Residues in Milk from Cows Fed 4-Amino-3,5,6-trichloropicolinic Acid

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Three dairy cows were fed rations containing the potassium salt of 4-amino-3,5,6-trichloropicolinic acid at various rates from 10 to 1000 p.p.m. and samples of their milk were analyzed for herbicide residues. Measurable residues (0.05 p.p.m.) first appeared in the milk of one cow after feeding at the 150-p.p.m. level. Average residues of 0.05 and 0.2 p.p.m. were found in milk from the three cows fed 300 and 1000 p.p.m., respectively, for 2 weeks. Residues disappeared rapidly from milk when the compound was withdrawn from the diet, becoming undetectable within 58 hours.

Tordon herbicides (Dow Chemical Co.), containing the active ingredient 4-amino-3,5,6-trichloropicolinic acid, show promise in the control of deep-rooted perennial weed species in small grains (Gantz and Warren, 1966; Haagsma and Wiffen, 1966; Nalewaja, 1966) and in the eradication of a variety of woody plants and noxious weeds in pasture lands (Gantz and Laning, 1963). The former use results in residues in grain at harvest in the range of 1 p.p.m. or less (Bjerke *et al.*, 1967) while the latter can result in residues in grass reaching several hundred parts per million (Getzendaner and Herman, 1967). Thus feed and forage ingested by dairy cows could contain the herbicide in widely varying amounts. This study was undertaken to determine the extent to which the compound is secreted into milk by cows fed graduated amounts of the herbicide.

EXPERIMENTAL PROCEDURES

Four Holstein dairy cows, each weighing from 1135 to 1252 pounds, were confined in stanchions and conditioned for 2 weeks to a basal ration of equal parts (by weight) of a grain mixture and chopped alfalfa hay. The grain rations were fed from 5-gallon pails while the cows were being milked. When all grain was consumed, the hay was made available in feed troughs specially designed to prevent scattering. The same rations were fed twice daily at about 6:00 A.M. and 6:00 P.M. During the conditioning period it was determined that 10 pounds of grain plus 10 pounds of hay was the maximum each animal would consume at each feeding without leaving a significant amount of hay.

The formulation of 4-amino-3,5,6-trichloropicolinic acid used in this study and the method of incorporation into the diet were as previously described (Kutschinski and Riley, 1969).

At the conclusion of the conditioning period, two cows were continued on control rations while two were fed the compound at the level of 10 p.p.m. for 6 days, then at 30 p.p.m. for 8 days. While maintaining one control, three cows (including the other control) were then fed the herbicide for consecutive 2-week periods at levels of 100, 150, 300, and 1000 p.p.m. The 1000-p.p.m. level is equivalent to a rate of 18 mg. per kg. per day. Each feeding period began with the 6:00 P.M. ration on Friday. At the conclusion of the final feeding period the herbicide was withdrawn from the diets and the cows were maintained on basal rations for 5 days.

Beginning 10 days prior to feeding the subject compound and continuing throughout the test, milk was sampled by combining ½ pint from the evening milking with an equal amount collected the following morning. Samples were taken Sunday P.M. plus Monday A.M. and Wednesday P.M. plus Thursday A.M. during the first week of each new feeding schedule and Sunday P.M. plus Monday A.M., Tuesday P.M. plus Wednesday A.M., Wednesday P.M. plus Thursday A.M., and Thursday P.M. plus Friday A.M. during the second week. All milking was done by machine and evening samples were stored overnight in a refrigerator.

The average daily milk production for each cow, calculated over one-week periods near the beginning, middle, and end of the test period is shown in Table I. Animal weights at the beginning and end of the experiment are also given. No adverse effect due to ingestion of the herbicide was noted in any of the animals.

ANALYTICAL METHODS

Most milk samples were analyzed within a few hours after the morning sampling. Those necessarily held under refrigeration for up to 48 hours were mixed thoroughly before sampling. Two related analytical procedures were used. Method I is simple and rapid but requires continual standardization during chromatography because of the adverse effect of co-extractives from milk on detector sensitivity. Method II includes a cleanup step which largely eliminates this effect.

All of the milk samples were analyzed by the simpler method. Several samples were also analyzed by Method II to confirm the adequacy of the method without cleanup.

The apparatus, reagents, and gas chromatographic details are essentially as reported for the analysis of cereal grains (Bjerke *et al.*, 1967). Only newly developed extraction and cleanup methodology is reported here.

Method I. Pipet 2.0 grams of well-mixed milk into a 4-dram screw-cap vial and add 4 ml. of distilled water. Add 2 drops of H_3PO_4 (pH<2), 4 grams of NaCl, and

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Table I. Milk Production and Body Weights of Cows

Week of	Average Daily Milk Production, Lb.				Body Weight, Lb.			
	Cow 3[a]	Cow 1	Cow 2	Cow 4	Cow 3[a]	Cow 1	Cow 2	Cow 4
12/11/66	49	39	67	55	1135	1139	1169	1242
1/ 8/67	67	40	53	55				
2/19/67	58	46	58	50	1127	1140	1153	1262

[a] Control cow.

4.0 ml. of ether. Cap the tube, shake vigorously for 2 minutes, and separate the phases by centrifugation. Pipet 2.0 ml. of the ether extract into a 5-ml. volumetric flask. Add 1 ml. of ethereal diazomethane reagent (approximately 18 mg. per ml.), 0.5 ml. of benzene, and a boiling chip, and boil off the ether on a steam bath. Cool to room temperature and dilute to 5.0 ml. with benzene. Chromatograph 2.0 μl. of the benzene solution and determine its concentration by referring the height of the peak obtained to a calibration curve prepared as previously described (Bjerke *et al.*, 1967).

Method II. Extract the milk with ether as in Method I. Prepare a 1-gram column of Woelm basic alumina, activity grade I, in ether. Pipet 2.0 ml. of the ether extract onto the column and allow it to run through. Follow, successively, with 20 ml. of ether and 20 ml. of acetone. Discard the effluent. Elute the column with 20 ml. of 0.25*M* $NaHCO_3$ solution, collecting the eluate in a 60-ml. separatory funnel. Acidify with 1.8 ml. of 5*N* H_2SO_4 and dissolve 4 grams of NaCl in the solution. Extract the solution successively with 20 and 10 ml. of ether, combining the extracts in a 50-ml. beaker. Add about 0.1 gram of Na_2SO_4, concentrate the ether solution to 1 to 2 ml. on a steam bath, and quantitatively transfer it to a 5-ml. volumetric flask, using ether for rinsing. Add a boiling chip and concentrate to about 1 ml. on a steam bath. Cool to room temperature. Complete the analysis as in Method I, beginning with addition of diazomethane reagent.

Representative chromatograms resulting from analyses of milk by the two methods, from both the control cow and those fed the herbicide, are shown in Figure 1.

BLANKS AND RECOVERIES

Samples of control milk obtained from all four cows before feeding the herbicide were analyzed by the procedures just described. In addition, milk from the cow maintained as control was sampled on the same schedule as the test animals throughout the experiment. No significant blanks occurred.

The efficiencies of both methods were determined by fortifying control milk with known amounts of the compound and applying the respective analytical procedures. Table II summarizes the results of these analyses, indicating an average recovery of 97% for the rapid method and 99% when the cleanup procedure was employed.

Figure 1 shows chromatograms from fortified milk.

RESULTS AND DISCUSSION

Table III shows the results of analyses of individual milk samples from the three test cows at various ingestion levels. Results by the two methods agreed closely. A residue of significant magnitude (0.05 p.p.m.) first appeared in milk from one cow ingesting the herbicide at the 150-p.p.m. level, although the compound was detectable when 100 p.p.m. was fed. Residues were secreted into the milk of one cow at levels distinctly above those of the other two. No known factor accounts for this difference. On the average, at the higher feeding

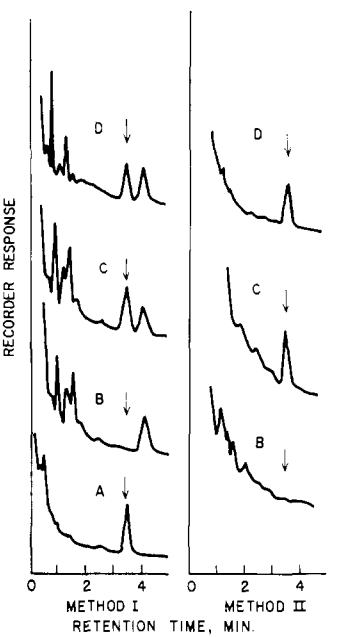

Figure 1. Representative chromatograms

Arrows indicate retention time of methyl 4-amino-3,5,6-trichloropicolinate
A. Ester equivalent to 0.02 ng. of 4-amino-3,5,6-trichloropicolinic acid
B. Control milk
C. Milk fortified at 0.05-p.p.m. level
D. Milk from cow fed 150 p.p.m.

Table II. Recovery of 4-Amino-3,5,6-trichloropicolinic Acid from Milk Fortified with Known Amounts

P.P.M. Added	Method I[a] P.p.m. found	Method I[a] % recovery	Method II[b] P.p.m. found	Method II[b] % recovery
0.05	0.05	100	0.05	100
	0.05	100	0.05	100
0.10	0.10	100	0.095	95
	0.10	100	0.10	100
0.20	0.19	95	0.195	98
	0.19	95	0.20	100
0.25	—	—	0.25	100
0.30	—	—	0.30	100
0.50	0.48	96	0.50	100
	0.475	95	0.495	99
1.0	0.96	96	—	—
	0.97	97	—	—
		Av. 97		99

[a] Rapid method (no cleanup).
[b] Method with alumina column cleanup.

Table III. Residues of 4-Amino-3,5,6-trichloropicolinic Acid in Milk from Dairy Cows

P.P.M. in Diet	Days Fed at Given Level	Apparent P.P.M.[a] Residue Cow 1	Cow 2	Cow 4
100	2	<0.05	<0.05	<0.05
	5	<0.05	<0.05	<0.05
	9	<0.05	<0.05	<0.05
	11	<0.05	<0.05	<0.05
	12	<0.05	<0.05	<0.05
	13	<0.05	<0.05	<0.05
150[b]	2	<0.05	<0.05	<0.05
	5	<0.05	<0.05 (<0.05)	<0.05
	9	<0.05	<0.05 (0.05)	<0.05
	11	<0.05	0.05 (0.05)	<0.05
	12	<0.05	0.05	<0.05
	13	<0.05	<0.05	<0.05
300[b]	2	0.05	0.08	0.05
	5	<0.05	0.08	<0.05
	9	0.05	0.07	<0.05
	11	0.06	0.07	<0.05
	12	0.05	0.06	<0.05
	13	<0.05	0.07	<0.05
1000[b]	2	0.14	0.23	0.16
	5	0.18	0.24	0.11
	9	0.15	0.29	0.13
	11	0.18	0.25	0.13
	12	0.21	0.26	0.16
	13	0.15	0.27 (0.27)	0.15
	15	0.15 (0.15)	0.27 (0.28)	0.11

[a] Values in parentheses are corroborative results obtained using Method II. All other data were produced using Method I.
[b] Cows previously fed for 2 weeks at each lower level.

Table IV. Disappearance of 4-Amino-3,5,6-trichloropicolinic Acid in Milk from Cows Fed 1000 P.P.M. after Withdrawal from Diets

Hours after Withdrawal	Apparent P.P.M. Residue Cow 1	Cow 2	Cow 4
0	0.17[a]	0.26[a]	0.14[a]
10	0.16	0.24	0.14
24	T[b]	0.10	T
34	T	0.05	T
48	N[c]	T	N
58	N	N	N
72	N	N	N

[a] Residues in milk from cows while ingesting 1000 p.p.m. of compound in their diets (average of all determinations for each cow during 15-day feeding period).
[b] T. Trace, response detected but not quantitatively determinable.
[c] N. None, response indistinguishable from control.

levels, the residue in milk was approximately 0.02% of the concentration in the diet.

Table IV shows the results of analyses of milk from the three cows, fed at the rate of 1000 p.p.m. of 4-amino-3,5,6-trichloropicolinic acid, when sampled at intervals after withdrawing the herbicide from their diets. Residues disappeared from the milk very rapidly after ingestion of the compound ceased. No measurable amount was secreted 24 hours after withdrawal from two cows, nor 48 hours after withdrawal from the third animal which had consistently produced higher residues during the test. Residues for all animals fell below the detection limit within 58 hours.

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