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Effects of Sodium Nitrite on Feral Swine and Non-Targets
Principal Investigator: Justin Foster

Clayton Wolf
Director
Wildlife Division

Carter Smith
Executive Director
Texas Parks and Wildlife Department

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ABSTRACT

Toxicants have been shown to be an effective control measure for feral pigs. One such toxicant, sodium nitrite, was labeled the "Achilles Heel" of feral pigs because of their unique sensitivity to the substance relative to Australian mammals. In the United States, where no toxicants are labeled for use and where pig populations continue to expand their range and abundance, further research is needed for developing more cost effective control tools. Given the qualities of sodium nitrite (i.e. readily available, inexpensive, existing data regarding effects on mammals, etc.) and a recent application for registry with the United States Environmental Protection Agency, it was prudent to conduct further investigations into the qualities of the toxicant. The objective of this study was to determine relative sensitivities of feral pigs, raccoons, and white-tailed deer. We conducted oral gavage trials at the Kerr Wildlife Management Area, Hunt, Texas. Absorbance coefficients for hemoglobin derivatives were estimated at four wavelengths (535, 585, 594, and 626 nanometers). Median lethal doses were estimated with Up-And-Down procedures and were further assessed with fixed dose trials at 113 mg/kg. Control specimens survived anesthesia and water gavage. Raccoons were more sensitive than pigs or deer with MLD and LD_{50} values estimated at 50 mg/kg and 58 mg/kg respectively. Raccoons expired more rapidly than pigs or deer with a mean time to death of 42.0 ± 7.2 minutes. Our data indicate that raccoons are more sensitive to sodium nitrite than feral pigs. Conversely, deer were less sensitive than feral pigs. Although pigs are not uniquely sensitive, their sensitivity coupled with a target specific delivery system puts them at low risk of intoxication. We recommend that sodium nitrite continue to be evaluated as a candidate toxicant with emphasis on non-target risk and target specific delivery systems.

PERFORMANCE REPORT

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I. Objectives:

1. Quantify the minimum lethal dose (MLD) and median lethal dose (LD_{50}) of gavage delivered sodium nitrite (SN) to feral swine (pigs, swine), white-tailed deer (deer), and raccoons.
2. Determine dose dependent adverse effects of SN on pigs, deer, and raccoons.
3. Compare the relative toxicity of SN on pigs, deer, and raccoons.
4. Assess recovery of pigs, deer, and raccoons from SN intoxication.
5. Identify hazards of SN to non-target species and suggest countermeasures (e.g. delivery systems, alternate toxicants) to mitigate risk.

This work tested the hypotheses that feral pigs are more sensitive (i.e. have higher median and minimum lethal doses) to SN than deer or raccoons.

II. Background:

Feral swine (*Sus scrofa*) have expanded their range into 32 of the United States (U.S.) (USDA NFSMS, 2009) with over 2 million occurring in Texas alone (Nettles 1997, Pimentel et al. 2000). Possessing the greatest reproductive potential of all free-ranging mammals in the U.S. (Hellgren 1999), feral swine numbers continue to increase. As their range has increased, so has information implicating feral swine as a pest. While considered a valuable resource for sport hunting, the damage to private and public property and natural resources is hardly justifiable (Seward et al. 2004). Negative impacts to natural resources (agriculture, commercial pork industry, wildlife, wildlife habitat, private property, and soils) are well documented (Hutton et al. 2006). The Texas Agrilife Extension Service estimates statewide annual economic damage at $51.7 million (Higginbotham et al. 2008). Pest status and range expansion have increased the need for population control measures and long-term monitoring.

Legal control measures in the U.S. include exclusion (electric and conventional fencing), hunting, aerial gunnery, trapping, etc. (Reidy et al. 2008). Each of these methods presents its own logistical and financial limitations along with varying degrees of success. Unfortunately, many statements regarding effectiveness are anecdotal at best, lacking cost / benefit data. Available data indicate that broadcast eradication applications show the

greatest potential for cost-effective control; especially the use of toxicants (M. J. Bodenchuk, USDA Wildlife Services, personal communication). Coblentz and Baber (1987) estimated poisoning was 11 times cheaper than shooting and 80 times cheaper than trapping. McCann and Garcelon (2008) suggested that eradication was a preferable alternative to long-term suppression. Broadcast toxicants (Wafarin and Compound 1080) have proven effective and economical, reducing feral swine by < 73% in some areas in Australia. Questions about the humaneness of these toxicants led to further research that demonstrated that SN is potentially more humane and target specific (Cowled et al. 2008). No toxicants are registered for use as feral swine toxicants with the U.S. Environmental Protection Agency (USEPA).

Cowled et al. (2008) labeled SN as the "Achilles Heel" of feral swine management because of its MetHemoglobin (MetHb) producing qualities. They suggested that swine may have lower levels of MetHb reductase than other mammals and may be more susceptible to SN intoxication. However, pilot studies (J. Foster, Texas Parks and Wildlife Department, unpublished data) indicate that non-target species [raccoons (*Procyon lotor*) and white-tailed deer (*Odocoileus virginianus*)] may be equally sensitive. As target specificity is a requirement of pesticide registration, it is important to demonstrate the effects (or lack thereof) of the pesticide upon animals (non-targets) that could potentially consume the active ingredient.

The raccoon has been identified as the most likely non-target in U.S. trials (Fletcher et al. 1990, Campbell and Long 2008). Additional research is needed to demonstrate a low MLD of SN in feral swine relative to that of non-targets. This would allow for selective removal of swine from the ecosystem and mitigates negative impacts to other species.

Approval for broadcast delivery of toxicants and contraceptives by the EPA is contingent upon the development of a species specific delivery system or active ingredient. Few studies have investigated specificity of baits, mechanisms, or active ingredients in the U.S (Campbell et al. 2006). Although claims of successful species specific delivery have been made overseas, these successes have yet to be replicated in trials in the U.S. (Campbell et al. 2006). As non-target species differ with ecoregion, it is important to demonstrate efficacy and cost effectiveness of feral swine specific delivery systems in multiple ecotypes (T.A. Campbell, USDA-National Wildlife Research Center, personal communication). In the U.S.A., the raccoon and other mammals have made species specific delivery difficult (Fletcher et al. 1990).

III. Procedures:

We conducted acute oral toxicity trials at the Kerr Wildlife Management Area (Texas Parks and Wildlife Department) Hunt, Texas (KWMA). All procedures were approved by the KWMA institutional animal care and use committee. Raccoons and pigs were wild-caught on the premises. Captive-born and raised deer specimens came from the Donnie Harmel Deer Research Facility (KWMA). No injured or ill animals were used in trials. Specimens were fasted for 4 hours before dosing and then provided a daily ration of feed. Water was provided ad libitum.

Raccoons and pigs were anesthetized with isoflourane. Animals were considered sedated when withdrawal symptoms to pain stimuli (e.g. ear pinches) were absent. Deer were not anesthetized as they are tolerant to similar stress during annual research activities. Moreover, an increase in handling time from their annual regimen would have been counterproductive to the positive effects of anesthesia. Blood was collected 1 minute prior to gavage and at death to assess methemoglobin levels. Jugular puncture was used for deer and raccoons and tail puncture was performed on pigs. All blood samples were stored in EDTA, placed on ice, and analyzed for methemoglobin levels and oxygen saturation on an IL-682 co-oximeter within 1 hour of collection. Reagent grade 99% sodium nitrite (Scholar Chemistry, Henrietta, New York) was dissolved to required concentration per kilogram of animal body weight in 15 ml of water and administered by orogastric gavage.

Dose progression followed Acute Oral Toxicity guidelines (USEPA 2002). Specimens were immediately returned to cages or pens and were monitored constantly for $\leq$ 4 hours and then daily for 2 - 14 days (raccoons 2 days, deer & pigs 14 days). Behaviors, signs of intoxication, and time to death were recorded. Death was confirmed by cessation of heartbeat and corneal reflex. A heart puncture was performed immediately after death. Death by sodium nitrite toxicosis was assessed by observation of clinical signs and confirmed by blood methemoglobin values. Recovered specimens were euthanized. All specimens were subjected to gross necropsy.

Pilot trials were conducted to assess starting doses for acute oral toxicity trials on raccoons and deer. It was essential that starting doses be below the true LD_{50} for each species. Therefore doses for these trials started at 90 mg/kg (i.e. MLD for pigs) and decreased until 2 consecutive sublethal results were observed. This protocol was chosen to elucidate sensitivity of the deer and raccoons relative to the minimum lethal dose of the feral pig (90 mg/kg) (Deeb and Sloan 1975). Pilot trials indicated that starting dose should be 49 and 131 mg/kg for raccoons and deer respectively.

Three individuals of each species served as controls for gavage trials. Protocol for control specimens were identical to protocol for treatment groups respective of species (i.e. the only difference was that SN was not administered during control trials).

Raccoons

Raccoons were captured with live traps. Occupied trap was weighed with a 0.1 kg graduated digital scale and then placed in an open circuit induction chamber. Isoflurane and pure oxygen were administered at 7% and 15L/minute until moderate sedation was achieved. The raccoon was then removed from the chamber and fitted with an anesthesia mask. Anesthesia was maintained with 2–3% isoflurane and 1.5-3 L/min oxygen through the duration of the procedures. Subsequent to gavage of sodium nitrite solution, specimens were immediately returned to separate 60 cm^3 cages to be monitored. All procedures were conducted in a building with a temperature between 16º and 22º C.

We used adult male raccoons in acute oral toxicity trials because pilot trials indicated that males may be more sensitive than females and may be more representative of minimum lethal doses for the species. Minimum lethal dose estimates would be useful in assessing risk to non-target species. Raccoons (n = 6) were dosed according to the Up-And-Down Method (UDP) (USEPA 2002). Doses were increased or decreased at regular intervals dependent upon the results of previous tests. Dosing was stopped when stopping criteria were met. Median lethal dose and confidence intervals were calculated using maximum likelihood and profile-likelihood estimators respectively in AOT425StatPgm.

A fixed dose trial was conducted to compare dose effects across species. In this trial, raccoons (n = 5) were dosed with 113 mg/kg according to the procedures described above. Specimens were monitored according to the methods described previously. Dose effect and mean time to death were calculated.

White-tailed deer

Deer were manually restrained in a squeeze chute and sodium nitrite was administered via esophageal gavage. Specimens were immediately returned to a 68 m x 48 m pen to be monitored. All procedures were conducted outdoors at temperatures between 15 and 30º C.

We used adult male deer aged 2 (n = 1) and 6 (n = 5) in acute oral toxicity trials because this was the most optimum control of sex and age variables of deer available for the procedure. Dosing followed UDP guidelines. Dosing was stopped when stopping criteria were met. Median lethal dose and confidence intervals were calculated using maximum likelihood and profile-likelihood estimators respectively in AOT425StatPgm.

Fixed dose trials were also conducted to compare dose effects across species. In this trial, deer (n = 5) were dosed with 113 mg/kg according to the procedures described above. Specimens were monitored according to the methods described previously. Dose effect and mean time to death were calculated.

Feral Pigs

Pigs were captured in a trap designed for this project (J. Foster unpublished data). This "Trap 'N' Gas" unit doubled as an anesthesia chamber and facilitated hands-free induction anesthesia (Figures 1, 2). Once sedated with light anesthesia, a mask was affixed to provide maintenance anesthesia. Sodium nitrite was administered via oral gavage and specimens were returned to the 7 m x 16 m pen for monitoring. Procedures were conducted between 14º and 26º C.

We used male (n = 5) and female (n = 1) pigs as available for acute oral toxicity trials. Six pigs were dosed in a UDP trial. Median lethal dose and confidence intervals were calculated using maximum likelihood and profile-likelihood estimators respectively in AOT425StatPgm. Fixed dose trials were also conducted to compare dose effects across species. In this trial, pigs (n = 5) were dosed with 113 mg/kg according to the procedures

described above. Specimens were monitored according to the methods described previously. Dose effect and mean time to death were calculated.

Figure 1. Pig held captive in the Trap 'N' Gas without anesthesia door in place.

Figure 2. Trap door side of the Trap 'N' Gas with immobilizing plunger in place.

IV. Results:

Absorption Coefficients of Hemoglobin Derivatives: Instrument Calibration

Absorbance coefficients were calculated for white-tailed deer (Table 1), feral swine (Table 2), raccoons (Table 3), and javelina (*Tayasu tajacu*) (Table 4).

Table 1. Hemoglobin derivative absorbance coefficients for white-tailed deer. Hbr (reduced hemoglobin), HbO_2 (oxyhemoglobin), HbCO (carboxyhemoglobin), and HbMet (methemoglobin).

Absorption Coefficients				
Species	**Hbr**	**Hb02**	**HbCO**	**HbMet**
Wavelength				
535.0	0.6500000	0.9400000	1.0000000	0.4381000
585.2	0.5779163	0.6186383	0.3381496	0.2339503
594.5	0.3860169	0.1471087	0.1272143	0.2101850
626.6	0.0825853	0.0120163	0.0204717	0.2535364

Table 2. Hemoglobin derivative absorbance coefficients for feral swine. Hbr (reduced hemoglobin), HbO_2 (oxyhemoglobin), HbCO (carboxyhemoglobin), and HbMet (methemoglobin).

Absorption Coefficients				
Species	**Hbr**	**Hb02**	**HbCO**	**HbMet**
Wavelength				
535.0	0.6500000	0.9400000	1.0000000	0.4381000
585.2	0.5820899	0.6186383	0.3370236	0.2269531
594.5	0.3926950	0.1471087	0.1262087	0.2035868
626.6	0.0830183	0.0120163	0.0200043	0.2489883

Table 3. Hemoglobin derivative absorbance coefficients for raccoons. Hbr (reduced hemoglobin), HbO_2 (oxyhemoglobin), HbCO (carboxyhemoglobin), and HbMet (methemoglobin).

Absorption Coefficients				
Species	**Hbr**	**Hb02**	**HbCO**	**HbMet**
Wavelength				
535.0	0.6500000	0.9400000	1.0000000	0.4381000
585.2	0.5554944	0.6186383	0.3328939	0.2343339
594.5	0.3727591	0.1471087	0.1272339	0.2109674
626.6	0.0854294	0.0120163	0.0209436	0.2537367

Table 4. Hemoglobin derivative absorbance coefficients for javelina. Hbr (reduced hemoglobin), HbO_2 (oxyhemoglobin), HbCO (carboxyhemoglobin), and HbMet (methemoglobin).

Absorption Coefficients				
Species	**Hbr**	**Hb02**	**HbCO**	**HbMet**
Wavelength				
535.0	0.6500000	0.9400000	1.0000000	0.4381000
585.2	0.5679508	0.6186383	0.3453622	0.2239785
594.5	0.3832934	0.1471087	0.1352184	0.2013196
626.6	0.0910984	0.0120163	0.0269017	0.2460959

Acute Oral Toxicity

Control Specimens

All control specimens survived water gavage. Clinical signs of anesthesia in raccoons and pigs included unconsciousness and lack of withdrawal from pain stimuli. All 6 raccoons and pigs recovered from anesthesia rapidly $\leq$ 7 minutes. All 9 specimens recovered fully from handling procedures. No gross abnormalities were detected in post-mortem examinations.

Sodium Nitrite Trials

Median lethal doses and mortality rates for a fixed dose were estimated (Table 5). Raccoons were most sensitive to SN with MLD and LD_{50} values estimated at 50 mg/kg and 58 mg/kg respectively.

The MLD and LD_{50} for pigs was 113 and 133 mg/kg respectively. Minimum lethal dose in deer was 90 mg/kg and LD_{50} was 154 mg/kg (Table 5).

Table 5. Estimated gavage delivered median lethal dose (LD_{50},), minimum lethal dose (MLD) with 95% confidence intervals.

Species	MLD	LD_{50}	95% CI for LD_{50}
Raccoon	50	58	44.4 – 70.9
Feral Pig	113	133	102 – 304
Deer	90*	154	126.4 - 198

*Three of five yearling deer died in a pilot trial (J. Foster unpublished data**)**

Fixed dose trials at 113 mg/kg produced $\geq$ 1 lethal effect in all species (Table 6). One hundred percent or raccoons died in less than an hour (n = 5, SD = 42.0 ± 7.2). Methemoglobin levels were $\geq$ 85% in all specimens that died from SN toxicosis (Table 6).

Table 6. Outcome, time to death, and methemoglobin levels of fixed dose (113 mg/kg) trials.

Species	*n*	Outcome (dead/survived) 113 mg/kg	Mean Time to Death (minutes)	Mean % Methemoglobin Level at Death
Raccoon	5	5/0	42.0 ± 7.2	85.7 ± 6.0
Feral Pig	5	3/5	131.3 ± 55.8	87.5 ± 2.8
Deer	5	1/5	120.0 ± 0	n/a

V. Analysis:

Our data indicate that pigs are more or equally sensitive to SN than deer. We estimate the gavage delivered LD_{50} of sodium nitrite in deer at 154 mg/kg. As food delivered doses may require 3 times the potency to be lethal, we assume the bait delivered LD_{50} in deer at approximately 399 mg/kg. If baits were formulated at a 10:1 ratio (i.e. 80g bait with 8g of sodium nitrite), then consumption of 2.8 baits would be lethal to 50% of 56.7 kg (125 lb.) deer. The MLD for yearling deer observed in a pilot study was 90 mg/kg (J. Foster unpublished data) so we estimate the minimum bait delivered dose at 270 mg/kg. Given an 80g bait with 8g active, a 56.7 kg (90 lb.) yearling would need to consume at least 1.9 baits for mortality to occur.

Raccoons were more sensitive to sodium nitrite than deer and feral swine. Up-And-Down trials indicate the gavage delivered LD_{50} of sodium nitrite in raccoons at 58 mg/kg. The food delivered LD_{50} is approximately 174 mg/kg. Therefore consumption of 0.09 baits would have a lethal effect on 50% of 4 kg raccoons. The observed MLD gavage dose for raccoons was 50 mg/kg. It is likely that bait delivered doses > 150 mg/kg would begin to produce lethal effects in raccoons. Consumption of > 0.08 of an 80 gram bait may produce a lethal effect in raccoons.

Feral swine were more sensitive to SN than white-tailed deer and less sensitive than raccoons. Median lethal gavage and food delivered doses were estimated at 133 mg/kg and 399 mg/kg respectively. Consumption of 2.2 baits would result in 50% mortality in 45 kg (100 lb.) pigs. The minimum lethal gavage dose observed was 113 mg. Similar to Cowled et al. (2008), we predict that bait delivered doses would require > 400 mg/kg to be

effective. One point nine baits would be the minimum needed to produce lethal effects in 45 kg pigs.

Our data indicate that sodium nitrite at pig lethal doses is potentially lethal to deer and raccoons. As no specimens expired or appeared ill after 48 hours of observation, our research supports London et al's. (1967) assessment that sublethal exposure poses no adverse affects to pigs. However, gavage dosages are indicative of relative species sensitivity and should only be used as a factor in risk prediction. Risk assessment should also include the probability that a non-target consumer would consume SN at a food administered lethal dose, which may be 3 times higher than gavage doses. Moreover, probability of non-target consumption is independent of species sensitivity. A very sensitive species remains at low risk if it is very unlikely to consume a lethal dose. Risk to a species then is a function of species sensitivity, amount of toxicant consumed, and probability of consumption.

In the case of white-tailed deer, one of the most socio-economically important species in the state, risk of death from consumption of sodium nitrite laden bait, when delivered from a hog specific feeder, is very low. A vegetarian diet and lack of dexterity to manipulate complex feeders (Long et al. 2010, Justin Foster unpublished data) make it unlikely that deer would consume meat based SN laden baits to produce a significant mortality rate.

Raccoons, the most probable non-target consumer of feral pig baits, are only at slightly higher risk than deer even though they are much more sensitive. In this case, risk of sodium nitrite intoxication is minimal because raccoons lack the strength to operate doors on hog exclusive feeders (Long et al. 2010, Justin Foster unpublished data). Their high sensitivity to SN however emphasizes the need to reduce spills of the baits through mechanical compromise of the feeder. Feeders must be constructed to resist damage from any species that would attempt to utilize them.

In conclusion, our data demonstrate that SN shows promise of reducing the cost of feral swine control in Texas. It is lethal, fast acting (< 180 minutes) and can be delivered solely to pigs. We recommend that research supporting the requirements of the application process for registry as a pig toxicant with USEPA be supported by stakeholders. Because SN is patented for use in pig baits, this effort should be in collaboration with the patent holder and all of their American collaborators (Texas Parks and Wildlife, USDA-Aphis-Wildlife Services, USDA-Aphis-National Wildlife Research Center). This would include small (< 10 acres) and large (> 10 acres) scale studies. Research topics and literature reviews should include target specificity of feeders; bait preference by pigs and non-targets; stabilization of active ingredients in bait; environmental effects; secondary consumers (i.e. bioaccumulation); efficacy; and novel control devices and solutions.

Prepared by: Justin Foster **Approved by:** Roxanne Loegering

TPWD, Principal Investigator Federal Aid Coordinator

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