Feeding Studies with Randox in the Dairy Cow

by

L. E. St. John Jr., and D. J. Lisk

Pesticide Residue Laboratory, Department of Food Science
New York State College of Agriculture, Cornell University
Ithaca, N.Y. 14850

Randox or CDAA (2-Chloro-N,N-diallylacetamide) is a pre-emergence herbicide which controls grasses and other weeds in forage and other crops. While Randox has been administered to various farm animals to study its toxicity (U.S.D.A., 1967), studies of the metabolism of the compound in animals have not been published. In the work reported, the herbicide was fed to a lactating cow to study its fate if ingested as a residue in forage.

Methods and Materials

A Holstein cow weighing 478 kilograms and with a daily milk production of 14.1 kilograms was catheterized and fed Randox at the 5 ppm level (based on a daily ration of 22.7 kilograms) for 4 days. The pure 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

Rumen Fluid. The stability of Randox in the presence of rumen fluid was studied. One milliliter of a solution of Randox in acetone (500 µg per ml) was thoroughly mixed with 100 ml of fresh filtered rumen fluid and held at 38° C. At measured intervals 5 ml of 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 benzene and 94 ml of 5% sodium sulfate solution. Five µl of the upper benzene layer was analyzed for Randox by electron affinity gas chromatography.

Liver. The stability of Randox was studied in the presence of the 10,000 X g supernatant fraction of fresh beef liver which contains microsomes and soluble enzymes. A portion of beef liver was immersed in 0.25M 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

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sucrose solution was prepared using a Dounce homogenizer. The homogenate was centrifuged at 10,000 X g max for 30 min. Incubation mixtures contained 5 μg of Randox (10 μl of a 500 μg per ml solution in chloroform), 25 μmol of magnesium chloride, 95 μmol of tris buffer, pH 7.4, 20 μmol of glucose-6-phosphate, 1.5 μmol of TPN, and 1 ml of the enzyme (10,000 X g supernate) preparation in a total volume of 5.0 ml. Incubations were carried out in a 25-ml capped Erlenmeyer flask at 37° C in an atmosphere of air for 30 min. The flasks contained a borosilicate marble 0.5 in. in diameter and were mechanically shaken 100 times per minute on a reciprocating shaker during incubation. (These samples as well as the controls, which included either no enzyme or no substrate, were carried through the procedure in triplicate.) After 30 min the reactions were terminated by the addition of 3 ml of acetone and each incubation mixture was transferred to a 100-ml volumetric flask using 10 ml of acetone for rinsing. Five milliliters of benzene were added, the flask was made to volume with 2% sodium sulfate solution, and was then shaken vigorously for 1 min. Part of the upper benzene layer (5 μl) was analyzed for Randox by electron affinity gas chromatography.

Extraction And Isolation Of Randox

Milk and Feces. Randox was extracted from milk and feces using acetonitrile followed by isolation using column chromatography on Florisil by the method of the Monsanto Co. (Pesticide Petition No. 0F0901, 1969).

Urine. Twenty five grams of urine was extracted by blending with 100 ml of chloroform. Water was removed by centrifugation and the chloroform solution was filtered and evaporated to dryness. The residue was dissolved in benzene. The latter benzene solution (as well as the column chromatographic eluates from the milk and feces samples) were analyzed by electron affinity gas chromatography.

Gas Chromatographic Analysis. Final analysis was made using a Barber-Colman Model 10 gas chromatograph equipped with an electron affinity detector. The detector was a battery-operated No. A-4071, of 6 cm^3 volume and containing 56 μCi 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 was 10,000. The columns were U-shaped, made of borosilicate glass, 6 mm i.d., 1.83 m long and containing a 1 to 1 by weight mixture of 10% OV-17 on 80 to 100 mesh Chromosorb W . The operating temperatures for the column, flash heater, and detector were 160, 250, and 235° C, respectively, and nitrogen (166 cm^3 per min) was the carrier gas. The retention time for Randox was 3.8 min.

Results

Residues of intact Randox were not detected in milk, urine, or feces. The compound was stable in rumen fluid

during 24 hours of incubation. It was no longer detectable, however, after 30 minutes in the presence of the beef liver 10,000 Xg supernatant fraction. The recoveries of Randox added to control samples are listed in Table 1. Attempts were made to detect diallyl amine and 2-chloro acetic acid as possible metabolites of Randox in the liver fraction following incubation but results were inconclusive.

Table 1
Recoveries of Randox from Control Samples

Sample	Added, ppm	Recovery, per cent	Estimated Sensitivity ppm
milk	0.04	68, 100, 100, 65, 90	0.01
	0.1	85, 88, 78	
	0.2	85, 78, 80	
urine	0.08	120	
	0.2	100, 107	
	2.0	100	
feces	0.2	80, 94, 100, 90	
	0.25	90	

Randox rapidly decomposes as a residue in plants. The 5 ppm feeding level used in this study is therefore an exaggerated dose. It is concluded that under typical field conditions use of the herbicide on forage plants would not result in residues of intact Randox in milk.

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