Radiochemical Analysis and Purity. The Van Slyke wet-combustion method for radioactivity analysis as described by Neville (*5*) was employed to oxidize the radioactive organic materials to carbon dioxide. The carbon-14 dioxide was collected in an ionization chamber. The ionization current produced in the chamber by the radioactive gas was determined on a vibrating-reed electrometer (Model 30, Applied Physics Corp., Pasadena, Calif.) This current was converted to microcuries of radioactivity by applying a previous calibration of the instrument obtained with a sample of radioactive benzoic acid which was standardized against a radioactive sodium carbonate solution as provided by the National Bureau of Standards.

The radiochemical purity was determined by an isotopic dilution analysis (*1*). A known quantity of pure nonradioactive Sevin was added to a carefully weighed sample of the radioactive preparation. The mixture was repeatedly recrystallized from *p*-xylene, by the procedure described above, until a constant specific activity was obtained. From this result and the known amounts of radioactive and nonradioactive Sevin used, it was calculated that the radiochemical purity of the preparation was 99.7%.

Recovery of Radioactivity. To the mother liquors from the recrystallization of the radioactive Sevin, nonradioactive Sevin was added as a carrier. Evaporation to dryness and recrystallization from boiling *p*-xylene, as before, gave additional pure radioactive Sevin, of a lower specific activity. Mother liquors from this treatment were hydrolyzed back to 1-naphthol by heating with an excess of 5% sodium hydroxide solution. After acidification of the hydrolyzate with 10% sulfuric acid, ether extraction, and drying of the extract over anhydrous sodium sulfate, evaporation of the ether gave the impure naphthol. Purification by vacuum sublimation yielded 1-naphthol-1-C^{14} of melting point 92–3° C. This procedure permitted recovery of nearly all of the original radioactivity in useful material.

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INSECTICIDE ANALYSIS

Colorimetric Determination of 1-Naphthyl *N*-Methylcarbamate in Agricultural Crops

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A colorimetric method of analysis for the insecticide 1-naphthyl *N*-methylcarbamate (Sevin) is described. Alkaline hydrolysis of Sevin produces 1-naphthol which is reacted with p-nitrobenzenediazonium fluoborate to produce a color with maximum absorption at 590 mμ. The method responds in the range of 5 to 40 γ of Sevin.

Sevin (1-naphthyl *N*-methylcarbamate) is an insecticide recently introduced by Union Carbide Chemicals Co. A cholinesterase inhibition method of analysis was recently published by Zweig and Archer (*4*). A more specific colorimetric method of analysis for this compound and one of its breakdown products was developed at the Berkeley and Yakima laboratories. The method is based upon the coupling of a diazonium salt with 1-naphthol, a product of the alkaline hydrolysis of Sevin.

Sevin is extracted from plant tissues with chloroform. The chloroform is evaporated and the residue taken up in a 1 to 1, by volume, water-methanol solution. Fruit waxes are removed by filtration of the cooled water-methanol solution. The filtrate is made alkaline with sodium hydroxide to hydrolyze Sevin to 1-naphthol. After acidification with phosphoric acid, the 1-naphthol is extracted with chloroform (*3*). The chloroform is evaporated, and the residue is taken up in methanol. Sodium hydroxide is added followed by *p*-nitrobenzene diazonium fluoborate to produce a color which measured at 590 mμ by spectrophotometry.

Preparation of Sample

Apple samples are ground in the presence of anhydrous sodium sulfate to produce a dry crumbly mass and are tumbled for 30 minutes with chloroform. Leaf samples are extracted for surface deposits by shaking them for 5 minutes with chloroform.

Pear, peach, and grape samples are homogenized with chloroform in a Waring Blendor, separated, and the chloroform is dried by passage through anhydrous sodium sulfate. The peaches did not give completely satisfactory results (Table I).

Procedure

Evaporate an aliquot of the chloroform extract to about 3 ml. in an evaporative concentrator (*1*). Using chloroform, wash the concentrated solution into a 50-ml. beaker. Evaporation is continued just to dryness with an air stream only. Continued blowing of air after removal of solvent resulted in the loss of Sevin; however, incomplete removal of chloroform gave erratic results. Take up the residue in 5 ml. of anhydrous meth-

anol with gentle warming. To this solution, add 5 ml. of a 2% aqueous ammonium chloride solution and cool in freezer for 5 minutes. After cooling, filter into a 250-ml. separatory funnel using a paper such as S&S 589 or Whatman No. 1. (Use of finer papers should be avoided as filtration time increases.) Rinse the paper twice using a total of 5 ml. of a 1 to 1, by volume, methanol-water mixture cooled to about 5° C. Add 25 ml. of 0.5*N* aqueous sodium hydroxide to the solution in the funnel and mix by shaking. After 3 minutes, add 10 ml. of 19.6% by volume aqueous phosphoric acid solution and mix. Extract the solution with 25 ml. of chloroform by shaking it for 1 minute. Transfer the chloroform phase to a 50-ml. beaker and evaporate to dryness as before, observing the same precautions. The residue is transferred to a 10-ml. volumetric flask with 5 ml. of methanol, using gentle warming to facilitate solution.

The colorimetric reaction is produced by the addition of 1 ml. of 0.5*N* sodium hydroxide followed by 1 ml. of 0.01% methanolic solution (w./v.) of *p*-nitrobenzenediazonium fluoborate. The diazonium salt reagent must be made twice daily and kept in the refrigerator. No appreciable deterioration occurs in the first 3 hours, but a noticeable reduction in absorbance at 590 mμ was observed after 3 hours (Table II). Make up to a 10-ml. volume with methanol and filter the solution through paper into colorimetric or spectrophotometric tubes. Allow the color to develop for 30 minutes. A rapid increase in color occurs in the first 30 minutes but only a slight increase at the end of 2 hours (Table III). The readings are consistent and reproducible when taken at the 30-minute interval. Read absorbance or transmittance at 590 mμ using methanol as a reference.

A standard curve is prepared using a methanolic solution of Sevin containing 10 γ per ml. Aliquots from 0.5 to 4.0 ml. are introduced to 10-ml. volumetric flasks, and the volume is made to 5 ml. with methanol. The procedure is then the same as that described for the samples after introduction to the volumetric flasks.

Interfering colors that appear in analyzing for Sevin in pears can be eliminated by the use of an alumina (Fischer Adsorption Alumina)–celite column 2 to 1, using chloroform as the eluent (*2*). Standard curves obtained from the two mentioned laboratories showed uniform and similar results, and the method can be used to analyse for as little as 5 γ.

Table I. Recoveries of Sevin Added to Indicated Materials

Sample Amount, G.[a]	Sevin Added, γ	Sevin Found, γ	Recovery, %
Apples			
30	0.0	1.3	..
30	12.0	11.8	87
30	..	1.5	..
30	12.0	12.3	90
20	..	1.0	..
20	10.0	10.9	99
20	..	1.1	..
20	10.0	10.8	97
Apple Leaves			
36.6	..	1.1	..
36.6	10.0	11.8	107
36.6	..	0.9	..
36.6	10.0	10.4	95
36.6	..	1.1	..
36.6	10.0	11.1	100
36.6	..	1.0	..
36.6	10.0	11.2	102
Peaches			
20.8	..	4.0	..
20.8	20.8	21.0	81
20.8	..	3.0	..
20.0	20.8	31.0	134
10.0	..	0.3	..
10.0	5.0	4.3	80
10.0	10.0	9.3	90
10.0	10.0	9.4	91
Pears			
10.0	..	7.5	..
10.0	10.0	17.5	100
10.0	..	12.5	..
10.0	10.0	22.0	95
Grapes			
10.0	..	2.2	..
10.0	5.0	6.0	76
10.0	10.0	9.7	75
10.0	2.5	4.0	72
20.0	..	5.0	..
20.0	20.0	26.0	105
20.0	20.0	23.0	90
20.0	20.0	27.0	110

[a] Sample amounts for apple leaves given in square centimeters.

Table II. Effect of Time on Diazonium Salt Stability Prior to Color Development

Hours					
0	1	3	5	6	23
Absorbance at 590 Mμ with 20 γ Sevin					
0.362	0.322	0.315	0.280	0.275	0.242

Table III. Effect of Time on Absorbance at 590 Mμ

Sevin, γ	Minutes 0	15	30	45
	Absorbance			
Blank	0.004	0.004	0.004	0.003
5	0.098	0.099	0.106	0.109
10	0.182	0.191	0.198	0.203
20	0.362	0.378	0.390	0.397
30	0.515	0.560	0.567	0.580
40	0.655	0.720	0.745	0.760

Table I shows recoveries of Sevin added to plant extractives.

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