Metabolism of 2-Chloro-4,6-bis(isopropylamino)-*s*-triazine (Propazine) and 2-Methoxy-4,6-bis(isopropylamino)-*s*-triazine (Prometone) in the Rat. Balance Study and Urinary Metabolite Separation

J. E. BAKKE, J. D. ROBBINS, AND V. J. FEIL

The excretion of Propazine-^{14}C and Prometone-^{14}C labeled in the triazine ring was determined following administration of a single oral dose to rats. Prometone-^{14}C activity was quantitatively recovered in the urine and feces within 72 hours after treatment. Propazine-^{14}C activity was excreted more slowly, with tissue residues remaining 12 days after treatment. No activity could be detected in the expired CO_2 with either ring-labeled compound. Ion exchange chromatography of the urine revealed at least 11 metabolites from Prometone and 18 from Propazine.

Very limited attention has been given to the mammalian metabolism of herbicidal triazines. St. John *et al.* (1965) found 1% of the administered dose of nonlabeled Simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] in the urine and none in the milk of the dairy cow over a 4-day period. Plaisted and Thornton (1964) mentioned the ion exchange chromatographic separation of rat urinary and fecal metabolites of ring-labeled Simazine.

The present study reports the rates and routes of excretion, the tissue levels, and the ion exchange chromatographic separation of the metabolites of the ^{14}C-labeled herbicidal triazines, Propazine and Prometone.

Materials and Methods

Radiolabeled Compounds. Propazine-^{14}C; 2-chloro-4,6-bis(isopropylamino)-*s*-triazine-2,4,6-^{14}C. Isopropylamine (2.36 grams, 0.04 mole) was added to a cold solution (0° C.) of 1.84 grams (0.01 mole) of cyanuric chloride-2,4,6-^{14}C (about 200 μc.) in 40 ml. of acetone. After addition of isopropylamine, the temperature was increased to 45° C. for 5 minutes, then cooled to 0° C. The Propazine was precipitated by adding 50 ml. of water, and after filtration was recrystallized twice from hot 95% ethanol to yield 2.20 grams [m.p. 216–17° C. (sealed capillary) (authentic m.p. 217–18° C.); specific activity 9.33×10^{-2} μc. per mg.]. This preparation was shown to be identical with authentic Propazine by comparison of the infrared, ultraviolet, and mass spectra, and the retention time on gas-liquid chromatography (2% Carbowax on Chromasorb W, 60/80 mesh, oven temperature 175° C., nitrogen carrier gas at 30 ml. per minute).

2-Chloro-4,6-bis(isopropyl-2-^{14}C-amino)-*s*-triazine. A solution of 1.84 grams (0.01 mole) of cyanuric chloride in 30 ml. of acetone was cooled in an ice bath. Over a period of 5 minutes, 16 ml. of 2.5*N* sodium hydroxide and a solution of 1.91 grams (0.02 mole) of isopropyl-2-^{14}C-amine hydrochloride (about 500 μc.) in 16 ml. of water were added simultaneously, with stirring. After the additions were completed, the reaction was stirred at 0° C. for 30 minutes, at room temperature for 4 hours, and then was allowed to stand at room temperature for 16 hours. The product was filtered, dried in a vacuum desiccator, and recrystallized from 90 ml. of hot 95% ethanol to yield 1.37 grams [m.p. 215–17° C. (sealed capillary); specific activity 2.04×10^{-2} μc. per mg.]. The purity of the preparation was determined by comparison of the infrared spectrum and retention time on gas-liquid chromatography with authentic nonlabeled Propazine. The radiopurity of both Propazine-^{14}C preparations was determined by thin layer chromatography on silica gel-HF using the solvent systems of Shimabukuro *et al.* (1966), which gave a single radioactive spot with a thin-layer scanner.

Prometone-^{14}C; 2-methoxy-4,6-bis(isopropylamino)-*s*-triazine-2,4,6-^{14}C. Ring-labeled Prometone was prepared by refluxing the ring-labeled Propazine (100 mg.; specific activity 9.33×10^{-2} μc. per mg.) in absolute methanol containing 20.3 mg. of sodium hydroxide until the Propazine peak disappeared upon gas-liquid chromatography (see above conditions). The Prometone-^{14}C had the same retention time as an authentic sample of nonlabeled Prometone. The yield was 98%, based on activity.

Metabolism Cages. Male rats of the Sprague-Dawley strain were housed in all-glass metabolism cages (Roth *et al.*, 1948) (Delmar Scientific Laboratories, Chicago, Ill.). Air was drawn through the cages and traps at 400 to 500 ml. per minute. The exhaled CO_2 was trapped in scrubbers containing ethanolamine in 2-methoxyethanol (1:7 v./v., 25 ml. per hour of collection time). In all cases, two scrubbers were connected in series.

Radioanalysis. A liquid scintillation spectrometer set up for channels-ratio quench determination was used for all radioanalyses. Two counting formulations were used: solution A for counting water-soluble samples, consisting of 7.0 grams of PPO (2,5-diphenyloxazole), 100 grams of naphthalene, 300 mg. of POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene], and enough reagent grade dioxane to bring the volume to 1 liter; and solution B for counting the CO_2 trapping solution, consisting of 8.25 grams of PPO per liter of reagent grade toluene. Ten milliliters of solution B and 8 ml. of the CO_2 trapping solution comprised the counting solution.

Metabolism and Radiation Research Laboratory, U.S. Department of Agriculture, Fargo, N.D.

Combustion Analysis. Freeze-dried, homogenized feces (50 mg.) were burned by the oxygen-flask technique. After combustion, the flask was cooled, and 10 ml. of the CO_2 trapping solution were added. An 8-ml. aliquot was counted with 10 ml. of counting solution B.

Homogenized, freeze-dried tissues were compressed into pellets (0.5 to 1 gram) and burned in a Parr bomb suitably equipped with a controllable-leak shutoff valve on the exit port. The CO_2 from the combustion was trapped in 50 ml. of the trapping solution by slowly releasing the pressure. When the pressure had been released, air was pulled through the bomb and traps at 300 ml. per minute for 5 minutes. The 50-ml. trap was backed up by a 25-ml. trap. Eight milliliters of each trap were counted with 10 ml. of solution B. In all cases, 98% of the activity was found in the first trap. Recoveries of 98 to 100% were obtained by adding labeled Propazine to freeze-dried control tissues. Samples were burned if duplicates differed by more than 5%. The detectability limit with the ring-labeled compounds was less than 1 p.p.m. of Propazine or Prometone equivalent on a freeze-dried tissue basis.

Sample Preparation. Urine was collected daily and freeze-dried after quantitation of the activity. The radioactivity was quantitatively extractable from the freeze-dried solids with absolute methanol. Preliminary studies showed that all samples (urine, feces, and tissues) could be freeze-dried without loss of radioactivity.

Feces were collected daily, homogenized with water, and freeze-dried prior to combustion. All tissues, except the hide, were homogenized and freeze-dried before combustion analysis. A weighed sample of the hide with the hair was burned without drying.

Ion Exchange Chromatography. Aliquots of the urine methanol extracts containing 2 to 3 $\times$ 10^5 d.p.m. were dried, taken up in 3 ml. of water, and adjusted to pH 3 with 6*N* HCl. An aliquot containing from 1.5 to 2 $\times$ 10^5 d.p.m. was placed on a 0.6 $\times$ 130 cm. column of bead-form, strong cation exchange resin (Chromabeads, Type A, Technicon, Inc.). The column was jacketed with water circulating at 65° C. The column preparation, equilibration, and regeneration, as well as buffer preparation (pH 2.875, 3.80, and 5.00 sodium citrate buffers) and buffer sequence in the variable-gradient device, were those given in the instruction manual for the automatic amino acid analyzer (Technicon Chromatography Corp., 1962) and similar to the system reported by Piez and Morris (1960), with the following exceptions: The column was monitored by a continuous-flow liquid scintillation detector; the detergent and bacteriostat were not included in the buffers; and after the end of the normal buffer cycle (675 ml.), the elution was continued with a two-chamber gradient consisting of 75 ml. of the pH 5 buffer in the first chamber and 75 ml. of 0.2*N* NaOH containing 0.6 mole per liter of NaCl in the second chamber.

The column flow rate was 0.5 ml. per minute, and 10-ml. fractions were collected. The fractions containing activity were quantitated by liquid scintillation in counting solution A.

Internal standards of valine-^{14}C and arginine-^{14}C were used with each run to determine elution reproducibility. The retention volume of the column (19 ml.) was determined with sodium benzoate-^{14}C, using 0.2*N* NaOH as the eluent.

Animal Treatment. Corn oil was saturated with Propazine-^{14}C by warming in a hot water bath. The solution was cooled and the undissolved material centrifuged. An aliquot of the oil was assayed for activity and each rat given a 1- to 1.5-μc. (3-ml.) dose by stomach tube. The dose level ranged from 41 to 56 mg. per kg., which is well below the rat LD_{50} of 5 grams per kg. (Frear, 1963).

Prometone-^{14}C was administered in the same manner, except that 20% ethanol (95%) in corn oil was used to solubilize the compound. Each animal was given 3 ml. containing 0.75 μc. (20.8 to 25.3 mg. per kg.; LD_{50}, 2.9 grams per kg.).

Ultraviolet, infrared, gas-liquid, and thin-layer chromatographic analyses showed that the compounds were not affected by heating in the corn oil.

The animals were placed in the metabolism cages immediately after dosing and given food and water *ad libitum*.

Results

Excretion of Prometone and Propazine and/or their ^{14}C-labeled metabolites was most rapid during the first 24 hours, decreasing to trace amounts at 72 hours (Figure 1). No radioactivity could be detected in the respiratory gases with either ring-labeled compound. The Prometone-^{14}C activity was quantitatively recovered in the urine (91.1%) and feces (9.1%) within 72 hours after dosing, with no detectable activity in the tissues. Ring-labeled Propazine-^{14}C activity appeared

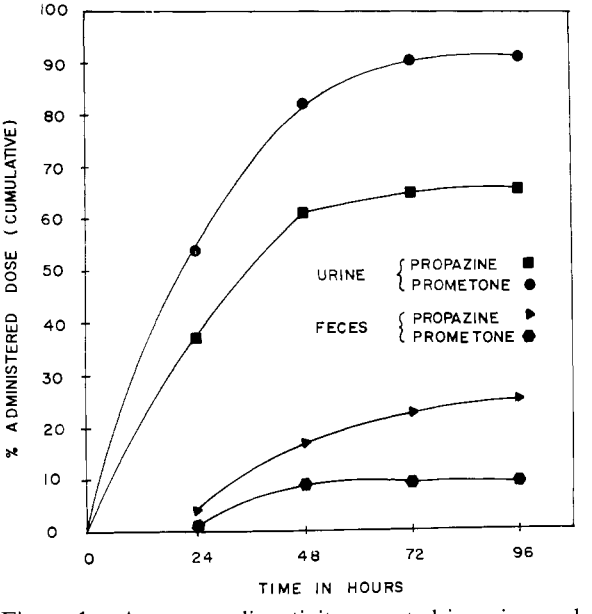

Figure 1. Average radioactivity excreted in urine and feces of male rats after a single oral administration of ^{14}C-ring-labeled Propazine or Prometone

Five rats in each experiment

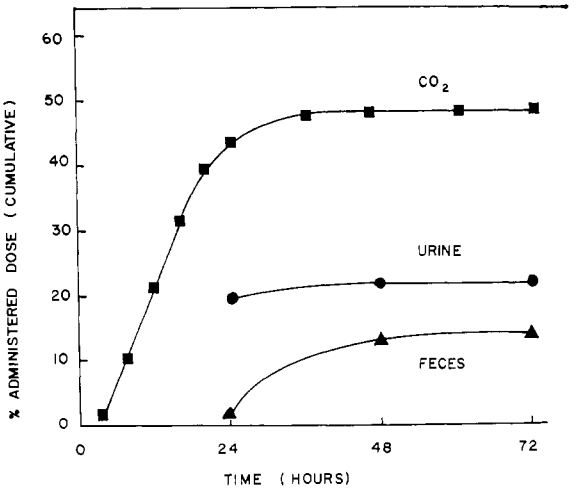

Figure 2. Average radioactivity excreted in urine, feces, and expired CO_2 of two male rats after a single oral administration of ^{14}C-isopropyl-labeled Propazine

Table I. Radioactive Residues Detected in Rat Tissues after a Single Oral Administration of Ring-Labeled Propazine-^{14}C[a,b]

Tissue	Tissue Residues, P.P.M. of Propazine-^{14}C Equivalents (Dry Wt. Basis)				
	2 days[c]	4 days[c]	6 days[c]	8 days[c]	12 days[c]
Liver	52.1	39.3	30.5	20.4	...
Lung	51.3	35.3	34.2	30.3	...
Spleen	46.7	31.3	34.1	25.7	...
Kidney	45.8	30.8	26.6	17.6	...
Heart	50.7	32.9	38.9	26.6	...
Brain	33.6	19.8	...[d]	13.0	...
Carcass	...	...	...	...	5.2 (1.56)[e]
Viscera	...	...	...	...	3.6
Hide	...	...	...	...	9.53[e]

[a] Dose range 41 to 56 mg. per kg.
[b] Pooled samples from 2 rats.
[c] Time of sacrifice.
[d] Sample lost.
[e] Fresh tissue basis.

more slowly in the urine and at a lower total excretion (65.8% in 72 hours) than Prometone; the fecal excretion was higher (23% in 72 hours). Tissue residues (Table I) of Propazine activity decreased from an average of 46.7 p.p.m. (Propazine equivalents) after 2 days to 22.3 p.p.m. after 8 days, and the total thoracic and abdominal viscera after 12 days contained 3.6 p.p.m. These data show a progressive and continued elimination of Propazine from the animal. Tissue residues were fairly evenly distributed throughout the organs examined, except for the brain, which consistently had a lower concentration. The Propazine-^{14}C activity in the combined body tissues (column 5, Table I) shows the hide and hair to have a concentration of Propazine-^{14}C residues approximately six times that of the eviscerated carcass.

The two animals held for 12 days after dosing with Propazine-^{14}C demonstrate the total activity recoveries. The recovery was 94.3%, distributed as follows: urine, 72.5%; feces, 15.6%; hide and hair, 3.35%; carcass, 2.22%; and viscera, 0.1%.

The average excretion of activity from two rats given 3.2 μc. of Propazine labeled at the 2 positions of the isopropyl groups is given in Figure 2. After 72 hours, 89% of the administered dose was found in the urine, feces, and CO_2, with 5.2% being recovered from the carcass with viscera, and 1.8% from the hide with hair.

Ion exchange chromatography indicates at least 11 urinary metabolites from Prometone and 18 from Propazine (Figures 3 and 4). Five metabolites exhibit similar retention volumes for these two compounds.

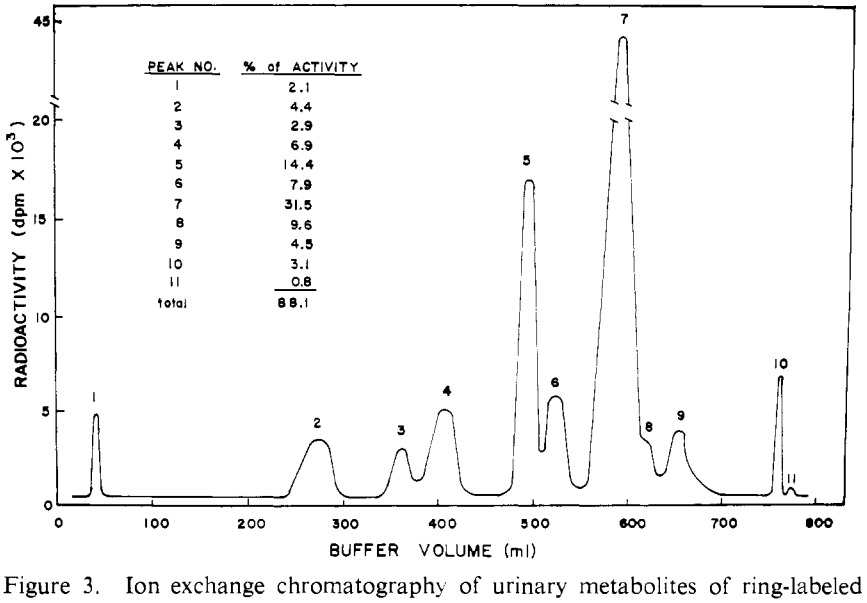

Figure 3. Ion exchange chromatography of urinary metabolites of ring-labeled Prometone

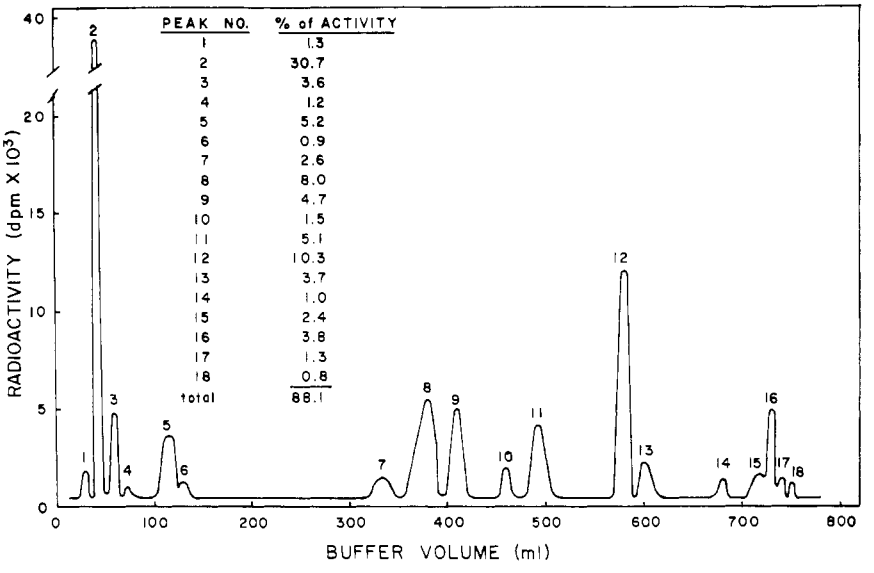

Figure 4. Ion exchange chromatography of urinary metabolites of ring-labeled Propazine

Discussion

Prometone and Propazine are rapidly absorbed from the gastrointestinal tract and eliminated by rats, mainly in the urine and to a lesser extent in the feces. Prometone-^{14}C activity was completely excreted in the urine and feces, with no detectable tissue residues after 72 hours.

Propazine-^{14}C tissue residues decreased with time, with small quantities were found after 12 days. There appears to be a higher concentration of Propazine activity in the hide and hair (9.5 p.p.m.) as opposed to the carcass (1.56 p.p.m.). This activity was not contamination on the hair, since no decrease in hide activity was observed after washing the pelts with detergent solutions and methanol, and no activity was recovered in the washings. All other tissues examined showed fairly uniform distribution of the activity with a progressive decrease with time.

Cleavage of the triazine ring of either Propazine or Prometone is not indicated, as has been proposed for plant metabolism (Montgomery and Freed, 1964), since no $^{14}CO_2$ could be detected in the expired air. Ring cleavage after hydrolysis of the chloro group of Propazine or the methoxyl group of Prometone would probably be metabolized to a carbamic acid and, subsequently, to $^{14}CO_2$ and a biguanide.

The appearance in the expired air of one half of the activity from the isopropyl-labeled Propazine indicates that at least one of the isopropyl groups is removed from the triazine ring. Also, preliminary data on ion exchange separations of the urine from these rats has shown that the major portion of the urine activity is not associated with fractions containing activity from the ring-labeled Propazine. Therefore, it is assumed that more than one half of the isopropyl groups are removed. The absolute extent of dealkylation has not been determined. Also, the metabolic fate of the amino groups is unknown.

The metabolism of Propazine and Prometone appears to be complex, as indicated by the number of components found in the urine by ion exchange chromatography. Assuming the absence of ring cleavage, the number of metabolites is greater than would be predicted for a specific conjugation of hydrolyzed and/or dealkylated products. A more complex or nonspecific detoxication mechanism is indicated, since preliminary studies with acid hydrolysis of the Propazine urinary metabolites only decreased the number from 18 to at least 14. The identification of these metabolites is in progress.

Literature Cited

Frear, D. E. H., "Pesticide Index," College Science Publishers, State College, Pa., 1963.
Montgomery, M., Freed, V. H., J. Agr. Food Chem. **12,** 11 (1964).
Piez, K. A., Morris, L., *Anal. Biochem.* **1,** 187 (1960).
Plaisted, P. H., Thornton, M. L., *Contrib. Boyce Thompson Inst.* **22,** 399 (1964).
Roth, L. J., Leifer, L., Hogness, J. H., Langham, W. H., *J. Biol. Chem.* **176,** 249 (1948).
Shimabukuro, R. H., Kadunce, R. E., Frear, D. S., J. Agr. Food Chem. **14,** 392 (1966).
St. John, L. E., Jr., Ammering, J. W., Wagner, D. G., Warner, R. G., Lisk, D. J., *J. Dairy Sci.* **48,** 502 (1965).
Technicon Chromatography Corp., Chauncey, N.Y., "Buffers for Micro-Column Amino Acid Analysis," Res. Bull. **10** (1962).

Received for review November 17, 1966. Accepted April 19, 1967. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.