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SHORT COMMUNICATION

Second-hand toxicity: A feasibility study of implant-mediated secondary poisoning in stoats

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Abstract: Secondary poisoning via toxic prey is a critical tool for controlling stoats in New Zealand. However, current approaches experience limited carcass persistence and risk sublethal dosing. This study evaluated implant-mediated secondary poisoning using a small, spherical, pH-responsive toxic implant. Sodium fluoroacetate (1080) implants coated with a reverse enteric polymer were tested in vitro, indicating that coating thickness influenced implant stability at neutral pH and release behaviour under gastric conditions. Implants were stable at subcutaneous pH for ≥ 7 d, demonstrating their likely persistence in implanted prey. In vivo trials showed that stoats readily consumed mouse carcasses containing implants (resulting in ≥ 50% mortality), however, some implants passed through the digestive tract intact, suggesting that variable stomach pH or fast gastric emptying times impacted toxin release. While further experiments are required to characterise the gastrointestinal transit time of stoats, these results demonstrate a proof-of-concept for implant-mediated secondary poisoning of stoats

Keywords: implant, invasive predators, pH-responsivity, secondary poisoning, stoat control

Introduction

New Zealand's native fauna faces significant threat from invasive mammalian predators, such as stoats (*Mustela erminea*) (Russell et al. 2015). In 2016, the New Zealand Government announced Predator Free 2050, the goal to eradicate rats (*Rattus* spp.), possums (*Trichosurus vulpecula*) and mustelids (*Mustela* spp.) by 2050 (New Zealand Government 2016). Stoats are a target for nation-wide eradication under the Predator Free 2050 initiative (Russell et al. 2015) and are among the most difficult to eradicate from large and remote areas due to their neophobic behaviour and high reinvasion potential (King et al. 2003; Smith & Weston 2017; Murphy et al. 2019).

Control of stoats currently includes trapping and poison baiting, with the latter generally considered more effective, particularly for large-scale deployment (e.g. Elliott & Kemp 2016). Importantly, poison baiting programs do not rely on the uptake of poisoned baits by stoats and instead exploit the susceptibility of stoats to secondary poisoning; stoats are killed when they eat 1080-poisoned prey such as rodents or possums (Brown et al. 1998; Murphy et al. 1999; Robertson et al. 2019; Dilks et al. 2020). Limitations associated with the current approach to secondary poisoning include: (1) poisoned prey may not persist long enough for stoats to find them, (2) regulations preventing rodenticide baits from being deployed near human habitation, (3) low abundance of poisoned prey reducing the effectiveness of secondary poisoning, and (4) sublethal dosing due to either degradation of the poison in the carcass or incomplete consumption of the carcass (Brown et al. 1998).

Brewer et al. previously designed a 1080-loaded "Population Protecting Implant" (PPI) aimed at targeting "problem individuals"—feral cats that repeatedly prey on vulnerable native animal populations following reintroduction (Brewer et al. 2022; Brewer et al. 2023a, b). The PPI was a small (c. 2 mm diameter, 10 mm length), rod-shaped implant containing a poison core and an outer coating composed of a reverse enteric copolymer that was stable at neutral, subcutaneous pH and dissolved at low, gastric pH. The PPI was designed to be subcutaneously injected into native animals prior to reintroduction, where the reverse enteric coating would be stable and prevent poison release. If the native animal was consumed by a problem individual, the coating would rapidly dissolve in its gastric environment and release the 1080 contained in the core, thereby offering a degree of selectivity for problem individuals (Manning et al. 2021; Brewer et al. 2022). For the PPI, stability in the subcutaneous environment, as well as the rate of poison release in the gastric environment, was highly dependent on the thickness of the polymer coating, allowing the performance of the implant to be modulated.

We proposed that similar implants could be exploited for use in secondary poisoning, herein referred to as secondary poisoning implants (SPIs). Wild-caught rodents could be administered a SPI and released for predation by stoats. While

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the SPIs are stable, the rodents would be attractive and toxic to stoats. The potential for sublethal poisoning would be reduced because the toxicity of the SPIs would remain constant while protected within the coating, rather than degrading over time. A thin reverse enteric coating with short-lived stability could be used to ensure that if no predation occurs, the coating will eventually fail, releasing the encapsulated 1080, and killing the implanted rodent. The invasive rodents would therefore not persist in the environment. Compared to feral cats, the smaller size of stoats requires SPIs that are smaller than the PPIs, to ensure the implant is consumed without rejection. The average body weights of stoats (males, 356 g; females, 222 g; King 1991), and their low tolerance to 1080 (LD_{50} and LD_{90} values of 0.49 and 0.70 mg kg^{-1}, respectively; Spurr 2000), suggest that the 1080 loading could be reduced more than ten-fold (i.e. LD_{90} doses of 0.15–0.25 mg) and the implant size reduced without reducing lethality. Encapsulation and targeted delivery of 1080—using far lower quantities than conventional baits—may reduce non-target risk.

The aims of this study were to: (1) manufacture small, spherical SPIs comprised of a 1080 core encapsulated within a thin, short-lived reverse enteric copolymer coating, to selectively release the poison upon ingestion by a stoat or following a predetermined period of time in a rodent (e.g. 2–4 weeks), and (2) conduct in vivo ingestion trials to determine whether stoats would consume implanted rodents and whether the SPIs will release the poison within the stoat.

Methods

Preparation and characterisation of implants

The SPI cores were prepared via wet granulation and extrusion-spheronisation (see Appendix S1 in Supplementary Materials) of a formulation containing 1080 (51% w/w), microcrystalline cellulose (MCC; 24% w/w), lactose (24% w/w), and rhodamine B (1% w/w) (refer to Appendix S1 for a more detailed description of the SPI manufacture and characterisation). Microcrystalline cellulose was used as a spheronisation aid and lactose as a filler. Rhodamine B is a pink/red dye used as a proxy for 1080 when monitoring 1080 release in vitro and has been successfully used in previous work (Brewer et al. 2023a). Due to the purity of the 1080 available (89.6%), the actual 1080 content of the formulation was c. 46% w/w. Based on the 1080 purity, implant size, and conservative density calculations, the amount of 1080 per SPI would be in the range of 0.45–0.54 mg. Therefore, based upon reported 1080 toxicity data (Spurr 2000), on average each implant would contain approximately twice the LD_{90} of a male stoat.

To obtain individual SPI cores with consistent size and 1080 content, 34 g of the bulk material was sieved into SPI cores (12.3 g) with diameters of 1.18−1.25 mm (Fig. 1a). Samples of the uncoated SPI cores were imaged using a microscope, measured using ImageJ software, and found to be consistent with the sieve sizes (Table 1).

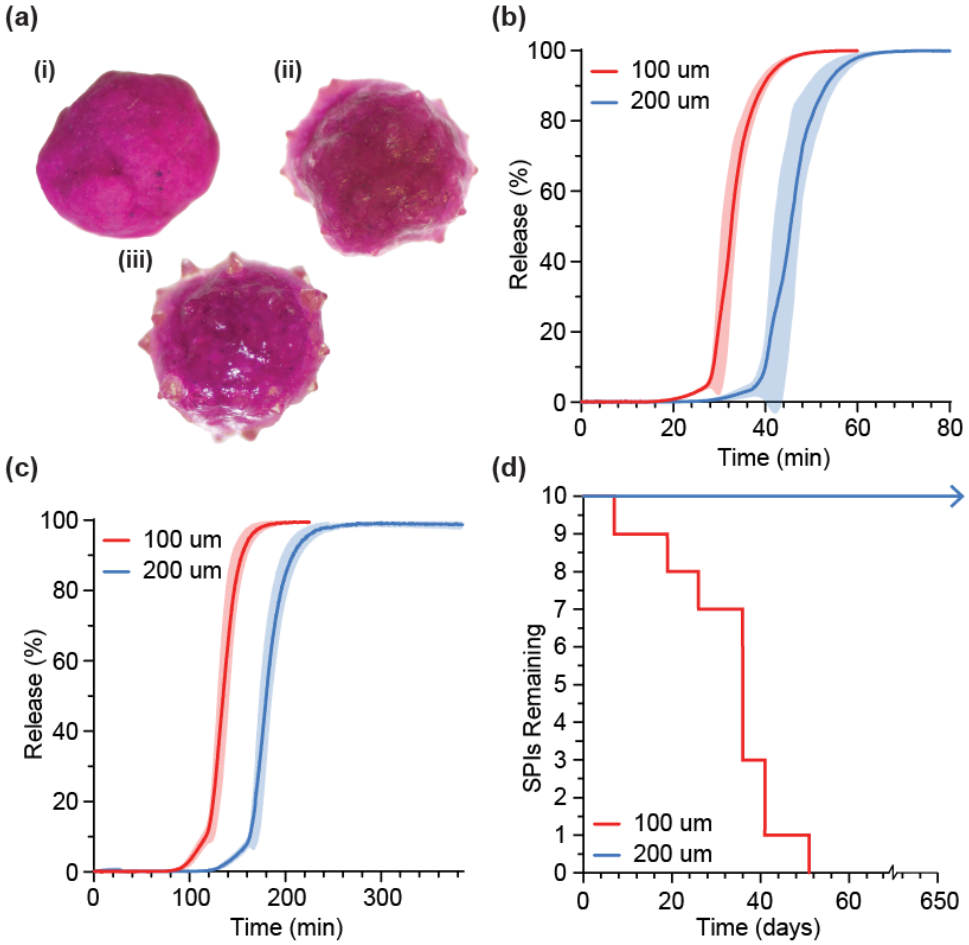

Figure 1. (a) Representative images of (i) an uncoated SPI core, and SPIs coated with (ii) 100 and (iii) 200 µm-thick polymer coatings. The images shown were brightness adjusted, and the backgrounds were removed using Adobe Photoshop (v22.4.1). In vitro release profile of rhodamine B from coated SPIs at 37 °C in pH (b) 1.5 and (c) 1.7 release media. Release is shown as the mean (solid line) ± standard deviation (shaded area) (n = 5). (d) In vitro stability results of coated SPIs over 650 days.

Table 1. Summary of SPI dimensions and in vitro release results for coated SPIs at pH 1.5 and 1.7. In vitro release results are reported as mean ± standard deviation ($n = 5$). Initial release time (t5%) is defined as the time for 5% of the core contents to release. Complete release time (t90%) is defined as the time for 90% of the core contents to release.

Coating thickness (µm)	*n*	Minimum feret diameter (mm)			Core release at pH 1.5 (min)		Core release at pH 1.7 (min)	
		Ave.	Std. Dev.	%RSD	t5%	t90%	t5%	t90%
Uncoated	30	1.24	0.05	3.9				
100	108	1.44	0.08	5.3	27.9 ± 1.2	39.9 ± 1.5	105.3 ± 5.2	153.1 ± 7.4
200	120	1.61	0.09	5.7	39.9 ± 3.6	52.4 ± 3.4	150.2 ± 5.5	205.7 ± 11.4

The SPI cores ($n > 8500$) were spray coated with the pH-responsive polymer through a series of coating and drying cycles during which the mass (Appendix S1) and dimensions of the SPIs (Table 1) were monitored. The coating mass increased linearly at a rate of 1.37 µg min^{-1} ($R^2 = 0.9995$), with c. 49% of the sprayed formulation deposited on the SPI cores (Appendix S2). The polymer coatings were generally smooth and free of defects, though small protrusions were observed (Fig. 1a). Some SPIs stuck together during coating (c. 25% regardless of the coating thickness, $n > 100$). These were removed and excluded from testing. Previous work involving the PPIs demonstrated that implant stability at subcutaneous pH increases with coating thickness (Brewer et al. 2022; Brewer et al. 2023a, b). Therefore, to measure the longevity of SPIs implanted subcutaneously in rodents, and the rate of toxin release in the stomachs of stoats, we selected SPIs with polymer coating thicknesses of 100 and 200 µm (Table 1).

In vitro release and stability

The dissolution rate of the SPIs was investigated in vitro at pH 1.5 and 1.7 by monitoring rhodamine B release from the core via UV-visible spectrophotometry (refer to Appendix S1 for a more detailed description of the release and stability studies). These pH values were selected to represent the lowest and mid-range values for the gastric pH of ferrets (*Mustela putorius furo*), another member of the mustelid family (pH 1.6–1.9; Fox et al. 1993). Release from the SPIs was strongly influenced by coating thickness and the pH of the release media. It was observed that a 2-fold increase in the coating thickness resulted in a c. 1.4-fold change in the initial release time, and a 1.3–1.8-fold change in the complete release time at both tested pH values (Table 1; Fig. 1b, c).

The stability of the SPIs was assessed in vitro using a phosphate-buffered saline solution (pH 7.4, 37 °C) (Fig. 1d). Coating failure was determined by the observed release of rhodamine B into the receiving solution. All SPIs ($n = 10$) with a 100 µm coating failed within 50 days (range = 7–50 days), while all 200 µm coated SPIs ($n = 10$) were still stable at the end of the experiment (650 days).

In vivo ingestion trials

Twelve wild-caught stoats were housed individually in pens and offered dead mice implanted with SPIs with 100 and 200 µm polymer coatings, inserted subcutaneously between the shoulder blades using a trocar (See Supplementary Material). To confirm that stoats would ingest the dead mice, a pilot group of six stoats were initially offered a dead (non-toxic) mouse. Each stoat consumed a mouse and was subsequently offered a mouse carcass implanted with a 200 µm SPI. The stoats were left undisturbed for 3 h before being checked to avoid interrupting feeding and were then monitored for symptoms of toxicosis every 2 h until 12 h after the implanted mouse was introduced. The following morning, the fate of each stoat (killed or not killed) was recorded, and the pen searched to confirm mouse and implant ingestion (Table 2).

Table 2. Outcomes of stoat ingestion trials indicating whether stoats consumed the mouse, ingested the SPI, and whether the SPI resulted in death.

Stoat ID	Sex	SPI coating thickness (µm)	Mouse eaten?	SPI ingested?	Stoat killed?	Notes
1	M	200	Y	Y	Y	
2	M	200	Y	Y	Y	
3	M	200	Y	Y	Y	
4	F	200	Y	Unknown	N	No symptoms of toxicosis
5	F	200	Y	Y	N	SPI passed undigested (found intact in faeces)
6	F	200	Y	Y	N	SPI passed undigested (found intact in faeces)
7	F	100	Y	Y	Y	
8	F	100	Y	Y	Y	
9	M	100	Y	Y	Y	
10	M	100	Y	Y	Y	
11	M	100	Y	Y	N	SPI passed undigested (found intact in faeces)
12	M	100	Y	N	N	SPI found uneaten in stoat's nest box

Results

All six stoats offered 200 µm SPIs consumed the mice, however, only 50% of the stoats were killed. Of the surviving stoats, two individuals passed the SPIs intact (Fig. S5; Supplementary Material). It could not be determined whether the third surviving stoat had ingested the SPI, as the SPI was not recovered. It was unclear whether these results were due to differences in the stoats' gastric emptying time (GET) or damage to the SPI during feeding that disrupted the SPI coating and resulted in the rapid, non-pH mediated release of 1080. The ingestion trial was repeated on a new cohort of six mice, with 100 µm SPIs and 60% of the stoats were killed following ingestion of the mice. Of the two surviving stoats, one passed the SPI intact and the other appeared not to have consumed the SPI.

In vivo gastric pH testing

Due to the stoats passing SPIs intact, the pH of the stoat ($n = 6$) gastric environment was tested post-mortem using colorimetric pH indicator strips, with measurements taken following tissue collection, c. 30 min post-mortem (see Supplementary Material). These measurements found a significantly higher pH than initially expected; fasting state: c. pH 6 and fed state: c. pH 3–4 (cf. 1.6–1.9 for ferrets; Fox et al. 1993).

Discussion

Unlike the PPIs, the spherical SPIs were found to develop a spiky appearance, which suggested that coating progressed via an island growth-type mechanism (Wang et al. 2022), likely caused by the much larger batch size ($n > 8500$ SPIs, cf., $n \leq 1000$ PPIs) resulting in insufficient drying and the polymer coating formulation having too high a viscosity. While the surface features of the SPIs indicated that the spray coating could be improved, a sub-optimal coating was not considered problematic if reproducible failure rates could be obtained for the resultant SPIs.

Consistent with previous reports (Brewer et al. 2022), we found the thickness of the polymer coating significantly altered the stability of the SPIs at pH 7.4. From our data, the 100 µm-coated SPIs appear to be the most appropriate for secondary poisoning applications, as rodents implanted with the SPIs will only persist for two months before toxin release. Accordingly, any negative environmental effects due to the activity of the rodents would be limited.

We were unable to find reported gastric pH and motility data for stoats, and so available data for ferrets—a mustelid closely related to the stoat (Schwarz et al. 2003)—were used as a reasonable approximation. We conducted testing at pH 1.5 and 1.7, as these pH values were thought to represent both low (pH 1.5) and moderate (pH 1.7) conditions (Fox et al. 1993). The release times for the SPIs at pH 1.5 were consistent with the PPIs (Brewer et al. 2022) and suggested complete dissolution would occur within the GET (1.5–2.5 h). At pH 1.7, the SPIs would not fully dissolve within the GET. However, a lethal dose (i.e. 50% release, 0.23–0.27 mg) could be achieved within the GET (c. 2.3 h) for the SPIs bearing a 100 µm coating, followed by the continued release of 1080 throughout the intestine, as the SPI core is highly water-soluble. In contrast, implants with a 200 µm coating would be unlikely to initiate release at pH 1.7.

Fox et al. (1993) tested the gastric pH of live ferrets under anaesthesia, using a paediatric pH probe; therefore, it is likely these measurements are significantly more accurate than our measurements taken post-mortem. The combination of post-mortem testing and use of an imprecise colorimetric pH test strip make it unlikely that accurate gastric pH values were obtained for the stoats. Therefore, to determine the effects of stoat gastric pH on SPI performance, further investigation is required to obtain accurate gastric pH and motility data for live stoats in both the fed and fasted states.

In addition, because the SPIs were injected into dead mice, there was no skin turgor or fibrous tissue capsule formation. As hypothesised in previous work (Brewer et al. 2022), a fibrous tissue capsule may anchor the implant at the implantation site and prevent dislodgement, thereby increasing the likelihood of consumption. The absence of fibrous tissue capsule formation in this work may have caused the SPIs to become dislodged during feeding and prevented ingestion. However, this is an unlikely failure mechanism following implantation in live rodents.

Our approach was similar to that of Nichols et al. (2022), who used carcasses of rats that had died from consuming cereal baits containing a lethal dose of 1080 to target stoats that were resistant to other control methods. The study by Nichols et al. underpins our work through the observation that toxic carcasses are consumed by stoats, with lethal effect. However, the use of SPIs has some key advantages:

(1) a targeted dose of 1080 can be contained within the SPI, as it does not require consumption by a vector animal—for which an appropriate dose of 1080 may differ significantly—before use. By minimising the dose of 1080 to that which is sufficiently lethal for stoats, potential toxicity to non-target animals could be reduced. For example, the SPIs reported herein contain 0.45–0.54 mg of 1080, approximately half the lowest amount consumed by lethally dosed rats in Nichols' work (c. 0.9 mg), and more than a tenfold reduction compared to the highest amount (c. 6.9 mg) (Nichols et al. 2022). Further testing may be required to confirm the efficacy of this dose.

(2) SPIs could be inserted into live rodents, which may be more attractive to stoats and increase the likelihood of ingestion of a lethal dose of 1080, thereby increasing the effectiveness of a secondary poisoning campaign. With failure engineered to occur after a designated period, implanted rodents that are not consumed would perish, and their disruption of the local ecosystem would be minimised.

We envisage that SPIs could be used in the final stages of stoat eradication projects, when removing the last surviving individuals can be costly in terms of time and effort, and sometimes unachievable with existing tools. Another potential application may be in response to stoat incursions into pest-free islands or sanctuaries. For example, a stoat that reached pest-free Rangitoto-Motutapu Island survived for over a year, despite a network of over 600 traps (DOC 2021).

These results provide a proof-of-concept that mice with subcutaneous toxic SPIs could be used as an eradication tool for stoats. However, further development is needed to optimise the polymer coating parameters and/or thickness to ensure failure at a designated time point and overcome difficulties with SPIs passing through stoats undigested. As the implant coating was originally optimised to release within the gastric environment of feral cats (*Felis catus*), if the gastric pH of stoats is significantly greater or the GET is faster, the coating will require re-optimisation for rapid release at a higher pH.

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Additional information and declarations

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Ethics: All procedures were conducted under approval 23/10/03 from Manaaki Whenua – Landcare Research Animal Ethics Committee.

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Supplementary material

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