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REVIEW ARTICLE

Toxicology and ecotoxicology of para-aminopropiophenone (PAPP) – a new predator control tool for stoats and feral cats in New Zealand

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Abstract: Para-aminopropiophenone (PAPP) paste was approved as a stoat control agent in New Zealand by the Environmental Protection Authority in August 2011 and for feral cat control in November 2011. PAPP was originally researched in Europe and the USA as treatment for cyanide and radiation poisoning. Over the last 10 years, our research has focused on several factors, including determining its toxicity to predators, field effectiveness for controlling stoats and feral cats, animal welfare profile, toxicology, ecotoxicology, and understanding and reducing non-target risks. PAPP has been developed specifically for the control of stoats and feral cats because of the special sensitivity displayed by these species. Its toxicity is mediated by the induction of methaemoglobinaemia (the ferric state of haemoglobin). Normally, methaemoglobin levels in the blood are below 1%. Levels of methaemoglobin in the blood above 70% are usually fatal, creating a lethal deficit of oxygen in cardiac muscle and the brain. In stoats and feral cats, death after a lethal dose usually occurs within 2 h after eating bait, with clinical signs first appearing in 10 to 20 min for stoats and at around 35 min for cats. Animals become lethargic and sleepy before they die, hence PAPP is relatively humane. A simple antidote exists, namely methylene blue. Further, birds display a lack of toxicity to PAPP when compared with other vertebrate pesticides. A paste containing 40% PAPP has been developed for use in meat baits in New Zealand. A toxic dose for stoats and feral cats is achieved when pea-sized amounts of paste are delivered in 10–20 g meat baits. When meat baits containing PAPP are applied in bait stations in field settings, stoat and feral cat numbers can be rapidly reduced. However, there has been limited practical experience with PAPP to date, especially when compared with alternative tools such as traps or sodium monofluoroacetate (1080) baits. Additional practical experience should enable the effective use of PAPP as a tool to help protect native species from introduced predators. In the future, PAPP will be developed in long-life bait and in a resetting toxin delivery system.

Keywords: efficacy; mode of action; non-target effects; vertebrate pesticide

Introduction

Stoats (*Mustela erminea*) were shipped to New Zealand from Britain in the 1880s and released on farmland in the hope that they would control rabbit (*Oryctolagus cuniculus*) numbers. Unfortunately they soon spread throughout the country, including to places where there were no rabbits (King & Murphy 2005). Cats (*Felis catus*) came with the European explorers and settlers from 1769 onwards and well established feral populations were reported in the 19th century. Cats were also introduced to many offshore and outlying islands (Gillies & Fitzgerald 2005). Both stoats and feral cats have been implicated in the ongoing decline of native New Zealand threatened species and ongoing control will be required to avert further extinctions (Dowding & Murphy 2001).

Para-aminopropiophenone (PAPP) has been developed as a Vertebrate Toxic Agent (VTA) specifically for the control of stoats and feral cats because of the special sensitivity displayed by these species. It has the potential to play a major role in the protection of threatened species. It is a compound with the formula $C_9H_{11}NO$ and a molecular weight of 149.19. Prior to the registration of PAPP for stoat and feral cat control, 1080 was the only toxin registered in New Zealand for feral cat control, while trapping and secondary poisoning from using 1080 or anticoagulant toxins for possum and rat control were the only means of controlling stoats. Trapping is often labour-intensive and expensive (Brown 2003). In this context, the need for an effective humane poison for stoats and feral cats was identified (Murphy et al. 2005, 2007), and is consistent with aspirations for continued improvements in the welfare aspects of pest control technology (Littin et al. 2004). PAPP has been developed in New Zealand to address this aspiration and need for a new tool for the control of stoats and feral cats. Paste containing PAPP as the active ingredient was approved as a stoat control agent by the New Zealand Environmental Protection Authority (EPA) in August 2011 and for feral cat control in November 2011. PAPP paste applied to fresh meat baits in bait stations has been shown to be effective for the control of both stoats and feral cats (Shapiro et al. 2010; Dilks et al. 2011; Murphy et al. 2011).

PAPP was not originally intended to be a vertebrate pesticide. Early research in Europe and the USA focused on

the potential of PAPP as a treatment for cyanide gas poisoning in the context of protecting soldiers during conflict (Rose et al. 1947; Bright & Marrs 1982). During 1960–80, i.e. the Cold War era, PAPP was further investigated as an antidote for human radiation poisoning (Oldfield et al. 1965).

Extensive research into PAPP has been conducted over more than 50 years, including in vitro and in vivo toxicology trials, metabolism studies, and experiments using human volunteers (Jandorf & Bodansky 1946; Rose et al. 1947; Oldfield et al. 1965; Fitzgerald et al. 1974; Bright & Marrs 1982, 1983, 1986, 1987; Marrs & Bright 1986, 1987; Marrs et al. 1991; Baskin & Fricke 1992; Scharf et al. 1992; Brown et al. 1993; Menton et al. 1997). It may seem strange that a compound originally intended as an antidote is now being used as a vertebrate pesticide. However, in early experiments it was observed that PAPP was specifically much more toxic to carnivores than to other species (Plzak & Doull 1963; Savarie et al. 1983; Scawin et al. 1984) and this has been confirmed, with birds and humans being less sensitive (Fisher & O'Connor 2007; Murphy et al. 2007).

The use of PAPP as a toxin for control of animal pests was first investigated in the 1980s by researchers in the U.S. Fish and Wildlife Service (Pan et al. 1983; Savarie et al. 1983; Schafer et al. 1983) as a tool for coyote (*Canis latrans*) control. In Australia, PAPP has been researched for its use for field control of feral cats, foxes (*Vulpes vulpes*) and wild dogs (*Canis familiaris*) (Marks et al. 2004; Fleming et al. 2006; Lapidge et al. 2007; Eason et al. 2010a). In New Zealand, PAPP has been studied in five species, namely ferrets (*Mustela furo*) (Fisher et al. 2007), dogs (Murphy et al. 2007), feral cats and stoats (Fisher et al. 2005; Eason et al. 2010a; Murphy et al. 2011) and weasels (*Mustela nivalis*) (Blackie et al. 2012). The initial drive in terms of completion of field trials and registration prioritised and focused on feral cats and stoats in part because of their pest status, particularly in the case of stoats and in part because of the proven susceptibility of these species to PAPP (Dilks et al. 2011; Shapiro et al. 2010; Murphy et al. 2011). The development of PAPP as part of the suite of tools for protecting native species in New Zealand, and its approval by the New Zealand EPA in 2011, was a big step forward. Its use should complement trapping and allow those involved in endangered species protection the ability to use a combination of tools. Most concerns in New Zealand regarding the use of poisons relate to fears about contamination of water supplies, possible sublethal effects on humans, welfare impacts, and non-target effects. In the following sections, the characteristics of PAPP are described relative to these concerns, including its mode of action and metabolism, acute toxicity in different species, its toxicokinetics and breakdown in the environment, sublethal effects, relative humaneness and other characteristics.

Mode of action

PAPP's primary mode of action is via a toxic effect on red blood cells, mediated by a toxic metabolite, para-hydroxylaminopropiophenone (PHAPP) (DeFeo et al. 1972). Following administration or ingestion in bait, PAPP is absorbed into the blood stream. PAPP is thought to undergo metabolic biotransformation to PHAPP. The basis for this is an analogy with the mechanism of methaemoglobinogenesis of analine and substituted anilines (Graffe et al. 1964; Kiese 1974). This toxic metabolite, although short acting, effectively oxidises haemoglobin to methaemoglobin (Graffe et al. 1964). This reaction can be rapid, peaking in 30 min or less (Goldstein & Doull 1973). Methaemoglobin is the oxidised form of haemoglobin, where the central iron is in the ferric (3+), rather than ferrous (2+) state. The ferric form of iron has a great affinity for cyanide, which it readily sequesters, and is the mode of PAPP's antidotal action.

However, methaemoglobin is also unable effectively to transport oxygen. Therefore, while a partial conversion of haemoglobin to methaemoglobin (15–30%) may be beneficial in a situation of cyanide exposure, the oxidation of haemoglobin to methaemoglobin is lethal if high levels of conversion (>70%) are induced, resulting in a lack of oxygen to the brain and heart, and death due to respiratory failure (Coleman & Coleman 1996). Normally, methaemoglobin levels in the blood are <1%. Symptoms are proportional to the methaemoglobin concentration in the blood and include skin colour changes (blue or greyish pigmentation), blood colour changes, and lethargy, with severe methaemoglobinaemia being fatal. Methylene blue will reverse the methaemoglobinaemia induced by PAPP and is considered an antidote to PAPP exposure (Bodansky & Gutmann 1947; Coleman & Coleman 1996).

PAPP belongs to a class of chemicals broadly classified as aromatic amines. In most species, aromatic amines ($R\text{-}NH_2$) are metabolised via acetylation of the amine moiety, prior to excretion. The N-acetyltransferases are cystolic enzymes found in all mammal species, with the exception of canids (Trepanier et al. 1997). N-acetyltransferases have been characterised in humans, rabbits, mice and hamsters (*Mesocricetus auratus*) (Vatsis & Weber 1997). By contrast, N-acetyltransferases activity is absent in the domestic dog (*Canis lupus familiaris*), red wolf (*C. l. rufus*), arctic wolf (*C. l. arctos*), coyote (*Canis latrans*), golden jackal (*C. aureus*), African wild dog (*Lycaon pictus*), arctic fox (*Vulpes lagopus*), kit fox (*V. velox*), and the silver fox (*V. vulpes*) (Trepanier et al. 1997). It is likely given their susceptibility that carnivores are also deficient in N-acetyltransferases.

Hydroxylation of PAPP to PHAPP has been demonstrated in vitro using rat, rabbit and human liver microsomes (Graffe et al. 1964; von Jagow et al. 1966; von Jagow & Kiese 1967; Coleman & Kuhns 1999) and in vivo in rats (Wood et al. 1991). The mode of action of PAPP was also studied by Wood et al. (1991) who examined the responses of rats, beagles (domestic dog) and cynomolgus monkeys (*Macaca fascicularis*) to PAPP administered orally. When PAPP was administered orally, methaemoglobin rapidly formed. However, when PAPP was administered directly into the blood, it did not induce methaemoglobin. On the basis of these results, it was considered that a hepatic biotransformation is responsible for converting PAPP to PHAPP and is the required pathway for activation of the compound. Biotransformation of PAPP by liver microsomes is now known to be dependent on several P-450 isoenzymes in the rat and in humans (Coleman & Kuhns 1999).

Figures detailing the mode of action for PAPP and the metabolic pathway for PAPP in different species are presented by Baskin and Fricke (1992) and Wood et al. (1991), respectively. Once conversion to PHAPP has occurred in the liver, it participates in a redox cycle in circulating erythrocytes. Methaemoglobinaemia is exacerbated in this process by intraerythrocytic NADPH (nicotinamide adenine dinucleotide phosphate) generated from glucose-6-phosphate dehydrogenase, which also reduces a secondary PAPP metabolite, *p*-nitrosopropiophenone (PNPP), back to the potent methaemoglobin inducer PHAPP (Baskin & Fricke 1992). These actions of PHAPP compete with the methaemoglobin

reductase system (Smith & Beutler 1966). Toxicity occurs when the oxidation process exceeds the rate of the reduction process, allowing high levels of methaemoglobin to be formed.

In contrast to the NADPH-dependent process, significant NADPH-reduction of methaemoglobin requires a co-factor or artificial electron carrier, such as methylene blue (Smith & Beutler 1966; Stolk & Smith 1966; Umbreit 2007). There are differences between species in the presence and relative activities of both of these systems. Rabbits and rodents appear to tolerate the pharmacological effect of PAPP quite well, owing to their relatively high NADPH-dependent capacity to convert methaemoglobin to haemoglobin. Conversely, cats and dogs are similarly more susceptible because of their lower NADPH-dependent capacity (Smith & Beutler 1966; Stolk & Smith 1966; Agar & Harley 1972; Rockwood et al. 2003). NADPH-dependent capacity in beagles is approximately two-thirds that of humans (Rockwood et al. 2003).

While examining species differences in methaemoglobin reductase systems, the human NADPH system was shown to possess the greatest capacity to up-regulate reduction of methaemoglobin in the presence of methylene blue (Smith & Beutler 1966). The degree to which methaemoglobin is reduced to haemoglobin in the presence of the antidote was 6-fold greater than in its absence, and exceeded that of other species tested. This is highly relevant in the context of assessing the risk PAPP presents to human health when used in baits as a vertebrate pesticide.

Early investigators into the effects of PAPP noted that lethal doses were far lower in some species (e.g. dogs and cats) than in others (Savarie et al. 1983; Sharf et al. 1992) for the reasons cited above. Methaemoglobinaemia can be induced in all species but reaches lethal concentrations most readily in carnivores (e.g. cats, dogs, foxes). This is consistent with the rapid onset of symptoms observed in cats, foxes and stoats, which usually become unconscious within 30–45 min (Marks et al. 2004; Eason et al. 2010b) following toxic doses. Gender differences in PAPP toxicity have also been reported, with female mice less susceptible and female rats and beagles more susceptible (Doull & Plzak 1963; Bright et al. 1987).

Acute toxicity

An extensive database on the acute toxicity of PAPP in a diverse spectrum of species exists. As with other poisons, reported LD_{50} values can be influenced by the method used to deliver the poison, and by environmental or laboratory conditions and protocols. Oral gavage of PAPP in several different carrier materials has been a standard dosing approach used by many researchers (Plzak & Doull 1963; Savarie et al. 1983; Scawin et al. 1984). Alternatively, PAPP has been fed to animals in measured quantities of foodstuffs or in prepared baits. Nevertheless, it is still relatively easy to see the substantial species variation that has been reported in the literature over the years. Table 1 shows acute oral toxicity LD_{50} values for a number of mammalian species. LD_{50} of PAPP in the two target pest species in New Zealand, namely stoats and feral cats, is <10 mg kg^{-1}. Cats and stoats are extremely susceptible, and as noted above, most other mammalian carnivores are highly sensitive to poisoning, with lethal doses that are far lower in these species than non-carnivorous mammalian species (Tables 1 & 2). There is no LD_{50} value for humans.

Table 1. Oral LD_{50} values available for PAPP on a range of mammal species. [a] Calculated 80% increase in methaemoglobin concentration, which correlated strongly with LD_{50} values (NWR 2006).

Animal	LD_{50} mg kg^{-1}	Reference
Cat (*Felis catus*)	5.6	Savarie et al. 1983
Coyote (*Canis latrans*)	5.6	Savarie et al. 1983
Brown bandicoot (*Isodon obesulus*)	6.4 [a]	NWR 2006
Dingo (*Canis familiaris dingo* hybrid)	8.5 [a]	NWR 2006
Stoat (*Mustela erminea*)	9.3	Fisher et al. 2005
Bobcat (*Lynx rufus*)	10	Savarie et al. 1983
Kit fox (*Vulpes velox*)	14.1	Savarie et al. 1983
Ferret (*Mustela furo*)	15.5	Fisher & O'Connor 2007
Spotted-tail quoll (*Dasyurus maculatus*)	24.8 [a]	NWR 2006
Red fox (*Vulpes vulpes*)	<25.2	Marks et al. 2004
Dog (*Canis familiaris*)	< 50	Vandenbelt et al. 1944
Dama wallaby (*Macropus eugenii*)	89	Fisher et al. 2008
Badger (*Taxidea taxus*)	c. 100	Savarie et al. 1983
Fat-tailed dunnart (*Sminthopsis crassicaudata*)	105 [a]	NWR 2006
Tasmanian devil (*Sarcophilus harrisii*)	120 [a]	NWR 2006
Raccoon (*Procyon lotor*)	142	Savarie et al. 1983
Stripped skunk (*Mephitis mephitis*)	>400	Savarie et al. 1983
Rat (*Rattus norvegicus* – male)	177	Savarie et al. 1983
Rat (Sprague-Dawley)	221	Pan et al. 1983
Rat (*Rattus* sp. – female)	224	Scawin et al. 1984
Rat (*Rattus* sp. – Porton-Wistar male)	475	Scawin et al. 1984
Mouse (*Mus musculus* – Swiss Webstar)	168	Pan et al. 1983
Mouse (*Mus musculus* – albino male)	233	Savarie et al. 1983
Mouse (*Mus musculus* – Porton albino female)	> 5000	Scawin et al. 1984
Brown antechinus (*Antechinus stuartii*)	>571 [a]	NWR 2006
Bush rat (*Rattus fuscipes*)	697 [a]	NWR 2006
Brushtail possum (*Trichosurus vulpecula*)	≥500	Fisher et al. 2008
Guinea pig (*Cavia porcellus* – Dunkin Hartley)	1020	Scawin et al. 1984

However, cynomolgus monkeys have been administered 150 mg kg^{-1} day^{-1} for 2 weeks without mortality (Lackenby 1987a), hence the single-dose oral LD_{50} for this species is clearly well in excess of 150 mg kg^{-1}, and it is probable that this is also the case for humans.

PAPP has been shown to be generally less toxic to birds than to mammalian carnivores (Table 2). In some investigations, the birds were administered PAPP directly to the stomach by oral gavage (Savarie et al. 1983; Schafer et al. 1982; Fisher et al. 2008). Further data have been generated in New Zealand to assess the toxicity to birds of the formulated PAPP paste presented in a meat bait (Eason et al. 2010c), as this is how PAPP is currently used for the control of both stoats and feral cats (Shapiro et al. 2010; Dilks et al. 2011; Murphy et al. 2011). Four bird species were assessed as representative non-target species. Forty Australian magpies (*Gymnorhina tibicen*), 20 blackbirds (*Turdus merula*), 20 mallard ducks (*Anas platyrhynchos*) and 21 weka (*Gallirallus australis)* were dosed orally with PAPP in the form of a 40% paste. The PAPP paste was added to meat as a delivery vehicle. The lethal dose to kill 50% (LD_{50}) of magpies was 1387 mg kg^{-1}, 174 mg kg^{-1} for blackbirds and 32 mg kg^{-1} for mallard ducks (Eason et al. 2010c). An LD_{50} of 568 mg kg^{-1} was determined for weka; however, this LD_{50} value underestimates the risk to weka as they were significantly affected at the lowest dose tested (62 mg kg^{-1}) (Eason et al. 2010c). Weka became subdued and lost their appetite until they were euthanased 30 h after dosing.

Eason et al. (2010c) confirmed that while ducks are more susceptible to PAPP than other birds, they are still less susceptible than stoats or cats and there is considerable interspecies variation in response to PAPP by birds. While birds are less susceptible to PAPP than stoats or feral cats, or mammalian carnivores in general, it appears some bird species are adversely affected and it will be important to limit their exposure when PAPP is used for predator control. The risk to any non-targets will be less with stoat bait than a cat bait, as only c. 13 mg PAPP is needed for stoats compared with c. 80 mg PAPP for cats (Murphy et al. 2007; Shapiro et al. 2010).

As a result of our understanding of PAPP's mode of action, variations in LD_{50} values can be viewed as a function of the differences in the metabolism of PAPP between species, in the metabolism and excretion of the degradation products (Wood et al. 1991), differences between species in the rates of haemoglobin oxidation (Smith & Beutler 1966), and to a lesser extent, in the relative capacity of species to reduce methaemoglobin to haemoglobin.

Of a range of iconic Australian native mammals assessed for the effects of orally administered PAPP, only the southern brown bandicoot (*Isoodon obesulus*) and spotted-tail quoll (*Dasyurus maculatus*) were more sensitive than dogs (NWR 2006; McLeod & Saunders 2013) (see Table 1). Of the two reptile species (*Varanus* sp.) assessed, only the goanna (*V. rosenbergis*) was more sensitive than dogs (NWR 2006; McLeod & Saunders 2013). This sensitivity of reptiles, as shown in Table 2, could have relevance to PAPP use in New Zealand and care with baiting strategies will be advisable. In a practical sense, the risk from the use of PAPP needs to be evaluated in the context of the impacts of stoats and feral cats on threatened species populations including reptiles.

With regard to fish species, QSAR analysis has been carried out to predict LC_{50} values for guppies (*Poecilia reticulata*), with reported values ranging from 23 to 623 mg L^{-1} (Karabunarliev et al. 1996). For fathead minnows (*Pimephales promelas*), predicted LC_{50} values ranged from 52 to 201 mg L^{-1} (Karabunarliev et al. 1996; Kulkarni et al. 2001). However, this research is of limited relevance to PAPP use as a predacide in New Zealand but is reported here for completeness.

Toxicokinetics and exposure risks

Wood et al. (1991) investigated the metabolism and excretion of radio-labelled PAPP in Sprague-Dawley laboratory rats (*Rattus norvegicus*), dogs and cynomolgus monkeys. PAPP was rapidly absorbed by all three species, with peak plasma

Table 2. PAPP oral LD_{50} values for bird and reptile species. [a] Calculated 80% increase in methaemoglobin concentration, which correlated strongly with LD_{50} values (NWR 2006).

	LD_{50} (mg kg^{-1})	Reference
Avian species		
Mallard duck (*Anas platyrhynchos* Pekin breed)	32	Eason et al. 2010c
Mallard duck (*Anas platyrhynchos* Pekin breed)	38	Fisher et al. 2008
Little Australian raven (*Corvus coronoides*)	130 [a]	NWR 2006
Red-winged blackbird (*Agelaius phoenicus*)	133	Savarie et al. 1983
Eurasian blackbird (*Turdus merula*)	174	Eason et al 2010c.
Common crow (*Corvus brachyrhynchos*)	178	Savarie et al. 1983
Black-billed magpie (*Pica pica*)	178	Savarie et al. 1983
Coturnix quail (*Coturnix coturnix*)	> 316	Savarie et al. 1983
Starling (*Sturnus vulgaris*)	> 316	Savarie et al. 1983
Weka (*Gallirallus australis*)	568*	Eason et al. 2010c
Silver gull (*Larus novaehollandiae*)	>1000 [a]	NWR 2006
Australian magpie (*Gymnorhina tibicen*)	1387	Eason et al. 2010c
Golden eagle (*Aquila chrysaetos*)	1/1 survived at 50, 100, 200, 400 mg kg^{-1}	Savarie et al. 1983
Reptile species		
Lace monitor (*Varanus varius*)	3	Frapell 2007 (cited in McLeod & Saunders 2013)
Rosenberg's goanna (*Varanus rosenbergi*)	12	Frappell & Andrewartha 2006 (cited in McLeod & Saunders 2013)

*While weka did not die at lower doses, they became subdued and lost their appetite at a PAPP concentration of 50 mg kg^{-1}.

concentrations at 15 min (male rats), 1 h (female rats), 30 min to 1 h (beagles) and 1 to 1.5 h (monkeys) after oral ingestion. As a result of metabolic and excretory processes (Wood et al. 1991), levels of plasma radioactivity decline rapidly, with plasma concentrations declining to very low levels by 72 h.

As described above (see Mode of Action) there are marked differences in the metabolic pathways in different species. In rats, PAPP was metabolised primarily by N-acetylation, in dogs, aliphatic hydroxylation predominated, whereas monkeys used both of these metabolic pathways. The increased capacity of primates to metabolise PAPP to non-toxic compounds when compared with canids is one reason why humans are at reduced risk of acute PAPP toxicosis.

The principal route of excretion of PAPP is via the kidneys in urine (between 65% and 90% of a dose), with excretion via faeces and in expired air contributing minimally (Tepperman et al 1946; von Jagow et al. 1966; von Jagow & Kiese 1967; Wood et al. 1991). Some PAPP metabolites were present in the bile of dogs and rats (Wood et al. 1991), but these appear to be reabsorbed via the intestine by enterohepatic recirculation and ultimately also excreted in the urine, as also happens in rabbits (von Jagow et al. 1966; von Jagow & Kiese 1967; Wood et al. 1991). Owing to the different metabolic pathways for PAPP between species, the forms of PAPP metabolites differ significantly (Tepperman et al 1946; von Jagow et al. 1966; von Jagow & Kiese 1967; Wood et al. 1991).

Wood et al. (1991) observed rapid excretion of PAPP via urine and faeces in rats, beagles and cynomolgus monkeys given oral doses of 0.5, 5, and 25 mg kg^{-1} PAPP, respectively. All animals were monitored for 120 h after PAPP administration. The majority of PAPP and its metabolites were excreted in urine and faeces within 24 h (93–96% in rats, 84% in dogs, and 84–95% in monkeys). Further details of the pharmacokinetics, including figures of plasma versus time concentration profiles and metabolic pathways, for these species can be found in Wood et al. (1991).

An elimination half-life for PAPP in plasma for rats has not been published. Phenones, compounds that are chemically related to PAPP, have relatively short half-lives in the blood (Paulet et al. 1963; Marino et al. 1997). For the purposes of this review, and to enable comparison with other vertebrate pesticides, the half-life for PAPP has been estimated using data on the concentration of radioactivity in plasma of Sprague-Dawley rats after oral gavage with ^{14}C PAPP over a period of 96 h, extracted from fig. 1 in Wood et al. (1991). A standard simple pharmacokinetic model was fitted and the elimination half-life was estimated to be c. 4 h (Elena Moltchanova, University of Canterbury, Christchurch, unpubl. data). By comparison, the active hydroxylated metabolite PHAPP has an exceedingly short half-life of c. 1 min (Wood et al. 1991). While no studies were found where depletion of PAPP was monitored to the point where it could no longer be detected, information on the pharmacokinetics of PAPP in rats, dogs and monkeys (Wood et al, 1991) is very comprehensive and it would appear that PAPP is likely to be completely eliminated in mammals in about 5 days.

By comparison, there is a huge variation in the way that different VTAs are absorbed, distributed, metabolised and excreted by mammals (Eason et al. 2008, 2011, 2012; Crowell et al. 2013). At one end of the spectrum there are compounds that are very water soluble, rapidly absorbed, well distributed and equally rapidly excreted, such as 1080 and cyanide. There are others that are extensively metabolised to more hydrophilic metabolites, such as cholecalciferol and diphacinone, and yet others that are lipophilic and poorly metabolised and exhibit unique receptor-binding characteristics, such as second-generation anticoagulants. The rapid metabolism and clearance of PHAPP and PAPP is a great advantage in the context of the application of PAPP as a prospective predacide because the risks of bioaccumulation in the food chain or secondary poisoning are significantly reduced.

The toxicokinetic information summarised above indicates that PAPP is likely to be slightly more persistent than cyanide in live animals but as, or less persistent than 1080, and substantially less persistent than anticoagulants. In conclusion, all species studied to date rapidly metabolise and excrete sublethal doses of PAPP. If recommended practices are followed in pest control operations, PAPP is unlikely to be present in game-meat, sheep or cattle for human consumption because of its comparatively rapid elimination. This means it will not bioaccumulate in the food chain, and secondary poisoning risks are low, particularly for the majority of bird species.

Toxicodynamics, welfare and antidote treatment

One of the key drivers for developing PAPP has been animal welfare. When delivered at a lethal dose, rapid induction of high levels of methaemoglobin can quickly induce unconsciousness and death with minimal symptoms of distress (Eason et al. 2010b). Rapidly induced anoxia is the cause of death and appears to be without appreciable pain or discomfort in much the same way as anoxia induced by carbon monoxide induces carboxaemia. Symptoms are proportional to the methaemoglobin concentration in the blood (Smith 1967, 1969; Rentsch 1968; Goldstein & Doull 1973).

In most species, peak methaemoglobin concentrations follow peak plasma PAPP levels by approximately 30–60 min (Paulet et al. 1963; Bright & Marrs 1982, 1983; Marino et al. 1997). This lag is a function of at least three processes — first, PAPP absorption; second, PAPP metabolism to an active metabolite; and third, the accumulation of PHAPP in red blood cells to a minimum effective concentration (Marino et al. 1997). The duration of the lag phase and the severity of symptoms will be dose dependent. During the lag phase, varying amounts of PHAPP will have oxidised a proportion of haemoglobin to methaemoglobin. Counteracting this, PHAPP and PAPP are metabolised to inactive derivatives prior to being cleared via the kidneys. Therefore, by the end of the first two hours, methaemoglobinaemia either will have reached peak (or lethal) concentrations, or have begun to decline because of enzymatic reduction of methaemoglobin to haemoglobin by methaemoglobin reductase, in which case the animal will recover. However, these outcomes and the duration of these processes and effects are dose, dosing vehicle, and species dependent.

Detailed observations in pen trials using 20 feral cats and 15 stoats were undertaken using meat baits containing toxic doses of PAPP (Eason et al. 2010b). Of the 20 cats, 18 died after eating PAPP bait while two survived. The cats showed a range of responses, with the onset of symptoms occurring between 35 and 112 min and death between 37 and 246 min. One animal that ate only a fraction of the dose of PAPP recovered. Cats exposed to lethal doses of PAPP lost consciousness without spasms or convulsions. All 15 stoats died quickly after eating the PAPP bait. The stoats showed first symptoms 6–40 min after ingestion and death between 15 and 85 min. There were

minimal signs of discomfort with poisoning; animals became quiet, lethargic, and then unconscious for a short period before death. There was no prolonged period of heavy breathing. These results confirmed the findings of earlier New Zealand studies in these two species (Fisher et al. 2005).

Although both cyanide and PAPP cause central nervous system anoxia, lethargy and death, it occurs through different mechanisms of toxicity. The sequence of events observed in both cyanide and PAPP poisoning are preferable, from a welfare perspective, to those seen with other poisons. In the simplified ranking system outlined in Table 3, cyanide is scored as a 'high' performing poison from a welfare perspective. Applying the same parameters to the effects of lethal doses of PAPP in feral cats and stoats, we would also consider PAPP to be a 'high' performer from a welfare perspective. The lethal response of feral cats and stoats to PAPP was rapid and appeared relatively free of the suffering that accompanies the use of some other toxins (Littin et al. 2002; Goh et al. 2005; Potter et al. 2006). The key parameters of note in welfare assessment of a VTA are the time to onset of symptoms, the duration of symptoms, and the severity of symptoms induced by toxic doses in target species, particularly up to the point where unconsciousness and insensibility occur. When these symptoms and times are compared with other VTAs (Table 3), it appears that PAPP is comparatively humane. Even in species such as rats where the sequence of PAPP-induced toxicosis is more prolonged when compared with stoats or feral cats, the severity of symptoms associated with PAPP-induced methaemoglobinaemia was determined to be relatively humane when compared with other rodenticides (Gibson et al. 2011).

Less susceptible animals that are accidentally exposed to PAPP suffer only partial methaemoglobinaemia that is transitory and causes no clinical symptoms (Gibson et al. 2011). Its effects can be reversed, even in late stages of toxicosis, by the administration of methylene blue, which is an antidote. Animals treated promptly and effectively with the antidote can fully recover, even from near-terminal late-stage toxicosis induced by PAPP. Such an antidote is not available for existing canid control agents such as 1080, which is considered less humane than PAPP for canid control in Australia (Southwell et al. 2011, 2013). The availability of an antidote is also considered advantageous where there is a risk of exposure of working dogs to bait.

The effectiveness of methylene blue as an antidote to PAPP-induced methaemoglobinaemia was assessed in dogs by Bodansky and Gutmann (1947) and Stossel and Jennings (1966), who showed that it was highly and rapidly effective in counteracting the symptoms of severe methaemoglobinaemia. Moreover, a majority of dogs that received the life-saving treatment recovered fully within hours and appeared physiologically normal days after the methylene blue intervention (Bodansky & Gutmann 1947). In addition, healthy dogs (anesthetised) who were given methylene blue intravenously, at doses exceeding 30 mg kg^{-1}, responded only with a rise in total circulating haemoglobin but were otherwise unaffected (Stossel & Jennings 1966).

The effectiveness of the antidote and its relatively wide therapeutic window make it the clinical treatment of choice. It remains the current best practice for the treatment of all methaemoglobinaemias. The treatment indication involves intravenous administration of methylene blue at 1–2 mg kg^{-1} of body weight formulated as an aqueous solution and administered over a 5-min period (WHO & CEC 1993; Greenberg 2001; Boylston & Beer 2002). Doses of methylene blue should not exceed 7 mg kg^{-1}. This is also the dose range that is used in veterinary medicine in the treatment of methaemoglobinaemia (Bodansky & Gutmann 1947). Treatment with the antidote is contraindicated at methaemoglobin concentrations below approximately 30%, which generally do not result in clinical symptoms and resolve themselves naturally once the causative agent is removed. Doses may be administered intravenously or orally following dilution of a stock solution.

Table 3. Summary of average times until onset of clinical signs of toxicosis, duration of key symptoms during sickness behaviour, and time to death in possums (*Trichosurus vulpecula*) following ingestion of poison baits (adapted from Eason et al. (2010a), with data included for PAPP).

Toxin	Average time until onset of sickness	Sickness behaviour	Average duration	Average time until death	Reference
Cyanide	3 min	Ataxia, impaired coordination, breathlessness, muscular spasms. Unconscious after 6.5 min	15 min	18 min	Gregory et al. 1998
PAPP	<45 min	Lethargy	30–60 min	40–80 min	Eason et al. 2010b
Zn_3P_2	1.5 h	Vomiting, epigastric pain, ataxia, breathlessness	2.4 h	4.0 h	Eason et al. 2012
1080	Approx. 2 h	Anorexia, ataxia, occasional retching, spasms, breathlessness, laboured breathing	9 h	5 h	Littin et al. 2009
Phosphorus paste	6 h	Retching, vomiting, hunched posture, intermittent repositioning, ataxia	19 h	25 h	O'Connor et al. 2007
Brodifacoum	14 days	Reduced feeding, ataxia, haemorrhages, prolonged lying down	7 days	21 days	Littin et al. 2002

Sublethal effects and exposure risk in humans and animals

Risk assessment is improved when fundamental research provides information about the fate, effects, and risk of exposure to PAPP. Hence, pharmacokinetics and the mechanism of toxicity are important in understanding the response of an animal or humans to a foreign compound. PAPP clearly has the potential to kill and cause transient sublethal effects in humans, but exposure of individuals or communities to amounts of PAPP that would cause such effects is most unlikely when it is used carefully for predator control. The acute toxicity of PAPP is well understood, and the toxic manifestations of sublethal methaemoglobinaemia have been extensively studied as described above (see Mode of Action). These are further explored below. As noted above, methaemoglobinaemia results in tissue anoxia, leading to respiratory failure and death when sufficiently high doses are ingested. In the following sections, we cite publications that show that any sublethal toxicological effects associated with chronic exposure to PAPP are also likely to be linked to induction of methaemoglobinaemia.

Multi-dose studies in animals have been conducted to shed light on additional potential sublethal effects and possible target organ toxicity, using periods of exposure of 14 and up to 30 days' duration (Doull & Plzak 1963; Baskin & Fricke 1992: Blickenstaff et al. 1994). While most studies have focused on the effect of PAPP on red blood cell function, other effects have been recorded. In a review paper, Baskin and Fricke (1992) report results from earlier unpublished trials (Lackenby 1987a, b) on the sub-acute oral toxicity of PAPP in laboratory rats and cynomolgus monkeys exposed to a 14-day treatment period, followed by a 14-day treatment-free period. In both studies, standard haematology, clinical chemistry, urine analysis, and pathology were evaluated. The rat study consisted of four treatment groups, which were dosed daily with 0, 35, 90 or 140 mg kg^{-1} for males and 0, 20, 50 or 130 mg kg^{-1} for females (Lackenby 1987b). Minor histopathological changes observed in PAPP-treated rats returned to control levels following cessation of treatment. In the cynomolgus monkeys (both sexes) dosed daily with 17, 50 or 150 mg kg^{-1} of PAPP, serum chemistry parameters, including lactate dehydrogenase (LDH) levels, increased (Lackenby 1987a). These changes are consistent with an effect on the liver. After treatments ceased, the abnormal serum chemistry values returned to control levels within 28 days. Baskin and Fricke's (1992) review concludes that the pathological and histopathological effects seen with PAPP treatment were to be expected as consequences of high methaemoglobin concentrations, implying an indirect rather than a direct effect of PAPP on the liver. The study on PAPP in this primate species is particularly important with regard to risk assessment for humans and it is notable that the doses of 150 mg kg^{-1} day^{-1} PAPP given to monkeys for 14 days were an order of magnitude greater than the single-dose LD_{50} in susceptible species such as stoats.

PAPP toxicology has also been studied in humans. Sublethal doses of PAPP administered did not adversely affect perception, as measured by visual rod-cell threshold in a dark setting at sea level or high altitudes post-exercise (Bodansky & Hendley 1946). Administration of low doses of PAPP did not decrease the oxygenation of working muscle in men exercising lightly (bicycle ergometer) with PAPP-induced methaemoglobin concentrations of between 7.5% and 15%. However, as the level of methaemoglobin progressed to concentrations above 20%, higher blood lactate levels were recorded in the subjects, with methaemoglobin levels of 21.7% and 27.1% but only at high workloads (Tepperman et al 1946).

Paulet et al. (1963) showed that sublethal doses (80 mg or 100 mg) administered orally to 51 human volunteers produced methaemoglobin concentrations ranging from 2% to 48%. Peak methaemoglobin concentrations were typically reached 1–2 h post-ingestion and produced few if any side effects. There was no evidence of physical, intellectual or psychological impairment, appetite was not suppressed, and renal systems were normal. Furthermore, ventilation rate, arterial pressure, and electrocardiograms were unchanged pre- and post-ingestion, during the expected period of toxicosis in 20 subjects and only slightly altered in two subjects. Haemolysis was not observed even at relatively high methaemoglobin concentrations of 48%. LD_{50} data are not available for humans but, in sublethal studies, oral doses of up to 10 mg kg^{-1} have been administered with few, if any, side effects other than methaemoglobinaemia. Therefore, the available data appear to indicate that PAPP is less acutely toxic to humans than to canids.

The carcinogenic potential of PAPP has been evaluated by in vitro and in vivo testing (Table 4). PAPP is not mutagenic and is unlikely to cause cancer, in part because of its lack of mutagenicity and in part because its use for vertebrate pest control should not involve significant exposure or absorption of the compound by non-target species including humans. PAPP tested as non-mutagenic in the Ames test, mouse micronucleus test, and the human lymphocyte test. In the forward gene mutation mouse lymphoma test, results were ambiguous, implying possible weak mutagenic activity. However, in studies to clarify the genotoxicity and mutagenicity of PAPP, it was shown not to be mutagenic (Table 4).

PAPP clearly has the potential to kill and cause transient sublethal effects in humans and, arguably, additional toxicology studies could provide more information for risk assessment. Equally, or probably more importantly, in a practical sense the use of PAPP must continue to include safeguards that focus on those individuals handling PAPP and operational safeguards to

Table 4. Summary of genotoxicity tests for PAPP.

Test system	Result	Reference
Mouse micronucleus	No increase in micronuclei. Not mutagenic	Asquith 1988a,b
Metaphase analyses in human lymphocytes	No clastogenicity. Not mutagenic	Cordery 1988a,b
Forward gene mutation in mouse lymphoma	Weak indication of mutagenicity	Thompson 1988
Ames test	Not mutagenic	Baskin & Fricke 1992
Unscheduled DNA synthesis in rat	No evidence of unscheduled DNA synthesis. Not mutagenic	Baskin & Fricke 1992

prevent exposure of people to PAPP. Exposure of individuals or communities to amounts of PAPP that would cause effects should be most unlikely when it is handled and used carefully for predator control.

Fate in the environment

PAPP appears to have similar biodegradability and solubility as cyanide and 1080. PAPP is water soluble, so if any bait is displaced from a bait station the PAPP will be easily leached into the soil by rain. PAPP is mobile in soil and is reported to be biodegradable by microorganisms (Southwell et al. 2011) though further details are not provided. This information needs to be viewed in the context of how PAPP will be used. Because stoat numbers are low relative to other pests such as rats or possums, very few baits are used, hence limited environmental contamination will occur and contamination of waterways is unlikely. McLeod and Saunders (2013) report low toxicity to soil invertebrates.

With correct use of bait stations, the risk of contamination is considered very low, and would most likely only occur from deliberate or accidental spills directly into water. For example, in a recent trial, 90 baits were placed out, one per station, at one time over an area of c. 800 ha. Each bait contained c. 0.04 g of PAPP in meatball-type bait (Dilks et al. 2011). In total, there were no more than 4 g of PAPP in the field at one time, thus soil or water contamination would have been limited.

Methods of PAPP application

There has been a sustained effort to improve predator control and increase native species protection in New Zealand, as well as to improve target specificity and reduce non-target mortality. The development of PAPP for predator control has been consistent with this trend. The currently registered product containing PAPP is a soft, green paste that contains 410 mg kg^{-1} PAPP as the active ingredient. It comes in pre-loaded syringes packed in a secure carry-box with instructions. Baits are prepared by enclosing a small amount of paste in green-dyed raw minced-meat to form a small meatball. These meatballs are placed in bait stations. Meat baits containing PAPP must be treated as potentially poisonous to non-target species and must be handled as carefully as any other type of toxic bait. To date, field experience with PAPP is limited, but where it has been used it has been very successful. Trials undertaken in Waitutu Forest, Southland, achieved a reduction in stoat abundance of 83– 87% over five nights. These results indicate that PAPP is an effective toxin for stoats in the field and has the potential to provide a significant new tool for conservation of native species (Dilks et al. 2011). Similar results have been obtained with radio-collared feral cats (Shapiro et al. 2010; Murphy et al. 2011). Long-life baits are being researched and a resetting toxin delivery system to achieve long-term suppression of stoat populations have been shown to be effective in pen trials (Blackie et al. 2012). Stoats have been killed in enclosure trials by a paste containing PAPP sprayed onto the chest and stomach by a resetting systems triggered by the stoats passing through a tunnel (Blackie et al. 2012), and the first field trials to successfully demonstrate that PAPP delivered in a resetting device could reduce stoat abundance were completed in 2013 (Ministry of Business, Innovation and Employment in collaboration with Connovation Ltd and the Department of Conservation).

Conclusions

PAPP is the first toxin registered for stoat control in New Zealand and the first new toxin registered for mammalian pest control anywhere in the world for at least 20 years. The approval of PAPP paste as a stoat and feral cat control agent in New Zealand was achieved through collaboration between Connovation, Lincoln University, and the Department of Conservation. The development of PAPP for predator control in New Zealand also owes a lot to earlier work by researchers investigating the mode of action and toxicological properties of PAPP, as well as its potential for animal pest control.

The availability of PAPP as a new toxin reinforces the concept of the pest control toolbox, where a variety of methods are available for use to protect threatened bird species. With the sustained trapping of stoats over many years in some areas, there is growing concern that trap shyness is developing: having another option such as the use of PAPP will strengthen the ability of pest control agencies and conservationists to better control stoats and feral cats, although selection of the most appropriate tool depends on factors such as site location and ease of access.

In this review, we have reported research spanning 60 years and have focused on several factors, including determining the toxicity of PAPP to predators, its field effectiveness for controlling stoats and feral cats, its animal welfare profile, toxicology, ecotoxicology, and understanding its non-target risk. It has been developed specifically for the control of stoats and feral cats because of the special sensitivity of these species to PAPP. Its toxicity is mediated by the induction of methaemoglobinaemia. The benefits of PAPP use in conservation and pest control should be weighed up alongside the risks of using PAPP and alternative techniques for pest control. Even though there are advantages for PAPP over other toxins, considerable care must be taken when using PAPP to ensure that the risks of its use are outweighed by the ecological benefits achieved. The advantages and disadvantages of PAPP are summarised simply below.

Advantages	Disadvantages
Simple antidote	Not broad spectrum
Humane (very rapid action)	
Low environmental impact and secondary-poisoning risk	

However, there has been limited practical experience with PAPP to date, especially when compared with alternative tools such as traps for pest control or baits containing 1080. The conditions of use imposed by the EPA and the Ministry for Primary Industries (MPI) have restricted the use of PAPP for native species protection so far, in 2013. A review of these conditions has been initiated with both agencies. Innovation needs to be stimulated to encourage improvement in endangered species protection. Unfortunately, regulatory impediments are currently restricting PAPP use to licensed operators. Ideally, researchers need to work closely with regulators, to ensure that vertebrate pest product registrations and regulations on the use of new tools enable conservation. Additional research efforts and practical experience, coupled with more appropriate

regulation on its use, should enable the effective use of PAPP as a tool to help achieve better conservation outcomes within New Zealand. At the time of writing, PAPP can only be used in fresh meat baits, but long-life baits and resetting toxin delivery systems should enable PAPP to be used more effectively for the long-term suppression of predators.

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