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ABSTRACT: Under an Environmental Protection Agency Experimental Use Permit, a pelleted bait containing 0.0025% (25 ppm) of the new anticoagulant difethialone was tested to determine the effectiveness in controlling Norway rats *(Rattus norvegicus)* and house mice *(Mus musculus)*. Sixteen (16) individual field studies were conducted in five (5) geographical locations of the United States. The results were conclusive in showing that difethialone bait formulated at 25 ppm was both palatable and efficacious in controlling both Norway rats and house mice under actual field conditions.

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INTRODUCTION

Difethialone is the first representative of a new anticoagulant chemical family called hydroxy-4 benzothiopyranones (Lechevin and Poch, 1988). Difethialone being of French origin (Lipha SA), the pharmacological and toxicological properties were reported by Lechevin (1986), as well as the activity of the compound in commensal rodents (Lechevin 1986, 1987b) and on several field species (Lechevin 1987a).

LiphaTech, Inc. sought and was granted an Environmental Protection Agency Experimental Use Permit in 1989 to allow the field testing of the compound as a requirement for the EPA registration of difethialone for control of Norway rats and house mice. These data were submitted as support for an Application for Pesticide Registration of difethialone pellets for control of rodents (rats and mice) in and around the periphery of homes, industrial, commercial and public buildings in urban areas, and inside homes and agricultural buildings in non-urban areas.

The U.S. Environmental Protection Agency requires the successful completion of the following field studies in the United States:

1) Norway Rats:
   5 indoor studies (one in each of the 5 regions) 2 outdoor studies (different regions)
2) House Mouse:
   5 indoor studies (one in each of the 5 regions) 1 outdoor study (any region)

A field trial must meet the following EPA requirements:

1) Efficacy data must show a 70% or greater reduction in the target population.
2) At least two (2) acceptable methods of pretreatment and posttreatment population censusing must be conducted.
3) The posttreatment census must be followed immediately by three (3) days of snap trapping.
4) Snap trapping must indicate a rate of no more than one (1) target animal captured per 10 snap traps set per night

METHODS

Suitability of the test site is often the most difficult aspect of a rodent field trial. Several guidelines must be met before a site is deemed suitable, these are:

1) Adequate rodent infestation (20-100) rodents per site.
2) Infestation by a single rodent species.
3) Cooperation of individuals owning/controlling the test site.
4) Reasonably isolated rodent infestation to prevent reinvasion.
5) Minimal hazard to nontarget species.
6) Minimal chance of contamination of food, water or the environment.
7) Lack of other chemical controls applied within the past 30 days.
8) Relatively free of human or domestic animal disturbance.
9) Lack of competitive feed on the site.

Once a potential trial site was located, the cooperator completed a Pretrial Site Evaluation which included several preprinted forms which are: Evaluation of Potential Rodenticide Trial Sites, General Site Description, Control History at General Site, Specific Trial Site Characteristics and Hazards at Specific Trial Site. Once a site was found suitable, a trial was initiated mindful of the following EPA requirements:

1) General and specific site maps are necessary, including locations of census points, toxic bait placements, traps, and recovered carcasses.
2) All raw census data including pre and posttreatment and snap trapping data.
3) Amounts of test material (difethialone) distributed at the test site.
4) Summary of climatic data obtained from the hygrothermograph and local weather station during the trial period.
5) Summary information concerning reduction in activity expressed as percent reduction for each census technique.
6) Snap trapping data, expressed as the number of target rodents trapped per 10 traps per night.

In addition to the EPA requirements, trials were conducted utilizing the normal parameters of field testing methodology (Kaukeinen 1979) which included, but were not limited to:

Familiarization Period

Verification of rodent species present by live-trapping, utilization of tracking boards, and/or conducting thorough searches for rodent signs and active points. In addition, the establishment of at least two (2) censusing techniques which included:

1) Food consumption.
2) Tracking determinations
3) Live-trap, mark, release and recapture.
4) Determination of active burrows.
5) Presence and quantity of droppings.
6) Actimeter counts.
7) Visual counts of rodents.
8) Determination of gnawing.
9) Water consumption.

Pretreatment Census

The pretreatment census was conducted for at least three (3) days after sufficient stabilization of activity patterns following the Familiarization Period. A specific map was drawn indicating pretreatment census points. Data from at least two (2) census techniques were collected on a daily basis.

Pretreatment Lag Phase

To minimize any possible effects of preconditioning, a lag phase of three (3) days with no disturbance between the pretreatment census and the treatment phase is required for all trials.

Treatment Phase

Difethialone 0.0025% (25 ppm) pelleted bait was distributed in either "tamper-proof bait stations where rodent activity was evident, placed directly in burrows in pre-weighed packages, or presented in such a manner so that the bait would not be accessible to children, pets, domestic animals, or wildlife. Bait was not placed in areas where there is a possibility of contaminating food, or surfaces that come in direct contact with food. Difethialone bait was provided in quantities consistent with the proposed Directions for Use (i.e. 4-16 ounces per placement for rats; 1/4-1/2 ounce per placement, up to 2 ounces per placement at high activity areas for mice.

Bait stations were placed at different locations from those used for census baiting. The duration of the bait exposure was extended as long as there was evidence of bait consumption that was attributed to the target species. Food consumption was recorded daily. If burrows were treated, consumption was not monitored, but the total amount of bait placed was recorded.

Moisture control stations, similar to those that held the bait, were placed in the census area to determine daily moisture pick-up or loss by the bait. These stations were inaccessible to both target and nontarget species.

Toxic bait stations and/or snap traps were distributed around the perimeter of the trial site to minimize the invasion of peripheral animals. This buffer baiting followed the same schedule as baiting in the census areas.

Posttreatment Lag Phase

After the toxic bait was removed, a three (3) day lag period was utilized where no disturbance took place. The lag phase allowed a time period for sick animals to die or recover so that the posttreatment survey did more accurately reflect the effects of the test bait.

Posttreatment Census

Posttreatment census techniques remained the same as those utilized during the Pretreatment Census. The posttreatment census was conducted for at least three (3) days and data from at least two (2) census techniques was collected on a daily basis.

Snap Trapping Phase

Immediately following the Posttreatment Phase (including live trap methods if used as a census technique), three (3) days of snap trapping was initiated using appropriate rat and/or mouse traps.

Approximately as many snap traps were used as there were baiting points with a minimum of ten (10) traps per night providing a minimum of at least 30 trap nights. Specific site maps were prepared indicating locations of snap traps and recovered carcasses.

Data Evaluation

Data for the census methods used were reported in a percent reduction of activity and the related percent control of the rodent population. Census data was presented by the following:

$$100 - \frac{\text{posttreatment census data}}{\text{pretreatment census data}} \times 100 = \%\ \text{reduction}$$

RESULTS AND DISCUSSION

Indoor Norway Rat Trials

Seven (7) individual indoor Norway rat field trials were conducted (one trial was replicated and is therefore counted as two trials). Site description, duration, census method and percent reduction per census technique is shown in Table 1. Duration range was 13 to 32 days with a mean of 18.0 days with an average reduction of Norway rat population of 96.2%. Mean percent reduction by repetitive census methods is as

Table 1. Norway rat indoor field trials with site description, duration, census method, and % reduction.

Site	Duration (days)	Census Method	% Reduction
Grain elevator	15	Tracking patches	97.0
		Visual count	94.7
		Sound	
Farm equipment & seed storage warehouse (simulated)	15	Food consumption	100[a]
		Tracking patches	100[a]
		Actimeter	100[a]
		Carcass count	100[a]
Wood & sheet metal feed storage	20	Food consumption	91.1
		Tracking patches	73.2
Horse/storage barn	16	Food consumption	91.9
		Tracking patches	96.0
Bird coop	32	Food consumption	100
		Tracking patches	90.4
		Active burrow counts	93.1
Swine keep	13	Food consumption	98.1
		Tracking patches	100

[a]Average of two replications.

follows: 1) Tracking Patches—93.8%; 2) Food Consumption—96.9%; and 3) Actimeter—100.0%.

In the swine keep trial site during the Snap Trapping Phase, one (1) trap was sprung and one (1) rat was captured which equates to 0.31 target animals. All other snap traps at all trial sites were unsprung equating to 0.0 target animals which confirms the positive control results indicated by the census methods.

It should be noted that a possible non-target incident occurred in one field trial conducted in a bird coop. The incident occurred during the posttreatment lag phase, when several chickens died. Two (2) peacocks also died during the posttreatment and snap-trap phases. Residue analysis of a dead peacock proved negative for difethialone. The death of these birds was not the result of anticoagulant poisoning.

Outdoor Norway Rat Trials

Two (2) individual outdoor Norway rat field trials were conducted. Site description, duration, census method and percent reduction per census technique is shown in Table 2. Duration range was 11 to 13 days with a mean of 12 days with an average reduction of Norway rat population of 83.5%. Mean percent reduction by repetitive food consumption census methods was 86.7%.

In the exterior grain mill trial site during the Snap Trapping Phase, the number of traps applied was purposely increased from 10 traps to 20 traps to provide additional information relative to the control observed. During the Snap Trapping Phase, one adult Norway rat was captured on the first night of trapping. One (1) house mouse was captured on the final night of trapping, and was counted as a sprung trap. No other rats were observed during the remainder of the phase. Using 20 traps (60 trap nights; 7 of which were sprung), the number of target rodents captured per 10 trap nights was 0.19. The same number of sprung traps, but with the number of traps decreased to only 10 traps (30 trap nights), equates to 0.42 target rodents captured. All other snap traps at all trial sites were unsprung equating to 0.0 target animals which confirms the positive control results indicated by the census methods.

No non-target exposures were noted in any of the trials.

Indoor House Mouse Trials

Eight (8) individual indoor house mouse field trials were conducted (one trial was replicated and is therefore counted as two trials). Site description, duration, census method and percent reduction per census technique is shown in Table 3. Duration range was 12 to 26 days with a mean of 21.125 days with an average reduction of house mouse population of 95.9%. Mean percent reduction by repetitive census methods is as follows: 1) Food Consumption—97.3%; 2) Tracking Patches—94.0%; and 3) Actimeter—94.0%.

In the farm equipment seed storage warehouse (simulated) site during the Snap Trapping Phase, one (1) mouse was captured per repetition which equates to 0.28 target rodent per repetition. In the hog farrowing trial site during the Snap Trapping Phase, one (1) mouse was captured which equates to 0.11 target animals captured per 10 traps set per trap night. All other snap traps at all trial sites were unsprung equating to 0.0 target animals which confirms the positive control results indicated by the census methods.

No non-target exposures were noted in any of the trials.

Outdoor House Mouse Trial

Only one (1) outdoor house mouse trial was conducted as required by EPA Guidelines. Finding a suitable outdoor site for house mouse trials proved to be a nearly impossible task, therefore, no further outdoor trials are planned as of this writing. The site consisted of a corn storage crib. The duration of the test was 28 days. Percent reduction of the mouse population by census technique is as follows: 1) Food Consumption—86.9%; 2) Tracking Patches—89.7%; and 3) Fecal Count—86.4%. All snap traps set as part of the Snap Trapping Phase were unsprung equating to 0.0 target animals which confirms the positive control results indicated by the census methods. No non-target exposures were noted in the trial.

Table 2. Norway rat outdoor field trials with site description, duration, census method, and % reduction.

Site	Duration (days)	Census Method	% Reduction
Junk pile/ exterior grain mill	13	Active burrow counts	75.0
		Food consumption	92.8
Exterior of grain mill	11	Tracking patches	85.6
		Food consumption	80.6

Table 3. House mice indoor field trials with site description, duration, census method, and % reduction

Site	Duration (days)	Census method	% Reduction
Grain storage building	22	Food consumption	99.7
		Tracking patches	96.6
Farm equipment seed storage warehouse (simulated)	21	Food consumption	97.0[a]
		Tracking patches	88.3[a]
		Actimeter	94.0[a]
		Carcass count	97.0[a]
Sheet metal horse barn	20	Food consumption	99.6
		Tracking patches	99.1
Abandoned pigeon coop	12	Food consumption	99.2
		Tracking patches	97.5
Turkey holding building	23	Food consumption	95.9
		Tracking patches	90.8
Unused sheep pen	24	Food consumption	100.0
		Tracking patches	100.0
		Trap/mark/release	100.0
Hog farrowing farm	26	Food consumption	89.4
		Tracking patches	85.7

[a]Average of two replications.

CONCLUSION

The experimental rodenticide difethialone was evaluated against free ranging indoor/outdoor populations of Norway rats and house mice under a variety of conditions where natural food sources were abundant Rodenticide formulations are

considered effective in the field when they demonstrate a minimum 70% reduction in activity when measured by two independent methods, and by capture of no more than 1 target rodent per 10 traps set. Difethialone pellets at the concentration of 25 ppm exceeded the EPA criteria, showing excellent bait consumption and population reduction in resident populations of target rodents at the same time showing low hazards to non-target species if used according to proposed label directions.

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