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BROMETHALIN—A PROMISING NEW RODENTICIDE

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ABSTRACT: Bromethalin is a unique highly potent rodenticide exhibiting a mode of action different from anticoagulant rodenticides. Bromethalin provides a lethal dose to rodents in a single feeding with death generally delayed two to three days. Rodents do not discriminate against bromethalin bait; therefore, excellent bait acceptance is achieved with no prebaiting. Field studies have shown bromethalin bait to be highly efficacious against Norway rats and house mice under a variety of field conditions. Laboratory and field trial data indicate bromethalin is effective against known anticoagulant-resistant rodent populations. Toxicological data indicate bromethalin bait is relatively safe to nontarget species as well as to the environment.

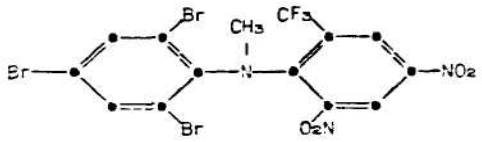
INTRODUCTION

The discovery of resistance in rats to anticoagulant rodenticides (Boyle 1960, Jackson and Kaukeinen 1972) stimulated investigators at Lilly Research Laboratories to search for a replacement rodenticide with a unique mode of action different from anticoagulants. This research led to the discovery of the toxic nature of certain classes of diphenylamines (Dreikorn 1978) and, through the development of a structure-activity relationship, to the subsequent discovery of bromethalin (Dreikorn 1980, Dreikorn et. al. 1979).

Bromethalin is a unique, highly potent, single -feeding rodenticide. While providing a lethal dose at the initial feeding, death is often delayed two to three days, but further feeding is inhibited. Documentation for the registration of bromethalin in the U.S. has been submitted to the EPA.

PHYSICAL CHARACTERISTICS

Technical bromethalin is a pale yellow, odorless, crystalline solid. Bromethalin is soluble in many organic solvents but essentially insoluble in water (<10 parts per billion). Technical bromethalin is stable under ordinary storage conditions.



$C_{14}H_7Br_3F_3N_3O_4$ Molecular weight: 577.88

N-methyl-2,4-dinitro-N-(2,4,6-tribromophenyl)-6-(trifluoromethyl)benzenamine

EFFICACY

In order to determine the efficacy of bromethalin against commensal rodents (Norway rats, Rattus norvegicus; roof rat, Rattus rattus; and house mouse, Mus musculus), the following studies were conducted: (A) acute oral LD_{50} (B) laboratory nonchoice feeding; (C) laboratory choice feeding (D) field efficacy; and (E) efficacy against anticoagulant-resistant rodents.

A. Acute Oral LD_{50}

The acute oral LD_{50} values for solubilized bromethalin technical material have been determined for commensal rodents (Table 1). LD_{50} values indicate that bromethalin is highly toxic to rodents; however, susceptibilities may differ between sexes and among species.

B. Nonchoice Efficacy Studies

Nonchoice efficacy studies with bromethalin bait against the Norway rat, roof rat, and house mouse are summarized in Table 2. Results indicate 100 percent mortality at >10 ppm for Norway rats and >20 ppm for the roof rat and house mouse. Average days until death ranged from 5.1 to 1.8 (10-100ppm) for Norway rats, 10.0 to 4.3 (5-25ppm) for roof rats, and 6.6 to 3.2 (20-50ppm) for mice. The amount of

Table 1. Acute oral LD_{50} (mg/kg) for bromethalin.

Species	Sex	Strain	LD_{50}	(95% Confidence limits)
Mus musculus	Female	ICR	8.13	(7.00-9.76)
Mus musculus	Male	ICR	5.25	(3.87-6.58)
Rattus norvegicus	Female	Wistar	2.01	(1.47-2.41)
Rattus norvegicus	Male	Wistar	2.46	(2.11-2.84)
Rattus rattus	Male/Female	Wild	6.60	(4.80-8.61)

Table 2. Nonchoice efficacy studies with bromethalin bait.

Species	Strain	Conc.(ppm)	No. dead/ No. tested	Avg. days until death	Bromethalin consumed, mgs/rodent
Rattus norvegicus	Wistar	5	2/5	7.0	0.52
Rattus norvegicus	Wistar	10	5/5	5.2	0.38
Rattus norvegicus	Wistar	15	5/5	4.4	0.32
Rattus norvegicus	Wistar	25	5/5	3.2	0.39
Rattus norvegicus	Wistar	50	5/5	2.4	0.50
Rattus norvegicus	Wistar	100	5/5	1.8	0.96
Mus musculus	ICR	10	0/5	---	0.34
Mus musculus	ICR	20	5/5	6.6	0.26
Mus musculus	ICR	30	5/5	5.2	0.24
Mus musculus	ICR	40	5/5	3.2	0.20
Mus musculus	ICR	50	5/5	3.2	0.28
Rattus rattus	Wild	5	2/10	10.0	0.43
Rattus rattus	Wild	10	8/10	9.1	0.50
Rattus rattus	Wild	15	9/10	7.5	0.39
Rattus rattus	Wild	20	10/10	5.8	0.34
Rattus rattus	Wild	25	10/10	4.3	0.36

toxicant per rodent resulting in complete mortality ranged from 0.32 to 0.96 mg in Norway rats, 0.34 to 0.36 mg in roof rats, and 0.20 to 0.28 mg in mice. As a result of these studies, bait concentrations between 50 and 100 ppm (2 1/2 to 5 times the lethal concentration) were designated for evaluation in choice feeding tests. At these concentrations, only a portion of the consumption need consist of bromethalin bait to be lethal.

C. Choice Efficacy Studies

Choice feeding studies with bromethalin bait against the Norway rat, roof rat, and house mouse were conducted at concentrations of 50 to 100 ppm, based upon results from previous nonchoice studies (Section B). The choice tests results are summarized in Table 3. Mortality of at least 95% occurred with only one day's exposure to the toxicant. Average days until death ranged from 2.7 to 3.7 in mice, 2.6 to 4.1 in Norway rats, and 2.5 in roof rats. Bromethalin bait was readily accepted by these rodent species, constituting about 50% of the total consumption. There were no indications of bait shyness, and pre-baiting was not required to achieve excellent acceptance.

D. Field Efficacy

Both indoor and outdoor field trials against Norway rat and house mouse populations were conducted under an EPA Experimental Use Permit (EUP) in a number of geographical regions in the U.S.A. (Spaulding and Jackson 1982). In all cases 0.005% bromethalin bait (65% cornmeal, 25% rolled oats, 5% sugar, 5% corn oil) was used, sometimes in bait stations, otherwise in plastic packs.

Three census techniques, rather than two required by the EPA, were generally utilized before and after bromethalin treatment in order to estimate control of the target rodent population. Three days of snaptrapping were conducted at the end of each trial to provide another parameter in determining efficacy. Lag periods were used both before and after bromethalin baiting to avoid preconditioning effects. Bromethalin bait usually was available until feeding ceased.

Field trial data indicated that 0.005% bromethalin was exceptionally effective against Norway rats and house mice in a variety of habitats (Tables 4 and 5). Bromethalin bait acceptance was excellent; no signs of bait-shyness were observed. In situations where competitive animal feeds were readily available, bromethalin baits were well accepted. Bromethalin treatments ranged from 7 to 30 days; mean treatment periods of 14 days for Norway rats and 16 days for house mice provided excellent control in most situations.

Table 3. Choice efficacy studies with bromethalin bait.

Species	Strain	Conc. (ppm)	No. dead/ no. tested	Avg. days until death	Percent acceptance of Bromethalin bait	Bromethalin consumed (mg/kg)
Rattus norvegicus	Wistar	50	20/20	2.8	57.1	2.40
Rattus norvegicus	Wistar	50	20/20	3.3	51.5	3.16
Rattus norvegicus	Wistar	50	20/20	4.0	47.5	3.11
Rattus norvegicus	Wistar	50	19/20	3.9	51.4	3.59
Rattus norvegicus	Wistar	50	20/20	4.1	48.9	3.16
Rattus norvegicus	Wistar	50	19/20	2.6	52.9	3.23
Rattus norvegicus	Wistar	50	18/20	2.8	49.3	2.62
Rattus norvegicus	Wistar	100	20/20	2.6	51.3	4.75
Mus musculus	ICR	50	19/20	3.1	54.4	7.47
Mus musculus	ICR	75	18/20	2.8	52.9	12.20
Mus musculus	ICR	100	18/20	2.7	65.5	12.50
Rattus norvegicus	Wild	50	19/20	2.8	48.9	1.79
Rattus norvegicus[1/]	Wild	50	20/20	3.6	56.2	2.65
Mus musculus	Wild	50	20/20	3.3	45.5	5.10
Mus musculus[1/]	Wild	50	19/20	3.4	47.5	3.47
Rattus rattus	Wild	50	20/20	2.5	46.0	2.27

[1/] One-day choice test.

Table 4. Efficacy summary of 0.005% bromethalin against the Norway rat (*Rattus norvegicus*).

Indoor trials	Census techniques	Pre-treatment index (Daily Avg.)	Post-treatment index (Daily Avg.)	Percent reduction	Rodents/10 traps/night	Treatment period (days)	Recovered carcasses
Hog barn (Ohio)	Feed consumption (g)	44.8	3.7	91.7			
	Tracking boards (tracks)	34.5	2.3	98.3	0.3	28	15
	Burrow Activity (#)	13.8	0.5	96.4			
	Live-trapping (#)	25.0	1.0	96.0			
Livestock barn (N. Carolina)	Feed consumption (g)	664.1	3.5	99.5			
	Tracking boards (tracks)	358.0	1.0	99.7	0.0	15	16
	Burrow activity (#)	13.0	0.0	100.0			
Dairy farm (Pennsylvania)	Tracking Boards (tracks)	198.2	16.7	91.6			
	Dropping counts (#)	81.7	1.3	98.4	0.0	14	8
	Burrow activity (#)	4.0	0.0	100.0			
Poultry farm (Rhode Island)	Feed consumption (g)	252.0	0.3	99.9			
	Tracking boards (tracks)	386.7	19.0	95.1	0.2	14	20
	Burrow activity (#)	23.0	2.0	91.4			
Duck farm (California)	Feed consumption (g)	2522.0	45.7	98.2			
	Tracking boards (tracks)	68.3	13.7	80.0	0.0	20	18
Feed mill (Texas)	Feed consumption (g)	489.6	99.5	79.7			
	Tracking boards (tracks)	646.0	285.3	55.8	0.6	16	12
	Dropping counts (#)	120.0	7.0	94.5			
Outdoor Trials							
Sheep pens (Indiana)	Feed consumption (g)	281.3	9.7	96.6			
	Tracking boards (tracks)	32.7	0.0	100.0	0.0	11	13
	Burrow activity (#)	49.0	0.0	100.0			
Hog farm (Kansas)	Feed consumption (g)	631.0	6.1	99.0			
	Tracking boards (tracks)	270.8	7.2	97.4	0.1	14	21
	Burrow activity (#)	58.0	2.0	97.1			

Table 5. Efficacy summary of 0.005% bromethalin against the house mouse (Mus musculus).

Indoor trials	Census techniques	Pre-treatment index (Daily Avg.)	Post-treatment index (Daily Avg.)	Percent reduction	Rodents/10 traps/night	period (days)	Recovered carcasses
Hog barn (Ohio)	Feed consumption (g)	363.0	31.3	91.4			
	Tracking boards (tracks)	509.7	221.5	56.5	1.5	7	87
	Burrow activity (#)	20.0	1.0	93.4			
Hog barn (Ohio)	Feed consumption (g)	38.3	0.4	99.9			
	Tracking boards (tracks)	285.8	0.0	100.0	0.0	30	7
	Live-trapping (#)	28.0	0.0	100.0			
Storage barn (S.Carolina)	Feed consumption (g)	22.3	0.0	100.0			
	Tracking boards (tracks)	245.8	3.5	98.6	0.0	12	15
	Live-trapping (#)	9.0	0.0	100.0			
Feed mill (Rhode Island)	Feed consumption (g)	36.0	4.2	88.3			
	Tracking boards (tracks)	584.5	234.8	59.8	0.2	21	9
	Live-trapping (#)	9.0	0.0	100.0			
Feed room (Pennsylvania)	Feed consumption (g)	49.6	1.9	96.2			
	Tracking boards (tracks)	207.1	73.3	64.6	0.1	18	10
	Live-trapping (#)	40.0	2.0	95.0			
Storage bldg. (Texas)	Total feed consumption (g)	46.9	15.8	66.3			
	Total feed consumption by mice (g)	29.5	7.9	73.2	0.5	18	1
	Tracking boards (tracks)	472.6	13.0	97.2			
Storage bldg. (Texas)	Feed consumption (g)	34.8	1.8	94.8	0.0	18	2
	Tracking boards (tracks)	99.9	3.9	96.1			
Farm supply store (California)	Feed consumption (g)	23.5	2.8	88.1			
	Tracking boards (tracks)	106.7	13.3	87.5	0.0	17	5
	Water consumption (ml)	162.7	99.0	39.1			
Outdoor trials							
Outdoor pen (Ohio)	Feed consumption (g)	62.9	0.8	98.7			
	Tracking boards (tracks)	283.4	0.0	100.0	0.0	7	10
	Live-trapping (#)	61.0	0.0	100.0			

Greater than 90% reduction of rodent population indices was obtained in most trials. The relative value of various population indices in these field evaluations is considered by Spaulding and Jackson (1982).

Particular attention was directed towards obtaining information concerning primary (accidental) or secondary poisoning hazards to nontarget species during the field trial program. No incidents of such poisoning were detected.

ANTICOAGULANT RESISTANT RODENTS

Laboratory determinations of bromethalin efficacy against resistant Norway rats and house mice required that test animals first be demonstrated warfarin-resistant by the standard World Health Organization tests (WHO 1970). Following a 30-day period on toxicant-free diet, such animals were subjected to standard EPA choice feeding efficacy tests with bromethalin (individually caged rats and penned mice). These test data indicate the efficacy of bromethalin against resistant animals (Table 6). Ninety percent of the test animals were killed, and consumption patterns were similar to those observed in other choice efficacy tests. The discrimination by female rats against the bromethalin bait is thought to be related to the use of insect-infested bait.

A field trial was conducted with 0.005% bromethalin bait against a resistant population of house mice in a poultry house. Resistance had been verified by using the standard WHO laboratory procedure. About two-thirds of the mice tested were warfarin resistant. Control was achieved under extremely difficult conditions (i.e., extremely large population with an abundant food supply in complex physical environment). The population in an adjacent poultry house actually increased during the trial period while feeding upon warfarin bait (Ashton et al. 1982).

Table 6. Summary of bromethalin choice test data with warfarin-resistant (WHO standard) rats and mice.

Species	No./ sex	Average 3-day consumption (g) EPA placebo	Bromethalin (0.005%)	Mortality	Bromethalin consumed (mg/Kg)
Norway rats (individually caged)	10M	8.2	7.0	10/10	1.0
	10F	21.3	7.7	9/10	1.5
	Total	14.8	7.4	19/20	1.2
House mice (pen test)	10M	3.4	3.3	8/10	5.9
	10F	1.6	1.3	10/10	2.6
	Total	2.5	2.3	18/20	4.3

TOXICOLOGY

Hazard Evaluation

Bromethalin is highly toxic in its pure state and for this reason is manufactured in a closed system. However, once diluted to a 0.1% concentrate or 0.005% final bait, the hazard to man and nontarget species is greatly reduced. There were no dermal, ocular irritation, and inhalation toxicity hazards at the 0.1 and 0.005% concentrations tested (Table 7) (Van Lier et al. 1980).

Table 7. Acute hazard evaluation of bromethalin.

Test	Tech material	0.1%	0.005%
Oral (rats) - LD_{50}	2.0 mg/kg	4305 mg/kg	5000 mg/kg
Dermal toxicity (rabbits)	3/6 deaths @ 200 mg/kg	No deaths @ 200 mg/kg	No deaths @ 200 mg/kg
Dermal irritation (rabbits)	None	None	None
Ocular irritation (rabbits)	Slight	Slight	Slight
Inhalation - LC_{50} 1hour exposure (rats)	.024 mg/L	No effect at maximum obtainable concentration	No effect at maximum obtainable concentration

Acute Nontarget Species Toxicity Studies

The acute toxicity (LD_{50}/LC_{50}) was determined for a number of nontarget species, both terrestrial and aquatic (Table 8). Results indicate that technical bromethalin is toxic to most species at ranges from 2-13 mg/kg. Feeding studies with quail and mallards resulted in LC_{50}s of 210 and 620 ppm, respectively. Species selectivity is obtained with bromethalin baits, since rodents will consume larger quantities of food per unit body weight than larger animals. No-effect levels also were determined for a number of species.

Table 8. Bromethalin toxicity data from acute and short-term feeding studies conducted with terrestrial and aquatic species.

Species	LD_{50}/LC_{50}	No-effect level
Dog - oral	4.7 mg/kg	-
Cat - oral	1.8 mg/kg	-
Monkey - oral	5.0 mg/kg	-
Rabbit - oral	13.0 mg/kg	-
Adult Quail - oral	4.6 mg/kg	1.0 mg/kg
Quail - dietary	210 ppm	6.25 ppm
Mallard - dietary	620 ppm	25 ppm
Pigeon - dietary	-	>250 ppm
Earthworms	-	100 ppm
Bluegill	120 ppb	18 ppb
Trout	>33 ppb <80 ppb	33 ppb
Daphnia	27 ppb	9 ppb

All aquatic data were obtained by introducing bromethalin into the test media with acetone, which allowed the true water solubility of bromethalin (less than 10 ppb) to be exceeded. The no-effect level is equal to or greater than the water solubility of bromethalin. It was concluded that a significant aquatic environmental hazard does not exist with the use of bromethalin.

Secondary Toxicity

Groups of rats were fed either 0.005% bromethalin bait or a placebo bait (without bromethalin) for 16 hours. This dose of bromethalin was sufficient to result in the death of ≥ 95 percent of the treated rats. Ninety control rats per sex and 70 treated rats per sex were used. The surviving rats were killed by cervical dislocation; all skins were removed and the carcasses ground. The resulting material was apportioned into 600-g blocks which were packaged, labeled, and frozen. Some dogs (high dose) were fed 600 g of treated rat meat daily. Other dogs were fed 600 g of rat meat daily consisting of three parts control meat with one part treated meat. Control dogs were fed 600 g of control rat meat daily.

Six beagle dogs were conditioned to a control rat meat diet for two days prior to being fed either the control, low, or high dose level diets. The dogs were maintained on the test rat meat diets for two weeks. None of the dogs showed any signs of toxicity attributable to bromethalin. No pathologic changes in nervous tissues were noted. It was concluded that the dogs consuming rats treated with bromethalin bait did not receive a sufficient amount of toxicant to produce any detectable signs of bromethalin toxicity. This study provides data to show that target animals that have ingested a lethal dose of bromethalin do not present a secondary hazard to predators.

Mode of Action

Signs of bromethalin toxicity can be divided into two categories: acute and chronic. Acute effects include tremors, one or two episodes of clonic convulsions, prostration, and death, usually within 18 hours. These effects occur when technical bromethalin is administered in a soluble form and given at a dose 2-fold or greater than the LD_{50} or by generous bait consumption. Chronic effects, which include lethargy, hind-leg weakness, loss of muscle tone and paralysis, occur with a single dose equal to the LD_{50}, with multiple smaller doses, or with sublethal dietary administration. Several acute and dietary studies have shown that, at sublethal doses, these effects are reversible if administration of the toxicant is discontinued.

Experiments in the physiological and biochemical mechanisms of action suggest that bromethalin uncouples oxidative phosphorylation in central nervous system mitochondria (Van Lier and Ottosen 1981). This could lead to a decreased production of ATP, a diminished activity of Na^+/K^+ ATPase, and a subsequent fluid buildup manifested by fluid-filled vacuoles between the myelin sheaths (Van Lier and Ottosen 1981). This vacuole formation in turn leads to an increased cerebrospinal fluid pressure and increased pressure on nerve axons, yielding a decrease in nerve impulse conduction, paralysis, and death.

The cerebral edema produced by sublethal doses of bromethalin can be ameliorated by treatment with an osmotic diuretic and corticosteroids. Intravenous infusion of hypertonic urea 24 hours after bromethalin reduced cerebrospinal fluid pressure (CSFP) to near control levels after 30 minutes. Elevated CSFP returned to normal in seven days after cessation of bromethalin feeding and could be reduced more rapidly by corticosteroid treatment (Cherry et al. 1982).

BAITING STRATEGY

Bromethalin is a unique, single-feeding rodenticide. It differs from anticoagulants in that continuous bait exposure is not necessary for effective control. There has been no evidence of bait-shyness with bromethalin, as seen with typical acute rodenticides, thus exposure to bait need not be limited. These unique properties necessitate the development of an effective baiting strategy for bromethalin.

Bait placement and timing are crucial in order to achieve effective rodent control with bromethalin bait. Initial placements of bait should be renewed at intervals of several days, but continuous bait availability (as with anticoagulants) is not required. However, bait needs to be exposed long enough for all animals in the area to feed. Bait quantities will be about one-third that used with anti-coagulants, since an animal ingesting a lethal dose does not feed again.

Social hierarchies in a population may control rodent feeding patterns. Low-ranking animals often are prevented from feeding initially, and movement into certain areas may be restricted. Several waves or surges of feeding activity have been observed during the first and sometimes second week of a baiting period (Spaulding and Jackson 1982). This may represent subdominants coming to baits for the first time--animals that received a sublethal dose and recovered coming back to feed--or immigrants arriving at feeding points. Knowledge of such patterns is important to efficacious use, and bait exposure may need to be extended accordingly.

CONCLUSIONS

Bromethalin has been shown to be an effective rodenticide in both laboratory and field studies against important commensal rodents. Properly used, it poses no danger to nontarget species. Bromethalin offers distinct advantages over currently used acute and anticoagulant rodenticides because of its unique mode of action.

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