A REVIEW OF THE TOXICOLOGY AND ECOTOXICOLOGY OF PARA-AMINOPROPIOPHENONE (PAPP) IN RELATION TO ITS USE AS A NEW PREDATOR CONTROL TOOL 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. Our research over the last 10 years has focused on several factors, including determining its toxicity to predators, its field effectiveness for controlling stoats and feral cats, its animal welfare profile, toxicology, ecotoxicology, and understanding and reducing non-target risk. It has been developed specifically for the control of stoats and feral cats because of the special sensitivity of these species. Its toxicity is mediated by the induction of methaemogobinaemia (the ferric state of haemoglobin). Normally, methaemoglobin levels are <1%. Levels of methaemoglobin above 70% in the blood are usually fatal, creating a lethal deficit of oxygen in cardiac muscle and the brain. Death after a lethal dose in stoats and feral cats usually occurs within 2 hours after eating bait with clinical signs first appearing in 10 to 20 mins. The animals become lethargic and sleepy before they die, hence PAPP is relatively humane. A simple antidote exists, namely methylene blue and PAPP lacks of toxicity to birds when compared to other vertebrate pesticides. A paste containing 40% PAPP has been developed for use in meat baits. 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 following prefeeding, 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 for pest control or baits containing sodium

monofluoroacetate (1080). Additional research efforts and practical experience should enable the effective use of PAPP in New Zealand as a tool to help achieve greater conservation outcomes. 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; predator control; para-aminopropiophenone (PAPP); toxicology; vertebrate pesticide.

INTRODUCTION

The toxic nature of para-aminopropiophenone (PAPP) was first noted when it was being investigated as a treatment for cyanide and radiation poisoning. PAPP has an empirical formula $C_9H_{11}NO$ and a molecular weight of 149.19. It is also known as 1, 4 para-aminopropiophenone, 1-propanone, 1-4 aminophenyl and 4-aminopropiophenone. The active ingredient is a light white powder, with a melting point of 140°C and a boiling point of 482°C.

In New Zealand, the control of introduced terrestrial pest species e.g., possums (*Trichosurus vulpecula*), rats (*Rattus* sp.), mice (*Mus musculus*) and rabbits (*Oryctolagus cuniculus*), is effectively conducted using sodium fluoroacetate (1080) on the mainland and brodifacoum on offshore islands. Trapping is often used to control feral cats (*Felis catus*), stoats (*Mustela erminea*) and ferrets (*Mustela putorius*). 1080 sometimes encounters public opposition (Hansford 2009), while second generation anticoagulants like brodifacoum have a long half-life and bioaccumulate and have welfare concerns associated with their use (Eason et al. 2010). Prior to the registration of PAPP for stoat and feral cat control, 1080 has been the only toxin registered for feral cat control, and the only means of controlling stoats has been trapping. Trapping is often labour-intensive and expensive (Dilks et al. 2011). In this context the need for an effective humane poison for stoats and feral cats was identified (Murphy et al. 2005; Murphy et al. 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 as a new tool for the control of stoats and feral cats . PAPP containing paste was approved as a stoat control agent by the Environmental Protection Authority in August 2011 and for feral cat control in November 2011. Paste containing PAPP applied to fresh meat baits in bait stations has been shown to

be effective for the control of both stoats and feral cats (Dilks et al. 2011; Shapiro et al. 2011).

PAPP was not originally intended to be a vertebrate pesticide and early research in Europe and the USA focused on the potential of PAPP as treatment for cyanide and then for radiation poisoning (Rose et al 1947; Bright and Marrs 1982) in the context of protecting soldiers during conflict from the harmful effects of cyanide gas. In the period 1960-80, during the "cold war" era, PAPP was further investigated as an antidote for human radiation poisoning.

Extensive research has been conducted over a period of more than 50 years, including in-vitro and in vivo toxicology trials, metabolism studies and experiments in human volunteers (Jandorf and Bodansky 1946; Rose et al. 1947; Oldfield *et al*. 1965; Fitzgerald *et al*. 1974; Bright and Marrs 1982, 1983, 1986, 1987; Marrs and Bright 1986, 1987; Marrs et al. 1991; Baskin and 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, it is noteworthy that in early experiments it was observed that PAPP was found to be specifically much more toxic to carnivores than other species and this has been confirmed in more recent studies, with birds and humans being less sensitive (Savarie et al. 1983; Fisher and O'Connor 2007; Murphy et al. 2007).

The use of PAPP as a toxin for control of animals pests was first investigated in the 1980's by researchers of the United States (U.S.) Fish and Wildlife Service (Pan et al. 1983; Savarie et al. 1983; Shafer 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. 2010). 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. It should complement trapping and allow those involved in endangered species protection the ability to use a mix of tools. Most concerns in New Zealand regarding to the use of poisons relate to fears regarding contamination of water supplies, possible sub-lethal effects on humans, welfare impacts and non-target effects. In the following sections the characteristics of PAPP will be described, relative to these concerns including its mode of action and metabolism, acute toxicity in different species, its toxicokinetics and break down in the environment, sub-lethal, relative humaneness and other characteristics.

MODE OF ACTION

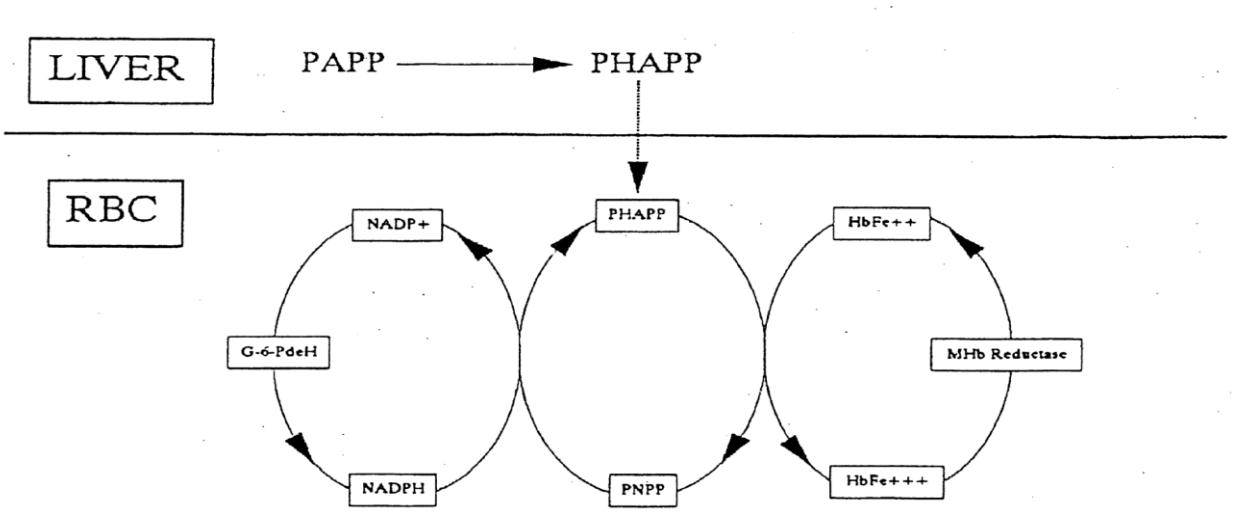
The primary mode of action of PAPP is through a toxic effect on red blood cells, mediated by a toxic metabolite, para-hydroxylaminopropiophenone (PHAPP) (DeFeo *et al*. 1972). Following administration or ingestion in bait PAPP is absorbed into the blood stream. Graffe et al., (1964) have shown that PAPP undergoes metabolic biotransformation to PHAPP and that this toxic metabolite oxidises haemoglobin to methaemoglobin. This reaction can be rapid, peaking in 30 minutes or less (Goldstein and Doull, 1973). The metabolite, though short acting, is a highly effective oxidiser of haemoglobin to methaemoglobin. 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 to effectively 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 (>70-90%) are induced, resulting in a lack of oxygen to the brain and heart and death due to respiratory failure. Normally, methaemoglobin levels in the blood are <1% and symptoms are proportional to the methaemoglobin concentration in the blood and include skin colour changes (with blue or grayish pigmentation) and 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.

PAPP belongs to a class of chemicals broadly classified as aromatic amines. In most species, metabolism of aromatic amines ($R\text{-}NH_2$) occurs via acetylation of the amine moiety, prior to excretion. The N-acetyltransferases (NAT) are cystolic enzymes found in all mammal species, with the exception of canids (Trepanier *et al*. 1997). Two major NAT enzymes, NAT1 and NAT2, have been characterised in humans, rabbits, mice and hamsters and a third functional enzyme (NAT3) has been characterised in the mouse only (Vastis and Webber, 1997). In contrast, NAT activity is absent in the domestic dog, red wolf, arctic wolf, coyote, golden jackal, African wild dog, arctic fox, kit fox, silver fox (Trepanier *et al*. 1997). Hydroxylation of PAPP to PHAPP has been demonstrated *in vitro* using rat, rabbit and human liver microsomes (Graffe et al. 1964; von Jagow and Kiese 1966; von Jagow et al. 1966; Coleman and 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

and cynomolgus monkeys (*Macaca fascicularis*) to PAPP administered orally who found that when PAPP is administered orally, there was a rapid formation of methaemoglobin. However, when PAPP is administered directly into the blood, it did not induce methaemoglobin. Based on 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 (CYP 2C11, 2E1 and 3A) and by at least CYP 2E1, the 2C series and 3A4 in man (Coleman and Kuhns, 1999).

Once conversion to PHAPP has occurred in the liver it participates in a redox cycle in circulating erythrocytes termed the "Kreisprozess" (see Figure 1). Methaemoglobinaemia is exacerbated in this process by intraerythrocytic NADPH generated from glucose-6-phosphate dehydrogenase that also reduces a secondary PAPP metabolite *p*-nitrosopropiophenone (PNPP) back to the potent methaemoglobin inducer PHAPP (Baskin and Fricke 1992). These actions of PHAPP compete with the methaemoglobin reductase system (Smith and Buetler 1966) and the toxicity occurs when the oxidation process exceeds the rate of the reduction process allowing high levels of methaemoglobin to be formed.

Figure 1 The Kreisprozess. Intraerythrocytic recycling of PHAPP and *p*-nitrosopropiophenone (PNPP) to bring about the simultaneous oxidation of haeme Fe^{2+} to Fe^{3+}. The reaction is dependent on glucose-6-phosphate dehydrogenase (G-6-PdeH) for the generation of reducing equivalents necessary to convert PNPP to PHAPP (adapted Baskin and Fricke 1992).



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

While examining the 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 and 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 the 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 investigations into the effects of PAPP noted that lethal doses were far lower in some species (e.g., dogs and cats) than others (Savarie et al. 1983; Sharf et al. 1983) 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 are usually unconscious within 30-45 minutes (Marks et al. 2004; Eason et al. 2011) 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 and Plzak 1963; Bright et al. 1987).

ACUTE TOXICITY

There is an extensive database on the acute toxicity of PAPP in a diverse spectrum of species. As with other poisons, the LD_{50} values that have been reported can be influenced by the method used to deliver the poison, and by environmental or different laboratory conditions

and protocols. Oral gavage of PAPP in several different carrier materials has been a standard dosing style used by many researchers (e.g., Scawin et al., 1984; Savarie et al., 1983, Plzak and Doull, 1962). Alternatively, PAPP has been fed to animals in measured quantities of food stuffs or in prepared baits. Table 1 shows acute oral toxicity LD_{50} values for a number of mammalian species. For laboratory rodents species (i.e., mouse and rat), the LD_{50} values range between 117 mg/kg and > 5000mg/kg. Fisher et al. (2008) reported the LD50 values for possums to be ≥ 500 mg/kg.

In the target pest species in New Zealand, namely stoats and feral cats the LD_{50} of PAPP is < 10 mg/kg. Cats and stoats are extremely susceptible, and as noted above most other carnivores are also highly sensitive to poisoning and the lethal doses are far lower in these species than others (Savarie et al. 1983; Sharf et al. 1983). There is no LD_{50} value for humans; however cynamolgus monkey have been administered 150 mg/kg/day for 2 weeks without mortality, hence the single dose oral LD_{50} for this species is clearly well in excess of this 150 mg/kg and it is probable that this is also the case for humans.

Table 1: Oral LD_{50} values available for PAPP on a range of animal species

Animal	LD_{50} mg/kg (95% C.I.)	Reference
Cat	5.6	Savarie *et al*, 1983
Stoat	9.3	Fisher *et al*, 2005
Coyote	5.6	Savarie et al, 1983
Bobcat	10	Savarie et al, 1983
Kit fox	14.1	Savarie et al, 1983
Ferret	29	O'Connor, 2002
Dog	26 - 43	Murphy *et al*, 2007
Dog (*Canis familiaris*- male)	30-50	Vandenbelt *et al.* 1943
Wallaby	89	O'Connor, 2002
Rat (*Rattus Norvegicus* - male)	177 (119-262)	Savarie *et al*, 1983
Rat (Swiss Webstar)	221	Pan *et al*, 1982
Mouse (*Mus musculus* - male)	233 (186-292)	Savarie *et al*, 1983
Rat (female)	224 (169-308)	Scawin *et al*, 1984
Rat (male)	475 (89-2525)	Scawin *et al*, 1984
Mouse (female)	> 5000	Scawin *et al*, 1984
Possum	≥ 500	O'Connor, 2002
Guinea pig	1020 (760-1520)	Scawin *et al*, 1984

PAPP has been shown to be generally less toxic to birds than to mammalian carnivores (see Table 2). However the birds were administered PAPP directly to the stomach by oral gavage (Savarie et al. 1983; Schafer et al. 1983; O'Connor 2001). Further data has been generated in New Zealand to assess the toxicity to birds of the formulated PAPP paste presented in a meat bait (Eason et al. 2010), as this is how PAPP is currently used for the control of both stoats and feral cats (Dilks et al. 2011; Shapiro et al. 2011). Four bird species were assessed, Australian magpies (*Gymnorhina tibicen*), blackbirds (*Turdus merula*), mallard ducks and weka (*Gallirallus australis*) 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 orally dosed 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, for blackbirds it was 174 mg/kg and for mallard ducks it was 32 mg/kg (Eason et al. 2010). An LD_{50} of 568 mg/kg was calculated for weka; however, this LD_{50} value underestimates the risk to weka as they were affected at the lowest dose tested (62 mg/kg). Weka became subdued and lost their appetite until they were euthanized 30 hrs after dosing. Whilst birds are less susceptible to PAPP than stoats or feral cats, it appears some bird species are adversely affected and it will be important to limit their exposure.

Table 2: PAPP oral LD_{50} values for bird species

Avian Species	LD_{50} (mg/kg)	Reference
Duck (Pekin, mallard)	32	Eason et al.
Duck (Pekin, mallard)	~38	O'Connor, 2002
Eagle	> 50	Savarie *et al*, 1983
Blackbird	174	Eason et al.
American crow	178	Schafer *et al*, 1983
Blackbilled magpie	178	Schafer *et al*, 1983
Crow	>178	Savarie *et al*, 1983
Magpie	178	Savarie *et al*, 1983
Magpie	~1300	Eason et al.
Quail	316	Schafer *et al* 1983
Quail	> 316	Savarie *et al*, 1983
Starling	316	Schafer *et al* 1983
Starling	> 316	Savarie *et al*, 1983
Weka	568*	Eason et al
Australian magpie	1387	Eason et al

*While the weka did not die at lower doses, it was observed at a PAPP concentration of 50 mg/kg that this species became subdued and birds lost their appetite

Eason et al. (2010) have confirmed that whilst ducks are more susceptible to PAPP than other birds, they are still less susceptible than stoats or cats and that there is considerable interspecies variation in response to PAPP by birds. Whilst birds appear to be less susceptible to PAPP than mammalian carnivores, some species are still 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).

The toxicity of PAPP (LD_{50}) via the oral route is characterized by the relative sensitivity of mammals and birds which fall into three general groups:

1. LD_{50} <50mg/kg, with cats the most sensitive followed by stoats>coyote, bobcats>kit foxes, ferrets, ducks and dogs;
2. LD_{50} 100-500mg/kg, encompassing the majority of species tested; and
3. LD_{50} >1000mg/kg, represented by female mice and female guinea pigs and magpies.

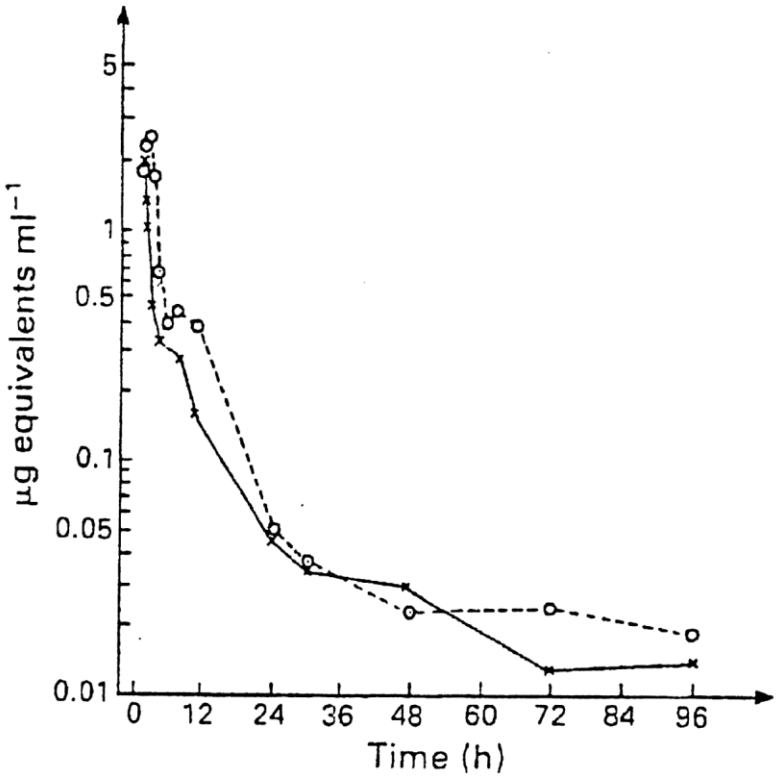
As a result of our understanding of the mode of action of PAPP these variations in the LD_{50} values can be viewed as a function of the differences in the metabolism of PAPP between species and in the metabolism and excretion of the degradation products, PAPP and PHAPP (Wood *et al*. 1991), differences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

Limited data has been found for PAPP with respect to toxicity to terrestrial invertebrates. An OECD 207 earthworm toxicity study determined the EC_{50} (7 day) was > 86 mg/kg (the highest concentration tested). The 14 day data indicated toxicity effects at PAPP concentrations of 24.4 mg/kg (80% survival) and 86 mg/kg (35% survival). An EC_{50} (14 days) was extrapolated as being 61 mg/kg. The relevance of this data is that toxic effects on terrestrial invertebrates are most unlikely to occur when PAPP is used discretely in predator control programmes as these soil concentrations are unlikely to be attained, and if they were it would be in localized areas.

TOXICOKINETICS AND EXPOSURE RISKS

Wood et al. (1991) investigated the metabolism and excretion of radio-labelled PAPP in Sprague-Dawley rats (*Rattus norvegicus*), dogs and monkeys (*Macaca fascicularis*), and found that PAPP was rapidly absorbed by all three species, with peak plasma concentrations at 15 minutes (male rats), 1 hour (female rats), 30 minutes to 1 hour (beagles) and 1 to 1.5 hours (monkeys) after oral ingestion. Levels of plasma radioactivity decline rapidly (see Figure 2).

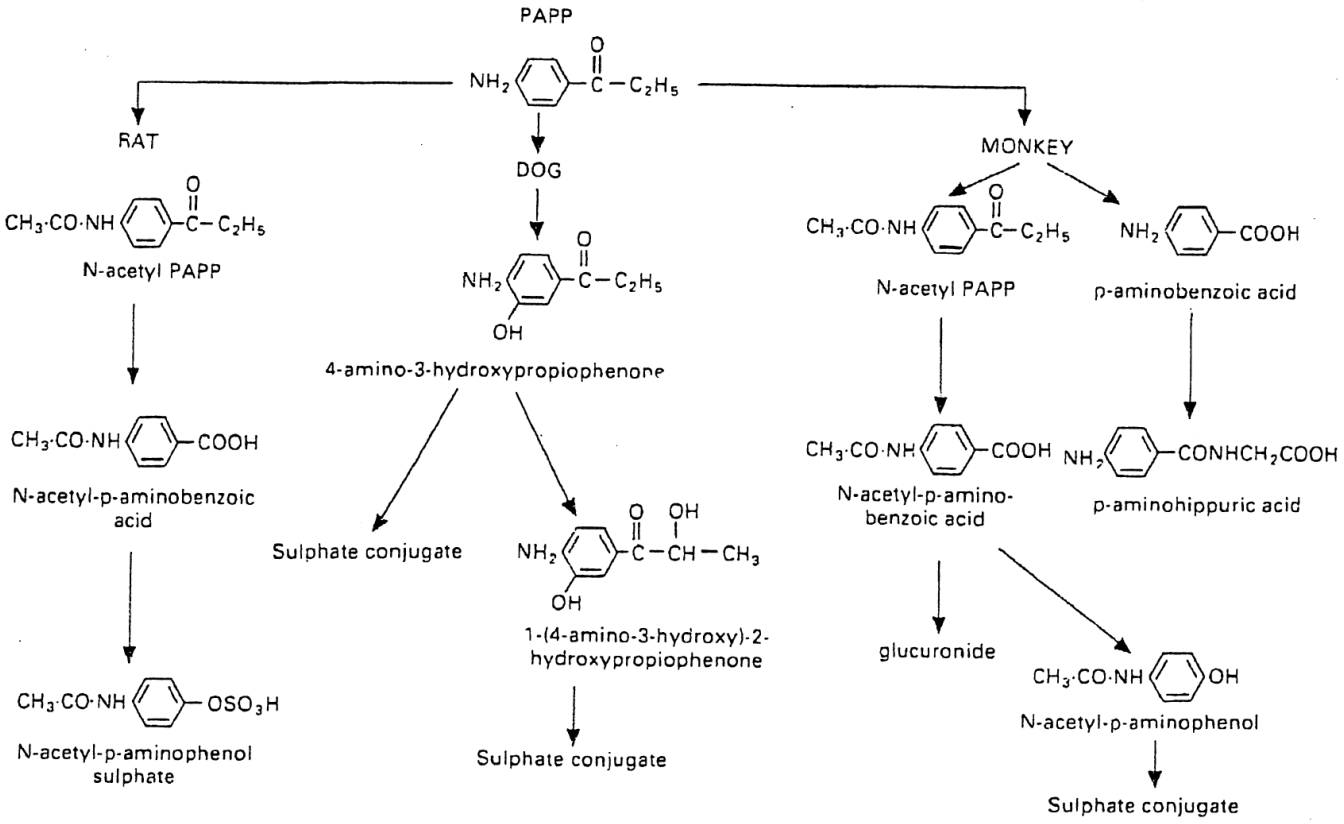
Figure 2 Mean concentration of radioactivity in plasma of rats after gavage with ^{14}C PAPP (adapted from Wood et al.1991).



As described above in the section on Mode of Action there were marked differences in the metabolic pathways in different species. In rats PAPP was metabolised primarily by N-acetylation, whereas in dogs aliphatic hydroxylation predominates and monkeys used both of these metabolic pathways. This increased capacity of primates to metabolise PAPP to non-toxic compounds compared to canines is a 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%), with excretion via faeces and in expired air contributing minimally (Tepperman and Bodansky, 1946; von Jagow and Kiese, 1966; von Jagow *et al*. 1966; Wood *et al*. 1991). Some PAPP derivatives were present in the bile of dogs and rats (Wood et al. 1997), but these appear to be reabsorbed and ultimately also excreted in urine of rats, dogs and rabbits (von Jagow and Kiese, 1966; von Jagow *et al*. 1966; Wood *et al*. 1997). Due to the different metabolic pathways for PAPP between species the forms of PAPP metabolites differ significantly (see Figure 3). (Tepperman and Bodansky, 1946; von Jagow and Kiese, 1966; von Jagow *et al*. 1966; Wood *et al*. 1991).

Figure 3 Differential metabolic pathways for the detoxification and excretion of PAPP in the rat, dog, and monkey



Wood et al. (1991) observed rapid excretion via urine and faeces, which was monitored for 120 hours after an oral dose of PAPP given to rats (5 mg/kg PAPP), beagles (0.5 mg/kg PAPP) and cynomolgus monkeys (25 mg/kg PAPP). The majority of PAPP and its

metabolites were excreted in urine and faeces within 24 hours (93 to 96% in rats, 84% in dogs, and 84 to 95% in monkeys). Excretion of trace amounts was detected in urine and faeces for all species at 120 hours (5 days).

Table 3 Percentage excretion of PAPP in rats, dogs and monkeys over time.

	Time (hr)	**Male rat (n=4)**	**Female rat (n=4)**	**Dog (n=4)**	**Male monkey**	**Female monkey**
Urine	0-6	57.6 +/-	46.4 +/-	50.9 +/-	72, 77	54, 69
	6-24	24.4 +/-	42.1 +/-	25.3 +/-	13, 17	16, 15
	24-48	1.2 +/- 0.7	2.4 +/- 1.0			
Faeces	0-120	9.1 +/- 0.3	3.7 +/- 1.2	7.9 +/-	0.4, 1.9	13.9, 1.2

Data from Wood *et al*. (1991)

PAPP and other phenones have relatively short half-lives of between 1 and 3 hours (Paulet *et al*. 1963; Marino *et al*. 1997). By comparison, the active hydroxylated metabolite PHAPP has an exceedingly short half-life of approximately 1 minute (Wood *et al*. 1991). This rapid metabolism and clearance is a great advantage in the context of the application of PAPP as a prospective predacide as the risks of bioaccumulation in the food –chain or secondary poisoning are significantly reduced. The pharmacokinetics of PAPP have been extensively studied in animal models (Marino *et al*. 1997) and whilst no studies were found where depletion of PAPP was monitored to the point where it could no longer be detected it would appear that PAPP is likely to be completely eliminated in about 5 days.

A comparison has been made (Table 4) between the persistence of different VTA's following sub-lethal doses. There is a huge variation in the way that the different vertebrate pesticides are absorbed, distributed, metabolised and excreted. 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 such as cholecalciferol, and diphacinone which are extensively metabolised to more hydrophilic metabolites, and others which are lipophilic and poorly metabolised and exhibit unique receptor binding characteristics. To help distinguish between different compounds and add some clarity, Eason *et al* (2012) have classified the vertebrate pesticides into 4 groups based on their persistence in sub-lethally exposed animals:

Group 1:-Sub-lethal doses of these poisons are likely to be substantially excreted within 24 hours. e.g. cyanide, zinc phosphide, and 1080. Whilst most of a sub-lethal dose of all these

poisons is likely to be substantially excreted within 24 hours, in the case of 1080, complete excretion of all residues may take up to 4 to 7 days. It is appropriate to consider PAPP as a member of this grouping.

Group 2:- Residues resulting from sub-lethal doses of these poisons are likely to be substantially cleared from the body within 2 to 4 weeks. e.g. pindone and diphacinone.

Group 3:- Residues resulting from sub-lethal doses of these toxins are likely to be cleared from the body within 2 to 4 months. e.g. cholecalciferol and coumatetralyl.

Group 4:- Residues resulting from sub-lethal doses of these poisons may not ever be completely cleared from the body. e.g. bromodiolone, brodifacoum, difenacoum and flocoumafen.

PAPP is likely to be slightly more persistent in live animals than cyanide or zinc phosphide but less persistent than all the other toxins including 1080.

Table 4: Summary of VTA actives with comparison of pharmacokinetics and expected persistence of residues in target species (adapted from Eason et al. 2012)

Group	Active ingredient	Half-life values	Likely persistence of residues after sub-lethal exposure
1	cyanide	+	12 to 24 hours
	zinc phosphide	+	12 to 24 hours
	para-aminopropiophenone	+	4 days
	1080	< 11 hours	7 days
2	pindone	2.1 days	4 weeks
	diphacinone	3 days	6 weeks
3	cholecalciferol	10-68 days	3 months
	coumatetralyl	50-70days	4 months
4	brodifacoum	130 days	24 months or longer

	bromodiolone	170 days	24 months or longer
	flocoumafen	220 days	24 months or longer

+ No published value but likely to be < 12 hours

In conclusion all species rapidly metabolise and excrete sub-lethal doses of PAPP and 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 as illustrated in Table 4. This means it will not bioaccumulate in the food chain and secondary poisoning risks are low, particularly for birds.

TOXICODYNAMICS AND WELFARE

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 death with minimal symptoms of distress. Rapidly induced anoxia is the cause of death and appears to be without appreciable pain or discomfort in much that same way as anoxia induced by carbon monoxide induces carboxaemia. Symptoms are proportional to the methaemoglobin concentration in the blood (Smith, 1967, 1969; Goldstein and Doull, 1973; Rentsch, 1968).

Peak methaemoglobin concentrations in most species follows peak plasma PAPP levels by approximately 30 - 60 minutes (Paulet *et al*. 1963; Bright and Marrs, 1982; Bright and Marrs, 1983; Marino *et al*. 1997). This lag is a function of at least 3 processes: firstly PAPP absorption, secondly PAPP metabolism to an active metabolite, and thirdly the accumulation of PHAPP in the 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 PHAPP will have oxidised proportion of haemoglobin to methaemoglobin. Counteracting this, PHAPP and PAPP are being metabolised to inactive derivatives prior to being cleared via the kidneys. Therefore, by the end of the first and second hours (although it is dose, dosing vehicleand species dependent) methaemoglobinaemia will have either reached peak (or lethal) concentrations, or begun to subside due to the enzymatic reduction of methaemoglobin to haemoglobin by methaemoglobin reductase in which case the animal will recover.

Recent pen trials with 20 feral cats and 15 stoats have been undertaken using meat baits containing toxic doses of PAPP (Eason et al. 2010). Of the 20 cats, 18 died after eating the PAPP bait and two survived. The cats showed a range of responses with onset of symptoms occurring between 35 and 112 min and death between 37–246 min. One animal that only ate 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. For stoats the first symptoms occurred from 6 to 40 min after ingestion and death between 15 and 85 min. There were no signs of discomfort, stress or vomiting associated with poisoning; animals became quiet, lethargic, and then unconscious for a short period before death. These results confirmed the findings of earlier New Zealand studies in these two species (see Table 5)

Table 5: Effects and time to mortality for stoats and feral cats

Reference	Species	Onset of symptoms	Duration of symptoms prior to unconsciousness	Time to death	Signs prior to unconsciousness
Fisher, O'Connor, Murphy (2005)	Stoat	20 min	~ 15 -20 min	40 min	Lack of co-ordination and lethargy
Eason et al. (2010)	Stoat	17 min	~ 20 -27 min	44 min	Lack of co-ordination and lethargy, sleepy; no nausea or vomiting
Eason et al. (2010)	Feral cat	36 min	~ 40 – 46 min	82 min	Lack of co-ordination and lethargy, sleepy, short period of retching in some animals (one minute); reduced by low fat bait

It is noteworthy that cyanide and PAPP cause central nervous system anoxia, lethargy and death, albeit through different mechanisms of toxicity. This sequence of events with both cyanide and PAPP are preferable to those seen with other poisons. In the simplified ranking system outlined in Table 6, 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 it as 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. When these symptoms and times are compared to other VTA toxins (Table 6) it is clear PAPP is comparatively humane. Intoxicated stoats and feral cats become lethargic before lapsing into unconsciousness followed by death.

Table 6: Summary of mean times to onset of clinical signs of toxicosis, duration of key symptoms during sickness behaviour, and time to death in possums following ingestion of poison baits from 4 separate publications (adapted from Eason et al. 2012 with data included for PAPP)

Toxin	Mean time until onset of sickness	Sickness behaviour	Mean duration	Mean time until death	Reference
Cyanide	3 min	Ataxia, impaired co-ordination, breathlessness, muscular spasms-unconscious after 6.5 minutes.	15 min	18 min	Gregory et al. 1998
PAPP	<45 min	Lethargy	30-60 min	40-80 min	
Zn_3P_2	1.5 h	Vomiting, epigastric pain, ataxia breathless	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	14d	Reduced feeding, ataxia, hemorrhages, prolonged lying down	7 d	21 d	Littin et al. 2002

m=minutes, h= hours and d= days

Less susceptible animals that are accidentally exposed to PAPP suffer only a partial methaemoglobinaemia that is transitory and causes no clinical symptoms. 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 antidotes are not available for existing canid control agents such as sodium fluoroacetate ("1080"). 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 (1946) and Stossel and Smith (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 and Gutmann, 1946). In addition, healthy dogs (anesthetized) who were given methylene blue intravenously at doses exceeding 30mg/kg responded only with a rise in total circulating haemoglobin but were otherwise unaffected (Stossel and Smith, 1966). A more recent study has examined PAPP effects on dogs when intravenously or orally followed by administration of methylene blue (per comm Simon Humpreys). The effectiveness of the antidote and its relatively wide therapeutic window make it the clinical treatment of choice and it remains current best practice for the treatment of all methaemoglobinaemias. The treatment indication involves intravenous administration of methylene blue 1-2mg/kg of body-weight formulated as an aqueous solution and administered over a 5 minute period (Anon, 1993; Greenberg, 2001; Boylston and Beer, 2002). Doses of methylene blue should not exceed 7 mg/kg. This also the dose range that is used in veterinary medicine in the treatment of methaemoglobinaemia (Bodansky and Gutmann, 1946). 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

SUB-LETHAL EFFECTS AND EXPOSURE RISK IN HUMANS AND ANIMALS

Risk assessment is improved when fundamental research provides information into 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 sub-lethal 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 sub-lethal methaemoglobinaemia have been extensively studied as described above in the "Mode of Action" section, and 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 sub-lethal toxicological effects associated with chronic exposure to PAPP are also likely to be linked to induction of methaemoglobinaemia.

Multi-dose studies in animals to shed light on additional potential sub-lethal effects and possible target organ toxicity have been undertaken, with periods of exposure of 14 and up to 30 days duration (Doull and Plzak, 1963; Baskin and Fricke, 1992: Blickenstaff et al., 1994). Whilst most studies have focused on the effect of PAPP on red blood function, other effects have been recorded. In a review paper, Baskin and Fricke (1992) report the sub-acute oral toxicity of PAPP in rats and monkeys from earlier unpublished reports. The authors assessed studies in these species that had similar experimental designs, with 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 for males and 0, 20, 50 or 130 mg/kg for females. Histopathological analysis of the spleens revealed a dose-related increase in erythroid hyperplasia, sinusoidal enlargement, erythrophagocytosis, and pigment deposition. Pigment was also evident in the Kupffer cells of the liver and in the renal proximal tubular epithelial cells of rats in the highest dose group. The pigment was still present in the liver, kidney, and spleen of rats in the highest-dose groups at the end of the treatment-free period. The hyperplasia and enlargement, however, had returned to control levels following cessation of treatment. Sub-acute toxicity was studied in cynomolgus monkeys (both sexes) dosed daily for 17, 50 or 150 mg/kg of PAPP. Serum chemistry parameters of the treated animals showed increased LDH (lactate dehydrogenase) levels for

the highest dose group after four days of treatment. After 10 days, bilirubin levels were increased in all of the treatment groups, while LDH was elevated in the 50 and 150 mg/kg dose groups. Female animals showed elevated GOT (glutamic-oxaloacetic transaminase) and GPT in the highest dose group. These changes are consistent with an effect on the liver. After cessation of treatments, the abnormal serum chemistry values had returned to control levels. In summary, the review by Baskin and Fricke (1992) concludes that the pathological and histopathological effects seen with PAPP treatment were to be expected consequences of high methaemoglobin concentrations, which implies the increases in GOT, LDH and bilirubin represent 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 noteworthy that the doses of 150 mg/kg/ day 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. Sub-lethal doses of PAPP administered did not adversely affect perception as measured by visual rod-cell threshold in dark adaptation at sea level or high altitudes post exercise (Bodansky and Hendley, 1946). Nor did administration of low doses of PAPP decrease the oxygenation of working muscle in men exercising lightly (bicycle ergometer) with PAPP induced methaemoglobin concentrations of between 7.5% to 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 (Teppermann and Bodansky, 1946; Teppermann *et al*. 1946). Moreover, Paulet *et al*. (1963) showed that sub-lethal doses (80mg or 100mg) administered orally to 51 human volunteer subjects produced methaemoglobin concentrations ranging from 2% to 48%. Peak methaemoglobin concentrations were typically reached between 1-2 hours 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 in 20 subjects and only slightly altered in 2 subjects. Haemolysis was not observed even at relatively high methaemoglobin concentrations of 48%.

LD_{50} data is not available for humans, but in sub-lethal studies oral doses of up to 10mg/kg of mass have been administered with few if any side-effects other than methaemoglobinaemia.

Therefore the available data appears to indicate that PAPP is less acutely toxic to humans than canids.

Scawin (et al, 1984) also observed that specific abnormal structures (Heinz bodies) develop in blood associated with PAPP exposure. Heinz bodies are small irregular, purple granules in red blood cells caused by damage of haemoglobin molecules. Significant differences between species responses to sub-lethal doses were observed. PAPP caused haemolysis in rats (Beutler and Mikus, 1961) and Heinz body formation in mice and rats in a dose dependent manner, but none of these toxicities were apparent in the guinea pig (Scawin *et al*. 1984; D'Mello, 1986) or man (Beutler and Mikus, 1961). All these animal and human studies indicate that there are no significant or severe chronic systemic toxic or target organ effects from prolonged sub-lethal doses of PAPP, other than secondary effects of methaemoglobinaemia.

The cancer–inducing potential of PAPP has been evaluated by *in-vitro* and *in-vivo* testing. The weight of evidence from the genotoxcity study results cited below indicates that PAPP is not mutagenic and is not likely to cause cancer. The Ames test demonstrated that PAPP in the presence or absence of Araclor-induced rat liver microsome fraction (S-9) was not mutagenic. PAPP has been determined as non–mutagenic in the mouse micronucleus test (bone marrow assay to detect chromosome anomalies) which encompassed PAPP oral administration over the dose range 300mg/kg to 1.2gm/kg. PAPP did not increase the formation of micronuclei in the bone marrow of mice (Asquith, 1988). A chemical that results in increased micronuclei is considered to be capable of inducing structural and /or numerical chromosomal damage indicating PAPP is not mutagenic.

Metaphase analysis of human lymphocytes treated with PAPP (125, 250, 500, 100ug/ml) in the absence of S-9 did not significantly increase chromosomal aberrations and therefore was not a clastogen. Forward gene mutation using mouse L5178Y lymphoma cells treated with PAPP over the range (1.6 to 1000ug/ml) in the absence of S-9 was not significantly increased indicating that PAPP is not mutagenic. In the presence of S-9 both the number of mutant colonies and the mutation frequency were significantly increased indicating PAPP exhibits possible mutagenic potential.

The unscheduled DNA synthesis test demonstrated that orally administered PAPP over the range (25 to 400mg/ml – 80% of LD50, rats) did not induce unscheduled DNA synthesis.

In conclusion PAPP tested as non–mutagenic in the Ames test, mouse micronucleus test, the human lymphocyte test. In the forward gene mutation mouse lymphoma test there were ambiguous results possibly implying weak mutagenic activity. However in studies to clarify the genotoxicity and mutagenicity of PAPP it was shown not to be mutagenic including the Ames test and Unscheduled DNA synthesis test. These studies are summarized below.

Table 7 Genotoxicity test for PAPP

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

In an older *in vivo* study, the effect of PAPP on the carcinogenicity of methylcholanthrene on mouse epidermis (Orechowski et al. 1965) was investigated. This paper reported that PAPP did not alter or increase the occurrence of tumours, and therefore provides evidence that PAPP is not a carcinogen.

There are limited developmental toxicology studies on PAPP. A study by Schafer (et al., 1982) provides an indication that PAPP does not appear to have any effect on the fertility of male quail. However, the reporting of the study design was insufficient to evaluate if the marginal effect apparent on fertility (eggs 80% fertile) and gonad size (an apparent 28% increase in size) were significant or important effects.

PAPP clearly has the potential to kill and cause transient sub-lethal effects in humans and arguably suggests 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 individually handling PAPP and operational safeguards to prevent exposure of people to PAPP. Exposure of individuals or communities to amounts of PAPP that would cause such effects should be most unlikely when it is used carefully for predator control.

FATE IN THE ENVIRONMENT

PAPP is water soluble. This means that if any bait falls out of a bait station the PAPP will be easily leached into the soil by rain. PAPP is mobile in soil and readily biodegradable with unpublished OECD test results showing it degrades within a month (pers comm. Simon Humphreys). Hence, PAPP appears to have similar biodegradability and solubility as cyanide and 1080 and as stoat numbers are low when compared to other pests such as rats or possums very few baits are used. As these baits will be in a secure bait station limited environmental contamination will occur.

As PAPP is water soluble, there is always a risk to waterways from its use. However, PAPP has been developed for use in bait stations, which will be placed away from waterways. With correct use of bait stations, the risk of contamination is considered very small, and would most likely only occur from deliberate or accidental spills directly into water. For example, in the recent Waitutu trial 90 baits were placed out (one per station) at one time over an area of approx 800 hectares and each bait contained approximately 0.04g of PAPP in meatball type bait (Dilks et al.2011). In total there was no more than 4g of PAPP in the field at one time so 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 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

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. Pre-feeding with non-toxic green-dyed minced meat for one to two weeks before laying bait maximizes bait uptake. Meat baits containing PAPP must be treated as potentially poisonous to non-target species and must be handled as carefully as other types of toxic bait. To date the field experience with PAPP is limited but where it has been used it has been used very successfully. Trials have been undertaken in Waitutu Forest, Southland, and stoat abundance reduced by 83% to 87% over 5 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 management of native species (Dilks et al. 2011). Similar results have been obtained with radio–collared feral cats in North Island trials (Shapiro et al. 2010, Murphy et al 2011). In the future PAPP should be used in the fresh meat baits proven to be effective in field trials. Long-life baits are being researched and a resetting toxin delivery system to achieve long-term suppression of stoats is showing promise (Blackie et al 2012).

CONCLUSIONS

PAPP is the first toxin registered for stoat control in NZ and the first new toxin registered for mammalian pest control anywhere in the world for at least twenty years. 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. This was achieved through collaboration between Connovation Ltd, Lincoln University and the Department of Conservation. PAPP's development for predator control in New Zealand also owes a lot to earlier researchers investigating the mode of action and toxicological properties of PAPP as well as its potential for animal pest control. PAPP works as a red blood cell toxin, by preventing haemoglobin from carrying oxygen and stoats and feral cats quickly become lethargic and then unconscious following ingestion of PAPP baits and die shortly afterwards. Stoats are responsible for approximately half of kiwi chick deaths in many areas through New Zealand. Cats also to a lesser extent prey on kiwi chicks. The combined effect of these predators results in only 10% of kiwi chicks surviving to the age of six months. Young kiwi chicks are vulnerable to stoat predation until they reach about 1 - 1.2 kg in weight, at which time they can usually defend themselves. The availability of this new toxin reinforces the

concept of the pest control toolbox where a variety of methods are available for use to protect kiwi and other native birds. The selection of different tools depends on factors such as the ease of access and site location of the area being managed. PAPP will strengthen the ability of pest control agencies to better control stoats and feral cats.

In this review we have reported research spanning 60 years and have focused on several factors, including determining its toxicity 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. Its toxicity is mediated by the induction of methaemogobinaemia. 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 is taken when using PAPP to ensure that the risks of its use are outweighed by the ecological benefits achieved from its use. The advantages and disadvantages of PAPP are summarized simply below.

Advantages	Disadvantages
Simple antidote	Not broad spectrum
Humane (very rapid action)	
Low 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 EPA and MPI have seriously restricted the use of PAPP for native species protection 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. 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 rather than have their use stifled to the detriment of the environment. Additional research efforts and practical experience, coupled

with more appropriate regulation on the use of PAPP should enable the effective use of PAPP in New Zealand as a tool to help achieve greater conservation outcomes. At the time of writing this review PAPP can only be used in fresh meat baits but long-life baits and resetting toxin delivery systems should also enable more effective use of PAPP.

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