Evaluation of new toxins for mustelid control

DOC SCIENCE INTERNAL SERIES 56

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Published by
Department of Conservation
P.O. Box 10-420
Wellington, New Zealand

DOC Science Internal Series is a published record of scientific research carried out, or advice given, by Department of Conservation staff, or external contractors funded by DOC. It comprises progress reports and short communications that are generally peer-reviewed within DOC, but not always externally refereed. Fully refereed contract reports funded from the Conservation Services Levy (CSL) are also included.

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ISSN 1175–6519

ISBN 0–478–22282–3

This report originated from work carried out under Department of Conservation investigation no. 3125. It was prepared for publication by DOC Science Publishing, Science & Research Unit; editing by Lynette Clelland and layout by Ruth Munro. Publication was approved by the Manager, Science & Research Unit, Science Technology and Information Services, Department of Conservation, Wellington.

CONTENTS

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A B S T R A C T

Sustainable, humane, and cost-effective stoat control is essential in New Zealand to protect our native fauna from predation. The long-term aim of this research is to develop a new tool for conservation that is designed with specificity and humaneness as the most important attributes. This project began by evaluating the sensitivity of captive stoats and ferrets to a range of potential mustelid toxicants. Ferrets and stoats were susceptible to MNT (a compound being developed for predator control in Australia). Preliminary observations indicate that death from MNT is relatively humane in mustelids, with no obvious signs of distress or pain observed. The non-target species tested were all susceptible to MNT but for possums and wallabies this was at higher concentrations than for stoats and ferrets, whereas for ducks it was similar. Further research, to determine the toxicity and relative humaneness of MNT to stoats and susceptibility of non-target species, is required to develop this toxin into an effective stoat control tool.

Keywords: stoat control; target-specific toxicants; non-target susceptibility; humaneness.

© July 2002, New Zealand Department of Conservation. This paper may be cited as:
O'Connor, C.E. 2002: Evaluation of new toxins for mustelid control. *DOC Science Internal Series 56*. Department of Conservation, Wellington. 14 p.

1. Introduction

The susceptibility of target and non-target animals to potentially mustelid-specific toxicants was evaluated by Landcare Research, Lincoln, for the Department of Conservation from July 1998 to June 2001.

2. Background

Mustelids, especially stoats (*Mustela erminea*) and ferrets (*M. furo*), are major predators of many bird species in New Zealand. Control strategies for mustelids, using poisoned baits, have previously relied on the use of sodium monofluoroacetate (1080) or anticoagulant rodenticides. While effective, the widespread use of 1080 is controversial, due to its lack of target specificity, and anticoagulants are under increasing scrutiny as a result of persistent environmental residues. The Department of Conservation (DOC) has indicated a need for a more target-specific toxicant for mustelid control and, as a first step towards achieving this goal, Landcare Research prepared a report for them in 1998 entitled 'Mustelid-specific toxicants'. This report identified a range of compounds to which mustelids appear to be particularly susceptible. These were, typically, veterinary products or human pharmaceuticals (medicines) that were reported to have caused death in individual pets, mainly ferrets.

In particular, some methaemoglobin-inducing agents (oxidants) and non-steroidal anti-inflammatory agents (NSAIDs) were identified as promising. In at least one study, ferret erythrocytes (red blood cells) were reported to be relatively sensitive to oxidative stress, leading to the conversion of haemoglobin to methaemoglobin, which is incapable of binding oxygen (Davis et al. 1993).

3. Objectives

- To evaluate the sensitivity of captive stoats and ferrets to new, potentially mustelid-specific toxicants in single-dose acute toxicity studies.
- To evaluate the sensitivity of captive stoats, possums, wallabies, and ducks to the Australian toxicant MNT.

4. Methods

Approvals from the Landcare Research Animal Ethics Committee (99/4/1, 99/5/1, 00/6/6, 01/3/3) were obtained before beginning each part of this research.

4.1 ACUTE TOXICITY IN FERRETS

First-generation captive-bred, adult ferrets were used in all these tests. Ferrets were individually housed in cages (50 × 50 × 100 cm) under an outside shelter at the animal facility at Landcare Research. Each ferret was supplied with a hessian sack for nesting and extra protection from the weather, and fed a rotation of dead day-old chick, mince and dog roll in the afternoon, with free access to water.

Initially, ferrets were tested in toxin susceptibility studies to determine their relative sensitivity to each of four compounds. Four treatment groups of animals were tested at different dose levels and a control. The toxicant was administered in the morning prior to the animals being fed, as a solution, by oral gavage (stomach tubing) in an appropriate vehicle, under light anaesthesia with fluothane. Animals were closely monitored for 30 days and all mortality and times to death recorded. Any animal showing greater than 25% body weight loss or behavioural signs of extreme distress or pain was euthanased immediately by anaesthesia with fluothane and then cardiac puncture with an overdose of Pentobarb 300.

Humaneness of each new toxicant was evaluated in the groups of animals used in the efficacy trials. Preliminary behaviour observations were made to record primary signs of toxicosis and, particularly, any behaviours indicating pain or distress. If there were not high mortalities at the initial doses tested, other compounds were investigated.

4.1.1 Acetaminophen

The first agent tested was a methaemoglobin-inducing agent acetaminophen. There are known species differences in susceptibility to acetaminophen with the hamster and mouse being more sensitive than the rat, guinea pig, rabbit (Davis et al. 1974) and possum (Eason et al. 1999). Cats are the most susceptible of any species studied with an LD_{50} < 40 mg/kg (Wickstrom & Eason 1999). The LD_{50} of most other species is in the range 150–1000 mg/kg (St Omer & McKnight 1980; Gaunt et al. 1981). Acetaminophen would only prove useful as a species-specific toxicant if mustelids were in the highly susceptible range (< 200 mg/kg).

Twenty-one ferrets (11 male, 10 female; mean weight: 0.98 ± 0.04 kg) were tested in groups of five (six at the highest dose) at reasonably low concentrations of acetaminophen: 0, 50, 100, and 200 mg/kg. The human pharmaceutical, Panadol double-strength, colour-free liquid (SmithKline Beecham) was the test solution at a concentration of 250 mg acetaminophen/ 5 ml. Nanopure water at the equivalent volume for 200 mg/kg (4 ml/kg), was used for the control animals. The limit of 200 mg/kg was chosen as the upper

limit to include ferrets as 'a susceptible species' and hence ensure minimal risks to other, non-target, species, which generally have a much higher LD_{50}. The mean air temperature was 16.4°C during testing.

4.1.2 Non-steroidal anti-inflammatory agents

The second types of compound tested, as above, were the non-steroidal anti-inflammatory agents. The compounds tested were acetylsalicylic acid, as Extra Strength Aspro® Clear (Roche) 500 mg tablets dissolved in nanopure water, and Nurofen® for children (Boots Healthcare) containing 100 mg ibuprofen/5 ml suspension. There are few data available on the sensitivity of other species to these compounds, although in children doses of acetylsalicylic acid over 150 mg/kg are considered an overdose (Meyers et al. 1980). Three females for each treatment group of 250 and 500 mg/kg doses for each compound (medium to high doses: Wickstrom 1998) were tested. These pilot trials were aimed at determining the toxicity to ferrets of concentrations that could, practically, be included in a bait. The mean air temperature was 8.5°C during testing.

4.1.3 MNT

Because ferrets were susceptible to acetaminophen (see results), a compound (Mustelid New Toxin: MNT) being developed for predator control in Australia (which we believed had a similar toxicological effect) was tested. (The 'in principle' agreement in place during these trials has led to the preparation of a Memorandum of Understanding between the Department of Natural Resources and Environment, Victoria, Australia; Landcare Research; and DOC to test this compound on mustelids.)

Forty-seven ferrets (15 male, 32 female; mean weight: 0.90 ± 0.04 kg) were tested at six dosages of MNT (0, 12.5, 25, 30, 40 and 50 mg/kg (Table 1)), after an initial pilot trial with six animals showed promise. Ferrets were dosed with 0.5–2.5 ml/kg MNT, in a crystalline form, dissolved in monopropylene glycol (MPG). Control animals were dosed with MPG. The mean temperature on the days of testing ranged from 4.2 to 17°C.

4.2 ACUTE TOXICITY IN STOATS

Wild-caught stoats were housed in cages (60 × 150 × 90 cm) under an outside shelter at the animal facility at Landcare Research. Each stoat was supplied with a nest box (40 × 33 × 15 cm) containing shredded paper as nesting material. Stoats were fed a rotation of dead day-old chicks, chicken pet mince and mutton/beef pet mince in the afternoon, and had free access to water.

The most promising toxicants were tested in the same manner as on ferrets. With stoats, only two groups of five to six animals were tested, one control and one treatment group, as information on the appropriate dose level was provided from the ferret data. There is little comparable toxicology data between these two species, but it was assumed that because they are closely related (the same genus), they would have similar susceptibility to toxicants.

Five stoats (3 male, 2 female; mean weight: 260 ± 32 g) were dosed with acetaminophen at 200 mg/kg, alongside five stoats dosed with water (2 male, 3

female; mean weight: 265 ± 14 g), as controls. Twelve stoats were tested with MNT, six at 25 and six at 50 mg/kg (10 male, 2 female; mean weight: 302 ± 19 g) alongside nine control stoats dosed with MPG (8 male, 1 female; mean weight: 310 ± 23 g). The mean temperature on the days of testing ranged from 9.5–17.6°C.

Again, humaneness of each potential toxicant was evaluated in the groups of animals used in the efficacy trials. Detailed observations of behaviour were made for signs of toxicosis and, particularly, any indication of pain or distress.

4.3 NON-TARGET ANIMAL SUSCEPTIBILITY TO MNT

At DOC's request the emphasis of this project in the final year (00/01) was changed, from the original objective to evaluate the relative humaneness of these new toxicants in captive stoats and ferrets, to evaluating the sensitivity of three non-target species to MNT. This was conducted as part of the agreement DOC (and Landcare Research) has with the Australian group. The humaneness research is still viewed as important, but is proposed for future years.

The relative susceptibility of three non-target species (possums, wallabies and ducks) to MNT was tested. Groups of six animals were tested at a time, in either a treatment or control group. All animals were orally gavaged, under light anaesthesia (or restraint for ducks), and closely monitored for signs of toxicosis. Control animals were dosed with the carrier solution only (MPG). The first treatment group (n = 6) received a dose of 500 mg/kg, 100 times the LD_{50} recorded for cats (5.6 mg/kg). In general, highly susceptible species have an LD_{50} < 20 mg/kg and most species tested have an LD_{50} 100–200 mg/kg. If there were no deaths then testing ceased, and the lethal dose would have been recorded as > 500 mg/kg. If there were any deaths, further groups of six animals were tested at a dosage incrementally decreasing (i.e. 250, 125, 62.5 mg/kg ...) until a dose level was found where no deaths were recorded. The number, and time, of deaths were recorded for each dosage level.

4.4 STATISTICAL ANALYSES

Approximate LD_{50} values for MNT were calculated for those species where sufficient data were available. The LD_{50} values and their 95% confidence intervals were estimated from logistic regression models fitted in SYSTAT 7.0.

5. Results

5.1 ACUTE TOXICITY IN FERRETS

5.1.1 Acetaminophen

There was 50% mortality at the highest (200 mg/kg) dosage tested, and no mortality at any of the other doses. The three ferrets died within 2 days of dosing. Lethargy and inappetance were observed in most ferrets in the 24–48 hours following dosing at all concentrations (except 0 mg/kg).

5.1.2 Non-steroidal anti-inflammatory agents

There were no signs of illness and no deaths following acetylsalicylic acid dosing in ferrets. Ferrets dosed with 500 mg/kg ibuprofen were lethargic within 2 hours, one animal vomited and another was unconscious for several hours, but there were no deaths.

5.1.3 MNT

There were no deaths at the lowest dose (12.5 mg/kg), 55% of ferrets died at 25 mg/kg and 91% at 50 mg/kg MNT (Table 1). The estimated LD_{50} for ferrets from these data was 29 mg/kg (95% CI: 22–37 mg/kg). There was no evidence of a sex difference in susceptibility to MNT.

At all doses of MNT, ferrets generally recovered from the anaesthesia within 5 minutes, showed some reduced coordination initially before progressing from lying, to sternal recumbent to a prostrate posture 50 minutes after dosing. Animals also showed changes in breathing from rapid breathing initially to shallow, slow breathing nearer death. The mean time to death was 149 minutes. None of these postural or breathing changes are indicators of pain. All animals became pale and many shivered, including those that recovered (recovery was seen approximately 5 hours after dosing).

TABLE 1. NUMBER OF FERRETS TESTED, PERCENT MORTALITY AND MEAN TIME TO DEATH (± SE) FOR EACH MNT DOSE TESTED.

DOSE (mg/kg)	NUMBER OF FERRETS TESTED	MORTALITY (%)	TIME TO DEATH (min)
0	6	0	–
12.5	7	0	–
25	11	55 ± 15	233 ± 134
30	6	33 ± 19	132 ± 73
40	6	83 ± 15	291 ± 153
50	11	91 ± 9	257 ± 80

5.2 ACUTE TOXICITY IN STOATS

5.2.1 Acetaminophen

There were no signs of illness and no deaths following acetaminophen dosing in stoats.

The NSAIDs were not tested on stoats because they showed no toxicity in ferrets.

5.2.2 MNT

There was one death in the control group (2 days after dosing) and in the 50 mg/kg group (death in 4 minutes), where the effects of anaesthesia combined with oral gavaging can not be ruled out. All five of the other 50 mg/kg treated stoats died within 50 minutes (mean: 41 ± 2.6 minutes). At 25 mg/kg MNT all six stoats died, also within 50 minutes (mean: 37 ± 3.6 minutes).

Stoats dosed with MPG (controls) recovered from the anaesthetic within 3 minutes, and immediately showed normal active behaviours, including grooming, before lying in a curled posture. Stoats showed a very consistent pattern after dosing with MNT. For example, stoat 172 dosed at 25 mg/kg woke from anaesthetic at 4 minutes and was uncoordinated, lay curled (normal posture) 5 minutes, distinctive blue colouration to lips by 10 minutes, lying/prostrate from 10 minutes, laboured breathing from 22 minutes, and dead 31 minutes later. Again, there were no indications of stoats feeling any pain with only postural or breathing changes observed.

5.3 NON-TARGET ANIMAL SUSCEPTIBILITY TO MNT

5.3.1 Possums

Eighteen acclimatised wild-caught brushtail possums (*Trichosurus vulpecula*), were used in this trial (9 male, 9 female; mean weight: 3.3 ± 0.2 kg). Possums were housed in individual wire cages (350 × 200 × 200 cm) in temperature-controlled rooms (19 ± 5°C) under natural-day-length fluorescent lighting.

The possums tested had 50% mortality at 500 mg/kg MNT (1 male, 2 female), and no deaths at 0 or 250 mg/kg MNT (Table 2).

All possums dosed with MNT became pale with a distinct 'blue' nose 30–60 minutes after dosing. Those animals that succumbed lay prostrate within 5 hours of dosing, dying 24–48 hours later (animals were unconscious for at least some of this time). Two animals that recovered had some external bleeding, from the nose or anus, 12–24 hours after dosing.

5.3.2 Wallabies

Forty-eight male dama wallabies (*Macropus eugenii*) (mean weight 5.5 ± 0.4 kg) were captured in the Rotorua district and transported to Guus Knopers' farm, Te Puke. Wallabies were housed in an enclosed pasture pen (6 × 6 m) with an enclosed shed (2 × 6 m) for shelter, and fed calf muesli and carrots for the duration of the trial. The mean temperature on the days of testing ranged from 15 to 23°C.

TABLE 2. NUMBER OF POSSUMS, WALLABIES, AND DUCKS WITH PERCENT MORTALITY FOR EACH MNT DOSE TESTED, AND APPROXIMATE LD_{50} VALUES FROM THESE DATA.

	DOSE (mg/kg)	NUMBER OF ANIMALS	MORTALITY (%)	ESTIMATED LD_{50} (mg/kg)
Possums	0	6	0	
	250	6	0	
	500	6	50	≥ 500
Wallabies	0	18	0	
	31.25	6	0	
	62.5	6	33	
	125	12	83	
	250	6	100	89
Ducks	0	12	0	
	15.625	6	17	
	31.25	6	67	
	62.5	6	100	
	125	6	83	
	250	6	100	
	500	6	100	38

There were mortalities at all doses of MNT tested, except 0 and 31.25 mg/kg (Table 2). These data indicate an LD_{50} of 89 mg/kg (95% CI: 63–118) for wallabies.

The symptoms seen in wallabies following dosing with MNT were blue mouth and excessive salivation, with breathing becoming more shallow and irregular closer to death. Most wallabies remained comatose until death (on average 20 ± 6 hours later). There was occasional limb flexing in some comatose animals. The animals that recovered appeared to have impaired coordination for varying lengths of time (< 24 hours) before recovering.

5.3.3 Ducks

Forty-eight Pekin ducks (*Anas platyrhynchos*) (18 male, 30 female; mean weight: 2.34 ± 0.1 kg) were housed in groups of at least six in pens (32 × 3 × 2 m) outside at the animal facility at Landcare Research. Their diet consisted of a grain mix, duck pellets and fruit with free access to water. The mean temperature on the days of testing ranged from 2.7 to 17.6°C.

There were mortalities at all doses of MNT tested (Table 2); hence the LD_{50} estimated from these data is 38 mg/kg (95% CI: 20–73). The high susceptibility of ducks to MNT was unexpected, as much higher LD_{50} values (133 to > 300 mg/kg) have been estimated in other bird species.

Within 5 minutes of dosing, ducks went and sat in shade/shelter, already with some incoordination whilst walking. After 10 minutes they had laboured breathing (beak open gasping for air) and were lethargic until death 2–12 hours later. Some ducks appeared distressed with head waving and neck arching observed.

6. Conclusions

- Ferrets, but not stoats, were susceptible to acetaminophen at the low doses tested.
- Human non-steroidal anti-inflammatory agents (acetylsalicylic acid and ibuprofen) were not toxic to ferrets at the doses tested.
- Ferrets were susceptible to MNT with an approximate LD_{50} of 29 mg/kg (95% CI: 22–37 mg/kg).
- Stoats were highly susceptible to MNT, with 100% mortality at 25 mg/kg.
- Preliminary observations indicate that death from MNT is relatively humane in mustelids, with no obvious signs of distress or pain observed.
- The non-target species tested (possum, wallaby and duck) were all susceptible to MNT. The lethal dose for possums and wallabies was higher than for stoats and ferrets, but that for ducks was similar.

7. Recommendations

This research has identified a potential new toxin for stoat control. The development of MNT as a new, humane, effective toxin for stoat control, with low non-target risks, requires further research to:

- determine the toxicity of MNT to captive stoats to calculate an accurate LD_{50}, including performance of MNT in a bait;
- evaluate the relative humaneness of MNT in captive stoats;
- estimate the susceptibility of non-target species to MNT, initially by desk-top extrapolation of available data to identify key information gaps, and critical species for future testing;
- determine the palatability and effectiveness of a toxic bait, containing MNT, for stoat control.

8. Acknowledgements

I thank Grant Morriss and Andrea Rhodes for assisting with the trials; Guus and Heddie Knopers for providing facilities, wallabies and wallaby care; and Julie Turner, Penny Willcock, Siobhan Kay and Michael Wehner for the husbandry and care of all other animals. Thanks also to Ray Webster for statistical advice, Penny Fisher, Elaine Murphy and Charles Eason for comments on earlier drafts, Christine Bezar for editing, and Wendy Weller for final word processing of this report.

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