Redevelopment of a Rat Specific Rodenticide Norbormide

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ABSTRACT: Norbormide is a rat specific toxicant. It causes vasoconstriction of small arteries and vasodilation of large arteries in rats, which results in a rapid fall in blood pressure and death from heart failure. It is an extraordinary compound in that it is only toxic to rats. The lack of toxicity of this compound to birds and other mammals is unique. It was originally researched in the 1960s and initially marketed in the USA. Problems with taste aversion slowed its continued use and sales it and was largely forgotten when anticoagulant rodenticides became more effective and popular. Following the emergence of anticoagulant resistance in some populations of rodents, residues of the second-generation anticoagulants in wildlife and concerns regarding humaneness, interest in non-anticoagulants, such as norbormide, has revived. Research has been conducted to help identify and understand a formulation of norbormide which is palatable, effective, and fast acting in rats. Further research is underway to determine methods for large scale synthesis of an improved form of norbormide. Field trials are planned in 2018/19. The ability to target rats with no risk to non-target species presents considerable advantages in many settings and warrants further investment and completion of the current scale-up phase of research and development.

KEY WORDS: norbormide, Norway rat, rat specific, *Rattus norvegicus,* rodenticide, rodent control

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INTRODUCTION

Norbormide is an acute acting selective rat toxicant. It was discovered during routine screening of a variety of agents for pharmacological activity at McNeil Laboratories, Fort Washington, Pennsylvania, USA. Norbormide was synthesized in the search for a novel anti-rheumatic drug. It was considered of doubtful value for that purpose and was further researched as an appetite suppressant initially in house mice (*Mus musculus*) and cats (*Felis catus*), with no toxic effects, and then in rats (*Rattus norvegicus* and *R. rattus*). The differences in susceptibly were so striking it was at first thought that an error had been made. However, the extreme susceptibility of rats was confirmed when the experiments were repeated. (Russell 1965, Roszkowski 1965). It was further researched and introduced to delegates attending the 149th National Meeting of the Chemistry Society in Detroit, Michigan in 1965 as a rare species-specific rodenticide (Poos et al. 1966) and marketed as Raticate and Shoxin. Its use declined in the 1970s as anticoagulant toxins became more popular. Taste aversion had limited its effectiveness, and field efficacy results were generally poor (Prakash 1988). This paper specifically focuses on research aimed at advancing the development of an effective form of norbormide.

Prior to the introduction of anticoagulants all rodenticides were acute-acting toxicants. Rodenticide research advanced significantly between the 1940s and 1990. Sodium fluoroacetate (1080) was developed in the 1940s, first generation anticoagulant rodenticides in the 1950s and 1960s, and cholecalciferol and second generation anticoagulant rodenticides in the 1970s and 1980s, partly to overcome resistance to first generation anticoagulants that occurred where there had been prolonged use of first generation compounds.

Anticoagulant rodenticides have been a major advance for the control of rodents globally for both crop protection and conservation (Russell and Broome 2015). The slow onset of action following ingestion of anticoagulant baits helps ensure that even wary rodents will ingest sufficient toxic bait to cause death. Hence, it is not surprising that after the introduction of warfarin and the other anticoagulants, the importance of the non-anticoagulants was reduced, at least for commensal rodent control (Eason et al. 2017). This applied to norbormide as well as the older poisons such as strychnine and arsenic, despite the unique species specificity of norbormide. Following the emergence of physiological resistance in some populations of rodents, even to second generation rodenticides, the discovery of residues of the second-generation anticoagulants in wildlife (Crowell et al. 2013, Young and De Lai 1997, Stone et al. 1999, US EPA 2004 and 2008), and questions about the humaneness of second-generation anticoagulants (Littin et al. 2002, Mason & Littin 2003), interest in non-anticoagulants, or at least less-persistent 'low residue' pesticides, has revived and research on a range of acute acting toxicants including norbormide has advanced (Eason et al. 2017).

Norbormide causes vasoconstriction (narrowing) of small arteries and vasodilation (widening) of large arteries in rats (Roszkowski 1965), which causes a rapid fall in blood pressure. Death probably results from circulatory

disorders and heart failure (Cavalli et al. 2004, Ricchelli et al. 2005). As it is acute-acting it is likely to be more humane than most other rodenticides, because of the relatively short time to death and duration of symptoms of poisoning when compared with anticoagulant rodenticides and cholecalciferol. As noted above, norbormide is highly toxic to members of the genus *Rattus* compared with other mammals or birds (Roszkowski et al. 1964). Rats are 150-fold and 40-fold more sensitive to norbormide than house mice and guinea pigs (*Cavia porcellus*) respectively, while most other mammals and birds tested are >200-fold less sensitive (Roszkowski et al. 1964).

Two New Zealand research teams, one currently funded by the Department of Conservation Predator Free 2050 fund supporting Landcare Research with Orillion (formerly ACP Ltd), and the second through investment by Invasive Pest Control Ltd., are looking at different ways of improving the effectiveness of norbormide and producing it in forms which are more palatable (pers comm., Duncan MacMorran, Invasive Pest Control Ltd; Jay-Smith et al. 2016). If either or both groups are successful, it will be a huge advance for targeted pest control with no non-target impact.

In this paper, we describe the progress of the Invasive Pest Control Ltd. initiative. Challenges recognised before embarking on research on the redevelopment of norbormide included maintaining high toxicity, understanding chemical characteristics of norbormide and norbormide synthesis that might influence effectiveness and incorporating the norbormide formulation into a bait matrix to produce a product that would be sufficiently attractive to all rats to deliver a lethal dose. Our approach to advance norbormide in its current form versus producing a new chemical entity which could release norbormide *in-vivo* was driven in part by our wanting to use the pre-existing preclinical toxicology, metabolism and safety database that already exists in peer reviewed journals in future registration dossiers.

METHODS

The efficacy of norbormide as a rodenticide has been re-assessed in Sprague Dawley laboratory strain Norway rats and wild captured ship rats. This research and development programme has several steps:

1. Invasive Pest Control Ltd acquired norbormide from several different suppliers globally.
2. The palatability and effectiveness of norbormide from different sources was compared.
3. The characteristics of different batches of norbormide produced by different manufacturers was researched by chemical analyses (Jay-Smit 2016).
4. Further research scale batches were produced and tested to generate a better understanding of the optimal characteristics of norbormide in terms of synthetic steps, isomer profile and particle size.

The efficacy of different batches of norbormide have been screened. Rats have been dosed orally with norbormide in a palatable paste in two-choice and no-choice trials. Over 250 rats have been dosed in a series of trials and the optimum percent of norbormide in bait reassessed at 1% and 2%. Earlier research with norbormide has shown that similar concentrations in bait were likely to be effective (Prakash 1988).

For these palatability and efficacy trials rats were lightly fasted overnight and then presented with toxic bait. For no-choice trials rats were presented 10 g of bait containing norbormide. For two-choice trials rats were presented with 10 g of bait containing norbormide alongside 10 g of non-toxic bait. Monitoring continued for up to 12 hours following administration of baits.

All animals were weighed, the amount of toxic bait consumed was measured and the symptoms of poisoning and time to death recorded.

RESULTS

Norway rats typically weighed between 250 to 500 g and ship rats 100 to 240 g. There was no obvious correlation between body weight and survival. Symptoms of poisoning were similar in all trials. The rats became subdued, and paws and nose area became very pale and they were increasingly unresponsive prior to death.

Significant differences in efficacy of different batches from different manufacturers have been determined. Batches were rejected that killed very few (15% mortality) to a moderate number (50% mortality) of a group of rats in no-choice trials. A result from one of the less effective forms of norbormide and the most effective batch are presented in Table 1.

The most effective batch was subjected to further assessment. The effective batch of norbormide killed >90% in no-choice and two-choice trials with Norway rats at 1% in bait. Norbormide appeared to be equally effective at 1% and 2% in no-choice trials, but was less effective at 2% in the two-choice trial (see Table 2). Further screening showed this batch of norbormide to be effective with ship rats (Table 3), despite the published LD_{50} being much higher for ship rats versus Norway rats (see Table 4).

During 2017 characteristics of the synthesis of norbormide have been further investigated. Further research batches were generated at laboratory scale by The University of Auckland, New Zealand to enable a better understanding of the optimal characteristics of norbormide and factors in terms of synthetic steps, including re-crystallization steps, different purification techniques, isomer profile and particle size that influence effectiveness of these batches versus a positive control. The positive control was the effective batch obtained from a commercial manufacturer for which results are reported above in Table 1-3. Further results from research batches versus the positive control are presented in Table 4. Norway rats were used and these trials were no-choice tests. A total of ten treatments were tested. A high-level description of the different forms tested is given. Further details cannot be provided at this stage because of commercial sensitivities.

Table 1. Percentages of livestock losses by category.

Cause of Death	Beef Cattle		Sheep	
	USDA (2015)	UC Survey (2016)	USDA (2014)	UC Survey (2016)
Predators	4%	35%	29%	52%
Diseases and Natural Causes	96%	65%	71%	48%

Table 2. Toxicity to Norway rats of an effective batch (EB) at 1% in a no choice trial and 1 and 2% in a two-choice comparative trial.

Dose	Average Bait Eaten	Mortality	Average Time to Death
EB 1% no-choice	1.9 g	93 %	2.5 hrs (30 mins to 8 hrs)
EB 1% two-choice	1.3 g	93 %	2.7 hrs (30 mins to 8 hrs)
EB 2% no-choice	1.7 g	93 %	2.1 hrs (30 mins to 6.5 hrs)
EB 2% two-choice	0.6 g	67%	3.1 hrs (30 mins to 9 hours)

CONCLUSIONS

Our experiments show that there are considerable differences in the effectiveness of norbormide from different manufacturers. This could in part explain why norbormide fell from favour in the 1970s after mixed results in field trials (Prakash 1988). Effective forms of norbormide have been identified and further testing in field trials is now warranted.

Earlier research (Rennison et al. 2007) and recent comparison of research scale batches has significantly enhanced our understanding of the optimal characteristics of norbormide and optimal physico-chemical characteristics of norbormide that influence effectiveness. These factors as suggested by Jay-Smith et al. (2016) are likely to be influencing the rate of dissolution and absorption, thus impacting on overall bioavailability and subsequently toxicity. As a result of these new insights we are closer to an optimum process of producing norbormide at scale, but further research and testing work on this is needed in 2018.

Norbormide meets many of the desirable features of an ideal rodenticide. These are: (i) specifically lethal to the target species, (ii) relatively humane (iii) orally active and rapidly absorbed, (iv) relatively short half-lives in blood and tissues vs. other rodenticides (many have long half-lives), (v) not persistent in the environment, (vi) does not lead to secondary poisoning, (vii) no antidote required.

Table 3. Toxicity to ship rats of an effective batch (EB) of Norbormide.

Dose	Average Bait Eaten	Mortality	Average Time to Death
EB 1% no choice	5.3 g	95 %	1.9 hrs (30 mins-5 hrs)

Table 4. Toxicity to Norway rats of different experimental batches of norbormide versus EB.

Type of Norbormide	Mortality
EB positive control	80%
Best match positive control	95%
Alternative close match to positive control	80%
2nd Alternative close match to positive control	80%
Different re-crystallization approach	30%
Different syntheses approach	30%
Direct route to re-crystallization	20%
Unpurified negative control	20%
Negative control variation to best match	10%
Manufacturing technique considered likely to produce an unpalatable form	10%

Norbormide is unique in that an antidote is not required. Norbormide's acute toxicity profile includes no toxicity to birds and companion animals. This is extremely important for conservation work or rodent control in agricultural or urban settings (see Table 5).

Symptoms in rats are mild and short-lived compared with other rodenticides. Norbormide has no chronic toxic effects on non-target species. For example, following treatment for up to 60 days, dogs tolerated a dose equivalent of 1000 ppm without ill effect. At 10,000 ppm they lost appetite and looked ill (Roszkowski et al. 1964). Pharmacokinetic studies indicate norbormide is readily metabolized and unlikely to be persistent (Ravindran et al. 2009a, 2009b) and secondary poisoning studies conducted by Russell (1965) showed no ill effects in cats, dogs, and pigs. Humans given large doses exhibited a slight decrease in blood pressure which normalized after two hours (Hayes and Laws 1991).

Given these positive attributes, it is not surprising that there have been several attempts to increase the effectiveness of norbormide. An encapsulation approach is reported by Nadian and Lindblom (2002) and prodrug forms of norbormide have been developed that aim to delay the action of the toxicant and increase palatability by masking the taste (Rennison et al. 2012). Our approach has been somewhat different and is not without similar challenges. Campbell et al. 2015 suggested that at the current rate of development, it is expected new forms of norbormide could be registered and available for field use within the next five years. The ability to target rats with no

Table 5. Toxicity to different species (from Roszkowski et al. 1964 and 1965.)

Species	LD^{50} mg/kg
Norway rat	5-15
Ship rat	52
Hamster (*Cricetus spp*)	140
Guinea-pig (*Cavia porcellus*)	640
House mouse	2,250
Rabbit (*Oryctolagus cuniculus*)	>1,000
Cat	>1,000
Dog (*Canis spp*)	>1,000
Duck (*Anas platyrhynchos*)	>1,000
Pig (*Sus scrofa*)	>1,000
Pigeon (Columbidae)	>1,000
Turkey (*Meleagris*)	>1,000
Sheep (*Ovis aries*)	>1,000

risk to non-target species presents considerable advantages in many settings and does warrant further investment and completion of the current scale-up phase of research and development.

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