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## PEN TESTS OF METHYL ANTHRANILATE AS A BIRD REPELLENT IN WATER

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**Abstract:** Two commercial (ReJeX-iTR brand) formulations of methyl anthranilate (MA), at concentrations of 0.10 - 0.5096 (0 -0.32% active ingredient [a.i.]), were highly effective in repelling mallards (*Anas platyrhynchos*), and ring-billed gulls (*delawarensis*) from pools of water in pen tests. For mallards, pool entries and bill contacts with water in MA-treated pools 1.4 and 4.0% of the levels in untreated pools during a 2-choice test, and 4.2 and 8.8% of the levels in untreated pools during 1-choice test. For gulls, the repellency levels were even higher, with activity levels in treated pools being < 1% of levels untreated pools during 1- and 2-choice tests. We recommend further pen tests to determine minimum effective concentrate levels and a field test to determine responses of free-ranging birds.

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In a variety of situations it is desirable to discourage birds from entering bodies of water. For example, gulls, waterfowl, and other bird species often flock to temporary pools of fresh water at airports after heavy rains, creating a safety hazard for aircraft (Blokpoel 1976, Buckley and Gurien 1986). Also, federally-protected waterbirds are sometimes attracted to settling and tailing ponds containing oil or toxic chemicals (Sturgess et al. 1989, Hallock 1990). The development of an environmentally safe compound that could be added to water to repel birds should have wide utility.

Methyl anthranilate (MA), a chemical with demonstrated bird repellent properties (Mason et al. 1989), is a likely candidate for such use. MA, which has a grape-like odor, occurs in numerous plant species, is used in the perfume and food industries, and is GRAS [listed] (generally recognized as safe) by the United States Food and Drug Administration (Jenner et al. 1964, Code of Federal Regulations 1988). Our objective was to evaluate 2 commercial formulations of MA (ReJeX-iTR brands) as bird repellents when added to pools of water, using captive, wild birds (mallards and ring-billed gulls).

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### **METHODS**

The experiments were conducted during September and October 1990 at Plum Brook Station, a 2,200-ha fenced facility operated by the National Aeronautics and Space Administra

tion in Erie County, Ohio. Birds used in the tests were cap in funnel traps or by rocket net in northern Ohio during J through September 1990.

### **Mallard Experiment**

Eight 8-m x 4-m corrals, each with an attached 2.5-m x 2.5-m x 2.0-m shaded holding pen, were set up on mowed grass in an area isolated from human disturbance. Each corral had 20.8m-diameter or 21.0-m-diameter plastic pools filled with 40 L or 90 L, respectively, of water (10-12 cm deep). Two pinioned mallards were placed in each holding pen and released daily for 9 hours into the corral to acclimate to the test condition for 2 days. Each corral contained a pan of cracked corn, millet, and commercial duck food.

On test day 1, 1 of 2 formulations of MA encapsulated into a food-grade starch or polymer matrix (ReJeX-iTR CN121 or ReJeX-iTR CN123, Table 1) was applied to fresh tap water (0.5% w/w) in a randomly selected pool in each corral at 0800. The water depth was measured to nearest ml and the 2 mallards were released in the corral. One of 4 observers (2 corrals per observer) watched each corral for 120 20-second intervals (40 min total) during the next 2 hours. The observer recorded the number of mallards in each pool (pool use) during each 20-second interval and the total number of times a bill touched the water (i.e., drinking or bathing activity) in each pool. At 1600 hours, the water depth was remeasured and the mallards were returned to their holding pen where they were provided food, but no water. This routine was maintained on days 2, 3, and 4. The mallards were kept in their holding pens on day 5 (with drinking water and food). On day 6, they were released into the corrals with only the MA-treated pool available. The birds were observed as before and the experiment was then terminated.

Table 1. Mean number of mallards and mean number of bill contacts with water in each of 2 swimming pools, 1 with methyl anthranilate (MA)-treated water and 1 with untreated water, during 120 20-second observation periods on each of 4 consecutive days, 19-22 September 1990. Each pen held 2 mallards.

MA formulation	pens	Mean no. of mallards in pools	Day	Mean no. of bill contacts with water	MA Control	control pool
ReJeX-iTa	4	1		2.8	17.0	23.3
76.5						
CN121'		2		0.5	38.8	3.0
158.8						
	3			0.0	29.3	4.0
137.5						
	4			0.8	31.3	0.8
103.3						
	x			1.0"	29.1 <sup>b</sup>	7.9°
119.0°						
ReJeX-iTR	4	1		0.0	15.8	3.3
35.5						
CN123'		2		0.0	50.3	1.5
130.3						

' Approximately 16% MA, applied to pool at concentration of 1 part formulation (by weight) to 200 parts water (0.50% concentration of formulation; 0.08% concentration of MA).

<sup>b</sup> Treatment means are significantly ( $P < 0.01$ ) different,  $F = 47.3$ , 1 and 3 df; day effect and day x treatment interaction are not significant ( $P > 0.10$ ),  $F = 0.3$  and 1.3, 3 and 18 df.

<sup>°</sup> Treatment means are significantly ( $P < 0.01$ ) different,  $F = 47.6$ , 1 and 3 df; day effect is not significant ( $P > 0.10$ ,  $F = 0.8$ , 3 and 18 df); day x treatment interaction is significant ( $P < 0.01$ ,  $F = 6.2$ , 3 and 18 df).

<sup>d</sup> Approximately 64% MA, applied to pool at concentration of 1 part formulation (by weight) to 200 parts water (0.50% concentration of formulation; 0.32% concentration of MA).

Treatment means are significantly ( $P = 0.04$ ) different,  $F = 12.5$ , 1 and 3 df; day effect and day x treatment interaction are not significant ( $P > 0.05$ ),  $F = 3.1$  and 2.7, 3 and 18 df.

<sup>f</sup> Treatment means are significantly ( $P < 0.01$ ) different,  $F = 42.9$ , 1 and 3 df; day effect and day x treatment interaction are not significant ( $P > 0.10$ ),  $F = 0.9$  and 2.0, 3 and 18 df.

### Gull Experiment

Methods were the same as in the mallard experiment except that: (1) the tests took place in the holding pens (and not the corrals) because the gulls could fly; (2) only 1 MA formulation (ReJeX-iTR CN123) was tested; and (3) the 1-choice test with only MA-treated pools available lasted 4 days instead of 1 day. Following these tests, a 2-choice test with the ReJeX-iTR CN123 formulation at a concentration of 0.1% (20% of the level used in all previous tests) was run on 7 days over a 9-day period, using a new group of gulls. Four pens (replications) were used. The gulls were fed fresh fish daily.

Randomized block analyses of variance, with repeated measures (days), were used to compare pool use and bill contacts with water between MA-treated and untreated pools in the 2-choice tests. Efficacy of the 2 formulations was compared in the 2-choice test with mallards by a 2-way, repeated measures analysis of variance in which the response variable was the difference in pool use or bill dips between the treated and untreated pools in each pen on each day. Paired t-tests were used to compare the net change in water level between MA-treated and untreated pools, and to compare mean pool use during the 4-day period when treated pools were available and during the 1-day (mallards) or 4-day (gulls) period when only MA-treated pools were available. A square-root transformation was performed on the response variables to normalize the distribution of data.

## RESULTS

### Mallard Experiment

Both formulations of MA were highly effective ( $P < 0.04$ ) in keeping the birds from swimming, drinking, or bathing in the MA-treated pools during the 4-day, 2-choice test (Table 1), and in the subsequent 1-day, 1-choice test (Table 2). There was no difference ( $P > 0.50$ ,  $F = 0.3$ ; 1 and 6 df) in effectiveness between the 2 formulations. During the 4-day, 2-choice test, 98.5% of the pool entries and 96.1% of the bill contacts with water (drinking or bathing activities) were in the untreated pools. There were no significant ( $P > 0.05$ ) day effects; the treatment x day interaction was significant ( $P < 0.01$ ) for bill contacts with water with ReJeX-iTR CN121 (Table 1). During the 1-day, 1-choice test, when only MA-treated water was available, pool use and bill contacts were only 3.8% and 8.8%, respectively, of the levels during the previous 4 days when untreated water was available.

Untreated pools averaged a 7- to 9-mm decline in water depth over the 4-day test period compared with an increase (due to rain) of 2 mm for the pools treated with either MA formulation. These significant ( $P < 0.01$ ) differences also indicated greater bird use of untreated pools compared with MA-treated pools (Table 3).

### Gull Experiment

The repellency of the MA formulation was even more pronounced than in the mallard experiment. During the 4-day 2-choice test with 0.5% ReJeX-iTR CN123, more than 99% of the pool entries and bill contacts were in untreated pools (Table 4). Water depth also declined more ( $P = 0.03$ ) in untreated pools ( $x = -16$  mm) than in MA-treated pools, ( $x = -3$  mm) during the 4-day period. During the subsequent 4-day, 1-choice test, only a single incidence of pool use and 83 bill contacts with water were recorded compared with 620 pool uses and 8,846 bill contacts with water (virtually all in the untreated pools) during the 4-day, 2-choice test (Table 5). In the following 7-day, 2-choice test with the reduced (0.1 % w/w) concentration of ReJeX-iTR CN123 formulation, no pool entries and only 21 bill contacts with water were recorded in MA-treated pools

(Table 4). During both 2-choice tests, there were significant ( $P < 0.02$ ) day effects, and treatment x day interactions for pool entries and bill contacts (Table 4).

Table 2. Mean number of mallards in swimming pools and bill contacts with water during 120 20-second observation periods on days 1-4 in which the birds had a choice between a control and a methyl anthranilate (MA)-treated pool, and on day 6 when the birds had only the MA-treated pool available, 19-24 September 1990. Each pen held 2 mallards.

MA formulation (days 1-4) <sup>b</sup>	No. of available pens (day 6) <sup>b</sup>	Mean no. of responses/day	
		MA control pools	MA pool only
ReJeX-iTR	4	30.1 <sup>°</sup>	3.0 <sup>°</sup>
CN121			
No. of bill contacts with water			
ReJeX-iTR	4	39.9 <sup>°</sup>	0.0 <sup>°</sup>
CN123			
No. of bill contacts			
		127.1 <sup>°</sup>	122.1 <sup>°</sup>

See Table 1 for concentrations. <sup>b</sup> Rainfall of 8 mm on days 1-4, 0 mm on day 6. <sup>°</sup> Means are significantly ( $P = 0.02$ ) different,  $t = 4.64$ , 3 df. <sup>°</sup> Means are significantly ( $P = 0.04$ ) different,  $t = 3.62$ , 3 df. <sup>°</sup> Means are significantly ( $P = 0.04$ ) different,  $t = 3.60$ , 3 df. <sup>f</sup> Means are significantly ( $P < 0.01$ ) different,  $t = 11.90$ , 3 df.

Both formulations were highly aversive to entries were recorded in ReJeX-iTR CN121 during 5 days of testing with mallards compared in untreated pools during 4 days of testing. CN123, only 6 entries were recorded in treated 120 days of testing with mallards and gulls compared; entries in untreated pools during 15 days of testing CN123 contained 4 times the methyl anthran weight) than did ReJeX-iTR CN121 (16% by wt

The significant day effects and day x treatment measured during the tests with gulls were probably reduced gull activity in control pools during days (Table 4). There was no trend of increased gut treated pools over time.

Both formulations partially settled on the bottom. The water appeared only slightly cloudy on days 1 turned orange by day 4, making the bottoms of pools obscured. This color change may have enhanced the smell of the treatments, acting as an aversive agent (Lipcius et al. 1980). However, bird response to the MA pools was also highly negative on days 1 and 2 when there was no color change. Furthermore, although color can influence bird use of water, the strong levels of repellency demonstrated in this study have not been induced by color alone (Li et al. 1980).

In conclusion, ReJeX-iTR formulations containing LMA added to water at concentrations of 0.1-0.59%, are highly repellent to mallards and ring-billed gulls in preliminary tests. Additional tests should be run with MA at lower concentrations to determine the minimum effective level for repellency. In addition, a field trial with free-ranging birds should be conducted, perhaps at an airport with an established problem of birds flocking to temporary pools of water.

Table 3. Mean water depth (mm) in pools either treated with methyl anthranilate (MA) formulation or left untreated in 1990, each with 2 mallards, over a 4-day period, 19-22 September 1990.

MA formulation (days 1-4) <sup>b</sup>	No. of pens	Treatment pools		Control pools	
		Mean depth at start of day 11 <sup>c</sup>	Mean depth change after day 3	Mean depth at start of day 1	Mean depth change after day 4
ReJeX-iTR	4	112	118	114	113
CN121	4	110	115	113	112
110106	106 <sup>b</sup>	-7			110
ReJeX-iTR	4	110	115	113	112
CN123	4	110	115	113	112
107103	101 <sup>°</sup>	-9			110

<sup>b</sup> Rainfall of 5 mm during day 1 and 3 mm during days 3 and 4. <sup>c</sup> Means are significantly ( $P < 0.01$ ) different,  $t = 7.36$ , 3 df. <sup>°</sup> Means are significantly ( $P < 0.01$ ) different,  $t = 19.54$ , 3 df.

Table 4. Mean number of ring-billed gulls and mean number of bill contacts with water in each of 2 swimming pools, 1 with methyl anthranilate (MA)-treated water and 1 with untreated water, during 120 20-second observation periods on each of 4 consecutive days, 29 September-2 October 1990, and on each of 7 days over a 9-day period, 10-18 October 1990. Each pen held 2 gulls.

Percent ReJeX-iTR CN123 (MA) concentration 0.50 (0.32)	Day	Rainfall (mm)	Mean no. of gulls in pools (n = 4)		Control Pool	Mean no. of bill contacts with water MA Control pool pool
			MA pool	(n = 41)		
	1	0	1.0		57.8	0.8 794.0
	2	5	0.0		17.3	0.0 285.0
	3	0	0.0		54.5	0.0 827.0
	4	0				
	X				<b>304.8</b>	
			0.3		38.5'	0.22 552y.7
0.10 (0.06)	1	16	0.0		0.0	0.0 0.0
	2	8	0.0		0.0	0.0 0.0
	3	0	0.0		4.0	1.3 84.5
	6	0	0.0		15.0	0.8 201.3
	7	0	0.0		17.8	2.5 193.8
	8	0	0.0		34.5	0.8 381.8
	9	12				-(~
	x		0(~		105	0.8a1~d

' Treatment means are significantly ( $P < 0.01$ ) different,  $F = 61.2$ , 1 and 3 df; day effect and day x treatment interaction are significant ( $P < 0.02$ ),  $F = 6.1$  and 4.6, 3 and 18 df. <sup>b</sup> Treatment means are significantly ( $P < 0.01$ ) different,  $F = 55.7$ , 1 and 3 df; day effect and day x treatment interaction are significant ( $P < 0.02$ ),  $F = 5.3$  and 5.0, 3 and 18 df.

Treatment means are significantly ( $P = 0.02$ ) different,  $F = 20.2$ , 1 and 3 df; day effect and day x treatment interaction are significant ( $P < 0.01$ ),  $F = 11.0$  and 9.6, 6 and 36 (if. <sup>d</sup> Treatment means are significantly ( $P = 0.02$ ) different,  $F = 18.7$ , 1 and 3 df; day effect and day x treatment interaction are significant ( $P < 0.01$ ),  $F = 9.5$  and 9.4, 6 and 36 df.

Table 5. Mean number of ring-billed gulls in pools and bill contacts with water during 120 20-second observation periods on days 1-4, when the gulls had a choice between a control and a methyl anthranilate (MA)-treated pool, and on days 6-9 when the birds had only the MA-treated pools available, 29 September October 1990. Each pen held 2 gulls.

NO Response of variable pens		Mean no. of responses/day	
		MA' & control pools available (days 1-4) <sup>b</sup>	MA' pool only available (days 6-9) <sup>b</sup>
No. of gulls in pools	4	38.8 <sup>c</sup>	0.1 <sup>c</sup>
No. of bill contacts with water	4	552.9 <sup>d</sup>	5.2 <sup>d</sup>

' Approximately 64% MA, applied to pool at concentration of 1 part formulation (ReJeX-iTR CN123) by weight, to 200 parts water (0.50% concentration of formulation; 0.329% concentration of MA).

<sup>b</sup> Rainfall of 5 mm on days 1-4, 18 mm on days 6 - 9.

Means are significantly ( $P < 0.01$ ) different,  $t = 10.0$ , 3 df. <sup>d</sup> Means are significantly ( $P < 0.01$ ) different,  $t = 6.2$ , 3 df.

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