March 1988

STATUS OF BROMETHALIN OUTSIDE THE UNITED STATES

S. R. Spaulding
Ciba-Geigy Animal Health/Hygiene, Basel, Switzerland

H. Spannring
Ciba-Geigy Agricultural Research Centre, St. Aubin, Switzerland

STATUS OF BROMETHALIN OUTSIDE THE UNITED STATES

S.R. SPAULDING, Ciba-Geigy Animal Health/Hygiene, CH-4002 Basel, Switzerland.

H. SPANNRING, Ciba-Geigy Agricultural Research Centre, St. Aubin, Switzerland.

ABSTRACT: Bromethalin has been extensively researched over the past decade in the United States, Switzerland, England, Denmark, and France. United States EPA registrations were received in 1982 and commercial pelleted formulations containing 0.01% bromethalin were developed and introduced in the USA by Ralston Purina (ASSAULT[1]*) in 1985 and Velsicol (VENGEANCE[1]*) in 1986. Ciba-Geigy is currently developing new formulations under the tradename DORATID[R] for use outside the United States. Bromethalin acute toxicity and 14-day subchronic studies are reviewed and data from recently completed 90-day subchronic studies required for registration outside the US are presented. Pharmacodynamic studies have shown that bromethalin acts as an uncoupler of oxidative phosphorylation, thus interrupting the vital production of ATP necessary to maintain essential metabolic functions. Laboratory and field trial data are presented from Switzerland, France, England, and Denmark that indicate the effectiveness of new bromethalin formulations against anticoagulant resistant and susceptible rodents. A comparative rodenticide pen testing system is described from which test results confirm bromethalin's quick action and feed consumption efficiency when compared to second-generation anticoagulants.

Proc. Vertebr. Pest Conf. (A.C. Ciabb and R.E. Marsh, Eds.),
Printed at Univ. of Calif., Davis. 13:64-69, 1988

INTRODUCTION

The worldwide development of bromethalin has been an expensive and lengthy process lasting over a decade. It has involved the dedicated work of people from a variety of disciplines including chemists, biologists, toxicologists, formulation scientists, regulatory specialists, and marketers.

The search for a novel rodenticide with a mode of action different from anticoagulants began in the mid-1970's when leads from a fungicide screening program at Lilly Research Laboratories indicated the rodenticidal potential of a group of compounds classified as diphenylamines (Dreikorn and O'Doherty 1984). After several years of screening and structure-activity relation tests, bromethalin was identified as the compound with the greatest biological potential. At the 1979 British Crop Protection Conference, Dreikorn (1979) presented the first biological results from laboratory tests with white rats and mice. Biological and toxicological testing continued at Lilly until sufficient data were assembled to submit an application for an Experimental Use Permit (EUP). In 1980, the EUP was issued by the Environmental Protection Agency (EPA) and a field trial program was initiated in conjunction with the Center for Environmental Research at Bowling Green State University. By the end of 1981, 17 successful field trials in Norway rat and house mouse infested sites were completed and the EPA granted bromethalin registration for use in and around buildings in 1982.

Marketing rights were subsequently licensed by Eli Lilly to Ralston Purina and Velsicol in the United States and Ciba-Geigy for all countries outside the United States. Ciba-Geigy initiated development in 1984 under the tradename DORATID[1]*. During the following year, ASSAULT[1]* was introduced into U.S. markets by Ralston Purina, and Velsicol followed in 1986 with the introduction of Vengeance. Over the past 4 years, Ciba-Geigy has focused on formulation development of grain-based baits and a bonded weather-resistant block, as well as conducting several additional toxicology studies required to meet European registration requirements. This work is now coming to an end and registration was initiated in 1988 in selected European countries.

It is the intent of this paper to review the status of bromethalin and to discuss efficacy and toxicology studies conducted outside the United States over the past 4 years.

REVIEW OF SELECTED BROMETHALIN TOXICOLOGICAL PROPERTIES

Toxicological aspects of bromethalin have been previously reviewed by Jackson et al. (1982), Spaulding et al. (1985), and Spaulding (1987). This brief review of toxicological data focuses on acute and subchronic toxicity and aspects related to pharmacodynamics, pharmacokinetics, and metabolism.

Acute Toxicity

Bromethalin acute LD_{50} values for the active ingredient in target and non-target species range from 1.8 mg/kg (cat) to 13.0 mg/kg (rabbit). LD_{50}'s of 2.3,6.7, and 6.6 mg/kg have been determined for the Norway rat, house mouse, and roof rat, respectively. The acute LD_{50} of the 0.01% finished bait is >2000 mg/kg.

Subchronic Toxicity

Subchronic and chronic toxicity tests with pesticides are designed to assess the toxicity and human carcinogenicity risk to humans in prolonged exposure situations. The EPA required only 14-day feeding studies with bromethalin to

support registration in the United States; however, 90-day feeding studies were required for registration in several European countries. While these 90-day tests are certainly applicable in the case of herbicides and insecticides applied to crops, the scientific value of these studies with rodenticides is questionable due to completely different use patterns. Rodenticides are applied in discrete locations in relatively small quantities; therefore, the potential for human exposure or food contamination is minimal.

In several early toxicity studies with bromethalin (van Lieretal. 1980, van Lier and Ottosen 1981), histopathological signs of toxicity indicated vacuolation of the white matter of the brain and spinal cord, which appeared as a spongy degeneration. To determine whether this spongy degeneration was a reversible phenomenon, two 14-day subchronic reversibility studies were undertaken (van Lier et al. 1980, van Lier and Cherry 1988). Rats were dosed with 0,0.5,2 or 8 ppm bromethalin in one study and 0, 1, 3, and 5 ppm bromethalin in a second study. Brain, spinal cord and sciatic nerve tissues were examined histologically over an 8-week recovery period. Behavior and motor coordination were measured in the second study using a rotorod. All rats given 8 ppm bromethalin died during treatment or were killed when moribund. Leg weakness and depressed motor function were observed by day 7 in rats given 5 ppm bromethalin. Electron and light microscopic examination of rat tissues taken at various times during these studies demonstrated that the primary lesions which occurred were spongy degeneration of the CNS white matter. However, the incidence of spongy degeneration decreased sharply at two and four weeks posttreatment and was not evident at eight weeks posttreatment, demonstrating the reversibility of these lesions. Furthermore, histological examination of tissues revealed no evidence of cell necrosis.

In order to conduct 90-day subchronic toxicity studies with inherently toxic rodenticides, extremely low doses must be administered in order to keep test animals alive throughout the 90-day test period. These doses were established for bromethalin subchronic studies by conducting 4-week pilot studies first with rats and dogs. In definitive studies (Ciba-Geigy, unpubl. rept.), rats and dogs were dosed with 0,5,25, 125, and 200 (dogs only) mcg/kg bromethalin daily over 90 days.Lesions were evident only atdosesof > 125 mcg/kgand were characterized by the typical spongy degeneration of CNS white matter seen in the 14-day studies. Even under extended subchronic exposure, a "no observable effect level" (NOEL) of 25 mcg/kg was demonstrated in rats and dogs.

Pharmacodvnamics/Pharmacokinetics/Metabolism

Several studies have been conducted to investigate the mechanism of action, metabolism, tissue distribution and excretion of bromethalin in laboratory rats. Van Lier and Cherry (1988) recently described the mechanism of action of bromethalin. Based upon their results from several mechanistic studies, the following mode of bromethalin toxicity is proposed. Upon ingestion, bromethalin is metabolized to desmethyl bromethalin which uncouples oxidative phosphorylation in the mitochondria. The sodium/potassium gradient normally maintained by sodium-potassium ATPase is weakened, and fluid builds up in the cell. While most tissues and organs can expand slightly to accommodate this effect, a fluid build-up inside the central nervous system leads to increased pressure on nerve axons. Demyelination occurs and nerve impulse transmission is reduced. In the lethally intoxicated animal, this leads to inadequate transmission to respiratory centers with ultimate respiratory arrest and death. The entire process is dose-dependent and death generally occurs from 12-72 hours following ingestion.

Studies with radio-labelled bromethalin have provided insights into the pharmacokinetic and metabolic aspects of bromethalin (van Lier and Ottosen 1981, van Lier and Cherry 1988). One study using radio-labeled bromethalin resulted in disappearance of radiocarbon from plasma at a rate which indicated a half-life of about 135 hours. Other studies using radio-labeled bromethalin indicated excretion is primarily through the feces and that bromethalin is absorbed after oral administration and distributed primarily to the fat, liver, gastrointestinal tract, adrenals, ovaries and thyroid tissues.

LABORATORY EFFICACY STUDIES

Laboratory choice tests were conducted with a variety of 0.01% bromethalin grain-based and weather-resistant bonded block formulations using anticoagulant susceptible and resistant Rattus norvegicus and Mus musculus. The studies were conducted at the following international research institutes:

1. Ciba-Geigy Agricultural Research Centre - Switzerland
2. INRA, Laboratoire de la Faune Sauvage - France
3. Danish Pest Infestation Laboratory - Denmark
4. MAFF Tollworth Laboratories -U.K. Ministry of Agriculture, Fisheries and Food

Data from the Ciba-Geigy Agricultural Research Centre have been published in part by Spaulding (1987).

A pooled data summary of choice tests with bromethalin grain-based formulations using individually-caged wild R. norvegicus is presented in Table 1. A total of 422 rats were used in 27 different tests. Formulations included 0.01% bromethalin whole oats and wheat, cracked or pinhead oats, or oat flakes. Average percent mortality of 97% (range 94-100%) was achieved within an average of 2.3 days (range 1.4-4.0 days) following ingestion of bromethalin baits. Formulations containing bromethalin were consumed an average of 48% (range 30-71%) of the time compared to untreated alternative diets.

A pooled data summary of choice tests with bromethalin weather-resistant block formulations using individually-caged R. norvegicus is presented in Table 2. A total of 145 rats were used in 11 different tests. Formulations consisted of whole wheat or oats or cracked maize impregnated with 0.01% bromethalin and bonded together in a patented process. Laboratory tests have shown this formulation to be highly palatable to rats and resistant to moisture and heat. Average percent mortality of 96% (range 83-100%) was achieved within an average of 3.5 days (2.1-4.6 days) following

Table 1: Choice tests with 0.01% Bromethalin Grain Formulations Against Individually-Caged R. norvegicus.

Country*	Grain	N (No.Tests)	Bromethalin Consumption %	Mortality %	Days to Death
SWIT[1]	Oats	22(2)	47	95	2.9
SWIT[1]	Oats	100(7)	63	98	2.1
SWIT[1]	Oats	32(2)	33	94	1.9
SWIT[1]	Cracked Oats	100(5)	71	99	1.7
SWIT[1]	Cracked Oats	48(3)	57	96	1.4
SWIT[1]	Wheat	40(2)	30	100	2.4
FRANCE[2]	Oats/ Wheat	40(2)	30	98	1.8
DENMK[3]	Oat Flakes/Wheat	20(2)	43	100	4.0
GRBRIT[4]	Wheat/ Oats (pinhead)	20(2)	56	90	2.7
		N = 422	x = 48	x = 97	x = 2.3

*Source: 1. Ciba Geigy Unpublished Report
2. Grolleau. 1987. Proceedings Rome EPPO/FAO Conference on Rodents
3. Lund. 1986. Unpublished Data
4. Greaves. 1986. Unpublished Data

ingestion of the bromethalin blocks. Blocks containing bromethalin were consumed an average of 60% (range 47-82%) of the time compared to untreated alternative blocks.

A pooled data summary of choice tests with bromethalin grain-based formulations using family groups (8-20 mice/group) of wild M- musculus is presented in Table 3. Formulations included 0.01% bromethalin canary seed, cracked oats, or oat flakes. A total of 326 mice were used in 20 different tests. Average percent mortality of 97% (range 92-100%) was achieved within an average of 2.3 days (range 1.5-2.8 days) following ingestion of bromethalin baits. Formulations containing bromethalin were consumed an average of 59% (range 37-88%) of the time compared to untreated alternative diets.

A pooled data summary of laboratory tests with bromethalin grain-based formulations using anticoagulant-resistant R. norvegicus and M- musculus is presented in Table 4. A total of 170 resistant rodents were tested at research institutes in France, England, and the United States. The R.

Table 2. Choice Tests with 0.01% Bromethalin Weather-Resistant Block Formulations Against Individually-Caged R. norvegicus.

Country*	Grain	N (No.Tests)	Bromethalin Consumption %	Mortality %	Days to Death
SWIT[1]	Wheat	24(2)	72	100	2.1
SWIT[1]	Wheat	12(1)	67	100	4.1
SWIT[1]	Maize	24(2)	47	100	4.0
SWIT	Oats	15(1)	82	100	3.4
FRANCE[2]	Wheat/ Oats	40(2)	47	95	2.8
DENMK[3]	Wheat/ Oats	30(3)	47	83	4.6
		N = 145	x = 60	x = 96	x = 3.5

*Source: 1. Ciba-Geigy Unpublished Report
2. Grolleau. 1987. Proceedings Rome EPPO/FAO Conference on Rodents
3. Lund. 1986. Unpublished Data
4. Greaves. 1986. Unpublished Data

Table 3. Choice Tests with 0.01 % Bromethalin Grain Formulations Against Family Groups of M- musculus

Country*	Grain	N (No.Tests)	Bromethalin Consumption %	Mortality %	Days to Death
SWIT[1]	Canary Seed	30(3)	66	98	2.8
SWIT[1]	Oat Flakes	10(1)	88	100	1.5
SWIT[1]	Cracked Oats	106(8)	37	97	2.1
FRA[2]	Canary Seed/Oat Flakes	60(3)	54	92	2.3
DENMK[3]	Canary Seed/Oat Flakes	120(5)	48	100	2.6
		N = 326	x = 59	x = 97	x = 2.3

*Source: 1. Ciba-Geigy Unpublished Report
2. Grolleau. 1987. Proceedings Rome EPPO/FA Conference on Rodents
3. Lund. 1986. Unpublished Data

Table 4. Laboratory Tests with 0.01% Bromethalin Grain Formulations Using Anticoagulant-Resistant R. norvegicus (RN) and M- musculus (MM).

Country***	Species	N (# Tests)	Bromethalin Consumption %	Mortality %	Days to Death
FRA[1]	RN*	10	No-Choice	100	1.6
FRA[1]	RN*	20	30	80	3.4
GB[2]	RN	10	63	100	1.7
GB[2]	MM	30	51	87	2.4
USA[3]	RN	50	33**	95	4.0
USA[3]	MM	50	48**	90	4.0
		N = 170	x = 45	x = 92	x =2.9

*Bromadiolone-resistant
** 0.005% bromethalin in EPA diet
*** Source: 1. Grolleau. 1987. Proceedings Route EPPO/FAO Conference on Rodents
2. Greaves. 1986. Unpublished data
3. Jackson et al. 1982. Proc. 10th Vert. Pest. Conf.

norvegicus tested in France were determined resistant to bromadiolone. Average percent mortality of 92% (range 80-100%) was achieved within an average of 2.9 days (1.6-4.0 days) following ingestion of bromethalin baits. Formulations containing bromethalin were consumed an average of 45% (range 30-63 %) of the time compared to untreated alternative diets.

FIELD EFFICACY TRIALS

Seventeen (17) field trials were completed with 0.005% bromethalin EPA baits in primarily rural sites such as livestock barns, poultry farms and storage buildings in 5 geographically different regions of the United States (Jackson et al. 1982, Spaulding et al. 1985). Average percent reduction in rodent activity in these trials ranged from 90-98% in 8 R. norvegicus tests and from 85-99% in 9 M-musculus tests. Bromethalin treatment periods ranged from 7-30 days and there were no incidents of primary or secondary poisoning to non-target species during the trial program.

Field trials completed outside the United States are summarized in Tables 5 and 6 for R. norvegicus and M-musculus respectively. Nine (9) R. norvegicus infestations were treated with 0.005% or 0.01% bromethalin grain baits in Switzerland, Denmark, England, Ireland and Brazil. Average percent reduction in census feed consumption ranged from 83 to 100% after 3 to 14 days of exposure to bromethalin baits. Four (4) M. musculus populations were treated with 0.01% bromethalin grain baits in France and Switzerland. Average percent reduction in census feed consumption ranged from 98 to 100% with 3 to 16 days of exposure to bromethalin baits. One hundred-fifty (150) mouse carcasses were recovered during the trial in France. There were no incidents of primary or secondary poisoning to non-target species in these trials.

Table 5. Field Trials with 0.005% and 0.01% Bromethalin Grain Formulations Against Infestations of R. norvegicus.

Country	Site	Treatment Days	Reduction rodent activity %	Reference
SWIT	Indoor Pen	6	100	Ciba-Geigy Rept. II-44
DK	Swine Barns	4	83(75-93)*	Lund Unpubl Data 1986
GB**	Farm Bldg.	14	90(80-100)*	Meehan 1983 Proc. 6th BPCA Conf.
IRL.	Railway Station	3	100	Ciba-Geigy Rept. II-47
BRA**	Urban Slum	4	100	Carvalho 1985 Biologico

*Pooled Summary of 3 Trials
**0.005% Bromethalin Bait

Table 6. Field Trials with 0.01% Bromethalin Grain Formulations Against Infestations of M-musculus.

Country	Site	Treatment Days	Reduction rodent activity %	Reference
FRA	Farm Bldg.	3	98	Grolleau Unpubl Data.1987
SWIT	Indoor Pen	7	100	Ciba-Geigy Rept. II-42
SWIT	Swine Barn	3	98	Ciba-Geigy Rept. II -43
SWIT	Warehouse	16	99	Ciba-Geigy Rept. II-48

RODENTICIDE PEN TESTING

Test Procedure

An indoor pen test system was established so that indirect comparisons of rodenticides could be made under similar conditions. Indoor pens measuring 4x2x2 (8 m2) were con-

structed on a concrete base with stainless steel wire mesh. The bottom of the pens was covered with approximately 10 cm of saw dust and abundant harborage was provided in the form of overturned plastic containers. Wild male and female Norway rats were introduced into the pen and provided standard laboratory diet and water ad libitum. Rodents were allowed to acclimate for 10-12 weeks before testing and obviously dominant or submissive animals were removed. Twelve (12) bait trays containing either 2 kg of rodenticide or untreated grain were distributed throughout the pen. The position of the bait trays was alternated daily and consumption was measured. Dead animals were removed when located. A diagram of the testing facility is shown in Figure 1.

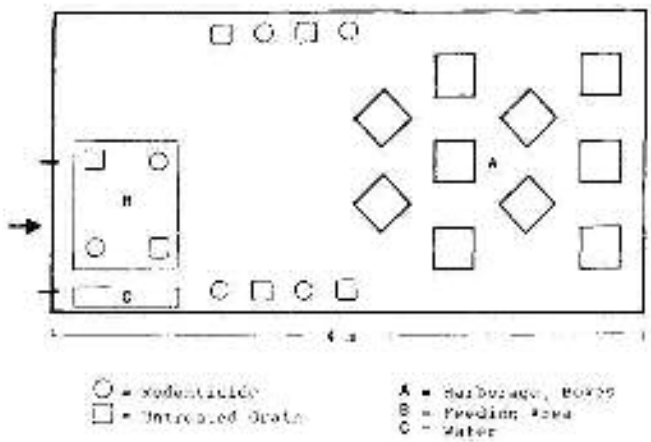

Fig. 1. Diagram of rodenticide test pens.

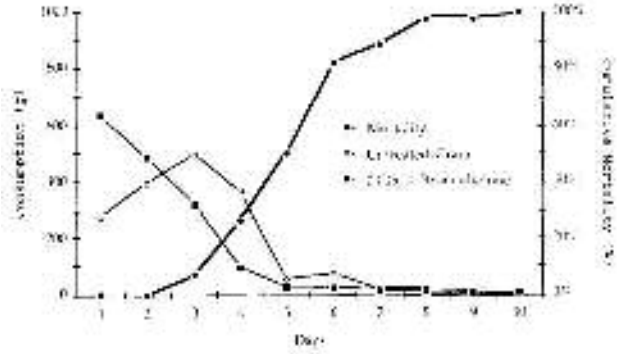

Fig. 3. Efficacy of 0.005% Bromadiolone oat baits in a pen trial with 92 wild Norway rats.

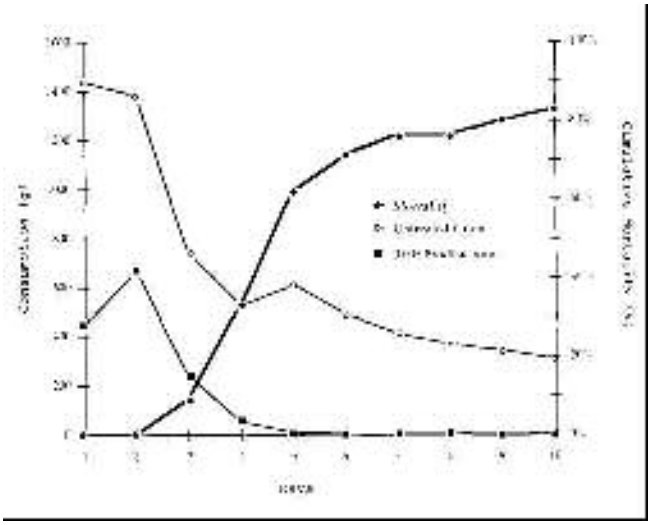

Fig. 4. Efficacy of 0.005% Brodifacoum pellets in a pen trial with 94 Norway rats.

Comparative Rodenticide Tests

The objective of these tests was to compare the palatability and efficacy of bromethalin with leading second-generation anticoagulants under controlled conditions.

Results from tests with 0.01% bromethalin and C.005% bromadiolone oat formulations and 0.005% brodifacoum commercial pelleted formulations are presented in Figures 2, 3, and 4, respectively.

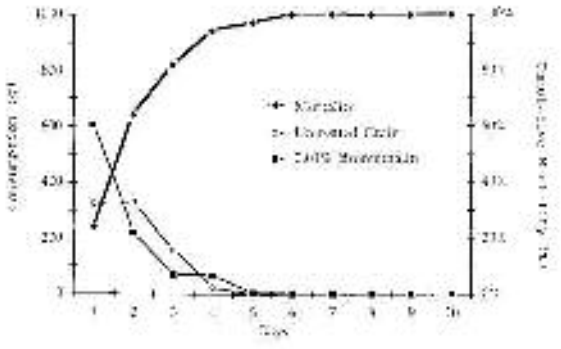

Fig. 2. Efficacy of 0.01% Bromethalin oat baits in a pen trial with 125 Norway rats.

A total of 836 g of untreated oats and 973 g of 0.01% bromethalin oat baits were consumed by 125 R. norvegicus in the bromethalin test pen over 6 days. This corresponds to 28.7 g of bromethalin bait/kg rat and 24.7 g untreated grain/kg rat. Complete mortality was achieved by the sixth day of baiting (x = 2.4 days).

In the bromadiolone test pen, a total of 1722 g of untreated oats and 1628 g of 0.005% bromadiolone oat baits were consumed by 92 R. norvegicus over 10 days. This corresponds to 99.1 g of bromadiolone bait/kg rat and 93.7 g untreated grain/kg rat. Complete mortality was achieved by the 10th day of baiting (x = 5.5 days).

A total of 6615 g of untreated grain and 1442 g of 0.005% brodifacoum pellets were consumed by 94 R. norvegicus over the 10-day test period. This corresponds to 60.1 g of brodifacoum bait/kg rat and 275.6 g of untreated grain/kg rat. Incomplete mortality of 83% (78/94) was achieved after 10 days of baiting. Rats which survived after 6 days fed almost exclusively on untreated grains rather than brodifacoum pellets.

Results from these tests illustrate differences between the two second-generation anticoagulants and bromethalin based upon their respective modes of action and formulation types. Rats continued to feed on the two anticoagulants over the 10 day test period resulting in at least 2 to 3 times more anticoagulant bait consumption and 3 to 11 times more untreated grain consumption when compared to results from the bromethalin test pen. Feed consumption in the bromethalin pen virtually stopped after 4 days, indicative of the efficiency of bromethalin baiting under semi-natural conditions. Dead rodents were rapidly observed within 24 hours after baiting with bromethalin and complete mortality was achieved by the 6th day as compared to 10 days with bromadiolone. Untreated grain was preferred over pelleted brodifacoum formulations leading to incomplete mortality with this product under these experimental conditions.

Overall, these results illustrate the quick kill and efficiency possible when utilizing bromethalin as an alternative to anticoagulant rodenticides for controlling Norway rat infestations.

CONCLUSIONS

The diphenylamine rodenticide bromethalin has been thoroughly researched over the past decade and commercial formulations have been safely used in and around buildings in the United States over the past 3 years. International development work has shown bromethalin to be effective against susceptible and anticoagulant-resistant rodents. Data required for European registrations are nearly complete and will be submitted to authorities in selected countries throughout 1988. Bromethalin's quick kill and stop-feed action make this rodenticide a highly efficient alternative to anticoagulants.

ACKNOWLEDGMENTS

The authors would like to thank G. Grolleau (INRA, France); M. Lund (PPIL, Denmark); and J. Greaves (Hereford, England) for their generosity in allowing us to use their unpublished data. We would also like to thank Mr. P. Bula from the Ciba-Geigy Agricultural Research Centre in St. Aubin, Switzerland, for his assistance in conducting laboratory and field tests.

LITERATURE CITED

CARVALHO, C.N. and F.A. MELLO. 1985. Field trial with a new rodenticide: bromethalin. Biologico 51(4): 334-336.

DREIKORN, B.A., G.O.P. O'DOHERTY, A.J. CLINTON, and K.E. KRAMER. 1979. EL-614, a novel new acute rodenticide. Proceedings British Crop Protection Conf.: 491-498.

DREIKORN, B.A. and G.O.P. O'DOHERTY. 1984. The discovery and development of bromethalin, an acute rodenticide with a unique mode of action. In: ACS Symposium Series #255. Pesticide Synthesis Through Rational Approaches, P.S. Magee, G.K. Kahn, J.J. Menn (Eds.). American Chemical Society .Washington, D.C. pp. 45-63.

CHERRY, L.D., M.D. GUNNOE, and R.B.L. VAN LIER. 1982. The metabolism of bromethalin and its effects on oxidative phosphorylation and cerebrospinal fluid pressure. The Toxicologist 2(1): 108.

G. GROLLEAU. 1987. Susceptibility of 3 commensal rodent species to bromethalin under laboratory conditions. Proceedings Rome EPPO/FAO Conference on Rodents (in press).

JACKSON, W.B., S.R. SPAULDING, R.B.L. VAN LIER, and B.A. DREIKORN. 1982. Bromethalin - apromising new rodenticide. Proceedings 10th Vertebrate Pest Conference, R.E. Marsh (ed.). Monterey, California, pp. 10-16.

MEEHAN, A.P. 1983. Some properties of bromethalin, a new rodenticide. 6th Br. Pest Control Ass.Conf., Cambridge. Sept.7-10. Session 5. Paper 11. 16 pp.

SPAULDING, S.R., R.B.L. VAN LIER, and M.E. TARRANT. 1982. Toxicity and efficacy of bromethalin. Acta Zoologica Fennica 173: 171-172.

SPAULDING, S.R. 1987. Bromethalin - an alternative to anticoagulants. BCPC Mono.No.37 Stored Products Pest Control: 137-147.

VAN LIER, R.B.L., L.D. OTTOSEN, G.K. HANASONO, and J.L. CARTER. 1980. Studies on the toxicity of EL-614, a new rodenticide. Presentation 19th Annual Meeting of the Society of Toxicology.

VAN LIER, R.B.L., and L.D. OTTOSEN. 1981. Studies on the mechanism of toxicity of bromethalin, a new rodenticide. The Toxicologist 1(1):114.

VAN LIER, R.B.L. and L.D. CHERRY. 1988. The toxicity and mechanism of action of bromethalin: a new single-feeding rodenticide. Fundamentals of Applied Toxicology (submitted for publication November 1987).