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## PERSISTENCE OF TARTRAZINE IN MARKING SHEEP WOOL

RICHARD J. BURNS<sup>1/</sup> AND PETER J. SAVARIE

### ABSTRACT

Tartrazine was examined as a possible replacement marker for rhodamine B in the Livestock Protection Collar. Test solutions were formulated in six combinations; tartrazine at 0.5% and 1.0% concentrations, with and without compound 1080, and with and without nigrosin black. Each solution was examined for persistence of color in the laboratory and when applied to the wool of dried sheep hide. Hide pieces were allowed to weather naturally or were sprinkled to simulate rain. Tartrazine alone was also tested at concentrations of 0.5% and 1.0% on the necks of sheep. Over a 3-month period, no fading was noted in the laboratory for any test solution. When applied to wool, no loss of color was attributed to 1080 or nigrosin black and no long-term advantage was detected for a 1.0% over a 0.5% tartrazine solution, except on the sprinkled pieces. Tartrazine usually provided an identifiable mark on the wool of dried hides and on live sheep for 1 to 2+ months, depending on test conditions. Tartrazine is an acceptable replacement for rhodamine B in the Livestock Protection Collar.

### INTRODUCTION

Compound 1080 (sodium monofluoroacetate) was registered by the U. S. Environmental Protection Agency (EPA) in July, 1985 (Registration #56228-22) for use in livestock protection collars (LPCs) to control coyote predation on sheep or goats (Moore 1985). The LPC consists of a 2-

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compartment rubber bladder containing a solution of compound 1080 and rhodamine B. The collar is fitted around the necks of sheep or goats exposed to coyote predation. The characteristic throat attack by coyotes usually results in punctures of the LPC and delivery of a lethal dose of solution to the attacking coyote (Connolly and Burns Accepted). Rhodamine B was included in the solution as a dye to help identify punctured or leaking collars.

Rhodamine B has been identified by EPA as requiring further study before being accepted as an inert ingredient in pesticide formulations (Moore 1987), and identification of a suitable alternative became desirable.

Tartrazine (FD & C yellow #5), recognized as a safe, nontoxic dye frequently used in coloring foods (Gardner 1979), could replace rhodamine B in LPCs if it produced a suitable stain on wool. This study assessed the potential of tartrazine for marking sheep wool, and determined if the color was influenced by the compound 1080 used in LPCs. To be satisfactory, the dye should be identifiable on wool for a minimum of 1 week under the environmental conditions prevailing in the area of intended use.

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### METHODS

Tartrazine was dissolved in distilled water at two concentrations, 0.5% and 1.0%. Portions of each solution were then formulated into two more test solutions by adding Compound 1080 (10 mg/ml) and compound 1080 (10 mg/ml) with 0.5% nigrosin black (a dye added to Compound 1080 by the supplier to identify their technical product). About 10 ml of each of the 6 test solutions were placed in individual 15

ml clear glass tubes. The tubes were kept in the laboratory at room temperature and away from direct sunlight to determine color stability.

To test color persistence on wool, 9 pieces (1 square foot each) of dried sheep hide were numbered and divided in half with a strip of 3/4 inch masking tape. The halves were labeled A (left) or B (right). Each half of each piece was treated by spraying 10 ml of one of the 6 solutions from a hypodermic syringe onto the wool and then smearing the application with a spatula. The pieces were attached to sheets of plywood with the treated (wool) side facing out, and placed outdoors in a chain-link fenced enclosure. The wool pieces were oriented toward the south to receive maximum exposure to sunlight and precipitation. Three replications of each treatment were tested.

In a similar test 9 other pieces of sheep hide were treated as above but subjected to weekly treatments of 1/4 inch of simulated rain from a lawn sprinkler. These samples were sheltered from sunlight.

Finally, each of 2 live sheep had 10 ml of a 0.5% tartrazine solution applied to the left side of its neck and 10 ml of the 1.0% tartrazine solution applied to the right side. Treatment was effected the same as on the wool of the dried hide. The sheep were kept in an outdoor pasture where they had continuous access to water and supplemental feed. They were checked daily for general welfare.

Chemicals used in the tests came from two sources. The tartrazine, nominally 90% pure, lot #875090 (dated 6-21-88), was from Ingredient Technology Corporation<sup>2/</sup>, Des Plaines, IL. The Compound 1080 was from lot #85949 (without nigrosin black) and #8027 (with nigrosin black) from Tull Chemical Company, Oxford, AL. The first lot was nominally 90% pure as

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<sup>2/</sup>Reference to trade names and manufacturers does not imply endorsement by the U. S. Government.

stated on the label. The second lot was determined 94% pure on 8-20-87 by colleagues at the Denver Wildlife Research Center, Denver, CO. All chemicals were weighed on an electronic analytical balance to the nearest thousandth of a gram, and the distilled water was measured in a graduated cylinder to the nearest milliliter.

Observations to detect color changes in the treatments were made weekly for the 6 solutions kept in the laboratory, the wool exposed to simulated rain, and the live sheep. The naturally weathered wool pieces were checked twice weekly. Observations continued for 2 months and were recorded on standard forms. Notes on weather conditions were recorded daily throughout the test periods, except for the laboratory and sprinkler tests. During observations, treated samples or sheep were compared to a treated non-weathered control and to a non-treated control. The tests were conducted at the Denver Wildlife Research Center's predator research site near Millville, Utah, between 9 December 1988 and 6 July 1989.

## RESULTS AND DISCUSSION

Initially, the yellow color of all 1.0% tartrazine solutions were slightly darker than the 0.5% solutions. None of the solutions kept in the laboratory showed any obvious signs of fading during the 2 months of observations. Three months after formulation, there was still no obvious fading in any of the test tartrazine solutions and the 1.0% formulations were still slightly darker than the 0.5% formulations. Both concentrations of tartrazine, and tartrazine with compound 1080, with and without nigrosin black, appeared to be color stable in the laboratory.

Weather conditions during the exposure tests with dried wool and live sheep showed 22 sunny, 15 cloudy, 19 partly cloudy, 8 snowy, and 2 rainy days. Observations of the exposed wool pieces suggested no difference in the rate of degradation of the marks among any of the 6 formulations (Table 1).

Differences in color intensity of marks produced by the 2 concentrations of tartrazine disappeared 4 to 5 weeks after treatment. All pieces treated with test formulations had readily recognizable marks 2 months after treatment, but the marks appeared faded when compared to non-exposed controls. Three months after treatment, the color produced by all 6 formulations had nearly disappeared and were difficult to detect.

The wool pieces treated by simulated rain received an average 1/4 inch in 5 min 52 sec (n = 9, range = 4 min 5 sec to 7 min 10 sec) or about 2.5 inches per hour. There was no clear difference in fading among formulations containing 1080, 1080 with nigrosin black, and tartrazine alone. The compound 1080 apparently had little influence on the persistence of tartrazine (Table 2). After 1 month, 7 of 9 (78%) samples at the 0.5% concentration showed evident marks, but 2 had faded substantially. At the same time, all marks from the 1.0% concentration were readily visible; only 2 of 9 (22%) had faded substantially. After 2 months, only 2 of 9 samples at the 0.5% concentration showed readily visible marks, compared with 7 of 9 treated at 1.0% tartrazine. These results indicated wool treated with 1.0% tartrazine remained darker and persisted longer than the 0.5% tartrazine under simulated rain.

The color intensity of marks produced on the neck wool of live sheep by the 1.0% and 0.5% tartrazine solutions appeared similar 3 to 4 weeks after treatment. The marks produced by both concentrations were readily visible 2 months after treatment (Table 3). At that time marks appeared faded, but were easily distinguished from unmarked sheep. Three months posttreatment, marks produced by both concentrations were barely discernible.

## CONCLUSIONS

We concluded that Compound 1080, with or without nigrosin black, did not alter the color persistence of tartrazine in any test. Initially the formulations with 1.0% tartrazine produced darker yellow marks than did 0.5% on the exposed wool pieces and on sheep necks. The differences were no longer evident within 3 to 5 weeks, and no long-term advantage for the higher concentration was indicated. On exposed wool and live sheep, tartrazine marks from both concentrations were readily discernible after 2 months, but by 3 months the marks had become unreliable.

Simulated rainfall on the tartrazine-stained wool caused the color to fade faster than other test conditions, but after one month nearly 80% of the 0.5%, and all of the 1.0% tartrazine treated samples still showed readily visible yellow color.

The tests encompassed a range of sunlight, simulated heavy rainfall, and natural weather conditions over periods of one or more months. We are confident that a 0.5% or a 1.0% solution of tartrazine from punctured or leaking collars could be readily detected on sheep or goats for about 4 to 8 times the one-week minimum under most weather conditions. Either concentration of tartrazine would be an acceptable substitute for rhodamine B in the LPC.

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Table 1. Subjective color ratings of naturally weathered tartrazine-marked wool pieces treated on 7 and 8 December 1988.

Dates	Color ratings of six tartrazine solutions					
	0.5% tartrazine			1.0% tartrazine		
	Alone	With 1080	With 1080 +nb <sup>b</sup>	Alone	With 1080	With 1080 +nb
12-09-88	111 <sup>c</sup>	111	111	111	111	111
12-13-88	111	111	222	111	111	222
12-16-88	111	111	222	111	111	222
12-20-88	111	111	222	111	111	222
12-23-88	111	111	222	111	111	222
12-27-88	---	---	---	---	---	---
12-30-88	111	111	222	111	111	222
01-03-89	313	123	313	113	113	223
01-06-89	333	233	333	223	233	223
01-10-89	333	233	333	223	233	223
01-13-89	333	333	333	333	333	333
01-17-89	333	333	333	333	333	333
01-24-89	---	---	---	---	---	---
01-27-89	333	333	333	333	333	333
01-31-89	333	333	333	333	333	333
02-03-89	444	444	444	444	444	444
02-07-89	444	444	444	444	444	444
02-10-89	444	444	444	444	444	444
03-10-89	555	555	555	555	555	555

<sup>a</sup>Color ratings: 1 = bright yellow; 2 = yellow; 3 = faded yellow; 4 = very faded yellow; and 5 = not readily visible.

<sup>b</sup>nb = nigrosin black.

<sup>c</sup>3 replications per treatment.

<sup>d</sup>No data collected.

Table 2. Subjective color ratings of tartrazine-marked wool pieces, subjected weekly to 1/4 inch of simulated rainfall, treated on 9 May 1989.

Color ratings of six tartrazine solutions						
Dates	0.5% tartrazine			1.0% tartrazine		
	Alone	With 1080	With 1080 +nb <sup>b</sup>	Alone	With 1080	With 1080 +nb
05-11-89	111 <sup>c</sup>	111	111	111	111	111
05-19-89	222	221	222	211	111	211
05-25-89	333	332	333	322	322	322
06-01-89	444	443	444	433	332	433
06-08-89	454	443	454	433	332	433
06-15-89	454	543	454	544	443	544
06-22-89	454	554	454	544	443	544
06-29-89	554	554	454	544	444	544
07-06-89	555	554	554	544	444	544

<sup>a</sup>Color ratings: 1 = bright yellow; 2 = yellow; 3 = faded yellow; 4 = very faded yellow; and 5 = not readily visible.

<sup>b</sup>nb = nigrosin black.

<sup>c</sup>3 replications per treatment.

Table 3. Subjective color ratings of tartrazine-marked neck wool on sheep treated with 0.5% and 1.0% concentrations of tartrazine on 7 December 1988.

Dates	Sheep	Color ratings									
		Left side (0.5%)					Right side (1.0%)				
		1	2	3	4	5	1	2	3	4	5
12-14-88	A	x					x				
	B	x					x				
12-21-88	A	x					x				
	B	x					x				
12-28-88	A	x					x				
	B	x					x				
01-04-89	A	x					x				
	B	x					x				
01-11-89	A		x					x			
	B		x					x			
01-18-89	A			x						x	
	B			x						x	
01-27-89	A			x						x	
	B			x						x	
02-01-89	A			x						x	
	B			x						x	
02-08-89	A			x						x	
	B			x						x	
03-10-89	A					x					x
	B					x					x

<sup>a</sup>Color ratings: 1 = bright yellow; 2 = yellow; 3 = faded yellow; 4 = very faded yellow; and 5 = not readily visible.