Enzymatic Estimation of Dimethyl 2,2-Dichlorovinyl Phosphate Spray Residues

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Recent interest in certain vinyl and chlorovinyl phosphates for possible use as insecticides has centered on the possible use of dimethyl 2,2-dichlorovinyl phosphate (DDVP) in Hawaii to control the oriental fruit fly and melon fly. The enzymatic method, now in use for estimating residues of demeton, has been modified for the determination of DDVP on subtropical fruits such as pineapple, guava, mango, avocado, and citrus. Residue determinations were also run on Lima bean plants, coleus, and geranium. The modified method consists in extraction of DDVP from plant residues with chloroform, evaporation of the extract in the presence of 20% aqueous alcohol solution, adjustment to pH 7, and measurement of the DDVP by its enzyme inhibition. This method, which is sensitive to 0.5 γ of DDVP, showed that DDVP residues virtually disappeared after 72 hours.

DIMETHYL 2,2-DICHLOROVINYL PHOSPHATE (DDVP) was recently discovered by Mattson, Spillane, and Pearce (*4*) as the dehydrochlorination product of dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate (Bayer L 13/59 or Dipterex). Its structure was established by Barthel, Alexander, Giang, and Hall (*1*) and independently at about the same time by Lorenz, Henglein, and Schrader (*3*). Interest in DDVP has centered about its high knockdown and toxicity to certain insect species, coupled with a considerably lower toxicity than parathion to warm-blooded animals. Preliminary tests indicate that DDVP is especially effective against the oriental fruit fly and the melon fly in Hawaii. Its possible use for the control of these and other insects made it desirable to estimate the residues of sprays containing this compound. Because of its high vapor pressure, the residues would be expected to disappear rapidly. The sensitive, but nonspecific, enzymatic method developed by Giang and Hall (*2*) for determining organic phosphorus insecticides has been modified to adapt it to the determination of DDVP residues on fruit and certain plants grown in the greenhouse.

Apparatus and Reagents

The apparatus has been described (*2, 5*).

DDVP used in making standard solutions was prepared and purified (boiling point 120° C. at 14 mm. of mercury) as described by Barthel and others (*1*).

Standard Buffer Solution. Dissolve 9.9831 grams (0.0542 mole) barbital (diethyl barbituric acid) and 50 ml. of 1*N* sodium hydroxide solution in water in a 1-liter beaker. Then dissolve in this solution 134.21 grams (1.8 moles) of potassium chloride and 1.633 grams (0.012 mole) of potassium dihydrogen phosphate. Using the pH meter, bring the solution to pH 8.1 with 0.1*N* hydrochloric acid (about 10 ml.), transfer it to a 1-liter volumetric flask, and make to mark with distilled water.

Acetylcholine chloride solution, 4 grams (0.22 mole, purified) in 100 ml. of distilled water.

Saline solution, 9 grams of sodium chloride in 1 liter of distilled water.

Standard cholinesterase solution (*2*).

Chloroform, redistilled.

Aqueous alcohol, 200 ml. of 95% ethyl alcohol made up to 1 liter with distilled water.

Procedure

Preparation of Standard DDVP Solution. Weigh into a small weighing cup 100 mg. of the purified DDVP and without delay drop the cup into a 500-ml. volumetric flask and dilute to volume with redistilled chloroform. Then transfer 25 ml. of the solution into another 500-ml. volumetric flask and dilute to volume with chloroform. This gives the standard solution containing 10 γ of DDVP per milliliter. With a 25-ml. microburet run the following aliquots into 150-ml. beakers: 2, 4, 8, 10, 12, 14, 16, and 20 ml. Make each beaker approximately to 50 ml. with chloroform and mix well. Add 50 ml. of aqueous alcohol, and put one or two clean glass beads into each beaker. Evaporate the chloroform on the steam bath until the chloroform layer has just (but completely) disappeared in the beakers.

Cool to room temperature. Adjust the solution in each beaker to pH 7.0 with 0.02*N* sodium hydroxide. Then, transfer each solution into a 100-ml. volumetric flask; rinse each beaker with the aqueous alcohol and with the rinsings dilute the solution in each flask to volume.

Preparation of Standard Curve. Place ten 10-ml. microbeakers on the bench near the constant-temperature unit, number them, and in each beaker place a magnetic flea (*2*). Reserve beakers 1 and 10 as the blanks. Into each of the blanks pipet 2 ml. of the aqueous alcohol, and into each of the other beakers pipet 2 ml. of one of the eight DDVP solutions. Thus, the quantities of DDVP in the eight beakers should be 0.4, 0.8, 1.6, 2.0, 2.4, 2.8, 3.2, and 4.0 γ.

From this point on run the determinations in the way previously described (*2*), but use 2 ml. each of the standard buffer and cholinesterase solutions. As directed in the previous paper, prepare a standard curve by plotting on a semilogarithmic paper the per cent inhibition against the micrograms of DDVP and drawing a straight line best fitting the points.

Extraction of DDVP from Plant Material. Chop the sample of plant material into small pieces and weigh an appropriate quantity for extraction. Place in a wide-mouthed glass jar the weighed sample and a measured volume of chloroform. Take a sample large enough to be representative, and use sufficient chloroform solvent to assure a complete extraction. Cover the jar tightly and tumble on a tumbling machine for at least 1 hour. Filter the extract through a plug of cotton, and evaporate a 50-ml. aliquot (more if necessary) in the manner described for preparation of the standard curve.

As it is desirable to have the final extract contain about 0.5 to 4.0 γ in 2 ml., it may be necessary to make a rough preliminary determination of the DDVP to be expected. Transfer 2 ml. of the extract, adjusted to pH 7.0, into a single

10-ml. beaker. Place a magnetic flea in the beaker, add 2 ml. of the aqueous alcohol, and evaporate on the steam bath until the chloroform is removed. Then add 2 ml. each of standard cholinesterase and buffer solutions, set the beaker in the 25° C. constant-temperature bath, and start the magnetic stirring for 30 minutes' incubation. Add 0.6 ml. of the substrate solution and note the delta pH (change) after 10 minutes. Multiply the delta pH by 6, and from the standard curve estimate the approximate concentration of DDVP in the plant extract. Adjust volume of extract accordingly.

From this point on estimate the DDVP present in the plant extract in the same manner as in the preparation of a standard curve. All determinations should be replicated.

Tests Made with Method

Recovery of DDVP from Chloroform Solution. Because DDVP has a high vapor pressure, it volatilizes appreciably when a chloroform extract is evaporated. While this method was being developed, it was found that if a small volume of the aqueous alcohol was added to this extract the DDVP tended to be held in the aqueous alcohol layer. Therefore, an experiment was set up in which different volumes of a chloroform extract of DDVP were evaporated on the steam bath in the presence of equal volumes of the aqueous alcohol, the residues were made up to 100 ml. in a volumetric flask, and appropriate aliquots were analyzed by the cholinesterase inhibition method. The results were compared with those obtained by analyzing aliquots of DDVP that had been dissolved directly in aqueous alcohol and not subjected to evaporation.

Alcohol and Chloroform, Ml. of Each	DDVP Added, γ	DDVP Recovered	
		γ	%
100	275.0	258.0	94
75	206.0	201.0	97
50	138.0	132.0	96
25	69.0	66.0	96
		Av.	96

Effect of Ratio of Solvent on Recovery of DDVP. To explore the effect of varying the ratio of aqueous alcohol to chloroform on the recovery of DDVP, 50-ml. aliquots of a chloroform solution were evaporated in the presence of different volumes of aqueous alcohol and each was then made to 100 ml. with the aqueous alcohol and analyzed by the cholinesterase inhibition method.

Aqueous Alcohol, Ml./50 Ml. Chloroform	Recovery from 26 γ of DDVP Added	
	γ	%
100	26.0	100
50	26.0	100
25	22.0	85
10	20.0	77

These data indicate that the volume of aqueous alcohol must be at least equal to that of chloroform to ensure a maximum recovery of DDVP.

Recovery of DDVP Added to Young Lima Bean Plants. Aliquots of a standard chloroform solution of DDVP were added to several 29.5-gram portions of chopped young Lima bean plants, extracted with 100 ml. of chloroform, and analyzed by the described procedure.

DDVP Added		DDVP Recovered		Recovery,
γ	P.p.m.	γ	P.p.m.	%
550	18.6	510	17.3	93
275	9.3	253	8.6	92
193	6.5	182	6.2	94
138	4.7	129	4.4	93
55	1.9	52	1.8	95
			Av.	93

DDVP Residues on Fruits and Young Plants. Residue determinations were run on the fruits of guava, mango, avocado, orange, and grapefruit, and the plants of Lima bean, coleus, and Easter lily (Table I). The fruit or plant was dipped for 30 seconds in a 0.1% solution of DDVP in aqueous alcohol and then left to dry at room temperature, which varied from 25° to 37° C. At various intervals up to 72 hours samples were taken for analysis. Entomological tests run by one of the authors (F.F.S.) indicated that the insecticidal effect of DDVP had disappeared after 48 hours, but it was still detectable by the enzymatic method after 72 hours.

Discussion

The method as described was developed primarily for the determination of DDVP in the minute quantities present in plant materials as a result of spray or dust applications. Although no interference has thus far been encountered from blank runs on plant material not treated with DDVP, it is nevertheless always important to run a control analysis on a sample of the untreated material. Chloroform was found to be the most suitable solvent for extracting DDVP from plants. Benzene and petroleum ether, commonly used to extract residues of chlorinated hydrocarbon insecticides, such as DDT and BHC, were unsuitable (as was diethyl ether), because they gave low recoveries. Alcohol, although a good solvent for DDVP, cannot be used for extracting purposes because of its miscibility with water.

The method as described is sensitive to about 0.5 γ of DDVP. By appropriate change of the buffer strength and the concentrations of cholinesterase and acetylcholine chloride solutions, it should be possible to increase the sensitivity of the method further.

Although the closely related insecticide Bayer L 13/59 is also an inhibitor of cholinesterase (I_{50} is approximately 40 γ), inhibition of this compound, when purified, is so much smaller under the described experimental conditions that it need not be considered as much of an interfering material in the determination of DDVP, which has an I_{50} of 2.78 γ, as indicated in the standard curve.

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Table I. DDVP Residues on Fruits and Young Plants

(Parts per million)

Hours after Dipping	Orange		Grapefruit		Mango		Avocado		Guava, Whole Fruit	Lima Bean Plant	Easter Lily Leaves
	Whole fruit	Peel	Whole fruit	Peel	Whole fruit	Peel	Whole fruit	Peel			
1	0.6	1.6	0.3	1.1	0.8	1.8	0.2	1.3	3.7	59.5	42.2
6	0.4	1.2	0.2	0.7	0.6	...	...	...	2.7	33.0	34.1
24	0.2	0.7	0.1	0.2	0.4	1.1	0.1	0.7	1.2	2.1	25.8
48	0.1	0.4	0	0.1	0.2	0.6	...	0.4	0.4	1.3	14.8
72	0.06	0.2	0	0.0	0.1	0.2	...	0.1	0.2	0.5	2.8