Bull. Environm. Contam. Toxicol. 26, 262-266 (1981)

Studies of Possible Bovine Urinary Excretion and Rumen Decomposition of Fenvalerate Insecticide and a Metabolite

Patricia C. Wszolek[1], Norman A. LaFaunce[2], Timothy Wachs[3], and Donald J. Lisk[1]

[1]*Toxic Chemicals Laboratory, New York State College of Agriculture and Life Sciences,* [2]*Department of Clinical Science, New York State College of Veterinary Medicine, and* [3]*Department of Chemistry, Cornell University, Ithaca, NY 14853*

Fenvalerate (Pydrin, Sumicidin, cyano(3-phenoxyphenyl)methyl 4-chloro-alpha-(1-methyl-ethyl) benzeneacetate) is a broad-spectrum insecticide, one of a group of promising synthetic pyrethroid compounds. Its metabolism in rats (OHKAWA et al. 1979) and its excretion in the milk and feces of dairy cows (WSZOLEK et al. 1980) have been studied. We report now on the analysis of urine for the elimination of fenvalerate and one of its major metabolites, 4-chloro-alpha-(1-methylethyl)-benzeneacetic acid, from a cow fed fenvalerate fortified grain. We also report the fate of fenvalerate incubated with bovine rumen fluid *in vitro*.

EXPERIMENTAL

Feeding and Sample Collection

Four portions of grain, each weighing fifty pounds (22.7 kg), were individually fortified with 227 mg of fenvalerate (Pydrin, SD43775) dissolved in 10 mL absolute ethanol and were thoroughly mixed. The amended grain, 10 ppm in fenvalerate, was fed to a catheterized, lactating, Holstein cow, one portion per day for four days. Urine samples were collected prior to feeding (control), each day during feeding and for six days afterwards. Samples were frozen prior to analysis.

Urine Analysis

Fenvalerate. Aliquots of urine (30-50 mL), pH~8, were extracted with hexane (3 X 30-50 mL distilled-in-glass, Burdick and Jackson, Muskegon, Michigan). Emulsions formed in some cases, and these were broken by the addition of 10 g sodium chloride plus centrifugation. Combined hexane extracts for each urine sample were washed with distilled water, dried over sodium sulfate and evaporated under vacuum to 1-10 mL. Fenvalerate was determined in the extracts by electron capture gas chromatography using a Tracor 222 instrument equipped with a 180 cm X 2 mm glass column packed with 3% OV-101 on 100/120 mesh Gas Chrom Q and a ^{63}Ni detector. Column, injector, and detector temperatures were 235° C, 285° C, and 310° C, respectively. Nitrogen was the carrier gas at a flow rate of 30 cc/min. Fenvalerate eluted as two chromatographic peaks (retention times 15.5 and 16.8 min) each representing one of the pairs of its enantiomers (LEE et al. 1978). In addition to the ten urine samples collected during the feeding period, three

0007-4861/81/0026-0262 $01.00

50 mL aliquots of control urine (first day of feeding) were amended with fenvalerate dissolved in methanol: 0.96 µg; 0.096 µg; and 0.096 µg, corresponding to about 20 ppb, 2 ppb and 2 ppb. These aliquots were carried through the extraction procedure to assess recovery of fenvalerate which was found to be 103%, 108% and 112% respectively. The detection limit was estimated to be 0.5 ppb of fenvalerate in urine.

4-chloro-alpha-(1-methylethyl)-benzeneacetic acid. Aliquots of urine (4 mL) were hydrolyzed with concentrated hydrochloric acid (0.4 mL) at 90°-100° C for 4 h. Hydrolyzates were extracted with ethyl acetate (Mallinckrodt, Analytical reagent, 3 X 6 mL) followed by ethyl ether (Mallinckrodt, analytical reagent 1 X 6 mL). Emulsions formed in some cases and were centrifuged. Combined extracts for each hydrolyzate were washed with distilled water, dried over sodium sulfate, and evaporated to about 5 mL under vacuum, and then to dryness under a stream of nitrogen. Residues were esterified according to the method of MIERZWA and WITEK (1977) with 1 mL of 20% (v/v) 2,2,2-trichloroethanol (Eastman Kodak Co., Rochester, NY) in trifluoroacetic anhydride (Pierce Chemical Co., Rockford, IL) plus 5 µL concentrated sulfuric acid at 95°-100° C for 20-30 min. The reaction mixture was reduced in volume under nitrogen, partitioned between 0.5M potassium hydroxide and isooctane and the isooctane layer was cleaned up on a micro-Florisil column (4 cm X 1 cm) topped with 1 cm of sodium sulfate. The column was first eluted with 30 mL hexane, and the trichloroethanol ester of authentic 4-chloro-alpha-(1-methylethyl)-benzeneacetic acid (Shell Chemical Co., SD44064) was eluted in the next fraction using 100 mL of 1% ethyl ether in hexane. This fraction was concentrated to 10 mL under vacuum. Electron capture gas chromatography followed using the same instrument and column described above except that operating temperatures were: 165° C (column); 235° C (injector); 300° C(detector). The 2,2,2-trichloroethanol ester derivative of 4-chloro-alpha-(1-methylethyl)-benzeneacetic acid eluted as a single peak with a retention time of 9.9 min. Control urine (day 1 of feeding) was amended with the authentic acid at three different levels: 7 ppm; 0.7 ppm; and 0.17 ppm, and these were carried through the above extraction and isolation procedures. The trichloro ester of the acid was detected above background by ECGC at the two higher levels of the acid in urine (7 ppm and 0.7 ppm), but not at 0.17 ppm. Recoveries were low: 20% (7 ppm level in urine) and 10% (0.7 ppm).

The trichloro-ester of the authentic acid and some of the final Florisil fractions of derivatized urine extracts were analyzed by negative ion electron capture chemical ionization mass spectrometry. A Finnigan Model 3300 gas chromatograph mass spectrometer with a System Industries 150 data system was used to obtain the spectra. The column was glass (150 cm X 2 mm) packed with 3% OV-101 on 100/120 mesh Gas Chrom Q and was programmed from 150°-250° C at 10° C/min. Methane, adjusted to give a pressure of 1 Torr in the ion source, served as both the carrier gas and the reagent gas for thermalizing electrons used in the capture process in the chemical ionization source (STAFFORD et al. 1978). The

negative ions were detected using a modification of a conversion dynode system previously described (HUNT et al. 1976).

Rumen Fluid Incubation

Rumen fluid was collected from a fistulated, nonlactating, Holstein into a Dewar flask and was quickly filtered through glass wool. This filtrate was poured into individual glass bottles, 50 mL fluid to each, which already contained 1 mL of an acetone solution of fenvalerate (96 µg). The resulting mixture in the bottles was approximately 2 ppm in fenvalerate, a concentration close to that estimated in the cow's rumen during the actual fenvalerate feeding study. The bottles were incubated at 39° C (the approximate natural rumen temperature) with gentle shaking and were covered with aluminum foil to exclude light. At specific times from 0 to 6 h, the contents of individual bottles were extracted with hexane (2 X 50 mL). Bottles were also rinsed with 10 mL acetone to aid in the removal of the hydrophobic fenvalerate, and the rinsings were added to the separatory funnel prior to extraction. Combined extracts for each bottle were washed with distilled water and dried over sodium sulfate. Fenvalerate concentrations were determined by ECGC as described above. Recovery of fenvalerate was approximately 60% from the fortified zero time rumen fluid. No GC peaks which could interfere with fenvalerate analysis were observed in rumen fluid incubated for 6 h with 1 mL acetone alone.

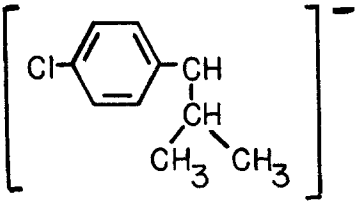
RESULTS AND DISCUSSION

Urine

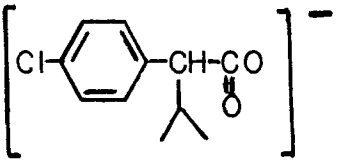
Intact fenvalerate was not detected in any sample of the urine excreted by the cow during the 10-day feeding study nor were there any chromatographic peaks observed in the control urine extract which would interfere with the determination. In our earlier study, significant amounts of fenvalerate were excreted in feces.

We were not able to identify unambiguously the presence of the acid metabolite in the urine. The control urine exhibited no chromatographic peaks at the retention time of the authentic acid ester derivative. Several of the urine samples from the feeding study did have a peak at the appropriate retention time. However, when the authentic compound was coinjected with the urine extracts in question, the peaks broadened in every case except for the urine from the fifth day of the feeding study and except for control urine adjusted to 0.7 ppm with the authentic acid. These latter two extracts were further examined by negative ion electron capture chemical ionization GCMS, a technique which is very sensitive for compounds which capture electrons, and the data were compared with the mass spectrum obtained for the 2,2,2-trichloroethanol derivative of the authentic acid under the same conditions. The spectrum of the authentic acid does not exhibit a molecular ion at m/e 342, but does contain characteristic peaks at m/e 167 and m/e 211 and their corresponding Cl^{37} isotope peaks at m/e 169

and m/e 213. The ion at m/e 167 presumably represents the structure



and the ion at m/e 211 the structure



Both structures would be characteristic of the acid portion of the derivative. These ions were found in the mass spectrum for the chromatographic peak occurring at the appropriate retention time in the extract of the control urine amended with the authentic acid at the 0.7 ppm level but not for the urine extract from the fifth day of feeding. Therefore, the presence of the acid in this urine sample could not be confirmed.

Rumen Fluid

The amount of fenvalerate extracted at specific times during the 6 h incubation with rumen fluid was constant. No significant degradation of fenvalerate was detected by the end of the short term incubation.

In our earlier work (WSZOLEK et al. 1980) we estimated that as much as 25% of the fenvalerate fed to the dairy cows could be excreted in their feces and about 0.5% in their milk. A smaller amount of the insecticide is apparently excreted in the urine, about 0.02% or less of the administered dose based on the total volume of urine collected during this study (about 290 liters). Although we could not identify the presence in the cow's urine of the acid expected to ba a major fenvalerate metabolite, 4-chloro-alpha-(1-methylethyl)-benzeneacetic acid, we can calculate that an upper limit of about 20% of the fenvalerate could be accounted for by possible excretion of this acid in urine.

When fenvalerate was fed to rats, rapid and complete elimination of the insecticide and its metabolites was observed using radiolabeled compounds (OHKAWA et al. 1979). Unmetabolized fenvalerate was the major product in the feces but only trace amounts were found in the urine some of which was attributed to contamination with fecal material. Our results are similar to these found for the rat. More than fifteen metabolites alone were found to derive from the acid portion of the insecticide fed to the rats with 4-chloro-alpha-(1-methylethyl)-benzeneacetic acid being the main species and some of the other species present only in trace amounts. A

similar situation may exist in the case of the cow but the large urinary volume dilution plus the presence of relatively large amounts of dietary metabolites make their analysis by gas chromatography very difficult.

Our in vitro rumen metabolism study suggests that fenvalerate is probably not quickly degraded in the bovine rumen. Any degradation of the insecticide probably takes place further along in the cow's digestive system. We cannot rule out, however, that significant amounts of fenvalerate accumulate in the animal's tissues and organs which we have not analyzed.

ACKNOWLEDGEMENTS

We are grateful to Shell Development Company for samples of fenvalerate and some of its metabolites including 4-chloro-alpha-(1-methylethyl)-benzeneacetic acid. We appreciate the assistance of Walter Gutenmann in feeding the cow and obtaining rumen fluid and the urine samples.

REFERENCES

HUNT, D. F., G. C. STAFFORD, F. W. CROW, and J. W. RUSSELL: Anal. Chem. 48, 2098 (1976).

LEE, Y. W., N. D. WESTCOTT, and R. A. REICHLE: J. Assoc. Off. Anal. Chem. 61, 869 (1978).

MIERZWA, S., and S. WITEK: J. Chromatography 136, 105 (1977).

OHKAWA, H., H. KANEKO, H. TSUJI, and J. MIYAMOTO: J. Pesticide Sci. 4, 143 (1979).

STAFFORD, G. C., J. R. RUHER, and M. S. STORY: Proc. Annu. Conf. Mass Spectrom. Allied Top., 26th, 459 (1978).

WSZOLEK, P. C., D. H. LEIN, and D. J. LISK: Bull. Environm. Contam. Toxicol. 24, 246 (1980).