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A Cage Efficacy Study of Sodium Nitrite Formulations for Rodent Control

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ABSTRACT: Rodents cause extensive damage to human and natural resources around the world. Rodenticides are heavily relied upon to reduce rodent populations and damage. However, some rodenticides are becoming less effective while others are becoming more restricted in their use. Additionally, there are growing concerns about the non-target effects of rodenticides and the humaneness of some rodenticides. In this study, we tested some formulations containing sodium nitrite, a salt that can be toxic in high enough concentrations. One of our previous studies indicated an LD₅₀ of about 246 mg/kg for various rodent species. It was also determined that rodents could eat enough sodium nitrite-laced food to consume a lethal dose if the concentration of sodium nitrite was high enough. However, in the current study, none of the formulations tested had hardly any efficacy at all ($\leq 20\%$) with wild-caught house mice and Norway rats in two-choice trials. While it appears that sodium nitrite may be an effective toxicant for some targeted species, such as feral swine, it appears that it will not be effective for problem rodents unless concentration and palatability issues can be resolved.

Key Words house mouse, *Mus musculus*, Norway rat, rodent damage, *Rattus norvegicus*, rodenticide, sodium nitrite

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Rodents cause significant damage to a variety of resources required by a growing human population (Witmer and Singleton 2010). Damage can be especially severe when rodent population densities are high (Witmer and Proulx 2010). When introduced to islands, rats and mice can cause substantial damage to flora and fauna (e.g., Angel et al. 2009). A variety of methods are used to reduce damage by rodents, generally framed within an Integrated Pest Management (IPM) strategy (Witmer 2007). One of the most important categories of available and effective tools is rodenticides (Witmer and Eisemann 2007).

Many commercial rodenticide baits are available on the market and many of these list house mice and commensal rats as targeted species (Jacobs 1994, Timm 1994a, 1994b, 1994c). Witmer and Moulton (2014) tested many commercial products, but found few (only 5 of 12 formulations tested) effective with house mice from the mainland US. While a wide array of rodenticides have been available for use in the United States (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 formulations and/or active ingredients that would remove or reduce some of the detrimental characteristics of many currently registered rodenticides (Eason et al. 2010a, 2010b; Eason and Ogilvie 2009; Schmolz 2010).

One potential new rodenticide is sodium nitrite. This chemical has wide uses in the food and pharmaceutical industries, but is known to be toxic at high enough doses. The LD50 for rats is in the range of 130-180 mg/kg (Cowled et al. 2008). It is being investigated as a feral pig (*Sus scrofa*) toxicant in Australia (Cowled et al. 2008, Lapidge et al. 2009), in New Zealand (Charles Eason, pers. comm.), and in the US (Snow et al. 2016). Some of the desirable attributes of sodium nitrite as a toxicant are that it is fast-acting, is considered humane, leaves no residues, has an antidote, and is rapidly degraded in the environment (Cowled et al. 2008, Lapidge et al. 2009). Cowled et al. (2008) reported that the symptoms in dosed pigs in the order of their occurrence were lethargy, dyspnoea (shortness-of-breath), reduced consciousness, and terminal seizures followed quickly by death. Some feral pigs vomited. The average time to death was 107 min (n = 10) when delivered by oral gavage (although 85 min if a delayed accidental death through handling a low-dosed animal is removed) or 140 min (n = 6) when a food bait is used and digestion is required. The mode of action of nitrite is the oxidization of the iron in oxyhemoglobin in red blood cells from the ferrous state to the ferric state to form methemoglobin (MetHb). MetHb is incapable of carrying oxygen and respiratory distress and cyanosis results with death occurring if the MetHb levels are high enough (Cowled et al. 2008, Smith and Beutler 1966). If the animal does not receive a lethal dose MetHb will undergo chemical reduction, through the action of MetHb

reductase, back to oxyhemoglobin, the rate of which differs between species (Smith and Beutler 1966, Agar and Harley 1972). Certain reducing agents such as methylene blue can accelerate that process and, hence, can be given as an antidote to nitrite poisoning (Lapidge et al. 2009).

We could find no literature on the use of sodium nitrite as a rodenticide. Hence, our preliminary studies (QA-1752; Witmer 2013) were to assess the potential of sodium nitrite as a rodenticide. The main objective of QA-1752 was to determine the LD50 of sodium nitrite in a variety of native and invasive rodent species, using oral gavage into the stomach. This was accomplished and while there was some variation across species and genders, the LD50 averaged about 246 mg/kg. The time-to-death was 41-55 minutes for 5 species, but somewhat longer (97 minutes) for Norway rats (*Rattus norvegicus*). The clinical symptoms observed in mice were lethargy, then loss of motor control followed by labored breathing with some gasping, and finally, spasms, coma and death. A secondary objective was a “proof-of-concept” small trial using the remaining animals to see if rodents could eat enough sodium nitrite-containing food bait in a single feeding to consume a lethal dose. A very simple food bait containing peanut butter, rolled oats, and encapsulated sodium nitrite (ESN) was presented to the rodents in a no-choice feeding trial. Additionally, all food was removed from the cages the afternoon before the ESN bait was to be added the next day so that the rodents were lightly fasted. Only 4-8 rodents of each species were available, so this was not really an efficacy trial and we varied the concentration of ESN as the various rodent trials based on the results of the previous oral gavage trial. We started with Richardson’s ground squirrels (*Spermophilus richardsonii*) and a 10% ESN bait; 3 of 5 animals died (60% efficacy). We next used house mice

(*Mus musculus*) and upped the concentration to 15% ESN; only 1 of 4 died (25% efficacy). For the remaining four species of rodents (*Microtus montanus*, *Rattus norvegicus*, *R. rattus*, *Cynomys ludovicianus*), we upped the concentration to 20% ESN; with 2 species we had 0% efficacy and with the other 2 species we had 50% efficacy. Hence, based on those preliminary results, we are mainly using a 20% ESN in the food baits tested in this study. We also concluded that additional research should be conducted to identify a highly palatable food bait and an appropriate sodium nitrite concentration that results in high mortality levels in rodents.

In this follow-up study, we conducted a preliminary evaluation of several potential food baits containing sodium nitrite as an oral rodenticide, using wild-caught house mice and Norway rats. The objective of this study was to identify effective new formulations of rodenticide food baits containing encapsulated sodium nitrite (ESN) for the control of house mice and rats. We hypothesized that some of the test food baits would exhibit a high efficacy (> 80% mortality) when presented to house mice and rats.

House Mouse Methods

House mice for this study were wild-caught mice from the Fort Collins, Colorado, area. Mice were kept in individual numbered shoebox cages in an animal room of the Invasive Species Research Building (ISRB). The weight, sex, and cage number of each mouse was recorded when they were brought into captivity. They were fed a maintenance diet of commercial rodent chow pellets (Lab Diet 5001) and received water ad libitum. They were provided with bedding and a den tube. There was a two-week quarantine period before the study began. There were 6 treatment groups with 5 or 10 animals (mixed genders) randomly assigned to each group. There was also a control group of 10 mice.

The 6 treatment groups are listed and described below.

1. A peanut paste block (20% ESN)
2. A peanut paste sachet (20% ESN)
3. Cracked wheat coated with ESN in oil (20% ESN)
4. Cracked wheat coated with ESN glued on (20% ESN)
5. Cooked rice with ESN absorbed (13% sodium nitrite; not encapsulated)
6. Peanut butter mixed with rolled oats (20% ESN)
7. Control (rats on maintenance diet and no ESN)

These were two-day feeding trials whereby the food is added in the afternoon and removed two afternoons later. Foods were replenished as needed. Foods were weighed at the start and at the end of the trials. When test foods were removed, they were replaced with the maintenance diet for a 2-3 day post-exposure observation period. The first trial was a no-choice trial with 5 mice per group in which the mice were lightly fasted before the treatment baits were added. All maintenance food was removed in the late afternoon. The next morning, the treatment baits were added.

The second trial was a two-choice trial with 10 mice per group. The mice always had access to the maintenance diet. We fed the mice a non-toxic food bait for two days to allow them to acclimate to a new food type. The non-toxic food bait for the peanut paste block and for the peanut butter and rolled baits was a mix of peanut butter and rolled oats, but did not contain ESN. The food bait for the rice bait was cooked rice that did not contain sodium nitrite. After 2 days, the non-toxic food bait was replaced with the ESN food baits for the next 2 days. When test foods were removed, they were replaced with the maintenance diet for a 2-3 day post-exposure observation period.

Mice on trial were examined twice daily by the study staff and their condition and any mortalities were recorded. Dead mice were weighed before disposal by incineration. All surviving mice were weighed and then euthanized and incinerated at the end of the study.

House Mouse Results

The results of the no-choice trial (trial 1) are presented in Table 1. Some mice (1-4 mice in each group of 5 mice) died in each treatment group. Consequently, efficacy in the treatment groups ranged from 20% to 80%. The two groups with the 80% efficacy were the treated rice group and the peanut butter-oats-ESN (PB-Oats) group. The mice in the peanut paste group and the PB-Oats group died relatively quickly (0.5-2 hrs), whereas, the mice that died in the other treatment groups took much longer to die (24-80 hrs.). We suspect that mice in the first two groups died as a result of ESN consumption (i.e., oxygen deprivation), whereas, the mice in the latter three groups died from not eating enough food/bait. All treatment mice lost weight over the course of the study with a range of -0.3 to -5.7g. In contrast, all control mice survived and gained some weight with a range of +0.7 to +2.6g. The mice in the rice treatment group lost the most weight with a range of -2.8 to -5.7g.

The results of one of the treatment groups in the two-choice trial are presented in Table 2. We only present the results of the PB-Oats group because that is the only treatment group in which some mice died. All mice in the other two treatment groups and the control group survived. Four of 10 mice in the PB-Oats group died for an efficacy of 40%. All these mice died relatively quickly (~0.75 hrs) suggesting that oxygen deprivation by consumption of the ESN was the cause of death. The mice that died all consumed ESN bait with a range of 0.04-0.11g of food bait consumed. This is similar

to the amounts consumed in the no-choice trial by the mice that died: 0.08-0.19g consumed. Hence, it appears that very little of the ESN bait needs to be consumed to be lethal. Any mice that did not die during the study were euthanized with carbon dioxide and incinerated at NWRC.

Norway Rat Methods

Norway rats for this study were live-trapped in the Fort Collins, Colorado, area. Rats were kept in individual numbered rat-sized, plastic shoebox cages in an animal room of the Invasive Species Research Building (ISRB) at the National Wildlife Research Center (NWRC) in Fort Collins, Colorado. They were fed a maintenance diet of rodent chow pellets, carrot or apple chunks, and received water ad libitum. They were provided with bedding and a den tube, and material to chew on (e.g., chew stick or wood chunks). There was a two-week quarantine period before the study was started. There were two tiers to this study. The tier 1 trial was a two-choice trial with rats receiving both the treatment bait and their normal maintenance diet. The four treatment baits used were produced by Connovation, New Zealand, and shipped to NWRC for the trials. Each of these four baits contained 20% encapsulated sodium nitrite (ESN). One bait was a peanut paste block and one bait was a peanut paste sachet. One bait had the ESN glued to grain and the fourth bait had the grain coated with oil containing the ESN. There were no other additives (such as flavors or sweeteners) added to the baits. There were 5 rats randomly assigned to each treatment group with a mixture of males and females in each group. There also was a control group of 5 rats. The weight, sex, cage number, and treatment of each rat were recorded before the initiation of the trial. A weighed and recorded amount of bait (37-40g) was added to each cage. The treatment baits were added to the cages on day one of

Table 1. Results of the no-choice bait with 20% ESN baits and 13% sodium nitrite rice, using wild-caught house mice.

Treatment	Mouse ID	Sex	Bait Weight (IN) g	Bait Weight (OUT) g	Amount Eaten (g)	Fate (A/D)	Time Until Death (hours)	Mouse Weight Change (g)
Peanut Paste Block ESN	PI04	F	19.49	19.40	0.09	D	1.5	-0.3
	PI20	M	19.55	19.47	0.08	D	2	-0.3
	PI34	F	19.54	12.87	6.67	A		-2.8
	PI54	M	19.95	15.20	4.75	A		-2.3
	PI64	M	18.76	18.71	0.05	D	1.5	-0.4
Peanut Paste Sachet ESN	PI84	F	13.30	13.24	0.06	D	28	-2.0
	PI19	M	13.36	13.15	0.21	D	24.5	-1.1
	PI43	M	12.44	3.58	8.86	A		-2.6
	PI50	M	13.33	13.17	0.16	D	24.5	-1.0
	PI59	F	12.78	10.00	2.78	A		-1.7
Glued Grain ESN	PI11	F	30.78	28.98	1.80	D	31.25	-2.0
	PI17	M	30.49	27.30	3.19	A		-2.4
	PI33	M	30.53	26.74	3.79	A		-2.8
	PI49	M	30.59	26.82	3.77	A		-2.4
	PI63	F	30.83	27.64	3.19	D	73	-1.3
Rice SN	PI08	F	26.30	25.97	0.33	D	80	-5.7
	PI16	F	30.21	29.54	0.67	A		-2.8
	PI42	F	25.07	25.21	-0.14	D	49	-4.1
	PI52	M	27.28	27.34	-0.06	D	24	-3.1
	PI61	M	30.87	32.16	-1.29	D	48	-4.9
Coated Grain ESN	PI07	F	30.42	28.51	1.91	D	72	-4.8
	PI21	F	31.50	27.71	3.79	A		-1.4
	PI40	M	31.34	28.62	2.72	A		-0.7
	PI55	F	30.73	28.52	2.21	A		-3.5
	PI65	M	30.03	28.90	1.13	A		-3.2
Peanut Butter Oats ESN	PI01	F	19.68	19.55	0.13	D	0.5	-0.3
	PI30	M	18.62	18.54	0.08	D	0.5	-0.5
	PI37	M	20.83	13.73	7.10	A		-0.8
	PI51	F	22.50	22.37	0.13	D	0.5	-0.9
	PI67	M	20.22	20.03	0.19	D	6.5	-0.6
Control	PI05	F	N/A	N/A	N/A	A		+1.4
	PI31	F	N/A	N/A	N/A	A		+1.6
	PI80	M	N/A	N/A	N/A	A		+2.6
	PI48	F	N/A	N/A	N/A	A		+2.1
	PI72	M	N/A	N/A	N/A	A		+0.7

Table 2. Results of the two-choice trial with the peanut butter-rolled oats bait with 20% ESN, using wild-caught house mice.

Treatment	Mouse ID	Sex	Bait Weight (IN) g	Bait Weight (OUT) g	Amount Eaten (g)	Fate (A/D)	Time Until Death (hours)	
Peanut Butter	PI03	F	19.70	19.66	0.04	D	0.75	
	PI12	M	18.03			A	N/A	
	PI22	M	20.08	20.00	0.08	D	0.75	
	PI48	F	19.28			A	N/A	
	PI68	F	21.43			A	N/A	
	Oats	PI73	F	20.03	19.96	0.07	D	0.75
	ESN	PI77	F	20.94			A	N/A
	PI82	F	23.82			A	N/A	
	PI89	M	20.95			A	N/A	
	PI95	M	21.79	21.68	0.11	D	0.75	

the trial and the rats were observed twice daily for the next 2 days. At the end of the second day of bait exposure, the rats were put into clean cages, back on the maintenance diet, and observed for 5 more days.

Because no rats died in the tier 1 two-choice trial, the tier 2 trial was conducted. This trial was a no-choice trial with 5 rats assigned to each treatment as previously described. For 2 of the treatment groups, the afternoon before the start of the trial, the rats were put in clean cages with no food; hence, they were slightly food deprived when the baits were added the next morning. One group of rats received the peanut paste ESN block, but it was first dipped in corn syrup (a sweetener). A second group of rats received the grain-coated ESN and a small amount of corn syrup was mixed with it before the bowl was placed in the rat cage. Each of these rats received 22-31g of the bait. The rats were observed twice daily for the next 2 days. A third treatment group received cooked rice that had been allowed to absorb sodium nitrite. The sodium nitrite concentration in the rice was

determined to be 13.3%. The rats in this third treatment group were given “placebo” cooked rice (containing no sodium nitrite) 2 days before the treated cooked rice was added so they could become familiar with the new food type. One day after the placebo cooked rice was added, the maintenance diet was removed from the cages of the third treatment rats to further encourage them to eat the placebo cooked rice. One day later, the sodium nitrite treated rice was added to each cage of the group 3 rats. Each rat received 50-51g of the treated rice. A fourth group of 5 rats served as the control group and continued to receive the maintenance diet. All rats were observed twice daily for the next 2 days after the treatment baits were added. At the end of the second day of bait exposure, the rats were put into clean cages with the maintenance diet and observed for 5 more days. Any rats that did not die during the study were euthanized with carbon dioxide and incinerated at NWRC.

Norway Rat Results

In the tier 1 trial (the two-choice trial) none of the treatment rats died (Table 3). Consequently, we did not determine the amount of food bait consumed. Because that trial was not successful, the tier 2 trial was conducted which was a no-choice trial (Table 4). None of the rats in the two 20% ESN treatment groups died even with the addition of some sweetener (corn syrup). Only one rat in the third treatment group died. That group had received the rice with sodium nitrite (13.3%) absorbed. Hence, the efficacy of all baits used in the 2 trials was very low (< 20%). The amount of food bait consumed in the tier 2 trial varied from 1.0g to 14.3g.

DISCUSSION

Overall, the results of this study with these sodium nitrite baits with wild-caught house mice were not very good. However, they were somewhat better than the results of the sodium nitrite baits with wild-caught Norway rats. Hence, while our original study (QA-1752; Witmer 2013) suggested that sodium nitrite had some potential as a new active ingredient for rodenticides, the latter two studies with mice and rats did not support that finding. We suspect that palatability may still be an issue even when encapsulated sodium nitrite (ESN) is used. Additionally, a higher concentration of ESN may be needed, but that may exacerbate the palatability issue. Additional research might be able to resolve these issues, but as it stands, it does not look promising for sodium nitrite to be a new active ingredient for rodenticides. Efforts to produce an effective toxic bait for invasive, feral swine using sodium nitrite have been more successful (e.g., Snow et al. 2016), perhaps in part because feral swine will eat more in a single feeding and, hence, are more likely to consume a lethal dose.

It appears that research to identify new, effective rodenticides will need to continue. Fortunately, researchers in several

Table 3. Results of 20% ESN baits with wild-caught Norway rats in a two-choice trial. Because no rats died, we did not determine the amount of bait consumption.

Treatment	Rat ID	Sex	Bait Weight (IN) g	Bait Weight (OUT) g	Fate (A/D)
Grain w/ Glue	PA01	M	40.02	N/A	A
	PA07	M	39.97	N/A	A
	PA21	F	40.12	N/A	A
	PA23	M	40.02	N/A	A
	PA73	F	40.03	N/A	A
Peanut Sachet	PA02	M	37.57	N/A	A
	PA10	M	37.18	N/A	A
	PA25	M	38.33	N/A	A
	PA27	F	39.16	N/A	A
	PA56	F	37.53	N/A	A
Grain w/ Oil	PA14	M	39.88	N/A	A
	PA28	F	40.18	N/A	A
	PA29	M	40.19	N/A	A
	PA34	M	40.09	N/A	A
	PA59	F	40.08	N/A	A
Peanut Block	PA04	M	37.18	N/A	A
	PA18	M	36.86	N/A	A
	PA31	M	36.66	N/A	A
	PA40	F	37.90	N/A	A
	PA61	F	37.44	N/A	A
Control	PA05	M	0	N/A	A
	PA19	M	0	N/A	A
	PA32	M	0	N/A	A
	PA41	F	0	N/A	A
	PA65	F	0	N/A	A

Table 4. Results of 20% ESN baits and a rice bait with 13.3% sodium nitrite with wild-caught Norway rats in a no-choice trial.

Treatment	Rat ID	Sex	Bait Weight (IN) g	Bait Weight (OUT) g	Amount Eaten (g)	Fate (A/D)
SN Rice (13.3%)(no-choice)	PA05	M	50.2	36.2	14.0	A
	PA41	F	50.0	52.1	-2.1	D*
	PA46	M	50.0	42.9	7.1	A
	PA55	M	50.5	36.2	14.3	A
	PA88	F	50.2	40.3	9.9	A
Sweetened 20% ESN Peanut Block (no-choice)	PA35	M	22.4	15.3	7.1	A
	PA39	M	23.5	22.5	1.0	A
	PA52	M	23.3	15.2	8.1	A
	PA91	F	23.5	17.0	6.5	A
Sweetened 20% ESN - Coated Grain (no-choice)	PA118	F	23.0	20.7	2.3	A
	PA38	M	28.7	23.9	4.8	A
	PA53	M	29.2	24.8	4.4	A
	PA68	M	31.4	24.0	7.4	A
	PA76	F	25.2	19.1	6.1	A
Control	PA112	F	29.3	22.7	6.6	A
	PA42	M	N/A	N/A	N/A	A
	PA54	M	N/A	N/A	N/A	A
	PA82	M	N/A	N/A	N/A	A
	PA86	F	N/A	N/A	N/A	A
	PA115	F	N/A	N/A	N/A	A

* placebo rice in treated rice

countries are pursuing this needed work with some promising results (e.g., Baldwin et al. 2016, Eason et al. 2010a, 2010b, Eason and Ogilvie 2009, Schmolz 2010, Witmer and Moulton 2014, Witmer et al. 2017).

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