



Primary poisoning risk for encapsulated sodium nitrite, a new tool for pest control

Lee Shapiro, Paul Aylett, Donald Arthur & Charles Eason

To cite this article: Lee Shapiro, Paul Aylett, Donald Arthur & Charles Eason (2017): Primary poisoning risk for encapsulated sodium nitrite, a new tool for pest control, New Zealand Journal of Zoology, DOI: [10.1080/03014223.2016.1264979](https://doi.org/10.1080/03014223.2016.1264979)

To link to this article: <http://dx.doi.org/10.1080/03014223.2016.1264979>



Published online: 04 Jan 2017.



[Submit your article to this journal](#) 



Article views: 20



[View related articles](#) 



[View Crossmark data](#) 

RESEARCH ARTICLE

Primary poisoning risk for encapsulated sodium nitrite, a new tool for pest control

Lee Shapiro^{a,b}, Paul Aylett^b, Donald Arthur^c and Charles Eason^{a,d}

^aCentre for Wildlife Management and Conservation, Ecology Department, Lincoln University, Lincoln, New Zealand; ^bConnovation Ltd, Auckland, New Zealand; ^cSelwyn Rakaia Veterinary Services, Dunsandel, Canterbury, New Zealand; ^dCawthron Institute, Nelson, New Zealand

ABSTRACT

Acute toxicity of sodium nitrite (NaNO_2) was assessed in chickens (*Gallus gallus domesticus*) and domestic mallard ducks (*Anas platyrhynchos domestica*) by oral gavage and in free-feeding trials with chickens, domestic mallard ducks, pigeons (*Columba livia f. domestica*), budgerigars (*Melopsittacus undulates*) and wētā (Family: Rhaphidophoridae). Free-feeding trials involved the presentation of toxic paste and pellet baits containing encapsulated NaNO_2 developed for the control of common brushtail possums (*Trichosurus vulpecula*) and feral pigs (*Sus scrofa*). The oral gavage LD_{50} value for NaNO_2 in solution was approximately 68.50 mg/kg (95% CI 55.00–80.00 mg/kg) for both chickens and ducks. In feeding trials, six out of 12 chickens consumed toxic paste bait and four of these birds consumed a lethal dose. When chickens consumed toxic paste bait, the LD_{50} value was approximately 254.6 mg/kg (95% CI 249.1–260.2 mg/kg). Of the other three species of birds presented with toxic baits only one duck consumed a lethal dose of paste bait. There was no evidence of wētā feeding on toxic baits.

ARTICLE HISTORY

Received 13 May 2016
Accepted 22 November 2016

KEYWORDS

Primary poisoning risk;
encapsulated sodium nitrite;
pest control; New Zealand

Introduction

Sodium nitrite (NaNO_2) has been researched in New Zealand, Australia and the USA as a potential vertebrate toxic agent (VTA) because high doses can lead to death in < 2 hours in target species. In New Zealand this research has focused on the control of brushtail possums (*Trichosurus vulpecula*) and feral pigs (*Sus scrofa*). As possums are a problem specific to New Zealand, the focus in other countries has been on controlling feral pigs because they are a major economic and ecological problem (Gentle et al. 2011; Barrios-Garcia & Ballari 2012; Engeman et al. 2016). Baits containing encapsulated NaNO_2 were registered to kill possums and feral pigs in New Zealand in 2013.

The suitability of NaNO_2 as a VTA relies on the target animals consuming a lethal dose as well as delivering the toxic bait in a safe manner to limit risk to non-target species. The key to ensuring that target species consume a lethal dose of NaNO_2 has been the development of an effective method of encapsulation (Shapiro et al. 2016); this enables the

extremely bitter and salty taste to be masked. The successful research and development of this new toxin for possum and feral pig control has relied heavily on this encapsulation method (Shapiro et al. 2015, 2016).

Research in pen and field trials demonstrated that an encapsulated form of NaNO_2 , when presented in palatable bait, is effective at killing possums and feral pigs (Shapiro et al. 2015, 2016). NaNO_2 toxicity is mediated through the elevation of methaemoglobin, a form of haemoglobin unable to transport oxygen, leading to methaemoglobinaemia and, in high enough doses, to cyanosis, cerebral anoxia and death (Chui et al. 2005; Bradberry 2011).

The potential risk of secondary poisoning to dogs (*Canis lupus familiaris*), cats (*Felis catus*) and birds, from consuming carcasses of possums poisoned with baits containing encapsulated NaNO_2 has also been assessed (Shapiro 2016). Carcasses from possums poisoned with baits containing encapsulated NaNO_2 were found to have a low risk of causing secondary poisoning to domestic dogs, cats and chickens (Shapiro 2016).

The use of VTAs to control pest animals involves a certain amount of risk. Risks to the environment and humans through bio-accumulation, through residues entering the food chain or being detected in wildlife (Eason & Spurr 1995; Eason et al. 1999; Booth et al. 2001; Spurr et al. 2005). There is also a risk to non-target species through the potential consumption of baits (primary poisoning) (Powlesland et al. 2000; Eason et al. 2011) or the consumption of carcasses of animals that have been poisoned (secondary poisoning) (Stone et al. 1999; Eason et al. 2011). These risks can be lowered through best-practice baiting strategies, including the selection of specific toxins for particular situations, amount of bait used, as well as through the delivery, i.e. in bait stations or smart new devices that enable target-specific delivery of VTAs (Blackie et al. 2016). Despite best-practice methodology, there will always be a level of risk of non-target species accessing toxic baits and it is important to quantify this level of risk during the development and registration of new VTAs.

To determine the risk that NaNO_2 poses to non-target species previous trials have involved feeding non-target wildlife baits containing NaNO_2 as well as orally gavaging wildlife with NaNO_2 . In Texas, raccoons (*Procyon lotor*) and white-tailed deer (*Odocoileus virginianus*) (both important non-target species in the USA), and feral pigs were orally gavaged with NaNO_2 (Foster 2011). Values of the median lethal dose (LD_{50}) were reported as 58, 154 and 133 mg/kg for raccoons, white-tailed deer and feral pigs, respectively (Foster 2011). Four native Australian species, Bennett's wallabies (*Macropus rufogriseus*), Tasmanian pademelons (*Thylogale billardierii*) (Fish & Statham 2009), brushtail possums (Fisher et al. 2009) and dama wallabies (*Macropus eugenii*) (Shapiro & Eason 2009), were all presented with baits containing NaNO_2 . Bennett's wallabies and Tasmanian pademelons did not consume any baits whereas possums and dama wallabies both consumed lethal doses. An Australian study calculated the potential risk of NaNO_2 to 28 marsupial and nine eutherian mammal, four reptile and two bird species (Lapidge & Eason 2010) and concluded that NaNO_2 is toxic to most species with an LD_{50} of approximately 60 mg/kg or greater in solution and a larger dose in food. To increase the safe use of this VTA the delivery method and bait type are of key importance to minimising risk to non-target species.

The main aim of this study was to determine the risk of primary poisoning from bait containing encapsulated NaNO_2 to a range of non-target species through a series of acute

toxicity trials. No previous non-target testing with NaNO_2 has been carried out in New Zealand with native species.

Materials and methods

For primary poisoning trials it is common and preferable to include non-native species as surrogates for native species (OECD 2010; Eason et al. 2013). Domestic mallard ducks (*Anas platyrhynchos domestica*) were chosen for trials reported here as they have previously been used in non-target primary poisoning trials in New Zealand (Eason et al. 2010) and they are commonly used in Organisation for Economic Co-operation and Development (OECD) guideline studies. Pigeons (*Columba livia f. domestica*) and budgerigars (*Melopsittacus undulates*) are also suggested as suitable species for oral toxicity testing (OECD 2010). Chickens (*Gallus gallus domesticus*) are a useful surrogate for weka (*Gallirallus australis*), a ground-dwelling bird species native to New Zealand that belongs to the rail family (Eason et al. 2013). Cave wētā (Family: Rhaphidophoridae), a native invertebrate, were also included as they are commonly found sheltering in bait stations and could potentially access baits in this manner and then be eaten by other non-target species including birds.

The methodology used for the oral gavage and free-feeding trials was adapted from that outlined in Eason et al. (2013). All the trials were carried out Lincoln University Johnstone Memorial Laboratory, Lincoln, Canterbury.

Gavage trial—chickens and domestic mallard ducks

Fifteen female domestic chickens were purchased from a commercial poultry farm (Lamond Poultry, Christchurch, New Zealand). They were weighed and housed individually in 1.5 m × 2 m outdoor enclosures constructed of plywood and wire mesh. Each cage had a wooden nest box filled with straw. The chickens were fed a commercial grain-based chicken feed and water was available *ad libitum*.

Fifteen domestic mallard ducks (ten males and five females) were purchased from a private breeder in Hororata (Canterbury, New Zealand). The ducks were weighed and fitted with coloured leg bands to aid identification and housed together in a 6 m × 6 m stall in a barn with sawdust flooring and straw bedding. They were fed a commercial grain-based duck feed with water available *ad libitum*. Three days before the trial 15 individual pens, each 2 m × 1 m were created with wire fences within the existing 6 m × 6 m stall and ducks were individually penned.

The outline for the gavage trial methodology was a modified version of the OECD guidelines for the testing of chemicals using the Up and Down Procedure to determine acute oral toxicity (OECD 2008). The Up and Down Procedure suggests that when no information exists on the toxicity of a substance to a particular species, then the starting dose should be 175 mg/kg with a dose progression factor of 3.2. The suggested starting dose of 175 mg/kg was used in these trials.

The chickens and ducks were each dosed in groups of three; the first group were each gavaged with 175 mg/kg. The NaNO_2 granules were dissolved in approximately 10 ml of distilled water immediately before each chicken or duck being dosed. Target doses were based on the weight of individual birds and so each bird was weighed directly before

dosing and the concentration of NaNO_2 in the solution was then calculated. All birds were orally gavaged by a veterinarian with the solution in a syringe that was delivered to the birds crop via a gavage tube. Once dosed, birds were returned to their pens and observed to determine the dose level for the following group. The time to the first symptoms being displayed, symptoms observed, and time to death were recorded for individuals. Birds were observed for signs of NaNO_2 poisoning including difficulty breathing, vomiting and diarrhoea, and methaemoglobinaemia (such as shortness of breath, cyanosis, lethargy, loss of co-ordination and loss of consciousness) (Eason et al. 2010). Necropsies were carried out on all birds that died.

When a dose group experienced mortality of two or three birds, the next group received a dose 1/3.2 times the initial dose—in this case 55 mg/kg. When a dose group experienced mortality of none or one bird then the next group received a dose 3.2 times the initial dose—in this case 550 mg/kg. The dose progression factor was abandoned once two different consecutive results were found, so when a dose group experienced mortality of two or three birds and the next dose group experienced mortality of none or one bird then the dose progression factor was abandoned. At this point a dose rate mid-way between the two previous doses was tested, and the mortality rule described above was used to determine whether the next midpoint dose was higher or lower than this dose.

The end point for these trials was a result of using the least number of animals possible while generating the most meaningful data possible. Although a conventional LD_{50} will provide robust data, these trials are out of favour for ethical reasons due to the requirement for testing large numbers of animals. It was possible to calculate an approximate LD_{50} using probit curve analysis (Finney 1971). Group sizes and the overall number of animals were kept as small as possible while still generating meaningful data. This reduction in numbers is in keeping with the '3Rs' principles (Russell & Burch 1959), namely the second of the 3Rs, reduction, which aims to use as few animals in research trials as necessary.

Free-feeding trials

Free-feeding trials were undertaken with chickens, domestic mallard ducks, pigeons, budgerigars and cave wētā. This involved presenting toxic paste and pellet baits containing encapsulated NaNO_2 and non-toxic paste and pellet baits, produced at Connovation Ltd (Auckland, New Zealand), to each species. Toxic baits were presented to each species for a 4 hour period. Although exposure to baits in the field will potentially be over a longer period, this period was chosen because it reduced the likelihood of animals consuming baits solely due to having no other food for an extended period of time. The toxic paste and pellet baits each consisted of 10% NaNO_2 , 0.5% encapsulant material and 89.5% non-toxic bait. A proprietary encapsulation technique (Connovation Ltd) was applied to NaNO_2 for the purpose of taste masking when delivered to target species as well as improving stability of NaNO_2 in toxic baits. The non-toxic paste bait formulation is outlined elsewhere (Shapiro et al. 2016) and the pellet bait formulation is a proprietary formulation. Paste and pellet baits containing encapsulated NaNO_2 will, from here on, be referred to as NaNO_2 paste and NaNO_2 pellet baits.

Chickens

Twenty-four female domestic chickens were purchased from a commercial poultry farm (Lamond Poultry). They were housed individually with housing conditions and husbandry identical to that described for chickens in the gavage trial. Chickens were assigned to two treatment groups and two non-treatment groups. For the initial non-toxic phase of the trial, chickens in Group 1 ($n = 12$) were each presented with 20 g of the non-toxic paste bait, Group 2 ($n = 6$) were each presented with 20 g of the non-toxic pellet bait, Group 3 ($n = 3$) were each presented with 20 g of the non-toxic paste bait and Group 4 ($n = 3$) were each presented with 20 g of the non-toxic pellet bait. This feeding regimen was carried out every second day during the week before the toxic trial. The chickens were fed non-toxic baits to acclimatise them to the bait formulation as this is routine procedure in pest control operations before deploying a toxic form of the same bait. Non-target species can potentially become acclimatised to non-toxic bait and be at an increased risk when toxic bait is deployed, so it is important to replicate what happens in control operations.

For the toxic phase of the trial chickens in Group 1 were each presented with 20 g of NaNO_2 paste bait, Group 2 were each presented with between 27.60 g and 29.70 g of NaNO_2 pellet baits, Group 3 were each presented with 20 g of non-toxic paste bait, and Group 4 were each presented with between 27.60 g and 28.50 g of non-toxic pellet baits.

The chickens were observed closely for bait consumption and any symptoms of poisoning every 10 minutes for the first hour and then every 20 minutes for the next 3 hours. They were observed for signs of NaNO_2 poisoning including difficulty breathing, vomiting and diarrhoea and methaemoglobinaemia (such as shortness of breath, cyanosis, lethargy, loss of co-ordination and loss of consciousness) (Eason et al. 2010). Baits from all groups were weighed immediately before feeding and again when baits were removed after the 4 hour exposure period. For individuals that consumed toxic bait, time to the first symptoms being displayed and the time to death were recorded.

All chickens were observed over 14 days following the trial. Post-mortem inspections were conducted on any birds that died during the trial. Chickens that consumed toxic bait and survived as well as three randomly selected birds from Groups 3 and 4 were euthanized after being observed for the 14 days following the trial and necropsies were conducted. An approximate LD_{50} for NaNO_2 paste bait was calculated for chickens in Group 1 using probit curve analysis (Finney 1971).

Domestic mallard ducks

Fourteen domestic mallard ducks (ten males and four females) were purchased from a private breeder in Hororata, Canterbury. Ducks were weighed and fitted with leg bands to aid identification. Ducks were penned under identical conditions as outlined for the gavage trials, including being initially housed in one group and then individually penned 3 days before the trial. Ducks were assigned to one of four groups, Group 1 ($n = 5$) were each presented with 50 g of NaNO_2 paste bait, Group 2 ($n = 5$) were each presented with 50 g of NaNO_2 pellet baits, Group 3 ($n = 2$) were presented with 50 g of non-toxic paste bait, and Group 4 ($n = 2$) were presented with 50 g of non-toxic pellet baits. In the week before presentation of NaNO_2 baits the ducks were presented non-toxic paste and solid baits every second day.

The presentation of baits and monitoring of the ducks, once exposed to bait, followed that outlined above for chickens. All ducks were observed for 14 days following the trial and any ducks that had consumed toxic bait and survived, as well as one randomly selected duck from each of Groups 3 and 4, were euthanized and post-mortem inspections were conducted on them. Baits from all groups were weighed immediately before feeding and again when baits were removed after the 4 hour exposure period.

Pigeons and budgerigars

Eighteen domestic pigeons (eleven males and seven females) and 16 domestic budgerigars (seven males and nine females) were purchased from private breeders in Canterbury. The pigeons were housed individually in cages approximately 1.5 m × 1.5 m × 1 m in size. All pigeons were fed a commercial cereal-based feed and budgerigars were also fed millet. Water was available *ad libitum*. The pigeons were given 1 week to acclimatise in cages before the trial.

Pigeons were assigned to one of four groups: Group 1 ($n = 6$) were each presented 50 g of NaNO₂ paste bait, Group 2 ($n = 6$) were each presented with 50 g of NaNO₂ pellet baits, Group 3 ($n = 3$) were presented with 50 g of non-toxic paste bait, and Group 4 ($n = 3$) with 50 g of non-toxic pellet baits. Budgerigars were assigned to one of four groups: Group 1 ($n = 6$) were each presented between 48.23 g and 49.96 g of NaNO₂ paste bait, Group 2 ($n = 6$) were each presented between 43.06 g and 49.37 g of NaNO₂ pellet baits, Group 3 were either presented with 48.86 g or 49.37 g of non-toxic paste bait, and Group 4 ($n = 2$) with either 44.06 g or 44.38 g of non-toxic solid bait. Every second day, during the week before the toxic trial, each bird was fed 50 g of either non-toxic paste or pellet baits depending on the type of bait they were scheduled to receive in the toxic trial.

In both budgerigar and pigeon trials, the presentation of baits and monitoring of birds, once exposed to bait, as well as necropsies followed that outlined above for ducks. Baits from all groups were weighed immediately before feeding and then when baits were removed after the 4 hour exposure period.

Cave wētā

Sixteen cave wētā were collected from a private property in Hororata, Canterbury and under these circumstances a permit for collecting this species was not required. Wētā were individually housed in wooden enclosures measuring 30 cm × 15 cm × 20 cm in a temperature controlled room. Room temperature ranged between 19.2°C and 19.9°C and the humidity between 55% to 71%. Wētā were acclimatised for a week before the trial commenced and kept under the same conditions as outlined by Barrett (1991). Wētā were assigned to one of three groups and presented bait for 14 days. Group 1 ($n = 7$) were presented between 12.111 g and 12.382 g of NaNO₂ paste bait, Group 2 ($n = 7$) were presented between 12.742 g and 13.204 g of NaNO₂ solid bait, Group 3 ($n = 1$) was presented 12.541 g of non-toxic paste and Group 4 ($n = 1$) was presented 11.248 g of a non-toxic pellet. Baits were weighed every second day and examined for any traces of feeding marks. NaNO₂ paste ($n = 2$) and pellet baits ($n = 2$) as well as non-toxic paste ($n = 2$) and pellet baits ($n = 2$) were also kept in cages without wētā to account for any bait weight fluctuations that were due to temperature or humidity. Fluctuations in the weights of baits in enclosures without wētā were then used to adjust any changes to baits in wētā cages to better gauge potential consumption by wētā.

At the conclusion of the trial all wētā were euthanized by freezing at -10°C and sent to Flinders Cook Technical Services Ltd (Auckland, New Zealand) to be assayed to determine if any trace of NaNO_2 could be detected. The preparation for each assay involved grinding wētā to a paste using a mortar and pestle, and then an extraction with distilled water. The minimum level of detection of the assay is $5\ \mu\text{g}/100\ \text{ml}$, the methodology is outlined in the Laboratory assays section below.

Laboratory assays

All assays of NaNO_2 paste and pellet baits, NaNO_2 , encapsulated NaNO_2 and individual wētā were carried out by Flinders Cook Ltd (Technical Services). The method of analysis was based on an internationally recognised analytical method described in Vogel (1979). All samples had their fat component removed using solvent hexanes that rinsed any hexane-soluble material (namely peanut oil) from the sample. The remaining solids (including NaNO_2 active) were treated with alkaline solution to dissolve the NaNO_2 encapsulant material to allow a colorimetric determination.

Encapsulated NaNO_2 used in paste and pellet baits, manufactured for the free-feeding trials, contained 95% weight/weight (w/w) NaNO_2 for the chicken, pigeon and budgerigar trials and 94% w/w NaNO_2 for the duck and wētā free-feeding trials. The NaNO_2 paste and pellet baits manufactured for the free-feeding trials contained 10% w/w NaNO_2 for the chicken, pigeon and budgerigar trials and 9.9% w/w NaNO_2 for the duck and wētā free-feeding trials.

Results

Oral gavage trial

An oral LD_{50} of $68.50\ \text{mg}/\text{kg}$ (95% CI $55.00\text{--}80.00\ \text{mg}/\text{kg}$) was calculated for both chickens (Table 1) and ducks (Table 2). Both chickens and ducks displayed symptoms of methaemoglobinaemia including lethargy, shortness of breath, loss of co-ordination and loss of consciousness. These symptoms were first observed on average after 18.25

Table 1. Chickens orally gavaged with NaNO_2 at four dose rates.

Bird	Weight (kg)	Dose (mg/kg)	NaNO_2 (mg)	Fate	First appearance clinical symptoms (min)	Time to death (min)
1	1.915	175	335	Died	12	19
2	1.767	175	309	Died	8	19
3	1.745	175	305	Died	15	19
4	1.708	115	196	Died	16	21
5	1.774	115	204	Died	32	34
6	1.961	115	226	Died	26	28
7	1.872	85	159	Died	16	20
8	1.902	85	162	Died	17	20
9	1.903	85	162	Died	16	26
10	1.726	55	95	Alive	27	–
11	1.759	55	97	Alive	8	–
12	1.842	55	101	Alive	26	–
13	1.702	Control	0	Alive	–	–
14	1.745	Control	0	Alive	–	–
15	1.675	Control	0	Alive	–	–

Table 2. Ducks orally gavaged with NaNO₂ at four dose rates.

Bird	Sex	Weight (kg)	Dose (mg/kg)	NaNO ₂ (mg)	Fate	First appearance clinical symptoms (min)	Time to death (min)
1	F	1.081	175	199	Died	4	20
2	M	1.168	175	215	Died	6	17
3	M	1.449	175	267	Died	10	18
4	M	1.530	115	185	Died	13	23
5	M	1.355	115	164	Died	6	27
6	F	1.149	115	139	Died	7	21
7	M	1.072	85	96	Died	18	48
8	M	1.226	85	110	Died	13	37
9	F	1.076	85	96	Died	17	42
10	M	1.380	55	80	Alive	11	–
11	M	1.381	55	80	Alive	12	–
12	M	1.238	55	72	Alive	7	–
13	M	1.774	Control	0	Alive	–	–
14	F	2.205	Control	0	Alive	–	–
15	F	1.265	Control	0	Alive	–	–

minutes (± 2.24 SE) for chickens and on average after 10.33 minutes (± 1.29 SE) for ducks (Tables 1 and 2). The average time to death for chickens was 22.89 minutes (± 1.77 SE). The average time to death for ducks was 28.11 minutes (± 3.79 SE).

Necropsy of chickens and ducks that died found that all the birds appeared cyanotic—they were very pale with a bluish discolouration of the skin and mucous membranes. Their blood had a dark brown coloration attributed to methaemoglobinaemia induced by NaNO₂. Necropsy of surviving birds carried out 14 days after being gavaged found no gross abnormalities and nothing of note.

Free-feeding trials

Chickens

Six of the 12 birds presented with NaNO₂ paste bait consumed between 0.5 and 20 g and four of these birds consumed a lethal dose (Table 3). Chickens displayed the same symptoms of methaemoglobinaemia observed in the oral gavage trials. These symptoms were first observed on average after 31.00 minutes (± 3.91 SE) (Table 3). The average time to death for chickens was 69.75 minutes (± 16.11 SE). An approximate dietary LD₅₀ of 254.6 mg/kg (95% CI 249.1–260.2 mg/kg) for chickens feeding on NaNO₂ paste bait has been calculated using probit curve analysis (Finney 1971). Findings of the necropsies of the four birds that died were identical to those from chickens that died in the oral gavage trial. Necropsy of the two birds that consumed toxic paste bait and survived and three birds fed non-toxic paste bait found no gross abnormalities and nothing of note.

Domestic mallard ducks

Two of the five ducks presented NaNO₂ paste bait consumed 1.3 g and 0.3 g, respectively (Table 4). The duck that consumed 1.3 g of NaNO₂ paste bait displayed the same symptoms of methaemoglobinaemia as the gavage trials and died after consuming approximately 99.3 mg/kg of NaNO₂. This calculation was based on the NaNO₂ paste bait containing 9.9% w/w NaNO₂. A dietary LD₅₀ for ducks feeding on NaNO₂ paste bait could not be calculated due to the small number of individual ducks that ate toxic baits. The necropsy of the one bird that died was identical to that for birds that died in

Table 3. Chickens free-fed with NaNO₂ paste (group 1) and pellet baits (group 2) and non-toxic paste (group 3) and pellet baits (group 4).

Bird	Weight (kg)	Group	Bait fed (g)	Bait eaten (g)	NaNO ₂ eaten (mg/kg)	Fate	First appearance clinical symptoms (min)	Time to death (min)
1	1.927	1	20.00	3.49	18.11	Alive	35	–
2	1.535	1	20.00	20.00	1302.93	Dead	32	35
3	1.567	1	20.00	0.00	0.00	Alive	–	–
4	1.676	1	20.00	5.99	35.73	Dead	43	72
5	1.754	1	20.00	0.50	2.85	Alive	–	–
6	1.766	1	20.00	0.00	0.00	Alive	–	–
7	2.026	1	20.00	0.00	0.00	Alive	–	–
8	1.343	1	20.00	0.00	0.00	Alive	–	–
9	1.577	1	20.00	0.00	0.00	Alive	–	–
10	1.843	1	20.00	0.00	0.00	Alive	–	–
11	1.932	1	20.00	17.10	88.50	Dead	23	114
12	1.732	1	20.00	20.00	1154.73	Dead	22	58
13	1.388	2	28.30	0.00	0.00	Alive	–	–
14	1.956	2	29.00	0.00	0.00	Alive	–	–
15	1.803	2	29.40	0.00	0.00	Alive	–	–
16	1.636	2	27.60	0.00	0.00	Alive	–	–
17	1.776	2	29.70	0.00	0.00	Alive	–	–
18	1.694	2	27.70	0.00	0.00	Alive	–	–
19	1.729	3	20.00	20.0	N/A	Alive	–	–
20	1.936	3	20.00	1.40	N/A	Alive	–	–
21	1.946	3	20.00	0.34	N/A	Alive	–	–
22	1.683	4	26.70	0.00	N/A	Alive	–	–
23	1.726	4	28.00	0.00	N/A	Alive	–	–
24	1.862	4	28.50	0.00	N/A	Alive	–	–

the oral gavage trials. Necropsy of the bird that consumed NaNO₂ paste bait and survived and three birds fed non-toxic baits found no gross abnormalities and nothing of note.

Pigeons

Three of the six pigeons presented NaNO₂ paste bait consumed between 0.18 g and 0.29 g (Table 5) and none of these birds displayed any symptoms of poisoning. All six birds in Groups 3 and 4 ate non-toxic paste or pellet baits with birds consuming an average of 1.69 g of paste bait and 0.29 g of pellet baits (Table 5). Necropsy of the three birds that

Table 4. Ducks free-fed with NaNO₂ paste (group 1) and pellet baits (group 2) and non-toxic paste (group 3) and pellet baits (group 4).

Bird	Sex	Weight (kg)	Group	Bait eaten (g)	NaNO ₂ eaten (mg/kg)	Fate	First appearance clinical symptoms (min)	Time to death (min)
1	M	1.296	1	1.3	99.3	Died	28	55
2	M	1.154	1	0	0	Alive	–	–
3	F	1.099	1	0	0	Alive	–	–
4	M	1.282	1	0.3	23.2	Alive	–	–
5	M	1.410	1	0	0	Alive	–	–
6	M	1.385	2	0	0	Alive	–	–
7	M	1.396	2	0	0	Alive	–	–
8	M	1.272	2	0	0	Alive	–	–
9	F	1.216	2	0	0	Alive	–	–
10	F	1.187	2	0	0	Alive	–	–
11	M	1.559	3	11.4	–	Alive	–	–
12	M	1.509	3	15.9	–	Alive	–	–
13	M	1.302	4	0	–	Alive	–	–
14	F	1.119	4	0	–	Alive	–	–

Table 5. Pigeons free-fed with NaNO₂ paste (group 1) and pellet baits (group 2) and non-toxic paste (group 3) and pellet baits (group 4).

Bird	Sex	Weight (kg)	Group	Bait eaten (g)	NaNO ₂ eaten (mg/kg)	Fate
1	M	0.351	1	0.00	0.00	Alive
2	F	0.313	1	0.00	0.00	Alive
3	M	0.317	1	0.18	56.78	Alive
4	M	0.344	1	0.29	84.30	Alive
5	M	0.377	1	0.21	55.70	Alive
6	F	0.296	1	0.00	0.00	Alive
7	M	0.364	2	0.00	0.00	Alive
8	F	0.281	2	0.00	0.00	Alive
9	F	0.299	2	0.00	0.00	Alive
10	M	0.312	2	0.00	0.00	Alive
11	M	0.337	2	0.00	0.00	Alive
12	M	0.339	2	0.00	0.00	Alive
13	F	0.272	3	1.50	0.00	Alive
14	F	0.288	3	2.33	0.00	Alive
15	M	0.354	3	1.25	0.00	Alive
16	F	0.294	4	0.26	0.00	Alive
17	M	0.311	4	0.46	0.00	Alive
18	M	0.301	4	0.15	0.00	Alive

consumed NaNO₂ paste bait and two birds fed non-toxic baits found no gross abnormalities and nothing of note.

Budgerigars

Three of the six budgerigars presented NaNO₂ paste bait consumed between 0.02 g and 0.03 g (Table 6) and none of these birds displayed any symptoms of poisoning. Both birds in Group 3 ate non-toxic paste bait, consuming an average of 0.26 g of paste bait. Necropsy of the three birds that consumed toxic paste bait and two birds fed non-toxic baits found no gross abnormalities and nothing of note.

Cave wētā

All wētā were alive at the conclusion of the trial and there was no evidence of wētā feeding on NaNO₂ paste or pellet baits. Wētā bite marks were observed on a single non-toxic pellet bait.

Table 6. Budgerigars free-fed with NaNO₂ paste (group 1) and pellet baits (group 2) and non-toxic paste (group 3) and pellet baits (group 4).

Bird	Sex	Weight (kg)	Group	Bait fed (g)	Bait eaten (g)	NaNO ₂ eaten (mg/kg)	Fate
1	F	0.0463	1	48.23	0.00	0.00	Alive
2	M	0.0529	1	49.69	0.03	56.71	Alive
3	F	0.0655	1	48.66	0.00	0.00	Alive
4	F	0.0517	1	49.69	0.02	38.68	Alive
5	M	0.0554	1	49.96	0.03	54.15	Alive
6	M	0.0469	1	49.21	0.00	0.00	Alive
7	M	0.0445	2	49.37	0.00	0.00	Alive
8	F	0.0671	2	46.36	0.00	0.00	Alive
9	F	0.0438	2	49.20	0.00	0.00	Alive
10	F	0.0439	2	49.34	0.00	0.00	Alive
11	M	0.0405	2	43.06	0.00	0.00	Alive
12	F	0.0421	2	47.81	0.00	0.00	Alive
13	F	0.0395	3	49.37	0.18	0.00	Alive
14	M	0.0380	3	48.86	0.27	0.00	Alive
15	M	0.0363	4	44.38	0.00	0.00	Alive
16	F	0.0373	4	44.06	0.00	0.00	Alive

All four bait types increased in weight over the course of the trial due to moisture; however, the weight fluctuations of baits in cages with wētā (Table 7) were comparable to those of baits left in cages with no wētā (Table 8). Assays performed on wētā detected NaNO_2 in one wētā from Group 2 (NaNO_2 pellet bait) and the assay detected $10 \mu\text{g}$ of NaNO_2 .

Discussion

An oral LD_{50} of 68.50 mg/kg (for chickens and ducks) and a dietary LD_{50} of 254.6 mg/kg (for chickens) shows that NaNO_2 is toxic to birds and that baits containing encapsulated NaNO_2 are potentially hazardous for non-target species. This is reinforced by unpublished study data on NaNO_2 , which calculated an LD_{50} of 120 mg/kg in blackbirds (*Turdus merula*) and 619 mg/kg in bobwhite quail (*Colinus virginianus*) (pers. comm. Simon Humphreys, IA-CRC). The LD_{50} for NaNO_2 is high for birds when compared with other vertebrate pesticides, but when manufacturing and using NaNO_2 paste and pellet baits, similar precautions to those applied to other VTAs that are ground laid should be applied. This includes colouring the baits green as well as baits being used in appropriate bait stations.

Lapidge and Eason (2010) suggested that when NaNO_2 baits are consumed slowly there is potentially a lower risk of poisoning due to methaemoglobin reductase being able to keep pace with the conversion of methaemoglobin back to oxyhaemoglobin.

Non-target testing has previously been carried out on another methaemoglobin-inducing VTA para-aminopropiophenone. Para-aminopropiophenone was registered in New Zealand for stoat (*Mustela erminea*) and feral cat control in 2011 (Eason et al. 2014). The oral LD_{50} value for ducks gavaged with this toxin was estimated as 32 mg/kg (95% CI $14\text{--}62 \text{ mg/kg}$) and the average time to death was 12.2 hours (Eason et al. 2010). The LD_{50} calculated for ducks gavaged with NaNO_2 was 68.50 mg/kg (95% CI $55.00\text{--}80.00 \text{ mg/kg}$) and the average time to death was 22.89 minutes ($\pm 1.77 \text{ SE}$). This illustrates the speed at which NaNO_2 is absorbed and metabolised and that small doses can be eliminated without lethal effects. This also further reinforces the hypothesis that NaNO_2 baits need to be consumed quickly to exert a lethal effect.

Table 7. Wētā free fed with NaNO_2 paste (group 1) and pellet baits (group 2) and non-toxic paste (group 3) and pellet baits (group 4).

Wētā	Group	Bait fed (g)	Bait remaining (g)	Weight change (g)	Fate
1	1	12.119	13.154	+1.035	Alive
2	1	12.203	13.102	+0.899	Alive
3	1	12.189	12.996	+0.807	Alive
4	1	12.220	12.792	+0.572	Alive
5	1	12.147	12.569	+0.422	Alive
6	1	12.382	12.806	+0.424	Alive
7	1	12.111	12.522	+0.411	Alive
8	2	13.014	15.874	+2.860	Alive
9	2	12.921	15.216	+2.295	Alive
10	2	13.204	14.358	+1.154	Alive
11	2	13.185	14.079	+0.894	Alive
12	2	12.944	14.072	+1.128	Alive
13	2	13.060	13.958	+0.898	Alive
14	2	12.742	13.827	+1.085	Alive
15	3	12.541	12.593	+0.052	Alive
16	4	11.248	11.475	+0.227	Alive

Table 8. Weight changes of NaNO₂ paste and pellet baits and non-toxic (NT) paste and pellet baits kept in cages without wētā for 14 days.

Bait	Bait type	Start weight (g)	End weight (g)	Change in weight (g)
1	Paste	12.492	13.214	+0.722
2	Paste	12.715	13.224	+0.509
3	Pellet	12.775	14.538	+1.763
4	Pellet	12.814	13.960	+1.146
5	NT paste	12.513	13.079	+0.566
6	NT paste	12.816	13.359	+0.543
7	NT pellet	10.419	11.544	+1.125
8	NT pellet	11.921	12.712	+0.791

Determining whether wētā had fed on NaNO₂ paste and pellet baits was difficult, but there was no mortality during the trial, possibly indicating that those baits are either unpalatable to wētā, that any potential effects were not observed, or that they are simply unaffected by NaNO₂. NaNO₂ residue at a very low level (10 µg) was detected in one wētā when assayed suggesting that the potential for bioaccumulation and secondary poisoning is also low. The concentration detected in this wētā was only just above the minimum detection level and was potentially the result of some bait material contaminating the wētā when collected at the conclusion of the trial. Based on the dietary LD₅₀ calculated for chickens, a 1 kg chicken would need to consume over 25,000 wētā (each with a residue of 10 µg) in quick succession to receive an LD₅₀ dose.

For many reasons, testing the toxicity of baits to native species is a very difficult process. As such, it is deemed more acceptable and practical to undertake non-toxic bait consumption trials. These are used to extrapolate potential consumption of toxic baits and compare this to the dietary studies carried out on non-native surrogate species like chickens and ducks. Four chickens and one duck consumed a lethal dose of NaNO₂ paste bait in free-feeding studies and this reinforces the need to ensure that NaNO₂ paste baits are used in a bait station to ensure access by non-target species is limited.

We conclude that there is a risk to birds from NaNO₂ paste baits. However, these baits have to be accessed and then eaten quickly to have a lethal effect. The risks to birds and other non-target species can be substantially reduced if baiting is carried out according to the suggested best practice (which is yet to be established). This includes baits being ground-laid in bait stations for possums (Shapiro et al. 2016) and feral pigs (Shapiro et al. 2015). Bait stations for feral pigs are self-closing and exclude non-target species like birds due to the weight of the self-closing lid. There is no evidence for NaNO₂ having insecticidal effects.

Acknowledgements

We acknowledge Dr Karley Hermans from Selwyn-Rakaia Veterinary Services for the oral gavage and necropsy of ducks and chickens and Dr James Ross for assistance with statistical analysis. Three anonymous referees and the editor Dr Chris Jones improved the manuscript. All pen trials were approved by the Environmental Protection Authority (HSC100058) and animal manipulations were approved by Lincoln University Animal Ethics Committee (AEC #412 Chickens, #413 Domestic mallard ducks, #478 Pigeons, #479 Budgerigars). Approvals were not required for invertebrate trials. Associate editor: Dr Chris Jones.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors acknowledge the funding support of TBfree New Zealand (Formerly The Animal Health Board) (R-80628-09, Primary poisoning risk to non-target species and fate of sodium nitrite in baits for possum control).

References

- Barrett P. 1991. Keeping wētās in captivity – a series of nine articles for schools and nature lovers. Wellington: Wellington Zoological Gardens. 17 p.
- Barrios-Garcia MN, Ballari SA. 2012. Impact of wild boar (*Sus scrofa*) in its introduced and native range: a review. *Biological Invasions*. 14(11):2283–2300.
- Blackie H, MacKay J, Barrett B, Inder S, MacMorran D, Bothwell J, Clout M, Eason C. 2016. A novel device for controlling brushtail possums (*Trichosurus vulpecula*). *New Zealand Journal of Ecology*. 40(1):60–64.
- Booth LH, Fisher P, Heppelthwaite V, Eason CT. 2001. Toxicity and residues of brodifacoum in snails and earthworms. DOC science internal series 143. Wellington: Department of Conservation.
- Bradberry S. 2011. Methaemoglobinaemia. Complications of poisoning. *Medicine*. 40(2):59–60.
- Chui JSW, Poon WT, Chan KC, Chan AYW, Buckley TA. 2005. Nitrite-induced methaemoglobinaemia – aetiology, diagnosis and treatment. *Anaesthesia*. 60:496–500.
- Eason CT, Fairweather A, Ogilvie S, Blackie H, Miller A. 2013. A review of recent non-target toxicity testing of vertebrate pesticides: establishing generic guidelines. *New Zealand Journal of Zoology*. doi:10.1080/03014223.2013.772067.
- Eason CT, Miller A, MacMorran D, Murphy E. 2014. Toxicology and ecotoxicology of PAPP for pest control in New Zealand. *New Zealand Journal of Ecology*. 38(2):177–188.
- Eason CT, Miller A, Ogilvie S, Fairweather A. 2011. An updated review of the toxicology and ecotoxicology of sodium fluoroacetate (1080) in relation to its use as a pest control tool in New Zealand. *New Zealand Journal of Ecology*. 35(1):1–20.
- Eason CT, Milne L, Potts M, Morriss G, Wright GRG, Sutherland ORW. 1999. Secondary and tertiary poisoning risks associated with brodifacoum. *New Zealand Journal of Ecology*. 23:219–224.
- Eason CT, Murphy E, Hix S, Henderson R, MacMorran D. 2010. Susceptibility of four bird species to *para*-aminopropiophenone (PAPP). DoC Research and Development Series 320. Wellington, NZ: Department of Conservation. 15 p.
- Eason CT, Spurr EB. 1995. Review of the toxicity and impacts of brodifacoum on non-target wildlife in New Zealand. *New Zealand Journal of Zoology*. 22:371–379.
- Engeman R, Cattaruzza R, Cattaruzza M, Fischer J. 2016. Photographic estimation of wild boar damage to alpine grazing pastures in the Carpathian Mountains of central Romania. *Environmental Science and Pollution Research*. 23(5):4949–4952.
- Finney DJ. 1971. Probit analysis. 3rd ed. Cambridge: Cambridge University Press.
- Fish R, Statham M. 2009. The attractiveness of the feral pig bait HOG-GONE® to Bennett's wallaby (*Macropus rufogriseus*) and Tasmanian pademelon (*Thylogale billardierii*). Unpublished report. Launceston, Tasmania: Tasmanian Institute of Agricultural Research.
- Fisher P, Brown S, Arrow J. 2009. Possum response to ingestion of a new toxin for feral pig control. Unpublished report. Lincoln, New Zealand: Landcare Research.
- Foster JA. 2011. Effects of sodium nitrite on feral swine and non-targets. Performance report for Texas Parks and Wildlife as required by Federal Aid in Wildlife Restoration Act. Federal Aid Grant No. W-132-R-9. 11 p.

- Gentle M, Phinn S, Speed J. 2011. Assessing pig damage in agricultural crops with remote sensing. Bureau of Rural Sciences Australian Pest Animal Management Program Final Report. 17 p.
- Lapidge S, Eason C. 2010. Pharmacokinetics and methaemoglobin reductase activity as determinants of species susceptibility and non-target risks from sodium nitrite manufactured feral pig baits. Report for the Australian Government Department of the Environment, Water, Heritage and the Arts. Canberra, Australia.
- [OECD] Organisation for Economic Co-operation and Development. 2008. OECD Guidelines for the testing of chemicals, Section 4: Health effects. Test number 425: Acute oral toxicity: Up-and-down procedure. 27 p.
- [OECD] Organisation for Economic Co-operation and Development. 2010. Test No. 223: Avian acute oral toxicity test. OECD guidelines for the testing of chemicals, Section 2. Paris: OECD Publishing. 25 p.
- Powlesland RG, Knechtmans JW, Styche A. 2000. Mortality of North Island tomtits (*Petroica macrocephala toitoi*) caused by aerial 1080 possum control operations, 1997–98, Pureora Forest Park. New Zealand Journal of Ecology. 24:161–168.
- Russell WMS, Burch RL. 1959. The principles of humane experimental technique. London: Methuen & Co. Special edition published by Universities Federation for Animal Welfare (UFAW), 1992.
- Shapiro L. 2016. The potential of sodium nitrite as a new tool for pest control from efficacy, safety and welfare perspectives [Unpublished PhD thesis]. New Zealand: Lincoln University.
- Shapiro L, Eason C. 2009. Summary of data from pre-feed and sodium nitrite bait trials with dama wallabies (*Macropus eugenii*). Unpublished report. Auckland, New Zealand: Connovation Research Ltd.
- Shapiro L, Eason C, Bunt C, Hix S, Aylett P, MacMorran D. 2015. Efficacy of encapsulated sodium nitrite as a new tool for feral pig management. Journal of Pest Science. doi:10.1007/s10340-015-0706-7.
- Shapiro L, Eason C, Bunt C, Hix S, Aylett P, MacMorran D. 2016. Encapsulated sodium nitrite as a new toxicant for possum control in New Zealand. New Zealand Journal of Ecology. 40(3): 381–385.
- Spurr EB, Maitland MJ, Taylor GE, Wright GRG, Radford CD, Brown LE. 2005. Residues of brodifacoum and other anticoagulant pesticides in target and non-target species, Nelson Lakes National Park, New Zealand. New Zealand Journal of Zoology. 32:237–249.
- Stone WB, Okoniewski JC, Stedlin JR. 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. Journal of Wildlife Diseases. 35:187–193.
- Vogel AI. 1979. Vogel's textbook of quantitative inorganic analysis. 4th ed. London: Longman; p. 356.