Residue of Cypermethrin and Its Major Acid Metabolites in Milk and Tissues from Dairy Bovines Treated with Cypermethrin

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A bovine feeding study was conducted to determine the magnitude of the residues of cypermethrin and its acid metabolites in milk, cream, kidney, liver, pectoral muscle, adductor muscle, peritoneal fat, and subcutaneous fat following daily oral administration of cypermethrin for 28 consecutive days. The dose levels were 5, 15, and 50 ppm (mg/kg) in the diet, which were equivalent to 1, 3, and 10 times the expected maximum intake level in animal feeds. In addition, two registered insect repellent eartags containing cypermethrin were fixed to each animal to establish the maximum conditions of exposure. The residue results were used to determine the extent of residue transfer from animal feeds into bovine milk, meat products, and meat byproducts. The residue decline rate was also measured during a recovery period following withdrawal of the test substance.

Keywords: *Cypermethrin; metabolites; residues; milk/tissues*

INTRODUCTION

Cypermethrin, a highly active synthetic pyrethroid insecticide, has been developed extensively by FMC Corp. for controlling a wide range of pests on many agricultural crops. Finite residues of either cypermethrin or its major acid metabolites may be contained in animal feed and could result in residue transfer into bovine milk and tissues. Detailed published discussions (World Health Organization, 1989) covered the use pattern of cypermethrin, its environmental and biological impact, and toxicity concerns associated with its use. The chemical name of the active ingredient is (±)-α-cyano(3-phenoxyphenyl)methyl (±) *cis*/*trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate. The major animal metabolites of cypermethrin have been identified as dichlorovinyl acid [DCVA; *cis*/*trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid] and *m*-phenoxybenzoic acid (*m*-PBA) (compound structures shown in Figure 1).

Although dairy bovine feeding with cypermethrin has been studied (Swaine and Sapiets, 1981a,b), no method recoveries of cypermethrin in milk and tissues have been reported. Most of the method recoveries for acid metabolites in tissues were inconsistent and below the normally acceptable range (70−120%). As a result, wide ranges of residues in tissues from the same treatment group were reported. The analytical methods developed in our laboratory (Chen et al., 1996) have been applied and proven reliable and reproducible in various bovine matrices. The residue transfer results determined from this bovine study, therefore, should closely represent the true residues in these matrices.

A total of five animal groups were included in this study; four groups (C, T-I, T-II, and T-III) consisted of three Holstein dairy bovines and one group (T-IV) consisted of four bovines. The control group (C) animals were fitted with eartags containing no cypermethrin and were given gelatin capsules without the test substance. The bovines in the first treatment group (T-I) were fitted with the eartags containing cypermethrin and were given gelatin capsules without the test substance. The other three treatment groups (T-II, T-III, and T-IV) were fitted with eartags containing cypermethrin and were dosed orally with cypermethrin in gelatin capsules at

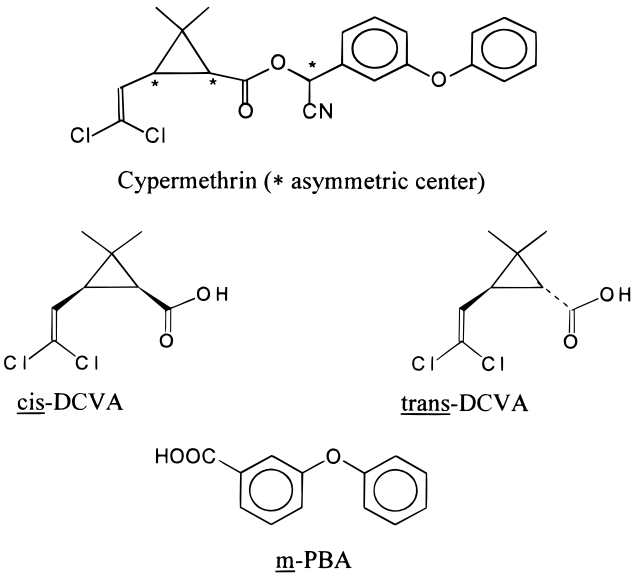

Figure 1. Structures of cypermethrin and three metabolites.

levels equivalent to 5, 15, and 50 ppm in diets (milligrams per kilogram of daily intake feed), respectively, for 28 consecutive days.

Milk samples were collected throughout the in-life period of the study; at sacrifice, tissue samples were collected. These milk and tissue matrices were analyzed to determine residues of cypermethrin and its acid metabolites, and the transfer factors from oral dosing to milk and fatty matrices were calculated. The extent of residue transfer from whole milk to cream was also determined. In addition, the residue decline rate was calculated on the basis of residue data generated after the termination of dosing program. All of the milk and tissue samples were stored frozen from collection, shipment through analysis.

Parent cypermethrin and acid metabolites were analyzed individually, using GC/ECD and GC/MSD. The limit of quantitation (LOQ) for milk samples was established at 10 ppb, with a limit of detection (LOD) at 2 ppb. For tissue and cream samples, the LOQ and the LOD were 50 and 10 ppb, respectively.

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Table 1. Cypermethrin Residues (Parts per Billion) in Bovine Milk Samples

	bovine identification no.																
	T-I (eartag only)				T-II (1×)				T-III (3×)				T-IV (10×)				
matrix	1	2	3	av	1	2	3	av	1	2	3	av	1	2	3	4	av
milk/test day																	
−1	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1, a.m.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1, p.m.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7	ND	ND	3
2	ND	ND	ND	ND	7	5	3	5	21	14	20	18	59	66	35	53	53
4	ND	ND	ND	ND	11	9	12	11	39	26	36	34	107	126	195	126	138
7	ND	ND	ND	ND	13	12	13	13	43	35	36	38	94	115	144	83	109
14	ND	ND	ND	ND	13	10	16	13	46	33	36	38	138	148	285	184	188
21	ND	ND	ND	ND	19	15	16	17	35	35	32	34	102	124	162	174	140
28	ND	ND	ND	ND	12	9	18	13	44	47	45	45	123	183	235	197	185
29	ND	ND	ND	ND	8	5	11	8	31	27	39	32	77	106	137	78	99
31[b]	ND	NA	NA	ND	2	NA	NA	2	10	NA	NA	10	22	NA	NA	28	25
34[c]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	15
skim milk	ND	ND	ND	ND	ND	ND	ND	ND	2	3	3	3	4	8	6	5	6
cream	ND	10	16	10	83	103	73	86	250	314	214	259	476	663	1009	905	763

[a] ND, nondetectable (<2 ppb for milk; <10 ppb for milk cream); NA, not applicable. [b] The first bovine of each group was allowed a 3-day recovery period. [c] The last bovine in T-IV group was allowed a 6-day recovery period.

Table 2. Cypermethrin Residues (Parts per Billion) in Bovine Tissue Samples

	bovine identification no.																
	T-I (eartag only)				T-II (1×)				T-III (3×)				T-IV (10×)				
matrix	1	2	3	av[a]	1	2	3	av	1	2	3	av	1	2	3	4	av
kidney	ND[b]	ND	ND	ND	ND	ND	12	ND	27	12	16	14	74	24	70	54	47
liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
muscle (pectoral)	ND	ND	ND	ND	15	ND	13	ND	50	79	38	58	133	104	199	120	151
muscle (adductor)	ND	ND	ND	ND	11	ND	ND	ND	18	41	14	28	59	28	41	34	34
fat (peritoneal)	ND	17	ND	11	182	101	150	125	493	444	527	485	1576	1350	1956	1421	1653
fat (subcutaneous)	ND	ND	ND	ND	91	63	88	76	296	462	315	388	1027	737	993	688	865

[a] Average residues in tissues were from two bovines (no. 2 and 3) sacrificed within 24 h of the last dosing. [b] ND, nondetectable (<10 ppb for tissues).

Table 3. Average[a] DCVA Residues (Parts per Billion) in Bovine Milk and Tissue Samples

	T-I (eartag only)		T-II (1×)		T-III (3×)		T-IV (10×)	
matrix	cis-	trans-	cis-	trans-	cis-	trans-	cis-	trans-
milk/test day								
−1	ND[b]	ND	ND	ND	ND	ND	ND	ND
1−34	NA	NA	NA	NA	ND	ND	ND	ND
skim milk	NA	NA	NA	NA	ND	ND	ND	ND
cream	ND	ND	12	ND	29	17	93	61
kidney	ND	ND	ND	22	ND	35	20	115
liver	ND	ND	ND	ND	ND	ND	11	25
muscle (pectoral)	NA	NA	NA	NA	ND	ND	ND	ND
muscle (adductor)	NA	NA	NA	NA	ND	ND	ND	ND
fat (peritoneal)	ND	ND	23	18	40	35	178	162
fat (subcutaneous)	ND	ND	ND	ND	24	25	100	93

[a] Average residues in tissues were from two bovines sacrificed within 24 h of the last dosing. [b] ND, nondetectable (<2 ppb for milk; <10 ppb for tissues and milk cream); NA, not analyzed or not applicable.

MATERIALS AND METHODS

Apparatus, Reagents, and Analytical Methods. Detailed information describing the apparatus, GC operating conditions, reagents, and analytical methods used for determining cypermethrin and its major acid metabolites in bovine milk, cream, liver, muscle, kidney, and fat samples were previously reported (Chen et al., 1996). The eartags used in this study were obtained from Y-TEX Corp. (Cody, WY). Both technical grade cypermethrin and treated eartags (each weighing ∼9.6 g) were assayed by the FMC Analytical Sciences Department and found to contain 95.7% and 7.0% cypermethrin by weight, respectively.

Test System. The test system consisted of 16 lactating Holstein dairy bovines. These 16 bovines were randomly assigned to their respective groups. Twelve days of quarantine served to acclimate the bovines to in-life facility conditions and allow time for them to be evaluated for suitability for the test. Bovine samples, including milk, cream, kidney, liver, pectoral muscle, adductor muscle, peritoneal fat, and subcutaneous fat, were collected at appropriate times and analyzed for cypermethrin residues.

In-Life Phase. The in-life portion of the bovine feeding study was conducted by Bio-Life Associates Ltd. (BLAL) in Neillsville, WI. During the in-life program, preparation of gelatin capsules, dosing procedures, feed consumption, milk production, tissue sample collection, body weight, and gross necropsy examination were carefully observed and recorded.

Gelatin Capsule Preparations. The cypermethrin test material was gently heated in a water bath (∼45 °C) before being weighed out and dissolved in acetone. Appropriate amounts of the solution were administered volumetrically to capsules containing ground grain. The acetone was allowed to completely evaporate in all capsules prior to dosing. The bovines were orally dosed with one gelatin capsule once daily for 28 consecutive days by a balling gun. The capsules were prepared weekly and were assayed by the FMC Analytical Sciences Department. The average cypermethrin contents in 5, 15, and 50 ppm dose groups were found to be 107, 334, and

Table 4. Average[a] *m*-PBA Residues (Parts per Billion) in Bovine Milk and Tissue Samples

matrix	T-I (eartag only)	T-II (1×)	T-III (3×)	T-IV (10×)
milk/test day				
−1	ND[b]	ND	ND	ND
1−34	NA	NA	ND	ND
skim milk	NA	NA	ND	ND
cream	ND	ND	ND	11
kidney	ND	ND	ND	36
liver	ND	ND	ND	ND
muscle (pectoral)	NA	NA	ND	ND
muscle (adductor)	NA	NA	ND	ND
fat (peritoneal)	ND	ND	ND	42
fat (subcutaneous	ND	ND	ND	14

[a] Average residues in tissues were from two bovines sacrificed within 24 h of the last dosing. [b] ND, nondetectable (<2 ppb for milk; <10 ppb for tissues and milk cream); NA, not analyzed or not applicable.

1154 mg per capsule, respectively. These dose levels were based on the highest feed consumption value recorded during the quarantine period (22.6 kg).

Feeding Study Design. Bovine milk samples were collected on test days −1, 1, 2, 4, 7, 10, 14, 21, 28, 29, 31, and 34. Milk was collected both morning and evening from every individual bovine. An aliquot of morning and evening milk was pooled on the basis of the weight ratio of production. On the days when the animals were sacrificed (test days 29, 31, and 34), only morning milk was available. On test day 1, however, the morning milk before actual dosing and the evening milk were collected separately. In addition, on test day 7, after regular milk sampling, the milk was separated into skim milk and cream by centrifugation. At the end of the dosing program, the eartags were removed and two animals from each group were humanely sacrificed within 24 h of the last dosing. One remaining animal from each group was sacrificed after a 3-day recovery period. The remaining animal from the highest dose group was sacrificed after a 6-day recovery period.

Experimental Design. A routine set of assays consisted of one or two control samples, one or two fortified control samples, and several treated samples. These samples were run concurrently through the same analytical procedures. The method recovery was determined by the results from the fortified control sample. Satisfactory recoveries at both LOQ and 10 times the LOQ levels demonstrated the accuracy of the method to measure the analysis in milk and tissue samples over a wide concentration range. All of the control samples were determined to be free of cypermethrin and its acid metabolite residues.

All of the milk and tissue samples collected were analyzed for cypermethrin, except for the morning milk from test day 1 (before dosing) and the milk samples from test day 10 (during plateau phase). For metabolite analyses, milk and muscle samples from T-III (3×) and T-IV (10×) groups were analyzed. Since no metabolite residue was detected in T-III group, samples from T-I (eartag only) and T-II (1×) groups were not expected to contain these residues and were not analyzed.

Storage Stability Program. The milk and tissue assays were completed within 6 weeks and 4 months after receipt, respectively. A laboratory-spiked storage stability study was conducted to determine the stability of cypermethrin, *cis*-DCVA, *trans*-DCVA, and *m*-PBA in/on bovine tissues and milk held under frozen storage conditions (∼−18 °C). Control samples of the bovine matrices (muscle, fat, liver, and milk) were subsampled by weighing into small glass vials or jars. The tissue subsamples (muscle, fat, and liver) were individually spiked with cypermethrin only or with the acid metabolites only at 0.5 ppm levels. The milk subsamples were spiked with the four test substances at 0.1 ppm levels. These spiking levels were set at 10 times the method LOQ, so that the residue levels could be determined in case degradation occurred. An equal number of tissue and milk control subsamples were stored without spiking to be used for background checks and method recovery determination. All of the subsamples were also stored frozen until analysis. Muscle and fat samples were analyzed at zero time and 3, 6, and 12 months after spiking; liver and milk samples were analyzed at zero time and 1 and 3 months after spiking.

Method of Calculation. The magnitude of cypermethrin and its acid metabolite residues in each sample was determined by an external standard calibration method (a single concentration) based on the average of all standards in an assay set. The standard solution was injected at the beginning of every set and subsequently after every two sample solutions. The amount of analyte was quantitated from the detector response transmitted to the data acquisition system. The response, as peak area, was calculated as nanograms (ng) of analyte on the basis of injection of standards.

Since all of the acid metabolite standards were derivatized as the pentafluorobenzyl ester as in the treated samples, no correction for the molecular weight was needed for calculations of metabolite residues in the treated samples. The calculations, therefore, were exactly the same for both cypermethrin parent and metabolites.

If all data points within a group to be averaged were ND, the average was ND. When a set of residue values to be averaged consisted of detectable and nondetectable residues, ND was assigned a value equal to half the method LOD for that analyte.

RESULTS AND DISCUSSION

Cypermethrin Residues in Bovines Treated with Eartag Only. Cypermethrin residues in milk samples from the first treated group, with eartag only, remained nondetectable (ND, <2 ppb) throughout the dosing period. Cypermethrin was detected only in only two cream samples (10 and 16 ppb) and one peritoneal fat sample (17 ppb). These results agree with other studies for cypermethrin residues in cream (residues ranged from 4 to 9.6 ppb in 18% of the samples, with the highest residue level found in the samples collected at test day 7). Likewise, nondetectable (<4 ppb) residues of cypermethrin were reported (Braun et al., 1985; Byford et al., 1986) in fatty tissues after 10 weeks of testing from bovines wearing impregnated eartags containing the same amount of cypermethrin. No metabolite residues were found in any of the bovine samples from this group.

Cypermethrin Residues in Milk. Cypermethrin residues in milk from other treated groups increased during the first few days of dosing. From test day 4 to 28, the residues reached mean plateau values of 13, 38, and 152 ppb for the 5 (1×), 15 (3×), and 50 (10×) ppm treated groups, respectively. These residue levels correlated well with the dose rate and also were comparable to other bovine feeding studies, which reported mean cypermethrin plateau values of 12 and 21 ppb (Croucher et al., 1985; Swaine and Sapiets, 1981a) for the 5 ppm dose group. On the basis of the above mean cypermethrin plateau values in the three dose groups, the amount of cypermethrin in the milk corresponded to ≈0.3% of the daily dose rate (the transfer factor = mean residue/dose rate). The residues in the milk did not show apparent accumulation and declined quickly during the recovery period without the test substance (∼25% remaining after 3 days and ∼15% after 6 days of recovery as shown in Table 1).

Metabolite Residues in Milk. Residues of either acid metabolite, *cis*/*trans*-DCVA or *m*-PBA, were nondetectable (ND, <2 ppb) in almost all of the milk samples analyzed (75 samples), with only trace amounts of *trans*-DCVA (3−4 ppb) found in four isolated milk samples from the highest dose group, but none was detectable after the recovery period.

Table 5. Metabolite Residues (Parts per Billion) in Bovine Cream and Tissue Samples

	bovine identification no.												
	T-I (eartag only)			T-II (1×)			T-III (3×)			T-IV (10×)			
matrix	1[a]	2	3	1[a]	2	3	1[a]	2	3	1[a]	2	3	4[b]
cis-DCVA													
cream	ND[c]	ND	ND	14	ND	17	34	31	22	86	79	103	106
kidney	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	18	22	ND
liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	11	11	ND
muscle (pectoral)	NA	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND
muscle (adductor)	NA	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND
fat (peritoneal)	ND	ND	ND	14	16	29	29	38	41	140	112	244	144
fat (subcutaneous)	ND	ND	ND	ND	ND	ND	34	25	23	84	86	114	52
trans-DCVA													
cream	ND	ND	ND	ND	ND	ND	20	16	14	57	53	66	69
kidney	ND	ND	ND	ND	17	26	ND	36	35	17	129	102	ND
liver	ND	ND	ND	ND	ND	ND	ND	11	ND	ND	24	25	ND
muscle (pectoral)	NA	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND
muscle (adductor)	NA	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND	11	ND
fat (peritoneal)	ND	ND	ND	12	11	26	24	36	34	107	109	214	101
fat (subcutaneous)	ND	ND	ND	ND	ND	ND	32	25	25	70	84	103	41
m-PBA													
cream	ND	ND	ND	ND	ND	ND	ND	ND	ND	14	ND	11	15
kidney	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	41	31	ND
liver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
muscle (pectoral)	NA	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND
muscle (adductor)	NA	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND	ND	ND
fat (peritoneal)	ND	ND	ND	ND	ND	ND	ND	ND	ND	17	26	58	19
fat (subcutaneous)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	16	13	ND

[a] The first bovine of each group was allowed a 3-day recovery period. [b] The last bovine in T-IV group was allowed a 6-day recovery period. [c] ND, nondetectable (<10 ppb for tissues and milk cream); NA, not applicable or not analyzed.

Table 6. Storage Stability Summary of Cypermethrin, *cis*/*trans*-DCVA, and *m*-PBA in/on Bovine Milk and Tissues at ~−18 °C

matrix/compd	nominal spike level, ppm	residues, ppm (% change from nominal) after storage for				
		0 time	~1 month	~3 months	~6 months	~12 months
muscle						
cypermethrin	0.5	0.52 (+4)	NA[a]	0.41 (−18)	0.42 (−16)	0.42 (−16)
cis-DCVA	0.5	0.53 (+6)	NA	0.55 (+10)	0.55 (+10)	0.56 (+12)
trans-DCVA	0.5	0.48 (−4)	NA	0.56 (+12)	0.53 (+6)	0.50 (0)
m-PBA	0.5	0.49 (−2)	NA	0.55(+10)	0.41 (−18)	0.38 (−24)
fat						
cypermethrin	0.5	0.48 (−4)	NA	0.54 (+8)	0.54 (+8)	0.51 (+2)
cis-DCVA	0.5	0.48 (−4)	NA	0.46 (−8)	0.55 (+10)	0.50 (0)
trans-DCVA	0.5	0.47 (−6)	NA	0.47 (−6)	0.54 (+8)	0.24 (−2)
m-PBA	0.5	0.48 (−4)	NA	0.48 (−4)	0.53 (+6)	0.49 (−2)
liver						
cypermethrin	0.5	0.57 (+14)	0.48 (−4)	0.51 (+2)	NA	NA
cis-DCVA	0.5	0.51 (+2)	0.52 (+4)	0.53 (+6)	NA	NA
trans-DCVA	0.5	0.50 (0)	0.52 (+4)	0.50 (0)	NA	NA
m-PBA	0.5	0.49 (−2)	0.52 (+4)	0.51 (+2)	NA	NA
milk						
cypermethrin	0.1	0.10 (0)	0.10 (0)	0.09 (−10)	NA	NA
cis-DCVA	0.1	0.11 (+10)	0.10 (0)	0.10 (0)	NA	NA
trans-DCVA	0.1	0.13 (+30)	0.10 (0)	0.10 (0)	NA	NA
m-PBA	0.1	0.13 (+30)	0.10 (0)	0.10 (0)	NA	NA

[a] NA, not analyzed.

Residues in Skim Milk and Cream. Skim milk from test day 7 showed only a trace of cypermethrin residue (2−8 ppb in 3× and 10× dose groups) and no detectable metabolite residue. The average cypermethrin residues in the cream were 10, 86, 259, and 763 ppb for the eartag and 1×, 3×, and 10× treated groups, respectively. The cream samples (average butter fat content 3.65%) contained ~93−95% of total cypermethrin residues from the whole milk for all dose groups. The transfer factor is 1.7% for cypermethrin from the daily dose rate to the cream based on the average residues. The magnitude of the parent and metabolite residues found in the cream was very similar to that in the subcutaneous fat.

Cypermethrin Residues in Tissues. Bovines from the 1× dose group had cypermethrin residues at or below the LOD (10 ppb) in all tissues except for fat samples, which contained 76 ppb in subcutaneous fat and 125 ppb in peritoneal fat. The transfer of test substance from oral dose to muscle was negligible, while the amounts of cypermethrin in the fat samples corresponded to ~1.5−2.5% of the daily dose rate.

For the 10× dose group, the average cypermethrin residue levels in tissues are as folows in decreasing order: peritoneal fat (1653 ppb), subcutaneous fat (865 ppb), pectoral muscle (151 ppb), kidney (47 ppb), adductor muscle (34 ppb), and liver (ND, <10 ppb). These cypermethrin residues followed a similar order but were ~5−10-fold lower than amounts previously reported (Swaine and Sapiets, 1981a,b). Within each group, the residue levels from the recovery animal were slightly lower than that from the animal sacrificed on day 29,

indicating that the cypermethrin residues were gradually excreted from the animal. However, the degradation rates of cypermethrin in fatty tissues (~80% remaining after 6 days, Table 2) were much slower than in the milk. Crawford et al. (1981a) reported that cypermethrin eliminated more slowly from fat than from other tissues.

Metabolite Residues in Tissues. Bovines from the 1× dose group had acid metabolite residues below the LOD (10 ppb) in all tissue samples except for kidney, which contained 22 ppb of *trans*-DCVA, and peritoneal fat, which had 23 ppb of *cis*-DCVA and 18 ppb of *trans*-DCVA.

For the 10× dose group, the order of average *cis*-DCVA residue in tissues was peritoneal fat (178 ppb) > subcutaneous fat (100 ppb) > kidney (20 ppb) > liver (11 ppb) > pectoral muscle = adductor muscle (ND, <10 ppb); the order of average *trans*-DCVA residue was peritoneal fat (162 ppb) > kidney (115 ppb) > subcutaneous fat (93 ppb) > liver (25 ppb) > pectoral muscle = adductor muscles (ND); and the order of average *m*-PBA residue was peritoneal fat (42 ppb) > kidney (36 ppb) > subcutaneous fat (14 ppb) > liver = muscles (ND, <10 ppb). Average metabolite residues can be found in Tables 3 and 4. These metabolite residues were much higher (3−10-fold) in fat and much lower (2−10-fold) in kidney, muscle, and liver in comparison with the previous study (Swaine and Sapiets, 1981b).

Degradation of individual cypermethrin isomers and metabolites has been studied extensively by other groups (Crawford et al., 1981b; Roberts and Standen, 1977, 1981). It has been observed that the order of degradation rates in various matrices was *trans*-cypermethrin > *cis*-cypermethrin and *m*-PBA > *trans*-DCVA > *cis*-DCVA. The residues of *cis*- and *trans*-DCVA found in cream and fat in this study correlated with these degradation studies except for kidney and liver, in which *trans*-DCVA residues were higher than *cis*-DCVA residues as shown in Table 3. Although kidney had much less cypermethrin than was found in the fat, it contained comparable residues of *trans*-DCVA and *m*-PBA to the fat samples. No detectable residue was found in any of the muscle samples except one (*trans*-DCVA, 11 ppb, 10× dose group). Metabolite residues in kidney, liver, and muscle in the recovery animal degraded rapidly, while the degradation rates of metabolites in peritoneal and subcutaneous fat were as slow as the rate of degradation of cypermethrin itself (see Table 5 for individual metabolite residues).

Storage Stability Data. In this bovine feeding study, analyses of muscle, fat, liver, and milk were completed within 4, 4, 3, and 1.5 months of cold storage, respectively. A storage stability study of cypermethrin and its acid metabolite residues in bovine milk and representative tissues (muscle, liver, and fat) was conducted previously in 1995 by Barrett and Pearsall. The results indicate that cypermethrin, *cis*-DCVA, *trans*-DCVA, and *m*-PBA are stable in bovine muscle and fat for at least 12 months and in liver and milk for at least 3 months when stored under frozen conditions (see Tables 6 and 7 for summaries of storage stability results and method recovery).

Chromatograms. Selected chromatograms of standard, control, and fortified control milk and fat samples have been reported elsewhere (Chen et al., 1996). Chromatograms of treated bovine milk and tissue samples in this study had similar matrix background to the respective control or fortified samples of the previous study.

Table 7. Method Recovery Summary of Cypermethrin, *cis/trans*-DCVA, and *m*-PBA from Fortified Bovine Milk and Tissues for Storage Stability Program

matrix/ compd	fortifn level, ppm	no. of analyses	recovery, % range	av	SD
muscle					
cypermethrin	0.25	3	89−111	99	
	0.5	5	89−113	99	±9
cis-DCVA	0.25	2	90−91	91	
	0.5	6	63−90	77	±9
trans-DCVA	0.25	2	83−96	90	
	0.5	6	80−117	94	±14
m-PBA	0.25	2	98−105	102	
	0.5	6	94−153	115	±25
fat					
cypermethrin	0.25	2	90−91	91	
	0.5	6	86−100	92	±6
cis-DCVA	0.25	2	88−93	91	
	0.5	6	70−82	75	±4
trans-DCVA	0.25	2	98−101	100	
	0.5	6	83−90	87	±3
m-PBA	0.25	2	100−118	109	
	0.5	6	86−121	110	±13
liver					
cypermethrin	0.25	2	90−108	99	
	0.5	4	80−107	97	±13
cis-DCVA	0.25	2	73−79	76	
	0.5	4	65−79	73	±6
trans-DCVA	0.25	2	82−83	83	
	0.5	4	70−90	80	±8
m-PBA	0.25	2	74−81	78	
	0.5	4	74−87	78	±6
milk					
cypermethrin	0.05	1	78		
	0.1	5	67−96	83	±13
cis-DCVA	0.05	1	82		
	0.1	5	65−89	77	±9
trans-DCVA	0.05	1	87		
	0.1	5	67−88	78	±8
m-PBA	0.05	1	80		
	0.1	5	73−90	80	±7

Conclusions. The results of this study, along with the acceptable and reproducible method recoveries, should reflect the true residues presented in the bovine milk, meat, and meat byproducts. The cypermethrin residues did not exceed 19 ppb in the milk samples from the 1× (5 ppm) dose group, the expected maximum intake level, and the acid metabolite residues were nondetectable. Furthermore, the residues of cypermethrin and its acid metabolites from the same dose group were at or below the LOD in all tissue samples except for the fat samples, which had up to 125 ppb of cypermethrin. Croucher et al. (1985) reported that most of the cypermethrin was eliminated in approximately equal proportions (~45% each) in the urine and feces of the bovines.

This study generated no apparent evidence that daily ingestion of cypermethrin as high as 50 ppm in diet for 28 days had any ill effect on the lactating dairy bovines. Body weight changes from initiation to termination in the treated groups were comparable to that of the control group. The lactation, feed consumption, and milk production in the treated groups were no different from those of the control bovines during the test and recovery periods. A liver abscess was noted in one of the treated bovines at necropsy. This finding appears to be incidental and not related to the test substance. All bovines appeared to be normal and active throughout the investigation. The gross necropsy performed on all study animals showed no abnormal or unexpected effects.

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