Metabolic Studies with Bexide (Herbisan) Herbicide in the Dairy Cow

Bexide [diethyl dithiobis (thionoformate)] was fed to a lactating cow at a level of 5 ppm in the feed. Analysis of milk, urine, and feces for Bexide and a possible metabolite, diethyl xanthate, was made by electron affinity gas chromatography. Residues of these compounds were not detected in the samples. Bexide could not be recovered in any form when it was incubated with rumen fluid, indicating instantaneous fixation, or less likely, degradation. Bexide did not degrade to diethyl xanthate in rumen fluid, since the latter metabolite was stable and could be recovered when it was added to rumen fluid.

Bexide [diethyldithiobis(thionoformate) or (ethyl xanthic disulfide)] is the active ingredient of the herbicide formulated as Herbisan, which is a product of Roberts Chemicals Inc., Nitro, W.Va. It effectively controls weeds in onions, beans, potatoes, sugar beets, and certain other vegetables. It also serves as a top and vine killer of potatoes. The synthesis of sulfur-35 labeled Bexide and its translocation in plants has been studied (Langston, 1955). In this paper Bexide was fed to a lactating cow to study the possible excretion and metabolism of the toxicant. Analysis for a possible metabolite, diethyl xanthate, (Paulin, 1968) was also made in animal excreta.

EXPERIMENTAL PROCEDURE

Feeding Experiment. A Holstein cow weighing 687 kg and with a daily milk production of about 18.7 kg was catheterized and fed Bexide at the 5-ppm level (based on a daily ration of 22.7 kg as fed) for 4 days. The 5 ppm feeding level was chosen as the maximum herbicide concentration that one would expect to find in herbage. The pure, recrystallized compound in acetone was thoroughly mixed with the evening grain. Morning and evening subsamples of the total mixed milk were taken 1 day prior to feeding (control sample), daily throughout the feeding period, and for 6 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.

In Vitro **Studies.** The stability of Bexide and a possible metabolite, diethyl xanthate, in the presence of fresh rumen fluid was studied. One ml of a solution of Bexide or diethyl xanthate in acetone (500 μg/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 5 ml of acetone were added. The mixture was filtered and the filter was rinsed with acetone to a total volume of 25 ml. One ml of the acetone filtrate was partitioned with 5 ml of hexane and 44 ml of 2% hydroxylamine hydrochloride solution. The upper hexane layer was analyzed by electron affinity gas chromatography.

Analytical Procedures. Extraction and isolation of Bexide and diethyl xanthate from milk, urine, and feces was as follows. Twenty-five grams (fresh weight) of the sample was blended with 65 ml of acetone. The mixture was filtered and the filter was rinsed with acetone to a total volume of 100 ml. Ten ml of the filtrate was partitioned with 5 ml of hexane and 90 ml of 2% hydroxylamine hydrochloride. The hexane layer was analyzed by electron affinity gas chromatography. Analysis of the hexane layer from these samples was also made using the flame photometric detector with the 394 mμ sulfur filter to detect other possible sulfur-containing metabolites which may not have been sensitive to electron affinity detection.

Determination. The electron affinity 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-in. chart paper, running 10 in. per hr. The electrometer gain setting was 10,000. The column was U-shaped, made of borosilicate glass, 6 mm i.d., 2 ft long, and containing 10% DC-200 on 80- to 100-mesh Gas Chrom Q. The operating temperatures for the column, flash heater, and detector were 200° (120° for diethyl xanthate), 250°, and 245° C, respectively, and nitrogen (60 cc per min) was the carrier gas. The retention times for Bexide and diethyl xanthate were 13 and 5.1 min, respectively. Analyses using the Tracor flame photometric detector were made with the 394 mμ sulfur filter. The column and operating temperatures were the same as those used with the electron affinity detector for diethyl xanthate analysis.

RESULTS AND DISCUSSION

Neither Bexide nor diethyl xanthate were detected in milk, urine, or feces samples. The method was sensitive to about 0.04 and 0.02 ppm of Bexide and diethyl xanthate, respectively. The recoveries of these compounds are listed in Table I. Diethyl xanthate, a suspected metabolite of Bexide, was the only compound which could be obtained from the manufacturer as a pure standard for this study. Analysis of milk, urine, feces, and rumen fluid was also performed using the sulfur flame photometric detector for other possible sulfur-containing metabolites of Bexide, but no significant peaks were detected when comparison was made with chromatograms of control samples. Hydrolysis of samples with acid or base to detect possible conjugates of Bexide (or moieties of it) was not performed, since Bexide would have been hydrolyzed to carbon disulfide and other products.

Bexide could not be recovered from fresh rumen fluid when the compound was added to the fluid, and the fluid was immediately extracted and analyzed. Bexide, when added to distilled water (5 ppm) which was then maintained at the rumen fluid incubation temperature (38° C) for 2 hr, could be recovered. The herbicide was therefore not believed to

Table I. Recovery of Bexide and Diethyl Xanthate from Samples

Sample	Added, ppm		Recovery, %	
	Bexide	Diethyl xanthate	Bexide	Diethyl xanthate
milk	0.1	0.1	90, 96	70
urine	0.1	0.2	106, 110	95
feces	0.2	0.2	72	46

have been lost by vaporization. Diethyl xanthate is a possible metabolite of Bexide in plants. If this product formed in rumen fluid, it might have either reacted further with the fluid or possibly have been lost by vaporization. Diethyl xanthate was, however, found to be stable and did not disappear when incubated with rumen fluid for 2 hr. Loss of diethyl xanthate by vaporization, fixation or metabolism was therefore ruled out. Bexide could not be recovered from rumen fluid which had been previously boiled to destroy microorganisms. It is possible that Bexide was nonenzymatically fixed by boiled rumen fluid components. The herbicide may also have degraded to produce a metabolite other than diethyl xanthate, which was not detected. In this regard it should be noted that catabolism of xanthates (including diethyl xanthate disulfide) and dithiocarbamates, when administered to men or guinea pigs, resulted in production of carbon disulfide in their expired air (Merlevede and Peters, 1965). Possibly Bexide or its rumen fluid fixation products are degraded in the rumen or at other points in the gastrointestinal tract to carbon disulfide and other products.

LITERATURE CITED

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Walter H. Gutenmann
Donald J. Lisk*

Pesticide Residue Laboratory
Department of Entomology
Cornell University
Ithaca, N.Y. 14850

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