Solubility of Carbon-14 DDT in Water

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A knowledge of the solubility of DDT in water was required to study the effect of dissolved and undissolved toxicant on bioassays with mosquito larvae. In the present study the DDT was analyzed radiometrically, undissolved particles (>41 A.) were removed by an average ultracentrifugal force of 84,150 G with the amount of impurity held to a minimum by avoiding the use of a large excess of solute. The solubility of DDT in water was found to be 1.2 p.p.b. or less at 25° C. Data on sizes of undissolved DDT particles and on the recovery of DDT after ultracentrifugation at 1 G are presented.

During investigations on the physical behavior of DDT in aqueous suspensions (*2*), it became necessary to know the solubility of DDT in water in order to evaluate the effect of dissolved and undissolved toxicant on mosquito larvae bioassays. Several solubility values are recorded in the literature. Neal *et al.* (*5*) reported 1000 p.p.b. at room temperature. Richards and Cutkomp (*6*) obtained 0.2 to 1.0 p.p.b. after limited ultracentrifugation and bioassay with mosquito larvae at 15° C. Roeder and Weiant (*7*) reported 10 to 100 p.p.b. at room temperature based upon symptoms of DDT poisoning in the ventral nerve cord of roaches while Gauvadan and Poussel (*3*) approximated 100 p.p.b. at 18° C. by nephelometry. Babers (*1*) obtained 37.4 p.p.b. at 25° C. from radiometric analyses. The determination of the solubility of DDT was undertaken to resolve these conflicting values.

The extraordinarily low solubility of DDT made the determination of its precise value an unusually difficult task, and sources of error not associated with the solubility study of a more soluble compound had to be eliminated. Minute, undissolved particles of DDT formed during the preparation of saturated aqueous solutions do not aggregate readily, so that the use of conventional methods, such as filtration or mild centrifugation, fails to remove all the particles and gives high results. Ultracentrifugation was therefore used in this study.

To permit the estimation of a few parts per billion, in the limited volume of sample available, through the use of prolonged ultracentrifugation, DDT was determined radiometrically by using a sample of high specific activity. In using this procedure it was found that a small percentage of radiolabeled impurity in the DDT markedly influenced the determination. This impurity was kept below the experimental error by avoiding the use of a large excess of solute. The purity of the carbon-14 DDT was established by paper chromatographic and radiometric analyses.

Another complicating factor was the tendency of DDT to accumulate at the interfaces of aqueous suspensions, with a consequent decrease in its concentration in the liquid (*2*). It was necessary to determine the loss of DDT from the filtrate at 1 G—no centrifugation—for several intervals of time, in order to evaluate the results of ultracentrifugation for the corresponding periods.

The solubility of DDT in water at 25° was not more than 1.2 p.p.b., a value that differs markedly from all but one of those reported.

Experimental

A Spinco Model L preparative ultracentrifuge equipped with a 26° No. 40 rotor was used. The samples were centrifuged at 25° ± 0.5° C. in stainless steel tubes fitted with aluminum caps. The loss of DDT due to codistillation (*2*) was prevented by using closed systems.

The diameter, d (cm.), of an undissolved particle of DDT that can be removed by ultracentrifugation from water at 25° C. may be calculated as follows:

$$d = \sqrt{\frac{370\ \eta}{\pi^2 T_s P_i (\sigma - \rho)}} \qquad (1)$$

where η is the viscosity of the medium (0.00894 poise), T_s is the precipitation time (minutes), σ and ρ are the specific gravities of DDT (1.556 grams per ml.) and of water (0.997 gram per ml.), and P_i is the performance index of the ultracentrifuge rotor,

$$P_i = \frac{(\text{r.p.m.})^2}{\log_e R_{max} - \log_e R_{min}} \qquad (2)$$

where r.p.m. is the revolutions of the rotor per minute, and R_{max} (5.9 cm.) and R_{min} (3.8 cm.) are the radial distances to the bottom of the top half and to the meniscus of the fluid.

Chromatography of DDT. About 20 γ of the *p,p'*-DDT-4-C^{14} (*3*)—specific activity 2.1 μc. per mg.—was chromatographed with dimethylformamide and heptane by the procedure described by Mitchell (*4*). The developed chromatogram was sprayed by the procedure of Winteringham (*8*) with potassium permanganate–benzidine reagent. The sprayed chromatogram was dried, sectioned, and counted in the proportional counter.

Preparation of Dispersion. The aqueous solution of the carbon-14 DDT was prepared in a glass-stoppered flask by removing the solvent at 30 to 40 mm. of mercury pressure from an acetone solution containing the appropriate quantity of carbon-14 DDT, and then sufficient water, redistilled from glass, was added to the residue. The mixture (15 p.p.b.) was heated and shaken for 1 hour in a water bath at 90° to 100° C. Then it was mechanically shaken at approximately 25° C. for 1 week and filtered through a fritted glass funnel (porosity 4.5 to 5 microns), and the filtrate was stored in a glass-stoppered flask at this temperature.

Methods of Sampling and Analysis. The flask with the filtrate was shaken to ensure a homogeneous dispersion of the small particles immediately before each pipetful was withdrawn either for analysis or for addition to the centrifuge tubes.

At the end of each experiment, the top half of the liquid was removed from all the ultracentrifuge tubes and combined. This was accomplished by means of a

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syringe equipped with a 40.5-mm., 18 gage stainless steel cannula that was inserted through the cap after the setscrew was removed. The syringe and cannula were finally rinsed with isooctane, which was added to the combined aqueous samples. The mixture was extracted with isooctane and the extract was analyzed radiometrically in the manner described by Bowman *et al.* (*2*). The top half of the liquid was analyzed because the particles removed from the top half are smaller than those removed from the entire liquid and the error caused by stirring back is minimized.

Distribution of Carbon-14 DDT at 1 G and after Ultracentrifugation. The decrease in concentration of carbon-14 DDT in the filtrate was determined after it had stood for various periods in the stainless steel, ultracentrifuge tubes at 1 *G*. Five groups of six tubes, each tube filled with 12.8 ml. of the filtrate, were capped. The liquid was sampled and analyzed immediately or after 1, 3, 6, and 12 hours.

The change in concentration of carbon-14 DDT at various centrifugal forces *vs.* time was also determined. Groups of six tubes were prepared as described above and centrifuged either at 5000 r.p.m. for 1 hour or at 39,400 r.p.m. for 3, 6, or 12 hours. The contents of the tubes were then sampled and analyzed.

Particle Size of Carbon-14 DDT in Filtrate. The diameters of the particles that could be precipitated by ultracentrifugation, as described above, were calculated by substituting the appropriate values of P_i and T_s in Equation 1. Particles larger than 1100 A. were removed during 1 hour of centrifugation at 5000 r.p.m. and those larger than 83, 59, and 41 A. during 3, 6, and 12 hours at 39,400 r.p.m. These calculated values were then related to the quantities of carbon-14 DDT that were lost from the filtrate during the various periods of centrifugation. The periods of acceleration and deceleration are short in the Spinco instrument and were disregarded in calculations because their influence is negligible. At 5000 and 39,400 r.p.m., average centrifugal forces of 1356 and 84,150 G were applied to the top half of the liquid.

Results and Discussion

The carbon-14 DDT used in this investigation was found by paper chromatography to contain a zone of impurity (more polar than DDT) that remained at the origin and reduced permanganate. This zone (57 counts per minute) and that of DDT (9757 counts per minute) at R_f 0.85 were the only zones that were detected radiometrically on the chromatogram. Mitchell (*4*) reported R_f 0.83 for DDT. The zone of impurity contained 0.58% of the total radioactivity detected on the chromatogram.

Preliminary experiments were made with filtrates from 1000 p.p.b. dispersions to determine the appropriate type of centrifuge tube, the effect of stirring back, and the approximate solubility of DDT in water. Adsorption of DDT from the filtrate by stainless steel tubes was found to be considerably less than by cellulose nitrate or polypropylene tubes, quantitative recovery of the insecticide being obtained only from the steel tubes. The fact that concentrations of DDT in the filtrate from the top and bottom halves of the steel tubes after prolonged ultracentrifugation were almost identical indicated that stir back was negligible. After prolonged centrifugation, the 1000 p.p.b. filtrate was found to contain radioactivity equivalent to 6.5 p.p.b. of DDT. However, as much as 5.8 p.p.b. of this could be due to the 0.58% radiolabeled impurity, depending on its water solubility.

In subsequent experiments, the initial concentration of DDT in the dispersion was reduced to 15 p.p.b. Only 0.09 p.p.b. (0.58% of 15 p.p.b.) could be impurity. The filtrate from this preparation contained 7.4 p.p.b. of carbon-14 DDT (corrected for impurity) and included undissolved particles less than 5 microns in diameter that the filter permitted to pass. The concentrations in the filtrate after standing in stainless steel tubes at 1 G for the intervals used in the centrifugation experiments are shown in Table I. When the filtrate was placed in the tubes and withdrawn immediately, the concentration decreased sharply, and then finally decreased during prolonged standing to about 42% of its original value. It is recognized that this affinity of the DDT for the walls of the tubes might have had some influence on the values for the solubility and particle sizes reported in this paper. However, since the change in concentration was less than that incurred by the precipitation of particles >1100 A. and the change during the 6- to 12-hour interval was small, no adjustment of the data was made for this effect.

The concentrations of DDT in the filtrate after various periods of ultracentrifugation are also shown in Table I. These results demonstrate the presence of undissolved particles of carbon-14 DDT in the submicron range, since a change in concentration was effected after particles larger than 1100 A. had been precipitated. After the 6-hour ultracentrifugation period, the DDT concentration remained substantially unaffected, changing only within experimental error (±0.12 p.p.b.) during the subsequent 6-hour centrifugation interval. Based upon the concentration of DDT remaining after removal of particles larger than 41 A., the solubility of DDT in water at 25° C. was approximated at 1.2 p.p.b., which must be considered as a maximum value. In another experiment using the filtrate from a dispersion initially 50 p.p.b., this solubility was confirmed when the value 1.0 p.p.b. (corrected for impurity) was obtained. Even the preliminary experiment with the 1000-p.p.b. suspension falls nicely into line, if the apparent DDT solubility value is corrected for 0.58% impurity assuming complete solubility of impurity: 6.5 (found) − 5.8 (impurity) = 0.7 p.p.b. This value is surprisingly close to the 1.2 p.p.b. found, considering that most of the radioactivity was due to impurity.

The impurity that caused the high apparent solubility value in the preliminary experiment may have been largely responsible for the high value reported by Babers (*1*) who also determined DDT's solubility radiometrically. It is surprising that the solubility reported by Richards and Cutkomp (*6*) approaches the value reported herein, since they used a much less sensitive method of analysis and apparently did

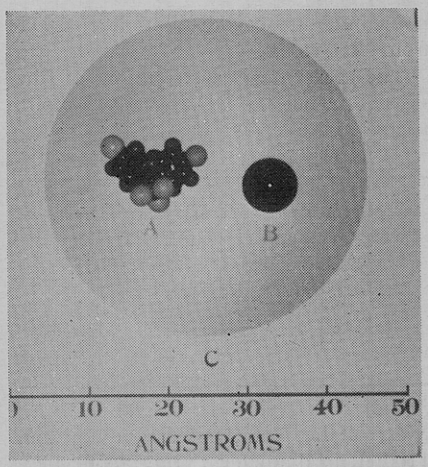

Figure 1. Relative sizes of the DDT molecule *A*, a sphere *B* equal in volume to *A*, and the smallest particle of DDT C that can be precipitated by ultracentrifugation under the experimental conditions employed

Table I. Concentration of Carbon-14 DDT in Filtrate, Initially 7.4 P.P.B., after Various Periods of Time in Stainless Steel Tubes

Hours	Carbon-14 DDT[a], P.P.B. After standing at 1 G	Carbon-14 DDT[a], P.P.B. After centrifugation
0	5.3	...
1	5.0	2.8[b]
3	4.1	1.8[c]
6	3.4	1.4[c]
12	3.1	1.2[c]

[a] Corrected for 0.09 p.p.b. impurity.
[b] At 5000 r.p.m.
[c] At 39,400 r.p.m.

not consider the codistillation of DDT from test systems.

The percentage distribution of the sizes of particles of DDT in the 15-p.p.b. preparation is shown below:

Diameter, A.	Per Cent
>50,000	50.7
1100–50,000	30.7
83–1100	6.6
59–83	2.7
41–59	1.3
7–41	8.0

Most of the particles were larger than 1100 A. in diameter. Only about 10% were in the range of 41 to 1100 A., but this quantity, if it were not removed from the filtrate, is sufficient to cause serious error in the solubility value.

Figure 1 shows a model of the DDT molecule—constructed from Fisher-Hirshfelder-Taylor atomic models—as compared with a sphere 7.1 A. in diameter that has a volume equivalent to that of the DDT molecule, and with the smallest particle, 41 A. in diameter, that could have been removed from the filtrate in the centrifugation experiments. The shape of the molecule is close enough to a sphere that Stokes' law may be used for the determination of particle size without appreciable correction. A centrifugation time in the No. 40 rotor of about 17.5 days at 39,400 r.p.m. is required to remove undissolved amicron particles of DDT larger than molecular size from the top half of the filtrate. Fortunately, stability to ultracentrifugal force was approached within 6 hours and it was not necessary to centrifuge for such an impractical period.

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INSECTICIDE RESIDUES IN MILK

Effects of Feeding Low Levels of Heptachlor Epoxide to Dairy Cows on Residues and Off-Flavors in Milk

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Heptachlor epoxide was fed to dairy cows at 0.5 and 1.0 p.p.m. of roughage intake. Analysis of the butter fat indicated residues reached a level of 0.38 and 1.94 p.p.m. for the two levels, respectively. These feeding rates simulate the residue of heptachlor epoxide shown to be present on alfalfa after practical application of heptachlor.

HEPTACHLOR is an effective insecticide for controlling many insects. It is recommended, particularly for the control of the meadow spittlebug and alfalfa weevil in the Northeast, at dosages ranging from 0.25 to 1 pound per acre, (Gyrisco *et al.*, *2*). Gannon and Decker (*1*) reported that heptachlor was converted to its epoxide on alfalfa and resulted in a residue of 2.42 p.p.m. when as little as 1 pound per acre of heptachlor was applied; 0.17 p.p.m. of the epoxide was still present after 21 days.

Heptachlor epoxide is more persistent and toxic than heptachlor. An experiment was conducted, therefore, to determine if heptachlor epoxide is excreted in the milk of cows fed levels of it, duplicating what comes from forage as a result of practical applications of heptachlor for insect control. Technical heptachlor epoxide was fed to dairy cows at levels of 0.5 and 1.0 p.p.m. of their average total daily roughage for a period of 2 weeks.

Materials and Methods

Three Holstein cows were assigned to each level of technical heptachlor epoxide and five cows were to serve as controls. The cattle were housed at random, in stables so constructed that no animal could steal food from her neighbor. Each cow was fed 40 pounds of hay, 50 pounds of silage, and grain of a good general herd mix at a rate roughly approximating 1 pound of grain for each 4 pounds of milk produced. All feed was carefully weighed out and portions not eaten were weighed and recorded. Thus, an accurate record of food intake was available for each animal over the 2-week standardization or pretest period and the following 2-week test period.

Each week the actual dosage of technical heptachlor epoxide fed to each cow was based on the dry weight of the previous week's intake of roughage. The amount of technical epoxide to be fed each cow to obtain the desired 0.5 or 1.0 p.p.m. dosage was weighed out carefully on a microbalance for each day, divided in half, and added to 1-pound lots of grain. Just prior to grain feeding for the herd each morning and evening, the pound of treated grain was fed in metal containers to each cow. Untreated grain was fed in a similar manner to the five control cows. Samples of milk consisting of 1 quart of fresh, well-mixed, raw milk were taken at the regular morning and evening milkings at −1, 1, 2, 3, 4, 5, 7, and 14 days, while the heptachlor epoxide was being fed, and again at 16, 18, 21, and 28 days after the initiation of the experiment.

Prior to feeding of the heptachlor epoxide and once a week thereafter, additional samples of milk were taken for butterfat, flavor, and odor tests.

The analytical procedure of Meyer, Malina, and Polen (*3*) was used, with several modifications, to determine hep-