Excretory Pathway of Terbacil (Sinbar) in Lactating Cows

Walter H. Gutenmann and Donald J. Lisk

A study of metabolism of the herbicide, 3-*tert*-butyl-5-chloro-6-methyluracil (Terbacil) in dairy cows is reported. At herbicide levels in the feed of 5 and 30 p.p.m., the intact compound was excreted in the milk at levels up to 0.03 and 0.08 p.p.m., respectively. No residues of Terbacil were found in urine or feces. The compound was stable when incubated with fresh rumen fluid, beef liver homogenate, and the 10,000 G max supernatant microsomal fraction of beef liver.

Terbacil, (3-*tert*-butyl-5-chloro-6-methyluracil), also known as Sinbar, is an effective herbicide for weed control in many crops including fruit, certain vegetables, and alfalfa. Studies of the fate of this compound in animals have not been published. In the work reported, Terbacil was fed to dairy cows to study its metabolism.

EXPERIMENTAL

Feeding Experiments. A Holstein cow weighing 1250 pounds and with a daily milk production of about 40 pounds was catheterized and fed Terbacil at the 5 p.p.m. level (based on a daily ration of 50 pounds) for four days. The pure, recrystallized compound in absolute ethanol was thoroughly mixed with the evening grain. Morning and evening subsamples of the total mixed milk were taken one day prior to feeding (control sample), daily throughout the feeding period, and for six days thereafter. The total daily urine and manure samples were similarly collected, weighed, mixed, and subsampled during the same test period. The manure samples were collected in specially constructed trays. All samples were immediately frozen prior to analysis. A second Holstein cow weighing 1375 pounds and with a daily milk production of about 55 pounds was similarly fed 30 p.p.m. of Terbacil in the ration for four days but only milk was collected.

In Vitro Studies. Stability of the herbicide was studied when incubated with rumen fluid. One milliliter of a solution of Terbacil in absolute ethanol (500 μg. per ml.) was thoroughly mixed with 100 ml. of freshly filtered rumen fluid and held at 38° C. At measured intervals, 5 ml. of the fluid were removed and mixed with 5 ml. of acetone and 1 ml. of 10*N* sulfuric acid. The remainder of the procedure beginning with successive extractions with chloroform was identical to that used for analysis of milk.

Possible metabolism of Terbacil in the presence of fresh beef liver homogenate was investigated. Fifty grams of fresh chopped liver were blended with 150 ml. of chilled 0.154*M* potassium chloride for 1 minute. To 1 ml. of the mixture in a series of test tubes were added 40 μl. of Terbacil in ethanol (500 μg. per ml.) and 4 ml. of 0.2*M* Tris HCl buffer, pH 7.2. The mixture was incubated at 38° C., and, at specific intervals, a tube was removed, 10 ml. of acetone were added, and the mixture was filtered. The filter was rinsed with 35 ml. of acetone, and the filtrate was extracted successively with 50, 25, and 10 ml. of chloroform. The combined chloroform layers were dried with sodium sulfate, evaporated, and the residue was dissolved in ethyl acetate. The latter solution was appropriately diluted and chromatographed.

The stability of Terbacil when in the presence of the 10,000 G supernate fraction of fresh beef liver was also studied. An Angus steer was sacrificed and the liver was immediately removed. A portion was immersed in 0.25*M* sucrose solution at 0° C. and all further processing for enzyme preparation was conducted in the cold (0–4° C.). A 20% liver homogenate in the sucrose solution was prepared using a Dounce homogenizer. The homogenate was centrifuged at 10,000 G for 30 minutes. Incubation mixtures contained 5 μg. of Terbacil, 5 μmoles of nicotinamide, 25 μmoles of magnesium chloride, 140 μmoles of phosphate buffer, pH 7.4, 20 μmoles of glucose-6-phosphate, 1.5 μmoles of TPN, and 1 ml. of the enzyme (10,000 G supernate) preparation in a total volume of 5.0 ml. Incubations were carried out at 37° C. in an atmosphere of air for 30 minutes. The remainder of the procedure was identical with that described for beef liver homogenate beginning with addition of 10 ml. of acetone.

Extraction and Isolation of Terbacil. The procedures used for extraction and isolation of Terbacil from the various body fluids and excreta include portions of methods described previously by Gutenmann and Lisk (1968) and Pease (1968). The herbicide was extracted from milk by blending 25 grams of the sample with 60 ml. of acetone. The mixture was filtered and rinsed with acetone to a total volume of 100 ml. The acetone filtrate was partitioned successively with 50, 25, and 10 ml. of chloroform and the chloroform layers were combined and dried by passing through a layer of anhydrous sodium sulfate. The chloroform was evaporated using a rotating evaporator and the residue was dissolved in 50 ml. of 1*N* sodium hydroxide. The alkaline solution was partitioned successively with 50 and 25 ml. of hexane and the hexane layers were discarded. The aqueous solution was extracted twice with 50 ml. of ethyl acetate and the organic layers were combined, dried with sodium sulfate, and evaporated to 5 ml. with air. Up to 8 μl. of the ethyl acetate

Pesticide Residue Laboratory, Cornell University, Ithaca, N. Y. 14850

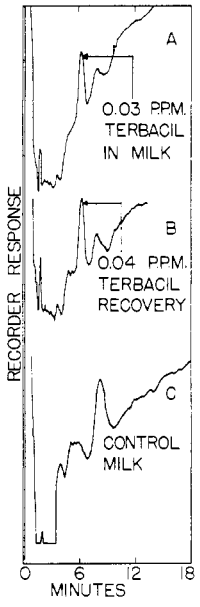

Figure 1. Chromatograms of (A) 0.03 p.p.m. of Terbacil in the fourth day P.M. milk (Table I), (B) the recovery of 0.04 p.p.m. of Terbacil from control milk, and (C) control milk at the 5 p.p.m. feeding level

solution were injected for gas chromatographic analysis. Milk was also analyzed by a procedure similar to that above except that the evaporated chloroform extracts were dissolved in ethyl acetate in which Terbacil was isolated by evaporative codistillation (Gutenmann and Lisk, 1968) prior to gas chromatographic analysis.

Possible residues of Terbacil in urine were determined as follows: Twenty-five grams of urine were blended with 1 ml. of 10*N* sulfuric acid and 60 ml. of acetone. The mixture was filtered and rinsed with acetone to a total volume of 100 ml. The remainder of the procedure was identical to that described above for analysis of milk.

Feces was analyzed by blending 10 grams of the sample with 75 ml. of ethyl acetate. The mixture was filtered and rinsed with the solvent to a total volume of 100 ml. The solution was evaporated to 10 ml. using rotary evaporation. Two milliliters of the solution were injected into a Kontes codistillation apparatus for isolation of the herbicide. Elution of the herbicide from the apparatus was performed as previously described (Gutenmann and Lisk, 1968). The effluent was collected, evaporated to 0.5 ml., and up to 8 μl. were analyzed by gas chromatography.

Determination. Final analysis for Terbacil was made by electron affinity gas chromatography. The gas chromatograph was a Barber-Colman Model 10 with a battery-operated, No. A-4071, 6 cc. electron affinity detector containing 56 μc. of radium^{226}. The recorder was a Wheelco, 0 to 50 mv., equipped with 10-inch chart paper, running 10 inches per hour. The electrometer gain setting was 10,000. The column was U-shaped, made of borosilicate glass, 6 mm. I.D., 2 feet long, and containing 10% Ucon polar on 80- to 100-mesh Gas Chrom Q. The operating temperatures for the column, flash heater, and detector were 200°, 250°, and 245° C., respectively, and nitrogen (60 cc. per minute) was the carrier gas. The retention time for Terbacil was about 6 minutes.

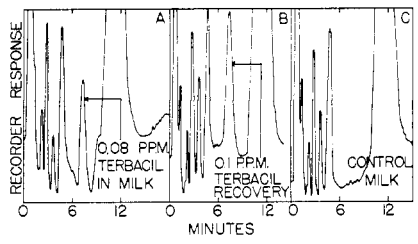

Figure 2. Chromatograms of (A) 0.08 p.p.m. of Terbacil in the fourth day A.M. milk (Table I), (B) the recovery of 0.1 p.p.m. of Terbacil from control milk, and (C) control milk at the 30 p.p.m. feeding level

RESULTS AND DISCUSSION

Table I lists the levels of Terbacil found at both feeding levels and the total daily excretion of the herbicide in the milk in milligrams. The sensitivity of the method for Terbacil in milk was 0.01 p.p.m. Proportionally more Terbacil was excreted at the lower feeding level. A similar lack of a linear relationship between the quantity of a toxicant ingested by a cow and total amount eliminated in milk has been observed with other compounds (Hardee *et al.*, 1964). The appearance of the herbicide exclusively in evening milk samples at the 5 p.p.m. feeding level and only (except on the fourth day) in the morning milk at the 30 p.p.m. ingestion level is notable but the metabolic significance, if any, is unknown. A variety of excretion patterns may have been observed if additional replicate cows had been included in the study. Figures 1 and 2 show chromatograms of milk containing Terbacil residues, Terbacil recovered from milk to which the herbicide was added, and control milk from the cows at both feeding levels.

Table I. Residues of Terbacil in Milk from Cows during and after Ingestion of Terbacil Herbicide

	Terbacil in Milk, P.P.M.			Daily Milligram Total Herbicide Excreted in Milk	
	Cow Fed 5 P.P.M.		Cow fed 30 p.p.m.		
Days	WCD[a]	CD[b]	WCD	Cow fed 5 p.p.m.	Cow fed 30 p.p.m
1 P.M.[c]	Nd[d]	Nd	Nd	...	...
2 A.M.	Nd	Nd	0.025	...	0.35
2 P.M.	0.025	0.03	Nd	0.22[e]	...
3 A.M.	Nd	Nd	0.02	...	0.30
3 P.M.	0.025	0.03	Nd	0.19[e]	...
4 A.M.	Nd	Nd	0.08	...	1.14
4 P.M.[f]	0.03	0.04	0.015	0.25[e]	0.18
5 A.M.	Nd	Nd	0.05	...	0.73
5 P.M.	Nd	Nd	Nd	...	...
6 A.M.	Nd	Nd	Nd	...	...
6 P.M.	Nd	Nd	Nd	...	...
7 A.M.	Nd	Nd	Nd	...	...
7 P.M.	Nd	Nd	Nd	...	...
8 A.M.	Nd	Nd	Nd	...	...
8 P.M.	Nd	Nd	Nd	...	...
9 A.M.	Nd	Nd	Nd	...	...
9 P.M.	Nd	Nd	Nd	...	...
10 A.M.	Nd	Nd	Nd	...	...
10 P.M.	Nd	Nd	Nd	...	...

[a] Analysis without codistillation step.
[b] Analysis with codistillation step.
[c] First day of feeding Terbacil.
[d] Not detectable.
[e] Daily total milligram herbicide calculated from medium p.p.m. level between that found by WCD and CD procedures.
[f] Last day of feeding Terbacil.

Table II. Recovery of Terbacil from Control Samples

	Terbacil Added, P.P.M.	Recovery, %
Milk (WCD method)	0.04	82
	0.1	100
Milk (CD method)	0.2	90
Urine	0.4	97.5
Feces	0.2	87.5, 70

The specific data in Figures 1 and 2 was obtained by the analytical procedure for milk which did not include the codistillation step.

The 5 and 30 p.p.m. feeding levels of Terbacil amounted, respectively, to daily totals of 113.5 and 681 milligrams of the herbicide ingested by the cows. The 5 p.p.m. level of Terbacil fed in this experiment represents an exaggerated dosage when compared to maximum residues of about 1.5 p.p.m. of the herbicide which have been determined on treated forage. (Rosen, 1968). One might consider that the excretion of an ingested toxicant in milk would be favored by feeding the entire dose in the evening grain (weighing 10 pounds) as compared to evenly mixing it with the entire daily ration weighting 50 pounds. This initial concentration effect could be expected to be largely nullified by the vigorous mixing which normally occurs in the rumen, the large capacity of this organ, and the residence time of ingested food in the rumen. The capacity of the rumen for a mature cow ranges up to 35 gallons (Dougherty *et al.*, 1965). About 24 hours is required for the complete exchange of rumen contents.

No residues of the intact herbicide were found in the urine or feces. The sensitivity of the method for Terbacil in urine and feces was 0.03 and 0.02 p.p.m., respectively. Attempts to determine possible urinary conjugates of Terbacil by acid hydrolysis of urine prior to extraction and analysis were unsuccessful. The recoveries of Terbacil from control samples are listed in Table II.

No decomposition of Terbacil was noted in the presence of rumen fluid for 8 hours or beef liver homogenate for 2 hours. The herbicide was stable when incubated with liver microsomes for 30 minutes as described.

ACKNOWLEDGMENT

The authors thank E. I. du Pont de Nemours and Co. for supplying pure samples of Terbacil.

LITERATURE CITED

Dougherty, R. W., Allen, R. S., Burroughs, W., Jacobson, N. L., McGilliard, A. D., "Physiology of Digestion In The Ruminant," Butterworths, Washington, D. C., 1965.
Gutenmann, W. H., Lisk, D. J., *J. Assoc. Offic. Anal. Chemists* **51,** 688 (1968).
Hardee, D. D., Gutenmann, W. H., Keenan, G. I., Gyrisco, G. G., Lisk, D. J., Fox, F. H., Trimberger, G. W., Holland, R. F., *J. Econ. Entomol.* **57,** 404 (1964).
Pease, H. L., J. AGR. FOOD CHEM. **16,** 54 (1968).
Rosen, D. E., E. I. duPont de Nemours and Co., Wilmington, Del., personal communication, 1968.

Received for review January 27, 1968. Accepted April 1, 1969.