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Insecticide Residues in Cattle Treated with a Cypermethrin, Chlorpyrifos, Piperonyl Butoxide-Impregnated Ear Tag

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Insecticide-impregnated cattle ear tags have provided economical control of the horn fly, Haematobia irritans (L.), for cattle producers throughout the United States. Since the development of the first ear tag, stirofos, in the late 1970's, increased technology has led to the development of more effective and longer-lasting synthetic pyrethroid ear tags. The first pyrethroid ear tags were released into the marketplace in the early 1980's and contained either permethrin or fenvalerate. These pyrethroid ear tags have provided >95% control of the horn fly for 16 weeks or longer (Schmidt and Kunz 1980; Knapp and Herald 1981). This, along with the widespread acceptance of these tags has led to an influx of pyrethroid insecticide ear tags available to producers.

Despite the labor-saving and cost-efficient control provided by these ear tags, cattle producers are now facing horn fly resistance to the pyrethroid ear tags throughout the southeastern United States (Sheppard 1984; Schmidt et al. 1985; Byford et al. 1985). As a result, new and/or alternate insecticide ear tags are necessary for control of these pyrethroid resistant horn flies. Therefore, a residue study was conducted to determine if an experimental cypermethrin, chlorpyrifos, and piperonyl butoxide combination ear tag had any deposition and/or duration of insecticidal residues in bovine tissue.

MATERIALS AND METHODS

Four Angus cattle (3 to 6 years of age), averaging 365 kg, were used in the trial. Three animals were tagged with two MAX-CON® (Y-Tex Corporation) insecticide (7% cypermethrin, 5% chlorpyrifos, and 3.5% piperonyl butoxide) ear tags per animal and one animal served as an untreated control. Each ear tag weighed ca. 9.6 g; consequently, each treated animal was exposed to 1.34, 0.96, and 0.68 g of cypermethrin, chlorpyrifos, and piperonyl butoxide, respectively.

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Tissue biopsies were performed on all animals at 10 and 12 weeks post-treatment. Perianal fat samples were obtained by routine surgical procedures at these intervals. At 14 weeks post-treatment, all animals were slaughtered and samples of diaphragmatic fat, omental fat, renal fat, muscle, heart, liver, and kidney were collected, placed in polyethylene bags, frozen, and held for residue analysis.

Sub-samples (5 g fatty tissue and 20 g muscle and organ tissue) of each tissue were removed. Following maceration in Na_2SO_4, the samples were extracted in petroleum ether and decanted through Na_2SO_4. Fat and tissue samples were cleaned-up by using the AOAC official method for multiresidues in fatty foods (PAM Vol. I, 211.14) utilizing petroleum ether-acetonitrile partitioning. Florisil column chromatography (PAM Vol. I, 211.14d) provided additional cleanup and separated the compounds of interest into three fractions: eluant I (6%) chlorpyrifos, eluant II (20%) cypermethrin, and eluant III (50%) piperonyl butoxide. The 50% eluant containing piperonyl butoxide underwent a bromination procedure to facilitate detection by gas chromatography. Bromination of the piperonyl butoxide was performed according to Meinen and Carlson (1982) which employed a florisil cleanup to eliminate the presence of interfering substances.

Residues were determined using the following three gas chromatographs: Hewlett-Packard Model 5880A, Perkin Elmer Model 3920, and Tracor Model 540. All were equipped with Ni^{63} electron capture detectors.

Chlorpyrifos was quantified on a 180 X 0.4 cm glass column packed with: 3% OV101 on Gas Chrom Q 80/100 mesh, column oven at 190°C, and a 5% methane in argon carrier gas at 100 ml per min. Further confirmation of chlorpyrifos included the use of a nitrogen phosphorous detector under the following conditions: hydrogen at 5 ml per min, air at 50 ml per min, element 235, detector temperature 300°C, oven temperature 190°C, carrier gas of prepurified nitrogen at 80 ml per min, and a 180 x 0.2 glass column packed with 4% SE30 / 6% OV210 on Chromosorb WHP 100/120 mesh. Cypermethrin was quantitated on 15% QF1 / 10% DC200 on Gas Chrom Q, 80/100 mesh, in a 180 x 0.4 cm glass column at 235°C with prepurified nitrogen carrier flow at 100 ml per min. Brominated piperonyl butoxide was determined on 3% Carbowax 20M on Chromosory G, AW, DMCS, H.P., 80/100 mesh, in a 120 x 0.2 cm glass column at 190°C with prepurified nitrogen flow at 60 ml per min.

Confirmations were done on 380 x 0.2 cm glass column packed with 4% SE30 / 6% OV210 on Chromosorb WHP, 100/120 mesh with 5% methane in argon flow at 120 ml per min, and on a 120 x 0.2 cm glass column packed with 5% OV210, 100/120 mesh Chromosorb W (AW-DMCS) H.P. with prepurified nitrogen as the carrier gas at 80 ml per min. Other gas chromatographic conditions were as

follows: injection temperature 200°C and detector temperature 350°C.

RESULTS AND DISCUSSION

Recoveries from spiked tissue samples were excellent. Cypermethrin recovery averaged 137% in the fatty tissue and 101% in the muscle and organ tissue. Interferences from co-eluting fat in eluant II made quantification of cypermethrin recovery difficult which may account for these high values. Similarly, chlorpyrifos recovery was 102 and 76% for the fatty tissue and muscle and organ tissue, respectively. Recovery of brominated piperonyl butoxide was 99% for fat tissue and 126% for muscle and organ tissue.

Cypermethrin, chlorpyrifos, and piperonyl butoxide residue data are presented in Tables 1 and 2. In this trial, 20 fatty tissue samples and 16 muscle and organ tissue samples were collected from the four animals. Preslaughter (10 and 12 weeks post-treatment) biopsy results indicated that detectable chlorpyrifos residues in the perianal fat ranged from 0.010 to 0.128 ppm. Neither cypermethrin nor piperonyl butoxide residues were detected (<0.04 ppm and 0.05 ppm, respectively) in any of the perianal fat samples. In the untreated animal, the only detectable residues were chlorpyrifos at 0.028 and 0.011 ppm at 10 and 12 weeks post-treatment, respectively

At the time of slaughter (14 weeks post-treatment), chlorpyrifos was the only residue detected for each tissue sample. Analysis for the fatty tissues showed that detectable chlorpyrifos residues ranged from 0.004 to 0.021 ppm. Among the muscle and organ tissues, chlorpyrifos residues ranged from none detected (<0.001 ppm) to 0.011 ppm.

The amount of chloropyrifos in perianal fat of treated cattle increased from 0.010 ± 0.003 ppm ($\bar{x}$ ± SE) at 10 weeks post-treatment to 0.040 ± 0.024 ppm at 12 weeks post-treatment. However, at neither time were residue levels significantly greater than background levels found in control animals ($P>0.05$, t-test). The levels of chlorpyrifos residues in treated cattle were highest in omental fat (0.38 ± 0.022 ppm), followed by diaphragmatic fat (0.029 ± 0.008 ppm) and renal fat (0.024 ± 0.006 ppm). These residues did not differ significantly from one another or from control levels ($P>0.05$, t-test). The residues of chlorpyrifos in fatty tissue were greater than levels detected in muscle and organ tissues. Muscle had higher amounts of chlorpyrifos (0.005 ± 0.003 ppm) than organ tissues. The organ tissues all had virtually the same residue levels (0.001 ± 0.001 ppm). Chlorpyrifos levels in the muscle and organ tissues of treated cattle did not differ significantly from residues in control animals ($P>0.05$, t-test).

Table 1. Residues[a] of cypermethrin, chlorpyrifos, and piperonyl butoxide in fatty tissues of cattle tagged with MAX-CON® insecticide ear tags.

Cow[b] #	Tissue Sample	Cypermethrin[c]	Chlorpyrifos[c]	Piperonyl Butoxide[c]
		10 Weeks Post-treatment		
5286	Perianal	N.D. @ 0.04	0.028	N.D. @ 0.05
3203	Perianal	N.D. @ 0.04	0.018	N.D. @ 0.05
3210	Perianal	N.D. @ 0.04	0.005	N.D. @ 0.05
3215	Perianal	N.D. @ 0.04	0.007	N.D. @ 0.05
		12 Weeks Post-treatment		
5286	Perianal	N.D. @ 0.04	0.011	N.D. @ 0.05
3203	Perianal	N.D. @ 0.04	0.010	N.D. @ 0.05
3210	Perianal	N.D. @ 0.04	0.100	N.D. @ 0.05
3215	Perianal	N.D. @ 0.04	0.010	N.D. @ 0.05
		14 Weeks Post-treatment		
5286	Diagphragmatic	N.D. @ 0.04	0.023	N.D. @ 0.05
3203	Diagphragmatic	N.D. @ 0.04	0.045	N.D. @ 0.05
3210	Diagphragmatic	N.D. @ 0.04	0.032	N.D. @ 0.05
3215	Diagphragmatic	N.D. @ 0.04	0.010	N.D. @ 0.05
5286	Omental	N.D. @ 0.04	0.014	N.D. @ 0.05
3203	Omental	N.D. @ 0.04	0.091	N.D. @ 0.05
3210	Omental	N.D. @ 0.04	0.018	N.D. @ 0.05
3215	Omental	N.D. @ 0.04	0.004	N.D. @ 0.05
5286	Renal	N.D. @ 0.04	0.021	N.D. @ 0.05
3203	Renal	N.D. @ 0.04	0.038	N.D. @ 0.05
3210	Renal	N.D. @ 0.04	0.018	N.D. @ 0.05
3215	Renal	N.D. @ 0.04	0.017	N.D. @ 0.05

[a] N.D. = None Detected
[b] Untreated Control = Cow #5286; Treated Animals = Cow #3203, 3210, and 3215
[c] ppm

Table 2. Residues[a] of cypermethrin, chlorpyrifos, and piperonyl butoxide in muscle and organ tissues of cattle tagged with MAX-CON® insecticide ear tags at 14 weeks posttreatment.

Cow[b] #	Tissue Sample	Cypermethrin[c]	Chlorpyrifos[c]	Piperonyl Butoxide[c]
5286	Muscle	N.D. @ 0.04	0.005	N.D. @ 0.05
3203	Muscle	N.D. @ 0.04	0.011	N.D. @ 0.05
3210	Muscle	N.D. @ 0.04	0.006	N.D. @ 0.05
3215	Muscle	N.D. @ 0.04	N.D. @ 0.001	N.D. @ 0.05
5286	Heart	N.D. @ 0.04	0.006	N.D. @ 0.05
3203	Heart	N.D. @ 0.04	N.D. @ 0.001	N.D. @ 0.05
3210	Heart	N.D. @ 0.04	0.002	N.D. @ 0.05
3215	Heart	N.D. @ 0.04	N.D. @ 0.001	N.D. @ 0.05
5286	Liver	N.D. @ 0.04	0.007	N.D. @ 0.05
3203	Liver	N.D. @ 0.04	N.D. @ 0.001	N.D. @ 0.05
3210	Liver	N.D. @ 0.04	0.003	N.D. @ 0.05
3215	Liver	N.D. @ 0.04	N.D. @ 0.001	N.D. @ 0.05
5286	Kidney	N.D. @ 0.04	0.002	N.D. @ 0.05
3203	Kidney	N.D. @ 0.04	N.D. @ 0.001	N.D. @ 0.05
3210	Kidney	N.D. @ 0.04	0.002	N.D. @ 0.05
3215	Kidney	N.D. @ 0.04	0.002	N.D. @ 0.05

[a] N.D. = None Detected

[b] Untreated Control = Cow #5286; Treated Animals = Cow #3203, 3210, and 3215

[c] ppm

The Environmental Protection Agency has established tolerances for cypermethrin, chlorpyrifos, and piperonyl butoxide in cattle fat, meat, and meat by-products at 0.05, 2.0 and 0.1 ppm, respectively. The levels of these compounds in bovine tissue in this trial were significantly less than the tolerances established by the EPA.

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