

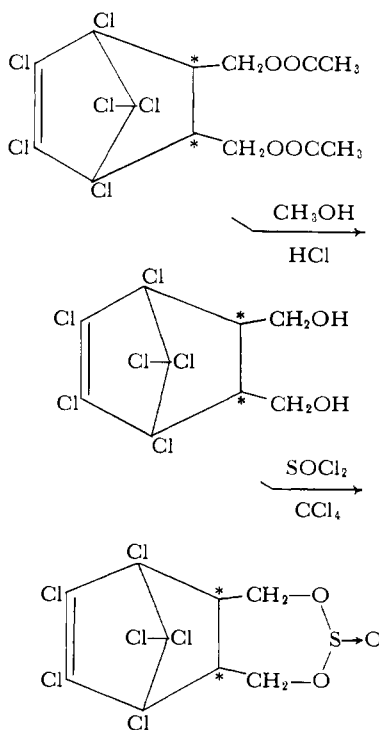
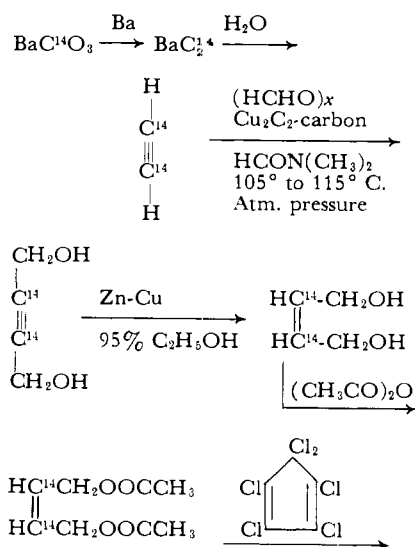
Thiodan-5a, 9a-C¹⁴

The insecticide 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide-5a,9a-C¹⁴ (Thiodan-5a,9a-C¹⁴) was prepared for use in biological studies which required that quantities as low as 1 part per billion be detectable. Starting with a 411-mc. quantity of barium carbonate-C¹⁴, the quantity of final product obtained was 210 mc., which represents a radiochemical yield of 51%.

THIODAN is a pesticide which is particularly effective on forage insects, potato insects, and most species of aphids. This synthesis of a radio-tagged material was undertaken to permit the direction of as little as 1 part of Thiodan per billion in biological studies. Because feeding experiments with cows were contemplated, the positions of the tagged atoms in the molecule had to be located to minimize any possibility of exchange during ruminant metabolism. These considerations therefore precluded the use of tritium, sulfur-35, or chlorine-36 as a tracer atom. The introduction of carbon-14 into a ring position in Thiodan, however, met the requirements.

A minimum of 75 mc. of Thiodan-5a,9a-C¹⁴ with a minimum specific activity of 2 μ c. per mg. was required for the biological studies.

The following scheme was adopted for the synthesis of Thiodan-5a,9a-C¹⁴:



The first two steps had already been carried out on a small scale (2). The other steps had been carried out with radioactive chemicals (7, 3, 4, 7, 8).

Thiodan was first prepared with nonradioactive chemicals, starting from barium carbonate. The intermediates and the products were characterized to verify their identities. A pilot batch was made at a low level of radioactivity to be sure that the radioactivity could indeed be attached to the Thiodan. More analytical data were obtained in that run than in the subsequent ones because it was desirable to conserve radioactivity.

It was deemed prudent to prepare

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the highly active material in two batches of approximately 200 mc. each as a precaution against accidental loss. Subsequent runs were made for the purpose of scavenging radioactive material.

Experimental

Each batch was kept separate throughout the preparation.

A. Barium Carbide-C¹⁴. The starting materials consisted of 1.78 grams of BaC¹⁴O₃ containing 200 mc. (19.64% C¹⁴) and 0.9 gram containing 210 mc. (75.5% C¹⁴). Fourteen grams of barium metal and 1.78 grams of barium carbonate-C¹⁴ were placed in an 18 × 150 mm. test tube by alternating 2-gram portions of thin metal shavings with approximately 0.3-gram portions of barium carbonate-C¹⁴. A stream of argon gas was directed into the tube while the charge was heated with a Fisher burner. The mixture momentarily became incandescent when it reacted.

B. Acetylene-1,2-C¹⁴. Barium carbide-C¹⁴ was warmed while 135 ml. of water were added. After the reaction subsided, the mixture was refluxed for 30 minutes with a hydrogen sweep. The acetylene-1,2-C¹⁴ was condensed in a glass spiral cooled with liquid nitrogen and then transferred quantitatively to a gas-holding bulb. Mass spectrographic analysis indicated 97.8% acetylene, 1.2% ethylene, and 1.0% ethane.

Some unconverted material (19 mc.) remained as BaC¹⁴O₃ and elemental carbon-14. These were recovered and converted to barium carbide-C¹⁴.

C. 2-Butyne-1,4-diol-2,3-C¹⁴. The catalyst was prepared by bubbling commercial acetylene into a mixture of 12 grams of Nuchar C-190-N activated

carbon suspended in a solution consisting of 200 ml. of water, 8 grams of cupric sulfate pentahydrate, 25 ml. of aqueous 28% ammonia, and 12.5 grams of hydroxylamine hydrochloride. The solids were filtered and washed thoroughly first with water and then with dimethylformamide. One gram of bismuth trioxide was added.

The catalyst, 6.3 grams of 95% paraformaldehyde (equivalent to 0.2 mole of formaldehyde), and 60 ml. of dimethylformamide were stirred in the reaction flask and the acetylene-1,2- C_2^{14} was added to the evacuated apparatus along with recovered acetylene from a previous run. The reaction mixture was heated in the presence of commercial acetylene at atmospheric pressure with only occasional stirring until the temperature reached 95° C. A reduced rate of stirring was used at first to avoid solution of an excessive quantity of acetylene which would desorb at a higher temperature. When desorption began, the stirrer was turned on full and after a short time the gas was reabsorbed. The reaction was maintained at 105° to 115° C. for 13 hours, while about 0.1 mole of commercial acetylene was absorbed. The absorption rate varied with the purity of the gas. The cooled reaction mixture was filtered onto a sintered glass disk built into the apparatus. The unreacted acetylene was condensed in a spiral with liquid nitrogen. The filtrate and dimethylformamide washings were vacuum-distilled through a small Vigreux column. The 2-butyne-1,4-diol-2,3- C_2^{14} distilled at 125° C. at 5 mm., and the last part was driven over with an electrically heated tape.

The same catalyst was used in the subsequent batches in order to conserve the radioactivity which remained on the catalyst. When the rate of absorption of gas fell off, addition of new catalyst and solvent did not increase the rate; nor did condensation of the gas with liquid nitrogen followed by evacuation of the system and evaporation of the gas back into the system.

When gas from the reaction mixture was condensed with liquid nitrogen and commercial acetylene was added to the reaction mixture, the absorption of gas was rapid. Analysis of the condensed gas by mass spectrometer showed the presence of 75% carbon dioxide, 1% allene or propyne, and 24% acetylene. The carbon dioxide was not radioactive, while the acetylene had a specific activity of 2.45 μ c. per mg. The gas was shaken with potassium hydroxide solution to remove carbon dioxide and the remaining acetylene-1,2- C_2^{14} was returned to the reactor. The source of the carbon dioxide was not known.

The yields of 2-butyne-1,4-diol-2,3- C_2^{14} from the principal runs were, respectively, 7.6 and 5.2 grams, specific

activity 15.0 and 24.8 μ c. per mg., total activity 114 and 129 mc., radiochemical yield from $BaC^{14}O_3$, 57 and 61.5%. Some additional activity was obtained by using the catalyst in a subsequent run, so that a total of 289 mc. of radioactivity was obtained in the form of butynediol from 411 mc. of $BaC^{14}O_3$ for a radio yield of 70%.

Commercial 2-butyne-1,4-diol (8.6 grams) was added to the extract along with the rest of the recovered dimethylformamide, and the mixture was distilled. This batch was carried along in the synthesis to scavenge radioactivity from other places in the synthesis.

D. *cis*-2-Butene-1,4-diol-2,3- C_2^{14} . Cupric sulfate pentahydrate (0.1 mole), dissolved in 600 ml. of water, was stirred with 0.5 gram atom of zinc dust until the color of the solution was no longer blue, and a zinc-copper couple had formed. The solids were filtered off and washed thoroughly with water.

Each batch of 2-butyne-1,4-diol-2,3- C_2^{14} dissolved in 600 ml. of 95% ethyl alcohol was stirred with freshly prepared zinc-copper couple and refluxed for 1 hour. The mixture was filtered and the product was concentrated by distillation. The residue which remained at 100° C. at 20 mm. gave the same infrared spectrum as *cis*-2-butene-1,4-diol.

E. *cis*-2-Butene-1,4-diol-2,3- C_2^{14} Diacetate. *cis*-2-Butene-1,4-diol-2,3- C_2^{14} was added to 0.2 mole of refluxing acetic anhydride during 1/3 hour. After 2 additional hours of refluxing, the product was distilled at 114° C. at 14 mm. The average material yield was 75%.

F. 1,4,5,6,7,7 - Hexachlorobicyclo-[2,2,1]-hept-5-ene-2,3-dimethanol-2,3- C_2^{14} Diacetate. Hexachlorocyclopentadiene (36 grams) and a batch of butenediol diacetate were stirred and heated under nitrogen at 165° C. for 8 hours. The unreacted portion was removed by distillation up to a kettle temperature of 165° C. at 1 mm. The first principal batch gave 24.5 grams of product and 17.8 grams of distillate.

The distillate of each previous batch was used in the subsequent batch, and the quantity of hexachlorocyclopentadiene was reduced by a corresponding amount.

In the scavenging batch, the distillate was subjected to second and third heating periods and additional radioactive product resulted.

G. 1,4,5,6,7,7 - Hexachlorobicyclo-[2,2,1]-hept-5-ene-2,3-dimethanol-2,3- C_2^{14} . Each batch of Diels-Alder adduct was alcoholized with 2 volumes of methanol per gram in the presence of 0.025 volume of concentrated hydrochloric acid per ml. of methanol by refluxing for 2.5 hours. The product was recrystallized with the aid of additional methanol and Darco G60 activated carbon. The yields of the princi-

pal batches were 13.4 and 12.7 grams.

After the scavenging batch had been crystallized from the mother liquors left from the preceding batches, evaporation of the mother liquor left a residue of 23 grams containing 32 mc. of radioactivity. This residue was mixed with nonradioactive 1,4,5,6,7,7-hexachlorobicyclo-[2,2,1]-hept-5-ene-2,3-dimethanol and the mixture was crystallized from methanol. The addition of nonradioactive material and crystallization were repeated. The final mother liquor had an activity of 24.8 mc. which was evidently not part of the desired compound.

H. 6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a - hexahydro - 6,9 - methano-2,4,3 - benzodioxathiepin 3 - oxide-5a,9a- C_2^{14} (Thiodan-5a,9a- C_2^{14}). Each product from G was refluxed with 1 ml. of carbon tetrachloride per gram while 0.27 ml. of thionyl chloride per gram was added during 0.5 hour. After the mixture had refluxed for 5 hours longer, the excess reagent and solvent were removed by distillation in vacuum up to a kettle temperature of 100° C. The product was sparged with nitrogen for a short time at elevated temperature in vacuum. The infrared spectrum of each product served to verify its identity (Figure 1).

The yield of the first principal batch was 14.6 grams, 3.7 μ c. per mg., 54 mc. total; and of the second principal batch, 14.4 grams, 5.91 μ c. per mg, 85 mc. total, 93.2% Thiodan. Other batches yielded additional quantities of Thiodan with less radioactivity. The total radiochemical yield for all of the batches combined was 210 mc., 51% of theoretical, from 411 mc. of barium carbonate- C^{14} .

I. Analytical Procedures for Radiochemical Assay of C^{14} -Tagged Intermediates and Thiodan. ACETYLENE-1,2- C_2^{14} . The sample was transferred by vacuum techniques to the combustion apparatus in which copper oxide at 300° C. oxidized the gas to carbon dioxide. The latter was absorbed in a magnetically stirred solution consisting of 5 ml. of standard 0.5N sodium hydroxide containing 117.7 mg. of sodium carbonate to dilute the activity. Following the addition of 5 ml. of 0.5M barium chloride solution, the excess alkali was titrated with standard 0.1N hydrochloric acid to a phenolphthalein end point. Subtraction of the latter titration from a blank titration gave the amount of alkali consumed by the carbon dioxide and corresponded to the quantity of acetylene.

Small aliquots of the barium carbonate were transferred to tared Millipore membranes mounted on a Tracerlab filter tower apparatus, filtered, washed with hot water, and vacuum-dried. The membranes were weighed, then mounted on Tracerlab ring and disk assemblies, and counted on the

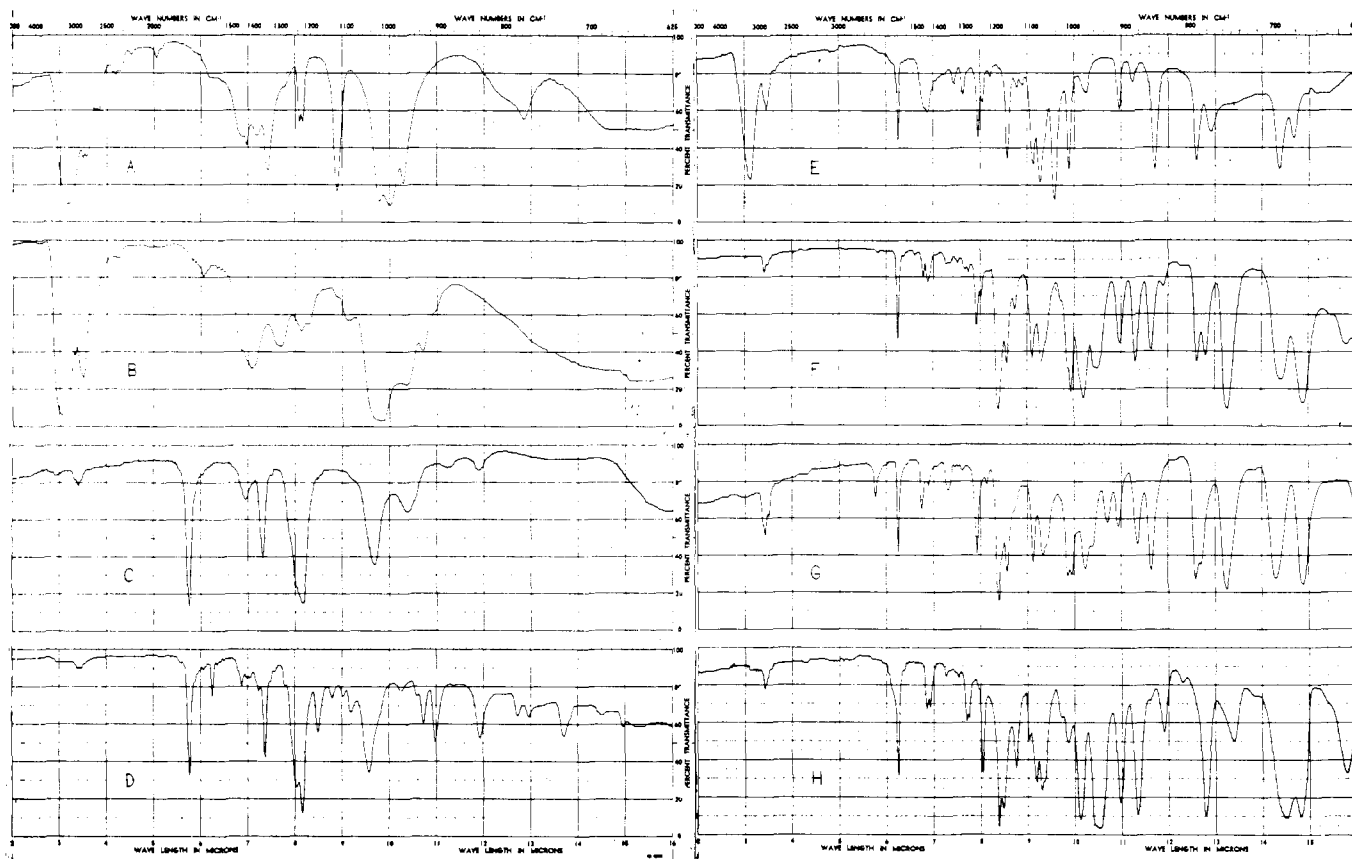


Figure 1. Infrared spectra

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| <p>A. 2-Butyne-1,4-diol (KBr)</p> <p>B. <i>cis</i>-2-Butene-1,4-diol (film)</p> <p>C. <i>cis</i>-2-Butene-1,4-diol diacetate (film)</p> <p>D. 1,4,5,6,7,7-Hexachlorobicyclo-[2,2,1]-hept-5-ene-2,3-dimethanol diacetate (solid)</p> | <p>E. 1,4,5,6,7,7-Hexachlorobicyclo-[2,2,1]-hept-5-ene-2,3-dimethanol (KBr)</p> <p>F. Thiodan, technical (solid)</p> <p>G. Thiodan, isomer, m.p. 108° C. (solid)</p> <p>H. Thiodan, isomer, m.p. 212° C. (KBr)</p> |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

top shelf of an end window counter. The radio-count was corrected for background, dead time, and self-absorption. The latter correction was made with barium carbonate prepared from an NBS solution of sodium carbonate containing 1280 decompositions per second per ml. Counts were reproducible to $\pm 2\%$.

OTHER INTERMEDIATES AND THIODAN-5a,9a-C₁₄. The other compounds were assayed using the apparatus and procedure of Thorn and Shu (6), followed by plating and counting the barium carbonate.

Because this procedure is relatively time-consuming and the substances to be analyzed were of relatively high radioactivity, a modification of the procedure of Schweibel, Isbell, and Moyer (5) was used. One milliliter of a dilute solution of the substance in dimethylformamide was counted in a stainless steel planchet under an end window counter whose efficiency was determined against a standard sample of benzoic acid-C¹⁴ dissolved in dimethylformamide. The standard, obtained from the New England Nuclear Co., contained 9860 decompositions per second per mg. and was counted at an average efficiency of 764 (\pm) 16 decompositions per minute per count per minute. Assay by this procedure gave

results within 2% of the results obtained by the combustion procedure.

A further check on this procedure was made by burning a sample by the method of Thorn and Shu. A portion of the barium carbonate which resulted was treated with acid to evolve carbon dioxide, which was then transferred to a 250-ml. ionization chamber. The activity was determined by the resistor leak method and gave results within 2% of the method of Schweibel *et al.*

J. Chemical Analysis of Thiodan. A 20- to 30-mg. sample of Thiodan was weighed in a 1-ml. beaker on a micro- or semimicrobalance and transferred to a 125-ml. iodine flask which contained 10 ml. of methanol and 2 ml. of methanolic 10% sodium hydroxide. The mixture was refluxed for 2 hours to hydrolyze the Thiodan to sodium sulfite. The mixture was allowed to cool and the condenser was washed down with 5 ml. of methanol and 10 ml. of water. The solution was made just acid to phenolphthalein by the dropwise addition of 5*N* sulfuric acid and then just alkaline with a few drops of sodium hydroxide to prevent loss of sulfur dioxide. The sample was transferred to a flask containing exactly 10.00 ml. of standard 0.05*N* iodine solution and 5 ml. of 5*N* sulfuric acid. Three 10-ml. washings with water were sufficient to complete

the transfer. The excess iodine was titrated with standard 0.02*N* sodium thiosulfate solution until only a light yellow color remained; then 5 ml. of 2% starch solution were added and the titration was continued until the blue color disappeared completely. Care was taken to use adequate stirring, a white background, and good lighting. A blank determination was also carried out. The Thiodan content was computed on the basis of the iodine consumed by the sulfur dioxide which was produced during hydrolysis of Thiodan.

K. Infrared Spectrophotometric Method for Determination of Low and High Melting Isomers of Thiodan. Thiodan exists in two isomeric forms (melting points 108° and 212° C.). The infrared spectrum of each isomer has a band near 8 microns which was used to estimate the quantity present. However, accurate results could not be obtained with samples of impure Thiodan. It was established that the isomer contents of the Thiodan-5a,9a-C₁₄ samples were roughly the same as the isomer content of commercial Thiodan.

The method involved making absorbance measurements of the samples dissolved in carbon disulfide at 7.95 and 8.05 microns for the low- and high-melting isomers, respectively.

A Baird Model 4-55 infrared spectrophotometer was employed at $2N$ slit program and $2X$ abscissa. Solutions for calibration were prepared using binary mixtures (by weight) of pure isomers made up to 5 ml. with carbon disulfide. The solutions were placed in a 0.2-mm. sodium chloride cell and the spectrum was scanned from 7.7 to 8.3 microns at the rate of 0.5 micron per minute. A base line was drawn between the transmittance maxima at 7.8 and 8.2 microns, absorbances were calculated, and graphs were plotted of absorbance at the respective wave lengths vs. concentration of the individual isomers. Both plots gave a straight line through all points.

Samples of Thiodan for analysis were treated in the same manner as the calibration standards.

L. Crystallization of Radio-Tagged Thiodan. Small samples of Thiodan-5a,9a- C_{12}^{14} were dissolved in warm hexane, filtered, and crystallized by cooling with ice water. Another crop was obtained in each case by further cooling with carbon dioxide ice, and the remaining mother liquor was evaporated to dryness. Each crop and residue were assayed chemically for Thiodan, a radio-count was determined, and the isomer ratio was estimated. The original samples assayed 85 to 93% Thiodan with a low melting isomer-high melting isomer ratio of about 2 to 1. Upon crystallization, the purer first two crops had higher

radioassays than the residues. These data indicated that the non-Thiodan portion was not radioactive. The various crops had different isomer ratios from the original samples, but no generalization could be made as to the direction of enrichment. When recrystallized Thiodan-5a,9a- C_{12}^{14} was crystallized with recrystallized commercial Thiodan of different isomer composition, the radioactivity of the resulting crops, which differed in isomer ratios, followed as would be expected. When radio-Thiodan and commercial Thiodan, neither of which had been recrystallized, were mixed and then crystallized from hexane, the radioactivity varied with the purity of the resulting crops. These data indicated that radio-Thiodan and commercial Thiodan had the same isomer content, and that the impurities in radio-Thiodan were not radioactive.

Because crystallization of Thiodan-5a,9a- C_{12}^{14} would alter the isomer content, it was preferable to use the unpurified material, and because the impurities did not appear to be radioactive, it was feasible to use the radio-Thiodan in biological experiments.

Acknowledgment

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the semimicroassay of Thiodan from a macroprocedure (9); and J. L. Kalinsky of the Radio-Isotopes Chemistry Section, Material Laboratory, N. Y. Naval Shipyard, Brooklyn, N. Y., for the use of the Cary Vibrating reed electrometer.

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INSECTICIDE TOXICITY

Preparation and Biological Activity of a Series of Halogenated Ethyl and Vinyl Dimethyl Phosphate Esters

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PRACTICALLY all of the work reported in the synthesis (1, 4-7) and biological activity (4, 5, 7) of insecticidally active halogenated ethyl or vinyl dialkyl phosphates has been done on chloro-substituted compounds with passing mention of the possibility of preparing mixed bromo-chloro compounds (6, 7). However, recently a report on the insecticidal properties of dimethyl 1,2-dibromo-2,2-dichlorophosphate was given by Gojemerac and Waples (2). This compound is available commercially under the name "Ortho-Dibrom" (California Spray Chemical Corp., Moorestown, N. J.).

The present report deals with the preparation and comparative toxicity to flies and rats of a series of tertiary

phosphate esters with two methyl groups as constant constituents. The variations in the tertiary group consisted of a series of bromo, chloro, and bromo-chloro-substituted ethyl or vinyl groups.

Compounds Synthesized

The compounds synthesized are shown in Table I, together with the method of synthesis. The tetrahalogenated compounds were prepared by bromination or chlorination of the dimethyl dichlorovinyl and dibromovinyl esters. The dichlorovinyl compound (I) was a specially purified commercial product (Montrose Chemical Co., Newark, N. J.). Dimethyl 2,2-dibromoethyl phosphate (IV), the bromine analog of DDVP (I),

was prepared by Perkow's reaction (7). The reaction was run in an ether solution at a lower reaction temperature (-10° to 0° C.) than that given by Perkow because the reaction was extremely vigorous. The reaction mixture was washed with aqueous sodium bicarbonate and several times with water. After drying with anhydrous sodium sulfate, the ether was removed under reduced pressure. Compounds II and V were obtained by bromination of I and IV. Brominations were done in carbon tetrachloride solutions with a slight excess of the calculated amount of bromine for a 24-hour period. Compounds III and VI were obtained by chlorinating I and IV. Chlorination was done by bubbling dry chlorine gas through carbon tetra-