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THE STATUS OF BROMADIOLONE IN THE UNITED STATES

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ABSTRACT: The anticoagulant rodenticide bromadiolone is used throughout the U.S. under a number of trade names. An expanded research program is underway within Chempar to examine the use of bromadiolone in commensal and field rodent control. Data are presented herein on the toxicology, metabolism, secondary hazards, efficacy, and formulation developments with bromadiolone. A new Maki 0.001% liquid bait is being tested and excellent control results obtained against Norway rats (*Rattus norvegicus*), roof rats (*R. rattus*), and house mice (*Mus musculus*). New Maki paraffin blocks containing 50 ppm bromadiolone have been developed and are soon to be on the market. Bromadiolone biogradability in the field and in animal tissues offers promise for expanded label claims for use in urban and field situations.

INTRODUCTION

The second-generation anticoagulant rodenticide bromadiolone was first introduced into the U.S. market in 1980 under the trade name MAKI. Details on its chemical structure and aspects of early development research are presented by Grand (1976) and Meehan (1978).

Summaries of early bromadiolone field testing in the U.S. were presented by Marsh (1977), Marsh et al. (1980), and Lechevin (1985). Since then, numerous research studies have been completed, most of which have not been published.

In the U.S. bromadiolone was first marketed under the trade name MAKI. Since then, through several licensing arrangements and subregistrations, many end-use baits containing the compound are now available in the PCO and consumer markets. Table 1 lists those trade names currently marketed and containing 0.005% of the compound.

Table 1. Products marketed in the U.S. containing bromadiolone.

Commercial Bromadiolone Products

- Maki Rat and Mouse Meal Bait
- Maki Paraffinized Pellets
- Maki Rat and Mouse Bait Packs (Pellets)
- Maki Paraffin Blocks
- Rat Arrest
- Mouse Arrest
- Contrac Rat and Mouse Bait
- Blitz One Feeding
- Rat Flip One Feed
- Mousebuster
- Ratfree
- Chacon One Shot Rat and Mouse Killer
- Just One Bite Rat and Mouse Bait
- Pied Piper Rat & Mouse Bait Packs (Pellets)
- Last-Stop Rat and Mouse Bait Packs (Pellets)
- NCH Paraffinized Pellets
- Rat-Tat-Tat II Rat and Mouse Bait Packs (Pellets)
- Starbar Trax-One

As a second-generation anticoagulant, bromadiolone is more active than products such as warfarin and chlorophacinone. Bromadiolone, however, is less toxic than brodifacoum and difenacoum. A summary of toxicity information on rodents and key domestic and wildlife species is presented in Table 2. The compound is not very soluble in water (19 ppm) (Lipha, undated). Data on bromadiolone toxicity to fish are given in Table 3.

Table 2. Toxicity of bromadiolone to various rodents, domestic animals and wildlife (Anon.)

Species	LD-50	LC-50
House mouse	1.750 mg/kg	--
Norway rat	1.125	--
Pine vole	3.900	--
Dogs(1)	15.000	--
Cats	25.000	--
Rabbit (oral)	1.000	--
Bobwhite quail	138.000	62.0 ppm
Mallard	--	110.0

(1) Maximum tolerated dose of 10 mg/kg. LD-50 is an estimate.

Table 3. Toxicity of bromadiolone to aquatic organisms.

Species	LC-50 (mg/L)		
	24 hrs.	48 hrs.	96 hrs.
Daphnia	8.8	0.24	--
Rainbow trout	3.9	2.4	1.4
Bluegill sunfish	4.4	4.1	3.0

FIELD TRIALS

Numerous field trials have been conducted through the U.S. to control commensal rodents with MAKI. Table 4 (Marsh et al. 1980) outlines major studies completed in the U.S. to support bromadiolone registration by the Environmental Protection Agency. As the efficacy data illustrate, the compound is very effective in reducing rodent numbers in various regions of the country.

Table 4. The results of field trials conducted for several rodent species with Bromadiolone (MAKI) in the U.S. (Marsh et al. 1980).

Species	Site	State	Efficacy (% mortality)
Norway rat	Horsebarn	New York	> 75%
	Grain elevator	Texas	70-80%
	Chicago Inner City	Illinois	86%
	Commercial buildings	California	99%
	Hog farm	Ohio	85%
	Farm home	Wisconsin	Near 100%
	Store	New Jersey	100%
	Feed lot	Nebraska	95%
	River bank	Minnesota	Near 100%
	Poultry farm	N. Carolina	89%
	Vacant lot	Massachusetts	> 90%
Apartment bldg.	Massachusetts	> 90%	
Norway rats & House Mice	Farm home	Wisconsin	68-85%
	Grain mill	Wisconsin	Near 100%
Roof rat	Residential block	Florida	100%
	Hospital	Texas	100%
	Residential block	California	100%
	Feed lot	California	85%
House mouse	Store room	New Jersey	100%
	Apartments	Massachusetts	Near 100%
	Home	Wisconsin	100%
	Grain mill	California	Near 100%
	Barn	Florida	> 75%
	Old field	Florida	> 90%
	Univ. campus bldg.	Kansas	Near 100%
	Office bldg.	Texas	Near 100%
	Warehouse bldg.	Texas	Near 100%

A more recent study by Salmon et al. (1984) resulted in a 96% rodent population reduction on a dairy farm. Table 5 outlines research completed on field rodent species throughout the U.S. The compound is very good at controlling virtually any pest species. Meehan (1985) reported on the good palatability of bromadiolone making it readily accepted by rodents.

Table 5. The results of field trials using Bromadiolone to control key field rodent species.

Species	Concentration	Site	State	Researcher	Efficacy(%)	Notes
Columbian Ground Squirrel	500	pasture	Montana	D. Sullivan	90.3	Handbait
	50	pasture	Montana	D. Sullivan	95	Bait Station
	50	pasture	Washington	L. Askham (1985)	70-80	Mid-Season
					100	Late-Season
California Ground Squirrel	200	pasture	California	R. Baker	78-91	Handbait
	50	pasture	California	R. Baker	79-80	Handbait
Pocket Gophers	300	pasture	California	R. Baker	62	Handbait
	400	pasture	California	R. Baker	62	Handbait
	500	pasture	California	R. Baker	85	Handbait
	175	clearcut	California	Tunberg et al(1984)	100	Handbait
Wood Rat	50	indoor	California	R. Baker	100	Buildings
	50	outdoor	California	R. Baker	100	
Meadow Voles	50	orchard	Washington	R. Hunter	100	
	50	orchard	Virginia	R. Byers	71	
	50	orchard	New York	Richmond and Miller (1980)	100	

Ground squirrel control ranged from 70 to 100% and varied according to time of application and species controlled (Table 5). Pocket gopher control results were more variable. Results using bromadiolone in a study by Tunberg et al. (1984) demonstrated that the rapid reinvasion of pocket gophers may hamper control efforts if made on a localized basis.

Vole control using MAKI ranged from 71 to 100% (Table 5). Research by Byers (1978, 1979, 1981), Byers et al. (1982), and Steblein et al. (1983) demonstrated the compound to be relatively consistent in reducing orchard mice problems. A major factor affecting efficacy is that of bait acceptance which varies in various parts of the U.S. No one formulation can meet all the needs within pest control.

FORMULATIONS

Currently MAKI is marketed as pellets, meal bait, and paraffin blocks. Several grain formulations are currently being tested. Submission of the registration support data is scheduled for late 1986. Two products which show promise are MAKI SOL and the new MAKI PARAFFIN BLOCK.

Liquid Bait

A 10 ppm end-use MAKI liquid bait is near test completion. When registered, the product will be sold under the name MAKI SOL and will be available in a liquid concentrate of 0.011% bromadiolone. Table 6 presents the result of laboratory testing of MAKI SOL against Norway rats and house mice. In rats, 100% mortality was achieved with a 5-ppm liquid bait; however, 10 ppm was required to attain the same level of control in mice. Dye-free baits increased acceptance significantly.

Table 6. Liquid MAKI bait laboratory evaluations using various concentrations (tap water as controls) and following EPA test protocol.

	Test no.	Days exposed	Color	Concentration (ppm)	Mortality (deaths/total)
Norway rats					
	84006	14	Red	50	25/25
	85004	12	Red	50	10/10
	85006	13	Red	10	8/10
	85006-B	9	Clear	10	20/20
	85009	8	Clear	5	10/10
House mice					
	85008-B	6	Clear	10	10/10
	85008-A	7	Clear	5	7/10
	85010-A	8	Clear	10	20/20
	85010-B	7	Clear	10	20/20

Field testing of MAKI SOL was completed in California (R. Baker, unpubl.). Table 7 summarizes the results with over 95% efficacy after 6 days and up to 99% after 12 days when used against roof rats (*Rattus rattus*). Additional test results are given in Table 8, again demonstrating the excellent control against three commensal species.

Table 7. Results of a field trial using MAKI SOL (50 ppm bromadiolone) liquid bait to control roof rats in a hay shed near Fullerton, California (R. Baker, unpubl.).

Census type	Efficacy (percent reduction)	
	Days after bait provided	
	6	12
Untreated water	95.7	99.7
Dry census bait (mash)	95.9	90.9
Electric counter	96.0	98.0

Table 8. Test data using 10 ppm MAKI SOL (liquid bait) to control three rodent species in a barn-type enclosure (R. Baker, unpubl.). Water consumption, census ration and electric counters were used to estimate efficacy.

Species	Number of test animals	Efficacy (% mortality)
House mice	40	100%
Norway rats	20	80%(1)
Roof rats	20	100%

(1) Test still in progress.

Paraffin Blocks

During 1985 a new paraffin block containing 50-ppm bromadiolone was developed. Test results are presented in Table 9. In Norway rats and house mice, acceptance was near 50% for both species, while acceptance of the incubated bait averaged in the range of 39%. Mortality in the test animals was 100%. Regional field testing is currently being organized for the three commensal species. It is anticipated that this new formulation will be available by early summer.

Table 9. Laboratory test results with a new 0.005% bromadiolone paraffin bait formulation used against Norway rats and house mice in standard EPA choice tests.

	House mice		Norway rats	
	1	2	1	2
Unincubated bait				
Test no.				
Acceptance(1)	52.6%	48.3%	48.6%	49.4%
Mortality(2)	20/20	20/20	20/20	20/20
Incubated bait (100°F, 100% humidity x 15 days)				
Acceptance	35.7%	38.2%	42.7%	37.7%
Mortality	20/20	20/20	20/20	20/20

(1) Test bait consumption / Test and placebo consumption

(2) No. rodents died / No. rodents in test

Pelleted and Meal Baits

In laboratory tests recently completed, MAKI was compared to other rodenticide products on the market. In these tests, a new MAKI pellet formulation attained an acceptance of 71.3% in males and 68.1% in female Norway rats and 100% mortality. Tests using the MAKI MEAL BAIT had an average acceptance of 50.8% (44.8-58.6) with 100% mortality when used against house mice. A study by Frantz (1982) showed MAKI baits to be highly palatable to Norway rats with acceptance results of 56.6% and 67% in two pelleted formulations. A summary of these results is presented in Table 10.

Table 10. Test results from 1985/86 using MAKI formulations against Norway rats and house mice.

	% Acceptance (1)			Mortality (2) (deaths/total)
	M	F	C	
Norway rats				
MAKI Pellets	71.3	64.0	68.4	20/20
MAKI Meal	61.2	48.4	54.6	20/20
House mice				
MAKI Pellets	26.7	57.2	43.6	20/20
MAKI Meal			50.8	40/40

(1) M=male, F=female, C=combined sexes

(2) No. rodents died / No. rodents in test

RESISTANCE TESTING

Historically, secondary-generation anticoagulants have been marketed as a tool to control warfarin- or cross-resistant rats and mice. As with resistance in most pest species, a compound that is too toxic may result in other environmental problems, while a product with less potency may control rats adequately but might be less susceptible to mice (Marsh 1977).

Early resistance testing results in the U.S. are presented in Table 11 and show Norway rats to be very susceptible to bromadiolone. Additional research on resistance was completed by Frantz (1982). Summary data (Table 12) from a study completed in Chicago demonstrated that a resistant Norway rat population could be reduced by 85.5% by using MAKI (Ashton and Jackson 1979).

Table 11. Laboratory tests using 0.005% bromadiolone bait fed to anticoagulant resistant Norway rats.

Test type	Resistance Type ¹	Sex	Number test animals	Consumption (g) Challenge	Test	Mortality (deaths/total)	Source
No-choice (6-day)	W	M	7	--	91.6	7/7	BGSU ² , unpubl.
	W	F	10	--	80.4	10/10	
Choice (15-day)	C	M	10	146.9	15.9	7/10	BGSU, unpubl.
	C	F	10	123.9	21.9	10/10	
No-choice (6-day)	C	M	5	31.0	73.0	5/5	BGSU, unpubl.
		F	5	70.5	30.2	5/5	
		F	5	29.1	61.6	5/5	

¹W=Warfarin resistant; C=Cross-resistant

²Bowling Green State University, W. B. Jackson

Table 12. Results of a field trial using 0.005% MAKI in an area of Chicago to control anticoagulant resistant rats. Approximately 71% of the rodent test population was considered resistant (Ashton and Jackson 1979).

	Number premises surveyed	Percent premises infested	Number of applications	Efficacy (% mortality)
Test 1 (Talon)	137	65	3	77.7%
Test 2 (Maki Pellets)	108	59	1	41.7%
Test 3 (Maki Meal)	128	59	3	85.5%
Control	168	64	-	-2.5%*

* % change

House mice are more difficult to control with anticoagulants. In France, laboratory tests using resistant mice revealed bromadiolone to be effective, as outlined in Table 13 (Lorgue, unpubl.). Tests recently completed in the U.S. (Table 14) resulted in 85% mortality in the resistant mice.

Table 13. Laboratory tests using 0.005% bromadiolone bait against house mice (Mus musculus) from France (Lorgue unpubl.).

Test type	No. test mice died / Total test mice	
	Males	Females
Nonresistant	Tank	
	27/27	15/15
	22/22	17/17
Individual Cages	11/11	6/6
	6/6	6/6
Totals	66/66	44/44
Warfarin-resistant	Tank	
	20/20	20/20
	17/17	17/17
Cages	3/3	11/12
	7/8	3/4
Totals	47/48	57/59

Table 14. Laboratory tests from February 1986 using MAKI (50 ppm) bait to control warfarin-resistant house mice (Ashton, unpubl.).

	Acceptance (percent)	Mortality (deaths/total)
Males	31.7	10/10
Females	37.0	7/10
Combined	<u>34.35</u>	<u>17/20</u>

Reports by Lund (1984) and Siddiqi and Blaine (1982) indicate the potential for house mice to develop resistance to bromadiolone after extended use.

NONTARGET HAZARD POTENTIAL

Of concern in the use of rodenticides is not only the activity, or toxicity, of a compound to various target species, but also its potential effect on domestic animals and wildlife (Poché and Sharp 1986). Toxicosis to rodenticides may occur by animals feeding directly on the bait or by consuming toxic-laden target species. Since anticoagulants have a delayed mode of action when compared with acute products, the tendency is increased for a rodent to accumulate a toxic load greater than the required lethal dose. Therefore, factors such as dose level (ppm) in the baits, methods of application, timing and rate of application of the bait, carriers and bait type, metabolism of the compound in biological systems, and fate of the product in the environment contribute to the potential impact of its use. A combination of factors interact in determining the fate of a compound in a target species. Data on residue levels, degradation, half-life, and metabolism of rodenticides are but a few important considerations in determining the relative safety of a product.

With bromadiolone, within 4 days after ingestion by rats, over 89% of the compound is eliminated through the feces via the bile duct (Table 15). Of the amount excreted, about 90% of the bromadiolone degrades into metabolites.

Table 15. Elimination of 14-C bromadiolone from rats in percentages of the dose administered. Test rats were gavaged with 5 mg/kg body weight bromadiolone (Lipha, unpubl.).

	Day				Total
	1	2	3	4	
Feces	58.90	20.25	8.65	1.30	89.10
Urine	0.42	0.19	0.06	0.07	0.74
Total	59.32	20.44	8.71	1.37	89.84(1)

(1) Only 10% was bromadiolone. Remainder was degraded metabolites.

Once a rodent ingests a rodenticide, the chemical is assimilated into the system and residue levels may appear in various tissues. Carbon-14 labeled bromadiolone studies in rats, demonstrated that within 48 hours after intubation, residue levels degraded within various body tissues an average of 61% (Table 16). The breakdown was most rapid in the carcass (81%) and slowest in fat tissues (27%) 46 hours later (HRC 1977).

Table 16. Concentrations of 14-C labeled bromadiolone (BDN) in rat tissues at 2 and 48 hours after intubation with 5 mg/kg of the compound (HRC 1977).

Organ	Concentrations of BDN in micrograms per gram of organ (ppm)	
	2 Hrs. (n=2)	48 Hrs. (n=4)
Liver	43.50	20.54
Kidneys	3.53	1.36
Lungs	1.60	0.49
Heart	1.25	0.25
Muscle	0.37	0.15
Fat	3.04	2.22
Carcass	5.95	1.11

Studies on bromadiolone tissue concentrations in Japanese quail (*Coturnix japonica*) over time were completed in the United Kingdom (Table 17). Adult birds, averaging about 235 g in weight, were gavaged with 1000 mg per kg body weight. At time intervals of 1, 2, 4, 7, 14 and 56 days after intubation four birds were sacrificed. The legs were removed at the hock joint and discarded, the birds plucked, and the whole carcass examined by HPLC for levels of bromadiolone. The average residue level was 286.5 ppm after 1 day. By day 4, the figure dropped to 0.19 ppm and after 7 days the birds contained an average of 0.065 ppm bromadiolone in the tissues. Extrapolation of these revealed the half-life of bromadiolone in quail tissues is about 4.5 days.

Table 17. Determination of tissue concentration of bromadiolone (BDN) in Japanese quail after a single oral dose of 1000 mg/kg (HRC 1980).

Sampling time (days)	Amount of BDN in tissue (ppm)
1	286.500
2	2.250
4	.190
7	.065
14	.092
56	.050(1)

(1) Limit of detection

A study with owls by Mendenhall and Pank (1980) demonstrated the hazard potential of using anticoagulants in field situations. Although laboratory studies do not always indicate a true picture of what one might expect in the field, such data are necessary to determine the "potential" hazard to nontarget animals.

As part of their study, Mendenhall and Pank (1980) fed bromadiolone-killed rats to barn owls (*Tyto alba*) (Table 18). The birds were fed only treated rats for periods of 1 to 10 days. Mortality was observed in one of the six owls which consumed bromadiolone-killed rats for 10 consecutive days during which it consumed 463 grams of rat tissues. Although one might interpret these data as being evident that bromadiolone is a potential hazard to raptors, one has to examine carefully the probability of a wide-ranging avian species to consume only bromadiolone-killed rodents for 10 consecutive days.

Table 18. Secondary toxicity of bromadiolone to barn owls (Mendenhall and Pank 1980).

Days Dosed	OWLS		RATS OFFERED		RATS EATEN			INTOX.
	Wt(g)	Sex	Total wt	Dose (mg)	Total wt(g)	Livers	Intestines	
1	460	M	118	2.65	52	1	0.8	-
3	450	M	358	6.60	281	3	3	-
3	425	M	228	3.96	146	3	2.8	-
6	490	M	625	11.11	295	5	4	-
10	540	F	1106	14.59	576	7.8	4.5	-
10	635	F	710	9.63	463	8.5	5.2	D(11)

The potential hazard bromadiolone poses to avian species is low (Grolleau and Lorgue 1984). As listed in Table 2, for example, the acute LD-50 of bromadiolone in bobwhite quail is 138 mg per kg. For a 50-ppm bait this translates to about 2.76 kg of MAKI. The propensity for bromadiolone to accumulate in avian tissues is not evident, as demonstrated in the rapid metabolism of the compound in Japanese quail.

A laboratory secondary hazard evaluation of bromadiolone was conducted in adult coyotes (Canis latrans) (Marsh, unpubl.). Fifty-ppm bromadiolone oat bait was fed to California ground squirrels (Spermophilus beecheyi) for 3 days in a choice test. As the test squirrels died, each was frozen and later fed to coyotes conditioned to feed on the sciurids. Each of four adult coyotes was fed one bromadiolone-killed squirrel daily for 5 consecutive days (Table 19). The protocol followed EPA recommendations for a worse-case situation in which in the canids consumed only rodenticide-killed squirrels. The coyotes were observed for 30 days posttreatment during which time none of the test animals died. Two, however, exhibited a reduction in food consumption, which returned to normal after 8 and 16 days into the observation period.

Table 19. Results of feeding bromadiolone-killed ground squirrels to adult coyotes (Canus latrans) for 5 consecutive days with no other food provided (UCD(1), unpubl.).

Animal Number	Sex	Weight (kg)	Wt. (g) of squirrels consumed	Toxicant (mg) consumed by squirrels	Toxicant (mg) consumed by coyotes	Results
B-1	M	13.6	3,199	22.5	18.0	Survived; no symptoms
B-2	F	7.9	2,165	22.5	15.75	Survived; feeding reduced 16 days
B-3	F	10.4	3,445	22.5	22.5	Survived; feeding reduced 8 days
B-4	M	7.5	3,133	22.5	22.5	Survived; no symptoms

(1) University of California, Davis; Rex Marsh, principal investigator

As with avian species, the potential secondary hazard of bromadiolone to coyotes is extremely low. In this study, which simulated exclusive feeding on bromadiolone-killed squirrels, no mortality was observed in adult coyotes.

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