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COMPARATIVE EVALUATION OF TAMPER-PROOF MOUSE BAIT STATIONS

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ABSTRACT: A method for the evaluation of mouse control using tamper-proof mouse bait stations was developed and efficacy trials conducted to determine if house mice (*Mus musculus*) would visit and consume rodenticidal baits located within these stations. All stations were rapidly investigated by mice. Variation seen between the individual stations related to animal variation and did not appear to be related to differences in the stations themselves. Station placement was more critical to mouse investigation and subsequent bait consumption from the station than were the various features used to prohibit non-target access.

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INTRODUCTION

The Environmental Protection Agency issued PR Notice 83-5 (Anon. 1983), citing over 1000 exposures per year, with 80% involving children under five years of age. This notice indicated the Agency's concern and specifically mentioned several bait stations that met EPA criteria of tamper-proof, based on safety considerations. NPCA responded to the Agency's lead by releasing a "Good Practice Statement" (Anon. 1985) which listed in some detail the areas where these stations should be used. Later a method was developed to evaluate rat entry to bait stations (Kaukeinen, in press). These stations were all rat-sized stations, with considerable differences in efficacy between station designs. In the period that followed, several manufacturers took the overall designs or tamper-proof qualities of these rat stations and scaled them down for mice. Adequate station performance was only assumed. EPA's concerns have arisen and been primarily limited to the non-target safety standpoint. The Agency has recently developed specific protocols for evaluation of tamper-resistance aspects to children (Jacobs and Gross 1987a), adults (Jacobs and Gross 1987b) and dogs (Jacobs 1987). There is nothing in the literature which indicates the relative performance of these stations, nor how they compare to non-tamper resistant stations.

While these new professional-use mouse station designs have emerged, several over-the-counter (OTC) retail manufacturers/distributors of rodenticide baits also proceeded to introduce pre-filled mouse bait stations. While EPA does not regulate devices, they are responsible for toxicants, and efficacy evaluations were required. Several studies were conducted at our laboratory to develop protocols and to support registrations for prefilled, tamper-proof mouse bait stations. We became interested in determining if activity seen in the OTC trials was similar to stations sold for professional use. The intent of this research was to determine efficacy and mouse activity levels in comparisons of tamper-proof professional-use stations, OTC tamper-proof stations and non-tamper-proof designs.

METHODS AND MATERIALS

Test Arenas

A 3 m by 3.7 m animal testing room was equally divided with a 1.5 m steel wall, creating 2 test arenas measuring 1.8 m by 3 m. No bedding was placed on the floor because of the need to count and recover feces as a census technique (see below). Each test arena was divided into 4 equal quadrants, designated 1 - 4. In quadrants 1 and 2 were placed single shipping pallets of 100 x 120 cm, covered with cardboard to serve as harborage. Additional harborage in the form of cardboard nesting boxes (23 x 16.5 cm) were placed near each corner of the pallets. Plastic pipes (105 x 10 cm) were located along two walls to provide further harborage for the mice. These pipes were held in place with masonry blocks (20 x 20 x 40 cm). Water was available via one-gallon chick waterers placed in a central area (quadrant 4). Food was placed at the junction of the quadrants in a container (19.5 x 10 x 9.5 cm), and was available ad lib. Fluorescent strip lighting was maintained on a 12:12 cycle using a 24 hour timer, with lights on at 06:00 hours. Temperature was maintained at 22 +/- 2 C, with 50 +/- 5% relative humidity, with approximately 10 air changes per hour.

Test Animals

Wild house mice (*Mus musculus*) were used in these studies, as they are the target species for the bait stations as evaluated. Previous work with this species in similar sized enclosures (Morris et al. 1983) indicated 10 adult mice (5 male and 5 female) per test arena could be successfully monitored. Mice of healthy appearance were randomly selected from stock maintained at ICI's Public Health Services Laboratory. These animals were live-trapped from a local source and held for a minimum of 3 weeks prior to selection. Mice were maintained with a commercial laboratory diet (Wayne Lab Blox, Allied Mills) and cracked corn, with water available ad lib. Selected adult mice were weighed, sexed and toe-clipped prior to release in the test arenas.

Stations

Stations evaluated were the American Cyanamid COMBAT™ station, The d-Con Mouse Killing Station, the Sherman Technology TACKLE™ station, the Bell Labs PROTECTA[®] mouse station and the Eaton's Mouse-sized tamper-proof station. Eaton's non-tamper-proof mouse bait station was also evaluated as a reference station (see Table 1). The COMBAT and d-Con stations containing 50 ppm brodifacoum wax blocks are currently available OTC, pre-filled with 50 ppm brodifacoum wax blocks. TACKLE bait stations are available directly from the manufacturer for professional use at the present time, but may be made available as a pre-filled OTC station in the future. The Bell and Eaton stations are primarily available to professional users of rodenticides. These later two stations were selected principally due to their availability from distributors in the southeastern US, an indication of their field use.

Prior to introductions, station were furnished either one or two TALON Weather Bloks, depending upon station design and bait capacity. TALON blocks were used for consistency since the COMBAT and d-Con stations are sold pre-filled with this formulation.

Trial Procedure

Discussions with EPA indicated the appropriate protocol for evaluation of these stations would be based on EPA OPP 1.220 (Standard Mouse Acute Place-Pack Dry Bait Laboratory Test Method Revised 11-15-80), with modifications. This protocol indicates test group size to be 20 animals in a test arena having a surface area of 17,000 to 25,000 cm² (18.3 to 26.9 ft²). We felt this population density was too high and opted to conduct 2 replicates, each with 10 animals per replicate within a test arena having 54,000 cm². Also, EPA recommended reducing bait placements from five 28 gram (minimum) placements as in the OPP 1.220 protocol, to 2 bait stations per test arena, for the station trials. Also, the place-pack protocol indicated a minimum of 5 days be used as an observation period after the treatment period. Previous experience with anticoagulants has shown mice will occasionally require greater than 10 days before mortality occurs (Rowe and Bradfield 1976, Redfern et al. 1976, Dubock and Kaukeinen 1978). As brodifacoum is an anticoagulant rodenticide, with first deaths normally being observed 3 - 5 days after exposure, we observed animals for 15 days. The EPA 1.220 protocol indicates the product is satisfactory if 90% mortality is observed, and this criteria was retained.

However, we felt sole use of the mortality criteria of the EPA protocol would give too little information to distinguish potential efficacy differences. We adopted additional techniques more normally used in field evaluations for these trials (Kaukeinen 1979); namely census methods comparing relative activity differences before and after treatment, such as activity counters, untreated diet consumption, tracking boards and feces counts. Also, toxic bait consumption from the bait stations was monitored daily during the 3 day test period and additional activity data provided by 2 activity counters fitted to each station.

Table 1. - Descriptions of mouse bait stations evaluated.

Station	Bait Station Description	
	Manufacturer	Remarks
COMBAT	American Cyanamid	OTC station pre-filled with two 20 g brodifacoum wax blocks. Refillable Tamper-proof
d-Con	The d-Con Co.	OTC station pre-filled with one 20 g brodifacoum wax block. Disposable Tamper-proof
TACKLE	Sherman Technology	Professional use station, folding with removable end caps. Refillable Tamper-proof
PROTECTA	Bell Laboratories	Professional use station, one piece unit with hinged lid, secured in place with a single allen screw. Refillable Tamper-proof
Eaton's	J. T. Eaton	Professional use station, removable lid secured in place with a single allen screw. Refillable Tamper-proof
Eaton's	J. T. Eaton	Professional use station, removable lid secured with plastic ties. (reference station). Refillable Non tamper-proof

CENSUS METHODS

Activity Counters

Actimeters™, fitted with fresh batteries at the start of each evaluation, were mounted in pairs in each quadrant. These devices are activated by a combination of heat and movement (Kaukeinen, *op cit*). An internal memory stores the accumulated counts until the unit is cleared. These were

located with viewing tubes approximately 10 cm above the floor and 2.5 cm from the wall. This gave an effective viewing diameter of approximately 6 cm. All Actimeters were used in the same location for each of the trials, unless Actimeter failure forced unit replacement. As these units cannot be "tuned", paired placements compensated for any slight differences in unit sensitivity. Daily, throughout all portions of the trial, these were monitored by plugging the Readout device into the Actimeter and noting the accumulated counts. This value was recorded on data sheets and reset to zero, for the next day's observation. Stations were also fitted with Actimeters located just inside the main portion of each of the entrances of the station. Units placed on stations were monitored only to verify entry during the treatment period.

Food Consumption

As previously indicated, one food container was centrally placed in each of the test arenas. This container was weighed daily to determine the amount of food consumption by the mice during the previous 24 hour period. Spillage was collected and weights adjusted accordingly. A minimum of 100 grams of diet was available to the mice at all times. During the conditioning period, the diet consisted of ground commercial rodent diet, while the test and observation diet was EPA Challenge Diet (65% ground corn, 25% ground oats, 5% IOX sugar, 5% corn oil). Feces were not removed from the food container, since they were judged not to be sufficient to affect food weight.

Tracking activity

Tracking activity was monitored using tracking boards. These were prepared by spraying 7.5 x 15 cm pieces of vinyl floor tile with a mixture of isopropyl alcohol (75%) and marking chalk (25%). Once dry, a smooth layer of chalk remained on the board and is easily removed by mice after walking on the tiles. A rating system ranging from 0 for no tracks to 5 for 100% of the board having tracks was used. Each arena received 5 tracking boards, with one in each quadrant and one adjacent to the food container. Each was observed daily, rated, and replaced with a fresh board for the next day. The COMBAT and d-Con stations were the first to undergo evaluation and used actual counts of tracks on the boards, up to 20. This proved to be inefficient, and subsequent trials used the rating system technique. Data for COMBAT were converted to the above rating system for comparison of tracking data.

Feces Counts

Visible feces were collected and counted daily. Each quadrant was counted separately, facilitated by using a mini-vacuum cleaner (Black and Decker, "Dustbustersm"). To reduce disturbance to mice, no attempts were made during the trial to recover feces deposited in inaccessible areas (i.e., under pallets or within nesting or harborage areas). Occasionally mice "kicked" feces out of these areas, but the effect of this activity was felt to be negligible and no attempts were

made to adjust the feces count.

Test Procedure

After all animals had been selected and placed into the test arenas, daily observations of activity commenced. At 16:00 hours, observations were made of all census methods. Animals were conditioned for 6 days. This interval was selected based on previous ICI studies that indicated mouse activity tended to stabilize within 6 days after introduction.

Following pretreatment, ground laboratory chow in the central feeders was removed and replaced with EPA Challenge Diet. Two stations were placed into each test arena in locations where pretreatment observations indicated high levels of mouse activity. Because of population variation, no attempts were made to standardize placement among trials and replicates. Given the limited home range of mice, such attempts would likely result in poor placement in some tests, resulting in additional variables or bias in the data, or both. After treatment, all bait stations were removed with food, water and census observations being maintained until all animals died or for 15 days.

RESULTS

All stations showed excellent control with a 3 day exposure, indicating mice rapidly investigated the stations and consumed lethal quantities of the brodifacoum bait located within. Treated bait consumption was variable and felt related to population rather than station differences. Bait consumption from the stations did not appear to follow any specific pattern (i.e. increasing consumption over time, see Figure 1). The mean of all 4 census methods was greater than 90% in all cases, and greater than or equal to 85% in all individual replicates.

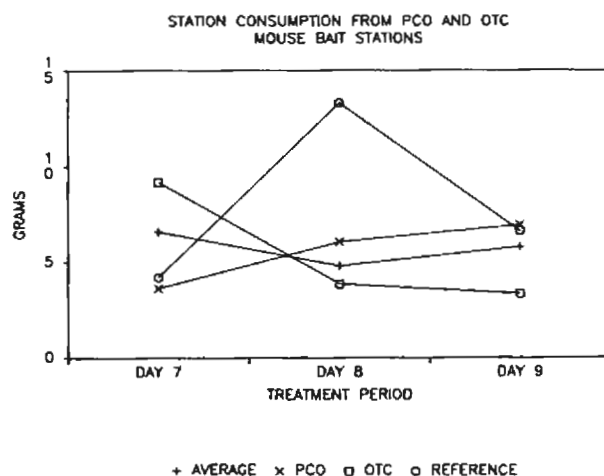


Fig. 1. Average consumptions of TALON wax block from PCO (PROTECTA, Eaton's, TACKLE), OTC (COMBAT, d-Con) and reference station.

Actimeter Counts

Generally, investigative behavior as revealed by Actimeter counts in the pretreatment period was initially high, then stabilized quickly during the later half of that period. Counts increased again when the treatment period began. Counts fell after that point to near zero at the final stages of post-treatment. In nearly every case, average actimeter counts increased to levels as high as early pretreatment counts when bait stations were introduced. This is not surprising, since mice are highly investigatory and will decrease their activities once they become familiar with a new object (Maruniak et al. 1974, Wolfe 1969). Decreasing activity observed during the final portion of treatment and post-treatment is consistent with brodifacoum poisoning. Actimeter counts failed to show any differences in activity between any of the stations evaluated. Overall activity reductions ranged from 93.2 - 99.9%.

Feces Counts

Visible fecal counts during pretreatment were generally consistent among tests. Large numbers of feces were found in the corners of quadrants 3 and 4 in all reps, indicating these were especially active areas. An entrance door and the room divider were present in these quadrants and mice were frequently investigating these arena features. It is interesting that these quadrants showed high activity, since they offered the least amount of harborage. Feces reduction ranged from 86.2 to 99.9%.

Tracking Boards

As with other techniques, tracking boards failed to show any differences between the stations evaluated. Reflected reductions in activity were excellent, ranging from 86.0 to 99.9%. Tracking boards are thought to be the least sensitive technique used in these studies, since surviving mice can repeatedly travel over one or more of the boards, inflating the board ratings.

Untreated Diet Consumption

Untreated diet consumption tended to stabilize by 3 days into pretreatment. Mean consumption of all trials was above 42 g for the remaining 3 days of this period. Consumption increased to 48.8 g on the first day of treatment, decreasing to 45.7 on day 2 and, 38.7 g on the final day of treatment. This increase in consumption parallels the change in untreated diets. Pretreatment diet consisted of ground laboratory chow and was replaced with EPA Challenge Diet which is more attractive to rodents. The lower level of diet consumption seen on the final day of treatment was related to animals beginning to decrease their intake due to rodenticide intoxication (see Figure 2). The reductions in feeding activity, as derived from comparing the final 3 days of the pretreatment period with the last 3 days of the post-treatment interval, ranged from 88.6 to 99.9%.

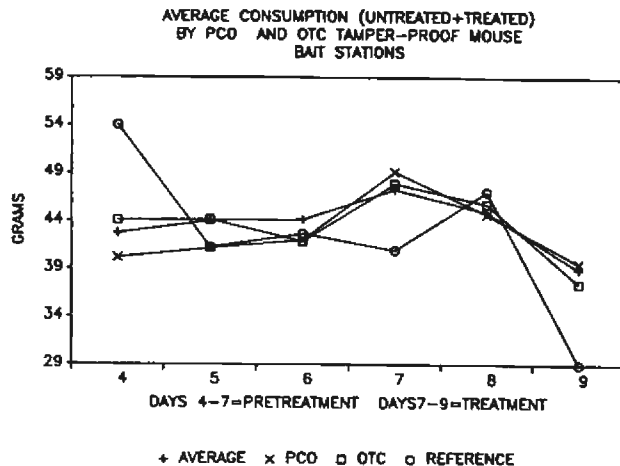


Fig. 2. Average untreated and treated consumption from PCO, OTC and reference stations from the last 3 days of pretreatment to the final day of treatment.

Bait Consumption from Stations

The quantity of bait removed from each station was variable due to differences in station placement within the testing arena, and in feeding behavior of the mice in each trial. As previously indicated, station placement was made in the two quadrants showing the highest levels of activity during pretreatment. In some cases the most obvious location for station placement was directly beneath the pallet, but station size and attached actimeters prevented this, resulting in less than ideal station positioning in these cases. Average consumption per replicate (2 stations for 3 days) was 17.7 g. This was equivalent to an average dose of 3.54 mg/kg brodifacoum per mouse (25 g mouse). No bait particles were observed outside any of the station evaluated.

DISCUSSION

It appears there is little or no difference in these stations as related to mouse utilization and presence or absence of tamper-proof features. Mice entered the stations and consumed brodifacoum baits in sufficient quantities to show mortality in all test populations greater than or equal to the 90% minimum. Other rodenticide products having comparable palatability of TALON blocks, but different active ingredients with less toxicity to mice, might show unsatisfactory efficacy. For evaluations of such products, the exposure period would need to be extended, such as to the 15 day period recommended by the US EPA in tests of multiple feeding rodenticide products. The average of all four of the census methods showed a reduction in activity for all stations greater than 90% (see Table 2).

Table 2 - Average percent reduction in activity by various census methods and average mortality for each station.

Census Technique	% Reduction by Station					Ref.
	PROTECTA	Eaton	TACKLE	COMBAT	d-Con	
Actimeter	99.9	99.9	96.8	99.9 ¹	99.7	98.5
Feces	99.9	99.9	97.4	99.9	92.8	97.7
Tracking	99.9	99.9	99.2	99.9	86.0 ²	93.8
Cons.	99.9	99.9	94.0	99.8	88.6	92.9
Average	99.9	99.9	96.9	99.9	91.8	95.7
Mortality	100%	100%	95%	95%	100%	95%

¹Based on 1 rep due to malfunctioning actimeter.

²Based on 1 rep due to inflated tracking board ratings.

Modifications of the EPA Acute place-pack protocol resulted in a more stringent test than the original Agency protocol (Palmateer 1976). As mentioned previously, the suggested size of test enclosures in the EPA protocol may crowd mice, possibly introducing an additional source of mouse mortality through antagonistic behavior, particularly with males. Protocol OPP 1.220 also suggests making five placements of at least 28 grams of bait each (140 g minimum total), while the Agency suggested only two placements with stations. Because of the dimensions of two of the stations involved in these trials, only one 20 g block could be placed inside the station, thereby reducing the amount of available bait by 71% from the EPA recommendation of 140 g. All other stations were capable of holding two 20 gram brodifacoum blocks, but still had nearly 42% less bait available than the place-pack protocol recommended. Nevertheless, acceptable mortality was achieved using brodifacoum blocks. While apparently not a factor in these trials, territorial animals could prevent others from freely feeding from stations during such a limited exposure. Since anticoagulants require several days before symptoms develop, portions of the population may be denied access to the station, resulting in decreased efficacy. Since it appears internal station configuration has little or no effect on mouse entry and efficacy, simulated field evaluations conducted under controlled conditions with bait placements maintained until populations had declined would be appropriate to determine efficacy. This would more closely approximate actual use patterns.

Until recently, no tamper-proof station designs were available OTC and their use by the general public will result in fewer exposures. While concern has been expressed over the use of second generation anticoagulants by non-professionals, hazardous exposure to first generation anticoagulants that are presently not available in stations, can be considered an equal or far greater risk than protected place-

ments of more toxic materials. Generally, OTC sales for mouse control products are to individuals interested in controlling the occasional mouse, and are not involved in intensive saturation baiting programs. Thus these individuals are unlikely to hire a professional to eliminate a problem that they can take care of themselves with purchase of 1 or 2 units of bait. Many PCO's have recognized this and have begun to sell small pre-packaged quantities of some pesticides directly to the homeowner. The commendable efforts to reduce the number of accidental exposures of children and pets from mouse control products through the use of tamper-proof designs does not appear to have affected mouse efficacy based on these evaluations. As a result, these effective baiting systems can be successfully utilized by the homeowner and the professional alike, reducing overall pesticide exposure while reducing noxious pest mouse infestations in home, industrial, agricultural and other commensal situations.

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The Actimeter Systems (for monitoring rodent activity) mentioned on pages 102-104 are produced and sold by Virgil Duncan, 1908 Ridge Road, Raleigh, NC 27607.