the rate of herbicide inactivation; it would tend to minimize differences among soil types caused by the effects of moisture and aeration on microbial activity. Loss of chemicals by leaching was eliminated by the experimental conditions. Since the solubilities of these herbicides in water vary from about 5 p.p.m. for simazine to about 3200 p.p.m. for simetone, the relative residual activities would probably be quite different for field applications where leaching by rainfall or irrigation water occurs. Differential volatilities would probably also influence the pattern of residual activity in the field.

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RADIOLABELED INSECTICIDES

Synthesis of *N*-Methylcarbamates via Methyl Isocyanate-C^{14} and Chromatographic Purification

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The increasing interest in *N*-methylcarbamates as insecticides prompted their radiolabeling. A convenient radiosynthesis procedure is reported for Sevin, Hercules 5727, Bayer 39007, Bayer 37344, and Zectran. Acetyl-C^{14} chloride and sodium azide were reacted to yield methyl isocyanate-C^{14} which was then reacted with the appropriate phenol. A two-compartment reaction tube with a break-seal was utilized. Yields on a 0.5-mmole scale were routinely 40 to 70%. Chromatographic procedures for isolating the *N*-methylcarbamates from their reaction mixtures are reported.

Several *N*-methylcarbamates possess desirable characteristics for use as insecticides. Sevin, 1-naphthyl-*N*-methylcarbamate, has been widely used. Others of particular current interest include the *N*-methylcarbamates of *m*-isopropylphenol (Hercules 5727, Union Carbide 10854), *o*-isopropoxyphenol (Bayer 39007), 4-methylthio-3,5-xylenol (Bayer 37344), and 4-dimethylamino-3,5-xylenol (Zectran). Intensive synthesis and screening programs now underway on this type of compound may yield others with desirable properties for development as insecticides.

Radiosynthesis of the carbamate insecticides has been restricted to Sevin-C^{14} prepared from 1-C^{14}-naphthol (*5, 13*). Labeling of the methyl or carbonyl group appeared desirable based on the following considerations: The synthetic procedure might be applicable for radiolabeling a variety of *N*-methylcarbamates; the *N*-methylcarbamoyl group is the toxophoric group based on structure-activity studies (*8, 15*) and the finding that cholinesterase may be carbamoylated by this type of compound (*2, 16, 17*); *N*-methylcarbamates may hydrolyze to yield methyl isocyanate (*1, 4*) as an intermediate before formation of methylamine and carbon dioxide and the biological fate of this intermediate has not been investigated; and the problem of cleanup for C^{14}-counting is greatly facilitated with these labeling sites as methylamine-C^{14} and carbon dioxide-C^{14} can be readily released and trapped for counting.

A simple and efficient procedure for C^{14}-labeling of *N*-methylcarbamate insecticides is reported. The chromogenic reagents for phenols and chromatographic systems used in developing this procedure are also considered.

Materials and Methods

Chemicals. The acetyl-C^{14} chloride (Volk Radiochemical Co., Skokie, Ill.) used was labeled in the 1-position and varied in specific activity from 1 to 5 mc. per mmole. Use of the 2-C^{14} compound would yield the label in the methyl group of the *N*-methylcarbamate. The phenols (Table I) were obtained from manufacturers of the *N*-methylcarbamates, or by thermal decomposition or saponification of the *N*-methylcarbamates. The purified phenols were dried over phosphorus pentoxide before use. The benzene utilized was thiophene-free and dried over anhydrous sodium sulfate, and the triethylamine was the technical material (Distillation Products, Eastman Kodak Co.). Sodium azide was activated by triturating with hydrazine hydrate and then precipitating from an aqueous solution with cold acetone and drying the product according to a modified procedure described by Smith (*14*). It was held for up to 20 days before use, and thereafter activity was regenerated at any time by dissolving in hot water and precipitating with cold acetone and drying.

Samples of *N*-methylcarbamates were obtained from the following sources:

Table I. Properties of Certain *N*-Methylcarbamate Insecticides and Their Phenolic Hydrolysis Products

Property	1-Naphthol	m-Isopropylphenol	o-Isopropoxyphenol	4-Methylthio-3,5-xylenol	4-Dimethylamino-3,5-xylenol
N-Methylcarbamates					
Designation	Sevin	Hercules 5727	Bayer 39007	Bayer 37344	Zectran
M.p., ° C.[a]	142–144	71–73	89–91	121–123	91–93
Phenols					
B.p. or m.p., ° C.	96	222–224/762mm.	209–210/762mm.	61–64	93–95
Retention time, min.	14.1	4.1	3.5	16.9	11.0
Chromogenic reagents, sensitivity in μg. and color[b]					
Fluorescence	0.03	>10	1	1	1
p-Nitrobenzenediazonium fluoborate	0.1 (blue)	1 (orange)	0.3 (blue)	1 (blue)	>10
4-Aminoantipyrine and potassium ferricyanide	1 (orange)	10 (orange)	1 (orange)	>10	3 (blue)
Diazotized sulfanilic acid	0.1 (red)	3 (yellow)	1 (orange)	>10	>10
Gibbs' reagent	0.1 (blue)	1 (blue)	1 (blue)	3 (blue)	5 (orange)
Ferric chloride and potassium ferricyanide	0.1 (blue)	1 (blue)	0.1 (blue)	0.3 (blue)	0.1 (blue)
Luteoarsenotungstic acid	1 (blue)	>10	3	>10	0.3 (blue)
Paper chromatography, reversed phase, R_f					
Acetone–methanol–water (1:2:8)					
N-Methylcarbamate	0.6	0.41	0.79	0.29	0.21
Phenol	0.46	0.44	0.49	0.42	0.32
Acetonitrile–water (1:4)					
N-Methylcarbamate	0.60	0.27	0.72	0.22	0.16
Phenol	0.51	0.28	0.46	0.36	0.28

[a] Melting points are uncorrected. The temperature range given included the melting point of the known compound, of the C^{14}-product, and their mixed melting point.

[b] The maximum amount of compound tested was 10 μg. Where this amount was not detected by the chromogenic reagent, the sensitivity results are indicated as >10.

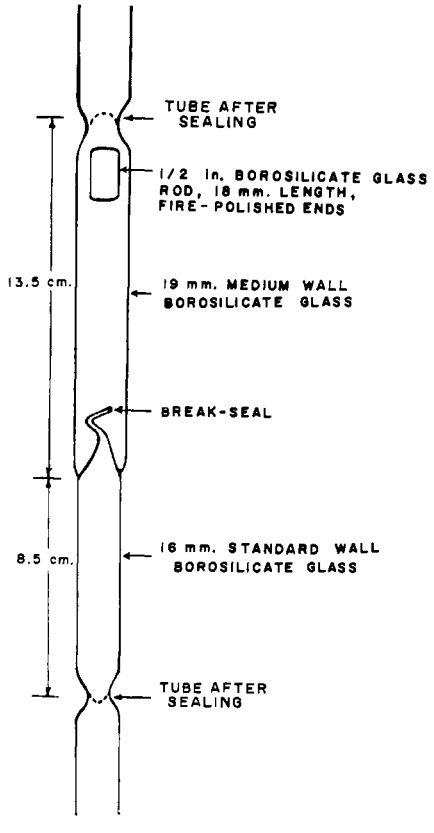

Figure 1. Break-seal reaction tube for preparing methyl isocyanate-C^{14} and coupling to yield substituted-phenyl *N*-methylcarbamates-C^{14}

Sevin from Union Carbide Chemical Co., Hercules 5727 from Hercules Powder Co., Bayer 37344 and 39007 from Chemagro Corp., and Zectran from Dow Chemical Co. The acetate esters were prepared by reaction in benzene of the phenols with acetyl choride in the presence of pyridine.

Apparatus. A borosilicate glass (Pyrex brand, No. 7740) tube consisting of two compartments separated by a break-seal (Figure 1) suitable for synthesis on a 0.5-mmole scale was utilized. This apparatus provided a separate chamber for preparation of methyl isocyanate which could be brought into contact with the phenol and catalyst in the other chamber by rupture of the break-seal with the glass slug. This compartmental arrangement was necessary to allow the acetyl chloride to react first with sodium azide and then undergo Curtius rearrangement to methyl isocyanate before contacting the phenol, so that the latter material would be acylated by methyl isocyanate and not by acetyl chloride.

Paper Chromatography. Whatman No. 4 chromatographic filter paper (2.5 × 22.5 cm.) was impregnated by dipping in a 5% acetone solution of Silicone oil DC 550 (Applied Science Laboratories, State College, Pa.). About 10 μg. of the compounds were spotted in acetone and the chromatograms developed with the polar mobile phase (Table I). The time for the front to ascend 20 cm. was about 1 hour for both systems. The acetate esters of the phenols had R_f values of less than 0.05 with the exception of 1-naphthyl acetate with an R_f of 0.14 to 0.16 in the two systems, and of *o*-isopropoxyphenyl acetate with an R_f of 0.24 in the acetone–methanol–water system and 0.14 in the acetonitrile–water system.

Chromogenic Reagents. The sensitivity of various chromogenic reagents for the phenols investigated is indicated in Table I. Papers spotted with the compounds were sprayed with 15% aqueous potassium hydroxide to hydrolyze the esters, dried, and subjected to the following treatments:

Fluorescence. Papers were observed directly under short wave length ultraviolet light (*3*).

p-Nitrobenzenediazonium fluoborate. The paper was sprayed with 1*M* acetic acid in methanol, dried, and oversprayed with 0.1% methanolic *p*-nitrobenzenediazonium fluoborate (*11*).

4-Aminoantipyrine and potassium ferricyanide. Papers were sprayed with 0.2% aqueous 4-aminoantipyrine followed by 0.8% aqueous potassium ferricyanide [method based on original studies of Emerson (*6*)].

Diazotized sulfanilic acid. Papers were sprayed with a solution prepared by adding 25 ml. of 5% sodium nitrite slowly at 0° C. to 5 ml. of sulfanilic acid solution (0.9 gram of sulfanilic acid and 9 ml. of concentrated hydrochloric acid, diluted to 100 ml. with water) (*3*).

Gibbs' reagent. Papers sprayed with 0.1% *N*,2,6-trichloro-*p*-benzoquinone imine in acetone (*3*).

Table II. Solvent Systems for Recovery of *N*-Methylcarbamates from Reaction Mixtures Added to Florisil Columns

Compound	*n*-Hexone–Ether Mixtures	Tubes Containing Phenol	Tubes Containing Carbamate
Bayer 39007	15 tubes 1:1, 20 tubes 0:1	4–8	20–26
Bayer 37344	15 tubes 2:1, 25 tubes 1:1	9–14	27–34
Hercules 5727	15 tubes 2:1, 20 tubes 1:1	6–9	22–27
Sevin	15 tubes 1:1, 20 tubes 0:1	5–9	21–27
Zectran	15 tubes 1:1, 20 tubes 0:1	7–12	19–26

Ferric chloride–potassium ferricyanide. Papers sprayed with 1% aqueous ferric chloride followed by 1% aqueous potassium ferricyanide and then glacial acetic acid (*9*). Esters of 4-dimethylamino-3,5-xylenol yield blue spots with this reagent without prior treatment with alkali, whereas with the other compounds examined only the free phenols or hydrolyzed esters yielded positive results. Papers were washed with water after color development to obtain permanent spots.

Luteoarsenotungstic acid. Papers were sprayed with a reagent prepared as follows: 20 grams of sodium tungstate dihydrate, 50 grams of arsenic pentoxide, and 200 ml. of water in a 500-ml. flask were refluxed for 90 minutes, cooled, and to this was added 50 grams of lithium sulfate monohydrate and 5 drops of bromine, and the solution was boiled for 10 minutes, and then diluted to 200 ml. with water and filtered.

Column Chromatography. Adsorption chromatography with Florisil and various mixtures of *n*-hexane and anhydrous ether yielded satisfactory separation of the *N*-methylcarbamates from their respective phenols (Table II). Eighty grams of Florisil (60–100 mesh, Floridin Co., Tallahassee, Fla.) were utilized with 2.5 × 35 cm. columns. Twenty-milliliter fractions were collected at a rate of 7 ml. per minute. The position of elution of components did not change in the range of 1 to 100 mg. of phenols and carbamates. The acetate esters eluted in the region of the phenols. Following the hexane–ether mixtures, methanol was run through the column to remove further C^{14}-labeled materials when chromatographing the crude reaction mixtures from synthesis of *N*-methylcarbamates-C^{14}.

Isolation of the *N*-methylcarbamates from the crude reaction mixtures was also possible but less satisfactory with the Florisil columns employing a gradient of benzene to benzene–ether (3 to 7) for elution. Zectran was most difficult to purify with this column. Another chromatographic system found to be satisfactory for Bayer 39007 and Sevin was a column consisting of 40 grams of dried celite and 40 ml. of 80% methanol, with *n*-hexane saturated with 80% methanol as the mobile phase.

The eluate fractions were tested for phenols or phenolic esters by spotting 10-μl. aliquots onto filter paper and spraying with alkali followed by the appropriate chromogenic reagents. Where the chromogenic reagent developed colored spots under alkaline conditions, the C^{14} was counted directly on the filter paper to approximate the degree of coincidence in elution or cochromatography of the phenolic materials and the radioactivity. Cochromatography was further established by determining the specific activity of each eluted fraction.

Gas-Liquid Chromatography. The phenols and their *N*-methylcarbamate and acetate esters were examined by gas-liquid chromatography with a column of 5% (w./w.) silicone oil DC 200 on Chromosorb W (both materials from Applied Science Laboratories, State College, Pa.). The column in a glass tube was 7 mm. by 2.0 meters. A Barber-Coleman Model 10 chromatograph with an ionization detector utilizing a strontium-90 source was used with an argon flow rate of 20 ml. per minute. The inlet pressure was 10 pounds per square inch and the outlet was at atmospheric pressure. Temperatures utilized were as follows: flash heater, 257° C.; column, 155° C.; and cell, 204° C. These conditions resulted in a quantitative thermal decomposition of the *N*-methylcarbamates to yield their respective phenols. The retention times for the phenols (Table I) are thus applicable to the carbamates as well. Methyl isocyanate had a retention time of 1.0 minute under these conditions. The acetates were readily resolved from the phenols and carbamates because of their longer retention times. The phenols used for radiosynthesis chromatographed as single components with the exception of *m*-isopropylphenol and *o*-isopropoxyphenol which had trace contaminants with retention times about 0.1 minute less than the major component. These contaminants were probably isomers of the respective phenols which were present in even the most highly purified samples of the phenols or carbamates available from the manufacturers. The contaminant in the sample of *m*-isopropylphenol was 1.5% *p*-isopropylphenol (*7*). The radiolabeled Hercules 5727 and Bayer 39007 would thus contain trace amounts of comparable impurities.

Standard Radiosynthesis Procedure

The following reaction sequence and conditions, as modified from Schroeter (*12*) and Metcalf *et al.* (*10*), were used for the preparation of *N*-methylcarbamates-C^{14}:

$$CH_3C(O)Cl + NaN_3 \xrightarrow[140^\circ\text{ C., 2 hours}]{\text{benzene}} CH_3NCO + NaCl + N_2$$

$$CH_3NCO \xrightarrow[\text{ROH } (C_2H_5)_3N]{100^\circ\text{ C., 2 hours,}} CH_3NHC(O)OR$$

The described reaction has been run about 50 times without technical difficulty. A good radiochemical hood is necessary as volatile radioactive compounds are used and may escape, particularly when the reaction tube is opened after completion of the synthesis. All operations from charging the radioactive material to the extraction of the reaction products are conducted in the hood with appropriate shielding.

Drying the Reaction Tube. The reaction tube (Figure 1) was thoroughly washed with water, acetone, and ether, dried at 110° C. for 15 to 30 minutes, and on removing from the oven both ends were closed with tight rubber stoppers fitted with calcium chloride tubes to prevent condensation during cooling.

Charing the Phenol and Catalyst. The calcium chloride tube on the shorter end of the reaction tube was then quickly replaced by a tightly fitted, dry, rubber stopper. This stopper and much of the tube were then covered by two separate layers of Parafilm (grade M, Marathon, American Can Co., Menasha, Wis.) or other comparable waterproof wrapping. The other compartment was charged with 0.75 mmole of the appropriate phenol, added in a manner to minimize contamination of the inside walls of the tube, and the calcium chloride tube was immediately replaced. The glass slug was now gently resting on the break-seal nozzle, and in this position the tube was lowered into an ice bath packed to within about one-half inch of the upper constriction. Next, the calcium chloride tube was removed and dry nitrogen was introduced slowly through a fine glass tube to displace most of the air. Two to three drops of the catalyst, triethylamine, were then added directly to the bottom of the compartment using a long, thin glass dropper. The reaction tube was then immediately sealed with an oxygen-gas flame.

Charging the Sodium Azide and Acetyl Chloride. The reaction tube was then removed from the ice pack, allowed to warm to room temperature, washed with acetone, the Parafilm removed, and the rubber stopper rinsed with acetone and then benzene, dried with an air jet, and replaced with a calcium chloride tube through which this compartment was evacuated with an aspirator pump. Sodium azide (activated, 0.75 mmole) was then

introduced with care to minimize contamination of the inside walls. The calcium chloride tube was quickly replaced, the apparatus packed to near the constriction level with ice as indicated before, and the air largely displaced with dry nitrogen. Acetyl-1-C^{14} chloride was then transferred from the sealed tube in which it was obtained to this compartment of the reaction tube. This was achieved by cooling the acetyl chloride tube in a dry atmosphere and opening the break-seal joint under 0.3 to 0.4 ml. of dry benzene in such a manner that the benzene entered the tube the moment the seal was broken. This benzene solution of acetyl-C^{14} chloride was then quickly and completely transferred directly into the bottom of the reaction compartment with a fine dropper and the reaction tube was immediately sealed as previously described.

Reaction to Yield Methyl Isocyanate. The tube was wiped dry, allowed to warm to room temperature, and transferred to a metal cylinder suitable to confine the solid ingredients in case of explosion. (Note: this over-all reaction was run about 50 times without explosion, but this precaution still appears warranted.) This cylinder was held in an oven at 140° C. for 2 hours.

Reaction of the Methyl Isocyanate with the Appropriate Phenol. After 2 hours at 140° C., the tube was removed and while still hot the break-seal was quickly ruptured by hitting suitably with the slug while the phenol compartment was upright. Appropriate shielding should be utilized during rupture of the septum. On breaking this nozzle, a whitish cloud usually engulfed the phenol compartment. The tube, in its metal jacket, was then held in the oven for 2 hours at 100° C. after which time it was removed and allowed to cool overnight.

Extraction of the Reaction Products. The outside of the tube was rinsed with chloroform, and then with the azide compartment up, it was heated rapidly at the extreme tip with a fine flame. The internal pressure resulted in forcing open a small hole through which the residual gases escaped. Immediately, 8 to 10 ml. of chloroform was injected into the tube with a syringe. The tip of the tube was then broken off, the break-seal partition further punched through with a glass rod, and an additional 15 to 25 ml. of chloroform used to rinse the tube thoroughly. The chloroform was dried with anhydrous sodium sulfate, filtered, and evaporated to yield crystals or a dark brownish semisolid, depending on the phenol. The residues were dissolved in chloroform and their infrared spectra examined to ascertain the approximate percentage yield.

Isolation of the *N*-Methylcarbamate. Adsorption chromatography on Florisil columns was utilized to separate the *N*-methylcarbamate from excess phenol and from other products formed in the radiosynthesis. Trace impurities (up to about 0.35% of the C^{14}) eluted in tubes 7 to 13, the region for elution of the acetate esters. The majority (80 to 95%) of the C^{14} subjected to chromatography eluted in the position associated with the *N*-methylcarbamate, and the remainder eluted with a methanol wash of the column. The C^{14}-labeled *N*-methylcarbamates eluting from the columns had infrared spectra identical with the known compounds. When microgram amounts of the radiolabeled compounds were added to the nonlabeled known materials and then subjected to rechromatography, the specific activity of the eluted fractions was constant, and no C^{14} eluted in positions other than that of the *N*-methylcarbamates. The melting points (Table I) and mixed melting points of the known and radiolabeled *N*-methylcarbamates were within one degree of each other. The chromatographic characteristics of the radiolabeled *N*-methylcarbamates on reversed phase paper systems (Table I) were the same as those of the known compounds.

Yields. Yields varied from 38 to 74%, apparently depending on the nature of the phenol, and the success in maintaining anhydrous reaction conditions and in quantitatively handling the small amounts of materials involved. Percentage yields for different radiosyntheses were as follows: Sevin—58, 42, 46, 57, and 60; Zectran—59, 59 and 65; Hercules 5727—37 and 45; Bayer 39007—74 and 56; and Bayer 37344—53 and 56.

Discussion

The *N*-methylcarbamates are susceptible to thermal decomposition to yield methyl isocyanate and their corresponding phenols. The characteristic pungent odor of methyl isocyanate is evident on heating dry substituted-phenyl *N*-methylcarbamates. This decomposition also occurs during boiling such materials in water. The reversible nature of this reaction allows a simple but inefficient method of radiolabeling *N*-methylcarbamates. Sevin could be labeled in the carbonyl position by heating a sealed ampule containing 0.02 to 0.1 mmole acetyl-1-C^{14} chloride, 0.03 to 0.2 mmole activated sodium azide, and 0.1 to 1.0 mmole nonlabeled Sevin for 1 hour at 150° to 160° C. The sequence of reactions as the temperature was raised included the formation of acetyl azide and its degradation to methyl isocyanate, followed by the thermal decomposition of Sevin. On cooling, the mixed labeled and nonlabeled methyl isocyanate recombined to yield the *N*-methylcarbamate, Sevin. Recovery of C^{14} in the labeled Sevin was about 70 to 90%, and chromatographic procedures were necessary to recover radiolabeled Sevin in a pure form. This procedure offers simplicity but obviously limits the specific activity obtainable in the final product to considerably below that of the starting labeled material, acetyl-1-C^{14} chloride. This limitation was circumvented by use of methyl isocyanate-C^{14} and 1-naphthol.

With the break-seal reaction tube, the optimal temperatures for the formation of methyl isocyanate and its reaction with 1-naphthol were examined. Different temperature combinations were tried between 100° and 160° C. with 10° intervals, and the optimal temperatures appeared to be about 140° C. for the first and 100° C. for the second reaction with a 2-hour period of heating at each temperature. The optimal reaction conditions as worked out with Sevin were applied directly to the other *N*-methylcarbamates.

The specific activity attainable is the same as that of the starting radiolabeled reactants. Acetyl bromide appeared to work as well as acetyl chloride. Specific activities of up to 5 mc. per mmole or 25 mc. per gram would be possible with samples of acetyl chloride currently available from commercial sources. The position of labeling, either in the carbonyl or methyl group, can be varied by using 1-C^{14} or 2-C^{14} acetyl chloride.

The advantage of any specific site of labeling depends on the study for which the tagged compound is to be used. Carbonyl-C^{14} derivatives should afford the greatest ease of handling, since they can be readily and quantitatively converted to carbon-14 dioxide for trapping and counting.

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INSECTICIDE ANALYSIS

Determination of Ronnel, *O,O*-Dimethyl *O*-(2,4,5-Trichlorophenyl) Phosphorothioate, in Sheep and Cattle Dip Solutions

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A method for the determination of ronnel, O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate, in sheep and cattle dip solution, is described. This method involves a single extraction of ronnel from solution with acetone and hexane. The compound is then hydrolyzed to liberate 2,4,5-trichlorophenol which is determined by a modified 4-aminoantipyrine method. By using a phosphate buffer containing 20% ethyl alcohol a stable dye solution is obtained. The use of alcohol in the buffer increases sensitivity of the method and increases the maximum amount of phenol that can be coupled with the 4-aminoantipyrine without obtaining hazy solutions or the formation of a precipitate.

RONNEL, *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate, trademark of The Dow Chemical Co. abroad, has found wide application for the control of parasites of sheep and cattle (*8*, *9*). Recently, ronnel has been used as a sheep and goat dip for the control of external parasites (*7*, *14*, *15*) and is now being tested experimentally as a cattle dip.

The concentration of the insecticide in the dip may be affected by a number of factors, such as selective carry-out, decomposition, settling, or evaporation of water. To maintain a constant level of the compound in the vat, it is necessary to have a method to determine the concentration of insecticide.

Two methods have been suggested for the analysis of ronnel dips. In the first (*5*), ronnel was hydrolyzed to liberate 2,4,5-trichlorophenol which was then isolated by steam distillation. The phenol was then determined by a modification of the antipyrine method (*13*). In the second (*12*), a multiple extraction of the dip with cyclohexane was employed to isolate the ronnel. Cyclohexane was removed by evaporation and the ronnel transferred to acetone and determined by titrating the solution with water until it became turbid. The amount of water employed was inversely proportional to the amount of ronnel present in the acetone solution.

A review of the problems encountered in using these two methods in the field suggested that the most satisfactory approach to the problem would be to use an extraction procedure to isolate ronnel from the dip and a modification of the antipyrine method to determine the 2,4,5-trichlorophenol liberated by the hydrolysis of ronnel.

Materials and Methods

Reagents. 4-Aminoantipyrine, 1%. Dissolve 1.00 gram of 4-aminoantipyrine (Winthrop Laboratories, New York, N. Y.) in 100 ml. of distilled water and store in a brown bottle. Make up fresh weekly.

n-Hexane, technical, 95 mole % (Phillips Chemical Co.).

Ronnel, purified. *O,O*-Dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate, 99% or better (The Dow Chemical Co.).

Phosphate buffer. Mix 70 ml. of 0.7*M* dibasic potassium phosphate solution (K_2HPO_4) with 30 ml. of 0.7*M* potassium dihydrogen phosphate (KH_2PO_4) in a 1-liter volumetric flask. Add 500 ml. of water, 200 ml. of absolute ethyl alcohol, and dilute to 1000 ml. with water.

Potassium ferricyanide, 1.4%. Dissolve 1.40 grams of $K_3Fe(CN)_6$ in 100 ml. of distilled water and store in a brown bottle. Make up fresh weekly.

Sodium hydroxide, 4*N*. Dissolve 40 grams of NaOH in about 150 ml. of water, and dilute to 250 ml.

Sodium methylate, 1*N*. Dissolve 54 grams of sodium methylate in methyl alcohol and dilute to 1000 ml. (Olin Mathieson Chemical Corp.).

Stock solution of ronnel. Dissolve 100 mg. of purified ronnel in 100 ml. of acetone.

Procedure. A 10-ml. aliquot of the dip solution is mixed with 10 ml. of acetone and 0.5 ml. of 4*N* sodium hydroxide in a 250-ml., glass-stoppered Erlenmeyer flask. To this solution is added 150 ml. of hexane, and the sample is vigorously shaken for 1 minute. After the two layers are completely separated, a 1-ml. aliquot of the hexane layer is transferred to an 18 × 150 mm. test tube. Two drops of 1*N* sodium methylate solution and a boiling chip are added, and the sample is heated in a boiling water bath until the hexane is distilled off. One milliliter of 50% ethyl alcohol is then added and the sample heated for an additional 5 minutes. After heating, the tube is cooled and 10 ml. of dilute phosphate