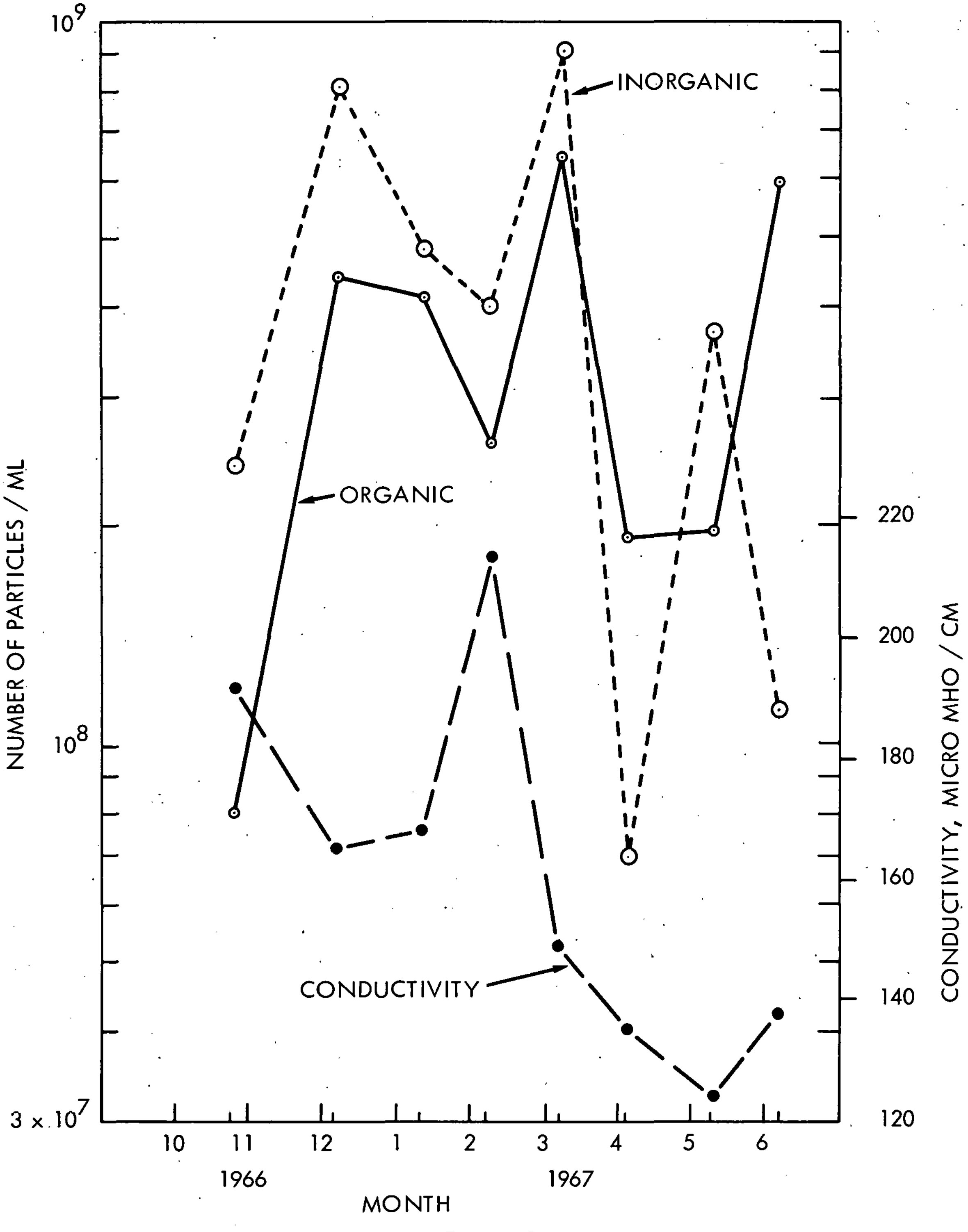
10^9
10^8
3×10^7
NUMBER OF PARTICLES / ML
INORGANIC
ORGANIC
CONDUCTIVITY
220
200
180
160
140
120
CONDUCTIVITY, MICRO MHO / CM
10
11
12
1
2
3
4
5
6
1966
1967
MONTH

Figure 2

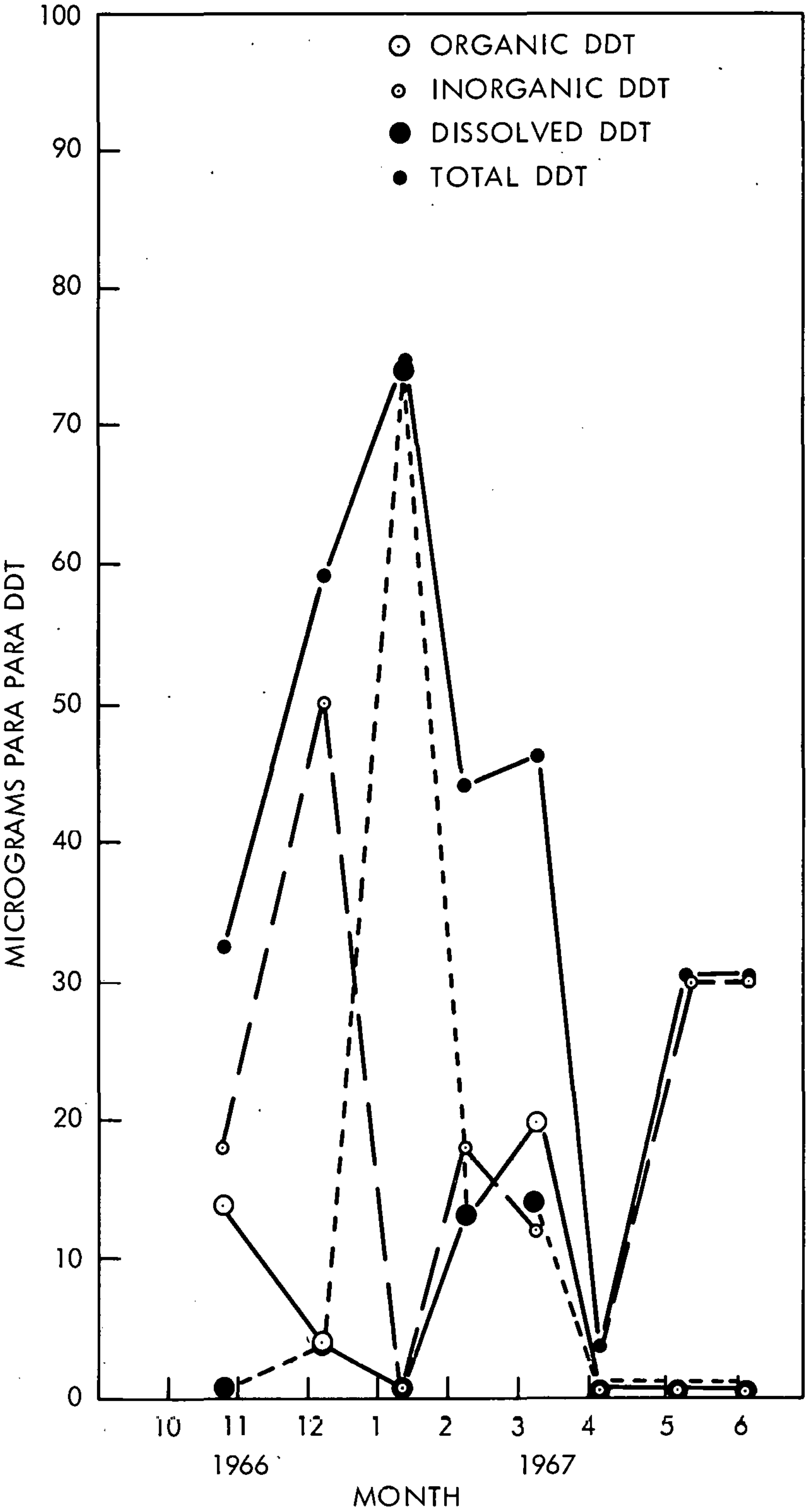
⊙ ORGANIC DDT
⊙ INORGANIC DDT
● DISSOLVED DDT
● TOTAL DDT
MICROGRAMS PARA PARA DDT
0
10
20
30
40
50
60
70
80
90
100
10
11
12
1
2
3
4
5
6
1966
1967
MONTH

Figure 3

Linear correlations were calculated for a number of combinations of variables using the statement:

$$b = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{N}}{\Sigma x^2 - \frac{(\Sigma x)^2}{N}} \text{ and } \tilde{y} = \bar{y} + b(x - \bar{x})$$

Where: x = the amount of DDT,

y = the fraction size,

N = the number of samples,

$\bar{x}$ or $\bar{y}$ = the mean, and

$\tilde{y}$ = the true value of y (13).

The linear correlation coefficient of these values was also calculated and all of these values are shown in table I along with values of Student's "t". The value of r, the regression coefficient of correlation of a linear correlation was calculated from the statement:

$$r^2 = \frac{\left[\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{N}\right]^2}{\left[\Sigma x^2 - \frac{(\Sigma x)^2}{N}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{N}\right]}$$

Where: x = the amount of DDT,

y = the fraction size, and

N = the number of samples (13).

The value of Student's "t" was calculated using the statement:

$$t = \sqrt{\frac{r^2(N-2)}{1 - r^2}} \quad (13).$$

In some cases correlations greater than 0.5 were obtained. There is some correlation between the microscope count of organic particles and both the amount of DDT associated with the organic fraction and with the total DDT, but there is none between the chart record area of organic particles and DDT. This may be due to the fact that the microscope count does not include organic detritus, very small, or rare forms as does the chart recording. The microscope estimate is mostly of bacteria, diatoms, and green algae in an intact condition. If true, this supports the hypothesis that active uptake plays an important role in DDT accumulation. There is a correlation between the amount of suspended inorganic material and the total DDT, but not between it and the DDT associated with the inorganic fraction. The total dissolved ions, determined by chemical analysis of the < 150 S fraction, is correlated with the DDT associated with that fraction. Additional correlations are shown in the table.

TABLE I

ISOLATED FRACTION VERSUS ITS DDT IN μg (Total μg)

x	y	N	b	r	t
Organic area	Organic DDT	8	0.46	0.12	0.29
Organic area	Total DDT	8	0.43	0.41	0.11
Organic count	Organic DDT	8	0.18	0.60	0.18
Organic count	Total DDT	8	0.67	0.60	0.15
Inorganic count	Inorganic DDT	8	0.14	0.27	0.68
Inorganic count	Total DDT	8	0.46	0.66	0.21
Conductivity μmho	< 150 S DDT	8	0.16	0.20	0.51
Conductivity μmho	Total DDT	8	0.27	0.38	0.10
Total ions	< 150 S DDT	6	0.14	0.52	0.11
Total ions	Total DDT	6	-0.14	0.44	0.97
Total Ca	< 150 S DDT	6	0.83	0.34	0.67
Total Ca	Total DDT	6	-0.14	0.46	0.10
Conductivity μmho	Total ions	6	-0.90	0.40	0.88
Conductivity μmho	Organic area	8	-0.18	0.26	0.66
Inorganic count	Conductivity μmho	8	0.11	0.11	0.27

Organic area = chart record area from the density gradient centrifugation.
Organic count = phase contrast microscopy count.
Inorganic count = phase contrast microscopy count.
Conductivity μmho = conductivity of < 150 S fraction in μmho/cm at 25°C.
Total ions = total Ca, Mg, P, K, and Si in mg/ℓ.
Total Ca = total Ca in mg/ℓ.

TABLE I

ISOLATED FRACTION VERSUS ITS DDT IN μg (Total μg)

x	y	N	b	r	t*
Organic area	Organic DDT	8	0.46	0.12	0.29
Organic area	Total DDT	8	0.43	0.41	1.1
Organic count	Organic DDT	8	0.18	0.60	1.8
Organic count	Total DDT	8	0.67	0.60	1.8
Inorganic count	Inorganic DDT	8	0.14	0.27	0.68
Inorganic count	Total DDT	8	0.46	0.66	2.1
Conductivity μmho	< 150 S DDT	8	0.16	0.20	0.51
Conductivity μmho	Total DDT	8	0.27	0.38	1.0
Total ions	< 150 S DDT	6	0.14	0.52	1.2
Total ions	Total DDT	6	-0.14	0.44	0.97
Total Ca	< 150 S DDT	6	0.83	0.34	0.72
Total Ca	Total DDT	6	-0.14	0.46	1.0
Conductivity μmho	Total ions	6	-0.90	0.40	0.88
Conductivity μmho	Organic area	8	-0.18	0.26	0.66
Inorganic count	Conductivity μmho	8	0.11	0.11	0.27

Organic area = chart record area from the density gradient centrifugation.
Organic count = phase contrast microscopy count.
Inorganic count = phase microscopy count.
Conductivity μmho = conductivity of < 150 S fraction in μmho/cm at 25°C.
Total ions = total Ca, Mg, P, K, and Si in mg/ℓ.
Total Ca = total Ca in mg/ℓ

*Corrected value based on the Student "t" equation.

The distribution of the DDT among the isolated fractions may depend on its mode of entry into the water. If the DDT were derived from a terrestrial application that later washed into the stream, one would expect that the DDT still would be associated with the particles which had adsorbed it before its entry into the stream. In this case most of the DDT would be expected to be found associated on suspended or colloidal inorganic particles, and DDT association with this fraction should be highest after a rainy period and especially following periods of crop dusting. There does seem to be a seasonal variation in the observed DDT distribution that can be roughly related to expected periods of crop dusting, growing seasons, and rain distribution.

The seasonal distribution of DDT in the organic fraction also appears to roughly follow the expected planktonic population variations with an obvious early spring increase.

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