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INSECTICIDE METABOLISM

Conversion of DDT to DDD by Bovine Rumen Fluid, Lake Water, and Reduced Porphyrins

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DDD is often detected as a residue in situations where only DDT has been used, and DDD appears to persist for unusually long periods. Studies with C^{14}-DDT incubated with bovine rumen fluid, lake water, and aqueous solutions of reduced porphyrins showed partial conversion to C^{14}-DDD. Conversion by bovine rumen fluid may explain certain DDD residues in milk, and conversion by lake water could account for the apparent extraordinary persistence of DDD in Clear Lake, Calif., because DDT may be available as a continuing precursor for DDD in these situations. The study with reduced porphyrins indicates a possible mechanism for this conversion in biological systems.

DDD [1,1-bis(*p*-chlorophenyl)-2,2-dichloroethane], also known as TDE, is a widely recognized and commonly used insecticide. Since it is a residual compound, its residues have been found on treated crops and in animals consuming such crops. However, DDD residues have also appeared in locations not treated with DDD and in animals under conditions where contact of the animals with DDD was unlikely or impossible. In 1963, Finley and Pillmore (*5*) reported the presence of DDD in a large number of water, soil, vegetation, and animal samples taken from an area where only DDT [1,1-bis(*p*-chlorophenyl) - 2,2,2-trichloroethane] had been used. Of 300 samples found to contain DDT, 70% also contained DDD. In another area treated with DDT, they found DDD as well as DDT in mule deer. During the first year after treatment with DDT, fat tissues from these deer showed more DDT than DDD, but sampling a year or more later revealed equal or greater amounts of DDD than DDT. DDD also appeared in birds, frogs, fish, and toads in yet a third area treated with DDT. Peterson and Robison in 1964 (*11*) showed that DDD was present in rats fed pure DDT. DDD was also reported to be present in mice by Barker and Morrison (*2*), who found DDD in DDT-treated mice after 2 to 8 days' incubation at room temperature following death.

DDD appears to be formed from DDT in various biological systems. Kallman and Andrews (*9*) demonstrated this conversion by yeast, using radiolabeled material. Allison *et al.* (*1*) found that other microorganisms accomplished the conversion, and Bridges *et al.* (*3*) found DDD in fish and crayfish taken from a pond treated with DDT. Peterson and Robison (*11*) noted DDD formation during incubation of DDT with a rat liver homogenate for 6 days, the part played by putrefaction being unknown. Recently, Castro (*4*) found that DDT was converted to DDD in the presence of ferrous deuteroporphyrin in an anhydrous and anaerobic solution consisting of isopropyl alcohol–acetic acid (1 to 1) under nitrogen saturated with potassium chloride.

In 1961, Heineman and Miller (*7*) reported the insecticide content found in 4000 milk samples collected throughout the United States. In the positive samples, 90% contained DDT, 12% contained DDD, and 43% contained DDE [1,1-bis(*p*-chlorophenyl)-2,2-dichloroethylene]. In 1963, Rollins (*12*) reported that DDD was found in a large number of milk samples collected in California and that DDD was rarely found in hay or other feed. Since milk samples containing DDD always contained DDT, he suggested, without definite experimental evidence, that DDT was degraded to DDD in the rumen of the cow.

The use of DDD to treat Clear Lake, Calif., for control of the Clear Lake gnat, *Chaoborus astictopus* Dyar & Shannon, has resulted in local controversy because of the persistence of the DDD

residues found in fish and birds living in the treated area. DDD was applied once annually in 1949, 1954, and 1957. In 1960, Hunt and Bischoff (*8*) reported that in the Clear Lake area large amounts of DDD were found in the fish, and they attributed western grebe losses to DDD poisoning. The Schechter-Haller method was used for determination of the DDD without correction for DDT because no DDT had been applied to the lake. DDD was still evident in Clear Lake wildlife in 1964. Since DDD application to the lake had been discontinued for 7 years and since DDT, but not DDD, was used in the interim in adjacent agricultural areas as reported by Swift in 1963 (*14*), it was considered that the conversion of DDT to DDD was a logical source of the DDD now found in the lake.

Identification of DDD as a DDT metabolite may involve a variety of analytical approaches. Since DDD is a constituent of technical DDT, it is necessary to establish that potential contamination by the DDD impurity does not contribute to residues reported as DDD throug h concentration of this impurity. It is not surprising that the conversion of DDT to DDD was not observed for many years, because the standard Schechter-Haller DDT analysis does not differentiate DDD from DDT. Finley and Pillmore (*5*) and Peterson and Robison (*11*) conclusively identified DDD as a metabolite of DDT in animal tissue by infrared analyses coupled with paper chromatography. Radiolabeled DDT was used by Kallman and Andrews (*9*) and others to study this conversion and was also employed in the present studies.

Experimental

In March of 1964, a sample of water was taken from the surface of Clear Lake at Lakeport, Calif., and incubated with ring-labeled C^{14}-DDT at a 0.01-p.p.m. level for 7 days at room temperature in a stoppered flask. The water was extracted with *n*-hexane, and this extract was chromatographed on paper, using the I/M system as reported by Menzel *et al.* (*10*). Seventy to 80% of the C^{14} material chromatographed in the position coinciding with the mobility of DDD. Cochromatography of this recovered material with authentic unlabeled DDD further supported its identity. Thin-layer chromatography (TLC), using silica gel G and two-dimensional development with *n*-hexane–ethyl ether (95 to 5 v./v.) followed by *n*-hexane–benzene (95 to 5 v./v.) (system suggested by F. A. Gunther, Department of Entomology, University of California, Riverside, 1964), was used to study further the conversion of DDT to DDE, DDD, and DDDE [1,1-bis(*p*-chlorophenyl)-2-chloroethylene]. Each of these materials was adequately separated by this chromatographic technique for identification; so this TLC system was used for the *n*-hexane extract of lake water. Radioautographs (using x-ray film) showed the presence of a spot with the same mobility as DDD, and cochromatography with unlabeled DDD demonstrated it to be DDD. Further confirmation was achieved by treating the metabolite recovered from Clear Lake water with ethanolic potassium hydroxide and cochromatographing the labeled degradated metabolite formed with authentic unlabeled DDDE. The resulting alkali-treated metabolite was found to be identical with DDDE.

Table I. Conversion of C^{14}-DDT to C^{14}-DDD on Incubation for 7 Days at Room Temperature with Clear Lake Water Samples

Source of Sample	DDD Content, %
Konocti Bay	10
Lakeport	0
Middle Lake	1
Middle Lake plankton	83
Sherman Oaks Arm	31
Sherman Oaks Arm plankton	95

Six water samples taken from different areas of Clear Lake in May of 1964 failed to show a uniform DDD content as the result of conversion of DDT to DDD (Table I). These samples were treated as described above. The conditions necessary for conversion of DDT to DDD by Clear Lake water were not investigated further, but it was observed that the extent of conversion was greater in the samples containing large amounts of plankton. However, we believe that variation in oxygen content of water in different areas of the lake and different rates of oxygen depletion on incubation, caused by different population levels of flora and fauna, may have contributed to the scattered results. Distilled water failed to show any conversion of DDT to DDD, nor did boiled distilled water under vacuum.

The effect of stagnating bovine rumen fluid on DDT also was examined.

Samples of rumen fluid were obtained from a fistulated animal at the University of California at Davis. These samples were collected 2 hours after feeding time and filtered through four layers of cheesecloth to remove large particles. C^{14}-DDT was added at a 0.04-p.p.m. level, and the nearly filled and stoppered flasks were incubated at room temperature for 0 to 24 hours prior to extraction.

The proportion of the total C^{14} recovered as DDD increased progressively with time (Table II). A boiled sample of rumen fluid incubated with C^{14}-DDT for 24 hours was inactive in the conversion to C^{14}-DDD. The identity of the DDD when present was confirmed by cochromatography on paper and TLC systems, and by conversion to DDDE and cochromatography of this C^{14}-labeled dehydrohalogenation product on TLC with authentic DDDE. In these experiments with rumen fluid, DDD was the only DDT metabolite found by chromatography.

Table II. Conversion of C^{14}-DDT to C^{14}-DDD on Incubation at Room Temperature with Bovine Rumen Fluid

Incubation Time, Hours	DDD Content, %[a]
0	0
3	11
6	15
9	35
12	45
24	65

[a] Average of two analyses—no more than 6% variation between samples.

To check the conversion of DDT to DDD in the presence of aqueous solutions of porphyrins, experiments were made with hematin and hemoglobin, two representatives of this group.

C^{14}-DDT was added to a Thunberg tube containing 5.0 ml. of boiled, distilled water and 100 mg. of hemoglobin. This was followed by the addition of 10 to 100 mg. of sodium dithionite ($Na_2S_2O_4$) to the side-arm bulb, and the system was degassed with a vacuum. Following the removal of the air, the contents of the tube were mixed and shaken at room temperature for 4 hours. The pH of this mixture was 6.5 at the start of the reaction and 6.6 at the end of 4 hours of incubation. The same experimental procedure was used with hematin, except that 5 ml. of 0.05*N* NaOH were added instead of distilled water to solubilize the 1.0 mg. of hematin used. Very little conversion took place unless the hematin was dissolved. The alkali produced an initial pH of 11.6, which dropped to 11.2 after 4 hours of incubation.

Following the 4-hour shaking, 15 ml. of acetonitrile were added to each 5 ml. of reaction mixture, and this admixture was extracted with 30 ml. of chloroform. The lower organic phase was dried by passage through anhydrous sodium sulfate. The total volume recovered from each reaction mixture was reduced by evaporation under an air stream; and an aliquot was chromatographed, as in the rumen fluid and lake water experiments. The criteria for identification of DDD were the same as those previously described.

The per cent of DDD found was based on total radioactivity recovered from the reaction mixture. In the case of hemoglobin and hematin this recovery ranged from 60 to 75%. Neither hematin nor hemoglobin produced any effect on DDT in the absence of sodium dithionite; and, although sodium dithionite alone did produce some DDD, it did not do so at the level found when

Table III. Conversion of C^{14}-DDT to C^{14}-DDD in Presence of Hemoglobin, Hematin, and Sodium Dithionite

Hemoglobin, Mg.	Hematin, Mg.	H_2O or 0.05N NaOH	DDD Content[a] at Various $Na_2S_2O_4$ Levels, %			
			0 mg.	10 mg.	15 mg.	100 mg.
100	0	H_2O	0[b]	16[b]	34[b]	20, 40
0	0	H_2O	0	1.8[c]	2.8[d]	8
0	1.0	NaOH	0	10[b]	...	...
0	0	NaOH	0	2[b]	...	...

[a] DDD (R_f = 0.36) was only one of products formed from DDT (R_f = 0.45). DDE (R_f = 0.57) but no DDDE (R_f = 0.52) was present in all alkaline incubation mixtures, and more polar materials (R_f = 0.00 and 0.22) were also present with hematin alkaline solution. Indicated R_f values are with TLC system using hexane-ether (95:5) solvent.
[b] Average of two separate analyses; individual amounts not deviating more than 0.7% in reported DDD content.
[c] Average of four analyses.
[d] Average of three analyses.

it was combined with hematin and hemoglobin (Table III). It was found that anaerobic conditions were essential for the production of DDD because no conversion took place unless the color remained red—the color of the reduced state of porphyrins.

Discussion

These studies extend the list of biological systems in which DDT is known to be converted to DDD to include water from Clear Lake, Calif., stagnating bovine rumen fluid, and aqueous solutions of reduced porphyrins.

The apparent unusual persistence of DDD in Clear Lake following use for gnat control may have resulted in part from formation of additional DDD from the DDT leaching into the lake from adjacent agricultural areas treated with DDT. DDD may have appeared in milk because of contamination of fodder with DDT, and conversion in the rumen prior to absorption and secretion into the milk.

DDD is not an end product but, rather, it is an intermediate in metabolism. But, in the case of the microorganisms used in this investigation, DDD appeared to be very stable, as was the case with microbial decomposition systems previously examined. In certain insects, DDD is known to be converted to DDDE (*13*), and to the hydroxylated material FW-152 [1,1-bis(*p*-chlorophenyl) 2,2-dichloroethanol] (*6, 15*). Peterson and Robison (*11*) have established that DDD can be recovered from DDT-treated rats and that DDDE can be found in DDD-treated rats. Further, they found stepwise degradation on feeding each product, in turn, along the sequence of saturation of the DDDE followed by additional dehydrochlorination and, finally, by hydroxylation and oxidation to form the acetic acid derivative, DDA [1,1-bis(*p*-chlorophenyl)acetic acid]. DDD appears to be more stable than DDT in certain biological systems. This point must always be considered in interpretation of earlier studies on DDT metabolism and residues, and in the design of further experiments on the metabolic conversion of the trichloroethane to the dichloroethane grouping.

The fact that porphyrins, under the proper reducing conditions, can convert DDT to DDD indicates one possible mechanism to explain this conversion in many biological systems.

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