Metabolic Studies with Gardona Insecticide in the Dairy Cow

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A feeding experiment was conducted to study the metabolism and excretion of Gardona [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate] insecticide in the dairy cow. At an insecticide level of 5 ppm in the daily ration, no residues of Gardona were found in milk or urine. A metabolite was found in hydrolyzed urine with a retention time identical to 1-(2,4,5-trichlorophenyl)ethanol. It corresponded to about 75.7% of the total Gardona fed. In the presence of beef liver 10,000 × *g* supernate, Gardona completely disappeared with production of a metabolite again with the retention time of 1-(2,4,5-trichlorophenyl)ethanol. About 0.6% of the total dose was found as the intact compound in feces. 2,4,5-Trichloroacetophenone, a possible metabolite of Gardona, was not detected in milk, urine, or feces. Gardona was stable when incubated with rumen fluid.

Gardona [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate] is an insecticide of the Shell Chemical Co. which effectively controls corn earworm, fall armyworm, codling moth, gypsy moth, houseflies, and arthopod parasites of poultry. The metabolism of Gardona has been studied in the dog and rat (Akintonwa and Hutson, 1967). Residues of the insecticide have been studied in tissues and eggs of chickens when the compound was used in chicken houses (Ivey *et al.*, 1969). Residues of Gardona were determined in tissues of sprayed cattle (Ivey *et al.*, 1968). Degradation of the compound in plants and soils has also been investigated (Beynon and Wright, 1969). This paper reports the results of a feeding study with the insecticide in a lactating dairy cow.

EXPERIMENTAL

A Holstein cow weighing 705 kg and with an average daily milk production of about 19.8 kg (3.7% butterfat) was catheterized and fed Gardona at the 5-ppm level (based on a daily ration of 22.7 kg) 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 Gardona in the presence of fresh rumen fluid was studied. One milliliter of a solution of Gardona 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 milliliter of the acetone filtrate was partitioned with 5 ml of hexane and 44 ml of 2% sodium sulfate solution. A portion of the upper hexane layer (10 μl) was analyzed for Gardona by electron affinity gas chromatography.

Liver. Possible metabolism of Gardona was studied in the presence of the 10,000 × *g* supernatant fraction of fresh beef liver which contains microsomes and soluble enzymes. 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* max for 30 min. Incubation mixtures contained 5 μg of Gardona (100 μl of a 50 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 × *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 2 ml of acetone for rinsing. Ten milliliters of hexane 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 hexane layer (5 μl) was analyzed for Gardona by electron affinity gas chromatography.

EXTRACTION AND ISOLATION OF GARDONA AND A POSSIBLE METABOLITE, 2,4,5-TRICHLOROACETOPHENONE

Milk and Urine. The sample (25 g) was blended with 70 ml of acetone for 2 min. The mixture was filtered and the filter was rinsed with acetone until the total filtrate volume was 100 ml. A portion of the filtrate (2 ml) was transferred to a 100-ml volumetic flask containing 10 ml of hexane. The flask was made to volume with 2% sodium sulfate solution and the contents were shaken vigorously for 1 min. Ten microliters of the upper hexane layer were analyzed by electron affinity gas chromatography.

Feces. The sample (25 g) was blended with 70 ml of acetone for 2 min. The mixture was filtered and rinsed with acetone until 100 ml of filtrate was collected. The entire filtrate was transferred to a 500-ml separatory funnel containing 100 ml of 2% sodium sulfate and 50 ml of hexane. The contents were shaken vigorously and the hexane was transferred to a 250-ml separatory funnel. The aqueous filtrate was again partitioned with a 25- and 15-ml portion of hexane. The combined hexane extracts were partitioned with two 20-ml portions of acetonitrile. The combined acetonitrile solutions

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were evaporated to dryness using a rotating evaporator, the residue was taken up in 10 ml of hexane, and 10 μl were analyzed by electron affinity gas chromatography.

HYDROLYSIS AND EXTRACTION OF POSSIBLE CONJUGATES IN URINE AND FECES

Five grams of urine or feces were transferred to a 100-ml volumetric flask, 20 ml of concentrated orthophosphoric acid was added, and the mixture was placed in a water bath at 90° C for 15 min. After cooling, 5 ml of hexane was added, the flask was made to volume with 2% sodium sulfate solution, and the contents were shaken vigorously. The hexane solution was 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 4% phenyldiethanolamine succinate on 80 to 100 mesh Gas Chrom Q and 15% QF-1 on the same support. This column is recommended for analysis of Gardona residues in crops and animal products by Shell Chemical Co. The operating temperatures for the column, flash heater, and detector were 200 (160 for urinary conjugates), 250, and 240° C, respectively, and nitrogen (60 cm^3 per min) was the carrier gas. The retention times for Gardona and 2,4,5-trichloroacetophenone were, respectively, 17.2 and 3.2 min.

RESULTS AND DISCUSSION

Residues of intact Gardona were not detected in milk or urine. Analysis of hydrolyzed urine showed a metabolite with the retention time of 1-(2,4,5-trichlorophenyl)ethanol (4.7 min). It appeared daily in urine from the first through the sixth day after dosing began. When correcting for the average % recovery (49%—see Table I) it amounted to 75.7% (343.7 mg) of the total equivalent Gardona (454 mg) fed. Metabolites were not observed in hydrolyzed feces samples. Gardona decomposed completely in the presence of the beef liver 10,000 × *g* supernatant fraction again with the production of a metabolite with the retention time of 1-(2,4,5-trichlorophenyl)ethanol (4.7 min). About 0.6% (2.76 mg) of the total insecticide dose was excreted in the feces. It appeared in feces collected on the second through the fifth day after insecticide feeding was begun. Residues of 2,4,5-trichloroacetophenone, a possible metabolite of Gardona, were not observed in milk, urine, or feces samples. Gardona did not decompose in the presence of rumen fluid for 3 hr. The recoveries of Gardona, 2,4,5-trichloroacetophenone, and 1-(2,4,5-trichlorophenyl)ethanol from various samples are listed in Table I.

Table I. Recovery of Gardona and 2,4,5-Trichloroacetophenone from Samples

Sample	Added, ppm	Recovery, %	Estimated sensitivity, ppm
	Gardona		
Milk	0.04	90	0.02
	0.1	92	
Urine	0.1	82	0.02
Feces	0.1	83	0.02
Rumen fluid	5	100	0.1
Liver 10,000 × *g* supernate	5	100, 95	0.1
	2,4,5-trichloroacetophenone		
Milk	0.1	81, 88	0.02
Urine	0.1	68, 72	0.02
Feces	0.1	77, 76	0.02
	1-(2,4,5-trichlorophenyl)ethano		
Urine	0.25	40	0.02
	0.50	52	
	0.75	55	
Feces	0.50	28	0.10

This study therefore indicates that Gardona may undergo hydrolysis in beef liver with major elimination of the chlorinated hydrolysis product as a urinary conjugate. It is possible that other metabolites may have been present in milk and excreta. In rats which were dosed with 2-chloro-1-(2,4,5-trichlorophenyl)[^{14}C]vinyl dimethylphosphate, 78% of the ^{14}C was excreted in the urine, 16.5% in the feces, and 0.5% in expired gases (Akintonwa and Hutson, 1967). Various of the following metabolites were found in the urine of rats and dogs receiving the tagged compound: 2,4,5-trichlorophenylethanediol glucuronide, [1-(2,4,5-trichlorophenyl)ethyl-β-D-glucopyranosid]uronic acid, 2,4,5-trichloromandelic acid, 2-chloro-1-(2,4,5-trichlorophenyl)vinylmethyl hydrogen phosphate, 2,4,5-trichlorophenylethanediol, and 1-(2,4,5-trichlorophenyl)ethanol. The major decomposition products of Gardona on apple foliage and fruit were conjugates of 1-(2,4,5-trichlorophenyl)ethanol with certain sugars (Beynon and Wright, 1969).

No chromatographic peaks for other metabolites were observed in milk or excreta in this study but it is possible that certain others were present and not determinable by the procedures used. Fifteen minutes at 90° C was found to be optimum for hydrolysis of urine or feces prior to analysis of the released metabolite. Shorter or longer periods of heating resulted in still lower recoveries possibly owing to vaporization losses. Hydrolysis with alkali yielded several interfering peaks.

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