NEW ZEALAND JOURNAL OF ECOLOGY

RESEARCH

Investigation of tutin, a naturally-occurring plant toxin, as a novel, culturally-acceptable rodenticide in New Zealand

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Published online: 28 November 2019

Auheke: He nui nga mātauranga a te Māori (Ngai Tūhoe) e pā ana ki nga momo hua tāokeoke (Toxins) e taea ana te whakarite hei rauemi tāwai i ngā riha kīrearea, pērā anō ki nga whiu takarangi o te tāoke 1080. I whakamātauhia e matou i nga ira tāoke o roto o te hua Tutu, ki rō taiwhanga pūtaiao. Mā te wero atu ki tētahi kiore (Norway Rat) i hua mai ngā mohiotanga o te nui me te momo o ngā tāokeoke kei roto i tēnei miro Māori, me te āhua o tēnei tāoke kia mau-rohā tonu tōna tuku whakahemo (Humaneness). Kei tua o te 55 mg kg^{-1} neke atu, te ine i tūtuki pai ai nga tāhawahawatanga o te miro Tutu, ā, e mau-roha tonu ana te kōhurutanga o te riha. Ko te whakatau kia kawea atu tēnei kaupapa ki nga ahurewa rangahau e taea ai te waihanga i tētahi mōunu tāokeoke, kia whakamātauria ki rō ngāhere. Hei tāpiritanga ki tēnei, he roa rawa te wā e pakari ai te whanaketanga mai o tētahi tākoe e rerekē ana ki te 1080, anō nei, mā ngā kawenga o te mātauranga Māori ki tēnei take e whanake tika ai te kaupapa nei.

Abstract: New Zealand has many introduced mammalian species that are managed as pests of conservation and/or economic importance, including four rodent species. Vertebrate pesticides are the most important rodent management tool, largely dominated by anticoagulants such as brodifacoum, and by the metabolic disruptor, Compound 1080. There has been considerable opposition to these pesticides, primarily based on concerns about environmental persistence and non-target effects; Maori have been particularly vocal in opposition. Maori have place-based knowledge about naturally-occurring plant toxins that could be used as culturally-acceptable alternatives to existing rodenticides. In the context of the research presented here, the term 'culturally-acceptable' refers to new pest control options that have been co-designed with Matauranga Maori experts that inherently include Maori ways of thinking, being, and acting. Tuhoe researchers in our study wanted to pursue the most promising natural toxic compound found in native plants as a suitable alternative to current vertebrate pesticides. Therefore, we undertook an oral gavage trial to assess the toxicity of tutin, the toxin active in tutu (*Coriaria arborea*), to the Norway rat, (*Rattus norvegicus*). Tutin was toxic to this species at a dose of 55 mg kg^{-1}, with a quick, humane death compared to other existing rodenticides. At a dose rate of 55 mg kg^{-1}, all animals of both sexes died within an hour, and once neurological poisoning symptoms commenced these animals were unconscious within 5–10 minutes. We conclude it is warranted to take the next logical research step, which is to prove whether this dose rate would be technically attainable in the field. Although for now New Zealand remains reliant on 1080 and anti-coagulants for mammalian pest control, efforts should continue to develop more targeted toxins and delivery systems. We recommend incorporating Matauranga Maori to identify alternative control tools that could lead to more culturally acceptable pest control.

Keywords: *Coriaria* spp., invasive species, Maori, tutu, tutin, *Rattus norvegicus*, vertebrate pest control

DOI: https://dx.doi.org/10.20417/nzjecol.43.37

Introduction

The fossil record suggests that before human settlement of New Zealand, the only terrestrial mammals were four species of bat, and a mouse-sized, ground-dwelling "Mesozoic ghost" mammal with no close affinities to any living groups (Tennyson 2010). Humans have intentionally or accidentally introduced many mammals, and 31 species are now established in New Zealand (King 2005). Many of these have significant impacts on the economy and on native biodiversity, and are managed as pests (Parkes & Murphy 2003). Four species of rodent have been introduced: the kiore (*Rattus exulans*) arrived with Māori settlers about 800 years ago (Wilmshurst et al. 2008); the ship rat (*R. rattus*), Norway rat (*R. norvegicus*) and house mouse (*Mus domesticus*), arrived with European ships in the late 18th and early 19th centuries (King 2005).

Until recently, the commonest technique for widespread control of rodents in New Zealand has been poisoning with anticoagulant toxins (Eason et al. 2010). However, in the past two decades, the use of anticoagulants has been re-evaluated, and there is currently a renewed interest in alternative toxins (Eason et al. 2017). This stems partly from an increasing incidence of pest resistance to anticoagulants (Cowan et al. 2017), and partly from concerns over their persistence in the environment (especially of second-generation compounds) and the associated risk of secondary poisoning of native animals (Young & De Lai 1997; Stone et al. 1999; Eason et al. 2002).

Compound 1080 (sodium fluoroacetate) is also widely used to control rodents and other vertebrate pests in New Zealand forests, but its use is controversial. In 2007, the New Zealand Environmental Risk Management Authority (ERMA) undertook an extensive 1080 reassessment process (ERMA 2007), that included a public submission process. A total of 1406 submissions were received from across New Zealand (a full list is available in Ogilvie & Miller 2007); twenty eight of which were from organisations and individuals that self-identified as Māori. These submissions indicated that the aerial application of 1080 poses cultural concerns (Ogilvie et al. 2010).

One approach to address Māori concerns is to use toxins that occur naturally in the forests where pests are being controlled. Using toxins from native plants could elicit stronger local Māori community support than existing options, because a toxin that the environment is adapted to, rather than a novel toxin, would be more acceptable to a holistic Māori-world view that incorporates kaitiakitanga (environmental guardianship). For Māori, place and whakapapa (genealogy) are important elements of kaitiakitanga (Walker et al. 2019). Therefore, where vertebrate pest control is deemed necessary to sustain native biodiversity, then deployment of a locally derived toxin is more likely to be culturally acceptable. Furthermore, increasing the availability of locally-sourced toxins would give new options for control, and so support putting the decision-making power back in the hands of communities, particularly for Māori communities as is the case presented here.

A number of compounds in currently available rodenticides are naturally present in plants, although none of these are derived from native New Zealand species. For example, bulb extracts and dried powders of the Mediterranean red squill (*Urginea maritima*) are highly toxic and have been used for rodent control (Verbiscar et al. 1986). Warfarin is a synthetic derivative of coumarin, a chemical found naturally in European woodruff (*Galium odoratum*), and at lower levels in liquorice (*Glycyrrhiza glabra*), lavender (*Lavandula angustifolia*), and various other plant species (Lam 1979, Wardrop & Keeling 2008). The primary natural source of strychnine is the plant *Strychnos nux vomica* found in southern Asia and Australia (Howard et al. 1968). Fluoroacetate occurs naturally in many plant species in Australia, South America, and Africa; these plants evolved the poison as a defense against browsing animals (Eason 2002, Eason et al. 2011).

In collaboration with representatives from Tūhoe, Pauling et al. (2009) identified six native plants that are recognised within Mātauranga Māori (Māori knowledge) for their biologically active properties, both as toxins and medicines. These species were ngaio (*Myoporum laetum*), karaka (*Corynocarpus laevigatus*), tutu (*Coriaria arborea*), porokaiwhiri (pigeonwood, *Hedycarya arborea*), kōwhai (*Sophora* spp.), and poroporo (bulli-bull, *Solanum aviculare* and *Solanum lacinatum*). To determine which of these species might have the most potential as a source of a vertebrate pest control agent, Pauling et al. (2009) devised a decision matrix using criteria that included the known environmental persistence and humaneness of toxins from all six plants, and key cultural and environmental values. This process highlighted tutu as having the most potential as a source of toxin for vertebrate pest control.

Historically, significant numbers of cattle and sheep have died from tutu poisoning (Parton & Bruere 2002). Many mammalian species are affected and there are reports of poisoning of cattle, sheep, pigs, elephants (Anderson 1968) and a dog (Graham & Cartridge 1961). Humans have also been poisoned by eating the attractive berries (Parton & Bruere 2002; Fields et al. 2014) and by eating toxic honey (McNaughton & Goodwin 2008), which can occur when bees collect honeydew from sap-sucking passion-vine hoppers (*Scolypopa australis*) that have fed on tutu.

The principal toxin in tutu is tutin, a crystalline glucoside ($C_{15}H_{18}O_6$, molecular weight 294.3, CAS Number 2571-22-4). Tutin has an excitatory picrotoxin-like effect that may cause bloat, and seizures. Treatment with barbiturates may be beneficial, but successful treatment is rare (Parton & Bruere 2002; Fields et al. 2014). The tutin molecule probably occurs in all parts of the tutu plant except the fleshy petals of the flowers (Poole 2009).

Our research explored the potential of tutin as a rodent toxin that is both effective in field operations and more likely to be culturally-acceptable to Maori. The objectives of the study were to estimate the lowest dose of tutin that would kill all animals in a sample of laboratory rats (*R. norvegicus*), and to provide information on the welfare aspects of tutin, associated with its potential use as a rodenticide.

Methods

Animal husbandry

Procedures involving the use of animals were carried out under Lincoln University Animal Ethics Committee approval (AEC# 426) and an existing protocol for the oral gavage of rats (SOP# 30-14). Sexually-mature adult laboratory rats were purchased from Otago School of Medicine, Christchurch, and housed in individual plastic rodent cages (200 mm wide × 400 mm long × 200 mm high) with a wire grate lid. They were kept in a controlled-temperature environment (20–22°C) on a 12-hour night/day cycle at the Lincoln University animal facility. All rats were acclimatised for 21 days prior to starting the trial,

weighed weekly, and had free access to water and cereal feed pellets (Weston Animal Nutrition, Rangiora, New Zealand).

Tutin sourcing

Pure tutin was extracted from wild-sourced plant material. A total of 5.1 kg of tutu berries collected from plants in the Maitai Valley, Nelson, New Zealand (41°16′33.24″S, 173°19′40.41″E), were lyophilised to give 1.4 kg of dry material. The dried material was powdered using a Waring® blender. Each 300 g portion of powder was extracted with 2.4 L of 1.25% $NaHCO_3$ by heating at 76°C for 30 min and leaving to soak overnight at 21°C. The mixture was then centrifuged at 3200 g for 10 minutes, the supernatant was collected and the pellet was re-extracted two more times with water, by shaking followed by centrifugation. The supernatants were combined and filtered through Whatman® 542 filter paper, total volume 3.3 L. The extract was concentrated to 1.3 L by rotary evaporation and then filtered through Whatman® 541 filter paper. Following this, 200 mL of isopropanol was added to the filtered extract and partitioned against 1.5 L ethyl acetate three times. The ethyl acetate extracts were combined and 200 g of Na_2SO_4 was added to remove water from the extract. The ethyl acetate extract was then chromatographed on a silica column eluted with hexane/acetone. The fractions containing tutin were combined and dried down (1.49 g). The tutin was chromatographed again on a silica column eluted with hexane/ethyl acetate. The fractions containing tutin were combined and dried down (1.3 g). The purified tutin was recrystallised from ethanol to give 1.2 g of tutin with a melting point of 213–214 °C. The purity of the tutin was found to be > 99% by LCMS, with the only observable impurity being < 1% dihydrotutin.

Rat dosing

The methods for this trial were based on the current guidelines released from the Organization for Economic Cooperation and Development (OECD 2001) which recommends the use of the 'up-down' procedure (UDP) as the most appropriate for assessing lethal dose ranges. This test procedure minimises the number of animals required to estimate the acute oral toxicity of a chemical as the stopping rules limit the number of animals in a test and sequential dosing introduces further efficiencies in animal use. Importantly, the guideline contains a requirement to follow the OECD guidance document on humane endpoints that reduces the overall suffering of captive animals used in toxicity tests.

As per recommendation under the OECD guidelines when there is no information available about the estimated slope of the dose-response curve, a dose progression factor of 3.2 was used for tutin in rats. Although 175 mg kg^{-1} is the recommended starting dose for a toxin with no previous toxicity history, previous trials with tutin involving mice estimated an LD_{50} of c. 5 mg kg^{-1} (McNaughton & Goodwin 2008) and very limited trials with rats estimated an LD_{50} of c. 20 mg kg^{-1} (Palmer-Jones 1947). Based on this, 55 mg kg^{-1} was considered as an upper limit for the current rat tutin trial with progressive doses of 18 mg kg^{-1}, 6 mg kg^{-1} and 1.8 mg kg^{-1}.

For each dose level, an appropriate quantity of tutin crystals was weighed and mixed into solution with the appropriate volume of deionized water. Actual tutin concentration for each dose solution was confirmed at Cawthron Institute (Nelson, New Zealand) using a validated LC-MS/MS method as described by Fields et al. (2014).

Rats were randomly selected and fasted for 12 hours before the trial commenced. In accordance with the UDP, five females were orally gavaged (dosing solution introduced directly into the stomach using an oral cannula, OECD 2001) with the highest dose (55 mg kg^{-1}) and one female was orally gavaged with the same volume of plain water (control). If all five rats died at the administered dose, then the next lower dose was administered to an additional five rats until we reached the dose where one or more rats survived, at which point the trial was discontinued. The female rat trial was conducted over 4 days. To minimise the number of animals used in the trial, the female rat trial would dictate the starting dose for the male rats, i.e. the male dose would commence at the lowest dose at which all female rats died and the dose levels would go up or down according to the results observed in the males. At each dose rate for each sex, one rat was given a dose of plain water (control rat).

Monitoring rat condition

Beginning at the time of dosing, each rat was monitored every 5 minutes using continuous focal sampling for the first 2 hours, followed by one observation each hour, for 8 hours. Thereafter, rats were monitored once per day, during daylight hours, for 4 days. During observations all behaviours and other obvious signs of poisoning were recorded. Where seizures occurred, start time, and length of seizure was noted. Between seizures, the level of responsiveness was tested. The response tests were: a threatening gesture (hand moving quickly towards the animal), click of fingers, clapping, or high-pitched squeak noise, palpation (using fingers) on the ear or tail, placement of rat on its back to observe righting response. A positive response to righting was recorded if the rat rolled back on to its feet/belly. If the animal remained on its back, cardiac rhythms were felt for, and respirations were observed. An animal with a lack of respirations for more than 1 minute with no cardiac rhythm was recorded as dead.

Statistical analysis

To address whether time to death was influenced by sex of the rat we ran a two sample *t*-test at the 55 mg kg^{-1} dose as in this test all males and females died. To compare between the 55 and 18 mg kg^{-1} doses we used a GLM with a gaussian link function to test for differences between dose and gender (we were unable to run this test at the 6 mg kg^{-1} dose because all the males survived). All analyses were done in GenStat, version 16.1 (VSN International, Hemel Hempstead, UK).

Results

Analysis of the tutin doses used in the trial and sent for concentration analysis shows that the actual concentrations given to the rats were within acceptable range compared to that of the nominal (calculated based on known tutin crystal concentration; Table 1).

Sex difference in tutin mortality

All rats died at the highest nominal dose of 55 mg kg^{-1} (Table 1); however, for female rats the average time to death was shorter ($\bar{x}$ ± SE; 24.2 ± 1.6 min) compared to that of males (31.6 ± 11.5 min), though not significantly different ($t(4) = -0.66$, $P = 0.54$). At the lower dose of 18 mg kg^{-1} all female rats died; however, the time to death almost doubled (41.2 ± 4.6 min) and four out of five male rats died with more than double the time to death (76.5 ± 28.4 min), though this average

Table 1. Experimental assessment of the toxicity of the neurotoxin tutin in *Rattus norvegicus*: nominal dose concentration, actual dose concentration (calculated from quantitative analysis of each dosing solution), sex, average time to death, number of rats used, and number of rats that died. At each dose rate for each sex, one rat was given a dose of plain water (experimental controls).

Nominal Tutin dose (mg/kg)	Actual tutin dose (mg/kg)	Sex	Average time until death ± SE mean (min)	Number of rats used	Number of rats died
55	56.1	female	24.2 ± 1.6	5	5
55	57.2	male	31.6 ± 11	5	5
18	19.4	female	41.2 ± 4	5	5
18	17.7	male	76.5 ± 28.4	5	4
6	7.3	female	67.4 ± 8	5	5
6	7.2	male	-	5	0
1.8	1.7	female	89	3	1
0	0	female	-	3	0
0	0	male	-	3	0

was strongly influenced by one male taking 161 minutes to die. For the 6 mg kg^{-1} dose group, all the females died (67.4 ± 8.1 min), while all male rats survived. For the female rats dosed at 1.8 mg kg^{-1}, one animal of three died (89 min). Male rats were not dosed at this level because they all survived 6 mg kg^{-1}. For those animals that died, there was a statistically significant difference in time to death between the 55 and 18 mg kg^{-1} dose levels ($F_{1,17}$ = 10.70, P = 0.004). We did not detect a significant difference between males and females ($F_{1,17}$ = 2.05, P = 0.171), although the relatively small sample sizes meant we lacked statistical power to detect anything other than large differences.

Physical symptoms

The onset of toxicosis was not immediate. Rats dosed with lethal amounts of tutin showed no symptoms until at least 5 minutes after dosing. In all cases, seizures were the first visible onset of symptoms.

At the 55 mg kg^{-1} dose, all animals, of both sexes, died within an hour and once neurological poisoning symptoms (Table 2) commenced these animals were quickly unconscious (5–10 minutes) with no response to threatening gesture, noise, palpations or righting response. At progressively lower doses, the time to displaying visible symptoms was shortened, time periods between these symptoms were lengthened and during these periods animals were sometimes conscious. All animals that survived had seizures similar to the other rats in their dose group that died, however, for the surviving animals, the seizures were shorter in length, with longer periods of recovery in between. The female in the 1.8 mg kg^{-1} dose group that died showed a similar pattern of seizures as the two females that survived. These two surviving females were inactive but fully responsive after five hours. To be consistent with the UDP we gavaged only three females for this dose group. They remained inactive although responsive, and did not eat or drink for 24 hours, but then fully recovered with no visible lasting effects.

Discussion

We conclude that tutin could be an effective rodenticide for *R. norvegicus* if a consistent dose of 55 mg kg^{-1} is achievable. However, the question remains over whether this is technically feasible in the wild. There are two key areas that need to be considered to assess the likely technical success of a new rodenticide; efficacy, and safety (Buckle & Eason, 2015). For efficacy, the primary test is that the rodenticide is toxic to target rodents. It must also be of a potency that allows sufficient active ingredient to be included within a bait, without making the bait unpalatable. The rodenticide should be equally effective against all individuals, independent of sex, age, and strain. Speed of action should be such that the onset of toxicosis does not occur before a lethal dose of poison has been consumed. Related to this, there should be minimal likelihood of a compound eliciting bait shyness.

For safety, there should be minimal chance of secondary poisoning. Ideal compounds would be rapidly broken down in the bodies of rodents, so that they are not secondarily toxic, and would have an available antidote. For non-target species, toxicosis would need to act at a speed that would allow time for observation of symptoms, diagnosis of the causative agent, and subsequent antidote administration. Desirable compounds would also have no teratogenic, oncogenic and carcinogenic properties. These compounds would be non-persistent in terrestrial or aquatic systems, have no likelihood of other unacceptable effects on the environment, and would have a humane mode of action in the target species.

In the present study, we were able to elicit new information on the toxicity of tutin to *R. norvegicus*, on the toxic effectiveness against different individuals within the species, on the speed of onset of toxicosis, on the humaneness of lethal toxicosis, and on the likely potency of raw tutu plant material as a toxin source.

Tutin toxicity, intra-species effectiveness, and speed of onset of toxicosis

Tutin was toxic to both male and female *R. norvegicus*, however there was an obvious difference in toxicity between the sexes, with female rats being more sensitive than males. There have been other documented differences between the sexes in their sensitivity to toxic compounds. For example, Kaur (2008) reports male ship rats (*Rattus rattus*) are more susceptible to cholecalciferol than females. Gaines and Linder (1986) also found many compounds where female rats were

Table 2. Symptoms of *R. norvegicus* after experimental dosing with the neurotoxin, tutin.

Dose (mg/kg)	Sex	Qualitative Summary of Symptoms	Min-max time to onset of symptoms (min)	Min-max time to onset of seizure and unconsciousness (min)	Min-max time from dosing to death (min)
55	Female (n = 5, survived; n = 0)	Seizures lasting up to one minute with intermittent 2-5 minute rest periods. No response to voice, touch or pain during seizures, otherwise responsive.	5–10	5–10	20–28
	Male (n = 5, survived; n = 0)	Symptoms started with belly press and inactivity, followed by pattern of seizures similar to female rats. Two rats had frothing from nose prior to death.	5–10	6–20	13–75
18	Female (n = 5, survived; n = 0)	Symptoms started with belly press and inactivity. Seizures lasting up to one minute with intermittent 2-5 minute rest periods. No response to voice, touch or pain during seizures, otherwise responsive. Three rats had vocalisations. One rat had frothing from mouth prior to death.	10–15	10–15	33–57
	Male (n = 5, survived; n = 1)	Symptoms started with belly press and inactivity. Less severe intensity seizures than at higher dose for up to 80 sec with rest periods. Lasted 12 min before death was accompanied by muscle spasms and strong twitching movements.	5–16	5–48	41–161
	Male (survived)	Symptoms started with hunched posture, had a single seizure lasting 30 seconds. Hunched position for 4 hours but responsive and alert.	5	48	Recovered after 18 hours.
6	Female (n = 5, survived; n = 0)	Symptoms started with belly press and inactivity. Less severe seizures lasting 2-3 sec with up to 8 min rest periods. One rat had three two-min periods of asphyxia. Vocalisation common.	10	8–24	49–95
	Male (survived) (n = 5, survived; n = 5)	Symptoms started with belly press and inactivity, one to three seizures lasting up to 45 sec followed by rest periods.	6–15	35–57	Recovered after 2–5 hours.
1.8	Female (n = 3, survived; n = 2)	Symptoms started with belly press and slow respiration rate. Minor twitching episodes followed by two seizures.	15	45	89
	Female (survived)	Symptoms started with belly press and slow respiration rate. Seizures lasting 2-3 sec. Over 5 hours had body rigidity and twitching and remained in lowered response state. At 5 hours was alert and responsive but inactive. 24 hours later was still alert but inactive.	10–15	37–43	Recovered after 48 hours.

more susceptible than males. Evidence suggests that some species have greater ability to metabolise toxins within liver microsomes (Lin & Lu 1997) and this can vary between the sexes. Rats have been presented as an example of gender difference in xenobiotic metabolism (Buckle & Eason 2015); generally, male rats have a higher capacity to metabolise xenobiotics than females. The difference between the sexes is due to females having fewer of the specific isoenzymes needed to metabolise these compounds (Hayes & Kruger 2014); this may well be the case for tutin in rats.

As reported, rats dosed with lethal amounts of tutin showed no symptoms of toxicosis until at least 5 minutes after dosing. This, at least theoretically, would mean that there is a 5-minute period of time in which individual rats could eat tutin bait before any stop-feed effect might commence. We have no evidence on whether or not tutin would be similarly effective against other species of rat.

Humaneness of toxicosis

Tutin was shown to be an acute-acting toxin with average time to death, at the 55 mg kg^{-1} dose, of 26 minutes for females and 31 minutes for males. This is shorter than any currently available rodent toxins including Compound 1080, brodifacoum and cholecalciferol that are currently used in New Zealand (Table 3). Due to the length of time animals remain conscious before death, and in that a period of potential suffering, these toxicants are considered to have lower animal welfare performance than faster-acting compounds like cyanide (Mason & Littin 2003, Eason et al. 2011). As tutin also has a shorter period of consciousness before death, it should be considered more humane than slower acting toxicants.

It is generally accepted that animal welfare is not compromised if the animal remains unconscious from the onset of symptoms until death (Mason & Littin 2003). For our tutin trials, the animals remained unconscious in the 55 mg kg^{-1} dose group, but at lower doses we observed longer time periods between seizures, and the animals regained consciousness during this period. Therefore, tutin could be considered more humane than all other presently available rodenticides in New Zealand, but only if administered at a dose of at least 55 mg kg^{-1}.

Potency of tutu plant material

Whole tutu plant material is a good natural source of tutin

Table 3. Symptoms and average time to death of current toxins used in bait for *R. norvegicus* control. Note that cyanide, a very fast-acting toxin, is not included in this table as it is not used for rodent control in New Zealand.

Toxin	Reference	Symptoms	Average Time to death (after oral gavage of lethal dose)
Brodifacoum	Littin et al. 2000 Eason & Ogilvie 2009	Lethargy, excessive external bleeding, internal bleeding, death from heart attack/bleeding out	7 days
Cholecalciferol (vitamin D_3)	Brown & Marshall 1988 Eason et al. 1994 Eason & Ogilvie 2009 Marshall 1984	Loss of appetite, lethargy, rapid breathing, death from renal or heart failure	3–7 days
Bromethalin (*used in USA only*)	Dreikorn 1978 (U.S. Patent 4,084,004) Eason & Ogilvie 2009	Loss of appetite, tremors, convulsions and prostration	2 days
Diphacinone	Bentley & Larthe, 1959 Eason & Ogilvie 2009	Labored breathing, muscular weakness, excitability, fluid in the lungs, and irregular heartbeats.	4–11 days
Warfarin	Bentley & Larthe, 1959 Eason & Ogilvie 2009	Extreme pallor of the skin, muscles, and all the viscera	5–10 days
1080	Eason et al. 2010	Labored breathing, seizures	5–10 hours
Strychnine	Eason & Ogilvie 2009 Osweiler et al. 1985	Restlessness and muscular twitching, convulsive seizures and violent muscular spasms	1–24 hours
Norbormide	Bova et al. 1996 Eason & Ogilvie 2009	Rapid fall in blood pressure, heart failure	8–24 hours
Zinc Phosphide	Marsh 1987 Eason & Ogilvie 2009	Lethargy, tachycardia, hunched posture	3–12 hours
Tutin	Current study	Severe seizures	24–31 minutes

(Connor 1977), with a highest measured tutin concentration of 3.7 mg g^{-1}, in new-growth parts of wild plants (Ogilvie et al. unpubl. data). Wild *R. norvegicus* in New Zealand typically have an average adult bodyweight of 300 g (King 2005). Therefore, to receive a 55 mg kg^{-1} dose, an average-sized individual would need to ingest 16.5 mg of tutin, or 4.45 g of new-growth plant material.

For acute-acting toxins, such as tutin, a bait is only likely to be effective if a lethal amount of toxin is ingested during an individual's first consumption of the bait (Buckle & Eason, 2015). There is a large amount of variation in the amount of bait that a rat will consume in one meal, but Le Magnen and Tallon (1967) in their study with 1500 observations of *R. norvegicus*, showed that 2.5 g was an average meal size. Clearly, it would not be possible to incorporate 4.45 g of tutu plant material into 2.5 g baits. On the assumption that tutin is the only toxic component in the naturally occurring plant material, naturally-sourced tutu plant material is of inadequate potency to be considered the toxic ingredient for a new rat bait.

However, further investigation is warranted because tutin may be more potent if the animal consumes the tutin in its natural form. A pharmacokinetic study of tutin in honey revealed that other toxic compounds, such as hyenanchin, may also be naturally present (Fields et al. 2014). However, it is important to note that to be present in honey, the toxins have been through the gut of plant hoppers and then honeybees, so there is uncertainty over whether they were originally present in the plant, or are metabolic products of plant hopper and honeybee digestion. Multiple toxic compounds can have a synergistic effect, resulting in higher toxicity (Hayes & Kruger 2014). It is also possible that concentrated tutin, extracted from raw tutu plant material, could form the basis of the toxic bait. Since the process used to concentrate tutin in this study was extremely expensive, further investigation is needed to develop economically viable means of tutin extraction.

Further considerations

Bait shyness

Tutin bait could cause bait-shyness. While we were not able to generate any new data in the present study, given the fast-acting nature of tutin toxicosis, it is likely that rats that consumed a sub-lethal dose of tutin bait, and subsequently became ill, would relate the illness to the tutin bait, and would therefore be shy of consuming it again. The obvious way to solve this problem would be to ensure that a lethal dose, of 55 mg kg^{-1}, was consumed in one sitting.

Secondary poisoning

There is currently no available information on secondary poisoning. Such poisoning is dependent on the stability of a compound within the digestive system of a target species. While this is unknown for rodents, there is documented evidence that tutin can remain stable having passed through the digestive system of invertebrates (Fields et al., 2014). This suggests that tutin ingested by a vertebrate may cause secondary poisoning, but further investigation is needed.

Potential antidotes

Ideal candidate toxicants will have an antidote, and for tutin, there are potential antidotes that come from Mātauranga

Māori. Māori hold knowledge that is specific to the tutu plant, including an antidote for tutu poisoning that is sourced from heart rot fungus (*Agrocybe parasitica*). The availability of an antidote would be a significant advantage in the development of tutin as a bait, although this would require considerable additional research.

Conclusions

It is clear there is more work required to develop an effective control tool. The next steps would be to incorporate a lethal quantity of tutin in a bait to (1) determine palatability of the baits to rats, (2) understand whether rats can smell or taste tutin in the bait, and (3) test the stability of tutin in potential bait formulations. Toxic baits could then be trialled in the field. It would also be good to test efficacy against other rat species (particularly ship rats *Rattus rattus*), as well as against brush-tailed possum (*Trichosurus vulpecula*). We contend that the findings of the present study give sufficient promise to warrant taking these next research and development steps. Furthermore, we feel that such steps are important as, if proven successful, tutin would provide a vertebrate pest control option that originated from Mātauranga Māori. Local New Zealand options for pest management in an otherwise controversial field, will have a higher likelihood of acceptance by local communities.

This research is part of a New Zealand effort to find alternatives to 1080 which is challenging as mammal responses to neurological toxins are complex, and suitable solutions may take considerable time to get right. New Zealand is therefore likely to remain reliant on 1080 as a pest control tool for the foreseeable future. Nevertheless, efforts should continue to develop more targeted toxins and delivery systems, including incorporating Mātauranga Māori, to identify and develop culturally acceptable pest control.

Acknowledgments

We thank Jan Gill for her assistance with animal husbandry and handling. Andrea Byrom, Tame Malcolm, Jacqueline Beggs, Deb Ogilvie, and Te Rerekohu Tuterangiwhiu are thanked for reviewing earlier versions of this manuscript. This work was funded by the New Zealand Ministry of Business, Innovation and Employment (MBIE) under Contract (LINX0902).

References

Anderson IL 1968. Tutu poisoning in two circus elephants. New Zealand Veterinary Journal 16: 146–147.

Bentley EW, Larthe Y 1959. The comparative rodenticidal efficiency of five anti-coagulants. The Journal of Hygiene 57: 135–149.

Bova S, Trevis L, Debetto P, Cima, L, Furnari M, Luciani S, Padrini R, Cargnelli, G 1996. Vasorelaxant properties of norbormide, a selective vasoconstrictor agent for the rat microvasculature. British Journal of Pharmacology 117: 1041–1046.

Brown LD, Marshall EF 1988. Field evaluation of Quintox (cholecalciferol) for controlling commensal rodents. Proceedings of the Vertebrate Pest Conference 13: 70–74.

Buckle AP, Eason CT 2015. Control methods: chemical. In A.P. Buckle, R.H. Smith eds. Rodent Pests and their Control. 2nd edn. Wallingford, CAB International. Pp. 123–154.

Connor HE 1977. The poisonous plants in New Zealand. New Zealand Department of Scientific and Industrial Research Bulletin No. 99. Wellington, G P Publications. 247 p.

Cowan PE, Gleeson DM, Howitt R., Ramón-Laca A, Esther A, Pelz HJ 2017. Vkorc1 sequencing suggests anticoagulant resistance in rats in New Zealand. Pest Management Science, 73(1): 262–266.

Eason C 2002. Sodium monofluoroacetate (1080) risk assessment and risk communication. Toxicology 181: 523–530.

Eason CT, Ogilvie SC 2009. A re-evaluation of potential rodenticides for aerial control of rodents. Wellington, Department of Conservation. 33 p.

Eason CT, Murphy EC, Hix S, MacMorran DB 2010. Development of a new humane toxin for predator control in New Zealand. Integrative Zoology 5(1): 31–36.

Eason C, 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, Shapiro L, Ogilvie S, King C, Clout M 2017. Trends in the development of mammalian pest control technology in New Zealand. New Zealand Journal of Zoology 44(4): 267–304.

ERMA 2007. The Reassessment of 1080: an Informal Guide to the August 2007 Decision of the Environmental Risk Management Authority. Environmental Risk Management Authority 28 p.

Fields BA, Reeve J, Bartholomaeus A, Mueller U 2014. Human pharmacokinetic study of tutin in honey; a plant-derived neurotoxin. Food and Chemical Toxicology 72: 234–241.

Gaines TB, Linder RE 1986. Acute toxicity of pesticides in adult and weanling rats. Fundamental and Applied Toxicology 7: 299–308.

Graham JM, Cartridge MEA 1961. Tutu poisoning in dogs. New Zealand Veterinary Journal 9 (2): 45.

Hayes AW, Kruger CL 2014. Hayes' principles and methods of toxicology. 6th edn. Boca Raton, CRC Press. 2111 p.

Howard WE, Palmateer SD, Nachman M 1968. Aversion to strychnine sulfate by Norway rats, roof rats, and pocket gophers. Toxicology and Applied Pharmacology 12: 229–241.

Lin JH, Lu AY 1997. Role of pharmacokinetics and metabolism in drug discovery and development. Pharmacological Reviews 49: 403–449.

Kaur G, Kocher DK, Sandhu HS, Brar RS 2008. Sex specific value of oral LD50 of cholecalciferol (vitamin D3) against house rat (*Rattus rattus*). Toxicology International 15: 143–144.

King CM 2005. Order Rodentia. In: King CM ed. The handbook of New Zealand mammals. 2nd ed. Oxford, Oxford University Press. Pp. 159–221.

Lam YM 1979. The toxicity of warfarin to the rice field rat, *Rattus argentiventer* (Robinson and Kloss). Malaysian Agricultural Journal 52: 177–181.

Le Magnen J, Tallon S 1966. The spontaneous periodicity of ad libitum food intake in white rats. Journal of Physiology, Paris 58: 323–349.

Littin KE, O'Connor CE, Eason CT 2000. Comparative effects of brodifacoum on rats and possums. New Zealand Plant Protection 53: 310–315.

Marsh RE 1987. Relevant characteristics of zinc phosphide as a rodenticide. In: Uresk DW, Schenbeck GL, Cefkin R eds, Great Plains Wildlife Damage Control Workshop Proceedings, University of Nebraska, Lincoln. Pp. 70–74.
Marshall EF 1984. Cholecalciferol: a unique toxicant for rodent control. Proceedings of the Eleventh Vertebrate Pest Conference: 95–98.
Mason G, Littin KE 2003. The humaneness of rodent pest control. Animal Welfare 12: 1–37.
McNaughton DE, Goodwin RM 2008. Reducing the threat Tutu toxic tutu honey poses to the New Zealand beekeeping industry and consumers. HortResearch Client Report No. 24884, Report to the Ministry of Agriculture and Forestry Sustainable Farming Fund.
OECD 2001. ICCVAM-Recommended test method protocol: The up-and-down procedure for acute oral systemic toxicity. Paris, OECD. 32 p.
Ogilvie SC, Miller A 2007. Māori perspectives on aerial application of vertebrate pesticides. Unpublished client report prepared for Pest Control Research Limited. 17 p.
Ogilvie, S, Miller, A, Ataria, J 2010. There's a rumble in the jungle: 1080–poisoning our forests or a necessary tool. Māori and the Environment: Kaitiaki, 251–261.
Osweiler GD, Carson TL, Buck WB, van Gelder GA 1985. Chemical and diagnostic veterinary toxicology. Dubuque, Kendall Hunt. 494 p.
Palmer-Jones T 1947. A recent outbreak of honey poisoning. Part III. The toxicology of the poisonous honey and the antagonism of tutin, mellitoxin, and picrotoxin by barbiturates. New Zealand Journal of Science and Technology 29: 121–129.
Parton K, Bruere AN 2002. Plant poisoning of livestock in New Zealand. New Zealand Veterinary Journal 50: 22-27.
Pauling C, Ogilvie SC, Miller A, Ataria JM, Waiwai J, Doherty J, Eason CT 2009. Mātauranga rakau paitini –naturally occurring toxins in New Zealand plants with potential for vertebrate pest control. Report prepared for Ngā Pae o te Māramatanga, University of Auckland. Lincoln University Wildlife Management Report No. 50. 30 p.
Parkes J, Murphy E 2003. Management of introduced mammals in New Zealand. New Zealand Journal of Zoology 30: 335–359.
Poole AL 2009. Tutu. In: McLintock AH ed. An Encyclopaedia of New Zealand. Originally published in 1966, www.TeAra.govt.nz/en/1966/tutu (accessed 21 January 2019).
Stone WB, Okoniewski JC, Stedlin JR 1999. Poisoning of wildlife with anticoagulant rodenticides in New York. Journal of Wildlife Diseases 35:187–193.
Tennyson AJD 2010. The origin and history of New Zealand's terrestrial vertebrates. New Zealand Journal of Ecology 34: 6–27.
Verbiscar AJ, Banigan TF, Gentry HS 1986. Recent research on red squill as a rodenticide. Proceedings of the Vertebrate Pest Conference 12: 51–56.
VSN International 2011. GenStat for Windows 14th Edition. VSN International, Hemel Hempstead, UK. www.GenStat.co.uk.
Walker E, Wehi P, Nelson N, Whaanga H 2019. Kaitiakitanga, place, and the urban restoration agenda. New Zealand Journal of Ecology. New Zealand Journal of Ecology 43(3): 3381.
Wardrop D, Keeling D 2008. The story of the discovery of heparin and warfarin. British Journal of Haematology 141: 757–763.
Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH 2008. Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. Proceedings of the National Academy of Science of the United States of America 105: 7676–7680.
Young J, De Lai L 1997. Population declines of predatory birds coincident with the introduction of Klerat Rodenticide in North Queensland. Australian Bird Watcher 17:160–167.

Received 17 March 2019; accepted 4 November 2019
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