Diphacinone and Cholecalciferol (D+C) as a Potent Low-residue Rodenticide

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ABSTRACT: Rodenticides such as brodifacoum are more potent than first-generation anticoagulants. However, their field, farm, and outdoor use in urban settings have been linked to bioaccumulation and non-target impacts for more than three decades. Product development strategies focused on baits that yield good control of pests without residue risks to wildlife are few. To fill this gap, a bait containing a combination of diphacinone at 0.005% and cholecalciferol at 0.06% (D+C bait) has been developed as a multispecies bait for NZ use, that is effective at killing rodents and also possums (*Trichosurus vulpecula*), which are resistant to the toxin effects of first-generation anticoagulants. This bait was approved by the NZ Environmental Protection Agency and the product registered by the NZ Ministry of Primary Industries in 2019. A new bait is being considered with a lower dose of cholecalciferol. A bait containing half or a quarter of the loading concentration of cholecalciferol would have an even better safety profile for rodent control alone. Cage trials indicate that cholecalciferol at 0.03% with diphacinone 0.005% is as effective as brodifacoum as a single exposure bait. Amounts of cholecalciferol as low as 0.015% significantly improve the effectiveness of diphacinone.

KEY WORDS: anticoagulants, cholecalciferol, diphacinone, diphacinone + cholecalciferol (D+C), nontarget hazard, rodenticides, residues

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INTRODUCTION

Brodifacoum is the most widely used slow-acting rodenticide worldwide and is highly effective. Unfortunately, brodifacoum and related compounds tend to bioaccumulate, and there are concerns regarding residues, food safety and non-target impacts (Eason et al. 1996, 1999, 2010a,b; Murray 2011, Langford et al. 2013, Christensen et al. 2012, Walker et al. 2014, Lopez-Perea et al. 2015, Young and de Lai 1997, Salim et al. 2015, Roos et al. 2021, Mercer et al. 2022). Partly because of these concerns and somewhat to maintain a pest control toolbox with a variety of tools for different applications, there has been a renewed interest in researching the combination of a first-generation anticoagulant with cholecalciferol (Witmer et al. 2014, Baldwin et al., 2016). This combination has been proven effective in rodents resistant to first-generation anticoagulants alone (Pospischil and Schnorbach 1994) and would have other applications. Diphacinone was preferred over other anticoagulants because of its short half-life (Crowell et al. 2013). Cholecalciferol (vitamin D3) was developed in the 1980s as a rodenticide (Marshall 1984, Tobin et al. 1993). More detailed reviews of these compounds' characteristics, international application, and toxicology can be found elsewhere (Prakash 1988, Hayes and Laws 1991, Eason et al. 2010a, Buckle and Eason 2015, Eason et al. 2017). This paper summaries some of the key features of the bait containing a combination of diphacinone at 0.005% and cholecalciferol at 0.06% that has been developed, and is now registered as a multispecies bait for NZ use, and is effective at killing rodents and also possums. These features are described in more detail in an earlier publication (Eason et al. 2019) hence they are only very briefly described here alongside an update of the registration status of this bait. Welfare, mode of action, residue risks and secondary poisoning were a keen focus of the regulatory assessments; hence these aspects are outlined below.

WELFARE

The average time to death for rats and possums poisoned with the combination of diphacinone and cholecalciferol was similar or shorter than for brodifacoum alone, and this is most apparent for possums. With second-generation anticoagulants, such as brodifacoum, as sole agents, sickness is protracted, particularly in possums, and the duration of sickness and time to death variable (Littin et al. 2000). The welfare of poisoned animals is improved by cholecalciferol shortening the time until death largely through potentiation of the anticoagulant action (see Table 1).

MODE OF ACTION

Diphacinone interferes with normal blood clotting by preventing vitamin K recycling which prevents the conversion of inactive precursors into active vitamin K-dependent blood-clotting factors (Thijssen 1995). Major haemorrhaging and death by respiratory or heart failure result

Table 1. Comparative average times to death for possums and rats. (adapted from Eason et al. 2019)

Anticoagulant	Average time to death (days)		Reference
	Possums	Rats	
Brodifacoum	20.1	7.2	Littin et al. (2000)
Diphacinone + Cholecalciferol	5.75	5.08	Eason et al. (2019)

following a lethal dose. Kerins et al. (2002) suggest that a low dose of cholecalciferol enhances the haemorrhagic effect of first-generation anticoagulants. This was the case in rats, with death in possums likely to be caused by a combination of haemorrhaging and hypercalcaemia (Eason et al. 2019).

RESIDUES

Many studies show that first-generation anticoagulants are less persistent than second-generation anticoagulants. For example, in a comparative study of rodenticides by Fisher et al. (2003), the hepatic elimination half-life of diphacinone was estimated at three days in rats versus several months for brodifacoum. Similarly, short half-lives have been recorded in pigs and deer (Crowell et al. 2013). Cholecalciferol (Vitamin D3) is metabolised to 25-hydroxycholecalciferol (25-OHD in the liver, with any surplus cholecalciferol either further metabolised and excreted in bile or stored in fat and muscle tissue (Parfitt et al. 1982, Holick 2003, Heaney et al. 2009). Because 25-OHD occurs naturally in animals, Fairweather et al. (2013) recommend excessive cholecalciferol consumption is indicated where 25-OHD residues in plasma or liver are at least four times as high as defined reference normal levels for particular species. As diphacinone and cholecalciferol are depleted at faster rates when compared with brodifacoum, when used together in cereal pellet bait, they are much less likely to lead to persistent residues and bioaccumulation in livestock or game than brodifacoum, and the secondary poisoning risk associated with the D+C bait is comparatively low (Eason et al. 2019).

DISCUSSION

In NZ, the requirements of the Hazardous Substances and New Organisms (HSNO) legislation must be met, along with the requirements of the Agricultural Chemistry and Veterinary Medicines (ACVM) Act. The registration process is challenging as approvals are required from both the NZ EPA and MPI; consultation with Māori is a prerequisite, and welfare considerations are a key component of the registration assessment process for vertebrate pesticides. However, the overall requirements are similar to those of the US EPA. New product development, in this context, is very challenging, in part because these data requirements are significant. A decade or more of research and development included cage and then field trials. The field trials, each of 200 hectares in size, targeting possums, ship rats (*Rattus rattus*) and mice (*Mus musculus*), achieved an average reduction in abundance of 94% for possums, 94% for ship rats and 80% for mice (Eason et al. 2019). These results underpinned the approval of D+C bait by the NZ EPA and MPI in 2019. To illustrate the time commitment and challenges of new product development, critical milestones for the development of the D+C baits are summarised below.

- 2008 – Fundamental & applied product development R&D
- 2000 to 2015 – Completion of cage and field trials on possums and rodents
- 2019 – Successful approval of registration dossiers with NZ Authorities (MPI/NZ EPA) for possum and rodent control
- 2022 – Extending registration of diphacinone 0.005% + cholecalciferol 0.06% to other pest species
- 2023/24 – Looking beyond NZ + exploring US & other markets for a D+C diphacinone 0.005% + cholecalciferol 0.015% bait targeting mice & rats

Looking to the future, unpublished laboratory trials indicate that a combination bait with cholecalciferol at half (0.03%) the concentration or quarter (0.015%) the concentration used in current bait is still effective for killing rats (pers. commun., Lee Shapiro, 2018). Hence where only rodents are being targeted, versus occasions where both possums and rodents are the targets, a bait with less cholecalciferol would be suitable, less hazardous and less costly. The development of this combination is now a consideration for further research and development.

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