

## Radioactive Sevin (1-Naphthyl-1-Carbon-14 *N*-Methylcarbamate), a Convenient Synthesis

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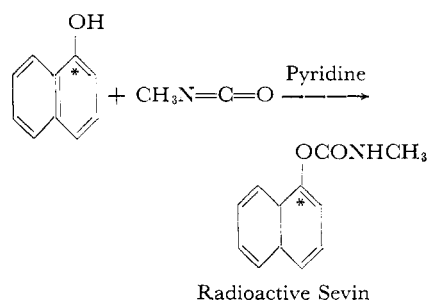
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The commercial introduction of Sevin as a broad spectrum insecticide of low mammalian toxicity has created interest in the study of the mechanism of its insecticidal action and its metabolism in insects. A convenient synthesis of the material labeled with radioactive carbon is described to provide a tool for these studies at normal dose levels.

SEVIN, A NEW INSECTICIDE with a broad spectrum of activity and a very low order of mammalian toxicity, was discovered in the Research Department of the Union Carbide Chemicals Co. (2). Extensive field testing and commercial use show its great promise against many pests of fruits and vegetables. It has been effective in forest insect control and gives excellent control of most major cotton pests (3).

Because of its rather simple chemical structure and its low mammalian toxicity, less than that of DDT, there is considerable interest in the mechanism of its insecticidal action, especially in its metabolism and assimilation in the insect organism. To study this action at normal dose levels, recourse must be made to tracer techniques and Sevin labeled with radioactive carbon-14 is required.

A simple, efficient synthesis from easily available starting materials was needed to provide the labeled material. The method developed was based on the reaction of radioactive 1-naphthol (Radiochemical Centre, Amersham, England, or Nuclear-Chicago Corp., Chicago, Ill.) with methyl isocyanate in the presence of a trace of pyridine as catalyst.



\* Denotes carbon-14 atom

### Experimental

**Reagents.** The commercial radioactive 1-naphthol was examined for the isomeric 2-naphthol, which if present in even a few per cent, leads to lowered yields of Sevin (4). A 2% solution of the radioactive naphthol in freshly distilled carbon disulfide was placed in a 1-mm. infrared, microcell having sodium chloride end windows. The solution was examined in a Baird Model 4-55 double-beam infrared spectrophotometer for the adsorption band at 13.45 microns which is characteristic of 2-naphthol. The absence of this isomer was conclusively demonstrated.

Benzene and pyridine were fractionally distilled, in an atmosphere of dry nitrogen, at atmospheric pressure. A solution containing 2 parts of pyridine in 100 parts of benzene by volume was prepared to provide both the solvent and the catalyst for the reaction.

Methyl isocyanate, prepared by the method of Slocombe *et al.* (5), was freshly distilled before use. It could be stored at  $-20^{\circ}\text{C}$ . in glass containers for periods up to one week without apparent deterioration.

The glass apparatus was dried at  $120^{\circ}\text{C}$ . in an oven before use. Moisture inhibits the reaction of methyl isocyanate and naphthol and leads to the formation of undesirable by-products.

**Synthesis.** An extensive series of small scale (1 to 2 mmole) experiments with nonradioactive materials was run in order to develop the apparatus design and optimum synthesis conditions. The highest product purity and radiochemical yield could be obtained by the following procedure.

1-Naphthol-1- $\text{C}^{14}$  (233.6 mg., 1.62 mmoles) was weighed into a 16-mm. diameter heavy-walled borosilicate glass tube 5 inches long, having an 8-mm. diameter constriction near one end to facilitate sealing. The pyridine-benzene solution (0.7 ml.) was added from a pipet. The reaction tube was attached to a vacuum manifold and was evacuated to below 1-micron pressure, while the reaction tube was kept partially immersed in liquid nitrogen. Methyl isocyanate (1.71 mmoles) in 5% excess over the theoretical requirement was distilled into the reaction tube. The tube was sealed at the constriction while still immersed in liquid nitrogen and attached to the manifold. It was then warmed to room temperature and placed upright in an oven heated to  $80^{\circ}\text{C}$ . After heating for 5 hours, the tube was cooled and opened. The solvent and unreacted isocyanate were removed by evaporation from a warm water bath under a stream of dry nitrogen. The crude Sevin (100% yield) was crystallized from hot *p*-xylene, by immersing the tube in a boiling *p*-xylene bath, and slowly adding solvent, until the product was just dissolved. Crystallization occurred on cooling the solution to  $15^{\circ}\text{C}$ ., just above the freezing point of *p*-xylene. The supernatant liquid was removed by inverted vacuum filtration with a fine grade fritted-glass filter stick attached to a suction flask. The Sevin was washed once with a minimum amount of cold *p*-xylene. Crystals adhering to the filter stick were combined with the product in the tube. After drying for several hours in a vacuum desiccator, 266 mg. (81.8% yield) of radioactive Sevin containing 2.9 mc. of carbon-14 were obtained, melting point,  $141\text{--}2^{\circ}\text{C}$ .

### 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 (7). 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 non-radioactive 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.

### Acknowledgment

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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  $m\mu$ . The method responds in the range of 5 to 40  $\gamma$  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  $m\mu$  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 War-

ing 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 (7). 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-