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Cage efficacy study of an experimental rodenticide using wild-caught house mice

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ABSTRACT: The availability and effectiveness of rodenticides in the US and elsewhere has been changing for various reasons. As a result, new rodenticide formulations and active ingredients are being investigated in the US and other countries. We conducted a cage efficacy study of a paste bait containing 4.4% alphachloralose. A commercial product of this nature is manufactured and used in parts of Europe. While the formulation we tested was effective (100%) in a no-choice trial with wild caught house mice, it was not effective in two-choice trials ($\leq 35\%$). We surmise that palatability may be an issue as the mice consumed very little of the paste bait. It was also clear that the paste bait is more effective at cooler temperatures. Future efforts could focus on identifying more palatable formulations.

Key Words alphacloralose, house mouse, *Mus musculus*, rodent damage, rodenticide

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Originally from the Middle East and Asia, house mice (*Mus musculus*) have followed humans around the world and are now found worldwide (Long 2003, Witmer and Jojola 2006). In many situations they live in a close commensal relationship with humans, but on many tropical islands and on portions of some continents, they are free-ranging and do not need the food and shelter provided incidentally by humans. House mice pose a threat to the native flora and fauna of islands (Angel et al. 2009, Burbidge and Morris 2002) and can cause significant damage to agricultural commodities and property (Long 2003, Timm 1994a). Most seabirds that nest on islands have not evolved to deal with predation and are very vulnerable to introduced rodents (Moors and Atkinson 1984). House mice are very prolific and populations have irrupted periodically to

cause “plagues” in places such as Australia and Hawaii (Long 2003). There has been an effort to eradicate introduced house mice from some islands with some successes (e.g., Burbidge and Morris 2002). Successful eradication rates for house mice, however, have lagged behind rates for rats (MacKay and Russell 2007). Three APHIS pesticide registrations for rodenticide baits (two with brodifacoum and one with diphacinone) are now available to allow rodenticide baiting of conservation areas to eliminate introduced rodent populations (Witmer et al. 2007). Unfortunately, the diphacinone formulation has not proven very effective for house mouse control (Pitt et al. 2011, Witmer and Moulton 2014). Studies in New Zealand have also shown that effective anticoagulant rodenticide formulations for house mice have

proven elusive (Fisher 2005, Morriss et al. 2008).

Many commercial rodenticide baits are available on the market and many of these list house mice as a targeted species (Jacobs 1994, Timm 1994a, 1994b). Witmer and Moulton (2014) tested many commercial products, but found few (only 5 of 12 formulations tested) effective with wild-caught house mice from the mainland United States (US). While a wide array of rodenticides have been available for use in the US, the continued use of some rodenticides is uncertain because of one or more issues such as toxicity, residue persistence, reduced effectiveness, hazards to non-target animals, environmental contamination, and humaneness (e.g., Cowled et al. 2008, Eason et al. 2010a, Mason and Littin 2003). As a result of this situation, there has been an increase in research on new products that would remove or reduce some of the detrimental characteristics of currently registered rodenticides (Baldwin et al. 2016, Eason et al. 2010a, 2010b; Eason and Ogilvie 2009; Schmolz 2010, Witmer et al. 2017).

One potential new rodenticide for the US is alphachloralose. This chemical is registered for use in the US as a bird anesthesia agent (Timm 1994b). However, it has been used in some European countries as a rodenticide (Cornwell 1969). Alphachloralose is a centrally active drug with both stimulant and depressant properties on the central nervous system. In rodents, it slows metabolism, lowering body temperature to a degree that may be fatal in small mammals. The smaller the body mass to surface area the more sensitive the animal; hence, house mice are very sensitive to alphachloralose intoxication especially at temperature lower than 15°C (Cornwell 1969, Timm 1994b). Generally, ataxia occurs in mice in 5-10 minutes following ingestion of the chemical. Then feeding

usually ceases within 20 minutes and mice are usually unconscious within 1 hour.

We could find very little literature on the use of alphachloralose as a rodenticide beyond the article by Cornwell (1969). If it is to be registered as a new house mouse rodenticide in the US, data sets on its cage and field efficacy must be submitted to the US Environmental Protection Agency (USEPA). Hence, we conducted a cage efficacy study with an alphachloralose (4.4%) food bait using wild-caught house mice to determine if the USEPA cage efficacy level of 90% would be achieved.

The objective of this study was to determine the efficacy of a rodenticide paste bait containing 4.4% alphachloralose. The efficacy was determined using a protocol recommended by the USEPA: EPA Laboratory Test Method 1.210: Standard Mouse Acute Placepack Dry Bait Laboratory Test Method with the bait removed from the sachet (USEPA 1991). The trial was a two-choice trial whereby the rodenticide bait was presented along with the USEPA challenge diet. The trial used wild-caught house mice. The USEPA required a cage efficacy of at least 90%.

METHODS

House mice for this study were wild-caught mice from the Fort Collins, Colorado, area. Mice were kept in individual numbered, plastic shoebox cages in a climate-controlled animal room of the Invasive Species Research Building (ISRB). They were fed a maintenance diet of rodent chow pellets and received water *ad libitum*. They were provided with bedding and a den tube. There was a 3-week quarantine period before the study began to help assure that animals were healthy, acclimated, and females were not pregnant.

The original, approved study protocol was amended to meet some requirements of the USEPA. This included:

1) following the USEPA Test Method 1.210 (paste bait removed from the sachets before being placed in the cages), 2) the room temperature was raised from 68°F (20°C) to 72°F (22.2°C), and 3) the room humidity was raised from ambient to 50% humidity. The study used individually-housed mice during the efficacy trial. There were 10 cages of male mice in the treatment group and 10 in the control group. There were also 10 cages of female mice in the treatment group and 10 in the control group. For the trials, each mouse was housed in a plastic shoebox cage with a den tube and bedding material. Mice were randomly assigned to the treatment and control groups although an effort was made to distribute mice of differing weights rather evenly so that no group is comprised of larger mice versus smaller mice. The weight, sex, cage number, and treatment of each mouse were recorded before the initiation of the trial.

On day 1 of the efficacy trial, all mice were placed in clean cages with no maintenance food. Pre-weighed foods were placed in 2 opposite corners of the cage in shallow bowls. For the treatment cages, one corner had a paste bait (sachet cover removed); the other corner bowl contained the USEPA challenge diet (USEPA 1991). The control mice were only presented with the USEPA challenge diet (as required by the USEPA). Remaining food in the bowls was replenished with weighed amounts as needed so that both food types were always available. After 2 days of bait exposure, the mice were put into clean cages with the maintenance diet for a 5-day post-exposure observation period. All remaining food in the dirty cages was removed and weighed. The total amount of foods consumed in each cage was determined by subtracting the remaining weight from that added over the course of the 2-day exposure period.

Mice were examined twice daily by the study staff and their condition and any mortalities

were recorded on animal health log sheets. Because the USEPA required death as an end point for this study, no intervention and euthanasia was used in this toxicity trial. Dead mice were placed in individual, labeled zip-lock bags and refrigerated for later incineration. All surviving mice were weighed, euthanized and incinerated at the end of the study.

The percent efficacy (i.e., mortality) of treatment groups and the control group was determined by the percent of animals that died during the trials in each group. Mouse weights were compared using *t*-tests. Food consumption of rodenticide bait versus the USEPA challenge diet and by males versus females was compared with *t*-tests. We also compared food consumption at the high versus low temperatures with *t*-tests.

RESULTS

Part 1 Trial (72°F, 22.2°C)

Of the 20 treatment mice in this two-choice trial, only one (a female) died. This equates to an efficacy of 5%. We noted, however, that 5 other treatment mice (2 males and 3 females) became “comatose” but recovered (sometimes it was a whole day or two later). Some went down very quickly after eating some bait. None of the 20 control mice died.

All mice tended to lose a gram or 2 of weight over the course of the 7-day trial (2 days exposure, 5 days post-exposure observation). Most mice ate relatively little of the bait, generally 0.1-0.4g. The one treatment mouse that died ate a little more (0.6g). Because of the poor performance of the paste bait in the part 1 trial, we did not tabulate the results like we did for the Part 2 and part 3 trials.

Over the course of the part 1 trial, the room temperature averaged 71.7°F (SD = 0.10) and the humidity averaged 49.5% (SD = 0.52).

Part 2 Trial (72°F, 22.2°C)

Because only 1 of 20 mice died in the two-choice trial (part 1 trial), we conducted a no-choice trial at the same room temperature. This was to make sure that there was an adequate concentration of the alphachloralose in the paste bait to cause mortality. Five mice were used (3 males and 2 females). The mice were lightly fasted by removing all food the afternoon before the paste bait was added the next morning. All mice became comatose during the day the paste bait was added. All five mice eventually died, but this varied from 1 to 6

days later (Table 1). The average alphachloralose bait consumption was 0.6g (SD = 0.1) with a range of 0.4-0.8 g. This average consumption was comparable to the amount eaten (0.6g) by the one mouse that died in the two-choice trial (part 1 trial). All 5 mice lost some weight over the course of the no-choice trial, probably because they stopped all feeding once they quickly became comatose and later died. The mice starting weights averaged 17.7g (SD = 1.4), while the end weights averaged 13.7g (SD = 0.9).

Table 1. Results of the no-choice alphachloralose feeding trial using wild-caught house mice.

Animal ID	Sex	Trial Start Mouse Weight (g)	Mean (SD) Start Weight (g)	Final Weight (g)	Mean (SD) Final Weight (g)	Comments
14	M	18.25		13.9		comatose; died 10/26/15
34	M	16.50		14.1		comatose; died 10/22/15
42	M	18.80	17.73 (1.4)	12.2	13.7 (0.9)	comatose; moving around 10/23 am; comatose 10/23 pm; died 10/26/15
10	F	16.00		13.6		comatose; died 10/24/15
23	F	19.10		14.5		comatose; moving around 10/26; died 10/27/15

Part 3 Trial (62°F, 16.7°C)

Because of the poor efficacy in the part 1 trial, we amended the protocol a second time. This was to repeat the previous trial, but at a lower temperature (62°F, 16.6°C). All other aspects of the trial were conducted as per the part 1 trial.

While the result were better than in the part 1 trial, they still were not very good. Only 7 of the 20 treatment mice died (4 males and 3 females; Table 2). This amounts to an efficacy of about 35%. No control mice died during this trial.

The treatment mice tended to gain a little weight over the course of the 7-day trial (2 days exposure, 5 days post-exposure observation), but only <1g (Table 2). The control mice tended to lose weight, but, again <1 g. There were no significant differences

($F = 1.91$; $p = 0.145$) in the starting weights of mice in the 4 groups (treatment males, treatment females, control males, control females).

As in the part 1 trial, most of the mice that died tended to eat a little more of the bait than the mice that lived, although the difference was not significant ($t = 1.75$; $p = 0.097$). Mice that died ate an average of 0.37g (SD = 0.18) of paste bait, while mice that lived ate an average of 0.22g (SD = 0.20) of paste bait (Table 3).

The amount of paste bait consumption did not vary significantly ($t = 0.65$; $p = 0.525$) between males (mean = 0.29g; SD = 0.23) and females (mean = 0.23g; SD = 0.182). However, both males and females consumed much more challenge diet than the paste bait (Table 3). For example, males consumed significantly more

Table 2. House mouse fates and weights in the two-choice alphachloralose trial at 62°F (16.7°C).

Group	Animal ID	Sex (M/F)	Start Weight (g)	Final Weight (g)	Comments	
Treatment Males	7	M	15.2	16.0		
	15	M	18.2	19.2		
	22	M	19.9	19.1	comatose 11/2; recovered 11/3 am	
	24	M	20.0	19.4		
	mean start weight 18.2g (SD = 1.7)	28	M	20.1	19.7	comatose 11/2; dead 11/3 am
		31	M	19.3	18.4	
	mean final weight 18.4g (SD = 1.1)	35	M	17.2	18.3	comatose 11/2; dead 11/3 am
		40	M	17.6	18.7	
		44	M	16.2	18.3	comatose 11/2; dead 11/3 am
		46	M	17.8	17.1	comatose 11/2; dead 11/3 am
Treatment Females	6	F	15.9	18.1	dead 11/3 am	
	8	F	12.6	12.4		
	12	F	16.2	16.1		
	16	F	17.0	17.4		
	mean start weight 17.2g (SD = 2.1)	20	F	19.6	21.4	dead 11/3 am
		32	F	18.5	18.4	
	mean final weight 17.7g (SD = 2.5)	38	F	16.6	18.4	
		47	F	17.2	16.0	
		49	F	19.5	18.1	
		52	F	18.8	20.4	dead 11/3 am
Control Males	4	M	22.0	19.3		
	21	M	16.6	16.6		
	26	M	18.9	18.5		
	27	M	19.2	19.0		
	mean start weight 19.7g (SD = 2.9)	29	M	17.8	18.5	
		33	M	23.6	23.0	
	mean final weight 19.3g (SD = 2.4)	37	M	22.8	22.3	
		43	M	16.8	17.5	
		45	M	22.9	22.1	
		50	M	16.5	16.0	
Control Females	1	F	21.9	21.1		
	3	F	18.8	19.2		
	5	F	17.4	17.3		
	17	F	21.4	17.7		
	mean start weight 18.1g (SD = 2.7)	18	F	17.9	17.7	
		19	F	20.0	19.0	
	mean final weight 17.2g (SD = 2.5)	25	F	18.8	17.5	
		30	F	17.4	16.5	
		36	F	13.4	12.3	
		39	F	14.3	13.9	

($t = 5.68$; $p < 0.001$) challenge diet (mean = 8.21g; SD = 4.40) than the paste bait (mean = 0.29g; SD = 0.23). Females exhibited the same pattern and both males and females consumed similar amount of paste bait and similar amounts of the challenge diet. Over the course of the part 3 trial, the room temperature averaged 62.6°F (SD = 0.11)

and the humidity averaged 50.3% (SD = 0.89).

When we compared the paste bait consumption by males at the higher temperature (part 1 trial) versus the lower temperature trial (part 3 trial), there was no significant difference ($t = 0.69$; $p = 0.502$). The same result occurred when the female bait consumption was compared between the

Table 3. House mouse fates and alphachloralose bait (AC) and challenge diet (CD) consumption by mouse in two-choice alphachloralose trial at 62°F (16.7°C). All food was added Nov 2 2015 and replaced and weighed Nov 4 2015; type of food: L=left side of cage; R=right side of cage.

Animal ID	Type of Food [Cage Size]	Container Weight (g)	Intake Container + Food Weight (g)	Intake Food Weight (g)	Additional Food Added (g) & Date	Outake Container + Food Weight (g)	Amount Eaten (g)	Fate (A/D) & Date	Comments
PL07M	CD [L]	6.1	16.1	10.0	10.2; 11/3/15	16.6	9.7	A	
PL07M	AC [R]	6.1	16.2	10.1		16.0	0.2		
PL15M	CD [R]	6.3	16.4	10.1	10.2; 11/3/15	12.1	14.5	A	
PL15M	AC [L]	6.1	16.0	9.9		15.9	0.1		
PL22M	CD [L]	6.0	16.1	10.1	10; 11/3/15	16.7	9.4	A	comatose 11/2; recovered 11/3 am
PL22M	AC [R]	6.0	15.8	9.8		15.6	0.2		
PL24M	CD [R]	6.1	16.0	9.9	10.1; 11/3/15	12.2	13.9	A	
PL24M	AC [L]	6.3	16.2	9.9		16.0	0.2		
PL28M	CD [L]	6.2	16.3	10.1		13.5	2.8	D; 11/3/15	comatose 11/2; dead 11/3 am
PL28M	AC [R]	6.1	17.1	11.0		16.8	0.3		
PL31M	CD [R]	6.1	16.2	10.1	10.2; 11/3/15	16.6	9.8	A	
PL31M	AC [L]	6.2	16.2	10.0		16.2	0.0		
PL35M	CD [L]	6.1	16.0	9.9		11.8	4.2	D; 11/3/15	comatose 11/2; dead 11/3 am
PL35M	AC [R]	6.1	16.5	10.4		16.1	0.4		
PL40M	CD [R]	6.3	16.5	10.2	10.1; 11/3/15	15.8	10.8	A	
PL40M	AC [L]	6.2	16.9	10.7		16.1	0.8		
PL44M	CD [L]	6.3	16.2	9.9		12.7	3.5	D; 11/3/15	comatose 11/2; dead 11/3 am
PL44M	AC [R]	6.1	17.0	10.9		16.8	0.2		
PL46M	CD [R]	6.1	16.1	10.0		12.6	3.5	D; 11/3/15	comatose 11/2; dead 11/3 am
PL46M	AC [L]	6.2	16.7	10.5		16.2	0.5		
PL06F	CD [L]	6.0	16.2	10.2		8.3	7.9	D; 11/3/15	dead 11/3 am
PL06F	AC [R]	6.0	16.8	10.8		16.2	0.6		
PL08F	CD [R]	6.2	16.2	10.0	10; 11/3/15	18.3	7.9	A	
PL08F	AC [L]	6.3	16.7	10.4		16.4	0.3		
PL12F	CD [L]	6.0	16.1	10.1	10.1; 11/3/15	15.6	10.6	A	

PL12F	AC [R]	6.0	15.8	9.8		15.7	0.1		
PL16F	CD [R]	6.1	16.0	9.9	10; 11/3/15	13.9	12.1	A	
PL16F	AC [L]	6.2	17.0	10.8		16.9	0.1		
PL20F	CD [L]	6.1	16.1	10.0		8.5	7.6	D; 11/3/15	dead 11/3 am
PL20F	AC [R]	6.2	15.9	9.7		15.8	0.1		
PL32F	CD [R]	6.0	16.0	10.0	10; 11/3/15	14.1	11.9	A	
PL32F	AC [L]	6.3	16.1	9.8		16.0	0.1		
PL38F	CD [L]	6.2	16.6	10.4	10; 11/3/15	15.1	11.5	A	
PL38F	AC [R]	6.0	16.5	10.5		16.4	0.1		
PL47F	CD [R]	6.2	16.2	10.0	10; 11/3/15	15.3	10.9	A	
PL47F	AC [L]	6.1	17.0	10.9		16.8	0.2		
PL49F	CD [L]	6.1	15.9	9.8	10.1; 11/3/15	16.2	9.8	A	
PL49F	AC [R]	6.2	16.2	10.0		16.0	0.2		
PL52F	CD [R]	6.1	16.3	10.2		8.5	7.8	D; 11/3/15	dead 11/3 am
PL52F	AC [L]	6.0	15.9	9.9		15.4	0.5		

two temperatures ($t = 0.98$; $p = 0.341$). Hence, males and females ate similar amounts of the paste bait, and those amounts were similar regardless of room temperature. A very different pattern occurred when the challenge diet consumption is compared between males at the two different temperatures. Males consumed significantly more challenge diet ($t = 2.93$; $p = 0.009$) in the higher temperature trial (mean = 14.4g; SD = 5.09) than during the lower temperature trial (mean = 8.19; SD = 4.43). The same pattern was observed for females at the two different temperature trials ($t = 8.76$; $p < 0.001$).

DISCUSSION

The cage efficacy of an alphachloralose paste bait provided by the Lodi, Inc., company of France was poor in both the high (72°F, 22.2°C) and low (62°F, 16.7°C) temperature trials. As expected, temperature does appear to make a difference in efficacy of this rodenticide; efficacy increased from 5% at the higher temperature to 35% at the lower temperature. We discussed this in various conference calls and there was some interest expressed in having a temperature effects study done. Based on the results of our cage efficacy trials at two different temperatures, it would seem that a temperature effects study could prove valuable. On the other hand, because the paste bait is only meant for use inside buildings, it would be most valuable to have a formulation that was effective at room temperature or perhaps at a somewhat lower temperature, but not substantially lower. In our no-choice trial, we had very good efficacy (100%), suggesting that the active ingredient was present in the paste bait and in adequate concentration to cause mortality, even when a relatively small amount (0.4-0.8g) of the bait was consumed. We did not do a chemical analysis of the paste bait, but relied on the certificate of analysis provided by the Lodi, Inc., company.

Odor and taste cues are important in the attractiveness of a rodenticide bait (Jackson et al. 2016, Witmer et al. 2014). The fact that very little of the paste bait was consumed by the mice suggests that improvement could be made in the formulation to increase the palatability. We thought that we were testing the commercial bait manufactured and sold in Europe in our trials. However, we later learned that a flavoring ingredient (hazelnut) used in the commercial product had been left out of the paste bait provided to us. We were told that this was at the request of the USEPA because hazelnut is not on their list of inert ingredients. It is possible that this explains the poor cage efficacy results in our trials and why so little of the paste bait was eaten. Perhaps one of the other tree nuts that are listed on the USEPA inert ingredients list could be used as a flavor and odor enhancer: almonds, peanuts, or walnuts.

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