Feeding Studies with Supracide in the Dairy Cow

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Supracide (S-[(2-methoxy-5-oxo-Δ^2-1,3,4-thiadiazolin-4-yl) methyl]-O,O-dimethyl phosphorodithioate) is an insecticide of J. R. Geigy, S. A., Basle, Switzerland which is useful for control of alfalfa weevils and other pests in orchards and vineyards. Using the ring labelled (carbonyl-^{14}C) compound, a considerable proportion of the insecticide was converted to $^{14}CO_2$ in rats (BULL 1968, ESSER and MULLER 1966, ESSER et al. 1968). In a cow-feeding study Supracide and its oxygen analog were not detected in milk and no significant tissue storage of residues were detected (CASSIDY et al. 1969a). Storage of the insecticide or its oxygen analog were similarly found negligible in a feeding study with bull calves (POLAN et al. 1969a). In other feeding studies Supracide or its oxygen analog were not found in milk, urine or feces or tissue of lactating cows (POLAN et al. 1969b) but decomposed in rumen fluid (POLAN et al. 1969c). At a concentration in the feed of 50 ppm or higher, residues of Supracide were detected in hens eggs (WISMAN and YOUNG 1970). Decomposition of Supracide in forage (CASSIDY et al. 1969b) and hay and silage (POLAN et al 1969c) and production of the oxygen analog in plants (CASSIDY et al. 1969b, MATTSON et al. 1969, BULL 1968) has been reported. In the work reported a lactating cow was fed a low concentration of Supracide to study its metabolism if ingested as a residue in forage.

METHODS AND MATERIALS

A Holstein cow weighing 477 kilograms and with a daily milk production of 14.2 kilograms was catheterized and fed Supracide 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 Supracide in the presence of rumen fluid was studied. One milliliter of a solution of Supracide in acetone (500 μg per ml) was thoroughly mixed with

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100g of fresh filtered rumen fluid and held at 38° C. At measured intervals up to 8 hours, 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 2% sodium sulfate solution. Five µl of the upper benzene layer was analyzed for Supracide by electron affinity gas chromatography.

Liver. The stability of Supracide 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 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 Supracide (10 µl of a 500 µg per ml solution in acetone), 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 Erlenmeyer flask at 37° C in an atmosphere of air for 30 min. The flasks 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 6 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 Supracide by electron affinity gas chromatography.

EXTRACTION, ISOLATION AND ANALYSIS OF SUPRACIDE

Milk and Feces. Supracide was extracted from weighed samples of milk (100 g) or feces (50 g) by blending with 200 ml of acetone. The mixture was filtered and the filter rinsed with 50 ml of acetone. (Fifty ml of water was added to the combined filtrates in the case of feces samples). The filtrates were combined and concentrated to about 60 ml using rotary evaporation. Five grams of sodium sulfate was added to the remaining aqueous solution and it was partitioned vigorously with 2 ml (5 ml in the case of feces) of benzene. The layers were allowed to separate using centrifugation when necessary. One ml (2 ml with feces) of the upper benzene layer was chromatographed on Florisil. The column was 1.6 cm (i.d.) containing 10 grams of Florisil with a 2.5 cm layer of anhydrous sodium sulfate above. The column was prewashed with 100 ml of hexane followed by 50 ml of 1% methanol in methylene chloride. After applying the benzene sample solution to the column, the column was eluted with

150 ml of 1% methanol in methylene chloride. The entire eluent was collected and concentrated to near dryness using rotary evaporation. Ten ml of benzene was added to the residue and the solution evaporated to dryness. The residue was dissolved in 2 ml (5 ml with feces) of benzene and up to 5 µl was analyzed by electron affinity gas chromatography.

Urine. Twenty five grams of urine was blended with 60 ml of acetone. The mixture was filtered and the filter was rinsed to give a combined filtrate volume of 100 ml. A 40 ml aliquot of the filtrate was concentrated (rotary evaporation) to 10 ml and was then partitioned vigorously with 2 ml of benzene and 88 ml of 2% sodium sulfate. Five µl of the upper benzene solution was analyzed by electron affinity gas chromatography.

EXTRACTION, ISOLATION AND ANALYSIS OF HYDROLYTIC PHOSPHORUS METABOLITES IN URINE

Possible hydrolytic phosphorus-containing metabolites of Supracide, such as dimethyl dithiophosphate and dimethyl thiophosphate, were determined by an adaptation of the method developed earlier (ST. JOHN and LISK 1968). To 5 g of urine was added 2 ml of 4 N hydrochloric acid, 38 ml of saturated sodium chloride and 5 ml of diethyl ether. The mixture was shaken vigorously and then centrifuged to clarify the layers. Two ml of the upper ether solution were removed, 0.22 ml of methanol were added and the solution was methylated with diazomethane following the procedure of Schlenk and Gellerman (1960). The excess diazomethane was removed with a gentle stream of nitrogen and the solution was diluted to 2 ml with benzene. Up to 5 µl of this solution was analyzed by alkali thermionic gas chromatography.

GAS CHROMATOGRAPHIC ANALYSIS

Final analysis was made using a Barber-Colman Model 10 gas chromatograph equipped with either an electron affinity or an alkali thermionic detector as noted above. The electron affinity 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 alkali thermionic detector was an adaptation of that described earlier (ST. JOHN and LISK 1968). The columns were U-shaped, made of borosilicate glass and 6 mm i.d. The column for the chromatography of Supracide contained a mixture of 2% OV-17 and 2% QF-1 on 80 to 100 mesh Gas-Chrom Q and was 0.61 m long. The operating temperatures for the column, flash heater and electron affinity detector were 185, 250 and 235° C, respectively, and nitrogen (400 cm^3 per min) was the carrier gas. The retention time for Supra-

cide was 4.3 min. The column used for the chromatography of hydrolytic phosphorus-containing metabolites contained 5% OV-17 on 80-100 mesh Chromosorb AW and was 1.83 m long. The operating temperatures for the column, flash heater and alkali thermionic detector were 110, 200 and 290° C, respectively, and nitrogen (180 cm^3 per min) was the carrier gas. The retention times for the methyl esters of dimethyl dithiophosphate and dimethyl thiophosphate were, respectively, 11.3 and 3.4 min.

RESULTS

Residues of intact Supracide were not detected in milk, urine or feces. The compound was stable in rumen fluid during 24 hours of incubation. At the end of 30 minutes incubation with the beef liver 10,000 X g supernatant fraction, degradation of the insecticides in three replicated samples was 82, 86 and 74 per cent. Analysis of urine showed the presence of dimethyl dithiophosphate and dimethylthiophosphate ions representing, respectively, 2.74 and 3.58% of the total Supracide dose. The recoveries of Supracide and metabolites are listed in Table 1.

Table 1. Recovery of Supracide and Metabolites from Control Samples.

Sample	Added, ppm	Recovery, Per Cent	Estimated Sensitivity, ppm
	Supracide		
Milk	0.02	85, 100	0.008
	0.05	70, 80, 94	
Urine	0.08	70, 88, 68	0.04
	0.2	100	
	0.4	79	
Feces	1.0	<50	0.5
Rumen Fluid	5.0	81, 81	
Liver (10,000 X g supernate)	5.0	90, 90, 90	
	Dimethyldithiophosphate as Supracide		
Urine	0.2	100	
	0.4	116, 104, 110	0.05
	Dimethylthiophosphate as Supracide		
Urine	0.4	73	0.05
	0.8	76	

The level of insecticide fed (a concentration in the feed of 5 ppm) appeared to be realistic based on harvest residues which can occur on harvested alfalfa (MATTSON et al. 1969). Considering the total amount of Supracide ingested, the cow used in this study received only about one fifth the quantity of insecticide which was administered to a cow using a balling gun in the study of Cassidy et al. (1969a). The absence of residues of intact Supracide in milk, urine and feces may be explainable by its obversed decomposition in the presence of the liver (10,000 X g supernate) fraction. Polan et al. (1969c) concluded that microbial decomposition of Supracide in the rumen was of relatively little importance and believe it is instead rapidly absorbed into the bloodstream where it would be carried directly to the liver. It would appear that Supracide can be safely used on forage for practical insect control without harmful residues appearing in milk.

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