C=C absorption band; 940 cm^{-1} assigned to C=C absorption band; 1040 cm^{-1} assigned to POC absorption band; and 1228 cm^{-1}, specific for Z isomer.

No significant differences in absorptivities were seen between the solution of the standard and respective radioactive preparation.

BIOASSAY METHODS

To determine if the radioactive polychlorophenyl vinyl phosphates were biologically comparable to pure authentic unlabeled reference standards the samples were bioassayed. Houseflies were tested by the topical method. By plotting the dosage *vs.* the percent mortality, the LD_{50} was found and the toxicity index was calculated. No significant differences in the LD_{50} were seen between the solutions of I, II, and III and the corresponding reference standards.

RESULTS

The polychlorophenyl vinyl phosphate molecule has been successfully specifically labeled in three positions. Typical reaction conditions, yields, purities, and specific activities of some of the preparations are given in Table IV. The reaction of trialkyl phosphite and polychloroacetophenone, as herein reported, gives mainly the bioactive Z isomer in a ratio of 10 (or higher) to 1 of the E isomer.

The main impurities in the technical material before purification were the E isomers in each instance plus dimethyl methyl phosphonate in III, TMP in II, and an unidentified ketonic material in I.

As the data show, liquid–liquid partition chromatography resolved these vinyl phosphates and allowed pure isomers to be isolated. The results of a typical separation are illustrated in Figure 3.

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Synthesis of ^{14}C-Benzenoid-Ring-Labeled Guthion

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A detailed method for synthesizing radiochemically pure Guthion (benzenoid-ring-UL-^{14}C) in good yield is described. The overall yield of labeled Guthion (specific activity 1.0 μCi/mmol) from the seven-step, three-trial synthesis was 80%. Each derivative of the synthesis was screened for composition and purity by conventional spectroscopic and radiometric procedures. This ring-labeled insecticide will be utilized as an aid in the elucidation of several currently reported unknown degradation products and will also undoubtedly prove to be a valuable aid in studying the metabolic patterns, distribution, and translocation of this compound in animals and plants.

Guthion {*O,O*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3-(4*H*)-yl methyl] phosphorodithioate} is a potent organophosphate insecticide toxic to insects and mammals by its ability to inhibit cholinesterase activity (Martin, 1966). Increasing amounts of Guthion have been used recently with the diminution in the use of chlorinated hydrocarbon insecticides (EPA, 1971).

Although two types of radiolabeled Guthion (^{32}P and ^{14}C at the methylene group) have been synthesized (Everett *et al.*, 1966), the synthesis of ring-labeled Guthion has been ignored and several metabolites still remain unidentified. Labeling of groups easily removed from the molecule by metabolic processes can be a great disadvantage in some cases. For this reason there is often justification for making the more difficult synthesis involved in the labeling of groups which are more central in the molecule, such as aromatic rings or ring substituents (Casida, 1969). This paper describes an extremely efficient method for synthesizing Guthion (benzenoid-ring-UL-^{14}C), of high chemical purity, that can be used directly in studies involving absorption, distribution, metabolism, excretion, and related physiochemical transformations.

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EXPERIMENTAL SECTION

Chemicals. Double-distilled reagent grade quality solvents were used throughout the synthesis (Figure 1). Technical *o*-nitroaniline (compound I) was purified by dissolution in hot ethanol–water (1 : 2) to which was added activated carbon, and the resultant slurry was clarified by vacuum filtration through a hot Büchner funnel dressed with 0.5 in. of Celite. The filtrate was chilled to 4°, seeded if necessary for crystallization, and then further chilled to −10°. Efficient agitation was used throughout the crystallization period. The fine bright orange-yellow crystals were collected by vacuum filtra-

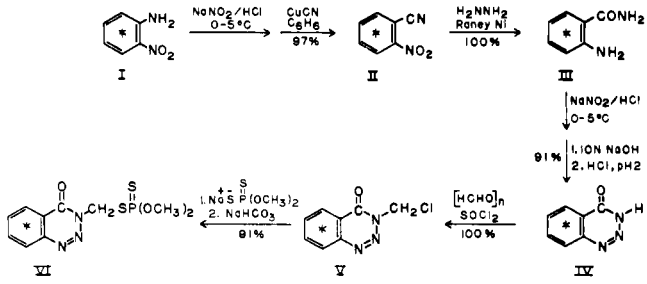

Figure 1. Reaction scheme for the synthesis of ring-labeled Guthion

tion, washed with ice water, and dried overnight in a vacuum desiccator. The dried crystalline product was found to be of high chemical purity and was used without further purification. The *o*-nitroaniline-(ring-UL-^{14}C), specific activity 7.03 mCi/mmol, was purchased from Mallinockrodt Nuclear, St. Louis, Mo., and diluted to 1.0 g with recrystallized *o*-nitroaniline (I).

Cuprous chloride was freshly prepared from 52.1 g, 209 mmol, of crystallized copper sulfate according to the method of Marvel and McElvain (1923). The reaction flask was purged with nitrogen during agitation periods as an aid to prevent darkening of cuprous chloride upon exposure to air. The resulting wet cuprous chloride was suspended in 83 ml of distilled water and converted to cuprous cyanide (Clarke and Read, 1941). The resulting cuprous cyanide solution (152 ml) was clarified by filtration and stored in the dark at 0–5°. This volume of cuprous cyanide solution was sufficient to react with 24.0 g, 174.0 mmol of *o*-nitroaniline. Stability was greater than 30 days.

Other chemicals and adsorbents included hydrazine hydrate (99%) (Mallinckrodt, St. Louis, Mo.), Raney nickel (99%) (Research Organic/Inorganic Chemical Co., Sun Valley, Calif.), *O,O*-dimethyldithiophosphoric acid (90%) (Watteree Chemical Co., Lugoff, S. C.), Celite 545 (Fisher Scientific Co., Fair Lawn, N. J.), and activated charcoal ("Darco" G-60) (Matheson Coleman and Bell, East Rutherford, N. J.). The sodium salt of *O,O*-dimethyldithiophosphoric acid was prepared by neutralizing a saturated aqueous solution of the acid with solid sodium bicarbonate. Heavy oily impurities were removed from the aqueous layer by partitioning them into chloroform and subsequently discarding the chloroform layer. After several chloroform washings the aqueous layer was chilled to 0–5° and the precipitated sodium *O,O*-dimethyldithiophosphate was isolated by vacuum filtration and desiccated. Other chemicals used were analytical grade.

Preparation of *o*-Nitrobenzonitrile-^{14}C (II). In a typical preparation, 1.0 g, 7.24 mmol of *o*-nitroaniline was placed in a 50-ml beaker together with 1.50 ml of concentrated reagent grade hydrochloric acid and sufficient cracked ice and water to provide a thin slurry at 0°. With stirring, 550 mg, 7.97 mmol of sodium nitrite in 1.5 ml of water was added to the cold suspension of *o*-nitroaniline hydrochloride. The temperature was maintained at 0–5° by the direct addition of cracked ice. Completion of the diazotization reaction was indicated by the formation of a clear pale-yellow solution. The solution was adjusted to pH 6 at 0–5° by cautiously adding 425 mg of anhydrous sodium carbonate. The cold solution was then introduced (over a period of 30 min) into a stirred mixture of 6.5 ml of cold cuprous cyanide solution and 75 ml of toluene contained in a 400-ml beaker. The mixture was maintained at 0–5° for 30 min by the addition of ice, followed by a 2-hr stir at room temperature. The mixture

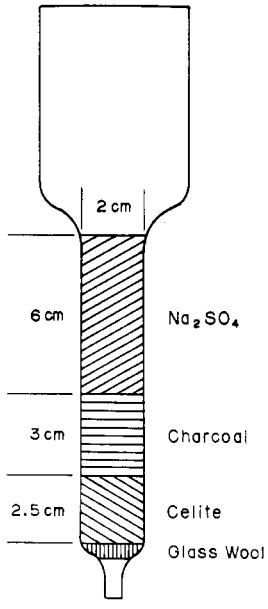

Figure 2. Schematic of clean-up column

was then transferred to a separatory funnel and the toluene layer was drawn off. The aqueous layer was washed with additional toluene, and the combined toluene extracts were evaporated to dryness at room temperature with a stream of dry nitrogen. The residual nitrile was dissolved in ethanol and clarified on the column illustrated in Figure 2. The purified product was isolated by crystallization from cold ethanol. The dried product weighed 1.08 g, yield 100%. The yields of three trials averaged 97%.

Preparation of Anthranilamide-^{14}C (III). Anthranilamide was prepared from *o*-nitrobenzonitrile by modification of a method described by Butler and Partridge (1959). *o*-Nitrobenzonitrile (1.08 g, 7.25 mmol) was dissolved in 100 ml of ethanol and 2.0 ml of hydrazine hydrate (99%). The mixture was heated to 45–50° and subsequently treated in a well-ventilated hood with small periodic charges ($\leq$10 mg) of Raney nickel. The temperature was maintained between 40 and 60° during the addition of the nickel catalyst. Completion of the reaction was indicated when no more hydrogen was evolved after the further addition of Raney nickel. The hot ethanolic solution was clarified under a nitrogen atmosphere on the column illustrated in Figure 2. Solvent percolation through the column was enhanced by the use of a vacuum filter flask. The column was not allowed to go dry because of the inherent fire danger of the nickel catalyst when exposed to air. The column was washed with an additional 300 ml of hot ethanol and the combined ethanol eluates were evaporated to dryness in a CALAB rotary vacuum concentrator. The dried product weighed 1.00 g, yield 100%. The yields of three trials averaged 100%. The spent filter cake was suspended in water and packaged wet for safe disposal.

Preparation of 1,2,3-Benzotriazin-4(3*H*)-one-^{14}C (IV). Sodium nitrite (733 mg, 10.0 mmol) in water (2 ml) was added with stirring to anthranilamide (1.0 g, 7.25 mmol) and hydrochloric acid (2.0 ml) suspended in ice–water. Again, completion of the diazotization reaction was indicated by the formation of a clear pale-yellow solution, and the temperature of the solution was maintained at 0–5° by the addition of ice. Cold 10 *N* sodium hydroxide was then added dropwise until the pH reached 8.5. Approximately 2 ml of base was required for ring closure. The resulting sodium salt slurry of the ring compound (IV) was treated with concentrated

hydrochloric acid to bring the pH to 2–4. The free 1,2,3-benzotriazin-4-(3*H*)-one was collected by vacuum filtration and washed with ice–water to remove residual traces of salt. The dried product weighed 0.93 g, yield 87%. The yields of three trials averaged 91%.

Preparation of 1,2,3-Benzotriazin-4-(3-chloromethyl)-one-^{14}C (V). This procedure is partially based on one described by Everett *et al.* (1966). 1,2,3-Benzotriazin-4-(3*H*)-one (0.93 g, 6.33 mmol) and 0.390 g of 95% paraformaldehyde were placed in a 50-ml round-bottomed flask with 30 ml of ethylene dichloride. The flask was fitted with a reflux condenser and the slurry was heated to 40°. Thionyl chloride (1.2 ml) was added to the stirred slurry through a dropping funnel, while the temperature was maintained at 40°. The reaction flask was then transferred to a 65° water bath and held there for a period of 4 hr. After cooling to room temperature, 10 ml of water were added and the mixture was neutralized with 10 *N* sodium hydroxide. The mixture was transferred to a separatory funnel and the ethylene dichloride layer was drawn off and clarified on the column illustrated in Figure 2. The aqueous layer was washed with two 50-ml aliquots of ethylene dichloride and this solvent in turn was added to the column. The column eluate was collected in a 250-ml vacuum filter flask, transferred to a preweighed 100-ml round-bottomed flask, and subsequently evaporated to dryness. The product weighed 1.23 g, yield 100%. The yields of three trials averaged 100%.

Preparation of Guthion-^{14}C (VI). The present procedure is an adaptation of the method described by Everett *et al.* (1966) to give somewhat better yields. Ethylene dichloride, 12 ml, was added to the 100-ml preweighed flask containing 1.23 g of 1,2,3-benzotriazin-4-(3-chloromethyl)-one (V). After heating to 50° with stirring, 5.5 ml of a solution of 0.05 g/ml of sodium bicarbonate and 12 ml of a 36% solution of *O,O*-dimethyl phosphorodithioate sodium salt in water were added. The stirred mixture was transferred to a separatory funnel. The ethylene dichloride layer was drawn off and the aqueous layer was reextracted with two 50-ml portions of ethylene dichloride. The combined ethylene dichloride extracts were purified by vacuum filtration through a column similar to that illustrated in Figure 2. The eluate was evaporated to dryness in a preweighed 100-ml round-bottomed flask. The pale-yellow oil weighed 2.06 g, yield 100%. The yields of three trials averaged 91.3%. The oily product was purified by recrystallization from methanol. Pure Guthion (80% overall, 1.0 μCi/mmol) was isolated in the form of pure fine white crystals.

Spectroscopic Measurements. Infrared spectra were obtained from potassium bromide pellets using a Perkin-Elmer Model 337 spectrophotometer. Mass spectra were performed for identification purposes. Fragmentation patterns were identical to standard spectra of similar benzotriazinones reported by Tore and Shadoff (1969). Spectra were obtained utilizing a Varian M-66 spectrometer.

Thin-Layer Chromatography. Radioactive Guthion was spotted on a Brinkmann silica gel F-254 precoated glass plate together with a reference standard of Guthion. The plate was developed in a 5:1 mixture of chloroform–acetone (Schulz *et al.*, 1970), and the spots were located under ultraviolet light (2537 Å). A single set of spots having an R_f value of 0.46 was visualized. The radiochemical purity of the labeled Guthion was determined by exposing the developed thin-layer plate to X-ray film (Kodak, No screen) for 4 days, after which the film was removed and developed. A single set of exposed spots having a common R_f was evident on the X-ray film, indicating a high degree of radiochemical purity.

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