

Table V. Rate of Degradation of TMTD in the Rumen of a Fistulated Animal

Water, %	Dry Matter, ^a Rumen	Intake TMTD, 8 A.M., CS ₂ μg.	TMTD Rumen, 2 P.M., CS ₂ μg.	TMTD, ^b % Degraded in 6 Hours	
85	14.3	50.1	10.5	79	
86	13.3	53.7	9.4	83	
80	19.0	37.6	8.8	77	
86	13.3	53.7	10.3	81	
85	14.3	50.1	10.7	79	
85	14.3	50.1	12.1	76	
85	14.3	50.1	10.0	80	
84	16.2	44.1	9.3	79	
86	13.3	53.7	11.8	78	
85	14.3	50.1	11.8	77	
86	13.3	53.7	11.6	78	
Av.	84.8	14.5	49.7	10.6	78.8

^a Capacity of rumen, 10 gallons; weight of ingesta, 95 pounds.

^b Calculations based on carbon disulfide/gram of dry matter.

allow the ruminant animal to tolerate considerable amounts of TMTD-treated corn without toxic effects. It seems reasonable to assume that considerable amounts of the volatile gases, carbon disulfide and hydrogen sulfide, could be released from the rumen by belching and aeration of the bolus during rumination, as the ingesta are estimated to remain in the rumen and reticulum for approximately 60 hours and the animal spends approximately 8 hours daily in the act of rumination (7). This would appear to reduce the concentration of carbon disulfide and hydrogen sulfide to a level which would not overload the detoxification processes of the body.

The metabolism of TMTD in the animal body was not investigated, as an unknown volatile substance was found to be naturally present in the blood and tissues which gave a reaction similar to carbon disulfide with the copper-amine reagent. These findings invalidated the distillation procedure for

the study of TMTD and its degradation products in animal tissues.

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ISOTOPE-LABELED INSECTICIDES

Ethion-P³²

THE PESTICIDE ETHION is used against a number of agriculturally important insects and mites. Feeding experiments with cows were contemplated in which it would be necessary to detect a few parts of ethion per billion. Since the molecule might be metabolized, tagging with tritium, carbon-14, or sulfur-35 could lead to ambiguous re-

sults. Tagging with phosphorus-32 seemed preferable because if metabolic decomposition occurred it would be indicated simply by the water-fat partition coefficient.

One difficulty with phosphorus-32 is its short half life (14.3 days) which requires that the synthesis and the subsequent experiments be done promptly.

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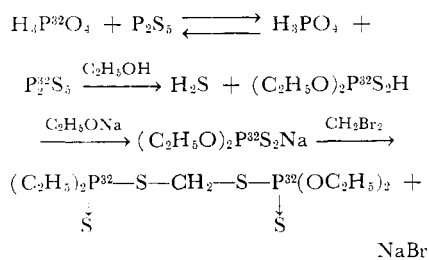
Also, since phosphorus-32 decays with the ejection of energetic beta particles, shielding is necessary.

The P³²S₅ was prepared by a method described by Casida (3). Although a radioyield of 80% was obtained in the preliminary experiments with weakly radioactive phosphoric acid, the yield in the principal run was about 45%.

The insecticide *O,O,O',O'*-tetraethyl *S,S'*-methylene bisphosphorodithioate- P^{32} (ethion- P^{32}) was prepared for use in biological studies. The product obtained from 3 curies of $H_3P^{32}O_4$ contained 780 mc. of P^{32} with a specific activity of 28.4 mc. per gram. When allowance is made for decay of the P^{32} (half life 14.3 days), this quantity represents a radioyield of 33%. Estimated vapor pressure of ethion was 1.5×10^{-6} mm. at 25° C.

The yield could probably have been improved by the addition of a small amount of nonradioactive phosphoric acid. The slight shift in the equilibrium to the left would have been more than overcome by the more efficient dispersion of the radioactive material in the reaction mixture.

The scheme of the synthesis was as follows:



In the preliminary run, 40 μ c. of $H_3P^{32}O_4$ in 150 ml. of 0.1*N* hydrochloric acid solution containing 100 mg. of 85% H_3PO_4 was used as the starting material for a reaction with 0.15 mole of P_2S_5 . The preparation of the $P_2^{32}S_5$ was otherwise the same as that described in the experimental section. The superior yield of 80% is attributable to the presence of phosphoric acid which distributed the radioactivity more effectively during the reaction.

Bowman and Casida (7) have reported the preparation of $(C_2H_5O)_2P^{32}S_2H$. The subsequent steps of this radioactive synthesis were a modification of the synthesis of Siwinski (6) with nonradioactive chemicals.

Experimental

All radiochemical yields were corrected for radioactive decay.

All work was done behind a $\frac{1}{2}$ -inch Lucite shield. One of the authors was exposed to a whole body radiation of 65 mr. during the week that radioactive materials were in the laboratory, while one hand received an exposure of 300 mr. These amounts are within the maximum permissible limits (4). Reduction of exposure could be attained by constructing an apparatus which would permit the operator to carry out the synthesis without the necessity for reaching behind the shield.

O,O-DIETHYL HYDROGEN PHOSPHORODITHIOATE- P^{32} . Three curies of P^{32} were obtained from the Oak Ridge National

Laboratory as $H_3P^{32}O_4$ in an 0.11*N* HCl solution. The 67.5 ml. of solution contained $44.51 \pm 3\%$ mc. per ml. of activity. The specific activity of the phosphorus which contained 0.156% P^{32} was 445,100 mc. per gram. The solution was evaporated in a 250-ml. three-necked flask by heating on a steam bath in a stream of filtered air. The residue was heated at 100° C. for a short time in vacuo.

The flask was next equipped with a thermowell and stopper in two of the necks while the third neck held a Y adapter. A stream of dry nitrogen was led into one arm of the Y and a reflux condenser was placed in the other. The top of the condenser held a tube filled with calcium chloride.

Phosphorus pentasulfide (technical quality, 0.0734 mole) was added, and the bottom of the flask was heated with a Fisher burner while the empty part of the flask was protected from the flame with a Transite shield. The mixture was boiled for 20 minutes and allowed to cool.

A Teflon-encased magnetic bar was added to the $P_2^{32}S_5$ flask and then a mixture of dry benzene (12 ml.) and absolute ethyl alcohol (0.289 mole). The mixture was stirred magnetically and heated under reflux with an oil bath for 3 hours. At this point, the kettle temperature had risen to about 100° C. Ethyl alcohol (3 ml.) was added, and the reflux was continued for 2 hours longer, by which time all of the solids had dissolved.

The reaction mixture was distilled through a 10 \times 1 inch Vigreux still, using an air-cooled condenser and a liquid nitrogen trap. After the solvent had been removed by gradually decreasing the vacuum to 25 mm. while the kettle temperature did not exceed 90° C., the *O,O*-diethyl hydrogen phosphorodithioate- P^{32} was distilled at 54° C. (1 mm.) up to a kettle temperature of 90° C., and collected in a 250-ml. three-necked flask. The n_D^{25} was 1.5068. The distillate (21.3 grams) contained 33% of the original radioactivity. Two 5-gram portions of $(C_2H_5O)_2PS_2H$ prepared previously were added to the residue in the kettle and distilled successively into the same receiver to chase any of the radioactive $(C_2H_5O)_2P^{32}S_2H$ which had not yet distilled. This increased the radioyield to 38% for the two steps, and to 85% yield for this step.

The nonuseful radioactivity remained in the distillation flask used for the preparation of $(C_2H_5O)_2P^{32}S_2H$ and represented mostly $H_3P^{32}O_4$ which failed to exchange with the P_2S_5 as well as some higher boiling organic materials.

O,O,O',O'-TETRAETHYL *S,S'*-METHYLENE BISPHOSPHORODITHIOATE- P^{32} (ETHION- P^{32}). The combined $(C_2H_5O)_2P^{32}S_2H$, amounting to 30.5 grams, was cooled in an ice-water bath while 68 ml. of 2.36*N* sodium ethylate (prepared under a nitrogen atmosphere) was slowly added until the pH reached 8 and then went to 9.3 (pH meter). Part of the ethyl alcohol (24 ml.) was removed by distillation under vacuum while the reaction flask was held in a bath of tap water.

Methylene bromide (0.01 mole) was then added, and the mixture was stirred and refluxed for 3 hours. The reaction mixture was cooled, brought to pH 9.3 again with a few milliliters of sodium ethylate solution, 1 ml. of methylene bromide was added, and the mixture was refluxed again for 2 hours.

The ethyl alcohol was removed by vacuum distillation until the kettle temperature reached 90° C. and the residue was washed twice with 45-ml. portions of water. The second wash water was extracted with 5 ml. of benzene which was added to the main oil layer. The oil was heated in vacuum to 80° C. at 1 mm., and then sparged with dry nitrogen at 90° C. for a short time. The product consisted of 27.5 grams of *O,O,O',O'*-tetraethyl *S,S'*-methylene bisphosphorodithioate- P^{32} , $(C_2H_5O)_2P^{32}-S-CH_2-S-P^{32}(OC_2H_5)_2$. Its

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specific activity was 28.4 mc. per gram, 4.50 days after the original $H_3P^{32}O_4$ was assayed, equal to a total of 780 mc. of recovered activity. The sample had n_D^{25} 1.5347; its chemical assay as ethion was 89.5% and its infrared spectrum was substantially the same as technical ethion. The radioyield for the last step was 87% and the over-all radioyield was 33%.

RADIOCHEMICAL ASSAYS AND CHROMATOGRAPHIC STUDIES. All radiochemical assays were run using an end-window mica (~ 2 mg. per sq. cm.) Geiger counter, mounted in an SC-10A Tracerlab radioassay sample holder which was connected to a 1095 Baird-Atomic scaler. The samples were counted in

stainless steel planchets as uniformly thin films (<2 mg. per sq. cm.).

$P_2^{32}S_5$ was assayed following hydrolysis with dilute NH_4OH which served to distribute the sample more uniformly.

The dithioic acid, which showed a significant vapor pressure and per se leads to contamination of the counter window, was assayed as the sodium salt, which is nonvolatile.

Ethion- P_2^{32} was assayed by counting a suitable aliquot of an ethyl alcohol solution.

Paper chromatograms were prepared with ethion- P_2^{32} by means of several solvent systems. When the chromatograms were autoradiographed, it was not possible to resolve clearly any impurities with the exception of a qualitative indication of a small amount of dithioic acid.

A silicic acid column chromatographic procedure (7) was employed to obtain additional information. Radiometrical assay of the ethion fraction indicated that the original sample had a purity of 90% which was in excellent agreement with the chemical assay of this fraction. The rest of the radioactivity was contained in five components present to the extent of 0.3 to 2.8%. These could not be further identified.

VAPOR PRESSURE OF ETHION. For the vapor pressure studies, the radioactive ethion was initially purified by means of a silicic acid column. Following the evaporation of the solvent, the residue which consisted of pure ethion-

P_2^{32} was brought to constant weight by heating in vacuo at 40° C.; the liquid was next transferred to a 1-ml. beaker which was then placed in a brass vessel. The latter was closed by a thin (1 ml.) plate equipped with a cylindrical orifice of known dimensions through which the vapor could effuse into a highly evacuated space (<0.1 micron) and condense upon the center of a chilled stainless steel coupon or disk. The latter was secured to the cold finger of a sublimation apparatus by means of high-vacuum grease. The technique is essentially similar to that of Carson *et al.* (2), except that the use of the demountable coupon lends itself, it is believed, to a more reproducible and accurate means of counting the distillate. Initially, the specific activity was determined by counting minute quantities of the purified radioactive ethion which had been weighed out on similar coupons with a microbalance.

The first half hour's reading was subtracted from that of a subsequent 1 hour's run. This automatically corrected for any uncertainty about just when the good vacuum took effect during the first few minutes of the run (5). The apparatus was calibrated using National Bureau of Standards benzoic- C^{14} acid. Runs were made at three constant temperatures—namely, 43°, 56°, and 67° C.—and the weight of the ethion was determined from the activity deposited on the coupon. A plot of log vapor pressure vs. $1/T$ gave a

straight line which fits the equation

$$\log_{10} P \text{ (mm.)} = 12.39 - \frac{5440}{T}$$

This equation gives 1.5×10^{-6} mm. for the vapor pressure of ethion at 25° C.

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ISOTOPE-LABELED INSECTICIDES

Preparation of Labeled 2-Ethylthioethanol, a Demeton Intermediate

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A small scale synthesis of 2-ethylthioethanol by the catalytic condensation of ethylene oxide and ethyl mercaptan is described. A S^{35} or C^{14} label may be incorporated in the alcohol, which is an intermediate in the preparation of demeton.

THE SYSTEMIC insecticide demeton, *O,O*-diethyl *O*-ethylmercaptoethyl phosphorothioate, is prepared conventionally by the reaction of diethylchlorothionophosphate with the sodium salt of 2-ethylthioethanol, or with the alcohol in the presence of copper and potassium carbonate (8). The first step in the

enzymatic-, acid-, or base-catalyzed hydrolysis of demeton results in formation of 2-ethylthioethanol (4). Labeling of sulfur or carbon in this alcohol is of interest in investigations of the reactions of the insecticide in biological systems.

2-Ethylthioethanol has been prepared by the reaction of ethylene chlorohydrin

with the sodium salt of ethyl mercaptan (3, 6, 9, 10), diethyl sulfate with the sodium salt of β -thioethanol (7), and ethylene oxide with ethyl mercaptan (2, 7). These procedures were examined to determine their practicality for labeling syntheses. In small scale preparations of labeled material, it is desirable