A Method for the Determination of 1-Naphthol in Urine

by M. T. SHAFIK, H. C. SULLIVAN, and H. F. ENOS
Perrine Primate Research Branch
Division of Pesticide Chemistry and Toxicology
Food and Drug Administration
Department of Health, Education, and Welfare
Perrine, Florida

Humans exposed industrially to the insecticide carbaryl (1-naphthyl N-methylcarbamate) excrete relatively large quantities of 1-naphthol conjugated either as the sulfate or glucuronide (1). A colorimetric procedure (2) is generally used to quantitatively determine 1-naphthol in human urine. However, this method lacks both the sensitivity and specificity necessary for determining the relatively small amounts of 1-naphthol excreted in the urine of agricultural workers exposed to low levels of carbaryl. The object of this investigation was to develop a rapid, sensitive method for the determination of 1-naphthol in human urine.

The 1-naphthol resulting from the hydrolysis of carbaryl has been used as an indirect measure of the residue level of the parent insecticide on a variety of agricultural crops (3,4). Argauer (5) described a procedure for chloroacetylating phenols and 1-naphthol for subsequent detection by electron-capture gas chromatography. In a more recent publication (6), Argauer utilized this procedure to determine a number of carbamate insecticides, but indicated that further modification was necessary if the method was to be extended to carbaryl. Since Landowne and Lipsky (7) had previously demonstrated that the monochloroacetate derivative enhanced electron-capture detection to a greater extent than a variety of other haloacetates, it was decided to adapt this derivatization procedure to the determination of 1-naphthol in human urine. To extend the sensitivity of the method, a cleanup step using silica gel adsorption chromatography is included for samples containing less than 1 ppm of 1-naphthol.

Experimental

Apparatus.[1] A gas chromatograph (MicroTek Model 220 equipped with a tritium detector) was operated under the following conditions: 180 cm x 0.4 cm i.d. glass column packed with 1.5% OV-17 and 1.95% QF-1 (w/w) on 100/120 mesh Chromosorb W (dimethyldichlorosilane, high performance); nitrogen carrier gas with a flow rate of 90 ml/min; column temperature 185°C; injection port, detector, and transfer line temperature 210°C.

Centrifuge, International size 2 with metal shield. International Equipment Co., No. 367.

[1] Use of trade names is for identification purposes only, and does not constitute endorsement by the U.S. Department of Health, Education, and Welfare.

Bulletin of Environmental Contamination & Toxicology
Vol. 6, No. 1, 1971, published by Springer-Verlag New York Inc.

Chromatographic columns, size 22, Kontes No. 420100; concentrator tubes, 25-ml glass-stoppered, $\overline{S}$ 19/22, Kontes No. 570050; condensers, $\overline{S}$ 19/22, Kontes No. 286810.

Solvents and Reagents. Benzene and hexane, pesticide quality; pyridine, Fisher, redistilled; hydrochloric acid, concentrated, Analytical Reagent; 1-naphthol, Eastman Organic Chemicals.

Chloroacetic anhydride, Eastman Organic Chemicals. Prepare a 2% solution in benzene.

Sodium sulfate, anhydrous. Extract with benzene in a Soxhlet apparatus for 4 hours. Dry and store at 170°C. For use, prepare a 3% aqueous solution.

Silica Gel, Woelm, activity grade I, Waters Associates, Inc. Dry adsorbent for 48 hours at 170°C and store in a desiccator. On the day of use, partially deactivate the silica gel with 1.5% water in the following manner. Add the necessary volume of water to a 125-ml glass-stoppered Erlenmeyer flask, rotating the flask to coat the sides with water. Add the weighed amount of silica gel, stopper the flask, and mix until the water is evenly distributed throughout the adsorbent. Allow to equilibrate for 2 to 3 hours with periodic shaking. Prepare the chromatographic columns just before use.

Use deionized or distilled water, pre-extracted with benzene, throughout the procedure.

Preparation of Standard Curve. From a stock solution of 1-naphthol in benzene, transfer 1-ml aliquots containing from 0 to 500 ng to a series of 25-ml concentrator tubes. Add 5 ml of benzene, 2 ml of the 2% chloroacetic anhydride solution, and 0.2 ml of pyridine. Mix thoroughly and allow the solutions to stand at room temperature for 10 minutes. Wash the solutions 3 successive times with 5 ml of water, centrifuging at 2000 r.p.m. after the last washing. Wrap the concentrator tube with a paper towel before placing in the metal shield for centrifugation. Carefully remove and discard all of the aqueous layer, using a disposable pipet fitted with a rubber bulb. Adjust the volume to 10 ml with benzene and inject 5 µl of each solution (0-250 pg) into the gas chromatograph. Prepare a standard curve by plotting response (peak height) versus concentration.

Procedure for Urine Samples. Pipet 5 ml of urine into a 25-ml concentrator tube, add 1 ml of concentrated hydrochloric acid, and mix well. Fit the concentrator tube with a glass-stoppered condenser and reflux the mixture in a steam bath for 90 minutes, cooling the condenser with circulating ice water. Remove from the bath and cool.

Wash down the sides and tip of the condenser with 2 ml of 0.1 N NaOH followed by 2 ml of benzene. Extract the urine twice with a total of 6 ml of benzene, centrifuging at 2000 rpm after each extraction, and transfer the organic extracts to a clean concentrator tube. Wash the benzene extract with two 3-ml portions of 3% sodium sulfate solution. Centrifuge at 2000 rpm and discard the aqueous layer. Proceed with the derivatization as follows: Add 2 ml of 2% chloroacetic anhydride solution and 0.2 ml of pyridine to the benzene extract. Mix well and let stand 10 minutes at room temperature. Wash the mixture 3 times with 5 ml of water to remove excess derivatization reagents. Centrifuge at 2000 rpm after the last washing and carefully remove all the water. Concentrate the sample to 0.5 ml in a water bath maintained at 40°C, using a gentle stream of nitrogen.

Silica Gel Cleanup Procedure. Add 1 g of partially deactivated silica gel to a chromatographic column which has been lightly plugged with a small piece of glass wool. Prewash the column with 10 ml of hexane and discard the wash. Transfer the concentrated benzene extract to the column with a disposable pipet. Rinse the tube with two 0.5-ml portions of 20% benzene in hexane and add the rinsings to the column. Elute with an additional 8.5 ml of 20% benzene-hexane and discard this eluate. Add 10 ml of 60% benzene in hexane to the column and collect the eluate in a 15-ml centrifuge tube. This fraction contains the 1-naphthyl chloroacetate derivative. Adjust volume to 10 ml with benzene. Inject 5 to 10 µl into the gas chromatograph, determine the peak height, and calculate the concentration from the standard curve.

It is recommended that the elution pattern of 1-naphthyl chloroacetate standard using the silica gel column be verified in each laboratory.

Results and Discussion

A standard of 1-naphthyl chloroacetate was prepared and purified using silica gel column chromatography. A comparison of the electron-capture detector response of 1-naphthol carried through the procedure and an equivalent amount of the purified 1-naphthyl chloroacetate indicates that essentially 100% conversion to the chloroacetate derivative is achieved.

Both infrared and mass spectral data were identical for the purified standard and a compound identified in the analytical procedure as the chloroacetate derivative of 1-naphthol (8). The source of 1-naphthol in this case was from the urine of an individual industrially exposed to carbaryl.

The recovery data summarized in Table 1 were obtained by analyzing human urine samples to which the sodium salt of 1-naphthol had been added. The samples were taken through the entire procedure including the acid hydrolysis and cleanup steps.

TABLE 1

Recovery of 1-Naphthol Added to Human Urine Samples

Amount Added (ppm)	Recovery (%)
0.02	92
0.02	92
0.02	89
0.1	89
0.1	92
0.1	93
0.5	92
0.5	95
0.5	95
1.0	91
1.0	93
1.0	93

Figure 1 illustrates chromatograms of human control urine with and without the addition of 1-naphthol. The peak in A represents 1-naphthyl chloroacetate equivalent to 50 pg of 1-naphthol.

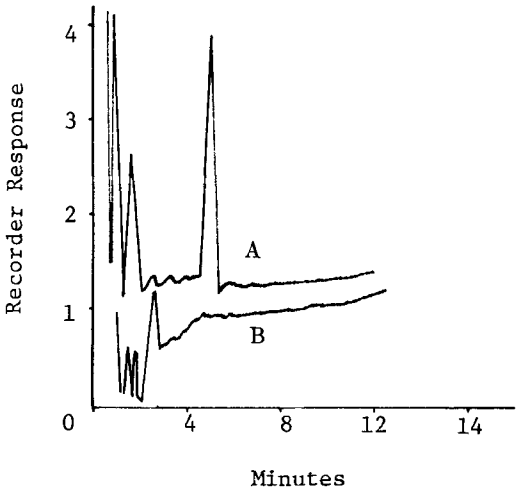

Figure 1. Representative chromatograms. A: Control human urine with 0.02 ppm 1-naphthol added, silica gel cleanup. B: Control human urine, no addition, with silica gel cleanup.

To determine the optimum time for acid hydrolysis, urine samples from the same highly exposed individual were hydrolyzed for times ranging from 30 to 120 minutes. On the basis of the data shown in Table 2, 90 minutes was selected as the length of time necessary to insure complete acid hydrolysis of conjugated forms of 1-naphthol at 100°C.

TABLE 2

Amount of 1-Naphthol Recovered from Urine as a Function of Acid Hydrolysis

Hydrolysis Time (min)	1-Naphthol (ppm)[a]
30	33
60	35
90	48
120	47

[a] Each value is the average of 3 determinations.

Samples of urine, obtained from formulators and farmers exposed to carbaryl at various levels, were analyzed in order to ascertain the applicability of the procedure to a wide range of concentrations of 1-naphthol. Concentrations varied from 6.2 to 78.8 ppm of 1-naphthol in the urine of persons who work as formulators of technical carbaryl at a manufacturing plant, while agricultural workers who used carbaryl for pest control purposes excreted from 0.07 to 1.7 ppm.

Evidence presented in Table 1 and the results of the analysis of urine from individuals exposed at high and low levels indicate that this method can be used to monitor exposure to carbaryl. The limit of detectability has been determined as 0.02 ppm. It is a rapid and sensitive method and the results are reproducible over a wide range of concentrations.

References

(1) BEST, E. M. and MURRAY, B. L., J. Occup. Med. 4, 507 (1962).

(2) CARPENTER, C. P., WEIL, C. S., PALM, P. E., WOODSIDE, M. W., NAIR, J. H., III, and SMYTH, H. F., JR., J. Agr. Food Chem. 9, 30 (1961).

(3) BUTLER, L. I. and McDONOUGH, L. M., J. Agr. Food Chem. 16, 403 (1968).

(4) BUTLER, L. I. and McDONOUGH, L. M., J. Ass. Offic. Anal. Chem. 53, 495 (1970).

(5) ARGAUER, R. J., Anal. Chem. 40, 122 (1968).

(6) ARGAUER, R. J., J. Agr. Food Chem. 17, 888 (1969).

(7) LANDOWNE, R. A. and LIPSKY, S. R., Anal. Chem. 35, 532 (1963).

(8) BIROS, F. J., presented at the ACS/CIC Joint Conference, Toronto, Canada, May (1970).