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# Potential secondary poisoning risks to non-targets from a sodium nitrite toxic bait for invasive wild pigs

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## Abstract

**BACKGROUND:** An acute and orally delivered toxic bait containing micro-encapsulated sodium nitrite (MESN), is under development to provide a novel and humane technology to help curtail damage caused by invasive wild pigs (*Sus scrofa*). We evaluated potential secondary risks for non-target species by: testing whether four different types of micro-encapsulation coatings could reduce vomiting by invasive wild pigs, testing the levels of residual sodium nitrite (SN) in tissues of invasive wild pigs, testing the environmental persistence of SN in vomitus, and conducting a risk assessment for scavengers.

**RESULTS:** Micro-encapsulation coatings did not affect the frequency of vomiting. We identified no risk of secondary poisoning for non-target scavengers that consume muscle, eyes, and livers of invasive wild pig carcasses because residual SN from the toxic bait was not detected in those tissues. The risk of secondary poisoning from consuming vomitus appeared low because ~90% of the SN was metabolized or broken down prior to vomiting, and continued to degrade after being exposed to the environment. Secondary poisoning could occur for common scavengers that consume approximately  $\geq 15\%$  of their daily dietary requirements of digestive tract tissues or undigested bait from carcasses of invasive wild pigs in a rapid, single-feeding event. The likelihood of this occurring in a natural setting is unknown. The digestive tracts of poisoned invasive wild pigs contained an average of ~4.35 mg/g of residual SN.

**CONCLUSION:** Data from this study suggest no risks of secondary poisoning for non-target species (including humans) that consume muscle, liver, or eyes of invasive wild pigs poisoned with a MESN toxic bait. More species-specific testing for scavengers that consume digestive tract tissues and undigested bait is needed to reduce uncertainty about these potential risks.

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**Keywords:** feral swine; non-target risk; pesticide; sodium nitrite; secondary poisoning; *Sus scrofa*; toxicant; vomit; wild boar; wildlife damage management

## 1 INTRODUCTION

Invasive wild pigs (hereafter: wild pigs; *Sus scrofa*; also referred to as feral hogs, feral pigs, feral swine, or wild boars<sup>1</sup>) are a damaging invasive species spreading throughout North America, Australia, South America, Africa, and many island nations.<sup>2,3</sup> Populations of wild pigs cause extensive damage to agricultural and natural landscapes, and are expensive to control using conventional methods of trapping or shooting.<sup>4–7</sup> Additionally, these methods have not mitigated damage across large regions.<sup>8,9</sup> Invasive wild pigs also serve as reservoirs of diseases,<sup>10,11</sup> reduce plant species diversity through rooting,<sup>12</sup> depredate sensitive species,<sup>13–15</sup> and destroy habitats for native species.<sup>16</sup> As a consequence of these negative effects, a cost-effective means for controlling wild pigs, in the form of a toxic bait, is being developed for use in the United States, Australia, and New Zealand.<sup>17,18</sup>

Until recently, no toxic baits for wild pigs have been registered for use in the United States. In 2017, a toxic bait containing the active ingredient warfarin was registered by the United States Environmental Protection Agency (EPA), but has not been approved for

use in any state. Warfarin causes death via diffuse hemorrhaging and is slow acting, therefore it has been deemed inhumane for wild

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pigs in Australia.<sup>19,20</sup> Two other toxic baits containing the active ingredients sodium fluoroacetate (1080) or yellow phosphorus, respectively, are used on wild pigs in Australia (yellow phosphorus under a single state permit only), although both baits have generated concerns about humaneness and hazards for non-target species.<sup>17</sup> Sodium nitrite (SN) was proposed as a new active ingredient for toxic bait for wild pigs that is considered humane with fewer non-target risks,<sup>17,18,21</sup> and has been recently registered for use in New Zealand on wild pigs and common brushtail possums (*Trichosurus vulpecula*).<sup>18</sup> Invasive wild pigs are highly susceptible to SN because they have lower levels of methemoglobin reductase enzymes compared to other mammals that protect against severe methemoglobinemia (i.e. blocking the uptake and transport of oxygen by red blood cells).<sup>17</sup> Severe methemoglobinemia quickly induces unconsciousness and causes rapid and humane death from anoxia with minimal symptoms of distress (e.g. symptomatically similar to carbon monoxide poisoning).<sup>22,23</sup>

The use of toxic baits containing SN brings forth benefits and challenges. The main challenge is that SN is a highly reactive compound that must be pre-formulated to prevent it from reacting with bait substrates to be shelf-stable and effective. However, this instability has two clear benefits. Once SN is exposed to the environment, its rapid breakdown into less toxic and more aversive forms via the nitrogen cycle reduces the risks of secondary poisoning for scavengers.<sup>21</sup> Also, SN does not bio-accumulate in tissues of animals because it is quickly metabolized or broken down,<sup>21</sup> making SN an all-or-nothing toxin in which repeated sublethal exposures by scavenging animals are unlikely to have debilitating effects.<sup>17</sup> Given these characteristics, a collaborative research effort between the Wildlife Services National Wildlife Research Center (NWRC, USA) of the United States Department of Agriculture, the Texas Parks and Wildlife Department (TPWD, USA), the Invasive Animal Cooperative Research Center (IACRC, Australia), Animal Control Technologies Australia (ACTA, Australia) Pty Ltd, and Connovation PTY Ltd (New Zealand) has developed an acutely toxic and highly effective lethal bait for wild pigs.<sup>24</sup> This bait contains 100 mg/g (10%) of SN incorporated into an oil-based matrix primarily comprised of peanut paste.<sup>25</sup>

Though the benefits of SN in toxic baits for wild pigs are clear, research assessing secondary poisoning risks associated with deployment of SN toxic baits in natural settings, with an emphasis on non-target scavenger species in North America, is required. Secondary risks are expected to be low, because consuming common brushtail possum carcasses poisoned with SN toxic bait in New Zealand revealed no risks for domestic dogs, cats, and chickens.<sup>26</sup> Other examinations of residual SN in carcasses of wild pigs were conducted via oral gavage using non-micro-encapsulated SN or prototype baits containing differing concentrations of SN,<sup>21</sup> and may not reflect actual residue levels resulting from the consumption of the current bait formulation. In addition, consumption of SN appeared to cause vomiting by some wild pigs,<sup>21</sup> which may affect lethality and generate risks of secondary poisoning for non-target species that could consume the vomitus. Prior to this study, it was not known if the frequency of vomiting or the potential risks of vomitus to non-target species could be reduced.

Our strategy for evaluating and attempting to minimize secondary risks incorporated the use of a micro-encapsulation coating around SN.<sup>18,27</sup> This coating is used to protect SN from interacting with bait substrates and to mask the salty taste of SN which animals find aversive at lethal concentrations. When wild pigs consume a lethal dose of the micro-encapsulated SN (MESN), the micro-encapsulated coating quickly breaks down inside the

digestive tract and releases SN into the blood to produce overwhelming methemoglobinemia. The timing and location of the release of SN in the digestive tract may affect the lethality, levels of residual SN in tissues and vomitus, and frequency of vomiting for wild pigs.

The objectives of this study were to evaluate the potential secondary risks of using MESN toxic bait to control wild pigs using four types of micro-encapsulation coatings. In particular, our goals were to reduce vomiting by wild pigs, reduce residual SN in carcasses of wild pigs, determine the environmental persistence of SN in vomitus, and evaluate potential risks of secondary poisoning for avian and mammalian scavengers. To meet these goals, we tested baits containing the four types of MESN on captive wild pigs, and evaluated for any reduction in vomiting and residual SN in carcasses. Results will be used to support the application for registration of a MESN toxic bait for wild pigs in the USA and Australia.

## 2 MATERIALS AND METHODS

We conducted the experimental trials in an outdoor research facility at the Texas Parks and Wildlife, Kerr Wildlife Management Area (KWMA), Hunt, TX, USA during January and May 2015. All wild pigs used in this study were wild captured from areas surrounding the KWMA. Upon arrival to Kerr WMA, we individually marked wild pigs with uniquely identifiable tags inserted in ears. The wild pigs were group housed in a 0.02 km<sup>2</sup> (5 ac) outdoor holding pen for  $\geq 2$  weeks prior to study initiation. This pen was constructed with steel-mesh perimeter fencing buried into the ground and contained naturally growing vegetation [e.g. oak (*Quercus* spp.) and cedar (*Juniperus ashei*) trees] and shade structures. We maintained the wild pigs on Bluebonnet® 18% Sow Ration Pellet (AC Nutrition, LP, Ardmore, OK, USA) provided at 3–5% of group body mass daily. This maintenance diet had a recommended feeding rate of 3% of body weight for growing swine. We provided water *ad libitum* from self-maintaining water troughs.

### 2.1 Test substances

We tested four formulations of proprietary micro-encapsulated coatings and a control formulation. This first treatment consisted of a water soluble, protein coating (Connovation Ltd, Auckland, New Zealand) applied to SN that is currently used in the prototype HOGGONE® (ACTA, Victoria, Australia) for the USA and Australia, and in Bait-Rite Paste® (Connovation Ltd) in New Zealand.<sup>18,28</sup> The second treatment consisted of a double coating of the first treatment resulting in a thicker coating in an attempt to delay absorption of SN into the bloodstream. The third treatment consisted of a soft wax coating that was insoluble in water (Southwest Research Institute, San Antonio, TX, USA). The fourth coating consisted of an acidic resistant resin specifically designed for enteric release of pharmaceuticals (Southwest Research Institute). Finally, the control treatment consisted of the first protein coating applied to sugar, instead of SN. The control group served as a baseline to assessing vomiting from non-toxic HOGGONE®.

### 2.2 Trial methodology

We tested each of the four treatments on  $n = 6$  wild pigs, and we tested the control treatment on  $n = 4$  wild pigs. Prior to the tests, we moved the wild pigs into a sorting pen and randomly selected three pigs per day for testing allowing for three constraints among the treatment groups: (1) equal sex ratio among all treatments,

(2) animal weights were between 20 and 40 kg to test subadults and adults, and (3) all animals appeared healthy and active. The selected wild pigs were fasted for 12 h prior to testing, although all animals had access to water and sparse vegetation in the pens.

We dosed each treatment animal with 400 mg/kg (body weight) of SN (not including the micro-encapsulation coating) which represented the estimated LD<sub>99</sub> for wild pigs.<sup>17</sup> The control animals were similarly dosed with 400 mg/kg (body weight) of sugar. Each treatment was hand-mixed into a bait matrix of peanut paste and crushed grains as found in HOGGONE®, so that the concentration of SN (or sugar) was 10% w/w of the finished bait. We loaded the freshly prepared bait into a ~3.2 × 45 cm polycarbonate pipe with rounded edges. The wild pigs were moved into a sorting chute and restrained with two animal control poles (The Ketch All Company, San Luis Obispo, CA, USA) secured around the upper snout. We inserted the pipe into the mouth of the focal wild pig and delivered the bait with a smaller, capped pipe. The bait was plunged slowly to allow the wild pigs to masticate and swallow. We observed each wild pig until mastication stopped to ensure that all bait was swallowed, and any bait dropped was redelivered. Dosing typically took ~10–20 min per animal.

After each animal wholly consumed the bait, we immediately moved it into one of three adjacent observation pens measuring ~1.5 × 2 m. Two observation towers were located directly above the observation pens so that researchers could observe each wild pig from ≤10 m. We observed the treatment animals continually until death or until 4 h had passed. After 4 h, the animals were checked every 15 min. After 8 h, the animals were checked approximately once per hour. Any treatment animals that survived for ≥24 h were humanely euthanized via cranial gunshot.<sup>29</sup> All control animals were humanely euthanized after 8 h. Research activities involving the handling of animals were approved by the TPWD Institutional Animal Care and Use Committee (protocol # 211010520151).

We recorded the time since dosing to first vomiting, death, and the frequency of vomiting for each animal. Once death occurred, we immediately collected five tissue samples (i.e. muscle from hind quarter, liver, eye, stomach, and small intestine). We rinsed the stomach and small intestine with water to remove any bait prior to freezing. We also collected two samples of undigested bait (i.e. stomach contents and vomitus if available). All samples were immediately vacuum sealed and frozen at approximately –12 °C to preserve the levels of residual SN. Vomitus was collected after the wild pig died to avoid disturbing the animal. The pens were thoroughly rinsed and scrubbed after each test to avoid any potential cross-contamination.

All tissue samples were analyzed by Southwest Research Institute using high-performance anion-exchange chromatography to quantify the concentration of residual nitrite. This method was calibrated using nine concentration points between 0.0001% and 0.02% of nitrite. The efficiency for recovery averaged 92.4% (SD = 22.2) and the method limit of detection was 0.00002% w/w. The undigested bait samples were analyzed by the USDA, National Wildlife Research Center (Fort Collins, CO, USA) using reverse-phase-ion-chromatography. This method was validated using samples containing 1% to 15% nitrite. The efficiency of recovery for nitrite averaged 92% (SD = 2.4%) and the method limit of detection was 0.00036% w/w. For all samples, if nitrite was not detected we reported the level to be consistent with the method limit of detection. We converted the concentrations of nitrite (mg/g) to SN to make inferences relative to the active ingredient.

## 2.3 Environmental persistence of SN in vomitus

After the above testing, we homogenized the samples of vomitus from all wild pigs for 15 min using a Brinkman Polytron PT 3000 homogenizer (Kinematica, Inc., Bohemia, NY, USA). We removed and placed approximately 2.1–4.7 g samples of the mixture into 132 individual plastic weigh boats (40 × 40 × 8 mm; Sigma–Aldrich, Darmstadt, Germany). We then immediately analyzed three randomly selected samples for an initial estimate of the residual concentration of SN (time  $t = 0$ ), using the methods described above for vomitus. We also immediately vacuum sealed and froze three samples at approximately –12 °C as controls for shelf-life in a freezer.

We split the remaining samples by placing half ( $n = 63$ ) into one of two environmental chambers (Conviron model E7/2; Conviron, Winnipeg, Manitoba, Canada). Each chamber was set to follow a 12-h photoperiod using ultraviolet and incandescent light, or darkness. We set the temperature and relative humidity (RH) to represent a hot and humid climate (e.g. typical of the south and south-east USA), or a moderate climate (e.g. typical of the midwest or west USA). Specifically, the hot and humid chamber was maintained at 35.2 °C (SD = 0.4) and 92.4% (SD = 1.9) RH during the day and 27.2 °C (SD = 4.1) and 98.6% (SD = 4.3) RH at night. The moderate chamber was maintained at 19.3 °C (SD = 0.4) and 59.6% (SD = 3.3) RH during the day and 11.3 °C (SD = 3.8) and 67.6% (SD = 10.2) RH at night.

From each chamber, we randomly selected and removed three samples at predetermined intervals. We examined for immediate trends by sampling at the hours of 1, 2, 3, 4, 5 and 6 post-initiation. Then, we examined for longer-term trends by sampling at the days of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 and 30 post-initiation. All samples were immediately vacuum sealed and frozen upon removal from the chambers at approximately –12 °C. At the end of the sampling interval, all samples were thawed and analyzed for concentration of residual SN using the method described above for vomitus.

We accounted for evaporative loss in the samples through time by calculating and dividing the mass of SN at time ( $t$ ) by the mass of SN at  $t = 0$ , to generate an overall proportion of SN relative to the beginning of the study. Mass at  $t = 0$  was calculated using the average concentration of SN detected from the six samples that were analyzed at time  $t = 0$ .

## 2.4 Data analysis

We compared the proportion of wild pigs that vomited among the four micro-encapsulation treatments using a Fischer Exact Test in Program R (v3.3.1; R Foundation for Statistical Computing, Vienna, Austria). Then, we compared the residual concentrations of SN in the tissues and undigested bait among treatments using generalized linear models. For these analyses we used the control animals as our reference samples. We considered all statistical and biological differences among the reference and treatment groups at the level of  $\alpha = 0.05$ .

For the environmental persistence of SN in vomitus through time, we compared the proportion of SN through time, relative to initial time ( $t = 0$ ), and between climatic conditions, using beta regression (package betareg) in Program R.<sup>30</sup> Specifically, we examined the interaction of time × climate to evaluate the cumulative effects of both conditions on the persistence of SN. We conducted two analyses of this interaction, the first to examine the immediate effects on persistence of SN during the first 6 h. The second analysis examined the longer-term effects for 30 days.



**Table 1.** Outcomes from invasive wild pigs consuming 400 mg/kg (body weight) of sodium nitrite in the toxic bait using four micro-encapsulation coatings in pens at the Kerr Wildlife Management Area, Hunt, TX, USA during 2014

| Micro-encapsulation treatment | N | Died | No. vomited | Time to first vomit (h) |      | Time to death (h) |      |
|-------------------------------|---|------|-------------|-------------------------|------|-------------------|------|
|                               |   |      |             | Mean                    | SE   | Mean              | SE   |
| Protein (single)              | 6 | 6    | 4           | 1.90                    | 0.20 | 2.37              | 0.24 |
| Protein (double)              | 6 | 6    | 3           | 2.26                    | 0.24 | 2.77              | 0.15 |
| Soft wax                      | 6 | 6    | 5           | 2.55                    | 0.23 | 2.93              | 0.28 |
| Resin                         | 6 | 5    | 5           | 9.99                    | 2.48 | 12.44             | 4.66 |
| Control                       | 4 | 0    | 0           | NA                      | NA   | NA                | NA   |

Control animals consumed 400 mg/kg (body weight) of micro-encapsulated sugar in a placebo bait.

**Table 2.** Means (mg/g) and standard errors (SE) of sodium nitrite (SN) detected in the tissues and undigested bait from invasive wild pigs after consuming 400 mg/kg (body weight) of SN in the toxic bait containing four micro-encapsulation coatings in pens at the Kerr Wildlife Management Area, Hunt, TX, USA during 2014

| Micro-encapsulation treatment | Residual sodium nitrite (mg/g) |        |                    |       |                    |        |                    |       |                     |       |                    |        |                    |      |
|-------------------------------|--------------------------------|--------|--------------------|-------|--------------------|--------|--------------------|-------|---------------------|-------|--------------------|--------|--------------------|------|
|                               | Muscle                         |        | Liver              |       | Eye                |        | Stomach            |       | Small intestine     |       | Stomach contents   |        | Vomit              |      |
|                               | Mean                           | SE     | Mean               | SE    | Mean               | SE     | Mean               | SE    | Mean                | SE    | Mean               | SE     | Mean               | SE   |
| Protein (single)              | 0.003 <sup>A</sup>             | 0.0009 | 0.006 <sup>A</sup> | 0.001 | 0.004 <sup>A</sup> | 0.0009 | 0.78 <sup>B</sup>  | 0.11  | 0.83 <sup>A,B</sup> | 0.29  | 11.51 <sup>B</sup> | 2.58   | 11.98 <sup>A</sup> | 2.23 |
| Protein (double)              | 0.003 <sup>A</sup>             | 0.0006 | 0.006 <sup>A</sup> | 0.002 | 0.002 <sup>A</sup> | 0.0002 | 0.82 <sup>B</sup>  | 0.15  | 1.63 <sup>B</sup>   | 0.57  | 12.74 <sup>B</sup> | 2.67   | 14.76 <sup>A</sup> | 3.47 |
| Soft wax                      | 0.001 <sup>A</sup>             | 0.0001 | 0.003 <sup>A</sup> | 0.001 | 0.006 <sup>B</sup> | 0.001  | 0.80 <sup>B</sup>  | 0.18  | 0.33 <sup>A,B</sup> | 0.85  | 9.31 <sup>B</sup>  | 2.37   | 9.56 <sup>A</sup>  | 0.49 |
| Resin                         | 0.002 <sup>A</sup>             | 0.0003 | 0.005 <sup>A</sup> | 0.003 | 0.005 <sup>B</sup> | 0.001  | 0.56 <sup>B</sup>  | 0.26  | 0.27 <sup>A,B</sup> | 0.80  | 13.93 <sup>B</sup> | 4.64   | 10.38 <sup>A</sup> | 1.93 |
| Control                       | 0.002 <sup>A</sup>             | 0.0001 | 0.005 <sup>A</sup> | 0.003 | 0.002 <sup>A</sup> | 0.0001 | 0.007 <sup>A</sup> | 0.003 | 0.03 <sup>A</sup>   | 0.004 | 0.008 <sup>A</sup> | 0.0001 | NA                 | NA   |

Superscript letters signify statistical differences among the micro-encapsulation treatments at the level of  $\alpha = 0.05$  within the sample groups.

For both analyses, we examined the parameter estimates ( $\beta$ ) and 95% confidence intervals (CIs) of the interaction for a lack of overlap of 0 to indicate statistical and biological influences on the persistence of SN.

## 2.5 Risk assessment

To our knowledge, we identified and evaluated the widest ranges of data available on sensitivity to SN for mammalian and avian species to provide insight on risk of secondary poisoning for scavengers that may consume tissues of wild pig carcasses. For mammalian species, we identified the lowest reported LD<sub>50</sub> value of 58 mg/kg (body weight) for raccoons (*Procyon lotor*)<sup>31</sup> and the highest reported value of 525 mg/kg (body weight) for black rats (*Rattus rattus*).<sup>32</sup> For avian species, we identified the lowest reported LD<sub>50</sub> value of 68.5 mg/kg (body weight) for domestic chickens (*Gallus gallus domesticus*) and domestic mallard ducks (*Anas platyrhynchos domestica*),<sup>33</sup> and the highest reported value of 619 mg/kg (body weight) for northern bobwhite (*Colinus virginianus*).<sup>34</sup> We used these ranges of LD<sub>50</sub> values to represent a general range of risk for mammalian and avian species that may scavenge carcasses of wild pigs. We calculated ranges of % live body mass that each class of scavenger would need to consume during a single-feeding event to be put at risk from SN, using the following formula:

$$\frac{\left[ \frac{\text{LD}_{50} \text{ value} \times \text{scavenger live body mass}}{\text{residual SN in IWP tissues}} \right]}{\text{scavenger live body mass}}$$

Finally, we used the values from above to calculate the % daily diet that a scavenger would need to consume during a

single-feeding event to be put at risk from SN. We calculated the mean and 95% CI for daily diet requirements (g) using allometric equations for field metabolic rates of dry matter food.<sup>35</sup> We multiplied the daily dry matter amounts by 3.33 g of fresh matter per 1 g of dry matter to convert dry matter to daily fresh matter intake.<sup>35</sup> Specifically, we calculated daily fresh matter intake for three common non-target species that may consume tissues or undigested bait from carcasses of wild pigs, including (1) an average sized coyote (*Canis latrans*; 15 kg) to represent a mammalian scavenger, (2) an average sized raccoon (7 kg) to represent a meso-omnivore, and (3) an average size turkey vulture (*Cathartes aura*; 2 kg) to represent an avian scavenger.

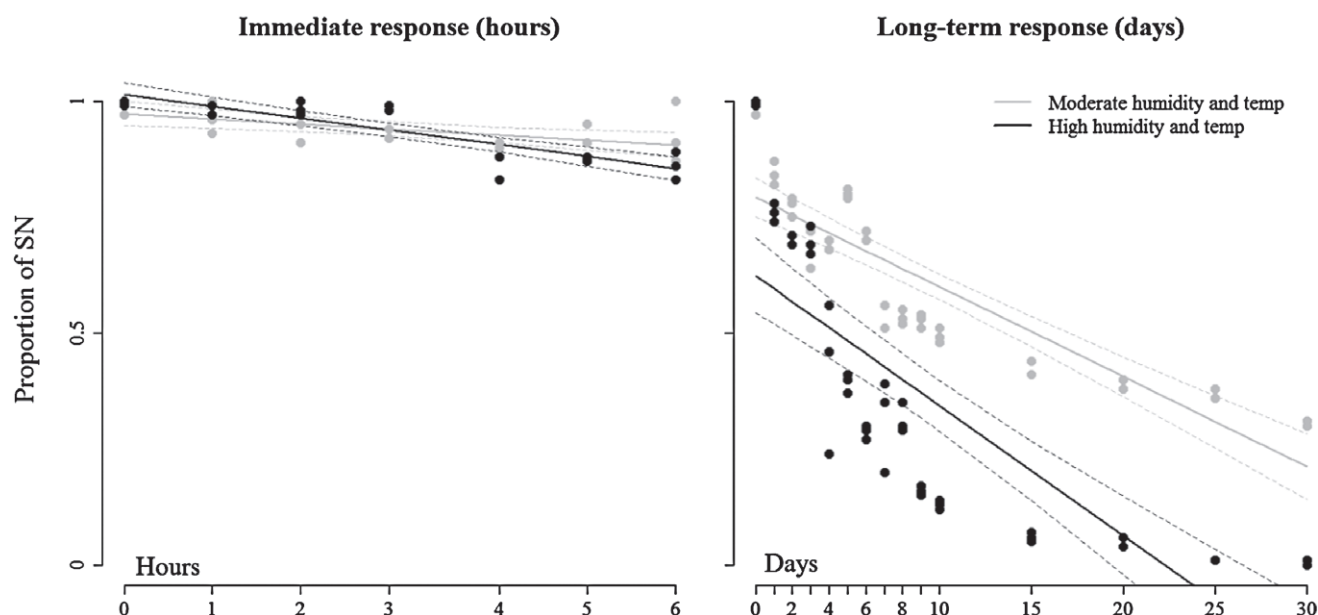
## 3 RESULTS

### 3.1 Micro-encapsulation treatments

All of the MESN treatments were 100% effective at killing wild pigs, except the resin treatment, where one animal survived (Table 1). We detected no differences in the proportion of wild pigs that vomited among the treatment groups ( $P = 0.766$ ), with a total of 17 of 24 (70.8%) wild pigs vomiting at least once. On average, the first vomiting occurred 2.3 h (SE = 0.15) post-consumption, quickly followed by death at 2.7 h (SE = 0.14), excluding the resin treatment group. None of the control animals showed any vomiting or symptoms of intoxication throughout the study.

### 3.2 Residual SN in tissues and undigested bait

We excluded the lone surviving wild pig from the resin treatment group from analyses of residual SN in tissues and stomach



**Figure 1.** Fitted linear regression lines (solid lines) with 95% confidence intervals (dotted lines) and associated data (points) showing reduction of sodium nitrite (SN) in the vomitus of the toxic bait through time under two environmental conditions collected from captive invasive wild pigs at the Kerr Wildlife Management Area, Hunt, Texas, USA. The toxic bait was comprised of 10% SN (100 mg/g SN) of which invasive wild pigs were dosed at 400 mg/kg (body weight) of SN. The level of residual SN at time  $t = 0$  when regurgitation occurred averaged 10.9 mg/g (SE = 0.07), equating to 1.09% SN in the vomitus.

contents. For all other wild pigs, the average levels of SN were not different between treatment and control animals in muscle ( $F_{4,22} = 2.18$ ,  $P = 0.105$ ; Table 2) and liver tissues ( $F_{4,22} = 0.53$ ,  $P = 0.716$ ). For the eye tissue, two of the treatment groups had higher levels of SN than in control animals ( $F_{4,22} = 4.15$ ,  $P = 0.012$ ), although all levels were low. For the stomach tissues, all treatments had higher levels of SN compared to control animals ( $F_{4,22} = 3.39$ ,  $P = 0.026$ ). For the small intestine tissues, only the double-coated protein treatment group had statistically higher levels of SN compared to control animals ( $F_{4,22} = 3.84$ ,  $P = 0.016$ ), albeit all treatment groups had noticeably higher levels of SN than control animals. For the stomach contents, all of the treatments had higher levels of SN compared to control animals ( $F_{4,22} = 2.92$ ,  $P = 0.044$ ). Lastly, there were no differences in the residual SN in vomitus among the treatment groups ( $F_{3,13} = 0.75$ ,  $P = 0.541$ ).

For the single pig that survived in the resin treatment group (26 kg, male), the SN had been metabolized or broken down, and was undetectable in tissues in  $\leq 24$  h. The animal appeared fully recovered at this time showing no symptoms of SN intoxication (e.g. labored breathing, incoordination, etc.). The muscle, liver, and eye all contained  $<0.003$  mg/g of SN each. The stomach contained 0.005 mg/g, the small intestine contained 0.014 mg/g, and the stomach contents contained 0.008 mg/g of SN.

### 3.3 Environmental persistence of SN in vomitus

The average concentration of SN in freshly regurgitated vomitus ( $t = 0$ ) was 10.9 mg/g (SE = 0.07), equating to vomitus comprised of 1.09% SN. Analysis of the first 6 h indicated that the interaction of time  $\times$  climate had a significant influence on the decrease of SN ( $\beta = 8.64$  95% CI = 7.62–22.60). In particular, vomitus in the high humidity and temperature climate had a greater decrease in SN than in the moderate climate through time, albeit both climates had slight decreases through time (Fig. 1). The same trend was even more evident in the longer-term analysis ( $\beta = 0.13$ , 95% CI = 0.09–0.17; Fig. 1). In the high humidity and temperature

climate, the SN had decreased nearly 100% in 25 days (Fig. 1). The moderate climate also had substantial decreases in SN, but less than the hot and humid climate.

### 3.4 Risk assessment

Digestive tracts (i.e. stomach, stomach contents, and small intestines) were the only components of wild pig carcasses we measured with elevated residues of SN (average = 4.35 mg/g, excluding wild pigs from the resin treatment group). Based on this average, we estimated that mammalian scavengers would have to consume 1.33–12.08% of their body mass of wild pig digestive tract during a single-feeding event to be put at risk of secondary poisoning. Similarly, we estimated that avian scavengers would need to consume 1.58–14.25% of their body mass during a single-feeding event.

Coyotes that consume an average of 1567 g (95% CI = 536–4579) of fresh matter daily would need to consume 13–116% of their daily dietary requirement (i.e. 200–1810 g) of digestive tract tissues or undigested bait in a single-feeding event to potentially be at risk of secondary poisoning from SN. Raccoons that consume 580 g (95% CI = 220–1530) of fresh matter daily would have to consume 16–147% of their daily dietary requirement (i.e. 100–800 g) in a single-feeding event to be at risk. Finally, turkey vultures that consume 228 g (95% CI = 89–584) of fresh matter daily would need to consume 14–125% of their daily dietary requirement (i.e. 30–280 g) in a single-feeding event to be at risk.

## 4 DISCUSSION

Overall, this study provided important information for understanding the potential secondary poisoning risks associated with wild pigs that consume a MESN toxic bait. None of the tested micro-encapsulation coatings appeared to offer less risk than the single-coated protein that is currently used to manufacture the

toxic bait, and currently used in New Zealand. The protein (single and double) and soft wax coatings had similar characteristics in time-to-first-vomit, time-to-death, residual SN, and efficacy. These three coatings appeared to release the SN similarly, and allow rapid absorption into the bloodstream resulting in a severe methemoglobinemia and quick death (mean = 2.7 h from dosing). The resin coating appeared to have a slower release of SN with higher variability, and subsequently had a delayed time-to-death (mean = 12.4 h). One wild pig survived in this treatment group, likely because the SN was metabolized quickly enough to counteract the variable release.

We found that  $\geq 50\%$  of fasted wild pigs vomited at least once after administering a rapid and lethal dose of the toxic bait in this study, regardless of which formulation of micro-encapsulation coating was used. The exact mechanism triggering the regurgitation could not be determined using this study design, but is likely related to the release of SN or the degradation of nitrite into nitric oxide gas following the breakdown of the micro-encapsulation coating and subsequent dissociation of SN.<sup>36</sup> Toxic baiting in more natural settings may reduce vomiting because wild pigs are unlikely to be fasted. Observations of vomitus in the field suggest that vomiting was infrequent in field trials in Australia.<sup>21</sup>

It is unknown whether non-target animals will consume vomitus of a toxic bait that contains SN. We suspect vomitus could be aversive to scavengers because the SN is exposed to the digestive tract, leading to a breakdown of the micro-encapsulation and therefore is no longer protected from degradation and detection by non-target animals. The micro-encapsulation coating dissolves (except resin coating) in the stomach of wild pigs when exposed to hydrochloric acid, digestive enzymes, and water; therefore, the vomitus likely acquires a strong salt flavor and exhibits aversive taste and odor from the SN breakdown. Ultimately, the residual SN oxidizes to harmless nitrates as part of the nitrogen cycle. Also, reduction of nitrite may occur and produce nitric oxide gas with a noticeable odor<sup>37</sup> that is likely aversive.

Our results show that the MESN toxic bait lost  $\sim 90\%$  of SN in the stomach of wild pigs prior to any vomiting (i.e. within 2.3 h). After 1 week of exposure to the environment,  $\leq 0.5\%$  of the original SN was detected in the vomitus of the MESN toxic bait, although this is conservative because no leeching of the SN occurred during our laboratory test (e.g. rain would rapidly remove SN residues). We were unable to accurately weigh the amount of vomitus from each wild pig because the vomitus was usually a liquid consistency and not all of it could be retrieved. Visual observations suggest that the amount of undigested bait in the vomitus varied widely but was often small relative to the amount of bait consumed. The consistency and often scarce amounts of vomitus expelled suggest that the risk of scavengers finding and consuming enough vomitus to ingest a rapid bolus dose of SN that would be required for a lethal sequel is low.

Our findings confirm that the majority of a wild pig carcass will not be hazardous to non-target species, similar to previous findings with MESN toxic baits.<sup>21,26</sup> The US Food and Drug Administration regulates that no more than 0.2 mg/g of SN be allowed as a food additive for human consumption in finished meat products.<sup>38</sup> Extrapolating this concentration to wildlife, we conclude that consumption of the muscle, liver and eye tissues of a wild pig carcass immediately after death is nonhazardous to non-target species, including humans. The digestive tract (i.e. stomach, stomach contents and small intestines) averaged  $\sim 22$  times this dose at the time of death, and therefore may pose some risk.

From our risk assessment we found that coyotes, raccoons and turkey vultures would need to consume approximately  $\geq 15\%$  of their daily dietary requirements of digestive tract tissues or undigested bait in a rapid, single-feeding event to be at moderate risk, based on LD<sub>50</sub> values reported for mammalian and avian species. This finding of is conservative for a few reasons. Primarily, the  $\sim 15\%$  value is based on the most conservative LD<sub>50</sub> estimates reported for mammalian and avian species, and then extrapolated. Some species may be less susceptible to SN based on higher levels of methemoglobin reductase enzymes for converting methemoglobin back to hemoglobin.<sup>39</sup> Using less conservative LD<sub>50</sub> values reveals that scavengers would need to consume more than their daily dietary requirements ( $\geq 116\%$ ) in a single-feeding event to be at risk. Second, the reported values of LD<sub>50</sub> were obtained from studies that used oral gavage to administer SN, and therefore represent more acute estimates of risk compared to free-feeding animals. For example, wild pigs and domestic chickens were approximately four times less sensitive to SN in free-feeding trials than in oral gavage trials.<sup>17,33</sup> Third, the residues of SN continue breaking down into less toxic forms (i.e. nitrate or nitric oxide) as carcasses age, therefore the digestive tracts likely become less toxic through time.<sup>21</sup> Turkey vultures and black vultures (*Coragyps atratus*) reportedly take  $\sim 24$  h to begin scavenging on pig carcasses in Texas.<sup>40</sup> Finally, this risk assessment assumes that scavengers would consume only the digestive tract during a single-feeding event, but likely scavengers would also consume other tissues (e.g. muscle) and dilute or minimize the amount of digestive tract consumed.

Scavengers would have to consume the digestive tract rapidly (e.g. within a single-feeding event) to experience toxic effects because SN toxicity is dependent on circulating methemoglobin concentration. If scavengers consumed the digestive tract slowly (e.g. over multiple hours) the SN will be metabolized and cleared from the circulation, and unlikely to be lethal. Most carcasses are not 100% consumed by scavengers,<sup>41</sup> lessening the risks that only digestive tracts are consumed. For example, black bears (*Ursus americanus*) typically avoid consuming digestive tracts,<sup>42</sup> and therefore likely experience low risk. Coyotes prefer to consume muscle tissue<sup>42</sup> but may consume digestive tracts during times of scarce resources, and therefore may experience some risk. Turkey vultures and black vultures will consume entire carcasses<sup>40</sup> but over a period of multiple days,<sup>43</sup> which therefore reduces their risk. Avian scavengers also did not prefer stomachs and stomach content from carcasses of wild pigs in Texas leaving those as the last items to be consumed.<sup>44</sup> A more thorough, probabilistic risk assessment accounting for all these factors is needed to quantitatively inform the risks of secondary poisoning for these scavengers.

Based on our findings, the risks of secondary poisoning for humans that may harvest a wild pig exposed to MESN toxic bait are negligible. Primarily, there is little opportunity for humans to harvest wild pigs exposed to MESN toxic bait because the time to death is  $< 3$  h. However, if a poisoned wild pig was harvested, consumption of muscle tissue would not be hazardous because SN residues are well below the recommended amount for human consumption.<sup>38</sup> In addition, a poisoned wild pig would be easily detected and consumption avoided because severe methemoglobinemia turns the blood a noticeable dark brown color.<sup>45</sup> The only risk to humans would occur if a human consumed the digestive tract of a poisoned wild pig. A 68 kg human would need to consume 0.91–8.22 kg of digestive tract during a single feeding to be put at risk of secondary poisoning, which is unlikely.



An important observation from this study involves the single wild pig that survived exposure to an expected lethal dose of the MESN toxic bait. By the time this animal was euthanized (i.e. 24 h post-ingestion), the animal appeared fully recovered and had no evidence of residual SN. This suggests that sublethal doses of SN are rapidly metabolized and do not bio-accumulate in the tissues. This is an important consideration for wild pigs, as well as the predators and scavengers of wild pigs that may be exposed to low levels of SN. No debilitating effects appeared to occur from sublethal dosing in this study, or in another study with other species.<sup>33</sup>

## 5 CONCLUSION

We found no evidence that use of alternative micro-encapsulation coatings would be beneficial compared to the currently used formulation of the MESN toxic bait. Vomiting appears to be a frequent outcome of MESN toxic bait delivered to wild pigs under these study conditions (i.e. ingestion of a rapid and lethal, bolus dose). However, the level of secondary risk from vomitus to non-target species appears low because ~90% of the SN has already left the bait, and continues to breakdown in vomitus once exposed to the environment. Carcasses of wild pigs contain low levels of residual SN and are not likely to be risky for scavengers to consume, except for the digestive tract. If mammalian or avian scavengers consume enough of the digestive tract secondary poisoning could occur. However, the risk is lessened because consumption would need to occur rapidly (i.e. during a single-feeding event) from a fresh wild pig carcass in which the SN has not had time to breakdown. Sublethal doses of SN are rapidly metabolized without debilitating effects.

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