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A SIMPLE AND SENSITIVE METHOD FOR THE DETERMINATION OF DICHLORVOS (DDVP) IN AIR[a]

by

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During trials in Nigeria of dichlorvos[e] as an insecticidal vapour for the control of mosquitos inside native huts it became necessary to estimate the substance in air at concentrations in the range 0.01 to 0.1 µg per litre. The determination of the dichlorvos by a procedure developed at the Communicable Disease Center, Savannah, Georgia, United States of America,[1] based on the colorimetric determination of phosphorus described by Chen et al.[2] was not satisfactory owing to the lack of suitable apparatus; the more specific method of Geiger & Fürer[3] proved unreliable for such small concentrations, especially as the air samples from native dwellings are frequently contaminated by smoke.

The specific inhibition of cholinesterase (ChE) by organophosphorus compounds before[4,5] or after[6,7] oxidation has already been used for the estimation of various other substances, while Giang et al.[8] have proposed the method for dichlorvos itself. The property of powerful and direct inhibition of human plasma cholinesterase in vitro is exploited in the present method to give great sensitivity and specificity.

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[e] "Dichlorvos" is the common name approved by the International Organization for Standardization (ISO) for the 2,2-dichlorovinyl dimethyl phosphate, also known as "DDVP"

Experimental

1. Reagents

(a) Standard solutions of pure dichlorvos. Dissolve 0.100 g dichlorvos in 100 ml acetone (solution A). Dilute 10 ml solution A to 100 ml with acetone (solution B).

(b) Buffer solution (Michel,[9]). Dissolve 1.237 g sodium barbital, 0.136 g potassium dihydrogen phosphate (KH_2PO_4) and 17.54 g sodium chloride in 900 ml distilled water, add 11.6 ml 0.1 N hydrochloric acid and dilute to 1 litre. The pH should be 8.0 at 25°C.

(c) Acetylcholine solution. Dissolve either 0.3 g acetycholine chloride or 0.4 g acetylcholine perchlorate in 10 ml water.

(d) Human blood plasma, best separated within 30 minutes from venous blood, with heparin as anticoagulant. The plasma keeps for at least a week at 4°C.

2. Special apparatus

(a) pH Meter capable of measuring changes of up to 2 pH units with a sensitivity of 0.01 pH units.

(b) Gas washing bottles, 250 ml capacity, fitted with No. 1 porosity sintered discs. The inlet and outlet tubes of the washing bottles should be fitted with spherical glass joints to permit connexion of two bottles in series.

3. Procedure

The dichlorvos is absorbed by drawing a measured volume of air (200-300 litres at 10 litres per minute for dichlorvos concentrations in the range 0.005-0.10 g per litre) through 50 ml water in each of two washing bottles connected in series.[a]

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[a] A suitable portable air-pump has been described by D. R. Johnston Report No. 186 and Addendum (1961), Tropical Pesticides Research Unit, Porton, England

(Rubber tubing should not be used to connect the two bottles; considerable loss may result if air containing dichlorvos is allowed to come in contact with rubber or plastic tubing or with grease.) Combine the two sample solutions and transfer to the laboratory as quickly as possible. (Under these conditions Mühlman & Schrader[10] found only one per cent. hydrolysis of dichlorvos after 6-1/2 hours at 30°C.)

Place in each of a number of 5-ml beakers 1 ml sample solution, 1 ml buffer solution and 20 µl human plasma. Shake the beaker for one minute; four minutes later add 0.2 ml acetylcholine solution and mix the contents of the beaker thoroughly. Measure the pH immediately; allow the reaction to proceed for 120 minutes at 25±1°C and note the pH again. The difference in pH is proportional to the ChE activity. The reaction time may be shortened to 60 minutes if the plasma is of high ChE activity.

Run blanks and standards at the same time by substituting distilled water and dichlorvos solutions of known strength for the sample solution. Dilute 1 ml standard solution B to 100 ml with water (solution C). Into each of a number of 100 ml volumetric flasks pipette 0, 1.0, 2.0, 5.0, 10.0 and 20.0 ml aliquots of solution C. (Different aliquots of the standard solution may be more convenient if the ChE activity of the plasma is low.) Dilute to the mark with water to give dilute standard solutions containing 0, 0.01, 0.02, 0.05, 0.10 and 0.20 µg dichlorvos per ml respectively. Place 1.0 ml portions of these dilute standard solutions in 5-ml beakers, add 1 ml buffer solution and proceed as described above.

Prepare a standard curve by plotting dichlorvos concentration (in the range 0-0.20 µg per ml) against the drop in pH (ΔpH) and use this to derive the concentration of dichlorvos in the sample solutions. It may be desirable in some cases to plot the curve on semi-logarithmic graph paper as this gives a closer approach to rectilinearity over part of the concentration range. A typical calibration curve is shown (Figure 1).

It is possible to simplify the technique by using in each beaker 1 ml of a mixture of 2 ml plasma with 100 ml buffer; this avoids the tedious pipetting of 20 µl volumes. Many samples may be handled together by suitable timing of the addition of the acetylcholine solution so as to keep the reaction time constant.

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FIG. 1

CALIBRATION CURVE, DETERMINATION OF DICHLORVOS IN AQUEOUS SOLUTION