DDT-Dehydrochlorinase for Identification of DDT in Soil

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It is well recognized that reports of insecticides in naturally occurring materials are most reliable if both the identity and concentration of the insecticide can be determined by two or more independent methods. This often necessitates the use of gas or thin layer chromatography, I.R., N.M.R., or mass spectroscopy and thus requires two or more, often expensive, pieces of equipment. An alternative to independent identification methods is the formation and identification of derivatives of the suspect insecticide.

Use of derivatives for DDT confirmation has the advantage that certain derivatives (eg. DDE) may be detected using the same equipment (eg. gas chromatograph) as the DDT. Several derivatives of DDT have been reported; the simplest to form being DDE. The equilibrium for this reaction favours the formation of DDE (Keq > 1,000, 1) so that essentially complete conversion to DDE is possible. The use of the enzyme DDT-dehydrochlorinase (E.C. 4.5.1.1.) has the advantage of converting DDT to DDE under relatively mild conditions so that non-DDT molecules are unlikely to be destroyed.

DDT-dehydrochlorinase used in these experiments was extracted from DDT resistant houseflies by homogenizing 100 g of adult flies in 200 ml 10 mM phosphate buffer containing 250 µg EDTA/ml (pH 7.4) using a Sorvall omni-mixer in an ice bath. After centrifuging at 20,000 g for 30 minutes the precipitate was rehomogenized and centrifuged. The supernatants were combined and the DDT-dehydrochlorinase partially purified as follows. The extract was adjusted to pH 5.0 by dropwise addition of 2 M acetic acid and centrifuged at 20,000 g for 10 minutes. The supernatant was fractionated by addition of solid ammonium sulfate; the fraction precipitating between 40% and 80% saturated ammonium sulfate was dissolved in phosphate-EDTA buffer and dialyzed against several changes of the buffer (10 mM phosphate, 250 µg EDTA/ml, pH 7.4) overnight. The volume at this point is about 70 ml, and it may be frozen in small batches for later use. Immediately before use the enzyme is diluted so that it is in 20 mM phosphate, 10 mM reduced glutathione buffer containing 500 µg EDTA/ml (pH 7.4). The exact amount of dilution permissible depends upon the enzyme activity and must be determined for each batch of enzyme. Further purification of DDT-dehydrochlorinase following the method of Dinamarca et al (2) can be carried out but is not necessary for the proposed use here.

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The following procedure used for confirming DDT identification by enzymatically converting DDT to DDE is based on the DDT-dehydrochlorinase assay procedure of Oppenoorth and Voerman (3). Into a screw cap culture tube place 1.0 ml of the sample containing from 0.001 µg to 10.0 µg DDT and evaporate to dryness by gently passing a stream of air into the tube. The residue is dissolved in 0.2 ml dimethyl sulfoxide. Add 2.0 ml of the DDT-dehydrochlorinase solution in 20 mM phosphate, 10 mM reduced glutathione buffer containing 500 µg EDTA/ml (pH 7.4). The tube is gased with nitrogen for 30 seconds and the screw cap attached. Incubate at 40 C for 30 minutes then add 2 ml of a saturated solution of Na_2SO_4 and 10 ml of a 2:1 cyclohexane: 2-propanol mixture and shake vigorously for 1 minute. The organic layer is transferred to 10 ml of demineralized or distilled water and shaken for 1 minute. The cyclohexane layer may now be injected into a gas chromatograph or, if the concentration of the insecticide is too low, the solvent may be evaporated and the residue dissolved in a smaller volume and injected into the GC. In preliminary experiments it was found that a single extraction with cyclohexane: 2-propanol was sufficient to extract all the DDT and DDE.

For insecticide detection we used a Varian Aerograph series 1200 gas chromatograph equipped with a 3 mm (O.D.) x 90 cm Pyrex column packed with 5% Dow-11 on chromosorb W HMDS, 60/80 and a 250 mc Tritium foil detector. The temperatures of the column, injector and detector were 190 C, 185 C, and 200 C respectively. Nitrogen was used as the carrier gas at a flow rate of 15 ml/sec.

The method outlined above was used to confirm the identification of DDT in material extracted from soil. The extraction method, provided by R.M. Tyo, was carried out by Dr. M.S. Tawfik (manuscript prepared, but under review) in this department and consisted of extraction of soil samples in acetone-acetonitrile followed by a clean-up through petroleum ether and an activated florisil column. Results in table 1 indicate that each of the samples contained a significant amount of material identified as DDT but not metabolizable by DDT-dehydrochlorinase.

TABLE 1

Treatment of soil samples with DDT-dehydrochlorinase.

Sample Number	Amount of material "identified" as DDT pecomoles/ml of sample. Before treatment	After treatment	Metabolized by DDT-dehydrochlorinase
Control	22,560.	0.	22,560.
1	73.	15.	58.
2	986.	680.	306.
3	180.	153.	27.
4	686.	364.	322.
5	854.	604.	250.
6	1,341.	486.	855.
7	632.	338.	294.

There exists the possibility that materials extracted from the soil along with DDT may inhibit DDT-dehydrochlorinase and thus prevent complete metabolism of the DDT present. To check this possibility aliquots of 3 of the soil samples used in table 1 (#2, 3, and 6) were evaporated to dryness after treatment with DDT-dehydrochlorinase and 564 pecomoles of DDT added to each aliquot and then the material was treated with DDT-dehydrochlorinase a second time. The results (table 2) indicate that in 2 of the 3 tests the added DDT was metabolized completely and hence that there was no inhibition of the enzyme by contaminants in the extract.

TABLE 2

Confirmation of DDT-like residue after addition of 564 pecomoles DDT to metabolized soil samples. Numbers in the body of the table are the pecomoles of DDT in the 1 ml sample.

	Soil Sample		
	#2	#3	#6
Before treatment	986	180	1,341
After treatment	680	153	486
Metabolized	306	27	855
After adding DDT	1,082	784	877
After retreatment	339	196	339
Metabolized	743	588	538

A comparison of the retention times of the peaks in the material extracted from soil with the retention times of polychlorinated biphenyl (PCB) standards suggested that most of the contaminents were PCB's. To check for the possible interference by PCB's with the enzymatic dehydrochlorination of DDT the confirmation procedure was run using 0.02 µg DDT with various known PCB's (Aerochlor 1221, 1242, 1248, 1254, 1260, 1262). The results in table 3 indicate that although PCB's interfere with the routine quantitation of DDT, they do not interfere with the enzymatic confirmation of DDT.

TABLE 3

Effect of known PCB's on the dehydrochlorination of DDT*†.

Material	No DDT-ase added "DDE"	No DDT-ase added "DDT"	Incubation with DDT-ase "DDE"	Incubation with DDT-ase "DDT"
Control (0.2 µg DDT)	31.4	567.6	745.8	0.0
1221 alone	60.6	149.4		
1221 + DDT	67.2	607.4	822.2	132.6
1242 alone	486.8	36.2		
1242 + DDT	478.8	624.2	493.4	84.4

TABLE 3 (contined)

Effect of known PCB's on the dehydrochlorination of DDT*†.

Material	No DDT-ase added "DDE"	"DDT"	Incubation with DDT-ase "DDE"	"DDT"
1248 alone	675.2	492.8		
1248 + DDT	709.8	1085.8	234.4	506.2
1254 alone	370.0	28.9		
1254 + DDT	354.4	658.0	453.0	50.6
1260 alone	98.7	349.4		
1260 + DDT	107.6	906.2	599.0	337.4
1262 alone	27.0	132.6		
1262 + DDT	76.2	848.4	634.8	189.2

* values expressed as pecomoles in 1 ml sample
† 0.2 µg or 564 pecomoles DDT added to each sample of PCB.

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