The Determination of 4,4′-Thiodiphenol in Human and Rat Urine as an Indication of Exposure to Low Levels of Abate

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The larvicide Abate® (0,0,0',0'-tetramethyl 0,0'-thiodi-p-phenylene phosphorothioate) has been registered for use in the control of mosquito larvae in nonpotable water. However, there are no specific methods available to determine exposure to Abate in humans and animals.

In an exposure study (1) carried out in Puerto Rico, Abate at a concentration level of 1 ppm was added to drums and cisterns which stored the drinking water for a community of 2,000 people. A total organic phosphorus method was used to analyze the urine from the exposed individuals; the results indicated that equilibrium levels were reached after about 20 weeks of exposure. The alkyl phosphate content in urine dropped rapidly to near control levels at the conclusion of the exposure study.

Abate residues have been determined colorimetrically (2) and gas chromatographically (3, 4, 5). Two of the reported methods are based on the determination of 4,4'-thiodiphenol, the hydrolysis product of Abate, by a spectrophotometric method (2) and a gas chromatographic method (4). All methods are designed to determine Abate in water with the exception of the method reported by St. John and Lisk (6) in which the methylated alkyl phosphate hydrolytic product of Abate was determined in soil extracts.

Metabolism studies have been performed to determine the metabolic fate of labeled Abate when administered orally to rats (7). The fecal (60%) and urinary (40%) routes were the principal means of elimination over a 48 to 72 hour period. Gross radioactivity levels in the blood were highest between 5 and 8 hours after ingestion, then decreased with a half-life of approximately 10 hours. In the feces and in fatty tissue, the radioactivity was due principally to unchanged Abate with minor amounts of its sulfoxide derivative. The principal urinary metabolic products were sulfate ester conjugates of the phenolic hydrolysis products 4,4'-thiodiphenol (24%), 4,4'-sulfinyldiphenol (2.5%) and 4,4'-sulfonyldiphenol (0.05%) (7). Thus the determination of the 4,4'-thiodiphenol in human or rat urine seems to be the best approach to determine low levels of exposure to Abate.

This paper presents a gas chromatographic method which has been developed to determine the extent of human and animal exposure to low levels of Abate.

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Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 4, 1970, published by Springer-Verlag New York, Inc.

Materials and Methods

Reagents

Tri-Sil concentrate (a 2:1 mixture of hexamethyldisilazane and trimethylchlorosilane), Pierce Chemical Co., Rockford, Ill.

Silica gel, 60-200 mesh, grade 950, Fisher Scientific Co., Pittsburgh, Pa.

Abate and 4,4'-thiodiphenol, both analytical standards, American Cyanamid Corp., Princeton, N.J.

Apparatus and Glassware

Microcolumn, i.d. 7 mm, Catalog No. K-420100, Size 22, Kontes Glass Co., Vineland, N.J.

Crucible holders, glass, top i.d. 25 mm.

Gas chromatograph, MicroTek Model MT 220 equipped with flame photometric detector, sulfur filter, 394 mμ, and a U-shaped aluminum column, 1/4" o.d. x 2', packed with 60-80 mesh Chromosorb W coated with 2.5% E 301 and 0.25% Epon 1001 (w/w). Temperatures: inlet, 190°C; column, 190°C; detector, 135°C; transfer line and outlet block, 200°C. Flow rates (ml/min): nitrogen, 140; hydrogen, 200; oxygen, 40. On-column injection was used for all analyses.

Analytical Procedure

Preparation of Standard Curves

Prepare four standard solutions of the 4,4'-thiodiphenol in chloroform or methylene chloride to contain 0.5, 1, 2, and 4 μg/ml.

Pipet a 1 ml aliquot from each standard solution into a 15 ml centrifuge tube. Add 0.25 ml pyridine and 0.2-0.3 ml Tri-Sil concentrate. Stopper, mix, and heat at 90°C in a heating block for 1 hour or leave overnight at room temperature. Add approximately 2 ml water, follow with 3 ml 1N H_2SO_4, and mix. After complete separation of the two layers, remove the water layer with a disposable pipet. Add approximately 5 ml distilled water and mix. Allow the two layers to separate and remove the water layer. Inject 10 μl of each standard corresponding to 5, 10, 20, and 40 ng of 4,4'-thiodiphenol into the gas chromatograph. Volumes up to 50 μl may be injected. Plot the response or peak height versus concentration on log-log graph paper. The detector response is linear up to 100 ng of the 4,4'-thiodiphenol, using the log-log scale. Eleven nanograms of the derivatized 4,4'-thiodiphenol produce a peak 1/2 full recorder deflection at 6.35×10^{-9} AMP full scale. The minimum detectable amount for 4,4'-thiodiphenol is 5 ng. A typical standard curve obtained in this fashion is shown in Figure 1. The gas chromatogram of the silyl ether derivatives of 4,4'-thiodiphenol, 4,4'-sulfinyldiphenol, and 4,4'-sulfonyldiphenol is shown in Figure 2.

Hydrolysis of the 4,4'-Thiodiphenol Urinary Conjugates

Pipet 1-10 ml of the urine sample into a 25 ml test tube. Slowly add a volume of concentrated HCl equal to half the volume of the urine sample and follow with approximately 0.5 g zinc dust. (The reducing medium is provided to minimize air oxidation of the 4,4'-thiodiphenol.) Place a reflux condenser on the tube and cool the condenser with circulating ice-cold water. Heat the tube in a heating block maintained at 120°C for 1 hour. Allow the tube to cool, wash down the sides of the condenser with about 2 ml of chloroform or methylene chloride, disconnect the tube, remove from the condenser, and proceed to the extraction and derivatization step.

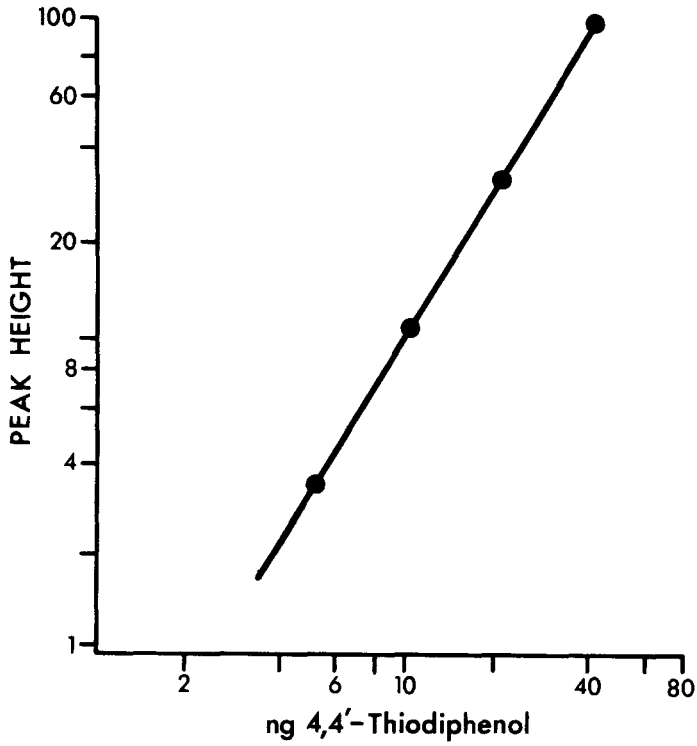

Figure 1. Standard curve of 4,4'-thiodiphenol TMS.

Extraction and Derivatization

Extract the hydrolyzed urine four times with a total volume of 10 ml chloroform or methylene chloride to remove 4,4'-thiodiphenol. Centrifuge the samples during the extraction if emulsions persist. Combine the extracts and transfer to a crucible holder containing about 25 g of anhydrous sodium sulfate and a loose plug of silanized glass wool. Collect the eluate in a 15 ml centrifuge tube.

Concentrate the extract to a volume of 1 ml with the aid of a gentle stream of clean, dry nitrogen and a heating block maintained at 50° C. Proceed with the derivatization step by adding the pyridine and Tri-Sil concentrate and wash to remove the excess reagents as mentioned under the preparation of standard curve.

Chromatographic Removal of Interfering Materials in Urine

Place a small loose plug of silanized glass wool in the tip of the micro-column. Slowly add 1.0 g of 60-200 mesh silica gel, gently tapping the column to insure uniform packing. Add 3 g of granular anhydrous sodium sulfate to the top of the column. Wash the column with 10 ml methanol; then condition the columns in a 130°C oven overnight before using.

Transfer the 1 ml volume of silanized chloroform extract quantitatively to the silica gel column and elute with 10 ml chloroform, collecting the eluate in a 15 ml centrifuge tube. Concentrate the eluate to a volume of 1.0 ml with the aid of a gentle stream of clean, dry nitrogen and a heating block maintained at 50 °C. Inject aliquots up to 50 µl in the gas chromatograph.

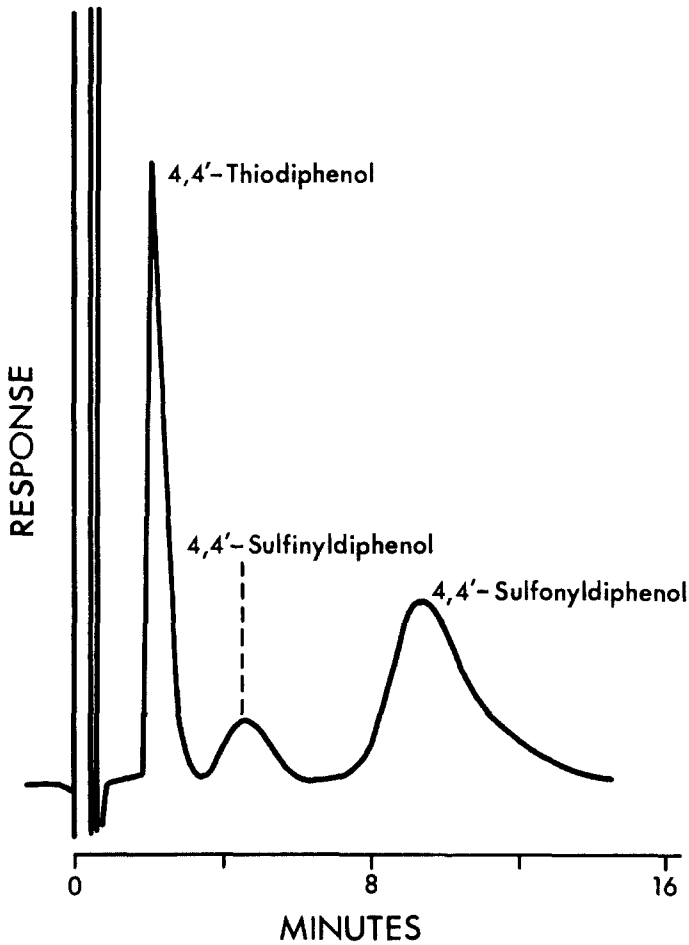

Figure 2. Chromatogram of the silyl ether derivatives of 10 ng 4,4'-thiodiphenol, 4,4'-sulfinyldiphenol undetermined, and 32 ng 4,4'-sulfonyldiphenol.

Recovery of 4,4'-Thiodiphenol from Human Urine

Five 10 ml aliquots of a human urine sample were fortified with 4,4'-thiodiphenol at the levels of 0.02, 0.05, 0.10, 0.50, and 1.0 ppm. The fortified samples were hydrolyzed, extracted, and derivatized, and the interfering materials were removed using the microcolumn cleanup described above.

The Determination of 4,4'-Thiodiphenol in Rat Urine

Two female Sprague-Dawley rats weighing 242 and 238 g were dosed by gavage with 9.6 mg of Abate (1/10 the LD_{50}) dissolved in peanut oil containing traces of ethanol. The dose was administered daily for three consecutive days. The animals were maintained in a stainless steel metabolism cage, and the urine was collected in a manner which minimized fecal contamination. Timed urine samples were collected before exposure and at various intervals thereafter; all urine samples were frozen until analyzed for 4,4'-thiodiphenol. Results are expressed as micrograms excreted per day.

Results and Discussion

The results of the analysis of the human urine sample fortified with 4,4'-thiodiphenol at the levels from 0.02 to 1.0 ppm using the suggested procedure are shown in Table I. The recoveries ranged from 75 to 87%, and levels as low as 0.02 ppm 4,4'-thiodiphenol were detectable.

TABLE I

Recovery of 4,4'-Thiodiphenol Added to 10 ml of Human Urine

Level of Fortification, ppm	Amount Recovered, ppm	Recovery, %
0.02	0.015	75
0.05	0.039	78
0.10	0.079	79
0.50	0.400	80
1.00	0.870	87

Appreciable amounts of 4,4'-thiodiphenol were found in the urine of rats dosed three times with a concentration of Abate equivalent to 1/10 the LD_{50}. The rate of urinary excretion of the phenol is shown in Figure 3; the results are expressed as micrograms excreted per day. Levels

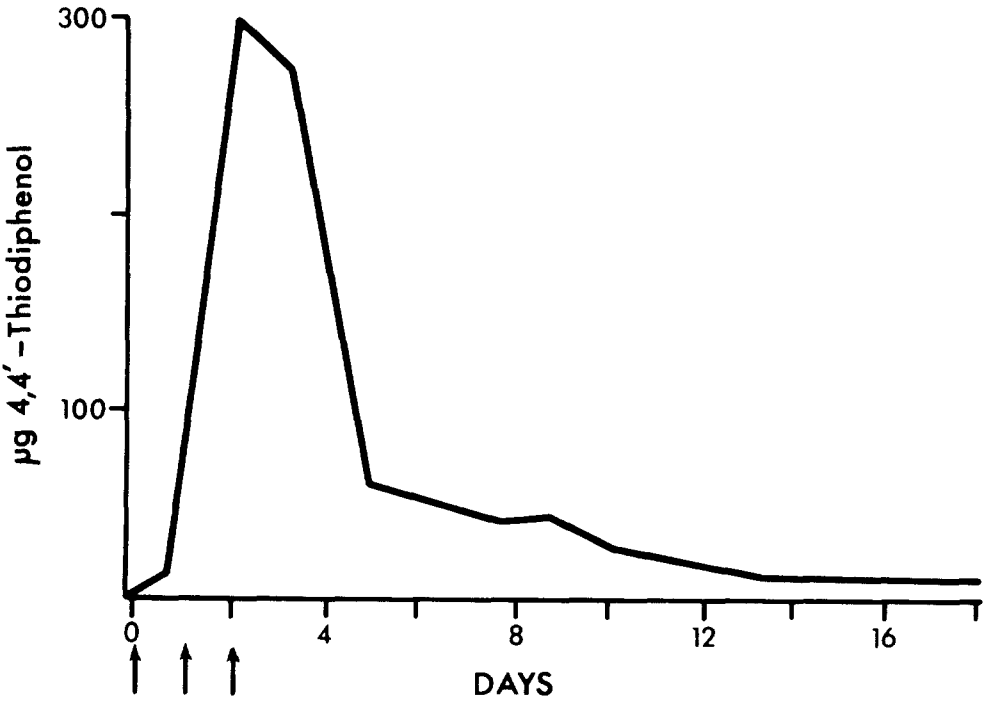

Figure 3. The rate of urinary excretion of 4,4'-thiodiphenol of rats fed three consecutive 1/10th LD_{50} dosages. Arrows indicate the time of dosage.

as high as 0.20 ppm of the 4,4'-thiodiphenol were still being excreted in the urine two weeks following the last dose. These data indicate that Abate or Abate metabolites may be stored in tissues of the rat. The possibility of storage has been reported by Laws et al. (8). These workers observed that the organic phosphorus level in human urine decreased slowly after the dosage was stopped and was still above control levels three weeks after the last exposure.

Small amounts of 4,4'-sulfinyldiphenol and 4,4'-sulfonyldiphenol residues in urine can be determined by extracting the acid-hydrolyzed sample with diethyl ether instead of chloroform. The ether extract is evaporated to dryness, redissolved in 1 ml chloroform, silanized, washed, and injected in the gas chromatograph without the silica gel cleanup step. The difficulty in obtaining a pure 4,4'-sulfonyldiphenol standard can be conveniently circumvented by oxidizing 4,4'-thiodiphenol with hydrogen peroxide; 10 to 20 µg samples of 4,4'-thiodiphenol can be oxidized quantitatively to the 4,4'-sulfonyldiphenol. Attempts to prepare a pure 4,4'-sulfinyldiphenol were unsuccessful.

The hydrolysis step, which includes a zinc and HCl medium maintained at 100°C, does not reduce either the 4,4'-sulfinyldiphenol or the 4,4'-sulfonyldiphenol residues present in the urine. Furthermore, these reagents serve to prevent the oxidation of the 4,4'-thiodiphenol. For this reason, a reducing medium is used in the hydrolysis step and solvents are evaporated with a stream of nitrogen instead of air.

The lower limit of detection using the suggested method and cleanup developed in this paper is 0.02 ppm.

Summary

A method for the determination of Abate in water has been modified to determine the 4,4'-thiodiphenol in human and rat urine. The method involves acid hydrolysis of conjugated residues, extraction, silylation, cleanup, and gas chromatography using the flame photometric detector equipped with a sulfur filter. The method can be used for the determination of the other Abate metabolites, 4,4'-sulfinyldiphenol and 4,4'-sulfonyldiphenol. The recovery of the 4,4'-thiodiphenol from fortified human urine was 75-87%. The minimum detectable amount is 0.02 ppm.

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