Investigations of Commensal Rodenticide Baits against Wild Norway Rats plus Additional Toxicology Data of Warfarin on Laboratory Norway Rats and House Mice

Jeff J. Mach
Genesis Laboratories, Inc., Wellington, Colorado

ABSTRACT: The use of warfarin in commensal rodenticides has been avoided by the pest control industry for many years because of concerns with low palatability, resistance, and its chronic toxicity requirements. It has been all but forgotten by the industry. Genesis Laboratories, Inc. evaluated warfarin in a currently-marketed commensal rodenticide, Kaput Rat and Mouse Bait (0.025% warfarin), in comparison to Maki pellets (0.005% bromadiolone) and Talon G pellets (0.005% brodifacoum) in a simulated field study design using warfarin-resistant wild Norway rats. Approximately 20 wild rats of varying ages and weights were randomly added to each of 3 study rooms, one for each product. After a 7-day acclimation period, the respective rodenticide baits were presented to the rats along with the EPA field rodent challenge diet or another alternate diet. The first death in each study room was recorded on Day 3 for Kaput, Day 13 for Maki, and Day 5 for Talon. Kaput acceptance was high, causing 9 deaths in the first 14 days. Again, because of poor acceptance of the Maki and Talon baits, the alternate diet was changed to milo on Day 22 and again to a no-choice regime on Day 28. At this point (Day 28), efficacy of the Kaput, Maki, and Talon rooms were at 85%, 20%, and 10%, respectively. 100% efficacy was achieved on Day 36 by Kaput, Day 38 by Maki, and Day 42 by Talon during the no-choice phase of the study. To investigate the warfarin toxicology profile further, we initiated another study with laboratory Norway rats and house mice in a no-choice regime with varying exposure periods (1, 2, 3, 4, and 5 days). At least two days of exposure was required to produce 100% mortality with the rats and 5 days of exposure was necessary for 100% mortality of house mice.

KEY WORDS: brodifacoum, bromadiolone, house mouse, Norway rat, *Mus musculus*, *Rattus norvegicus*, warfarin

Proc. 21st Vertebr. Pest Conf. (R. M. Timm and W. P. Gorenzel, Eds.)
Published at Univ. of Calif., Davis. 2004. Pp. 140-144.

INTRODUCTION

Scimetrics, Ltd. has introduced a new warfarin product and desired to compare its efficacy and palatability against some of the most common, comparable commensal rodenticides on the market used for Norway rat (*Rattus norvegicus*) and house mouse (*Mus musculus*) control. We were unable to find cereal bait formulations of other popular active ingredients so we were forced to use pelleted formulations. Typically, warfarin was avoided by the pest control industry because of concerns with low palatability, warfarin resistance, and its chronic toxicity (R. E. Marsh, pers. commun.). However, with the following studies, we have identified that it can still be used with effective results, even better than some of the market leaders.

The objective of this comparative study was to determine palatability and efficacy of Kaput Rat & Mouse bait (0.025% warfarin), Talon pelleted bait (0.005% brodifacoum), and Maki pellets (0.005% bromadiolone) in a simulated field environment. The objective of the second phase of the study was to provide additional data on the toxicology profile of warfarin on laboratory Norway rats and house mice. This data was desired to provide the sponsor with a thorough knowledge of how their warfarin product performs.

MATERIALS AND METHODS - SIMULATED FIELD STUDY

Test Substance

Each of the test substances, Talon pellet bait (lot# E29002), Kaput Rat and Mouse grain bait (lot# 2061103A), and Maki pellet bait (lot# 02203), were purchased from a distributor or the manufacturing company. They were logged into an ambient temperature storage locker for the duration of the study.

Test System

Sixty-three wild Norway rats were live captured from the Genesis Laboratories, Inc. breeding colony from July 8, 2003 to July 10, 2003. The date of birth or age of the rats is unknown, as they breed freely in the colony confines; however the rats were at least sub-adults. The founder population of the Genesis colony was acquired in July 2000 and had a population of approximately 100 animals of all ages, established from rats originally live-trapped in Chicago, Illinois, that had been documented as being "warfarin-resistant." Three past GLP studies at Genesis have identified three lots of rats from the same location in Chicago to be ~55% warfarin-resistant, according to the World Health Organization (WHO) protocol for determining resistance in Norway rats (WHO 1982). The protocol suggests that the rats must survive a 6-day, no-choice exposure to a 50-ppm warfarin diet. The WHO resistance test was not conducted immediately prior to use of these rats in this simulated field test, however, the historical data shows the level of resistance.

Husbandry

To simulate a "field" site in each study room, we added 8 straw bales, all adjacent to each other, and lightly covered the floor with wood shavings. Additional 4-in PVC tubing was added for shelter and to promote

colonization of each study room. The study room door and the water basin were partitioned away from the rest of the usable area. The study rooms were of the following areas: A2 (Maki, 20.5' × 22' = 451 ft^2), A3 (Kaput, 17' × 18' = 306 ft^2) and A5 (Talon, 16' × 23.5' = 376 ft^2). Tap water was available ad libitum by gravity-fed galvanized waterers. Harlan Teklad 8664 pelleted rodent diet (Harlan Holding, Inc., Wilmington, DE) was also supplied ad libitum. Because of the mode of action of the test substance (anticoagulant), individual rats were not physically marked with an identifier, as marking may have influenced the test outcome.

Heating was supplied by a thermostatically-controlled gas furnace. In addition, humidity was regulated by a Humidistat mounted on the gas furnace. The minimum and maximum readings were recorded daily for the duration of the test.

Fluorescent or incandescent bulbs provided lighting. An electric timer was used to maintain the light cycle, which was set for 14 hours light:10 hours dark. The average light intensity at floor level was 27.2 foot-candles (incandescent) in A2, 18.9 foot-candles (fluorescent) in A3, and 13.4 foot-candles (fluorescent) in A5. Light intensity was measured with a NIST traceable Extech Instruments model light meter and was within parameters set by the EPA.

Acclimation Period and Group Assignment

On July 8, 2003, the acclimation period began. All rats were transported from the breeding colony directly into the simulated field environment. The rats were randomly added to the rooms until 10 males and 10 females were housed in each of the test rooms, except for the Talon test room, which had 12 females and 11 males. The rats were acclimated under test conditions for 7 days. During the acclimation periods, the rats were observed daily to assess general physical condition, disease, and any abnormalities; and the attending veterinarian examined the rats on the first day of acclimation. All rats appeared healthy. The study rooms were randomly assigned to treatment by a computer-generated random numbers table.

Exposure Period

To initiate the exposure period, two trays of OPP field rodent challenge diet (OPP designation: 1.216, Standard *Peromyscus* Species Anticoagulant Dry Bait Laboratory Test Method, revision 7-4-91), and two trays of the respective bait were placed in opposite corners of the study room for a choice-test regime. The tray positions were switched daily to eliminate any positional bias. The trays were weighed approximately every 3 days to measure consumption of the diet. After Day 3, the field rodent challenge diet was changed to whole corn in an effort to increase consumption of the three rodent baits. Milo was used as an alternate diet after Day 22 for the same reason, and then the alternate diet was removed and the bait was the sole food source after Day 29, resulting in a no-choice test.

During the exposure period, the rats were observed daily to assess general physical condition and any signs of anticoagulant toxicosis. The straw bales were occasionally turned over or moved to facilitate the recovery of dead rats. Straw bales were carefully replaced in the original position.

Consumption

The bait and the alternate diet were weighed approximately every 3 days to calculate consumption. Consumption was weighed to the nearest 0.1 gram.

Mortality

Mortality was calculated for each commensal bait using the following formula:

$$\frac{\text{initial \# of rats - rats surviving}}{\text{initial \# of rats}} \times 100 = \%\ \text{mortality}$$

MATERIALS AND METHODS - WARFARIN TOXCITY STUDY

To explore the capabilities of warfarin further, and possibly dispel earlier literature suggesting extended exposure periods and poor palatability, the sponsor decided to conduct two studies with laboratory rats and mice. The toxicity of Kaput Rat and Mouse bait was examined with a multi-day, no-choice exposure of 1-, 2-, 3-, 4-, and 5-day periods. Five male and five female rats and mice were used for each respective treatment group. Rats were held individually and mice were held in groups of five. Stainless steel cages (511 cm^2) equipped with automatic watering systems were used for the test. Temperature and relative humidity was recorded daily. Consumption was conducted daily with an accuracy of 0.1 gram. Spillage was recovered and weighed. Day 0 body weights were recorded. After the respective exposure period, the animals were fed Harlan Teklad 8664 Rodent Diet for the remainder of the post-test period. An HPLC analysis of the bait verified the concentration of the Kaput Rat and Mouse bait at 0.0234% warfarin.

RESULTS AND DISCUSSION - SIMULATED FIELD STUDY

Test Substance

Although this test directly compares the palatability of the baits, it does not directly compare the efficacy of the active ingredients. The test does illustrate, based on the total consumption of the bait and the total efficacy, that the Kaput Rat and Mouse Bait out-performed the other two pelleted commensal rat and mouse baits. In addition, it showed warfarin as an effective active ingredient against "warfarin-resistant" rats.

Environment

All study rooms had similar temperatures during the exposure period, ranging from 21° to 25°C.

Consumption

The Kaput rats consumed 5,969.4 grams of challenge diet and 322.9 grams of the bait during the choice test. During the choice test, the Maki rats consumed 10,818.1 grams of challenge diet and 64.5 grams of the bait, and the Talon rats consumed 11,051.2 grams of challenge diet and 39.6 grams of the bait (Table 1, 2).

Consumption was observed within 3 days with the

Kaput and Talon bait, while it took up to 12 days before the Maki bait was consumed. Kaput bait consumption far exceeded that of the other baits, by at least 80%. In an effort to increase consumption of the baits, especially the Maki and Talon baits, we changed the "challenge diets" to less palatable diets. As a result, more bait was consumed, but consumption measurements indicated the alternate diet was preferred over the Maki and Talon baits. Even during the no-choice portion of the study, it appeared that in the Talon room, the rats were cannibalizing the first rats that died rather than eat the bait. In the Maki room, the rats were noted as chewing on the rubber molding one day after the no-choice test was initiated, while the bait remained uneaten. Only three rats survived the choice test in the Kaput room.

Observations

Test substance-related signs were observed in the treated group rats during the exposure and post-test period. The first symptoms of anticoagulant poisoning were observed on Day 3, when a dead rat with anticoagulant symptoms was found in the Kaput room. The first deaths in the Maki and Talon rooms were on Days 13 and 5, respectively. Eight of 20 rats had died in the Kaput room by Day 8, one on Day 12, and another 8 died between Days 20 and 25. Consumption within the other two study rooms was low, until they were forced to eat the bait in the no-choice portion of the exposure period. In the Maki room, deaths were recorded on Days 13, 16, 19, and 20. In the Talon room, deaths were recorded on Days 5 and 7. All other deaths were in the no-choice period for the Maki and Talon baits.

Mortality

During the first 28 days of the exposure period, the rats were exposed to bait and a challenge diet. The resulting efficacy was 10% mortality from the Talon bait, 20% from the Maki bait, and 85% from the Kaput bait (Figure 1). This illustrates that the Kaput bait was the most palatable and therefore, the most efficacious. During the no-choice exposure period, the remaining rats in each of the study rooms died. Cumulative mortality data are presented in Table 3.

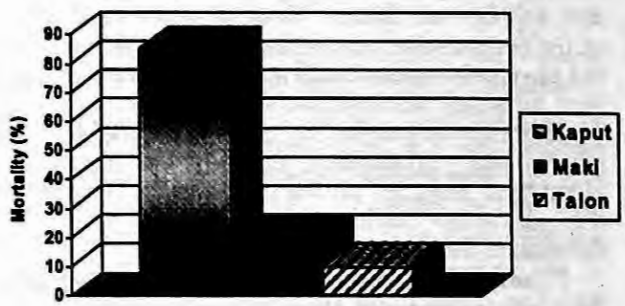

Figure 1. Comparative bait efficacy against wild warfarin-resistant Norway rats during the choice-test period of the simulated field test.

RESULTS AND DISCUSSION – WARFARIN TOXICITY STUDY

The daily consumption of the rats and mice was consistent until about the third day of the study, when the warfarin appeared to be exhibiting its effect and consumption slowed (Table 4, 5). The no-choice tests identified that 100% of the rats succumbed to warfarin with at least 2 days of exposure (Figure 2), and 100% of the mice succumbed to warfarin with at least 5 days exposure (Figure 3). Cumulative mortality data for the rat and mice are presented in Tables 6 and 7, respectively.

Table 1. Test substance consumption by three groups of wild warfarin-resistant Norway rats, each group made up of approximately 20 animals. Groups were maintained on one of the three test baits under free choice, followed by no-choice conditions until 100% mortality was achieved.

Treatment	Test Substance Consumption (g)				
	Choice Test				*No-choice Test*
	Oats and Rodent Diet (Day 0-3)	Corn (Day 4-22)	Milo (Day 23-28)	Total (Day 0-28)	(Day 29-41)
Maki	0.5	60.9	3.1	64.5	861.7
Kaput	50.8	270.3	1.8	322.9	134.7
Talon	13.5	22.7	3.4	39.6	1,779.2

Table 2. Challenge diet (non-toxic feed) consumption by three groups of wild warfarin-resistant Norway rats, each group made up of approximately 20 animals. Groups were maintained on one of the three test baits under free choice, followed by no-choice conditions until 100% mortality was achieved.

Treatment	Challenge Diet Consumption (g)			
	Oats and Rodent Diet (Day 0-3)	*Corn (Day 4-22)*	*Milo (Day 23-28)*	*Total (Day 0-28)*
Maki	1,142.5	7,601.5	2,074.1	10,818.1
Kaput	1,200.0	4,338.8	430.6	5,969.4
Talon	1,607.9	7,230.8	2,212.5	11,051.2

Table 3. Cumulative number of rats that died over the length of the simulated field test. The Maki and Kaput treatment groups consisted of 20 rats each, while the Talon treatment group had 23 rats.

Group	Study Day																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Maki	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	2	3	4	4
Kaput	0	0	0	1	1	2	2	6	8	8	8	8	9	9	9	9	9	9	9	10	11
Talon	0	0	0	0	0	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Group	Study Day																				
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Maki	4	4	4	4	4	4	4	4	4	4	4	5	9	12	14	16	19	20	-	-	-
Kaput	11	13	14	16	17	17	17	17	17	17	17	18	19	19	19	20	-	-	-	-	-
Talon	2	2	2	2	2	2	2	2	2	2	2	2	3	5	5	5	7	11	18	20	23

note: Day 28 is the end of the choice test and initiation of the no-choice test.

Table 4. Test substance (treatment) consumption (grams) by laboratory Norway rats during the multi-day no-choice exposure test with warfarin bait. Five male and five female lab rats were used in each treatment group.

Treatment group	Sex	Exposure day					Sum	Mean grams / day	a.i. (mg) / rat / day	Weight (kg) @ day 0	a.i. (mg)/ kg body weight / day
		1	2	3	4	5					
1-day	Male	22.7	-	-	-	-	22.7	22.7	5.312	0.240	22.133
	Female	19.3	-	-	-	-	19.3	19.3	4.516	0.244	18.508
2-day	Male	24.0	26.8	-	-	-	50.8	25.4	5.944	0.242	24.562
	Female	21.0	24.3	-	-	-	45.3	22.7	5.312	0.218	24.367
3-day	Male	25.1	31.1	26.9	-	-	83.1	27.7	6.482	0.271	23.919
	Female	21.8	25.0	21.7	-	-	68.5	22.8	5.335	0.218	24.472
4-day	Male	20.9	24.3	19.8	10.2	-	75.2	18.8	4.399	0.243	18.103
	Female	22.2	26.8	24.3	16.3	-	89.6	22.4	5.242	0.223	23.507
5-day	Male	18.8	22.9	23.4	14.1	12.5	91.7	18.3	4.282	0.244	17.549
	Female	18.9	22.0	22.1	16.3	4.4	83.7	16.7	3.908	0.215	18.177

Table 5. Test substance (treatment) consumption (grams) by laboratory house mice during the multi-day no-choice exposure test with warfarin bait. Five male and five female lab mice were used in each treatment group.

Treatment group	Sex	Exposure day					Sum	Mean grams / day	a.i. (mg) / mouse / day	Weight (kg) @ day 0 (kg)	a.i.(mg) / kg body weight / day
		1	2	3	4	5					
1-day	Male	13.0	-	-	-	-	13.0	13.0	3.042	0.021	144.857
	Female	12.1	-	-	-	-	12.1	12.1	2.831	0.020	141.550
2-day	Male	18.7	15.6	-	-	-	34.3	17.2	4.025	0.023	175.000
	Female	12.6	11.1	-	-	-	23.7	11.9	2.785	0.021	132.619
3-day	Male	13.9	12.5	12.1	-	-	38.5	12.8	2.995	0.024	124.792
	Female	13.1	18.8	11.1	-	-	43.0	14.3	3.346	0.020	167.300
4-day	Male	20.1	20.3	20.6	9.4	-	70.4	17.6	4.118	0.023	179.043
	Female	13.7	12.5	10.8	11.2	-	48.2	12.1	2.831	0.020	141.550
5-day	Male	11.7	13.7	16.6	6.8	2.3	51.1	10.2	2.387	0.022	108.500
	Female	15.0	16.7	7.2	13.8	1.7	54.4	10.9	2.551	0.021	121.476

CONCLUSIONS

The greatest efficacy achieved during the choice test was with the Kaput Rat & Mouse Bait with 85% mortality. Only during the no-choice portion of the simulated field test did the other baits reach acceptable mortality. The no-choice laboratory test also identifies warfarin as being 100% effective to rats in mice in as little as 2 days of exposure for rats and 5 days of exposure for mice. Warfarin has previously been identified by literature as being ineffective and unpalatable, but these tests prove that warfarin products can be used for effective control of commensal rats and mice, even against rats that are claimed to be "warfarin-resistant."

LITERATURE CITED

WHO (WORLD HEALTH ORGANIZATION). 1982. Instructions for Determining the Susceptibility or Resistance of Rodents to Anticoagulant Rodenticides. World Health Organization Publication WHO/VBC/82.843. 8 pp.

Table 6. Cumulative number of laboratory Norway rats dead during each of the days of the multi-day no-choice exposure test with warfarin bait. Five male and five female lab rats were used in each treatment group.

Treatment group	Sex	Study day															
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1-day	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Female	0	0	0	0	1	2	3	5	5	5	5	5	5	5	5	5
2-day	Male	0	0	0	2	4	4	4	4	5	5	5	5	5	5	5	5
	Female	0	0	0	0	1	1	3	4	5	5	5	5	5	5	5	5
3-day	Male	0	0	0	0	1	1	4	5	5	5	5	5	5	5	5	5
	Female	0	0	0	0	0	1	3	5	5	5	5	5	5	5	5	5
4-day	Male	0	0	0	0	2	2	2	5	5	5	5	5	5	5	5	5
	Female	0	0	0	0	2	2	2	5	5	5	5	5	5	5	5	5
5-day	Male	0	0	0	0	1	1	3	5	5	5	5	5	5	5	5	5
	Female	0	0	0	0	1	1	4	5	5	5	5	5	5	5	5	5

Table 7. Cumulative number of laboratory house mice dead during each of the days of the multi-day no-choice exposure test with warfarin bait. Five male and five female lab mice were used in each treatment group.

Treatment group	Sex	Study day											
		0	1	2	3	4	5	6	7	8	9	10	11
1-day	Male	0	0	0	0	0	0	0	0	0	0	0	0
	Female	0	0	0	0	0	0	0	0	0	0	0	0
2-day	Male	0	0	0	2	2	2	2	2	2	2	2	2
	Female	0	0	0	0	0	0	0	0	0	0	0	0
3-day	Male	0	0	0	0	0	2	3	3	3	3	3	3
	Female	0	0	0	0	0	0	0	0	0	0	0	0
4-day	Male	0	0	0	0	0	2	3	4	4	4	4	4
	Female	0	0	0	0	0	4	4	4	4	4	4	4
5-day	Male	0	0	0	0	1	3	5	5	5	5	5	5
	Female	0	0	0	0	0	0	4	5	5	5	5	5

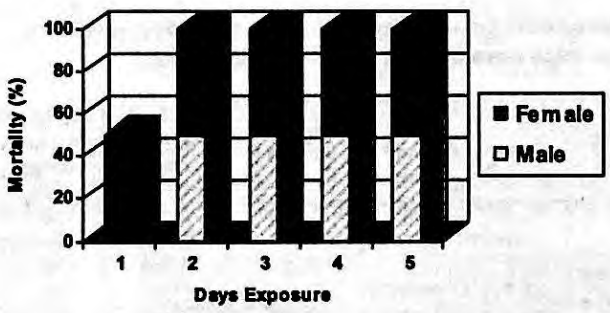

Figure 2. Mortality in laboratory Norway rats in a no-choice test of Kaput Rat and Mouse Bait (0.025% warfarin) during the multi-day exposure test.

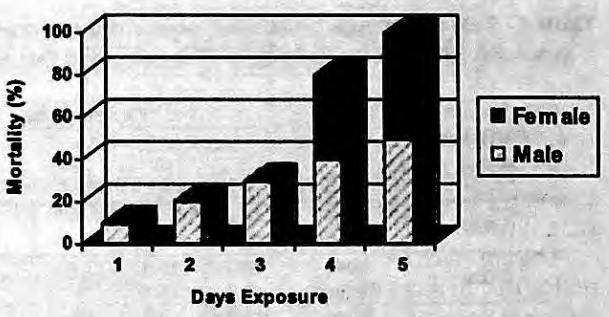

Figure 3. Mortality in laboratory house mice in a no-choice test of Kaput Rat and Mouse Bait (0.025% warfarin) during the multi-day exposure test.