Metabolic Studies with *O,O*-Diethyl *O*-(3,5,6-Trichloro-2-pyridyl) Phosphorothioate (Dursban) Insecticide in a Lactating Cow

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Dursban was absent from the milk and urine of a dairy cow fed 5 p.p.m. of the compound in the feed. A compound characterized by retention time as Dursban was found in the feces and represented 1.7% of the insecticide fed. Two metabolites were excreted in the urine which, following methylation, had retention times identical to the methyl esters of diethylthiophosphate and diethyl phosphate. They represented, respectively, 35.9 and 26.8% of the total insecticide fed on an equivalent basis. Dursban was stable when incubated with fresh rumen fluid or beef liver.

Dursban [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is a broad-spectrum insecticide which controls flies, stored product insects, aphids, mites, soil insects (Kenaga *et al.*, 1965), and animal parasites (Smith, 1966). Its many potential uses, certain of which could conceivably cause residues in cattle feed, prompted this study of its metabolism in the dairy cow.

The metabolism of Dursban has been studied in rats (Smith *et al.*, 1967) and goldfish (Smith *et al.*, 1966). Following a single dose of Cl^{36}-labeled Dursban, rats excreted 3,5,6-trichloro-2-pyridyl phosphate (75 to 80%), 3,5,6-trichloro-2-pyridinol (15 to 20%), and traces of intact Dursban in their urine and feces. Dursban accumulated in fatty tissues and was slowly liberated. The metabolites were identified by their R_f values using paper chromatography or gas chromatographic retention data. The following metabolites accumulated in the tissues of goldfish exposed to water containing C^{14}-labeled Dursban for 48 hours: 3,5,6-trichloro-2-pyridyl phosphate, ethyl-3,5,6-trichloro-2-pyridyl phosphorothioate, diethyl 3,5,6-trichloro-2-pyridyl phosphate, and Dursban. The metabolites were tentatively identified by their respective R_f values using paper chromatography. The metabolism of Dursban has not been studied previously in cows.

EXPERIMENTAL PROCEDURE

A Holstein cow was catheterized and fed 5 p.p.m. (based on a daily ration of 50 pounds) of pure analytical grade Dursban for 4 days. The compound in absolute ethanol was thoroughly mixed with the 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 4 days thereafter. The total daily urine and feces samples were similarly collected, weighed, separately mixed, and subsampled during the same test period. All samples were frozen immediately and held for analysis. All analyses of daily milk were made on a composite of the morning and evening subsamples.

Possible residues of free Dursban were extracted from milk, urine, and feces by blending 25 grams of the sample with 70 ml. of acetone. The mixture was filtered, and the filter was rinsed with acetone to a total volume of 100 ml. Five milliliters of this solution was partitioned with 5 ml. of the benzene and 90 ml. of 2% sodium sulfate solution. The upper benzene solution was analyzed for the insecticide using electron affinity gas chromatography. The column used was 6 feet long and contained 10% DC-200 on 80- to 100-mesh Gas Chrom Q. The gas chromatograph used was a Barber-Colman Model 10 with a battery-operated 6-cc. electron affinity detector containing 56 μc. of radium-226. The operating temperatures for the column, flash heater, and detector were 200°, 265°, and 235° C., respectively, and nitrogen (60 cc. per minute) was the carrier gas. The retention time of Dursban was 13.6 minutes.

A newly developed procedure (St. John and Lisk, 1968) was applied for the determination of possible hydrolysis and oxidized hydrolysis products of Dursban in urine. It was probable that diethyl phosphate and, possibly, diethyl thiophosphate might be produced and excreted. Urine was analyzed for these products.

Briefly, the method involved acidification and salt saturation of the urine sample followed by ether extraction. The ether solution containing the phosphate metabolites was methylated with diazomethane to produce the methyl esters of diethyl thiophosphate and diethyl phosphate. The ether solution of phosphate esters was chromatographed on a column 6 feet long containing 2% Ucon Polar on 80- to 100-mesh Gas Chrom Q at 105° C. and using a modified thermionic detector (Giuffrida *et al.*, 1966).

Stability of Dursban when incubated with fresh rumen fluid was studied. Fifty micrograms of Dursban in 1 ml. of absolute ethanol was thoroughly mixed with 100 ml. of filtered rumen fluid (0.5 p.p.m.) freshly sampled from a heifer, and the mixture was held at the normal body temperature (38° C.). At 13 measured intervals during 6 hours of incubation, the fluid was again mixed, and 5 ml. was removed and diluted to 26 ml. with acetone. One milliliter of the solution was transferred to a 50-ml. volumetric flask containing 1 ml. of benzene, and the contents were made to volume with 2% sodium sulfate solution. The mixture was thoroughly shaken, and the upper benzene solution was analyzed by electron affinity gas chromatography using the DC-200 column.

Possible metabolism of Dursban in the presence of liver from a freshly slaughtered cow was studied. The insecticide (50 μg.) in 0.1 ml. of ethanol was added to 1 gram of

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thinly sliced liver (50 p.p.m.) contained in a 100-ml. beaker with 10 ml. of 0.9% sodium chloride solution. The contents were maintained at 38° C. in a shaking water bath. At four specific intervals during 2 hours of incubation, a sample beaker was removed and the contents were blended with acetone and filtered and rinsed with acetone to a total volume of 50 ml. One milliliter of this solution was transferred to a 50-ml. volumetric flask containing benzene and 2% sodium sulfate, the mixture was partitioned, and the benzene solution was analyzed as in the procedure for rumen field.

RESULTS

No residues of Dursban were detected in the milk or urine samples. A peak having the retention time of Dursban appeared in the chromatograms of feces samples collected on the last three days during which Dursban was fed and the first day after. None appeared in feces samples taken on the second, third, and fourth days after insecticide feeding was discontinued. Based on peak height measurements, this totaled 1.7% of the total (454 mg.) Dursban fed. Figure 1 illustrates chromatograms of feces collected the third day following initiation of feeding and shows the peak and the control. Recoveries of Dursban added to control milk, urine, and feces prior to extraction at a level of 0.2 p.p.m. were 95, 120, and 82%, respectively. The method was sensitive to 0.04 p.p.m. of Dursban. The insecticide did not decompose detectably in fresh rumen fluid in 6 hours or in the presence of liver for 2 hours.

Analysis of urine following methylation revealed chromatographic peaks with retention times identical to those of the methyl esters of diethyl thiophosphate and diethyl phosphate. (Methylation of these compounds would yield, respectively, diethylmethyl thiophosphate and diethylmethyl phosphate.) The peak heights of these apparent metabolites represented, respectively, 35.9 and 26.8% of the total insecticide fed on an equivalent basis.

Figure 2 illustrates the appearance and disappearance of both peaks (calculated as equivalent milligrams of Dursban) in urine as a function of time following initiation of feeding Dursban. Figure 3 shows chromatograms of the tentatively identified diethyl thiophosphate and diethyl phosphate metabolite (methylated) peaks in urine on the second day after dosing began and in the control urine. The method was sensitive to about 0.2 p.p.m. of diethyl thiophosphate and 0.05 p.p.m. of diethyl phosphate in urine. Recovery of each compound added to urine in progressively increasing concentrations was linear when response (peak height) was plotted against the compound concentration added to urine (St. John and Lisk, 1968).

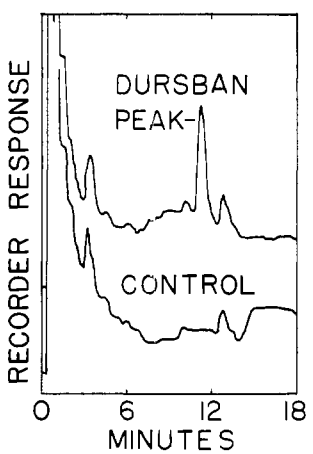

Figure 1. Chromatograms showing peak assigned to Dursban excreted in feces on the third day after feeding began and for the control

DISCUSSION

Trolene [*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate] insecticide is similar in structure to Dursban. Residues of P^{32}-labeled Trolene were found in milk, blood, and several tissues of a cow which received a single dose of

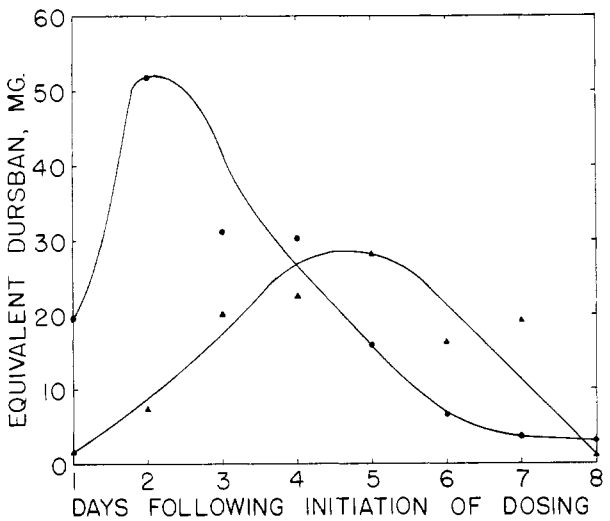

Figure 2. Formation and disappearance of the metabolites, probably diethyl thiophosphate (●) and diethyl phosphate (▲), in urine

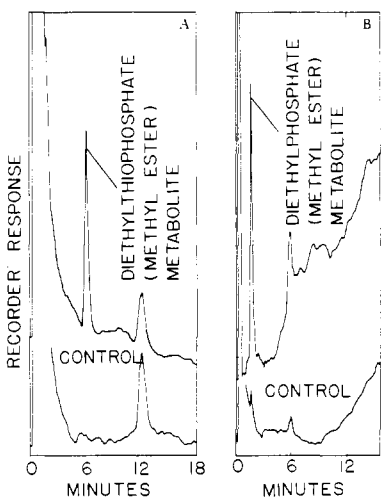

Figure 3. Chromatograms showing the apparent diethyl thiophosphate (*A*) and presumed diethyl phosphate (*B*) metabolite (methylated) peaks in urine on the second day following initiation of dosing and in control urine

70 grams of the compound (70 times the total quantity of Dursban fed) (Plapp and Casida, 1958). About 1% of the Trolene fed appeared to be eliminated intact in the feces based on solubility and radioactivity measurements. This agrees well with the urinary elimination of 1.7% of the total Dursban dose. Hydrolysis products of Trolene in stagnating rumen fluid were produced and characterized.

About 49% of the Trolene administered was excreted in the urine, based on total radioactivity counted. A total of 62.7% of the Dursban fed was accounted for in urine by the presence of the two metabolites characterized by retention time as diethyl thiophosphate and diethyl phosphate. Other workers have reported urinary excretion of similar hydrolysis products of organophosphorus insecticides when fed to ruminants. Thus, the metabolites, *O,O*-dimethylphosphoric acid, *O,O*-dimethylthiophosphoric acid, and *O,O*-dimethyldithiophosphoric acid, were identified in the urine of a cow which was fed P^{32}-labeled dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] insecticide (Dauterman *et al.*, 1959). Similarly, dimethylphosphoric acid was excreted in the urine of a ewe (Chamberlain, 1964a) and a goat (Chamberlain, 1964b) which was dosed with P^{32}-labeled Ciodrin (α-methylbenzyl 3-hydroxycrotonate dimethyl phosphate) insecticide.

The possibility of production and excretion of phosphorus-containing desethylated hydrolytic metabolites of Dursban (such as monoethyl phosphate or monoethyl thiophosphate) in urine was considered. Although pure standards of these compounds were not available for study, methylation of them would yield products differing from the respective diethyl compounds by only 14 molecular weight units (one CH_2 group). Conceivably, these very similar compounds would not have been adequately separated by gas chromatography. Desethylated phosphates would be more acidic and might not be extracted into ether unless the urine was acidified further. No peaks in close proximity to those of the observed metabolites were present in the chromatograms. Also, desethylation often constitutes a very minor metabolic pathway in mammals.

Assuming the identities of the metabolites of Dursban in urine are correct, about 64.4% of the total Dursban fed was accounted for. Although pure standards of other possible metabolites of Dursban were not available for investigation, it would appear that, although traces of Dursban residues might occur in the milk of a cow at greatly exaggerated feeding levels, the presence of Dursban in forage at levels below 5 p.p.m. would not constitute a hazard and could be recommended.

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