Encapsulated sodium nitrite as a new toxicant for possum control in New Zealand

Lee Shapiro1, 2, *, Charles Eason1, 3, Craig Bunt4, Steve Hix2, Paul Aylett2 and Duncan MacMorran2

1 Centre for Wildlife Management and Conservation, Lincoln University, PO Box 84, Lincoln 7647, New Zealand
2 Connovation Ltd, PO Box 58 613, Botany, Auckland 2163, New Zealand
3 Cawthron Institute, Private Bag 2, Nelson 7042, New Zealand
4 Department of Agricultural Sciences, Lincoln University, PO Box 84, Lincoln 7647, New Zealand
* Author for correspondence (Email: lee@connovation.co.nz)

Published online: 14 April 2016

Abstract: Sodium nitrite (NaNO2), a commonly used food preservative, has been researched in New Zealand for the control of brushtail possums (Trichosurus vulpecula). In sufficiently high doses, NaNO2 is toxic because it disrupts circulatory transport of oxygen. As NaNO2 is very bitter, encapsulation and mixing it through a highly palatable bait formulation is necessary to effectively deliver it to target pest species. In no-choice cage trials, 12/12 possums consumed a lethal dose of toxic paste bait and died on average after 95.6 minutes (±4.9 SE). In two-choice cage trials 7/8 possums consumed a lethal dose of toxic paste bait and died on average after 96.7 minutes (±11.4 SE). Two field trials targeting possums using this toxic paste in bait stations reduced their abundance by 81.2% (± 2.5% SE) and 72.7% (± 1.6% SE) respectively. NaNO2 paste, known as BaitRite, has been registered in New Zealand as a vertebrate toxic agent for controlling possums.

Keywords: brushtail possum; methaemoglobin; methaemoglobinaemia; NaNO2; vertebrate pesticide

Introduction

In New Zealand, common brushtail possums (Trichosurus vulpecula) are a threat to native biodiversity, through the damage they cause to flora and fauna (Innes et al. 2004; Glen et al. 2012; Nugent & Morriss 2013). They also threaten the primary sector through their role as the main wildlife vector of bovine tuberculosis (Mycobacterium bovis) (Coleman & Caley 2000; PCE 2011). Possums are controlled with a variety of traps and toxins, including ground-based control with the toxins cyanide, brodifacoum and cholecalciferol, as well as aerial control with sodium fluoroacetate (1080). Control work is often undertaken on or in close proximity to farmland. When these operations are undertaken, there is therefore a risk of secondary poisoning to non-target species, including working dogs, from carcasses of poisoned possums (Meenken & Booth 1997; Eason 2002; Eason et al. 2011). Research to minimise this risk has focused on developing vertebrate toxic agents (VTAs) that have low residue, low risk to non-target animals, and animal welfare as a key consideration (Morgan et al. 2013; Eason et al. 2014; Shapiro et al. 2016b).

One compound researched for possum control has been sodium nitrite (NaNO2), an inorganic salt commonly used to add colour and flavour to food for human consumption and as an antimicrobial agent in cured and processed meats (Binkerd & Kolari 1975; Hord et al. 2009). The chemistry and toxicology of NaNO2 is well understood due to the numerous documented cases of accidental poisoning of humans and animals (Counter et al. 1975; Bradberry et al. 1994; Gautami et al. 1995; Vyt & Spruytte 2006). NaNO2 has also been researched as a potential VTA for feral pigs (Sus scrofa) (Sullivan 1985; Cowled et al. 2008, Shapiro et al. 2016a). Research in New Zealand expanded on that of Cowled et al. (2008), carried out in Australia, and investigated the utility of NaNO2 as a potential control tool for possums and feral pigs.

NaNO2 has been referred to as a red blood cell toxicant (Eason & Ogilvie 2009) due to its mode of action. The protein haemoglobin, found in red blood cells and responsible for oxygen transport, has an alternate form called methaemoglobin (MetHb) and normally accounts for less than 2% of the total haemoglobin circulating at one time (Fan et al. 1987; Bradberry 2011). Ingestion of NaNO2 causes an elevation in the levels of MetHb (Beutler & Mikus 1961) and in high enough doses this leads to methaemoglobinaemia. Chui et al. (2005) describe methaemoglobinaemia as the potentially fatal condition where the oxidation of haemoglobin to MetHb negates its ability to bind and transport oxygen. Levels of MetHb <20% of total haemoglobin are usually asymptomatic (Bradberry 2011). At levels higher than this, symptoms of methaemoglobinaemia appear and, in humans and possums, include a bluish grey skin colour, lethargy, cerebral anoxia, chocolate-coloured blood, irregular breathing, loss of consciousness. Levels above 80% can be fatal (Fan et al. 1987; Brunning-Fann & Kaneene 1993; Fisher et al. 2008). The treatment of methaemoglobinaemia as outlined by Umbreet (2007) commonly involves the infusion of methylene blue, a compound routinely used to treat nitrate poisoning in cattle (Bolan & Kemp 2003). Following treatment with methylene blue, a rapid improvement is usually seen 30–60 minutes after its administration (Chui et al. 2005).

Lapidge and Eason (2010) noted from previous research that the lethal doses for humans, rats, and pigs, administered NaNO2 by oral gavage, were approx. 100 mg/kg. Based on this, a 3-kg possum would require 300 mg of NaNO2 for a lethal dose; however, this figure is based on oral gavage not delivered in bait. For the research reported here, we wanted to ensure there was a low chance of sub-lethally dosing possums and our aim was to exceed the oral gavage lethal dose several fold, paste baits containing 10% w/w sodium nitrite were trialled.

The low palatability of NaNO2 was observed in early cage trials carried out by Shapiro et al. (2009), where raw NaNO2 (10% w/w), mixed in paste bait, was fed to possums. Only four out of 12 possums consumed any bait, and in each case it was insufficient for a toxic effect to be observed. A proprietary encapsulation technique (Connovation Ltd) was applied to
NaNO₂ for the purpose of taste masking. The purpose of the research reported here was to determine the effectiveness of encapsulated NaNO₂ in a paste bait, containing 10% w/w of the active ingredient, for possum control.

Materials and methods

**Possum cage trials**

Twenty wild common brushtail possums were captured using Victor® leg-hold traps in Hororata, Canterbury, New Zealand in summer. Possums were housed individually in indoor cages at the Johnstone Memorial Animal Facility, Lincoln University. Cages were kept in a temperature controlled room (19°C ± 5°C) and were constructed of stainless steel and measured 110 cm × 55 cm × 60 cm; each had a plastic box for possums to use as a den. The room lighting was kept under natural day-length lighting. Possums underwent a health check on arrival during which they were weighed, sexed, and females were screened for pouch young. Possums were fed solid pellets, made of various grains, as well as fresh vegetables, and water was available *ad libitum*.

Possums were acclimatised for 1 week to ensure they were eating and that their weight remained stable. Once acclimatised, the pellets and vegetables were removed from cages and individuals were fed 50 g of a non-toxic paste (Connovation Ltd), consisting of a mixture of peanut butter (35%), kibbled wheat (20%), ground maize (15%), margarine (15%), and sugar (15%). This was undertaken on two occasions 3 days apart in the week leading up to the toxic trials. Possums received the standard pellet and vegetable diet on the days between being fed the non-toxic paste, apart from the 24 hours immediately before the toxic trials where they received no vegetables and half rations of pellets. Two cage trials were conducted. The first was a no-choice bait acceptance trial to determine whether possums would consume sufficient toxic bait to receive a lethal dose. The second, a two-choice trial, was to test whether possums would still consume a lethal dose of toxic paste when also presented with non-toxic paste. For both trials the length of exposure to toxic baits was determined from the acclimatisation period, on these two occasions all possums consumed the entire 50 g of non-toxic paste bait within 4 hours.

**No-choice trial**

Once acclimatised to the non-toxic paste, 12 possums (six male and six female; weight range 1.78 – 3.20 kg) were each presented with approx. 50 g of the NaNO₂ paste (Connovation Ltd). Toxic bait was placed in a metal feed tray with a single tray in each cage. Each possum was observed with a single LTL Acorn 5210A motion-detecting video camera left in the cage, and footage was observed at the end of the trial. Both bait types were weighed again after trays had been left in the cages for 4 hours. All monitoring of possums during this time was identical to that of the no-choice trial.

**Two-choice trial**

Once acclimatised to the non-toxic paste, eight possums (four male and four female; weight range 1.60 – 4.18 kg) were each presented with approx. 50 g of the paste bait containing NaNO₂ (Connovation Ltd) the same formulation as the no-choice trial. Each possum was also presented with approx. 50 g of the non-toxic paste. The two forms of the paste bait were placed in separate compartments of a metal feed tray and this was randomised to avoid any potential effect of animals being conditioned to feeding from a particular compartment. Each possum was observed with a single LTL Acorn 5210A motion-detecting video camera left in the cage, and footage was observed at the end of the trial. Both bait types were weighed again after trays had been left in the cages for 4 hours. All monitoring of possums during this time was identical to that of the no-choice trial.

**Possum field trials**

Two field trial sites were established on two privately owned farms located in Canterbury, New Zealand. Field trial site one was located approx. 15 km west of Little River on Banks Peninsula (43°79’ S, 172°70’ E). Site two was located approx. 25 km north-east of Little River on Banks Peninsula (43°72’ S, 173°08’ E). Each site consisted of a treatment and a non-treatment area located 1.5 km apart within each trial site, each area approx. 100 ha. Vegetation at both trial sites consisted of open pasture, mānuka (*Leptospermum scoparium*), tōtara (*Podocarpus totara*), various *Coprosma* species, the native tree nettle ongaonga (*Urtica ferox*), and regenerating scrubland.

The trial at site one took place over a 4-week period during June 2010, with an average overnight temperature during this period of 5.9°C (min 2.9°C and max 10.3°C). Total rainfall for the duration of the trial was 93.0 mm (NIWA, 2010). The trial at site two took place over a 4-week period from mid-June 2010 to mid-July 2010, with an average overnight temperature during this period of 5.4°C (min 2.2°C and max 9.5°C). Total rainfall for the duration of the trial was 72.6 mm (NIWA, 2010).

Relative possum abundance was measured before and after control using the NPCA Waxtags® protocol (NPCA 2010) in the treatment and non-treatment areas for both sites. Using this method, relative possum abundance is calculated as a percentage of the Waxtags® bitten by possums and recorded as a Bite Mark Index (BMI). In the treatment and non-treatment areas at each site Waxtags® were deployed on five lines with 20 tags per line and 10 m between tags. Waxtags® were left out for 7 nights and then retrieved.

In the treatment area of both sites bait stations were set up at approx. 100 m intervals, on lines spaced 150 m apart in areas where vegetation was sparse and 100 m apart in areas of thicker scrub. A total of 83 mini Philpro® bait stations were set out across site one and 65 across site two. Prefeeding at both treatment sites was carried out using non-toxic paste (Connovation Ltd), consisting of a mixture of peanut butter (35%), kibbled wheat (20%), ground maize (15%), margarine (15%), and sugar (15%). On three occasions, at 1-week intervals, approx. 200 g of this non-toxic paste was placed in each bait station. One week after the last pre-feed, any remaining non-toxic paste was removed from the bait stations and replaced with approximately 130 g of NaNO₂ paste (Connovation Ltd). This replacement paste consisted of 90% non-toxic paste and 10% w/w NaNO₂. Baits were checked every 2 days and replenished wherever there was less than half the original amount left in a bait station. Baits
were left out for 4 nights, after which time they were removed from bait stations.

Samples of the encapsulated NaNO₂ active and NaNO₂ paste were analysed by Flinders Cook Ltd (Technical Services) to confirm the concentration of NaNO₂ active before each of the trials. Samples of the encapsulated NaNO₂ contained 95% w/w NaNO₂ active and 5% encapsulant material. Samples of the NaNO₂ paste contained 10.0% ± 0.3% NaNO₂. The method of analysis was based on an internationally recognised analytical method described in Vogel (1979).

Results

No-choice efficacy trial
In the no-choice efficacy cage trial all 12 possums (100%; 95% binomial CI 73.5 – 100%) died after consuming NaNO₂ paste. Possums consumed an average of 9.49 g (± 1.36 SE) of bait, an average dose per possum of 360.8 mg/kg (± 49.75 SE) of NaNO₂ (Table 1). Clinical signs first appeared on average after 20.6 minutes (± 1.8 SE) and possums died on average 95.6 minutes (± 4.9 SE) after ingesting bait. Symptoms observed included pale noses, pale gums, lethargy, ataxia, slight tremors, collapse, and death.

Table 1. Time to appearance of symptoms, duration and time to death for possums that consumed NaNO₂ paste (cage trials).

<table>
<thead>
<tr>
<th>Possum</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Bait consumed (g)</th>
<th>Dose (mg/kg)</th>
<th>First appearance clinical symptoms (mins)</th>
<th>Time to death (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>2.87</td>
<td>11.15</td>
<td>388.50</td>
<td>15</td>
<td>104</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>2.46</td>
<td>8.07</td>
<td>328.05</td>
<td>35</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>3.02</td>
<td>14.01</td>
<td>463.91</td>
<td>15</td>
<td>107</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>1.78</td>
<td>6.04</td>
<td>339.33</td>
<td>22</td>
<td>89</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>2.22</td>
<td>6.27</td>
<td>282.43</td>
<td>23</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>3.05</td>
<td>6.84</td>
<td>224.26</td>
<td>20</td>
<td>130</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>3.20</td>
<td>6.39</td>
<td>199.69</td>
<td>30</td>
<td>114</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>2.09</td>
<td>6.97</td>
<td>333.49</td>
<td>16</td>
<td>72</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>3.08</td>
<td>8.96</td>
<td>290.91</td>
<td>20</td>
<td>96</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>2.38</td>
<td>5.04</td>
<td>211.76</td>
<td>15</td>
<td>103</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>2.56</td>
<td>21.54</td>
<td>741.41</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>2.96</td>
<td>12.62</td>
<td>426.35</td>
<td>16</td>
<td>95</td>
</tr>
</tbody>
</table>

Two-choice trial
In the two-choice cage trial seven of the eight possums (87.5%; 95% binomial CI 47.4 – 99.7%) consumed a lethal dose of NaNO₂ paste. Those seven possums consumed an average of 8.41 g (± 2.2 SE) of bait, an average dose per possum of 260.5 mg/kg (± 64.8 SE) of NaNO₂ (Table 2). Clinical signs first appeared on average after 24.0 minutes (± 2.9 SE) and possums died on average 96.7 minutes (± 11.4 SE) after ingesting bait. One of the eight possums did not consume a lethal dose of toxic paste (Table 2) but displayed clinical symptoms, including a pale nose and gums as well as being lethargic, for 45 minutes before recovering and was euthanased at the conclusion of the trial in line with our animal ethics approval document. Based on consumption, the relative palatability of the NaNO₂ paste was 66.3% compared with 33.8% for the non-toxic paste. Using the data generated in these two cage trials and from a previous pilot trial with four possums (Hix et al., 2010), an LD₅₀ for possums free feeding on paste bait containing NaNO₂ (10% w/w) was calculated as 121.6 mg/kg (95% CI 45.4 – 169.6 mg/kg).

Possum field trials
Before the toxic trial, possum abundance at site one was found to be 85.0% BMI (± 6.7 SE) in the treatment area and

Table 2. Time to appearance of symptoms, duration and time to death in possums presented non-toxic paste and NaNO₂ paste (cage trials).

<table>
<thead>
<tr>
<th>Possum</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Non-toxic paste consumed (g)</th>
<th>NaNO₂ paste consumed (g)</th>
<th>Toxic dose (mg/kg)</th>
<th>Appearance clinical symptoms (mins)</th>
<th>Time to death (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>2.35</td>
<td>0.36</td>
<td>13.66</td>
<td>581.28</td>
<td>16</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>2.85</td>
<td>0.26</td>
<td>8.85</td>
<td>310.53</td>
<td>31</td>
<td>115</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>4.18</td>
<td>0.84</td>
<td>17.84</td>
<td>426.79</td>
<td>29</td>
<td>107</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>2.68</td>
<td>13.16</td>
<td>3.87</td>
<td>144.40</td>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>2.40</td>
<td>0.05</td>
<td>5.11</td>
<td>212.92</td>
<td>26</td>
<td>105</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>1.60</td>
<td>12.59</td>
<td>1.72</td>
<td>107.50</td>
<td>19</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>3.57</td>
<td>0.00</td>
<td>7.81</td>
<td>218.77</td>
<td>33</td>
<td>145</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>3.48</td>
<td>43.37</td>
<td>2.85</td>
<td>81.90</td>
<td>16</td>
<td>Recovered</td>
</tr>
</tbody>
</table>
79.0% BMI (± 6.0 SE) in the non-treatment area. At site two possum abundance was found to be 77.0% BMI (± 6.1 SE) in the treatment area and 86.0% BMI (± 1.9 SE) in the non-treatment area. Post-monitoring, undertaken immediately after the toxic trial, found that possum abundance at site one in the treatment area had reduced significantly (t =11.09, P<0.01) to 16.0% BMI (± 1.0 SE). This represents a decrease in possum abundance of 81.2% (± 1.5 SE). Post-monitoring at site two found that possum abundance in the treatment area had also reduced significantly (t = 9.68, P < 0.01) to 21.0% BMI (± 1.9 SE). This represents a decrease in possum abundance of 72.7% (± 3.0 SE).

There was no significant change in possum abundance in the control area of site one (t =-2.44, p = 0.07) or site two (t = 1.20, p = 0.29). Post-treatment possum abundance was 85.0% BMI (± 4.5 SE) at site one and 81.0% BMI (± 1.0 SE) at site two.

Discussion
An encapsulated form of NaNO2 has been developed, which has been shown, in small-scale pen and field trials, to be palatable and effective for the control of possums when presented in paste bait. The encapsulation of NaNO2 has improved its effectiveness in possums compared with results from previous trials where possums were presented with unencapsulated NaNO2 in the same paste bait matrix (Shapiro et al. 2009).

Sufficient sodium nitrite needs to be ingested quickly to induce fatal methaemoglobinaemia and death. NaNO2 at high doses induces a relatively fast time to death, in possums, compared with conventional VTAs such as 1080, brodifacoum and cholecalciferol (McIlroy 1983; Jolly et al. 1993; Littin et al. 2000, 2002) but comparable to those times observed for stoats (Mustela erminea) and feral cats (Felis catus) poisoned with PAPP (Savarie et al. 1983; Eason et al. 2010).

Due to its use in food preservation, a large amount of data exists on the metabolism of NaNO2 by humans as well as numerous other species. The time for nitrite to be eliminated from the blood is expressed in terms of plasma elimination half-life (\( t_{1/2} \)). Results for various species, in minutes, were collated from previous research by Lapidge and Eason (2010) and reported for sheep (29), dogs (30), ponies (34) (Schneider & Eason 2000, 2002) but comparable to those times observed for stoats (Mustela erminea) and feral cats (Felis catus) poisoned with PAPP (Savarie et al. 1983; Eason et al. 2010).


