The behaviour, pathophysiology and pathology of brushtail possums (*Trichosurus vulpecula*) poisoned with 1080 or brodifacoum, and the implications for possum welfare.

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Abstract

Millions of brushtail possums (*Trichosurus vulpecula*) are poisoned yearly in New Zealand owing to damage caused to agriculture and the environment. They are nonetheless sentient animals capable of suffering, and legislative and ethical obligations, and public concern demand that only the most humane poisons are used (Chapter 1). Accordingly, the aim of this work was to evaluate the effects of two poisons used for killing possums in New Zealand, 1080 and brodifacoum, and the implications for animal welfare. Animal Ethics Committee approval was obtained for all work. A lethal dose of 1080 in carrot baits caused retching, vomiting and seizures in possums caged indoors (Chapter 2). Possums did not fully lose consciousness until death but were likely to have been in a reduced state of awareness beforehand. The first signs of poisoning were observed after an average of nearly 2 h and they died on average 11.5 h after consuming baits, giving a period of potentially reduced welfare of approximately 9.5 h. Six possums of eight that consumed a sublethal dose showed signs of sickness, indicating that some sublethal doses can reduce welfare. Alpha-chloralose (a sedative) and paracetamol (an analgesic) had no effect on the behaviour of caged, 1080-poisoned possums (Chapter 3). The consumption of 0.88 mg/kg brodifacoum in cereal pellet baits by caged possums caused widespread haemorrhages which may have led to weakness, sickness or pain (Chapter 4). Possums did not lose consciousness until death after an average of 21 days but were likely to have been in a reduced state of awareness for up to six days beforehand. Signs of poisoning were first seen after 14 days on average, meaning welfare was potentially reduced for about seven days. Following the consumption of 0.86 mg/kg brodifacoum in cereal pellet baits, blood clotting ability was reduced and all possums had internal haemorrhages, both within eight days of bait being offered (Chapter 4). Possums penned outdoors with space for a high level of activity and exposure to spring and summer weather died after a lethal dose of 1080 or brodifacoum at about the same times as possums caged indoors, and following similar preceding signs of poisoning (Chapter 5). This implied that the results from caged possums are applicable to wild possums in New Zealand at most times of the year. This work, together with that of others, suggests that 1080 is the second-most humane poison for possums and that brodifacoum is among the least humane (Chapter 6).

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Chapter 1
General introduction: the possum problem and the ethical and animal welfare implications of solving it

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Abstract

Possums remain the most important vertebrate pest in New Zealand by virtue of their significant impacts on the environment, threatened endemic plants and animals, and primary production. They are just one of nearly 50 vertebrate species officially considered to be pests in New Zealand. These pests, and the methods used to control them, impact on people, animals and the environment. Hence, any ethical consideration of vertebrate pest control must incorporate the interests of all three. Pest control is no different from any other arena where animals are subject to human interference: such interference must be both necessary and justified. Besides an ethical obligation to ensure that this is the case, strong public concern and legislation demand it. Necessity is judged in terms of whether intervention is required at all, whether killing is necessary if intervention is deemed necessary, and whether there are alternatives to pest control or killing. Non-lethal methods can be used in addition to lethal methods to improve the success of pest control programmes. Pest control is normally justified in terms of whether the benefits to people, animals and the environment accruing from control outweigh any harms caused to people, animals (including pests themselves) and the environment. However, judgements based on this kind of justification are fraught with challenges. Justification can only be strengthened by ensuring that *all* benefits in *all three* areas are maximised, and that *all* harms in *all* areas are minimised as far as possible. Specifically, seven principles ensure that direct, primary benefits are maximised: 1. The anticipated benefits of any pest control programme must be clear; 2. Control should only be undertaken if the benefits are realistically achievable; 3. Methods used must be those that kill the most pests and produce the least unwanted impacts; 4. Methods must be used in ways that ensure the kill rate is maximised while the unwanted impacts are minimised; 5. Whether or not the benefits were achieved must be evaluated at the cessation of any programme; 6. Follow-up steps must be taken to ensure that the benefits are maintained once control has ceased; 7. Pest control methods must be continually improved toward the goal of causing the least amount of harm and producing the maximal amount benefit. Additionally, indirect, secondary benefits must be maximised, for example by the use of by-products from the control (although arguments against this are discussed). The maxim that impacts on people, animal, *and* the environment must be minimised can lead to conflict when particular features of methods are more favourable in one area than another (e.g., if they are cheap but inhumane). In all cases however, the most humane control method possible must be

used, and we must seek ways to improve the humaneness of existing methods and to find new methods that are more humane. This requires a thorough evaluation of the animal welfare impacts of pest control methods. Traps have been and are being evaluated in this respect in New Zealand. This thesis describes part of a collaborative project to assess the behavioural and pathophysiological effects of poisons on possums, and the application of this information to an animal welfare evaluation. It focuses on sodium monofluoroacetate (1080) and brodifacoum, two of six poisons used for possum control in New Zealand.

Introduction

The work described in this thesis is an example of the application of animal welfare science to an issue of potential concern for animal welfare in New Zealand: namely, the use of 1080 and brodifacoum to kill brushtail possums ('possums', *Trichosurus vulpecula*). This thesis therefore incorporates an integrated synthesis of animal behaviour, physiology and ethics pertaining to the investigation of this animal welfare issue. This chapter describes the pertinent ethical and animal welfare background to this issue. The scientific background relevant to each investigation is described in the following chapters.

Possums and vertebrate pests in New Zealand

With high hopes of a future fur trade and no realisation of the problems they would one day cause, early residents first released brushtail possums in New Zealand in 1837 (Pracy 1962). Further releases and spreading continued until 1926. At that stage, there was concern that their numbers would decline, so limits were placed on hunting. This was reversed in 1947, and poison use was legalised for possum control after it was discovered what damage possums did in native forests (Clout & Erickson 2000). Since then, partially with the aid of further illegal distribution by hunters keen to collect government bounties for furs which were offered until the 1950s, possums have spread to cover all of New Zealand except for the most remote mountainous areas and some offshore islands (Clout & Erickson 2000). They are now known to have serious harmful impacts in several areas (Cowan 2001):

- They are predators of birds, chicks, eggs and invertebrates;

- They selectively defoliate trees and disturb ecosystem functioning by contributing to canopy collapse;
- They compete with native and domestic animals for food;
- They graze exotic forestry plantations;
- They are the main wildlife vector of tuberculosis and thereby indirectly threaten international agricultural trade;
- They create problems in urban areas, for example by nesting in houses and polluting water supplies;
- The methods used to control them can also have detrimental effects (e.g., non-target capture and poisoning, public opposition, threats to international trade caused by pesticide residues);
- The cost of controlling them is nearly NZ$50 million per year (Warburton *et al.* in press); this does not include the costs of lost production, repairing the damage they cause or research into their control.

This has led to possums being declared a 'wild animal' under the Wild Animal Control Act 1977, which means they can be hunted by anyone and any means. The other 'wild animals' listed under this act are thar, chamois, wallabies (no particular species is identified), all deer including wapiti and moose, and goats and pigs not kept in captivity as domestic animals, as listed, with their scientific names, in Table 1.

The Animal Health Board, the Department of Conservation and regional councils are responsible for possum control. Pest control contractors normally undertake the control operations, although anyone can buy possum control products off the shelf. Possums are mainly controlled by poisoning (aerial 1080, ground-based 1080, brodifacoum, cholecalciferol, pindone, phosphorus and cyanide are all used in different forms), by trapping (mainly with leg-hold and kill traps) and to a lesser extent by shooting (Eason *et al.* 2000; Montague & Warburton 2000; Cowan 2001). An electrocution trap, cage traps, snares and several other methods of control are also available for killing possums (Eason *et al.* 2000; Montague & Warburton 2000; Cowan 2001).

New Zealand's vertebrate pests and their control

Possums are only one of the nearly 50 introduced vertebrate species now legally considered to be pests in New Zealand. Most of these were introduced intentionally, and for nine of them, control is considered to be a high priority because of their unwanted impacts (Cowan & Tyndale-Biscoe 1997). Table 1 includes all vertebrate species currently recognised as pests in New Zealand by virtue of their listing as 'wild animals' under the Wild Animal Control Act and inclusion in Wild Animal Control Plans, their listing as 'unwanted organisms' or their inclusion in Pest Management Strategies under the Biosecurity Act 1993 (MAF 2002*a*,*b*), or their inclusion in other pest control plans (e.g., the Department of Conservation's Kaimanawa Horse Management Plan; DoC 2001). It also lists some considered to be animal pests by the Department of Conservation (DoC 2002). Several of these species are only considered to be pests in particular areas or situations, and may be valued (e.g., as game animals) or ignored elsewhere. Conversely, there are several animals that are considered to be pests in some areas but that are not managed or legally recognised as such and are not included in this table (e.g., dogs that prey on kiwi, *Apteryx australis*, in Northland).

As would be expected with such a wide range of pests, a variety of non-lethal and lethal methods are used to control them in New Zealand (reviewed by King 1990, 2001*a*; Sadleir 1994; Cowan & Tyndale-Biscoe 1997; Oogjes 1997; Eason & Wickstrom 2001; Eason *et al.* 2000; Montague & Warburton 2000). Non-lethal methods include translocation, repellents, exclusion and removal of food sources (the latter two methods could be considered 'potentially lethal'). Lethal methods include shooting, use of dogs ('dogging'), poisons and biological control involving disease agents, kill- and restraining-traps, and predation, although predation is not always a controlled or controllable method. The kill-traps include head- and neck-hold and electrocution traps; restraining-traps include leg-hold and cage-traps, where the animal is usually killed by the trapper after capture. There are several other methods used overseas including glueboards or sticky traps, snares, immersion traps and non-toxic feeds based on cellulose pellets (e.g., Thompson & King (eds) 1994; Mason & Littin 2003).

Table 1. Vertebrates officially listed as pests in New Zealand (see text for details).

Scientific name	Common name
Amphibians	
Limnodynastes dumerilii	Eastern banjo frog
Litoria aurea	Green and golden bell frog
Litoria raniformis	Southern bell frog
Litoria ewingii	Whistling tree frog
Fish	
Cyprinus carpio	Carp – European/ Koi
Cyprinus spp. (other than Koi)	Carp
Gambusia affinis	Mosquito fish
Ictalurus nebulosus (syn. *Ameiurus nebulosus)*	Catfish– brown bullhead
Leuciscus idus	Orfe
Scardinius erythropthalmus	Rudd
Tinca tinca	Tench
Birds	
Acridotheres tristis	Mynah
Corvus frugilegus	Rook
Gymnorhina hypoleuca and *Gymnorhina tibicen*	Australian magpie
Platycercus eximius rosella	Eastern rosella
Trichoglossus haematodus	Rainbow lorikeet
Mammals	
Bos taurus	Cattle (feral)
Capra hircus	**Goat (feral)*****
Cervus spp.	Deer
Cervus elaphus scoticus	**Red deer *****
Cervus nippon	Sika deer
Cervus elaphus nelsoni	Wapiti
Cervus timorensis	Rusa deer
Cervus unicolor unicolor	Sambar deer
Dama dama	Fallow deer
Odocoileus virginianus	White-tailed deer
Chinchilla laniger	Chinchilla
Equus caballus	Horse (feral)
Erinaceus europaeus occidentalis	West European hedgehog
Felis catus	**Cat (feral) *****
Hemitragus jemlahicus	Himalayan thar
Lepus europaeus occidentalis	European hare
Macropus rufogriseus	Wallaby – Bennett's/ red-necked
Petrogale penicillata	Wallaby – brushtailed rock
Macropus parma	Wallaby – Parma
Wallabia bicolor penicillata	Wallaby – swamp
Macropus eugenii	Wallaby – Tammar/Dama
Macropus spp.	Kangaroo
Mus musculus	Mouse – house
Mustela erminea	**Stoat *****
Mustela furo	**Ferret *****
Mustela nivalis vulgaris	Common/least weasel
Oryctolagus cuniculus	**European rabbit *****
Ovis aries	Sheep (feral)
Rattus exulans	Kiore (Polynesian rat)
Rattus norvegicus	Norway rat
Rattus rattus	**Ship rat*****
Rupicapra rupicapra	Chamois
Sus scrofa	**Pig (feral)*****
Trichosurus vulpecula	**Brushtail possum *****

*** These animals have been accorded a high priority for control according to Cowan and Tyndale-Biscoe (1997) owing to unwanted impacts on people, animals or the environment.

The impacts of pests and pest control methods

Pests can have desirable and undesirable impacts (e.g., King 1990, 2001*b*; Garden 1994; Sadleir 1994; Parkes 1995; Morrison 1996; Livingstone 1996; Cowan & Tyndale-Biscoe 1997; Montague 2000; Willis & Ling 2000), as can pest control methods (e.g., Eason *et al.* 1994; Eason & Spurr 1995; Nettles 1997; Eason *et al.* 1999). These can be conveniently considered as impacts on people, impacts on animals and impacts on the environment (i.e., that apart from animals). The impacts may be direct or indirect, intentional or unintentional, and may occur immediately or in the future. Because people, animals and the environment are interrelated and dependent on each other, these impacts are not mutually exclusive. For example, impacts on the environment can affect animals within that environment, and people who use that environment.

Impacts on people

Pests and pest control methods can impact on several areas that might feasibly be of concern to people, including aesthetics and emotions, economics, health, safety and comfort, cultural values, needs, valued resources (including animal and environmental resources), and recreation. For example, a pest may damage property so that it looks unattractive, or a pest control method may cause a death that looks distasteful. Specific features of pest control that impact on these areas include:

- Efficacy (the kill-rate of the control method);
- Economic features, including the costs of training, capital outlay, maintenance of equipment, and the negative impacts on domestic and international production, trade and related obligations, primary production industries and small industries such as possum fur and meat enterprises;
- Portability, ease and comfort of use, and safety.

Impacts on animals

For animals, chief concerns upon which both pests and pest control may impact are likely to include the survival of individuals and populations, reproduction and animal welfare. Specifically, the impacts of pest control methods on animals can be considered in terms of non-target and target animals. Non-target animals include community and family members and dependants that remain after target animals are killed. The main

impacts on non-target animals are accidental poisoning (primary, secondary or tertiary) or accidental capture, and the intended beneficial impact on the individuals and populations that the control measures are implemented to protect. Impacts on target animals include those due to the intended capture and/or death, and those due to unintended escape from traps and similar devices or survival following sublethal poisoning. Arguably, the impacts on the research animals used during the development and testing of control methods should also be considered in a complete ethical evaluation. For instance, research on the animal welfare impacts of pest control methods often requires the use of captive wild animals and lethal endpoints: in this case there is a clear conflict between the obligation to conduct this research and the obligation to avoid or minimise animal suffering that may be caused by this research (see Littin 1999 for further discussion).

The efficacy and practical features of pest control methods listed above can directly influence animal welfare:

- Efficacy: features of control methods or tools that contribute to efficacy also affect animal welfare. Examples include whether a trap is fit for its purpose (and therefore whether it captures the animal correctly), whether sublethal dosing occurs with a particular poison and whether shooting leads to injury without death;
- Practicality: the ease and comfort of use influence operator mishap or mishandling. This could directly influence animal welfare, for example by affecting the way traps are set.

The efficacy, practicality and cost of pest control methods can also indirectly influence animal welfare by influencing the choice of method in any given situation: a humane method is not likely to be chosen if it does not work, is difficult or unsafe to use or is expensive.

Impacts on the environment

Environmental properties on which pests and pest control may have an impact include the normal functioning of the environmental features (air, water, soil, plants) and the environment as a whole, including its biosecurity, biodiversity and sustainability.

Ethical issues with pest control

Because all pests, and the methods by which they are controlled, have significant impacts, both intentional and unintentional, on people, animals and the environment, any ethical consideration of pest control needs to include the interests of people, animals and the environment. Besides this, pest control is no different from any other occasion where animals are manipulated by people, and the animal welfare and ethical principles that apply to the use of animals in research (Sherwin *et al.* 2003; Smith & Boyd 1991; Mellor & Reid 1994; Mellor 1997, 1998) and to the farming of animals (Mellor & Stafford 2001; Wilson *et al.* 2001; Stafford *et al.* 2002) can also be applied to the killing or manipulation of animals as part of vertebrate pest control activities in New Zealand and elsewhere. It is argued below that this means that while the control of animals that threaten human health, safety or economic well-being, or that have a detrimental impact on the environment, is regarded by many as generally acceptable, it must be absolutely necessary in every case and it must be undertaken in ways that minimise the negative impacts and maximise the positive impacts on people, animals and the environment. In particular, if the action is likely to result in pain or distress for the animals affected, it is important that we seek ways to avoid or minimise such suffering. It is generally accepted that all vertebrates are sentient and are therefore capable of experiencing, at least, pain and distress, and some authors have argued that certain invertebrates should also be included. This is based largely on neuroanatomical and neurophysiological similarity to humans with respect to pain and other sensory mechanisms, behavioural responses to pain and distress, and the evolutionary significance of pain and distress to particular species (e.g., Bateson 1991; Broom 1998; Gregory 2000*b*; Kirkwood & Hubrecht 2001; Rutherford 2002; Sherwin 2001). Hence, we are ethically obliged to avoid or minimise suffering in pests of any of these species.

Public concern as an impetus for action

In accord with the ethical obligation to follow these principles, public concern and legislative requirements provide impetus for action, as follows. Firstly, most people express concern about the welfare of those animals which they believe can feel pain and can suffer, and, increasingly, many also express strong concern for the environment (Kirkwood *et al.* 1994; Broom 1999; Gregory 2000*a*, 2001). This has led to a demand that the negative effects of pest control on non-target animals, animal welfare and the

environment be minimised or avoided, as reflected by the following developments: increasing scrutiny by non-governmental organisations of vertebrate pest control (e.g., Loague 1993, 1994*a*,*b*; Lenghaus *et al.* 1994; Oogjes 1996, 1997, 1999; Ben-David 1996); movement towards sustainable business practices, which require consideration of the impacts of business on society and the environment, rather than just on finance (NZBCSD 2001); and legislative and policy development as discussed briefly below.

While public concern is undoubtedly worthy of consideration, the weight put on public concern in the vertebrate pest control context, as in other contexts, must be carefully evaluated for various reasons. Public views are not always consistent as they may vary over time, and within and between individuals and communities. Moreover, public views are influenced by several features such as context (e.g., hunting for sport versus for food) and the characteristics of animals or the environment (e.g., similarity to humans, appearance, perceived rarity and abundance; King 2001*b*; Garden 1994; Hickling 1994; Morrison 1996; Oogjes 1997; McInerney 1998; Warburton 1998; Marks 1999; Gregory 2000*a*, 2001), and they may be inconsistent with scientific knowledge or practical reality (Bateson 1991; Morrison 1996; Mellor 1999*a*; Gregory 2000*a*, 2001). For instance, the capacity of several species (e.g., rats, rabbits, cats) to feel pain and suffer is usually acknowledged if they are pets, but may be disregarded when they are designated as pests (Oogjes 1996, 1997; Broom 1999). The extent of this disregard, in some respects at least, is likely to be related to the threat that the pest is perceived to pose.

Legislation as an impetus for action

Secondly, New Zealand has several pieces of legislation that deal with aspects of pest control, including the impacts on animal welfare. In this it is no different from some other countries, including Australia (e.g., Oogjes 1999), the UK (e.g., Stroud *et al.* 1999) and Canada (e.g., Warburton 1998). For example, New Zealand legislation including the Wildlife Act 1953, the Wild Animal Control Act 1977 and the Biosecurity Act 1993 require and regulate pest control, the Resource Management Act 1991 aims to minimise the negative impacts of large-scale pesticide use on the environment, and the Hazardous Substances and New Organisms Act 1996 works to limit the risks that vertebrate pesticides pose to people and the environment.

With regard specifically to pest animal welfare, the Agricultural Compounds and Veterinary Medicines Act 1997 requires that all vertebrate pesticides be tested for their impacts on animal welfare, according to particular standards and guidelines modelled on international requirements (MAF 2002*c*). The Animal Welfare Act 1999 regulates traps and 'devices' (electrical or electronic devices used for the same purpose as traps), but specifically exempts from its requirements hunting and killing of pests by any other means, including fishing and the use of dogs for pig-hunting. Nevertheless, pests must not be killed in ways that are unusual or that cause unnecessary or unreasonable suffering (MAF 1999). Traps are regulated by provisions in the Act itself (e.g., that restraining traps be checked within 12 h of sunrise on the day after they were set) and by regulations made under the Act to prohibit or restrict traps if they are considered by the National Animal Welfare Advisory Committee (NAWAC) to cause unreasonable pain and distress. Trap tests to determine the degree of injury caused by restraining traps and times to death for kill-traps, and national guidelines on acceptable limits for these (NAWAC 2000) provide the basis for these decisions (Warburton & O'Connor 2002).

Is vertebrate pest control unavoidable? Is it necessary?

It has been argued that interference with animals in any situation by humans must be necessary and it must be justified (e.g., Smith & Boyd 1991; Banner *et al.* 1995; Gregory 1998*b*). Vertebrate pest control is no different, and if it is to be conducted, it must be necessary and it must be justified. Moreover, some forms of interference, including pest control, may never be considered justifiable, for example, because they cause harm of an unacceptable nature (e.g., Banner *et al.* 1995; Broom 1999).

The ethical obligation to ensure that pest control is necessary has two main implications. First, is it necessary to control the pests at all (Gregory 1998*a*; Marks 1999)? Second, is it necessary to kill the pests in order to control them?

With regard to first question, there are several reasons why pest control may be deemed to be unnecessary and inaction appropriate: 1) The pest might be better left to regulate its own numbers, rather than its population rebounding to the same or higher levels than those present before reduction during a control programme; 2) It might be better to wait until we more thoroughly understand the consequences of pest control, including those that are indirect and unforeseen (Hickling 1994; King 2001*a*; McDonald

& Larivière 2001). For instance, predators may begin to consume threatened native species when their preferred prey is eradicated as part of a pest control programme, as described by Norbury and Heyward (1996) for ferrets after rabbit control; 3) It might be argued that whatever we actively do to pests or the environment could have direct or indirect negative effects on us now or in the future, so that we should not interfere with Nature but should let it take its course. Moreover, the fact that we were responsible for most such animals becoming pests suggests that we cannot always predict the consequences of human intervention and should therefore not interfere further; 4) It might also be argued that human dominance on Earth, with all its attendant problems, is part of evolution and the natural rise and fall of species (Gregory 1998*a*), so that we should not interfere to correct problems, even those we have created.

An alternative view demands that pest control be undertaken: human understanding, however limited, brings with it an obligation to try to rectify problems however they were created, and furthermore, if interference by people caused the problem then people are responsible for rectifying it (e.g., Marks 1996; Spedding 2000). It can also be argued that we are obliged to control pests in order to protect people, other animals and valued features of the environment (Marks 1996; Muschamp 1996).

So what of the second question 'Is killing necessary for control?'. If we deem it necessary to interfere, we then need to consider whether killing *per se* is necessary, or whether non-lethal methods such as exclusion, relocation or the use of repellents would be more suitable. Even if killing *is* used as the preferred method of control, non-lethal methods should be used as well to ensure that all target pests are exposed to pest control methods and that other pests do not return to an area in which pest control has already been conducted (Mason & Littin 2003). For instance, immunocontraception could reduce the rate at which pest populations recover between lethal control operations, thereby reducing the required frequency of such operations (Warburton & O'Connor 2002).

Is vertebrate pest control justified?

Weighing the benefits against the harms

If we decide in principle in favour of intervention, we need to justify intervening in each case. An action is generally judged to be justifiable if it leads to greater benefits than

harms. In the context of vertebrate pest control this is often taken to mean that the anticipated positive impacts of a control programme on people, animals and the environment should be *greater* than the anticipated negative impacts on the pests (Hickling 1994; Kirkwood *et al.* 1994). However, such a justification could be considered ethically weak, because it implies that it is justifiable, without restraint, to obtain desired benefits (for example to people or a few rare, iconic animals in this case) at the expense of some victims (vertebrate pests) (Battye 1994, 1998). The justification can be strengthened ethically by making sure that the benefits of the action are maximised and the harms caused are minimised, so that the separation between the benefits and the harms is the *greatest* that can be feasibly achieved (Battye 1994, 1998). Ethically therefore, it is necessary to ensure that *all* of the anticipated positive impacts of control are maximised and that *all* of the anticipated negative impacts are minimised as far as can be feasibly achieved.

The weight given to the interests of people, animals (including pests) and the environment and the context in which the decision is made, are clearly central issues in such assessments (Morrison 1996; Oogjes 1997; Warburton 1998; Marks 1999; Gregory 2001). In some cases arguments will be so compelling that virtually no one would doubt the need to institute control measures to prevent or mitigate the effects: for instance, devastating plagues involving millions of mice which cause major harm to people, animals *and* the environment (Caughley *et al.* 1994), albeit short-lived, evidently must be controlled or the impacts at least mitigated. In other cases the justification is more complex and may seem to be less compelling: for instance, weighing the negative impacts on sentient pests against the negative impacts on insentient animals or insentient features of the environment such as threatened plants (Marks 1996, 1999; Singer 1997), or weighing the harm to numerous sentient pests against the benefits of protecting a few endangered sentient animals (Marks 1996, 1999).

Maximising the benefits

The benefits of a control programme may be divided into primary and secondary benefits. The primary benefits include the direct positive outcomes of successfully minimising the harms done by the target-species to people, animals and the environment. The secondary benefits include opportunities offered by a control

programme itself or by the utilisation of products of the control programme: for instance, creating a tourist trade in pest control or using by-products such as fur, fibre, horn, skin and meat (Gregory 1998*a*; Warburton 1998). However, it can be argued that secondary benefits must not provide the principal justification for killing a pest, and further, that they are not justified if they do not actually help to achieve the aim of pest eradication or control (e.g., if the pursuit of sport is used to justify deer control, but it does not reduce deer numbers; Eggleston *et al.* 2003). It can also be argued that a trade in products derived from pest control may subvert such control by making the pest a valuable resource, and would impede the drive for eradication by creating a desire to retain a sustainable population of pests (Nugent & Fraser 1993; Loague 1994*a*; Oogjes 1997). Also, international trade in several by-products of the capture of wild animals might not be desirable for the welfare of those animals, particularly in countries where the humaneness of control techniques is not monitored, and where threats to the survival of endangered species would be increased. Nevertheless, trading useable products from the justified control of pests that would otherwise be wasted is a benefit that should be considered in light of the ethical requirement to maximise *all* of the benefits.

Minimising the harms

Successful pest control programmes minimise the negative impacts of the pests on people, animals and the environment by intentionally killing, capturing, removing or excluding the pests or by otherwise neutralising their effects. However, many of the methods employed cause suffering to the target-species and they can also have unintended negative consequences for non-target species, as noted above. Accordingly, these and other harms to people, animals and the environment must be minimised. However, there may be some conflict between the interests of people, animals and the environment in this endeavour (Hickling 1994). For example, humane tools may not be the most the most efficacious (e.g., Warburton 1998).

Negative effects of pest control methods may be limited in practice by careful application of methods and good quality control (e.g., Colvin *et al.* 1988; Frampton *et al.* 1999; Powlesland *et al.* 1999; Morriss *et al.* 2000; Warburton & O'Connor 2002). For instance, the following could all seriously influence both the likelihood of occurrence and the severity of negative impacts on people, animals and the

environment, including animal welfare (Sadleir 1994; Morriss *et al.* 2000; Eason & Wickstrom 2001; Warburton & O'Connor 2002; Mason & Littin 2003):

- The manufacture of poison baits, including formulation, attractiveness, size, colour, and concentration of poison;
- The construction and mechanics of traps, including the clamping force and the use of exclusion devices;
- The use of poisons, including the density at which baits and bait stations are laid;
- The use of traps, including position and correct setting.

With regard to animal welfare, three strategies must be adopted in order to minimise the harms caused by pest control and enable us to behave with more ethical credibility (Mellor 1999*b*):

- We must use the most humane methods currently available. (Arguably we should first investigate, if not use, non-lethal methods, providing they are not less humane than lethal methods; Oogjes 1997.)
- We must seek to improve the humaneness of current methods;
- We must work to develop other more humane methods.

To the last point, it could be added that non-lethal methods should at least be investigated, if not used.

Principles for ethically sound vertebrate pest control

On this basis, seven major overarching principles can be derived to guide the design and execution of ethically sound vertebrate pest control programmes.

1. The harms, aims and benefits of each control programme must be clear.
Harms and benefits must be identified so that they can be minimised or maximised accordingly, and so that the necessity of the control can be determined. This requires a sound understanding of the impacts of the pest in each case. For instance, it would be purposeless to eliminate one pest species from an area if it merely allowed another pest to dominate with equally negative, although possibly qualitatively different, impacts. It must be decided whether the aim is to eradicate, or to reduce or avoid impacts of the pests, as the control method may be different or conflicting in each case (Oogjes 1997).

2. *Control must only be undertaken if the aims can be achieved.*

If the proposed benefits are not achievable the control program cannot be justified (Marks 1999), because the justification for that programme was the projected benefit. The certainty of benefit needs to be assessed and even if the harms are low, control should not be undertaken if the certainty of benefit is low (Hickling 1994).

3. *The methods that most effectively achieve the aims of the control programme must be used.*

The method must kill or deter the most target pests with the least harm to non-target animals, people and the environment. This means that the methods must be appropriate for the species and the situation (Loague 1993; King 1994; King *et al.* 1994; Oogjes 1997). The choice will therefore depend on knowledge of which methods can best achieve the aims with the target-species in their particular locations. For instance, aerial spreading of 1080 poison to kill possums in inaccessible areas compared to the manual placement of 1080 in bait stations.

4. *The methods must be applied in the best possible way.*

This is achieved by good quality control applied to, for example, the manufacture, selection, operation, placement, maintenance and effective use of devices, poisons and other components of each control method (e.g., King & Edgar 1977; King 1981*b*; King 1994; King *et al.* 1994; Moller *et al.* 1996; Short & Reynolds 2001; Warburton & O'Connor 2002).

5. *Whether or not each control programme actually achieved its precise aim must be assessed.*

In reality, control programmes do not always achieve their aims. Whether or not this is the case must be determined so that if necessary, methods can be changed to those that are more likely to achieve the desired aims. The real measure of success is whether a pest control programme reduces the negative impacts of pests, not merely whether the number of pests is reduced following control (e.g., King 1994; Oogjes 1999).

6. *Once the desired aims or benefits have been achieved, steps must be taken to maintain the beneficial state.*

If that were not done, the control programme and any suffering it causes would be purposeless.

7. Pest control methods must be continually improved toward the goal of causing the least amount of harm and producing the maximal amount benefit.

Without continually striving toward a 'gold standard' of zero harm and maximal benefit, the situation would remain as it is presently—far from ideal. All incremental steps towards this standard enhance our ethical credibility and help to meet obligations placed upon us by legislation and a concerned public (Mellor & Stafford 2001). A further implication is that the capacity of any animal to become a pest should be thoroughly researched before it is introduced or before its negative impacts increase to a level sufficient to cause harm. This would help to avoid negative impacts of new pests and related pest control measures in the future (Loague 1994*a*).

To aid adherence to these principles there are numerous practical reports and guides on the choice and effective use of killing and control methods and monitoring. This includes advice for the capture or killing of mustelids (e.g., King & Edgar 1977; King 1981*b*; King 1994; King *et al.* 1994; Moller *et al.* 1996), possums (e.g., Swan 1996; AWAC 1998*a*), cats (AWAC 1998*b*) and fish (Gregory 2000*b*). Documents that outline pest control strategies may also be useful (e.g., Parkes 1993*a*,*b*; Warburton 1995*b*).

Animal welfare and its assessment for vertebrate pest control

The ethical imperative to minimise harm and maximise benefit requires that harms and benefits can be assessed. One harm of concern is compromise to animal welfare.

Defining animal welfare

A definition and assessment methods devised for research animals (Mellor & Reid 1994) and later extended to farm animals (Mellor & Stafford 2001) is also applicable to pest control. It is suggested that when welfare is good, suffering is absent. Good welfare may be said to be present when the nutritional, environmental, health, behavioural and mental needs of animals are met. These areas represent five 'domains of potential welfare compromise'. Poor welfare is present when there is compromise in one or more of these domains. The domains are interconnected: compromise in the first four, largely physical domains either leads to or adds to compromise in the fifth, largely mental, domain. The fifth domain is comprised of the components of suffering, including anxiety, fear, pain, distress, sickness, hunger and thirst. Obviously the degree

of anxiety, fear, hunger and the like is important in an assessment of suffering, since animals would not survive without at least some degree of fear driving escape from predators, hunger driving eating and so on. Because one or more of these components arises from the use of many pest control methods, such an approach to defining animal welfare is evidently well suited to the vertebrate pest control context.

By contrast, definitions of animal welfare centred on fitness or survivability (e.g., Barnett & Hemsworth 1990; Broom 1991) have limited application to pest control as their purposes are generally antithetical to these views—the purpose of pest control is to reduce fitness and therefore survivability. Likewise, definitions focussing on the animals' demands and feelings (e.g., Dawkins 1990; Fraser & Duncan 1998) have limited application to pest control because we would expect that animals would not choose to experience the effects poisons have, and likewise that the feelings they experience after poisoning would be largely negative. This does not mean that these definitions have no application to pest welfare, however. For example, the aversiveness of pest control methods could be an indicator of their impact on animal welfare (Mason & Littin 2003).

Guidelines for assessing animal welfare

There are some published guidelines for assessing the impacts of pest control on animal welfare. These include an international trap standard developed by the International Standards Organisation (ISO 1999*a*,*b*), a New Zealand trap standard based on the international standard (NAWAC 2000), and UK (PSD 2001) and New Zealand guidelines (MAF 2000*c*; Littin & O'Connor 2002) for the assessment of the animal welfare impacts of vertebrate pesticides. In addition, several reviews of the animal welfare impacts of vertebrate pest control tools outline methods for their assessment (Rowsell *et al.* 1979; Kirkwood *et al.* 1994; Sainsbury *et al.* 1995; MAFF 1997; Broom 1999; Mason & Littin 2003). For instance, Kirkwood *et al.* (1994) suggest that welfare compromise caused to wild animals by humans can be evaluated using the number of animals affected, the type and intensity of harm (i.e., the level of stress, anxiety and fear, boredom and frustration, pain and discomfort, suffering, and disease), the duration of exposure to harm and the capacity of the animal to suffer as indicators of the severity of welfare compromise. They also provide a methodology to do this. Using the methodology of Kirkwood *et al.* (1994) to determine the welfare impacts on wildlife of

human interference including poisoning with anticoagulant rodenticides, Sainsbury *et al.* (1995) graded stress into three categories of severity: 'physiological stress', i.e., where a small amount of physical resources is put into maintaining normal functioning and the animal is not aware of it, 'overstress', i.e., where a significant level of resources are used and the animal remains unaware of it, and 'distress', i.e., where the animal is aware of the process and may experience negative side-effects. They categorised fear as present or absent, and pain was judged as 'pain' or 'severe pain' on the basis of human experiences of similar pathological effects.

These and similar ideas (Gregory 1998*a*; Broom 1999; Mason & Littin 2003) together suggest that four features are important in an assessment of the impacts of poisons on possum welfare, as follows.

Assessing the quantum of animal welfare compromise

An assessment of the total impact of a vertebrate pest control method on animal welfare needs to include consideration of *all* the effects of the pest control, intended and unintended, on *all* sentient animals impacted (as discussed earlier). There are four essential features of such an assessment: the capacity of the animals to suffer, the duration of all suffering, the intensity of all suffering, and the number of animals affected (e.g., Kirkwood *et al.* 1994; Gregory 1998*a*; Broom 1999; Littin & O'Connor 2002).

Capacity to suffer

This means the capacity of the species to suffer at all, with the general acceptance that all vertebrates can suffer (as noted earlier), and the capacity of the species in question to suffer in particular ways (e.g., pain compared to anxiety). The latter requires consideration of whether individual or species traits introduce or predispose animals to certain welfare consequences (e.g., Spedding 2000; Beaver *et al.* 2001). Such traits might include normal diet, food and water requirements, basal metabolic rate, normal pattern of reproduction and whether the animals are nocturnal or diurnal, solitary or social. Finally, animals must be conscious (i.e., not anaesthetised or comatose) to be capable of suffering.

In this thesis, I assume that possums are capable of experiencing pain and distress on the basis that the same arguments extended to other animals and described

earlier can be extended to them: they show behaviour that suggests their welfare is poor in situations where this could be expected [as described later in this thesis and by Spielman (1994), Eason *et al.* (1996) and Gregory *et al.* (1998, 2000)] and their neuroanatomy is sufficiently complex to suggest, at least, that they are capable of the conscious recognition of pain (see Beck *et al.* 1996 and Catania *et al.* 2000).

Duration of suffering

The duration of each potentially unpleasant effect such as retching, vomiting or physical trauma and the overall duration of suffering are important. The overall duration cannot be ascertained from the time to death alone because death can occur after a period of unconsciousness during which the animal does not suffer. Thus, the duration is the period from the onset of any signs of potential or actual suffering until permanent loss of consciousness or death, if death is not preceded by unconsciousness. It must be established that the loss of consciousness is permanent. Otherwise there is the possibility that unconscious animals may regain consciousness and could then suffer from the negative consequences of events that occurred during unconsciousness (e.g., physical trauma caused during seizures).

Intensity of suffering

The intensity of suffering needs to be assessed according to the nature of any suffering (e.g., thirst, hunger, cold, pain, distress, social isolation, fear) and the relative severity of each form of suffering (i.e., mild, moderate, marked, severe, or similar classification) as outlined, for example, by Kirkwood *et al.* (1994), Mellor and Reid (1994) and NAWAC (2000). It can also be helpful to consider the experiences of humans and other animals poisoned with the same or similar compounds in order to anticipate some possible types of suffering.

The number affected

An animal welfare assessment of vertebrate pest control must consider the total number of animals subjected to control, and the proportion of animals affected in different ways by each method. While it does not matter to the animal whether it is the only one that suffers or is one of many, the greater the number of animals involved the greater the difficulties of reducing the total quantum of welfare compromise (Spedding 2000).

A methodology for assessing the animal welfare impacts of poisons on possums

These requirements suggest that an experiment to assess the animal welfare impacts of a vertebrate poison must obtain all of the following information:

- The nature and duration of each effect and the overall duration of sickness (requiring the recording of the time at which dosing starts and finishes, and the time at which the signs of sickness are first and last seen);
- The number of animals experiencing each effect;
- The times at which unconsciousness and death occur.

The studies described in this thesis were designed to obtain this information for possums poisoned with brodifacoum and 1080. It is well recognised that sound animal welfare assessments are based on several indicators as opposed to a single indicator (e.g., Barnett & Hemsworth 1990; Broom 1991; Poole 1992; Broom & Johnson 1993; Mason & Mendl 1993). This means that behaviour, physiology, pathophysiology and pathology should all be considered. Hence, my studies were based on direct observation and published reports of the behaviour, pathophysiology and pathology of poisoned possums. In the case of vertebrate pesticides, a sound knowledge of the toxicodynamics (mode of action) and toxicokinetics (absorption, distribution, metabolism and excretion) can help understand the potential and likely animal welfare impacts.

Review of research toward humane pest control

It has been noted above that there are ethical and legal requirements to conduct research evaluating the animal welfare impacts of vertebrate pest control methods, and the information collected must fulfil those requirements.

European research and changes to legislation, both driven by concern for the animal welfare impacts of hunting and killing wild animals, began more than 50 years ago (King & Edgar 1977; Thompson 1994). Similar concerns drove research in Canada (Rowsell *et al.* 1979; Warburton 1998). More recently, the animal welfare impacts of several methods of vertebrate pest control or procedures that could be used in pest control, including hunting, fishing, trapping and poisoning, have been reviewed

(Kirkwood *et al.* 1994; Sainsbury *et al.* 1995; Broom 1999; Gregory 2000*b*; Mason & Littin 2003).

Similar concerns about the humaneness and effectiveness of the Lanes-Ace toothed leghold traps ('gin' traps) in New Zealand led to the introduction of Fenn traps for mustelids (ferrets, stoats and common weasels) in 1972 (King & Edgar 1977; King 1981*a*), and the development of a more humane cage trap for mustelids (King & Edgar 1977). In the early 1980s research was initiated into testing and improving the humaneness and target specificity of existing traps for possums while maintaining other desirable features (e.g., portability, trapper safety), and into developing new more humane traps (e.g., Warburton 1982, 1992, 1998; Dix *et al.* 1994; Warburton & Hall 1995; Warburton & Orchard 1996; Nutman *et al.* 1998; Warburton *et al.* 1999; Morriss *et al.* 2000; Warburton *et al.* 2000; Warburton & O'Connor 2002). Research has also aimed to improve the target specificity of traps without reducing their efficacy (e.g., Morriss *et al.* 2000). This led to the development and use of traps that cause less severe injuries, and which are now favoured by pest control managers (King & Edgar 1977; Sadleir 1994; Warburton 1992).

New Zealand researchers have also played a role in the development of updated international standards for trap efficacy and humaneness (Jotham and Phillips 1994; Warburton 1995*a*; Warburton *et al.* in press), and there is a draft New Zealand standard to ensure that traps meet acceptable national and international standards for animal welfare, user safety, trap efficacy and target specificity (NAWAC 2000). To date, several traps for possums, Norway rats, ferrets, stoats and feral cats have been tested according to this standard (Warburton & O'Connor 2002; Warburton *et al.* in press).

Biological control methods are being investigated worldwide. New Zealand research is on the immunosuppression of reproduction and the introduction of parasitic and other disease agents to control, for example, possums (Cowan 2000, 2001) and mustelids (King *et al.* 2001; McDonald & Larivière 2001). New Zealand work has also provided complementary support for Australian research into Rabbit Calicivirus Disease (RCD) before its illegal introduction into New Zealand in 1997 (Lenghaus *et al.* 1994; Morrison 1996; Norbury 2001). Animal welfare has been and remains an important factor in the appraisal process for selecting possible biological control options (e.g., Lenghaus *et al.* 1994; Fitzgerald *et al.* 2000).

With regard to vertebrate poisons, there is a growing amount of literature specifically on the animal welfare impacts of some control methods (e.g., Rowsell *et al.* 1979; Kirkwood *et al.* 1994; Sainsbury *et al.* 1995; MAFF 1997; Mason & Littin 2003). New Zealand and international research aims to improve existing vertebrate poisons or to develop novel ones which cause less animal welfare compromise, are safer for people and the environment, and are more target-specific (e.g., Eason 1996; Cook 1998; Frampton *et al.* 1999; Gregory *et al.* 2000; Willis and Ling 2000; Warburton & O'Connor 2002). For example, Marks *et al.* (2000) described methods for ameliorating the potential suffering of foxes (*Vulpes vulpes*) poisoned with 1080.

Until recently there was a dearth of published research on the animal welfare impacts of the vertebrate poisons used in New Zealand, so that at present, opinions and practices within the New Zealand pest control industry and among members of the public regarding humaneness are still based largely on perceptions rather than scientific evaluations. Thus, Department of Conservation staff do not permit the use of phosphorus (Sadleir 1994), whereas some regional councils still favour its use for possum control where there would otherwise be risks to dogs from eating possum carcasses containing 1080 residues (Eason *et al.* 1998; Eason & Wickstrom 2001). In addition, it is essential that the animal welfare impacts of *all* poisons be assessed in *all* species, because species-specific differences in absorption, distribution, metabolism, excretion and pathophysiological effects of poisons (e.g., Williams 1974; Walker 1978; Rozman 1988) make it inappropriate to extrapolate data from one species to another. Some authors have described the effects of some poisons for possums (e.g., Bell 1972; Morgan 1990), but the studies have been qualitative rather than quantitative, or do not contain all the essential information listed above.

These concerns and RNZSPCA opposition to the use of phosphorus (Loague 1994*a*) prompted the research programme of which my studies are part (Eason *et al.* 1998). Published and unpublished work from this programme at present apparently constitutes the sole body of work specifically on the animal welfare impacts of any vertebrate poisons currently used in New Zealand.

The overall aims of this research programme, of which my studies form a part, were to assess the animal welfare impacts of five of the six poisons used for possum control in New Zealand: cyanide (Gregory *et al.* 1998), 1080 (see Chapter 2), brodifacoum (Littin *et al.* 2002, and see Chapter 4) and cholecalciferol and phosphorus

(Eason *et al.* 1998). Pindone, an anticoagulant poison, is also used to kill possums in New Zealand but was not investigated as part of this programme as it is not widely used owing to its relative ineffectiveness for possums (Eason *et al.* 2000; Eason & Wickstrom 2001) and its mode of action is the same as that of brodifacoum. The programme was expanded to include some anticoagulant rat poisons, including brodifacoum (Littin *et al.* 2000).

Accordingly, the aims of this thesis are:

- To describe the behavioural changes seen in possums poisoned with 1080;
- To describe the behavioural, pathophysiological and pathological changes seen in possums poisoned with brodifacoum;
- To determine from these the possible impacts of these poisons on possum welfare;
- To determine whether some negative animal welfare impacts of 1080 can be mitigated;
- To assess the relative humaneness of the poisons most commonly used for killing possums in New Zealand.

Chapter 2 deals with the behavioural responses of caged possums to 1080 and the implications for animal welfare. *Chapter 3* is an account of a pilot study to assess whether additives could ameliorate the potential pain or distress caged possums might experience when poisoned with 1080. *Chapter 4* contains descriptions of the behaviour, coagulopathy and pathology of caged possums poisoned with brodifacoum, and an assessment of the implications for animal welfare. *Chapter 5* contains a description of the responses of penned possums to 1080 or brodifacoum, a comparison of these responses with those of caged possums, and an assessment of the implications of this comparison for the application of the outcomes of the studies described in this thesis to free-living wild possums. Finally, in *Chapter 6* some underlying methodological concerns and the application of these studies to the practical assessment of both the animal welfare impacts and the relative humaneness of vertebrate poisons are discussed.

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Chapter 2
Behaviour and time to unconsciousness of brushtail possums (*Trichosurus vulpecula*) after consuming a lethal or sublethal dose of sodium monofluoroacetate (1080), and the implications for possum welfare

Author's note: A condensed version of this chapter has been prepared to submit for publication as an article:

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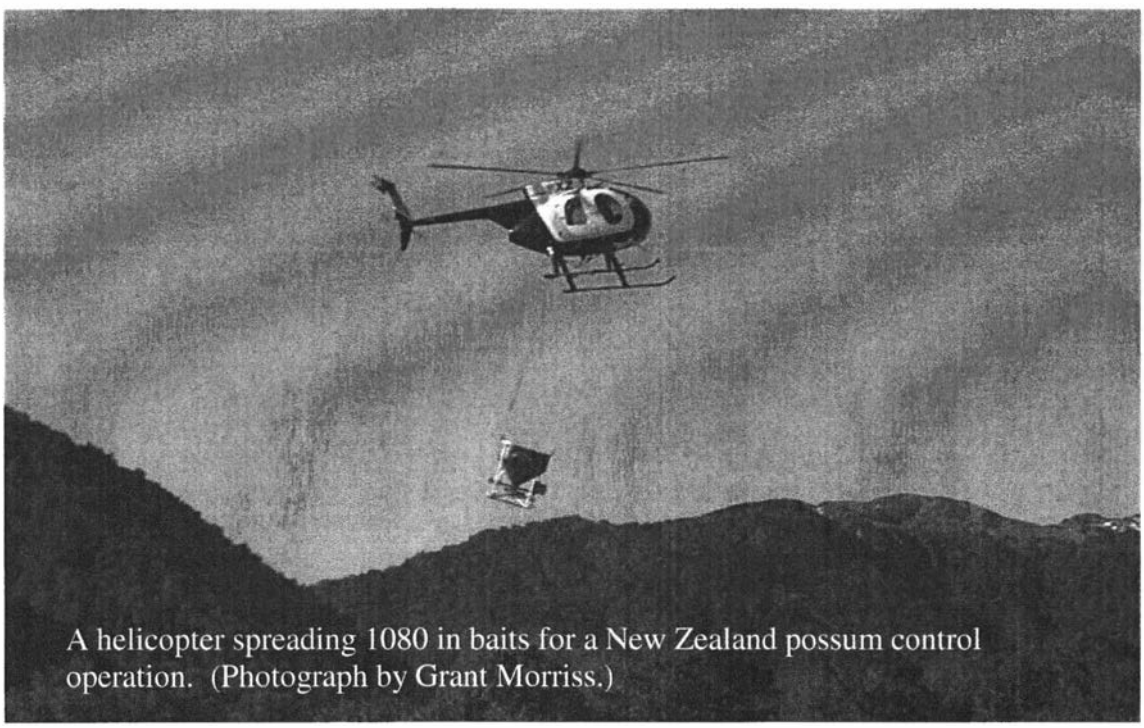
It has also been presented at a conference as follows:

Littin, K.E.; O'Connor, C.E.; Eason, C.T.; Gregory, N.G.; Mellor, D.J. (2002) How humane is 1080 for possums? (Abstract). In 'Animal Welfare: From Science to Solution, Programme of the International Two Day Conference held at Novotel, Hamilton, New Zealand, 27th–28th June, 2002.' p. 32.



A helicopter spreading 1080 in baits for a New Zealand possum control operation. (Photograph by Grant Morriss.)

Table of contents

Abstract

Sodium monofluoroacetate (1080) is the most commonly used poison for controlling brushtail possums (*Trichosurus vulpecula*) in New Zealand. Its general effects on the behaviour of possums have been described but not quantified, and there has been no work on the impacts of 1080 on possum welfare. The objectives of this study were to quantify the behavioural effects and time to loss of consciousness in possums fed a sublethal or lethal dose of 1080 in a commonly used bait, in order to assess the duration and nature of any sickness or suffering caused. In one experiment, eight possums that had consumed a lethal dose of 1080 in carrot baits (3.29 ± 0.16 1080 per kg body weight, compared to a LD_{50}[1] of 1.2 mg/kg) and eight that had consumed a sublethal dose (1.10 ± 0.14 mg/kg) were observed regularly until death or recovery. In two further experiments involving lethal doses, ten possums that consumed 3.39 ± 0.06 mg/kg and nine that consumed 3.67 ± 0.06 mg/kg of 1080 were handled to test their responses to stimuli and the presence or absence of reflexes, in order to determine the time of onset of unconsciousness. All possums which had consumed a lethal dose of 1080 died. In unhandled animals, death occurred after an average of 11 h 26 min ± 1 h 55 min. They had an altered appearance and adopted abnormal postures beginning 1 h 33 min to 2 h 24 min after dosing. Shortly after that, seven possums retched in up to six bouts, and three vomited, over a 27-min period. Possums then became incoordinated and later all were lying for more than 75% of each hour for a maximum period of 14 h 15 min before death. All possums experienced myoclonic spasms and tremors during this period. Five experienced tonic, clonic, and tonic-clonic seizures (occurring within 1 h 15 min of death in all but one possum), accompanied by vocalisation. The sublethal dose of 1080 caused two or more of abnormal appearance or posture, lethargy, ataxia, retching, myoclonic spasms or tremors, or bouts of prolonged lying in six possums. All sublethally dosed possums did not begin grooming until 19 h after dosing, or actively moving about or feeding until 26 h after dosing. One possum in this group died 18 h 15 min after dosing, experiencing two seizures within 30 min of death. In the remaining experiments, handled possums gradually lost most responses to stimuli, with the righting response, response to handling and palpebral and corneal reflexes remaining until death in most animals. This suggests that possums remained conscious and

[1] The median dose that kills 50% of the exposed animals.

therefore may have been capable of experiencing noxious effects of 1080-poisoning until shortly before death. Retching and vomiting are not likely to have caused substantial suffering as both were rated as only mild to moderate in severity, and were short-lived. However, tonic seizures may have caused pain, and tonic-clonic seizures may have caused distress or pain if possums sustained physical injuries during the seizures, or experienced post-ictal headache. The duration of behavioural change and the time to death following 1080 poisoning was intermediate compared to other poisons used to kill possums in New Zealand. Nevertheless, the possibility that possums may suffer distress during or after retching and seizures means that it may pose some risk to possum welfare.

Introduction

Sodium monofluoroacetate (1080) is the most widely used poison for controlling brushtail possums (*Trichosurus vulpecula*) in New Zealand (Eason *et al.* 2000), both in terms of the amount used and the land area covered. It is also used to kill other animals in New Zealand, and is registered for controlling animals in other countries including Australia (e.g., for foxes, *Vulpes vulpes*; feral cats, *Felis catus*; and dingoes, *Canis familiaris dingo*) and the USA (for coyotes, *Canis latrans*) (Burns *et al.* 1996; Short *et al.* 1997; NRA 2002). In the context of research, it is used to study brain metabolism and the importance of neuroglia for the functioning of the central nervous system (e.g., Paulsen *et al.* 1987; Fonnum *et al.* 1997).

Techniques for 1080 use have been reviewed elsewhere (Morgan & Hickling 2000), but briefly, the aim of control using 1080 is to either achieve initial knockdown of possum numbers, or to sustain low numbers after initial control. The main method of distribution in New Zealand since 1956 has been aerial sowing, but it is also used in bait stations or on the ground (Morgan & Hickling 2000). Carrot or cereal baits (usually containing 0.15% 1080) are spread aerially over areas of up to 20,000 ha at sowing rates of 10 kg/ha and 5 kg/ha respectively, or cereal, carrot, paste or gel baits are laid by hand in bait stations. Kills of 80–95% achieved within two days of an aerial operation are possible (Eason *et al.* 1993; Morgan & Hickling 2000). Given that possums are found at densities of up to 25/ha in favourable habitats (Efford 2000), one aerial operation over such habitat could kill almost 500,000 possums. With about 1,700 tonnes of 1080 used each year at a rate of 5 or 10 kg bait / ha (Morgan & Hickling 2000), it follows that

roughly 2–4 million possums are killed each year with 1080 in New Zealand. Although usage has decreased over the last five years, further decreases seem unlikely as the Animal Health Board aims to substantially reduce the incidence of tuberculosis, which will require greater usage of 1080 and the killing of a greater number of possums. Further, 1080 is still widely used by the Department of Conservation for aerial control of possums (Innes and Barker 1999) and has a number of advantages, discussed below, which help to ensure its continued use until suitable alternatives are found.

1080 is the sodium salt of fluoroacetic acid, a naturally occurring toxin present at harmful levels in at least four plant genera: *Acacia* and *Gastrolobium* (found in Australia), *Dichapetalum* (Africa) and *Palicourea* (South America) (Twigg 1994). It is found at lower levels in a range of other plants (Twigg *et al.* 1996). Co-evolution with these plants has conferred tolerance to its toxic effects on several animal species (Egekeze & Oehme 1979; Twigg 1994). It was first synthesised in 1896 but was not isolated from plants until around 1943 (Peters *et al.* 1981). It was already renowned for its toxicity to several species when first described as a rodenticide with the label 'Compound 1080' in 1945 (Chenoweth 1949).

Toxicity

1080 is potentially toxic to all animals including invertebrates, but the toxicity varies widely between species (e.g., Chenoweth 1949; Egekeze & Oehme 1979; Rammell & Fleming 1978; Osweiler *et al.* 1985; McIlroy 1981, 1986). In general, invertebrates, poikilotherms, primates and birds are relatively resistant to 1080 whereas other homeotherms are less resistant, with carnivores and rodents being particularly susceptible (Chenoweth 1949; Egekeze & Oehme 1979). For example, an LD_{50} of 500 mg/kg was reported for rainbow trout (Bauermeister *et al.* 1977) and 72 mg/kg for native ants (Booth & Wickstrom 1999) compared to 0.07 mg/kg for dogs (Eason & Wickstrom 2001).

There are several reported LD_{50}s for possums (reviewed by McIlroy 1983). A recent review gave an LD_{50} of 1.2 mg/kg for New Zealand possums (Eason & Wickstrom 2001). For higher median lethal doses, Morgan (1990) cited unpublished data indicating that an average dose of 2.3 mg 1080 per kg body weight killed 83% of

273 possums captured from throughout New Zealand, and McIlroy (1983, citing Batcheler 1982[2]) gave an LD_{95} of 2.5 mg/kg.

Advantages and disadvantages

A number of advantages have been used to justify the continued use of 1080:

- It can rapidly kill a high percentage of possums (e.g., Morgan & Hickling 2000);
- It is completely biodegradable and water soluble (e.g., Eason *et al.* 2000);
- It can be spread by air, allowing coverage over difficult or steep terrain (e.g., Morgan & Hickling 2000);
- It is relatively cheap compared to other poisons (Eason & Wickstrom 2001).

Conversely, its disadvantages include the following:

- Its toxicity to other animals, although non-target poisoning can be minimised by sensible practices during use (e.g., Chenoweth 1949);
- The need for a license (only granted to specific users, normally those working for pest control contractors or the Department of Conservation; NZFSA 2002*a*);
- There is no satisfactory antidote, although work continues in this area (Cook *et al.* 2001);
- Bait shyness can occur due to either innate aversion, or sublethal dosing causing learned aversion (e.g., Hickling *et al.* 1999). Sublethal dosing also has negative consequences for animal welfare (discussed further below);
- It has also been questioned whether manufacture will be discontinued and international supplies run out (Eason *et al.* 1993).

[2] Morgan (1990) cites unpublished data from Peters, and McIlroy (1983) cites a personal communication from J.A. Peters to Batcheler (1982). Presumably the source is the same for both data.

Public concern about 1080 use

Some of these features of 1080 have engendered strong public opposition to its use in certain areas (Morgan & Hickling 2000). For example, operations using aerial spreading of 1080 arranged by the Animal Health Board for the West Coast of the South Island have been delayed and prevented by the public bringing cases before the Environment Court under the Resource Management Act 1991. The strength of this public concern is partially responsible for a review of 1080 use currently being conducted in New Zealand by the Environmental Risk Management Authority. The reviewers will consider the availability of any significant new information on occupational exposure, mutagenicity, teratogenicity and ecotoxicity (which includes toxicity to non-target animals) of 1080, and the substantial increase in the amount imported and manufactured (ERMA 2001). Likewise, concern over deaths of non-target animals has stimulated a review in Australia by the National Registration Authority for Agricultural and Veterinary Chemicals (NRA). The NRA review will also cover animal welfare concerns (NRA 2002).

The main areas of concern to the New Zealand public appear to be:

- The perceived risk of environmental contamination;
- The death, and the perceived risk of death, of non-target animals, particularly dogs and valued wildlife;
- The perceived risk to public health caused by 1080 contamination of water supplies, farmed meat and game meat.

The evidence for and against these areas of concern is thoroughly reviewed elsewhere (e.g., PCE 1994, 2000; Eason 1997, 2002; Eason *et al.* 1994*a*, 1999, 2000), but can be briefly summarised, as follows.

Environmental contamination

1080 is highly soluble in water meaning that it can be readily leached from baits (Pelfrene 1991; Booth *et al.* 1999*b*). Complete, rapid biodegradation by soil microorganisms (King *et al.* 1994), aquatic plants (Ogilvie *et al.* 1996, 1998) and natural events such as rainfall (Booth *et al.* 1999*a*) usually occurs within one to two weeks in favourable conditions (Eason *et al.* 1999). This means that sustained

environmental contamination by 1080 is unlikely (Booth *et al.* 1999*a*; Eason *et al.* 1999; Eason *et al.* 2000). However, persistence in baits, carcasses and soil is influenced by factors affecting microorganisms and solubility, including bait type (Bowen *et al.* 1995), temperature, moisture, pH, and the organic content of the soil and water (Parfitt *et al.* 1995; Ogilvie *et al.* 1996). Persistence is prolonged in cold or dry conditions, with degradation extended to up to several months (Eason *et al.* 1993).

Non-target poisoning

The extensive toxicity of 1080 means that non-target animals may be at risk from primary or secondary poisoning. While sublethal doses are rapidly cleared and animals generally recover unharmed (described below), lethal doses can and do kill non-target animals during 1080 control operations (e.g., Spurr 1994*a*,*b*). It is presumably this toxicity that has stimulated restrictions or prohibitions on the use of 1080 in several countries. It is important, however, to separate the effects of 1080 on the *individuals* of a non-target species or living in an area from the effects on the *population* of that species or in that area (Innes and Barker 1999). Indeed, several studies have shown that the impacts of individual deaths on populations as a whole are minimal, and even that populations may increase where the benefits of a reduction in possum numbers outweigh initial individual losses of non-target animals (e.g., McIlroy *et al.* 1986; McIlroy & Gifford 1991; Spurr 1994*a*,*b*; Powlesland *et al.* 1999). In addition, the poisoning of non-target animals by 1080 can in fact provide a benefit when the poisoned animal are pests, such as ferrets, stoats and cats (Gillies & Pierce 1999; Murphy *et al.* 1999). For example, Moller *et al.* (1996) noted a decline in ferrets (*Mustela furo*) following a possum control operation using 1080 paste. Nevertheless, in cases where every individual animal is important, for example, because they are favoured game or an endangered species, any death is undesirable.

Primary poisoning

As described in Chapter 1, the risk of primary poisoning is influenced by several features of toxic baits and the way in which they are used. Quality control to ensure that such features meet standards based on thorough scientific research (reviewed by Morgan & Hickling 2000) reduces the risk of primary poisoning of non-target animals. For example, Powlesland *et al.* (1999) noted a loss of 43–55% of a population of North Island robins (*Petroica australis longipes*) in a 1080 operation using carrot baits that

had not had chaff fragments screened out, compared to an 8–10% loss in a later operation using screened carrot baits.

Secondary poisoning

Secondary poisoning of non-target animals is not a major threat when carcasses and any poison residue they contain decompose quickly. However, because 1080 acts quickly baits can collect in the stomach of possums and not be eliminated before death. This presents a risk to scavenging animals such as dogs and pigs. Meenken and Booth (1997) illustrated this risk: stomachs and intestines from possum carcasses collected 75 days after a 1080 drop in winter in New Zealand remained reasonably intact and contained an average of 4.90 mg 1080 per kg body weight. A 20-kg dog would only need to eat 570 g of this tissue to have consumed twice the LD_{50} for 1080 (based on an LD_{50} of 0.07 mg/kg for dogs reported by Eason and Wickstrom 2001), and Meenken and Booth (1997) concluded that four of the ten carcasses sampled would pose a substantial threat to such a dog. Feral pigs (*Sus scrofa*) apparently are not as susceptible to 1080 poisoning as dogs, so would be likely to rapidly eliminate 1080 rather than show clinical effects of poisoning (Hone & Kleba 1984). The lack of an adequate antidote (Cook *et al.* 2001), and the perceived inhumaneness of 1080 for dogs (see Marks *et al.* 2000 and Sherley 2002) exacerbate public concern.

Contamination of water and meat

Finally, there is some concern that 1080 could harm humans consuming contaminated drinking water or meat.

Water

Although 1080 operations are not allowed in water catchment areas or near known water sources, some baits may fall into water that eventually ends up in water supplies (Eason *et al.* 1999). Residues of 1080 in field-water are constantly monitored, and Eason *et al.* (1999) noted that 5.3% of 868 samples taken immediately after aerial operations up to 1998 contained residues of 1080: none of these samples was from reticulated drinking water supplies. Furthermore, in only three of these samples were the residues higher than the limit of 2µg/l recommended by the Ministry of Health, a level which is well below that required to provide a lethal dose to a human, or even to produce subclinical effects (Eason *et al.* 1999).

Meat

With regard to meat, while game meat sold for human consumption is subject to the Animal Products Act 1999 and is rigorously monitored for pesticide residues, game meat for personal consumption is not (NZFSA 2002*b*). Recreational hunters are advised not to take game from areas where 1080 has been used within a certain number of days, as specified on notices around hunting areas. In addition, 1080 must not be used near grazing livestock. In any case, a sublethal dose of 1080 is rapidly metabolised and eliminated by poisoned animals, reducing the risk of meat contamination: sublethal doses are normally excreted within 1–4 days (Eason *et al.* 1994*b*). For example, Eason *et al.* (1994*a*) reported that sheep and goats rapidly eliminated a sublethal dose of 0.1 mg/kg 1080 so that only trace amounts of 1080 were detected in the plasma 18 h after dosing in goats and 96 h after dosing in sheep. The 1080 concentration in the kidney, heart, muscle, spleen and liver tissue of the sheep was less than that in the plasma at 2.5h following dosing, and only trace amounts were recorded in these tissues after 96h. Even if humans eat contaminated meat, they are apparently at little risk of receiving a dose high enough to cause harm (Eason *et al.* 1999). For instance, Eason *et al.* (1994*b*) concluded that a 30-kg child would have to consume 500g of sheep meat within 2.5h of the sheep eating 1080 baits in order to ingest a lethal dose of 1080.

Humaneness concerns

While it was recommended in the past as being more humane than the older vertebrate poisons strychnine and arsenic (Bell 1972), 1080 has recently come under scrutiny for its questionable impact on animal welfare for some species. On the grounds of human symptoms and the aesthetics of violent seizures seen after 1080 intoxication in some animals, it has been suggested that 1080 is inhumane; others argue that it is not (reviewed by Oogjes 1996). In Australia, concern about the welfare of poisoned animals (particularly foxes; Sherley 2002), was partially responsible for the NRA review of 1080 use (NRA 2002). Although animal welfare concerns have not been specifically raised as an issue for possums in New Zealand, the then Animal Welfare Advisory Committee considered the impact of 1080 on animal welfare in the late 1980's, concluding that more research was needed to allow secure conclusions to be drawn (AWAC 1989, cited in Oogjes 1996). Additionally, the Agricultural Compounds

and Veterinary Medicines Act 1997, which is in the process of being implemented for vertebrate pesticides, demands that vertebrate pesticides be humane (s.19). Formal guidelines prescribe the quantitative data required for such assessments (MAF 2000).

It is essential to have a clear understanding of the toxicokinetics, toxicodynamics, pathophysiological changes, clinical signs and pathological findings at necropsy in order to evaluate the impacts of vertebrate poisoning on animal welfare (e.g., Gregory *et al.* 1996; Broom 1999; Kirkwood *et al.* 1994; Sainsbury *et al.* 1995; Littin & O'Connor 2002; Mason & Littin 2003; Chapter 1). These have been extensively studied and reviewed previously for several species poisoned by 1080 or related compounds (e.g., Chenoweth 1949; Atzert 1971; Rammell & Fleming 1978; Egekeze & Oehme 1979; Osweiler *et al.* 1985; Klaassen 1990; Pelfrene 1991; Eason *et al.* 1993; Eason *et al.* 1994*b*; Eason & Wickstrom 2001). Several studies also detail some effects of 1080 on possums (e.g., Bell 1972; Rammell & Fleming 1978; McIlroy 1982, 1983; Morgan 1990). However, these studies did not have the aim of quantifying behavioural responses and animal welfare impacts, so most of the data were qualitative only, or were otherwise not sufficient to allow a thorough evaluation of the animal welfare implications of 1080 poisoning. Because an understanding of these processes is essential for such an assessment, a brief account follows.

Toxicokinetics

Several authors have thoroughly reviewed the toxicokinetics of 1080 (e.g., Chenoweth 1949; Atzert 1971; Eason *et al.* 1994*b*). Briefly, 1080 is readily absorbed into the body through the mucosal surfaces and abraded skin, and less readily through intact skin. Peak concentrations are reached in possum plasma about 0.5 h after a sublethal dose (0.1mg/kg by gastric lavage; Eason *et al.* 1993).

1080 is rapidly cleared from the plasma and distributed through soft tissues and organs, with distribution dependent on blood flow (Eason *et al.* 1994*b*): in possums the plasma half-life of 1080 is about 9h (Eason *et al.* 1993). The tissue half-life is similarly short (e.g., from 11h in sheep muscle to 1.7h in mouse muscle; Eason *et al.* 1994*b*). Likewise, it is rapidly eliminated from invertebrates (e.g., 1080 residues in New Zealand native ants (*Huberia striata*) exposed to a sublethal (0.03%) 1080 solution declined from 5.51 mg/kg at one day after exposure to 0.27 mg/kg at seven days; Booth & Wickstrom 1999).

1080 is cleared quickly by the liver, then expired, excreted, or deposited in bone, as unchanged fluoroacetate, fluorocitrate, fluoride and various other metabolites (Eason *et al.* 1994*b*). For example, Sykes *et al.* (1987) injected mice with 1080 containing radiolabelled fluorine and showed that it was defluorinated and the fluorine deposited in bone.

Toxicodynamics

1080 has the same mode of action in all animals, essentially killing by interfering with the tricarboxylic acid cycle (TCA cycle; Fig. 1), causing the accumulation of citrate in plasma and tissues, and energy deprivation. The following brief account, unless otherwise noted, is based on reviews by Osweiler *et al.* (1985), Klaassen (1990), Clarke (1991), Pelfrene (1991), Eason *et al.* (1994*b*), Lauble *et al.* (1996) and Fonnum *et al.* (1997).

Following absorption, sodium monofluoroacetate is metabolised to fluoroacetate. This combines with coenzyme A (CoA) to produce fluoroacetyl coenzyme A, rather than the normal acetyl CoA. Fluoroacetyl CoA condenses with oxaloacetate in the TCA cycle to form fluorocitrate instead of citrate. Only one of the four isomers of fluorocitrate, (–) *erythro*-2-fluorocitrate, is toxic. It is defluorinated and bonds tightly with aconitase (aconitate hydratase), forming an enzyme-inhibitor complex rather than isocitrate (Lauble *et al.* 1996). The defluorination (which effectively detoxifies the fluorocitrate) and reversible bond are part of the reason why sublethal doses of 1080 can be metabolised and eliminated. The formation of the enzyme-inhibitor complex means that isocitrate is not available for the remainder of the TCA cycle, so the cycle eventually stops. Another isomer, (+) *erythro*-2-fluorocitrate, binds with aconitase, but the defluorination reaction defluorinates the fluorocitrate and releases oxalosuccinate, which goes on to form α-ketoglutarate (Lauble *et al.* 1996). Meanwhile, citrate that is formed from remaining acetyl CoA accumulates. Citrate accumulation itself inhibits both the TCA cycle and energy production via inhibition of phosphofructokinase (an enzyme essential to glycolysis).

Because most of the body's energy (in the form of adenosine triphosphate, ATP) comes from the oxidative phosphorylation of products of the TCA cycle along an electron transport chain coupled to the TCA cycle, cessation of the cycle causes energy deprivation. Furthermore, fluorocitrate may inhibit mitochondrial citrate transport by

interfering with a membrane transport protein (Kirsten *et al.* 1978), effectively ensuring citrate remains elevated. It may also inhibit succinate dehydrogenase (Fanshier *et al.* 1964) thereby inhibiting the conversion of succinate to fumarate (Fig. 1).

Death is usually either by progressive cardiac failure or ventricular fibrillation, or by progressive depression of the central nervous system and consequent respiratory or cardiac failure, or by respiratory arrest following severe seizures (Chenoweth 1949). For possums, times to death are noted variously as from five to 23 h following an oral dose of 1 mg/kg (Bell 1972); 5–97 h after various oral doses (McIlroy 1982); and 6–40 h following consumption of various doses of 1080 in carrot and cereal pellet baits (Morgan 1990).

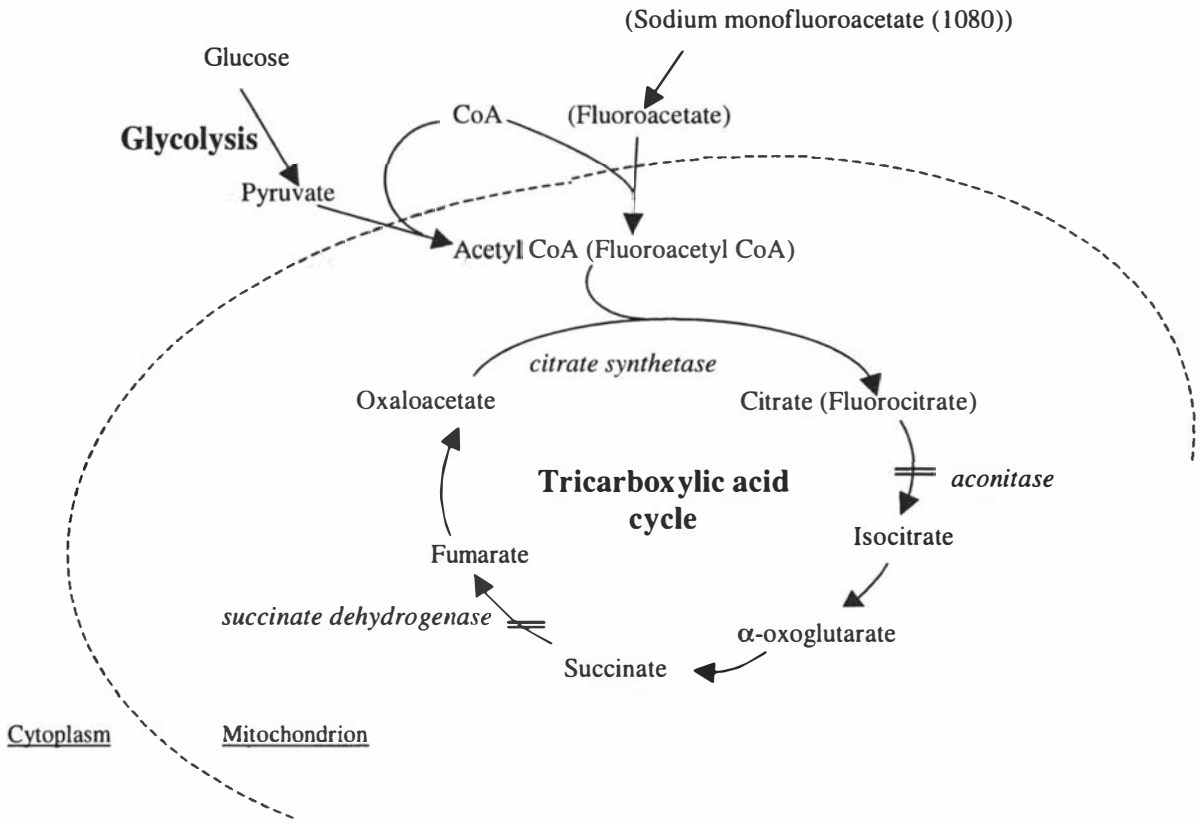


Fig. 1. Mechanism of interference by 1080 in the tricarboxylic acid (TCA) cycle. Parentheses enclose alternative components which are a result of 1080 entering the cycle. Interference is indicated by ═. Italics indicate critical enzymes. 1080 is converted into fluoroacetate which then forms fluorocitrate. The main action is fluorocitrate blocking aconitate hydratase and thereby stopping the TCA cycle. Fluorocitrate also inhibits succinate dehydrogenase.

Pathophysiological changes

Pathophysiological changes are essentially related to the sensitivity of particular organs to a reduction in energy. Organs and systems reliant on energy for membrane transport, chemical synthesis or mechanical work will therefore be affected by 1080. Hence one of either the heart or CNS is normally most affected (depending on species) and the other is little affected, if at all (Chenoweth 1949). Other organs with a high energy demand are also harmed, particularly the kidneys and testes, as described below. There is also some evidence from rats and mice that 1080 is teratogenic, but not mutagenic (Eason *et al.* 1999).

Other pathophysiological changes are due to the directly toxic effects of citrate, or other consequences of the cessation of the TCA cycle, such as the hypocalcaemia arising from ionised calcium being chelated by citrate (Chi *et al.* 1996, 1999), and the glucosuria, hyperglycaemia, lactic acidaemia and metabolic acidosis stemming from the reduced utilisation of glucose (Chenoweth 1949; Osweiler *et al.* 1985; Pelfrene 1991). All are reflected accordingly in biochemical changes, such as increased tissue and plasma citrate (especially in the kidneys) and decreased liver glycogen and glutathione (Chenoweth 1949; Schultz *et al.* 1982; Osweiler *et al.* 1985; Pelfrene 1991), and in clinical signs described later.

Cardiovascular effects of 1080

Pathophysiological changes involving the cardiovascular system are generally due to the direct action of 1080 on the cardiac cells and may also be due to hypocalcaemia causing depolarisation of neural and cardiac membranes (Pelfrene 1991). Change usually includes a depression in blood pressure due to negative inotropy and reduced systemic vascular resistance, a variety of arrhythmias which differ between species, tachycardia, premature ventricular contractions and ventricular fibrillation (Chi *et al.* 1996, 1999). Anoxic seizures following from cardiac effects may be confused for those occurring through direct effects on the nervous system (Chenoweth 1949).

Neurological effects

1080 is directly neurotoxic, having a local action on glial metabolism (Fonnum *et al.* 1997). Additionally, systemic abnormalities such as acidaemia, hypocalcaemia, hypoxaemia and membrane transport dysfunction contribute indirectly to its

neurotoxicity and further neurological dysfunction (Raabe 1981; Fonnum *et al.* 1997). For example, Largo *et al.* (1996) showed that the regulation of pH and of neurotransmitter and extracellular potassium levels may have been impaired following 1080 administration to rat brains.

Both fluoroacetate and fluorocitrate are taken up by the neuroglia (rather than the neurons), leading to cessation of the TCA cycle locally. Glial metabolism of glutamate to glutamine is inhibited, resulting in an accumulation of glutamate and γ-4-amino-butyric acid (GABA; Fonnum *et al.* 1997). The reaction during which glutamine is synthesised also fixes ammonia, so cessation of this reaction leads to an accumulation of ammonia, which is also neurotoxic (Raabe 1981; Fonnum *et al.* 1997). The primary outcomes of both of these events are energy deprivation and overexcitation (which exacerbates energy deprivation), leading to a cascade of events similar to that seen as a result of ageing, and ending in neurodegeneration and necrosis (Fonnum *et al.* 1997; C. J. Cook, C. T. Eason and M. Wickstrom, unpublished data). This in turn would contribute to further neurological dysfunction.

Renal and testicular effects

The kidneys and testes are also particularly sensitive to a cessation in energy supply, presumably because of their energy requirements for active transport and spermatogenesis, respectively. The resulting cellular necrosis affects functioning. In addition, the acidosis caused by the accumulation of citrate is likely responsible for some of the harm caused to the kidneys by 1080. Acute renal failure has been noted after 1080 poisoning in humans for example (Chung 1984; Chi *et al.* 1996, 1999), and damage to proximal convoluted tubules and widespread necrosis of the kidneys occurred in rats dosed with 1080 (Cater & Peters 1961). Parkin *et al.* (1977) also described chronic renal failure in a rabbit hunter repeatedly exposed to 1080 over ten years. Eason *et al.* (1994*b*) cited evidence for several reproductive and testicular effects, including reduced plasma testosterone, the degeneration of seminiferous tubules (and atrophy if exposure is prolonged) and depletion of spermatids.

Clinical signs

Clinical signs relating to pathophysiological effects of 1080 on target organs are seen after a latent period. This period corresponds to the transport of fluoroacetate into cells,

the conversion of sodium monofluoroacetate to fluoroacetate, the translocation of fluoroacetate, the conversion of fluoroacetate to fluorocitrate and the accumulation of fluorocitrate, and finally the disruption of cellular functioning (Chenoweth 1949; Osweiler *et al.* 1985; McIlroy 1982, 1983). Eason *et al.* (1994*b*) noted that the onset of clinical signs relates to the occurrence of peak plasma 1080 concentrations. The duration of the latent period before the first clinical sign is seen differs between species, and, to an extent, decreases as dose increases (Chenoweth 1949; McIlroy 1982, 1983). The latent period before the onset of the first clinical sign of poisoning in possums that have ingested a lethal dose of 1080 in carrot (0.14% w/w 1080) or cereal pellet baits (0.18% w/w) has been reported as 10–120 min and 40–165 min, respectively (Morgan 1990). McIlroy (1982, 1983) observed a latent period of one to 38 hours for possums following a range of doses, and Rammell and Fleming (1978) noted four to five hours. The time of onset and the nature of clinical signs also differ between species (see, e.g., reviews by Chenoweth & Gilman 1946; Chenoweth 1949; McIlroy 1982; Osweiler *et al.* 1985), as would be expected by the variability in target organ susceptibility to 1080 and response to an energy deficit noted above. These species differences in clinical signs may be due to species differences in:

- The degree and timing of the increase in citrate (Pelfrene 1991);
- Ability to utilise alternative sources of energy (e.g., α-ketoglutarate produced from oxalosuccinate; Lauble *et al.* 1996) and alternative means of energy production (e.g., glycolysis; Fonnum *et al.* 1997);
- Blood-brain barrier (Cook 1998);
- Metabolic rate and metabolism of xenobiotics;
- Systemic generation of nitric oxide (C. J. Cook, C. T. Eason and M. Wickstrom, unpublished data).

As a rule, herbivores are less sensitive to neurological effects, and most herbivores (including rabbits, goats, and horses; Chenoweth & Gilman 1946) tend to show clinical signs of poisoning related to cardiac failure. For example, rabbits become weak, decrease activity and remain relatively subdued for a substantial period before death, then experience cardiac arrhythmia, rapid weak pulse and ventricular fibrillation, associated with sudden violent clonic convulsions, opisthotonos, mydriasis, progressive relaxation, gasping and death (Chenoweth 1949; Osweiler *et al.* 1985). Other signs in

herbivores may include staggering, weakness, teeth grinding, moaning and cyanosis (Osweiler *et al.* 1985). Chenoweth and Gilman (1946) classified this group of signs as Class I.

Omnivores, cats, rhesus monkeys, pigs and birds may show a mixed response including signs of neurological and cardiac disturbance which are grouped in Class II (Chenoweth & Gilman 1946; Chenoweth 1949). For example, following a latent period, cats vomit, salivate and show mydriasis, hyperpnoea, hyperexcitability, tonic seizures and intermittent tonic-clonic seizures until death (Chenoweth & Gilman 1946). Possums show a similar range of signs, which would put them in this class. For example, McIlroy (1982, 1983) and Morgan (1990) reported hypersensitivity to noise or movements, followed by lethargic sitting or lying until death, interspersed in some possums with retching, incoordination and shivering, and, rarely, with brief convulsions associated with ejaculation, and squeaking. However, not all possums poisoned with 1080 necessarily show these signs, and the prevalence or incidence for each of the behaviours in possums has not apparently been published.

Carnivores and some other animals including guinea pigs and frogs (Chenoweth & Gilman 1946) tend to show signs related to CNS disturbance such as convulsions, (neurally-induced) gastric hypermotility and 'frenzied' or 'manic' running (Chenoweth 1949; Osweiler *et al.* 1985; Marks *et al.* 2000) and these signs have been classified as Class III (Chenoweth & Gilman 1946).

A further group of animals show Class IV signs, which include inactivity, bradycardia and bradypnoea (Chenoweth & Gilman 1946).

These are only generalisations however, and there are several examples of animals and individuals not following these tendencies. For instance, Schultz *et al.* (1982) described frenzied running in a sheep poisoned with fluoroacetate.

Humans

It is helpful to review human clinical signs in order to determine the possible range of effects that might be experienced by other animals. In a review of 38 cases of 1080 poisoning in humans, Chi *et al.* (1996) found that nausea and vomiting (which can lead to aspiration pneumonia) occurred in 74% of cases, and diarrhoea, agitation, abdominal pain and respiratory distress occurred in 20–30%. Symptoms noted by these and other

authors include chest pains, excessive salivation, gastrointestinal bleeding, headache, acute renal failure, uraemia, a range of neurological signs including nystagmus, apprehension, anxiety, verbosity, irritability, hyperactivity, mental confusion, dizziness, numbness or tingling, altered hearing and vision, tremors, twitching, ataxia linked to cerebellar damage, seizures, status epilepticus, coma, and a range of cardiac signs including tachycardia, ventricular fibrillation, ventricular arrhythmia, and respiratory distress (e.g., Trabes *et al.* 1983; Pelfrene 1991; Cai *et al.* 1997; Sanders 1997; Chi *et al.* 1996, 1999; Robinson *et al.* 2002).

Sublethal doses

Sublethal doses of 1080 are normally metabolised and excreted within 1–4 days (Eason *et al.* 1994*b*). Nevertheless, mild clinical signs may be seen, depending on the dose. For example, Schultz *et al.* (1982) observed that sheep given a range of sublethal doses of fluoroacetate exhibited tachycardia, hyperpnoea, incoordination, abnormal gait and other neurological signs, including convulsions in one animal.

Besides an ethical concern that vertebrate pests which experience ill-effects but do not die have suffered (if indeed they do suffer) unnecessarily, this situation is important for two main reasons. Firstly, while the primary effect of 1080 on intracellular energy processes may not be permanent and is not apparently cumulative between successive exposures, 1080 can cause permanent tissue damage in surviving animals, such as necrosis of myocardial fibres (reviewed by Eason *et al.* 1994*b*). Secondly, because clinical signs occur soon after poisoning, animals can associate the bait with the noxious experience and become bait shy (i.e., learn to avoid the same or different baits or poisons in the future; e.g., Hickling *et al.* 1999; O'Connor & Matthews 1999; Ross *et al.* 2000). This is a serious problem because it produces populations of animals that cannot be controlled using conventional baits. It can be overcome by the use of long-acting poisons such as anticoagulants, and novel baits or lures, and can be avoided by pre-feeding, appropriate lures to enhance bait acceptance, ensuring that baits contain adequate concentrations of poison, and alternating poisons and bait strategies (Henderson & Frampton 1999; Ross *et al.* 2000).

Pathological changes seen at necropsy

There are apparently no pathological changes specific to 1080 (Peters *et al.* 1981); changes seen are more a result of the consequences of 1080 poisoning rather than a direct effect of 1080 itself (Chenoweth 1949; Schultz *et al.* 1982; Pelfrene 1991; Eason *et al.* 1994*b*). For example, a human chemist who died after occupational exposure to 1080 had congested lungs, kidneys and liver, and focal interstitial myocarditis (Peters *et al.* 1981). Cyanosis of mucous membranes and other tissues, dark and congested liver and kidneys, and diffuse visceral haemorrhages are common findings in non-human animals, the heart is usually in diastole and there may be subepicardial and subendocardial haemorrhages (Osweiler *et al.* 1985; Eason *et al.* 1994*b*). Similar haemorrhages have been noted on the epicardium, endocardium, epiglottis and trachea in possums (see Eason *et al.* 1994*b*). Dogs and cats normally have an empty stomach, colon and urinary bladder (Osweiler *et al.* 1985). Histopathological changes have been recorded in a number of tissues including rat and lizard testes, cat brain and rat kidney (reviewed by Eason *et al.* 1994*b*).

Aims of this study

The overall aims of this study were to produce a more comprehensive list of the sickness behaviours in possums poisoned with either a lethal or sublethal dose of 1080 in a bait that is commonly used in New Zealand, and, for the first time, to quantify their duration and prevalence. Because possums may encounter a range of sublethal doses in the wild, which makes the assessment of the animal welfare impacts of sublethal doses difficult, the sublethal dose chosen was only representative of such a dose size. This chapter details three experiments. The purpose of the first 'hands-off' experiment was to describe the sickness behaviours that develop during 1080 intoxication in possums given a sublethal or lethal dose of 1080. The purpose of the second 'hands-on' experiment was to assess the onset of unconsciousness in possums poisoned with a lethal dose of 1080. As it appeared that the experimental methodology had influenced the outcome of this experiment, a third 'hands-off/ hands-on' experiment was undertaken to more clearly achieve the purpose of the second experiment. Using the results of these three experiments, a further objective was to ascertain whether 1080 caused pain, distress or suffering in the possums.

Materials and methods

All experiments were conducted with prior approval from the Landcare Research Animal Ethics Committee which acts under a Code of Ethical Conduct approved by the New Zealand National Animal Ethics Advisory Committee (projects 97/4/1, 97/6/2 and amendments).

Animals and housing

Brushtail possums (*Trichosurus vulpecula*) were trapped from tuberculosis-free areas in the South Island of New Zealand and transported to the animal facility. Male and female possums of mixed age were kept in individual wire cages (350 x 200 x 200 cm) with removable nestboxes (30 x 20 x 20 cm) in temperature-controlled indoor rooms (19 ± 5 °C) under natural daylength fluorescent light throughout experiments, unless otherwise noted below. Females were not lactating or carrying pouch young. Possums had free access to water and were fed fresh apples or carrots at least once a day throughout all experiments, except for the day immediately prior to dosing in each experiment. Cereal feed pellets (Weston Milling, Christchurch, New Zealand) were also freely available before each experiment, but were withdrawn 16–18 h prior to dosing on each occasion in order to encourage possums to eat the baits, and were replaced when possums had consumed the bait offered. Possums were either used after at least six weeks of acclimation to captivity in these conditions in order to reduce the effects of human observation and handling on experimental results, or within four days of capture so that behaviour better represented that of free-living, wild possums rather than habituated, captive possums. The time of acclimation is detailed for each experiment below.

Possums were weighed four days before dosing in the hands-off experiment, eight days before dosing in the hands-on experiment, and one day before dosing in the hands-off/ hands-on experiment. They were then weighed at death or at 48 hours after dosing.

Death was defined as the loss of palpebral and corneal reflexes, combined with the absence of a heartbeat on external palpation and/or relaxation of the iris causing mydriasis. It was confirmed by drying out and wrinkling of the cornea, which usually occurred within about 15 min of death.

Bait manufacture and 1080 exposure

Possums were fed 1080 in baits rather than orally dosed by gastric lavage. This was so as to better represent the field situation, and in particular to ensure that bait effects on toxicodynamics and toxicokinetics would not reduce the applicability of the results to the field. Baits were manufactured in a process simulating normal field production of 0.15% 1080 carrot baits (Morgan & Hickling 2000), as follows. A solution of 20% 1080 in water, blue dye and cinnamon oil was added to fresh carrots diced into approximately 6-g pieces and tumbled in a mixer for 10 minutes. Baits were immediately stored at less than 4 °C until use within 36 h. A sample of bait was kept at -20 °C for later determination of the concentration of 1080 using gas chromatography (DWRC 1989).

Possums were offered baits and the time at which possums started and finished eating baits and the amount eaten was recorded. In the hands-off experiment (described in detail below), one group of possums was offered what was intended to be a sublethal dose of 1080: 1.0 g carrot bait per kg body weight which was designed to provide about 1.5 mg 1080 per kg body weight, compared to the oral LD_{50} of 1.2 mg/kg for possums (Eason & Wickstrom 2001). These possums are hereafter referred to as 'sublethally dosed' (Table 1). Possums in the other group in the hands-off experiment and in all subsequent experiments were offered a lethal dose of 1080: 3.0 g carrot bait per kg body weight, which was designed to provide about 4.5 mg 1080 per kg body weight given a nominal concentration of 0.15 % . The actual doses consumed are detailed below.

Table 1. Description of the treatment for possums in each experiment

Experiment	Dose offered assuming 0.15% of 1080 in carrot baits		*n*	Type of behavioural observation
Hands-off	'Lethal'	4.5 mg/kg	8	Spontaneous behaviour
	'Sublethal'	1.5 mg/kg	8	
Hands-on	'Lethal'	4.5 mg/kg	10	Response behaviour
Hands-off/ hands-on	'Lethal'	4.5 mg/kg	9	Response behaviour

It was decided not to conduct necropsies after death owing to time constraints during the experiment, the fact that the pathology of 1080 in possums had already been assessed (see Eason *et al.* 1994*b*), and the anticipation that the non-specific pathology caused by 1080 would mean that these examinations would not give any further information about the animal welfare impacts of 1080.

Hands-off experiment

Thirteen possums trapped four days previously were offered a lethal dose of 1080 at 1400h; eight (five males, three females) consumed the bait offered. Twelve possums which had been trapped four days previously were offered a nominally sublethal dose of 1080 at 1800h; eight (four males, four females) consumed the bait offered. The remaining possums did not consume any bait and were excluded from the experiment. Poisoned animals were then immediately moved to new cages with no nest boxes in a room kept under continuous light to facilitate behavioural observations, and were not handled further.

Beginning 1.5 h after bait was offered, all possums were systematically observed at specific intervals by one of six observers experienced in observing possum behaviour. Inter-observer reliability was not assessed as it was considered that the behaviours were distinct enough to be recognised by all observers. Possums were observed sequentially once every 15 min for a 1-min period using continuous all occurrences focal sampling until death or until 37 h after dosing. At each observation, all incidences of all behaviours and other clinical signs of poisoning were noted, and observations were simultaneously recorded on videotape (many behaviours were difficult to see on the video recordings so video recordings were only used where necessary to confirm observations). Behaviours were later classified as follows:

- Crouching: sitting or standing in a hunched posture, often with the head down;
- Standing: standing still on all four legs;
- Grooming: scratching, licking, face washing;
- Moving about the cage or feeding: walking, jumping, climbing or exploratory behaviour; it was sometimes difficult to distinguish whether possums swallowed what they put in their mouths during exploratory behaviour, so moving and feeding behaviour were combined;

- Curled or sitting: curled in a ball lying on one side, or curled resting upright on the lumbar or sacral regions of the back, with the tail, forelegs and usually the head tucked into the chest or abdomen, or with the forelegs on the ground;
- Lying: on the side (laterally recumbent) or front (sternally recumbent).

Breathing rates were recorded for each possum approximately once every hour, if time allowed. Where possible, any abnormal behaviours and clinical signs of poisoning observed between 1 min observation periods were also noted. The first behaviour or posture noted during each 1 min period was also recorded separately as an instantaneous scan sample.

Hands-on experiment

Eleven possums were offered a lethal dose of 1080 at 0630h. Ten (seven male possums, 1 female possums, and two of unknown sex due to missing records) which had been acclimated to captivity for 7 or 17 weeks consumed the bait offered. These were handled regularly in tests to determine the time to loss of consciousness. The remaining possum did not consume any bait and was excluded from this experiment.

Tests were conducted on each possum beginning approximately 1.5 h after bait was offered, and continuing about every 1.5 h until death. These times are approximate because possums could not be tested simultaneously, but, each time, were tested separately in the order in which they finished eating baits. Possums were carried in their nestbox to a separate room and gently tipped out of the box for each test, then returned with nestboxes to their cages at the end of each test. Each test consisted of observations of gait, climbing ability and responses to a series of stimuli, made in the same order each time. If possums showed a strong positive response to any stimulus in any test they were not tested with further stimuli (i.e., all possums were not tested with all stimuli at each test). Tests were conducted as follows, in the order listed.

Gait was scored while possums moved freely in the room as 1 = unable to walk; 2 = imbalance or only able to walk or crawl a short distance when induced to move; 3 = imbalance or incoordination, but still able to walk; 4 = normal. Their ability to climb up a tree branch was then scored. A normal healthy possum would be expected to climb a branch when placed into the room. The scores were 1 = unable to climb; 2 = able to

climb with difficulty; 3 = easily able to climb. 4 = not motivated to climb branch, but may have been able or unable to do so.

An observer next approached and knelt over the possum and any escape attempt or defence behaviour was scored as a positive response. Typical defence behaviours in possums include a glare, raised paw threat, bipedal threat, swipe, lunge or pounce (Day *et al.* 2000).

Possums were then presented with a range of stimuli or manipulated as follows, and a distinct physical reaction to a stimulus was recorded as a positive response. The stimuli were (in order of presentation): sudden noise (a clap of the hands or click of the finger and thumb near the possum's ears but behind their visual range); threatening gesture (the back of a hand moved quickly toward the possum's face); ear, tail, and toe pinches (a brief pinch with fingernails on the pinna, tail tip, and web of a back foot); air blow (a quick blow of air onto the possum's face); handling (possum was grasped around the thorax).

The following reflexes were tested only after the possums were lying prostrate, and so not all possums were tested in this way. The righting response was tested by grasping and lifting a forelimb and ipsilateral hindlimb and placing the possum onto its back. A positive righting reflex was recorded if it rolled over onto its front of its own accord. Jaw tone was judged by the strength of the jaw when prised open with the fingers, and finally, palpebral and corneal reflexes were tested with a piece of straw.

Hands-off/ hands-on experiment

In this experiment, eighteen possums were offered a lethal dose of 1080 at 0400h. Nine possums did not eat any of the bait offered and were excluded from the experiment. Nine male possums which had been in captivity for 43, 49 or 61 weeks consumed the bait offered. They were moved into new cages immediately afterwards and were not removed for the remainder of the experiment. They were observed continuously without handling of any sort, and after they started to lie flat on one side, or the belly or back, their responses to the following stimuli were tested in the order listed approximately once every 15 min as described above, except that possums remained in their own cages. The stimuli and reflexes examined were, in order of testing, a threatening gesture, air blow, sudden noise, ear pinch, foot pinch, tail pinch, handling, righting, jaw tone, and corneal reflex. As with the hands-on experiment, responses were

always tested in the same order, but if possums showed a strong response to prior stimuli in any test they were not tested further, so all possums were not tested with all stimuli at each test.

Statistics

The difference between the times to death of males compared to females in the lethal dose group in the hands-off experiment was analysed with a Mann-Whitney U-test. In the other experiments, there were either no females or the number of females was too small for such an analysis. Wilcoxin tests for matched pairs were used to evaluate the difference between pre-treatment body weight and body weight at death in all four experiments. The dose of 1080 consumed was calculated from the amount of carrot bait consumed by each possum and the concentration of 1080 in the baits as determined by gas chromatography after the experiment. The dose consumed, body weight before treatment and at death or recovery, and times to death were compared across experiments using Kruskal-Wallis tests followed by a Dunn's Multiple Comparison post-hoc tests. Spearman Rank Correlations were used to investigate the relationship between time to death and dose of 1080 consumed, and between time to death and body weight at death in all experiments. Behavioural changes were not statistically analysed in this study owing to the low occurrences of most behaviours at each time point. Analyses were performed using Systat 7.0 (SPSS Inc.) and GraphPad Prism 2.01 (GraphPad Software Inc.) All times presented in the results were calculated from the time at which possums had finished consuming the required dose of 1080, or the time before death. All results are presented as means ± standard error of the mean (SEM) where appropriate, unless otherwise stated.

Results

The concentration of 1080 in the carrots baits was found to be 1.02 g/kg (0.102 %). The limit of detection was 0.003 mg/g (0.0003%) and the 95% confidence interval (CI) was ± 10%. There was an overall significant difference ($K_2 = 7.76$, $P < 0.05$) between the dose of 1080 consumed by possums offered a lethal dose in each experiment as calculated according to this bait concentration, with lethally dosed possums in the hands-off experiment and the hands-on experiment consuming slightly less (3.29 ± 0.16 mg 1080 per kg body weight and 3.39 ± 0.06 mg/kg, respectively) than possums in the

hands-off/ hands-on experiment (3.67 ± 0.06 mg/kg). However, only the difference between lethally dosed possums in the hands-off and hands-off/ hands-on experiments was significant ($P < 0.05$).

Hands-off experiment

Eight possums that were offered a lethal dose consumed 3.29 ± 0.16 mg 1080 per kg body weight in 8.65 ± 0.77 g bait within 5 min. All died after an average of 11 h 26 min ± 1 h 55 min after they had finished consuming 1080: half died between 4 h 39 min and 8 h 53 min, and half died between 14 h 5 min and 17 h 47 min. There was no significant difference in times to death between males and females ($U = 4.00$, $P > 0.05$, $n_{males} = 5$, $n_{females} = 3$). Eight possums offered a sublethal dose of 1080 consumed 1.10 ± 0.14 mg/kg 1080 in 3.03 ± 0.27 g carrot bait within a maximum of 2 min after starting to eat; the LD_{50} is 1.2 mg/kg for possums (Eason & Wickstrom 2001). One of these possums, possum #6, which had ingested 1.05 mg/kg, died 18 h 15 min after it had finished eating bait. The remainder survived.

Clinical signs of poisoning

All possums behaved normally prior to, and immediately after dosing (i.e., behaviour was similar to that of untreated, captive possums in neighbouring rooms and to that described for untreated possums in other studies in our program, e.g., Littin *et al.* 2002). All lethally dosed and some sublethally dosed possums then showed minor clinical signs of poisoning (Table 2) before exhibiting overt behavioural changes, as follows.

Lethally dosed possums

The first sign of poisoning in lethally dosed possums was a changed appearance, which included abnormal posture (possums often lay in abnormal positions or sat with their head hanging), sunken, glazed eyes and lowered ears. This was not recorded routinely, but was noted in four possums from 1 h 33 min to 2 h 24 min after they had finished eating carrot baits, which was from 4 h 12 min to 15 h 11 min before death. Possums also appeared to be less alert.

Beginning almost 3 h after dosing and finishing after 27 ± 12 min on average, seven lethally dosed possums were observed retching in 1–6 bouts of 2–17 rapid

retches. One of these possums vomited at each of three bouts, and two appeared to vomit once, regurgitating vomitus into the mouth without expelling it.

Within another 40 min (1 h 38 min–14 h 48 min before death), six lethally dosed possums appeared to be markedly incoordinated, showing unsteady head movements, unsteady walking, and rolling from an upright position onto one side. From then on, they generally remained lying (as described later) except for occasional seizures, as follows.

From about 4 h after poisoning until death 1 h 26 min to 14 h 48 min later, all eight lethally dosed possums exhibited 3–17 intermittent myoclonic spasms in single episodes involving the limbs or body. They also exhibited repeated episodes of tremors involving discrete muscles, areas of the body, or limbs, and engaged in leg paddling consisting of running movements with two or four limbs while lying on one side. Possums occasionally repositioned themselves between episodes, but generally remained lying.

Table 2. Time after dosing at which clinical signs of poisoning appeared in caged possums fed a sublethal or lethal dose of 1080 in the hands-off experiment. Times are the mean±SEM, and *n* = 8 for each sign.

Sign	Lethal dose		Sublethal dose	
	Time (h:min)	Number affected	Time (h:min)	Number affected
Onset of change in appearance	1:50 ± 0:09	4	3:52 ± 1:26	4
Onset of retching and vomiting	2:53 ± 0:13	7	12:37 ± 5:13	3
Onset of incoordination	3:37 ± 0:32	6	7:49 ± 3:19	5
Onset of spasms, tremors and seizures	4:05 ± 0:40	8	4:01 ± 0:15	3
Time to death	11:26 ± 1:55	8	18:15	1

In five possums this activity increased in duration and severity so that it resembled a *grand mal* seizure. Of these, three possums had one seizure each within 11 min of death, one had one seizure 1 h 15 min before death, and one had three seizures from 7 h 44 min to 15 min prior to death. Seizures comprised 1–3 bouts of tonic, clonic and tonic-clonic activity in the form of whisker twitching; stiffening of limbs with hunching of the shoulders; single or repeated spasms or jerks in limbs, head, abdomen or shoulder; leg paddling; rolling onto the back with a stiffened body; continuous body rolling; trembling; rigidity of the entire body; and tail wagging and tail circling. Possums were sometimes propelled into the air or along the floor by these movements. One of these possums also became cyanotic within 1 h prior to death. The duration of seizures was not always accurately recorded, and it was not possible to record the duration of seizures which continued longer than the 1-min sampling period, but most seizures lasted for more than 1 min.

Five lethally dosed possums vocalised during spasms, tremors or seizures. In two of these animals, it was loud and prolonged or repetitive during an episode. Noises were also frequently heard during retching or during breathing in terminal stages (squeaking, gasping and gagging noises).

Breathing rates did not show a clear pattern, but generally tended to increase if the possum was active or otherwise obviously alert, and decrease when possums were lying. It was difficult to distinguish breaths from other abdominal movements during spasms, tremors and seizures. Nevertheless it appeared that breathing rate increased briefly during some spasms and seizures, and that it was markedly depressed or intermittent for 15 to 33 min before death in five possums and for 4 h 30 min before death in another possum. The possums with depressed breathing rates were lying on one side or their back or belly during this time, and breathing was often accompanied by abdominal spasms and vocalisations (a gagging noise).

Sublethally dosed possums

Of the eight possums that consumed a nominally sublethal dose, including the one that died, six showed two or more clinical signs and one (#12) showed one sign, as follows. One (#12) was noted as lethargic, and four others (#2, 6, 9 and 11) adopted abnormal postures or had an otherwise abnormal appearance typical of a sick possum (as described for lethally dosed possums) beginning from 2 h 19 min to 6 h 49 min after

poisoning (Table 2), or 15 h 33 min before death in the possum that died (#6). In addition, four possums (#1, 6, 9 and 11) were noted on one or two occasions as ataxic (with unbalanced walking) beginning from 1 h 29 min to 18 h 48 min after poisoning. One sublethally dosed possum (#3) was also noted as hyper-responsive to noise. Three sublethally dosed possums (#1, 3 and 6) had up to three bouts of one to four rapid abdominal movements that may have been retches, beginning around 22, 12 or 4 h after dosing respectively. These movements looked very different from the rapid, repeated retching seen in lethally dosed possums. Finally, beginning from 3 h 33 min to 4 h 27 min after dosing and last seen from 13 h 51 min to 25 h 33 min later, three possums (#2, 6 and 9) had 9–17 brief episodes of spasms or tremors, mainly including the following: tremors of the head or limbs; tonic events appearing as sudden stiffening of limbs with hunching of shoulders or rigidity of the entire body; and single or repeated spasms, twitches or jerks in limbs, head, abdomen or shoulder; and mild limb paddling. The one possum that died (#6) had two convulsive episodes within half an hour before death that were similar in appearance to the seizures described in lethally dosed possums. This possum also twice vocalised briefly during a spasm, and had periods of abnormal breathing in association with spasms, tremors and seizures including breathing of irregular rate and depth described as gasping, gagging and laboured breathing. However, this possum and the other sublethally dosed possums did not show further clear changes in breathing rate except for a tendency toward an increase when possums were active and a decrease when possums were inactive, as described for lethally dosed possums.

Behaviour

Lethally dosed possums

Possums that had consumed a lethal dose of 1080 rapidly became inactive following poisoning, spending increasingly less time curled in the common resting position and correspondingly more time crouching or lying (Fig. 2). All possums were lying within 5 h after dosing (from 45 min to 14 h 15 min before death), and thereafter were seen lying in various positions for more than 75% of the time until near death when they were seen lying 100 % of the time. They were not seen standing, grooming, or moving about the cage or feeding from within two hours of poisoning, and only moved briefly to reposition themselves or when spasms, tremors or seizures occurred.

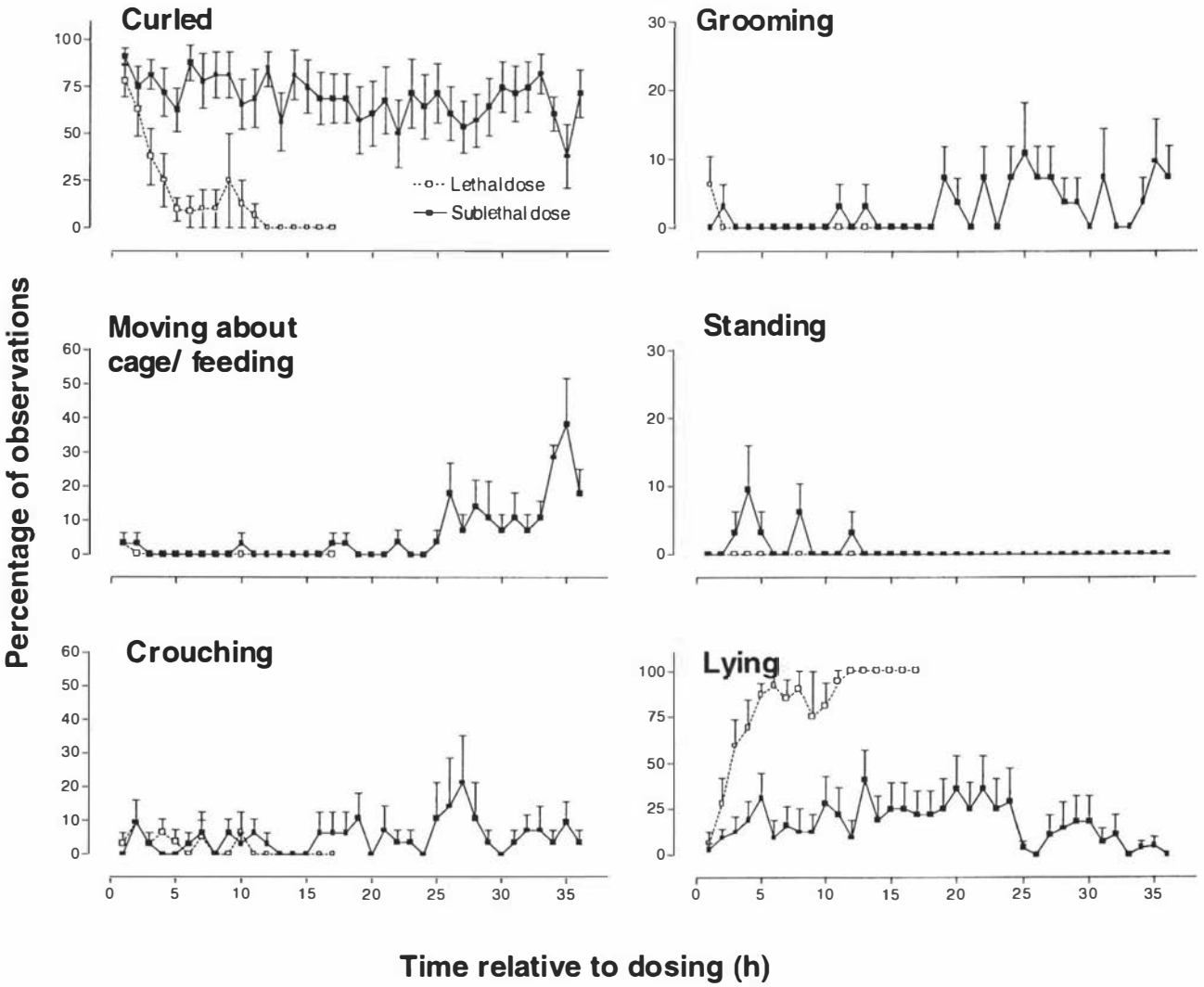


Fig. 2. Mean (±SEM) percentage of observations each hour in which caged brushtail possums were observed sitting or lying in a curled posture ('curled'), moving about the cage, crouching with hunched back, grooming, standing still on all four legs, or lying on the side, back or front ('lying'). Possums were fed a lethal (□) or sublethal dose (■) of 1080 in carrot baits at 0 h. Note different scales on y-axis.

Sublethally dosed possums

In contrast, sublethally dosed possums spent more than 50 % of the time after dosing curled in the normal resting posture and less than 50 % of the time lying, with only four sublethally dosed possums observed lying for more than four observations (1 h), beginning from 7 h 42 min to 15 h 36 min after dosing. One of these possums lay for this period of time on one occasion (#11), two lay for more than one hour on each of four occasions (#3 and #9), and one remained lying until death (#6). There was no clear

pattern in crouching and standing, but it was clear that sublethally dosed possums were only rarely observed grooming, or moving about the cage or feeding until these behaviours began increasing in frequency at an average of 19 h and 26 h after 1080 was consumed, respectively (Fig. 2).

Hands-on experiment

Ten possums consumed 3.39 ± 0.06 mg/kg 1080 in 9.03 ± 0.67 g of bait within 10 min and died 6 h 36 min ± 53 min later. This average time to death comprised nine possums that died between 4 h 8 min to 8 h 2 min after consuming 1080, and one remaining possum that died after 14 h (Table 3).

The times to loss of response for the hands-on and hands-off/hands-on experiments were calculated from the time at which a negative response was first noted. This means that the responses that were lost before death could have been lost at any time after the previous test, up to 1 h 30 min earlier.

The pattern of loss of responses to stimuli varied slightly between individuals, and not all possums lost responses to the whole range of stimuli in the test sessions before the last test, which was conducted between 25 min and 1 h 34 min before death. Possums also appeared to become fatigued during the tests, seeming more active and alert at the beginning of a test session and becoming lethargic and unresponsive to observers as the test progressed.

Nevertheless, it was clear that all possums but one lost their defence response first, at an average of just under 2 h after dosing (Table 3). Gait and climbing ability were affected around 30 min later, i.e., a score of 1 (unable to walk) or 2 (imbalance or only able to walk or crawl a short distance when induced to move) for gait or 1 (unable to climb) or 2 (able to climb with difficulty for climbing ability) was first noted at this time. The response to noise and a threatening gesture was next lost 1h later on average. The responses to a pinch on the ear, tail and foot were not lost in a clear order, but the ear pinch tended to elicit a response until a later time than the other two pinches. By contrast, the response to handling was lost last or not at all. Likewise, possums continued to show a response to a blow of air onto the face until the last testing session. The righting response, jaw tone and the eyelid and corneal reflexes were only tested in possums which were lying prostrate before the test. Of seven possums tested for

righting responses, two lost this response last and five did not lose it at all. Similarly, jaw tone was not lost before the last test in any of the six possums tested. Corneal and palpebral reflexes were both tested in two possums. One of these possums lost its palpebral reflex prior to death, while the corneal reflex was not lost in either case.

Table 3. Time before death at which ten caged brushtail possums lost reflexes or responses to stimuli after consuming a lethal dose of 1080 in carrot baits in hands-on experiment[ʃ].

Stimuli, response or reflex[§]	Mean (h:min)	Range (h:min)	Number of possums losing response before last test[††]
Defence response	4:48	2:50–6:50	10
Gait	4:21	1:34–11:20	10
Climbing ability	4:21	2:34–9:50	10
Noise	3:30	0:25–9:50	9
Threatening gesture	3:30	0:38–9:50	9
Tail pinch	2:45	0:25–9:50	9
Foot pinch	1:34	0:25–4:13	9
Ear pinch	1:26	0:25–2:58	7
Handling	0:53	0:38–1:13	3
Righting response	-	0:50, 1:13	2/7
Blow of air onto face	(not lost)	-	0
Eyelid reflex	-	0:50	1/2
Corneal reflex	(not lost)	-	0/2
Jaw tone	(not lost)	-	0/6
Last arena test	1:03	0:25–1:34	-
Time of death	6:36	4:08–14:00	-

[ʃ] First observation time at which response was *not* seen.

[§] See text for details of testing methods and stimuli used.

[††] Ten possums were tested unless otherwise indicated. Righting response, jaw tone, and eyelid and corneal reflexes were only tested in prostrate possums. This was partly for safety for human handlers, and partly because past observations showed that possums do not lose these responses to handling until after they become prostrate, so possums were not disturbed more than necessary before they became prostrate.

Hands-off/ hands-on experiment

Nine possums consumed 3.67 ± 0.06 mg/kg 1080 in 12.23 ± 0.43 g of bait within 26 min and died 6 h 37 min ± 1 h 7 min later. Seven of these died between 3 h 10 min and 6 h 20 min of poisoning, with the remaining two dying 9 h 41 min and 14 h 9 min after consuming 1080 (Table 4).

Table 4. Time before death at which ten caged brushtail possums lost reflexes or responses to stimuli after consuming a lethal dose of 1080 in carrot baits in hands-off/ hands-on experiment[ʃ]

Stimuli, response or reflex[§]	Mean (h:min)	Range (h:min)	Number of possums losing response before last test[†]
First arena test	3:30	0:17–10:45	-
Threatening gesture	2:53	0:17–10:29	9
Foot pinch	2:39	0:33–8:28	8/8
Tail pinch	2:04	0:33–4:50	7/8
Ear pinch	1:42	0:05–4:45	7/8
Noise	0:56	0:05–2:26	7
Righting response	0:56	0:05–2:04	5/7
Handling	-	0:20, 1:16	2
Blow of air onto face	-	0:00, 0:20	2
Jaw tone	-	0:05	1/5
Corneal reflex	-	0:00, 0:05	2/5
Last test	0:08	0:00–0:18	-
Time of death	6:37	3:10–14:09	-

[ʃ] First observation time at which response was *not* seen.

[§] See text for details of testing methods and stimuli used.

[†] Nine possums were tested unless otherwise indicated. All tests were conducted in the same order, but if possums showed a strong response to prior stimuli in any test they were not tested further, so possums were not all tested with all stimuli at each test.

Possums began lying on one side, or their back or belly within 3 h 35 min of dosing, so tests of responses to stimuli were started at this time. As with the hands-on experiment, the pattern of loss of responses to stimuli in the hands-off/ hands-on experiment varied slightly between individuals, and not all possums lost responses to the whole range of stimuli in the test sessions before the last test, which was conducted no more than 18 min before death. Conversely, some possums changed very rapidly from appearing normal to becoming prostrate, and so had lost most responses to stimuli by the time they were tested. One possum (#17) had lost responses to all stimuli except for a blow of air on the face by its first test. In addition, many possums lost responses then regained them at subsequent tests.

Despite this, all possums lost the response to a threatening gesture first, about 3 h before death, except for one possum which showed intermittent loss. Responses to ear, foot and tail pinches were lost next, although there was no clear order of loss, and all or nearly all of the eight possums tested lost these responses before death. The righting response was always lost at either the same test or before the remaining responses, but was not lost before death in two of seven possums tested. Handling was not lost in six of eight possums tested, and in the two remaining possums it was lost 1 h 16 min before death and 20 min before death, respectively. Finally, of five possums tested, jaw tone was only lost in one possum 5 min before death, and the corneal reflex was lost within 5 min of death in two possums.

In addition to these findings, one possum (#15) appeared to become hyper-responsive to noise. In previous tests it had shown a weak or no response to sudden noise, but nearly 10 h after finishing its dose, it became very responsive to noise and remained so until 5 min before death.

Times to death

There were no significant differences in the times to death between experiments in which possums were lethally dosed ($K_2 = 5.238$, $P = 0.073$). There was no significant effect of 1080 dose on time to death in any experiment, or in all experiments combined (r^2 ranged from 0.015 to 0.175, $P > 0.05$, $n = 8, 10, 9, 27$ respectively).

Body weight

There were significant differences between the four groups of possums in the three experiments in both pre-treatment body weight (Fig. 3; $K_3 = 12.07$, $P = 0.007$) and body weight at death ($K_3 = 8.97$, $P = 0.030$).

Pre-treatment body weight in the hands-off/ hands-on experiment was significantly higher than in lethally dosed possums in the hands-off experiment and in the hands-on experiment ($P < 0.05$). Body weight at death in the hands-off/ hands-on experiment was also significantly higher than that of lethally dosed possums in the hands-off experiment ($P < 0.05$), and tended to be higher than bodyweights after recovery in sublethally dosed possums or at death in hands-on possums. However, there was no correlation between pre-dosing body weight and time to death for possums given a lethal dose of 1080 in any of the experiments or in all experiments combined (r^2 range = 0.015–0.096, $P > 0.05$, n = 8, 10, 9, 27 respectively).

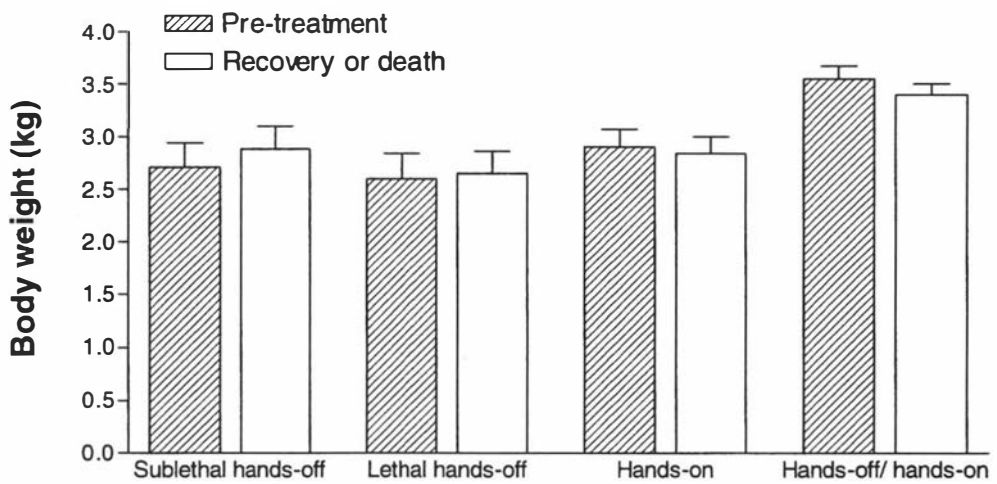


Fig. 3. Mean (±SEM) body weight of caged brushtail possums fed 1080 in carrot baits in four experiments. In the 'hands-off' experiment, they consumed a sublethal or lethal dose of 1080 in carrot baits, and were observed until recovery or death. In the 'hands-on' experiment they consumed a lethal dose, and were regularly handled to determine their response to stimuli in order to monitor the onset of unconsciousness. In the 'hands-off/ hands-on' experiment they consumed a high dose of 1080 and were handled to determine their response to stimuli as above only after they lay on one side, their back or belly for two prior observations.

Body weight at death was slightly but significantly lower than before treatment in the hands-off/ hands-on possums ($W = 39.00$, $P = 0.0195$, $n = 9$). In the remaining possums it remained relatively stable over time (W ranged from –22.00 to 28.00, P ranged from 0.078 to 0.297, $n = 7$, 7 & 9 respectively).

Discussion

1080 produced clinical signs of poisoning and overt changes in behaviour in all lethally poisoned possums and some sublethally poisoned possums. Response tests suggested that lethally dosed possums remained conscious until death or immediately before death. These data are discussed below.

Times to death

Possums that had consumed a lethal dose of 1080 in the hands-off experiment and were unhandled died about 11.5 h after consuming poisoned carrot baits, on average. Comparison with times to death in previously published studies is difficult owing the different dose rates and methods of administration (e.g., Bell 1972; Rammell & Fleming 1978; McIlroy 1982, 1983). However, in one broadly comparable study possums died 10–40 h (the median time was 12 h) following consumption of 1080 in carrot baits at a wide range of doses (2–60 mg/kg), although the exact time to death was not ascertained for all possums (Morgan 1990).

Possums that were lethally poisoned in the hands-on and hands-off/hands-on experiments in the current study and subsequently handled died about 6.5 h after poisoning. The difference in times to death between these experiments and the hands-off experiment was not significant. However, possums tended to die in two clusters in all experiments, either earlier or later, and more possums died later in the hands-off experiment than in the experiments in which possums were handled. This suggests that handling may have shortened the time to death.

Possums in the hands-on experiment appeared to become markedly fatigued while they were being tested for their responses to several stimuli. This may have affected the time to death in these individuals. After completing this experiment, a new experimental design was devised and used in the subsequent hands-off/hands-on experiment. Possums were not removed from cages, thereby avoiding extra handling, and were not subjected to so many tests. While this did not significantly affect the time

to death, the test protocol did not obviously exhaust any possums. This reinforces the importance of using a hands-off treatment to determine the time to death in any study designed to assess the animal welfare implications of vertebrate pesticides.

It is interesting to speculate about the reasons for the apparent early and late clusters in time to death. These two ranges may reflect different mechanisms of death, since animals that are predominantly neurologically affected by 1080 tend to die sooner than animals experiencing mainly cardiovascular effects (Chenoweth 1949). This would need to be confirmed by determining the manner of death in possums, for example, by means of physiological monitoring (e.g., by electroencephalogram), or postmortem examination. Unfortunately, it would not be definitive to base an analysis of the manner of death entirely on the presence or absence of seizures or other signs of neurological dysfunction as these can also occur secondarily to cardiac dysfunction and other aspects of 1080 toxicity (discussed below). Nevertheless, it could be useful to determine the cause of this difference and attempt to exploit it as a means of shortening the time to death (for example, by intentionally inducing convulsions in unconscious possums), thereby improving the humaneness of 1080 as a possum poison.

It is possible that the use of more animals or a repeat of this study could have confirmed whether there was a real difference between groups in the time to death. It was intended to have more animals, but several did not eat the bait offered. The handling protocol was again altered after this study to counter possible effects of handling on the time to death. This is discussed further in the following chapters.

If there was a real difference in time to death between the experiments, the period of acclimation might have contributed to it. It was four days in the hands-off experiment and more than three weeks in the other experiments, three weeks being the recognised time required for possums to acclimate to captivity (O'Connor & Day 1998). However, in another study in the programme of which the present study was part there was no significant difference between the times to death of phosphorus-poisoned possums that had been in captivity for four days compared to 30 days (C.E. O'Connor, L.M. Milne & N.G. Gregory, unpublished data).

Clinical signs and behavioural changes

Lethally dosed possums in the hands-off experiment generally appeared ill, showed a short period of retching and vomiting, then became lethargic and inactive,

predominantly lying thereafter and occasionally experiencing spasms and tremors, with these escalating into seizures before death. This is broadly similar to behavioural changes previously reported for possums poisoned with 1080 (McIlroy 1982; Morgan 1990). The behavioural changes and clinical signs in the current study appear to be similar to the mixed response seen in other Class II animals, as described by Chenoweth and Gilman (1946) and outlined in the introduction to this chapter. The occurrence of marked seizures in some possums in this study, and milder or less frequent neurological signs in other possums, in particular, puts possums in this class. This is important as these classes are still sometimes used to classify the responses of animals to 1080, and from this, to make inferences about its humaneness for different animals, with animals that show predominantly cardiovascular responses assumed to suffer less than animals showing more neurological signs (e.g., Sherley 2002).

The data that could be reported from this study are somewhat constrained by the method of observation, as the possums were observed using scan sampling, and focal sampling for a one-minute period. Scan sampling is useful for providing an impression of changes in behavioural state (e.g., active compared to lying), but does not allow accurate calculation of the occurrence of discrete or rare events (e.g., retching, spasms). By contrast, focal sampling allows such events to be counted, but only at the times when animals are observed. This meant that accurate timing of seizures and other events that extended beyond the 1-min observation period was not possible. In addition, the poor resolution of the video recordings allowed observation of gross behavioural changes, but did not allow assessment of finer signs, such as breathing, and did not allow behaviours that look generally similar (e.g., retching compared to abdominal spasms or tremors) to be distinguished. Finally, while all observers involved in this study recognised appropriate behaviours, the detail recorded and the way in which behaviours were described differed, making it difficult to determine some finer details in the study, such as whether lying was abnormal (e.g., unusual position, or prostrate) or normal (lying on one side). Such detail was also hard to determine from video recordings. Behaviours were classified in greater detail in later studies described in this thesis, allowing a better assessment of behavioural change.

The first clinical sign of poisoning, abnormal appearance, was observed in four lethally dosed possums up to 2 h 24 min after consuming 1080, or at a maximum of 15 h 11 min before death. Abnormal appearance included abnormal posture (often lying in

unusual positions or sitting with the head hanging), lowered ears, and sunken and glazed eyes, and was accompanied by lethargy. The study by Morgan (1990) similarly showed that possums withdrew and became increasingly lethargic from 10–120 min after first consuming a similar dose of 1080 in carrot baits. Also, there is evidence that such an abnormal appearance is a common sign of illness in possums (Gregory *et al.* 1996), rather than a specific reflection of 1080 poisoning. Certainly in later experiments in the programme of which this study was part, abnormal appearance was noted as the first clinical sign after poisoning by brodifacoum (Littin *et al.* 2002), and phosphorus or cholecalciferol (O'Connor 2000). It has also been noted in possums following poisoning with 3-nitropropionic acid (Gregory *et al.* 2000). Hence, it is likely that more possums than those reported showed this as a first sign in this study, but that it was not noticed because the signs were subtle and difficult to recognise to first-time observers. There is also a gradation of change in appearance, and individual features of this change need to be closely monitored in order to appreciate the gradual change. In later studies reported in this thesis, this was done by conducting a separate round of observations specifically to note these features. However, this was not possible in the present study owing to time constraints—there were eight possums being observed for one minute each, and only 15 minutes in which to observe them, record extra observations, and operate and move video equipment.

Retching occurred in several bouts over 27 min in total in seven of eight lethally dosed possums; three of these vomited. Three sublethally dosed animals showed behaviour that may have been retching, but the behaviour was not as distinctive and repetitive as that seen in lethally dosed possums and occurred much later, so instead may have been coughing, abdominal spasms or other spasmodic activity (e.g., hiccuping). Animals given an intraperitoneal injection of 1080 still vomit (McIlroy 1981). This could possibly be a result of 1080 causing vagal dysfunction and thereby stimulating the central vomiting centres. Retching and vomiting in the present study did not begin until nearly 3 h after dosing. As direct local irritation would presumably have caused retching at an earlier stage, these observations suggest that this vomiting was largely centrally-mediated, as suggested by the results of McIlroy (1981). Retching or vomiting at 40–200 min after dosing was reported by Morgan (1990) in around 3% of possums that had consumed a lethal dose of 1080 (the exact number affected by each was not stated). Vomiting was the least common of the two behaviours, and he noted

that it mainly occurred in animals that had consumed higher than median doses. This contrasts with the present study in which possums consumed about half of the dose consumed in Morgan's study, and most retched repeatedly. Given this finding, it is possible that retching is more widespread at lower doses than would be predicted by the findings of Morgan (1990).

Incoordination was seen next in six of eight possums, occurring at a maximum of nearly 15 h before death, and again has been previously described in possums by McIlroy (1983) and Morgan (1990). Incoordination can obviously not be observed in animals that do not move, so it is possible that more animals would have appeared incoordinated if more had been observed moving. Such incoordination is likely to be due to weakness following 1080-induced energy loss, or to neurological dysfunction, and hence specifically reflects 1080 poisoning rather than general sickness. There is evidence that 1080-induced damage to the cerebellum is linked to this incoordination: Trabes *et al.* (1983), using computer assisted tomography, provided direct evidence of cerebellar damage linked to ataxia and other cerebellar signs following 1080 ingestion by a human, and C. J. Cook, C. T. Eason and M. Wickstrom (unpublished data) measured supernormal levels of glutamate in the cerebellum around the time at which rats became incoordinated following poisoning with 1080.

Following this, lethally dosed possums tended to remain lying until death, moving only to reposition or if they experienced spasms, tremors or seizures. Given the action of 1080 to reduce available energy, this likely reflects weakness. However, like abnormal appearance discussed above, it could also be a general indicator of sickness, as described in this thesis and by other authors (e.g., Gregory *et al.* 1996, 1998, 2000).

Spasms occurred in all lethally dosed, unhandled possums, and in three sublethally dosed possums. Seizures occurred in five lethally dosed possums and the one sublethally dosed possum that subsequently died. McIlroy (1983) similarly reported brief convulsions. Spasms and seizures were not noted by Morgan (1990) however, although he did observe shivering, rapid breathing and ejaculation. It is possible that the ejaculation occurred during a tonic seizure that was not recognised as a seizure, or during relaxation following a seizure or prior to death. There is not enough information in the report in order to verify this.

In the present study, the duration of seizures was not accurately recorded, and often extended beyond the 1-min recording period. In an attempt to avoid this deficiency, future studies described in this thesis incorporated continuous *ad libitum* focal sampling of some possums for a longer duration. This meant that one possum could be observed for a particular period of time and all behaviours recorded.

The causal mechanism of seizures following 1080 poisoning appears to be complex, and has implications for animal welfare (as discussed below). Seizures could be due to the directly neurotoxic effects of 1080, such as overexcitation caused by the action of excess glutamate in the striatum (e.g., Fonnum *et al.* 1997; C. J. Cook , C. T. Eason and M. Wickstrom. unpublished data), ammonia toxicity (e.g., Raabe 1981), and several resulting or complicating features of neurological dysfunction (e.g., ionic imbalance due to reduced active transport; Largo *et al.* 1996). Alternatively, they could be due to indirect neurological effects such as subnormal levels of calcium in the cerebrospinal fluid (Hornfeldt & Larson 1990; Fonnum *et al.* 1997), or indirect systemic effects such as hypoxaemia and hypoglycaemia. It is probable that the seizures in at least three of the five lethally dosed possums in this study were caused by anoxia rather than being due to neurotoxic effects of 1080. This is because the seizures occurred some time after dosing, and within 11 min of death in these possums, when breathing was markedly depressed (see below). In one of the remaining possums, seizures occurred some time after dosing (around 10 h) and some time before death (over 7.5 h), and in the last possum, shortly after poisoning (around 2 h) and shortly before death (around 1 h). This makes it difficult to ascertain the cause.

Six of eight lethally dosed possums and one of eight sublethally dosed possums also showed intermittent abnormalities in breathing before death. While the patterns of change were not clear, respiration rate tended to increase during activity, including seizures, and decrease markedly before death. These abnormalities probably have different causes depending on the situation in which they were observed, and as with neurological dysfunction, the causal mechanisms following 1080 poisoning are complex. 1080 has direct effects on the brain, indicated for example by an increased glutamate activity in the nucleus tractus solidarius which C. J. Cook, C. T. Eason and M. Wickstrom (unpublished data) showed to correspond with cardio-respiratory depression in 1080-poisoned rats. 1080 also has systemic effects, including acidaemia, hypoxaemia and energy deprivation, and cardiotoxic effects (e.g., Osweiler *et al.* 1985;

Pelfrene 1991), all of which can influence breathing. Also, increased muscular activity during seizures could be expected to elevate the breathing rate. Again, as with seizures, breathing abnormalities have implications for welfare as discussed below, but this study did not provide the information to determine the cause of the abnormal breathing that was observed.

Breathing characteristics were difficult to observe during this experiment: possums were only observed for a 1-min duration once every 15 min, and all behaviour was recorded continuously during the minute, making it difficult to focus on changes in breathing rate. It was also difficult to observe on the recorded videos when they were reviewed. Future experiments should allow longer and more frequent sampling times for focal animals in order to record breathing characteristics more fully.

All sublethally dosed possums were observed moving about the cage or feeding more often from 26 h after dosing on average, and they were more often seen grooming from 19 h after dosing. This suggests that they had begun to recover from ill effects of poisoning by this time. McIlroy (1983) similarly reported that brushtail possums that were dosed with 1080 and did not die began recovering from 11.6 to 38.9 h after oral gavage. However, the observations in the current study could also be due to the possums becoming accustomed to observers, or the new room into which they were moved for observations, or to captivity, as they had only been in captivity for four days prior to this experiment. This could only be ascertained by further studies using acclimated possums. Nevertheless, these results show that possums had shown signs of recovery by at least this time.

Time to loss of consciousness

In this study, possums had their responses to a series of somatosensory, visual, auditory and painful stimuli tested in order to determine the pattern of loss of consciousness, as described by Gregory *et al.* (1998). The hands-on and hands-off/hands-on experiments had the aim of doing this, and some aspects of experimental design must be borne in mind while discussing these results.

First, possums were tested every 1.5 h or every 15 min in the hands-on and hands-off/hands-on experiments respectively, so time estimates were only accurate to this degree. This also meant that the possums were not always tested close to death. Possums did not lose all responses to stimuli and reflexes before they were last tested,

but they may have lost them before death, given the interval between the final test and death in each case. Moreover, the pattern of loss often differed between individuals, making interpretation difficult. Shorter intervals between testing would be preferable so that possums could be tested as close to death as possible. However, this means that group sizes must be kept small and tests simple, as they take some time to conduct.

Second, possums could not be tested simultaneously, so it was difficult to test possums at exactly 1.5 h or 15 min after poisoning if several of them finished baits at the same time. This could be changed in the future by staggering the times of baiting.

Third, while a positive response confirms consciousness, a lack of response does not necessarily indicate unconsciousness, and results must be carefully interpreted. For instance, paralysis would also prevent an overt physical response to stimuli.

The pattern of loss of responses was similar between the hands-on and hands-off/hands-on experiments. Awareness or consciousness can be considered as a spectrum rather than as an absolute (Piggins & Phillips 1998; Sommerville & Broom 1998), and the pattern of loss of neurological responsiveness in these experiments suggests a graded loss of awareness and brain function, as explained below.

Possums tested in a separate room in the hands-on experiments first failed to respond defensively to close human presence within five hours of death on average. Within the last 4.5 h before death on average, they became incoordinated, as indicated by a reduced ability to walk and climb. The ability to walk and climb were not tested in the hands-off/hands-on experiment. This time was about one hour later than the time at which the possums in the hands-off experiment appeared incoordinated. The discrepancy is relatively small, so could merely reflect inter-individual variation, especially as the ranges of times to incoordination overlapped. Alternatively, it could have been due to the error associated with the interval between observations, or it could have been because more subtle signs of incoordination could be observed in cages than in the arena tests. The loss of a defence response and incoordination could both have been caused by weakness or neurological dysfunction (e.g., cerebellar ataxia, impaired vision), as discussed for incoordination above, and probably did not reflect a reduction in consciousness.

The remaining responses were tested in both the hands-on and the hands-off/hands-on experiments. The second group of responses lost were those to ear, foot

and tail pinches. The loss of responses to pinches indicated disruption of the spinal pain pathways, or could have been due to an inability to respond due to weakness. However, some possums that had lost the response to the pinches remained responsive to several other stimuli and were obviously aware in some cases (e.g., they oriented towards observers, or moved voluntarily), implying that such possums may have had reduced awareness, but were not unconscious.

The responses to handling and righting were lost next, within about an hour of death in some but not all possums. The remainder retained these responses through to the last test. As with all of the responses however, they may have lost these two responses between the last test and death. The righting reflex requires normal vestibular, visual and proprioceptive function, implying maintenance of some degree of consciousness. The response to handling requires co-ordinated processing of sensory input, determination of physical threat, and motor output. Hence loss of this response would represent a substantial loss of consciousness. Indeed, Gregory *et al.*(1998) used this as an indicator of unconsciousness in cyanide-poisoned possums.

Finally, the response to a blow of air onto the face, and the corneal and palpebral reflexes were not lost in most possums. This implies only that facial nerve response pathways remained functional. Again, they could have been lost between the last test and death.

It might have been helpful to use EEG or brain imaging to more reliably assess the loss of consciousness. In fact, the possibility of using EEG was considered for this study. However, it was decided that the benefits of the information gained would be outweighed by the practical difficulties with establishing the use of EEG for possums, as apparently there have been no previous studies on its use in these animals. In addition, while the EEG can be used to determine abolition of brainstem functioning, its use to determine degrees of consciousness is difficult (Pallis 1983).

These observations suggest that, unlike cyanide poisoning (Gregory *et al.* 1998), but similar to brodifacoum poisoning (Littin *et al.* 2002), 1080 does not induce total loss of consciousness until shortly before death in possums. However, they are not likely to be fully aware for some time before death. Notably, this means that possums are likely to be capable of experiencing unpleasant effects until this time. This would not be a surprising result if possums were dying from cardiac failure rather than outright

neurological dysfunction, since in this case neurological function might only be slightly impaired before death, by the indirect effects of poisoning such as acidaemia and hypocalcaemia as discussed previously.

Body weight

Initial body weight differed between groups in this study: possums in the hands-off/ hands-on experiment weighed nearly 1 kg more on average than possums in the other experiments. However, there was no relationship between time to death and body weight, suggesting that this difference did not influence results. Bodyweight did not change before death or recovery in the hands-off and the hands-on experiments, but possums in the hands-off/ hands-on experiment weighed about 4 % less at death on average than they did before dosing. Such a small reduction in body weight is not likely to constitute a major welfare insult, and was presumably due to the cessation in feeding.

Implications for animal welfare

The main effects of concern regarding the welfare of 1080-poisoned possums are likely to be the retching and vomiting, and spasms and seizures if they occur while animals are conscious. While abnormal breathing, vocalisations and lethargy occurred, these are not likely to indicate poor welfare. The reasons for these statements are discussed below. The important features of any effects in terms of animal welfare are the proportion of animals affected, and the duration and intensity of the effects.

Retching and vomiting are likely to be unpleasant experiences for as long as they occur. Retching might cause some pain due to repeated muscular contractions causing a build-up of lactic acid if it were prolonged and severe enough. It occurred in seven of eight possums, implying that it is very likely that all possums poisoned in a similar way would experience it. However, possums experienced up to six bouts each and the overall duration was 27 min on average, so it could be considered as mild in severity, particularly when compared to the marginally more severe retching and vomiting seen after phosphorus poisoning in possums (O'Connor 2000) or the markedly severe vomiting seen in other poisoned animals, such as 1080-poisoned pigs (O'Brien *et al.* 1986). Vomiting could cause local irritation of the oesophagus and pharynx if it were prolonged, due to the acidity of the vomitus. However, it only occurred up to three times in this study, so is not likely to have caused substantial distress. It should be

noted, that the bait formulation may influence the likelihood of vomiting, as raised by Savarie *et al.* (1983) and discussed by Marks *et al.* (1999).

A complicating factor in this welfare assessment of retching and vomiting is nausea: retching and vomiting are commonly associated with a sensation of nausea in humans, and nausea was the most common symptom reported by 1080-poisoned humans in a survey of cases by Chi *et al.* (1996). There is some evidence that 1080-poisoned rats may also experience nausea (Cook 1998). Hence it is possible that possums felt nauseous for at least the average of 27 minutes during which retching occurred.

If there were an obvious behavioural sign indicating nausea that was associated with retching and vomiting, it might be possible to confirm the occurrence of nausea by giving an anti-nausea drug to possums and observing whether the drug abolished the behaviour. For example, Cook (1998) showed that a serotonergic receptor antagonist with anti-emetic and anti-nausea effects reduced behaviours associated with gastric upset in rats poisoned with 1080. However, possums in the current experiment did not show any specific sign that could be exclusively associated with nausea.

Seizures would not compromise welfare if possums were unconscious throughout and remained so afterwards. The seizures induced by 1080 are reportedly similar to those occurring in human hyperinsulinism and epilepsy, which are accompanied by unconsciousness or reduced awareness, and can still occur in sedated or anaesthetised animals (e.g., Chenoweth & Gilman 1946; Chenoweth & St John 1947; Loracher & Lux 1974; Raabe 1981). Some authors suggest that this means 1080-induced seizures do not cause animal welfare compromise (Gregory 1996; Williams 1996). However, 1080-induced seizures could be spinally-mediated (Hornfeldt & Larson 1990; Fonnum *et al.* 1997), which implies that animals may be able to remain conscious. In support of this contention, there is at least one report of an animal – a dog – poisoned with 1080 remaining conscious during tonic-clonic seizures (Foss 1948).

Although some spinal involvement is probable, it seems likely that the 1080-poisoned possums in this study were unconscious during most, if not all seizures for three reasons:

1) During the epileptiform seizures as were seen in a few possums in this study, severe neurological dysfunction would rule out consciousness. This does not mean that possums could not have then recovered consciousness, however;

2) Seizures occurred near death in most cases in this study, suggesting that they were a response to anoxia, as noted earlier, and therefore were likely to be accompanied by gross neurological dysfunction. However, this does not rule out the possibility that a different mechanism operated for the seizures that occurred earlier;

3) Possums in the hands-on and hands-off/ hands-on studies lost the response to handling and the righting response within an hour of death, suggesting a state of markedly reduced consciousness after that time. It is likely that any possums that experienced a seizure during this time (i.e., the majority of possums in this study) would not have been aware of it. Again, this does not rule out consciousness in any possums that experience seizures before this stage.

Notwithstanding this, despite animals generally being unconscious during tonic-clonic, epileptiform seizures, they may not necessarily have been unconscious during tonic seizures, and probably would not have been unconscious during spasms. This could be a concern if such episodes were prolonged as the sustained muscular contraction would be likely to be painful.

Finally, there is evidence that animals (and humans) can resume consciousness between successive 1080-induced seizures (Chenoweth and Gilman 1946; Foss 1948). This implies that they would be capable of experiencing negative consequences of the seizure, such as physical injury, or other unpleasant effects of the seizure such as post-ictal headache. In later studies in this thesis, attempts were made to assess the consciousness of possums immediately following resolution of 1080-induced seizures in order to further investigate this question (Chapters 3 and 5).

Reports following accidental or intentional 1080 intoxication suggest that in addition to retching, vomiting and seizures, humans poisoned with 1080 also experience epigastric pain (Chi *et al.* 1996), and some also experience breathlessness, distress or anxiety (e.g., Chi *et al.* 1996; Sanders 1997). It is difficult to ascertain whether these are concerns in possums, as follows.

With regard to evidence for epigastric pain, lethargy was seen in possums in this study. This is considered to be an indicator of distress and pain in a range of animals (Morton & Griffiths 1985; Sanford *et al.* 1986; Johnston 1996), which might also include possums. However, lethargy could also be due to weakness or reduced awareness, both of which are likely sequelae of the actions of 1080. If epigastric pain in particular were present, it would probably have been associated with crouching, as it is well recognised that crouching with a hunched back is a sign of gastric pain in companion animals (e.g., Hellyer & Gaynor 1998). It is also difficult to imagine a cause for this epigastric pain given the mode of action of 1080, and the fact that it is not caustic.

With regard to signs of poisoning that might cause outright distress, it is hard to make conclusions about breathlessness from this study. The abnormal breathing seen in this experiment was likely to be due to the direct effects of 1080 on the central respiratory centres (Chenoweth 1949; Holleran *et al.* 2001; C. J. Cook, C. T. Eason and M. Wickstrom, unpublished data), or to the hypoxaemia induced by 1080. Although hypoxaemia is a mild stimulus for breathlessness, when extreme, as after 1080 poisoning, it is likely to cause reduced awareness or unconsciousness. Also, the abnormal breathing was largely seen shortly before death when possums were probably in a reduced state of awareness, although not full unconsciousness. Further speculation is complicated by the fact that the observations of breathing in this study were inconsistent, as discussed earlier.

Vocalisation is taken to be an indicator of distress – often severe distress – in several animal species (e.g., Morton and Griffiths 1985; Sanford *et al.* 1986), this did not appear to be the case in the present possums for the following reasons. The vocalisations were likely caused by the rapid involuntary contraction of the abdominal muscles forcing air against a closed glottis during retching and seizures, rather than a voluntary response to pain. This suggestion is based on the fact that vocalisations only occurred during retching and seizures, and was not prolonged. In a previous unpublished observation, possums poisoned with strychnine vocalised loudly for prolonged periods during seizures, for which they remained fully conscious, and which were subjectively judged to be very painful. The vocalisations noted in the present study did not resemble those caused by strychnine in nature or duration (L. Milne, personal communication).

Finally, it is also possible that possums could become hypothermic, due to the reduced energy available for thermogenesis following 1080 poisoning. This is suggested by the findings of Misustova *et al.* (1969) that thermoregulation in mice was impaired by 1080, respiratory exchange reduced and metabolic rate lowered to the extent that they became temporarily poikilothermic following 1080 poisoning. It is also possible, of course, that the possums could have become hyperthermic if they had been unable to avoid high ambient temperatures while in a weakened state and unable to stabilise body temperature. Marked hypothermia may be linked with reduced consciousness (Mellor & Stafford 2003) and with a reduced or no ability to perceive cold, as it is in humans (Schimelpfenig & Lindsey 2000) and therefore would not constitute a threat to animal welfare. However, the onset of hypothermia may be associated with an unpleasant feeling of being cold and there is some indication that cold temperatures cause distress to possums, despite their fur. For example, Dawson (1969) reported that the body temperature of possums increased when ambient temperatures were above 33°C or below 15°C, and that respiratory rate increased at ambient temperatures above 30°C. Also, O'Connor & Day (1998) noted that brushtail possums become distressed below 8 °C ambient temperature. This suggests that temperatures outside this range could cause distress if the possum was unable to thermoregulate effectively.

Sublethal doses

At least four sublethally dosed possums showed two or more clinical signs, including spasms, abnormal appearance and, possibly, retching, which together suggest that they were affected by 1080. This included the one possum that died after consuming what was intended to be a sublethal dose. Two more may have been sick but the signs were not as clear. No sublethally poisoned possums experienced a seizure and recovered. This indicates that possums receiving a similar sublethal dose may become sick and experience some noxious effects of 1080, although these are likely to be mild. There was even some suggestion that the duration of sickness in sublethally dosed possums was more prolonged than in those that received a lethal dose of 1080: the one possum that died first appeared to be sick within 3h after poisoning and did not die until 15 h 33 min later, and the remaining possums that appeared sick were still judged to be so after the lethally dosed possums had died. This is in comparison to an approximate average duration of sickness of 9.5 h in lethally dosed possums, as described below.

Of course the effects of a sublethal dose are likely to depend on the exact dose consumed and whether it is enough to cause any toxic effects. Aspects of bait quality including size, concentration of 1080 in the bait and use of an appropriate lure reduce the chances of possums eating a sublethal dose. The noxious effects of a sublethal dose are undesirable not only in terms of possum welfare, but also because they induce bait shyness (Hickling *et al.* 1999; O'Connor & Matthews 1999; Ross *et al.* 2000), whereby possums will not take 1080 baits or similar baits when they encounter them in the future.

Humaneness of 1080

The first sign of poisoning, the appearance of sickness, occurred almost two hours after possums had consumed a lethal dose of 1080 and possums died after about 11.5 h. On the basis of that first sign, the maximum duration of sickness was about 9.5 h on the basis of average times, or ranged from about 4 h in one possum to about 15 h in another. Comparison of these results with those of other poisons shows that 1080 is the second fastest acting poison available for possum control, with an equivalently short duration of sickness: cyanide is the fastest, causing unconsciousness after 6 min and death after 14 min (Gregory *et al.* 1998), phosphorus takes 18 h (O'Connor 2000), cholecalciferol about 12 days (O'Connor 2000), and brodifacoum about 21 days with a duration of sickness of about seven days (Littin *et al.* 2002; Chapter 4). The degree of suffering caused by 1080 in possums could also be considered to be intermediate. This is distinct from the use of 1080 in canids where it has been considered to be unacceptably inhumane (see Marks 1996 and Marks *et al.* 2000). Nevertheless, it causes a short period of retching in possums which may be associated with nausea, and a prolonged period (over seven hours on average) of spasms, tremors and a few severe seizures after which possums may regain consciousness.

Although 1080 is evidently not the most inhumane poison available for possum control in New Zealand, its humaneness could be improved. This strategy has been investigated in rats (Cook *et al.* 2001) and foxes (Marks *et al.* 2000) and a pilot study with possums is described in Chapter 3 of this thesis. In any case, concerns about the humaneness of 1080 must be weighed against its advantages such as its superior efficacy, relatively cheap cost, and the fact that it can be spread aerially.

Effects of experimental design and environmental conditions

The duration of distress depends on the time at which the first sign appears, and the time at which the animal loses consciousness or dies. Anything influencing either of these three features of poisoning therefore influences the suffering it causes. Environmental and experimental features may influence these. One way of evaluating the influence of environmental effects on time to death would be to conduct field or pen studies. This possibility is evaluated in Chapter 5. With regard to 1080, ambient temperature and activity level are likely to be particularly important, and these would differ between natural conditions, and those in the present study using indoor cages.

Ambient temperature can have a profound effect on the toxicity and efficacy of 1080 (Misustova *et al.* 1969; Oliver & King 1983; Eastland & Beasom 1986; Veltman & Pinder 2001). It may also influence the time to death owing to the fact that the little energy available after 1080 poisoning is used up more rapidly during thermoregulation. For example, McIlroy (1983) noted a similar, but non-significant trend toward a lower LD_{50} (0.47 mg/kg cf. to 0.68 mg/kg, respectively) for brushtail possums from the East of Australia, at ambient temperatures of 10–11°C than at 22°C; the time until the onset of signs of poisoning (2.5–14.9 cf. 1.4–29.1 h, respectively), and the time until death (5.0–38.1 cf. 15.0–97.0 h, respectively) were also slightly truncated in the possums kept at colder temperatures. This is investigated in an experiment described in Chapter 5.

Secondly, the cages restricted activity to some extent. It is possible that in natural conditions increased activity and social interaction could alter the time to death through the consequent increased rate of energy-use, although the latter is probably limited in the relatively asocial possum (Day *et al.* 2000). In fact, as 1080 reduces available energy, increased activity would be expected to reduce the time to death and perhaps also shorten the duration of sickness. The tendency for shorter times to death in hands-on and hands-off/ hands-on possums in this study supports this. This suggestion is also investigated in an experiment described in Chapter 5.

With regard to further environmental and individual effects, McIlroy (1981) concluded that nutritional state, the presence or absence of pouch young, sex, and age (although pouch young appeared to be more sensitive than adults) did not affect the LD_{50} of 1080 for Eastern brushtail possums. However, stomach contents can influence the lethality of various poisons (Brown 1980; Ross *et al.* 2000), and water intake may affect the lethality of 1080 (Chenoweth 1949). Some authors have shown that the route

of administration and the bait type can also influence the lethality of 1080 (O'Brien *et al.* 1988; Frampton *et al.* 1999; Henderson *et al.* 1999) or the time to onset of clinical signs of toxicosis (Morgan 1990). Others have reported no effect (e.g., Chenoweth & Gilman 1946; Atzert 1971). For instance, McIlroy (1981) reported no difference in the LD_{50} between possums dosed by intraperitoneal injection or by mouth. This potential difference between bait types highlights the importance of the current study to quantify the behavioural changes seen in possums poisoned with 1080 in carrot baits. This is especially important since carrot baits are the most common bait type used in New Zealand. It also suggests, however, that the time course of the response of possums to 1080 in cereal pellets and other baits – particularly the more recently developed gel – should be investigated in order to ensure that the timing of behavioural changes is the same across all bait types.

One final experimental variable that may have impinged upon these results is the time of day at which possum were exposed to 1080. This differed between experiments in the current study owing to practical constraints. It is possible that circadian changes in both physiology and behaviour could have influenced the time-course of toxicosis. However, another author found no difference in the time to death between 1080-poisoned brushtail possums dosed in the morning and dosed in the evening up to 1 h after sunset (McIlroy 1983). Regardless, there is not sufficient data with which to investigate this more fully in the current study. For example, sufficient behavioural data were not taken in the hands-on and hands-off/hands-on experiments, in which 1080 exposure was at 0630h and 0400h respectively, in order to compare circadian rhythms in behaviour with those in the hands-off study, with 1080 exposure at 1400h or 1800h.

Acknowledgements and author's contribution

The design of this experiment was led by Neville Gregory (SARDI), Lynne Milne and Charles Eason (Landcare Research), although I had input into detailed changes once the experiment had commenced. Behavioural observations and tests were undertaken by myself, Charles Eason, Neville Gregory, Lynne Milne, Andrea Rhodes and Mark Wickstrom. Lynne Milne and Andrea Rhodes reviewed the video cassettes. I am grateful for statistical advice from Duncan Hedderley (Massey University) and Ray Webster (Landcare Research). This work was funded by the Agricultural and Marketing Research and Development Trust (AGMARDT)(9552), and the Foundation

for Science, Research and Technology, New Zealand (CO9632). In addition to the role enumerated above, I was entirely responsible for bringing together and considering the relevant literature, for evaluating the results and their initial interpretation, and, with advice from Cheryl O'Connor and PhD supervisors, for the refinement of the initial interpretation and the preparation of the manuscript for publication.

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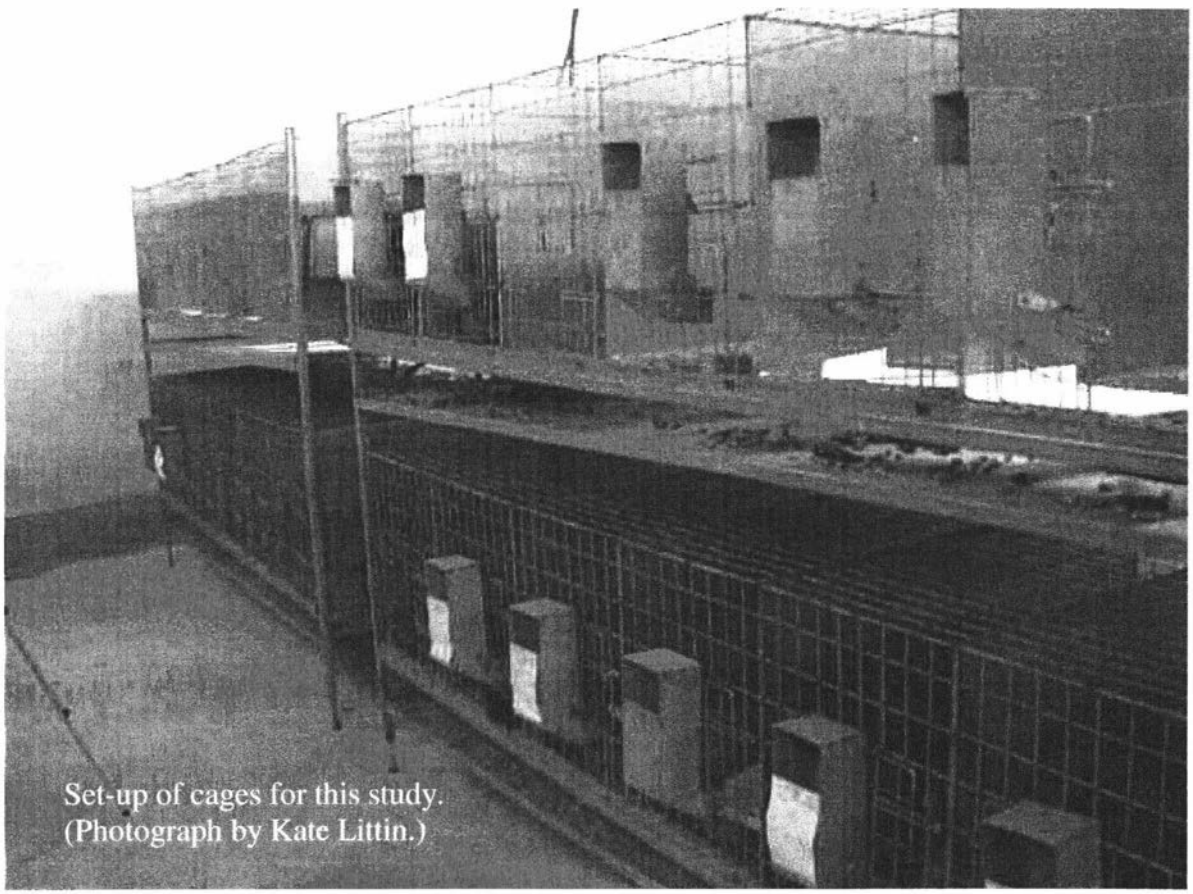
Chapter 3
Effects of alpha-chloralose and paracetamol on the behaviour of brushtail possums poisoned with 1080: a pilot study



Set-up of cages for this study.
(Photograph by Kate Littin.)

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Abstract

Previous studies of the humaneness of 1080 in brushtail possums revealed some effects of toxicosis that may be detrimental to possum welfare. These included seizures, retching and, possibly, epigastric pain and nausea. In this study, possums were treated with 1080, or 1080 immediately followed by drugs intended to mitigate these effects. Three groups of 10 anaesthetised possums were dosed by gastric lavage with either 1080 + water, 1080 + alpha-chloralose (a sedative) or 1080 + paracetamol (an analgesic). Possums were observed for clinical signs of toxicosis and behavioural changes from dosing until death. Possums given alpha-chloralose were not obviously sedated but the time spent lying did tend to increase sooner than other possums. There were no other differences between treatment groups. This lack of variance could be caused by several factors: 1) the durations of action of the agents might not have been long enough; 2) the dose of agents might have been inappropriate; 3) species-specific anomalies in metabolic rate and drug metabolism might be responsible; and 4) either 1080 does not cause pain at all, or it causes more pain than is alleviated by paracetamol, or it causes pain that cannot be alleviated by paracetamol. This study could be repeated with a higher dose of alpha-chloralose, or stronger, restricted drugs such as phenothiazine tranquillisers or opioid analgesics. In both cases however, economic costs, human safety and bait palatability would need to be closely investigated.

Introduction

Possums given 1080 in previous experiments showed signs of toxicosis that could indicate a reduction in possum welfare (Chapter 2). The main effects of concern were persistent retching and occasional spasms and seizures during which possums may have retained some degree of consciousness. It was also conjectured that 1080-poisoned possums may experience epigastric pain and nausea.

1080 remains an essential tool for possum control owing to the fact that it can rapidly kill high numbers of possums and can be spread by air to inaccessible places (Morgan & Hickling 2000), it is completely biodegradable and water soluble (Eason *et al.* 2000) and is cheap compared to other poisons (Eason & Wickstrom 2001). As yet, no alternatives have been found that surpass all of these advantages. Its use thus seems assured until such alternatives are found. Accordingly, it seems appropriate that methods of reducing the negative impacts of 1080 on animal welfare be investigated.

There are two main ways by which animal welfare compromise in poisoned animals can be mitigated or avoided:

- The duration of illness can be shortened by hastening the onset of unconsciousness or death, by either increasing the speed of action of the poison or inducing unconsciousness. In some cases, increasing the dose of poison may have this effect, or at least ensuring that a sufficient dose is ingested;
- The unpleasant effects can be prevented or reduced by the use of ameliorative agents, such as antiemetics, anxiolytics, analgesics and anti-convulsants;

For instance, influencing the metabolic rate or targeting a specific feature of the toxicodynamics of a poison could hasten the onset of death — enhancing the chance or rate of bleeding in anticoagulated animals is one example of the latter.

The idea of reducing or avoiding suffering caused by poisons is not new. For instance, there have been studies exploring the potentiation of warfarin and other coumarin anticoagulants in Norway rats, roof rats (*R. rattus*) and mice (*Mus musculus*) with compounds such as vitamin A (Krishnakumari & Muralidhara 1977), calciferol (Muktha Bai *et al.* 1978), L-histidine and vitamin K adsorbers (Muktha Bai 1979), non-steroidal anti-inflammatory drugs (Lewis *et al.* 1974; Yacobi *et al.* 1980; Sridhara & Krishnamurthy 1992) and sulphaquinoxaline (Rowe & Redfern 1965). There is also a substantial body of work on the effect of several drugs on the occurrence of haemorrhage in patients on warfarin treatment (e.g., Zweifler *et al.* 1966; Macon *et al.* 1970; Koch-Weser & Sellers 1971*a*,*b*; Levine *et al.* 2001). Several of these drugs could be used to hasten the onset of bleeding and death in anticoagulant-poisoned animals.

Additionally, Marks *et al.* (2000) described the amelioration of possible suffering in foxes (*Vulpes vulpes*) poisoned with 1080. Cook (1998) reported a reduction in behaviour related to anxiety and gastric discomfort in Norway rats (*Rattus norvegicus*) poisoned with 1080. Methods of reducing vomiting have been investigated as a means to ensuring that a lethal dose of poison is retained by pigs poisoned with 1080. For instance, O'Brien *et al.* (1986) found that thiethylperazine, prochlorperazine or metoclopromide did not reduce the frequency of vomiting in feral pigs (*Sus scrofa*) poisoned with 1080. Reducing vomiting would presumably benefit animal welfare.

There are apparently no studies of the mitigation of unpleasant effects of 1080 in possums.

There are some provisos to the use of mitigating agents. They may interact with poisons, although this could be a benefit or drawback, depending on their effects. For instance, if the compound reduces the unpleasant effects of a poison but makes the carcass toxic to non-target predators or scavengers, it would be difficult to promote the use of the additional compound. Likewise, it would be detrimental if animal welfare is made poorer: the compound should not itself cause additional detrimental effects, should not increase the severity of any existing effects, and should not prolong any suffering or increase its prevalence, even if the degree is reduced. In order to be useful in a practical situation, and in order for a more humane poison to be readily adopted by pest control regulators and operators, any agent added to a poison bait would have to be relatively cheap and readily available, and must not interfere with poison toxicity and efficacy. For instance, a compound found to be unpalatable would not be acceptable for pest control.

Alpha-chloralose and paracetamol (acetaminophen) were chosen for examination in a pilot study on the mitigation of some effects of 1080 that are detrimental to possum welfare.

Alpha-chloralose

Alpha-chloralose was chosen as a sedative for 1080-poisoned possums for the following reasons. As well as being long recognised and widely used as an anaesthetic in the past (Cornwell 1969), it is also registered as a rodenticide in the UK (Mason & Littin 2003) and elsewhere, and as an avicide in New Zealand (MAF 2000). It is thus readily available and does not require a license when used at concentrations in bait below 2.5% (NZFSA 2002), so it would provide a practical alternative to the use of prescription-only sedatives or anaesthetic agents. The LD_{95} for possums is known, giving a baseline from which doses may be calculated: Eason and Jolly (1992) determined it to be approximately 200 mg/kg. In previous studies (L. M. Milne, N. G. Gregory & C. T. Eason, unpublished data), 110.9–171.3 mg/kg oral alpha-chloralose produced stupor or unconsciousness in two out of three phosphorus-poisoned possums (the degree of consciousness was not tested), but did not reduce repeated retching. This was unexpected, given that 40–100 mg/kg IV is sufficient to produce 6–10 hours of

anaesthesia in cats and dogs (Lees 1972). As a consequence the LD_{95} was used for 1080-poisoned possums in this study to ensure sedation was achieved in all possums, and therefore to minimise their capacity to perceive undesirable states caused by poisoning.

Paracetamol

Paracetamol was chosen as an analgesic for 1080-poisoned possums. While opiate analgesics might have a more powerful analgesic action, they were avoided in this study on the basis that they are more expensive than paracetamol and they must be used under the supervision of a veterinarian, medical practitioner or similarly qualified person. In addition, Eason *et al.* (1999) have established the toxicokinetic profile of paracetamol in possums. Paracetamol is an analgesic and antipyretic drug with only weak anti-inflammatory properties (Jenkins 1987; Brune & Ulrich Zeilhofer 1999) that acts centrally and peripherally to produce analgesia (Jenkins 1987; Urquhart 1993; Brune & Ulrich Zeilhofer 1999). It is recognised as a weak analgesic only and is mostly recommended for treating mild pain conditions, fever, headache and the pain associated with cancer (Brune & Ulrich Zeilhofer 1999).

Tests of lethality to possums showed that paracetamol reached peak levels at a similar time to 1080, had a plasma half-life similar to 1080 (around 9 hours), and possums were able to maintain high rates of elimination and appeared resistant to hepatotoxic effects, even at high doses of 2000 mg/kg (Eason *et al.* 1999). Recommended doses for analgesia in dogs include 25–30 mg/kg orally every 4–6 hours (Taylor 1985), 15 mg/kg every 8 hours (Jenkins 1987) and 10–15 mg/kg orally every 6–8 hours (Boothe *et al.* 1999). Given the apparent ability of possums to maintain a high elimination rate irrespective of the dose (Eason *et al.* 1999), a higher dose was used for this experiment in an attempt to ensure adequate depth and duration of analgesia, although it is realised that the depth of analgesia is not necessarily related to the hepatic elimination. It could be anticipated that possums given paracetamol would cease to show behaviours that may be related to pain, such as head-pressing and crouching with a hunched back.

The main objective of this study was therefore to observe the influence of alpha-chloralose or paracetamol on the behaviour of possums poisoned with 1080, and from

this to determine whether they might be suitable candidates for further testing as mitigation agents for 1080.

Methods

This study was conducted with prior approval from the Landcare Research Animal Ethics Committee (project 99/9/1). Possums were orally dosed and then observed for behavioural changes until death. Tests of the loss of response to stimuli and the loss of reflexes were conducted in order to establish the time until loss of consciousness.

Animals and housing

Brushtail possums (*Trichosurus vulpecula*) were trapped from tuberculosis-free areas in the South Island of New Zealand and transported to the animal facility. Thirty male and female possums of different ages were used. Female possums were not lactating or carrying pouch young. Possums were housed in individual wire cages (350 x 200 x 200 cm) with removable nest boxes (30 x 20 x 20 cm) in temperature-controlled rooms (19 ± 5°C) under natural daylength fluorescent lighting, and were acclimatised for 7 weeks prior to this experiment. All possums had free access to water and standard cereal feed pellets (Weston Milling, Christchurch, New Zealand), and fresh apples and carrots were fed once a day throughout the experiment. Beginning two days prior to dosing, possums were observed three times daily in order to habituate them to behavioural observations.

Groups

Male and female possums were separately randomly assigned to one of three groups so that each consisted of four female and six male possums. Possums were then redistributed so that groups were balanced for body weight in order to eliminate this as a source of variance. There was no significant difference between groups in body weights of possums before treatment ($P = 0.8753$, $F_2 = 0.1339$) (Table 1).

Dosing

Dosing solutions were prepared in a laboratory within two hours of dosing, as follows:

- 1.8 mg/ml (0.18% w/v) 1080 solution in MQ water;

- 80 mg/g (8% w/w) suspension of alpha-chloralose (Sigma) in 0.5% Carbapol 941 solution;
- 200 mg/g (20%) suspension of paracetamol (Sigma) in 0.5% Carbapol 941 solution.

Table 1. Groups, body weights and doses given.

Group	Body weight (mean±SEM)	Dose of 1080 (mg/kg)	Dose of compound (mg/kg)
1080 + water	2.98±0.19	4.5	0.0
1080 + alpha-chloralose	2.98±0.17	4.5	200.0
1080 + paracetamol	2.86±0.17	4.5	500.0

Dosing occurred between 07:00 and 09:45 h. Animals were removed from cages for dosing and replaced in cages immediately after dosing. The 1080 + water group was dosed first, followed by 1080 + alpha-chloralose then 1080 + paracetamol. Individuals from each group were dosed in a random order.

All animals were induced and maintained under halothane-oxygen anaesthesia (4–5% Fluothane in oxygen, according to Landcare Research Standard Laboratory Procedure 7). They were weighed and then dosed by gastric lavage with 2.5 ml/kg of 1080 followed immediately by either 2.5 ml/kg of water, 2.5 ml/kg of alpha-chloralose or 2.5 ml/kg of paracetamol to provide the doses shown in Table 1.

No possums were dosed with alpha-chloralose or paracetamol alone. Similarly, there were no sham-dosed possums and no entirely unhandled possums. The reason for this was that this was a pilot study to screen for potential mitigating agents, rather than a full study to investigate the effects of mitigating agents on possum welfare. The purpose was to reduce the number of animals used in the pilot study, and, if appropriate, to more fully test any compounds that successfully mitigated the unpleasant effects of 1080 in a future study[3].

[3] Time constraints prevented further studies of these mitigating agents; this work will be continued by others at Landcare Research.

Behavioural observations

In order to determine behavioural changes and clinical signs of poisoning, following dosing, possums were observed using instantaneous scan sampling and *ad libitum* focal sampling, as follows. On replacement in cages, possums were observed continuously until they recovered from anaesthesia[4]. Upon recovery they were observed in the order in which they had been replaced in cages, once at 10, 20 and 30 min after replacement, then at 30 min intervals until 10:00 or 10:30h, whichever was the closest. Thereafter, all possums were observed using instantaneous scan sampling sequentially every 30 min until death, or until 16 h after dosing if death did not occur. On each occasion, the behaviour or posture was noted immediately. Postures recorded were sitting, standing, walking, grooming, crouching, lying on belly or back, lying on side and lying prostrate (completely flat) on the side, back or belly. All clinical signs of poisoning were also noted in all possums when seen during these observations.

Additionally, four possums (two male, two female) from each treatment group were observed continuously (except for approximately a 1-min period every 30 min when all possums were scanned) from the time of recovery from anaesthesia until death, or until 17 h after dosing. The incidences of all clinical signs, abnormal postures, and occurrences of retching, vomiting, spasms and seizures were recorded. The duration of any discrete events such as seizures was also recorded. This subset of focally sampled animals was introduced to improve on experimental design used in earlier experiments described in Chapters 2 and 4.

Time to loss of consciousness

Once they had become prostrate, or had been lying for one hour or longer, possums were tested to determine the time to loss of consciousness as follows. All possums, not only those used for focal observations, were tested. They were exposed to a range of stimuli or manipulated as described in the previous chapter, approximately once every 60 min. Some possums were tested more or less frequently if it was obvious that they appeared

[4] A separate group of possums was previously monitored during recovery from Fluothane-oxygen anaesthesia in order to familiarise the author with normal recovery. Possums opened their eyes and raised their heads 9±1 min (mean±SEM) after the mask was removed, stood at 11±2 min and locomotion was first observed at 12±2 min after mask removal. The main behaviours seen during recovery prior to locomotion were mild breathing irregularities, intermittent shivering, limb twitching and ataxia.

to have lost consciousness or were aware of the observer, respectively. A distinct physical reaction to a stimulus was recorded as a positive response. Possums remained in their own cages for the tests. The stimuli and reflexes examined were, in order of testing, an air blow (a quick blow of air onto the possum's face); a threatening gesture (the back of a hand moved quickly toward the possum's face); touch (the observer touched the possum's back); ear, tail, and toe pinches (a brief pinch with fingernails on the pinna, tail tip, and web of a back foot); jaw tone was judged by the strength of the jaw when prised open with the fingers; and finally, palpebral and corneal reflexes were tested with a piece of straw.

Responses were always tested in the same order and possums were all tested with all stimuli at each test unless they showed a strong or adverse reaction to previous stimuli in the test sequence.

Statistics

Times to death were compared using Kruskal-Wallis non-parametric ANOVA. The difference between the times to death of males compared to females in all groups combined was analysed with a Mann-Whitney *U*-test. The mean percentage of behavioural observations was calculated by averaging the number of occurrences of a behaviour seen during all observations made within a two hour period. This meant that n was 6 for the first two hours (because observations were taken 10, 20, 30 min after dosing, at the next nearest half hour, and at 30 and 60 min following that), and n was 4 for the remaining two hour blocks (because observations were taken every 30 min). All results have been presented as means ± SEM (standard error of the mean) unless otherwise stated.

Results

Times to death

Except for two possums dosed with 1080 + paracetamol which took more than 20 h to die, all possums died in 15 h 2 min or less and the time to death did not differ significantly between groups ($P = 0.798$; $K = 0.4516$) (Table 2). The overall mean (± SEM) time to death for all groups combined was 9 h 25 min ± 4 h 52 min. There was

no significant difference between the times to death of male and female possums (P = 0.2997, U = 83.00).

Behaviour changes

Feeding was not seen following poisoning and possums became more lethargic following poisoning of all three groups. Overall behavioural patterns were similar for each treatment group (Fig. 1).

Possums quickly reduced the time spent grooming and active to less than 10% of the time on average, within 2 h of poisoning. The time spent in the normal curled posture, or crouching decreased as the time spent lying on the side increased.

Possums dosed with 1080 + alpha-chloralose tended to spend more time lying on the side sooner than other possums, with a peak at 8 h. They then exhibited a slight tendency to spend more time lying prostrate than did the other possums, rather than spending time lying on the belly or back. For possums in the other two groups, lying on the side peaked at 8 h (1080 + water) or 10 h (1080 + paracetamol) after dosing, and the time spent lying on the belly or back, or lying prostrate, subsequently increased.

Clinical signs

In all possums, death was preceded by abnormal postures, then retching, prolonged lying, shivering, and spasms and seizures (Table 2).

Abnormal sleeping and sitting postures were seen first in a small number of possums from each group. Retching occurred next: one focal possum from each group retched (Table 2) and one treated with 1080 + water vomited once. Six other possums were heard retching and one of these vomited. The character and duration of bouts was similar for the focal possums treated with 1080 + water and 1080 + alpha-chloralose: each had three bouts of up to 12s in duration with up to 12 retches in each bout. The number and duration of bouts was not recorded for the possums treated with 1080 + paracetamol.

Shivering was next observed in three focal possums treated with 1080 + water and two focal possums treated with 1080 + alpha-chloralose. It was also observed later as part of spasms or seizures.

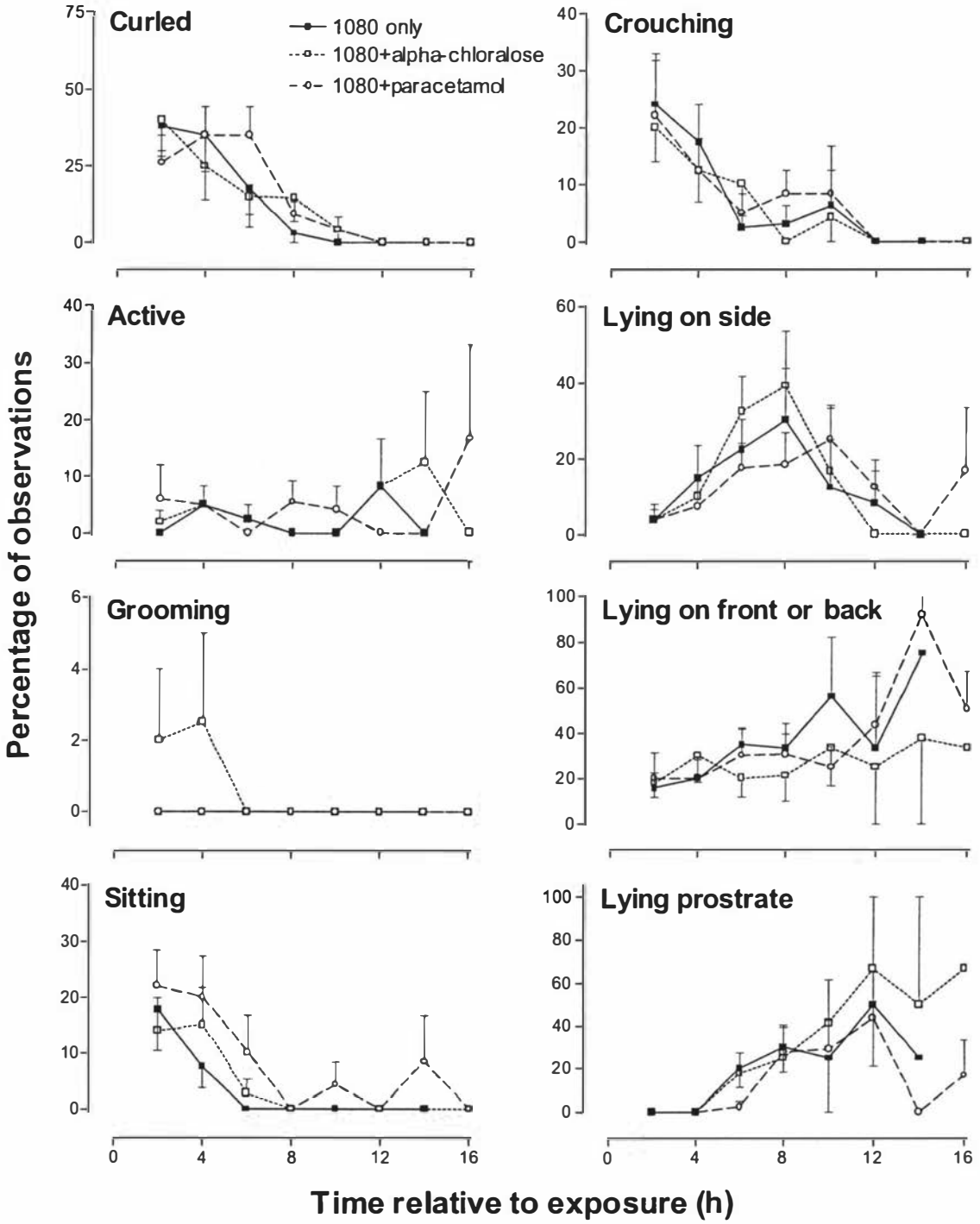

Fig. 1. Behaviour changes seen in possums given 1080 + water, 1080 + alpha-chloralose, or 1080 + paracetamol (note different scales on y-axes).

Following this, possums began lying on the side, back or belly for increasing amounts of time as described above. While lying, 29 of the possums were observed showing spasms or seizures over a period of up to 2 h 41 min before death (Table 2). The character of these spasms and seizures was discerned from observations on focal

possums as follows. All of the focal possums experienced intermittent myoclonic spasms in the limbs, tail or body; repeated episodes of tremors involving discrete muscles, areas of the body, or limbs; and/or leg paddling (running movements with two or four limbs while lying on one side). 1080 + water possums showed 1–5 bouts, 1080 + alpha-chloralose showed 1–2 bouts and 1080 + paracetamol showed 1–10 bouts.

In some focal possums, this activity progressed into tonic and tonic-clonic seizures with leg paddling, body rigidity, body rolling, tail flicking, wagging and twirling, whisker twitching, shivering and trembling, accompanied by abnormal breathing and apnoea. Three possums dosed with 1080 + water experienced 1–2 seizures, three possums dosed with 1080 + alpha-chloralose had 1, and two possums dosed with 1080 + paracetamol had 2–3 seizures. All such episodes were brief, lasting less than 1 min.

Table 2. Time to onset (mean±SEM) of clinical signs of poisoning in brushtail possums orally dosed with 1080 + water, 1080 + alpha-chloralose or 1080 + paracetamol, and number of possums showing each sign.

Sign	Treatment group					
	1080 + water		1080 + alpha-chloralose		1080 + paracetamol	
	No. of possums[‡]	Time to onset (h)	No. of possums	Time to onset (h)	No. of possums	Time to onset (h)
Incoordination	0	-	0	-	1	6:52
Abnormal posture[¶]	4	1:40 ± 0:01	2	0:40, 3:43	2	0:33, 2:55
Retching	1	3:07	1	2:54	1	1:55
Prolonged lying[¶]	10	4:15 ± 0:24	10	4:07 ± 0:27	10	4:55 ± 0:51
Abnormal vocalisation(s)	0	-	2	3:10, 8:31	1	8:11
Shivering[§]	3	4:38 ± 0.09	2	3:10, 3:13	0	-
Spasm(s)	4	4:57 ± 0:16[†] (1:32 ± 0:32 h before death)	4	6:04 ± 0:49 (0:19 ± 0:10 h before death)	4	5:28 ± 0:36 (1:55 ± 0:36 h before death)
Seizure(s)	3		3		2	
Death[¶]	10	8:21 ± 0:52 (range: 5:07–13:20)	10	8:38 ± 1:04 (range: 5:26 – 15:02)	10	11:15 ± 2:16 (range: 5:01–25:40)

[‡] $n = 4$ and data was taken from observations of focal possums, except where stated.

[¶] $n = 10$ since data for abnormal postures, prolonged lying and death are displayed for all possums.

[§]Shivering appeared to occur either on its own or associated with seizures or spasms. It seemed to occur early more often in 1080 + alpha-chloralose possums.

[†]This time represents the onset of the first spasm *or* seizure as they had no regular order of occurrence.

In addition, possums from all groups were heard vocalising abnormally. Of the focal possums, two dosed with 1080 + alpha-chloralose and one with 1080 + paracetamol were heard vocalising twice, once and three times, respectively. These vocalisations always occurred during spasms or seizures. No possums dosed with 1080 + water were heard vocalising.

Finally, abnormalities in the depth and frequency of breathing – including apnoea, hyperpnoea and laboured breathing – were observed in one focal possum from each group at different times throughout toxicosis. These were separate from the abnormalities in breathing that occurred during spasms or seizures.

Time to loss of consciousness

Responses were not lost in a consistent pattern, as some possums regained some responses that they had previously lost. This meant that a clear comparison between groups in the time to loss of responses was not possible. Nevertheless, it appeared that there were no differences in the loss of responses across groups. Additionally, it was possible to determine whether groups differed in their loss of responses within one hour of death, because several possums were tested with most or all stimuli between 58 and 38 min prior to death and several were similarly tested within 27 min of death: the data discussed below and shown in Table 3 are from these possums.

Table 3: Number of possums showing a positive response to stimuli at two stimulus response tests before death.

Stimuli, response or reflex[§]	Tested between 58 and 38 min before death	Tested from 27 min before death until death
Threatening gesture	1/5	0/4
Blow of air onto face	4/8	3/7
Noise	3/6	2/5
Ear pinch	1/7	1/5
Foot pinch	0/7	0/5
Tail pinch	1/6	1/4
Touch	3/8	1/5
Palpebral reflex	4/6	1/4
Corneal reflex	5/5	3/5

[§] See text for details of testing methods and stimuli used.

There were no obvious differences between groups, so results have been combined. Despite the small sample sizes, Table 3 shows that most possums had lost their response to a threatening gesture, a pinch on the ear, tail or foot, and a touch by 58–38 min prior to death, but had not lost their palpebral and corneal reflexes. The palpebral reflex was then lost by most possums within 27 min of death, but the corneal reflex maintained.

Discussion

Present observations

The main behavioural changes were similar in all three groups. All possums showed abnormal postures, then began retching or vomiting, became progressively lethargic and began lying with intermittent spasms and seizures. These changes occurred at similar times in each group. They were followed by loss of consciousness then death, at a similar time after dosing for all possums. As the onset of these behaviours followed by loss of consciousness and death occurred at similar times in each group, the duration of sickness was evidently similar for most possums.

It was anticipated that possums dosed with alpha-chloralose would become sedated or unconscious and that possums dosed with paracetamol would show less crouching and abnormal postures than possums dosed with 1080 + water. Despite behavioural changes not differing clearly between groups, 1080 + alpha-chloralose-dosed possums tended to spend more time lying on the side sooner than other possums. A further tendency was for them to spend more time lying prostrate, rather than lying on the belly, back or side. This may indicate a sedative effect of alpha-chloralose. However, possums were not obviously sedated, as evidenced by similar times to loss of responses to stimuli in all three groups (Table 3).

The occurrence of shivering was particularly interesting: it could be anticipated to follow alpha-chloralose intoxication, which normally causes hypothermia (see Mason & Littin 2003), but the presence of shivering in 1080 + water possums (Table 2) complicates interpretation of this. Steffey (2001) notes that shivering is common in animals recovering from halothane anaesthesia. However, earlier observations on possums recovering from halothane anaesthesia (K. E. Littin, unpublished data: see footnote 4 above) indicated that shivering occurred up to 12 min on average after the mask used for vapour delivery was removed. By contrast, possums in the current

experiment began shivering around 4.5 h (1080 + water) or 3 h (1080 + alpha-chloralose) after dosing. This suggests that the shivering was not an effect of the anaesthesia. Further, Morgan (1990) reported shivering in possums poisoned with 1080, suggesting that the shivering seen in the current study could be due to 1080 toxicosis rather than, or as well as, alpha-chloralose.

There are several possible causes of the apparent lack of effect of the mitigating compounds: 1) the durations of action of the mitigating agents may have been too short; 2) the dose may have been inappropriate; 3) there may have been species-specific anomalies in metabolic rate, and in drug and toxin metabolism; and 4) either 1080 does not cause pain at all, or it causes more pain than is alleviated by paracetamol, or it causes pain that cannot be alleviated by paracetamol. It is also possible that: 5) the number of animals was too low to be able to notice a difference in behaviours that have a low overall incidence; or 6) the halothane general anaesthetic used during dosing interfered with the actions of the drugs.

Firstly, it is possible (though unlikely in the case of paracetamol) that the peak activity of the mitigating agents occurred before the effects that they were intended to mitigate occurred. A 40–100 mg/kg dose of alpha-chloralose produces 6–10 hours of anaesthesia in cats and dogs (Lees 1972). Spasms and seizures occurred close to death which ranged up to 15 h in possums treated with alpha-chloralose, suggesting that the sedative effects of alpha-chloralose could have abated by the time these occurred. It seems unlikely that this explains the data from paracetamol-treated possums however, since it reaches peak levels at a similar time to 1080, and has a similar plasma half-life (Eason *et al.* 1999).

Secondly, possums may have been dosed with an inappropriate amount of alpha-chloralose or paracetamol. With regard to alpha-chloralose, in a previous study this dose induced sedation in possums that had been poisoned with phosphorus, so it seems unlikely that the dose was inappropriate. A possible explanation is that the lethargy and prolonged lying brought on by 1080 masked any further effect of the sedative, which would have been clearly obvious in phosphorus-poisoned possums that do not begin lying for prolonged periods until later in toxicosis (O'Connor *et al.* 2003). With regard to paracetamol, Eason *et al.* (1999) showed that possums are relatively resistant to the toxic effects of paracetamol. If this means that they metabolise the drug more efficiently than other animals, this could reduce its bioavailability and hence its

analgesic properties.

The third explanation above could be likely, given that species differences in toxin and drug metabolism are well known (Brown 1980; Osweiler *et al.* 1985; Smith 1997). It is possible that these compounds did not act in the way expected owing to some anomaly in the way that possums metabolise these particular drugs. Olkowski *et al.* (1998) and Ho *et al.* (1998) both found anomalies in the metabolism of xenobiotics by brushtail possums. This should be more thoroughly investigated in future studies with the same aim.

Fourthly, with regard to paracetamol, it is possible that possums poisoned with 1080 are not actually in pain and therefore their behaviour is not altered by an analgesic. While people poisoned with 1080 often report diffuse epigastric pain following 1080 ingestion (e.g., Chi *et al.* 1996), the mechanism for this is not clear, given the mode of action of 1080. This makes it hard to select an analgesic agent that targets the potential pain caused by 1080. Alternatively, 1080 may cause either marked pain that is not alleviated by paracetamol, being a relatively mild analgesic, or pain caused by inflammation that is again not alleviated by paracetamol with its weak anti-inflammatory properties. Paracetamol is recommended for headache in some instances (Brune & Urlich Zeilhofer 1999), suggesting that it may be expected to alleviate a headache induced by 1080. However, it would be hard to determine its effectiveness in this regard for possums poisoned by 1080, since head-pressing – one specific behaviour that may indicate headache – occurs very rarely, which would make it difficult to ascertain differences between treatment groups.

A fifth explanation is that the number of animals recorded performing some of the behaviours and exhibiting the clinical signs was low. This made comparison between treatments difficult and may have masked real differences between groups. This could have been rectified by using more animals. However, as this was a pilot study the number of animals was purposely limited, and the need for heightened resolution of the results for these behaviours was not anticipated.

Finally, it is possible that the halothane anaesthesia interfered with the action of alpha-chloralose or paracetamol. Certainly halothane interacts with several physiological systems and organs while animals are anaesthetised (Steffey 2001). However, it is cleared quickly (Steffey 2001), suggesting that the effects, if any, would

be minimal.

Comparison with 1080 findings in Chapter 2

A comparison of the results of the current study with those reported for 1080-poisoned possums in Chapter 2 is interesting. The general pattern and timing of behavioural changes and clinical signs were similar between these two studies. This lends support to the results of those experiments.

However, the time to death differed slightly between this experiment and the previous 1080 study described in Chapter 2. In this experiment, the first possum died 5 h 1 min after dosing in the current experiment and the last possum died 25 h and 40 min after dosing: an average of 9 h 25 min. By contrast, possums fed a lethal dose of 1080 in carrot baits in the previous experiment died between 4 h 39 min to 17 h 47 min after dosing (on average of 11 h 26 min). If this slightly lower time to death is a real effect, it could be an effect of dosing by gastric lavage rather than dosing by means of cereal pellet baits since absorption of 1080 from baits could be expected to take longer than absorption of 1080 from a liquid suspension as was used in the current study. Alternatively, it could be due to some interference by the halothane anaesthesia. For example, reduced body temperature is a known effect of halothane anaesthesia (Steffey 2001). Thermoregulatory compensation for reduced body temperature on recovery from anaesthesia may draw on energy resources and hasten 1080 toxicosis, as discussed in Chapters 2 and 5.

Where to from here?

Rather than finish with the unsatisfying conclusion that alpha-chloralose and paracetamol cannot be used to mitigate undesirable effects of 1080, it might be helpful to examine some alternatives. This study could be repeated with higher doses of alpha-chloralose, or stronger, restricted drugs such as phenothiazine tranquillisers or opioid analgesics. If doses were increased, the economic cost, toxic effects to human users, and bait palatability could be a problem. Safety and palatability problems could be remedied by changing the formulation of the mitigating compound or the bait in which it is carried, for example by encapsulation with an enteric coating.

Metabolic inhibitors may be a more promising way of decreasing the time to death in 1080-poisoned animals: Kemmerling (1996) reported that the sudden death in

cattle that have eaten the plant *Palicourea marcgravii* was due to the co-existence of two metabolic inhibitors, N- methyltyramine and 2-methyltetrahydro-beta-carboline, with a very low level of 1080. Ho *et al.* (1998) showed that possums have a reduced ability to metabolise midozalam, a drug metabolised by cytochrome P3A enzymes. They suggested that a novel poison for possums could combine a toxicant that targets cytochrome P3A enzymes and an inhibitor of cytochrome P3A enzymes.

There could be other possibilities. For instance, it seems feasible that drugs that alter energy metabolism themselves might increase the toxicity or speed of action of 1080, offering a means of improving its humaneness. For example, Parke and Williams (1969) state that salicylic acid may uncouple oxidative phosphorylation. Given the action of 1080 on energy metabolism, the addition of salicylic acid to 1080 may have the desired effect. Chenoweth (1949) makes a further suggestion: it could be possible to hasten the onset of 1080 toxicosis by increasing the chain length of 1080, thereby increasing lipid solubility and decreasing dissociation, and so increasing the speed of cell penetration. As solubility in water is one of the environmental advantages of 1080 however, this might not be a desirable option. Finally, some agents facilitating cholinergic transmission, such as neostigmine in mice, potentiate convulsant drugs (see Chenoweth 1949) and may shorten the onset of 1080 toxicosis.

Summary

In summary, alpha-chloralose and paracetamol did not appear to mitigate noxious effects of 1080. The lack of effect of alpha-chloralose and paracetamol with 1080 may be due to an effect of the dose, or a shorter duration of action of the mitigating agents compared to 1080. If the dose were increased, toxic effects, cost and bait palatability would be a problem with alpha-chloralose, and cost may be a problem with paracetamol. It may be necessary to use stronger, restricted drugs such as phenothiazine tranquillisers, or opioid analgesics, in order to mitigate possible pain and suffering caused by 1080.

Author's note and acknowledgements

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Chapter 4
Behaviour, coagulopathy and pathology of brushtail possums (*Trichosurus vulpecula*) poisoned with brodifacoum, and the implications for possum welfare

Author's note: This chapter has been published in an condensed form as the article:

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Condensed versions of this chapter in combination with data from experiments on rats (*Rattus norvegicus*) have been presented at two conferences and published, as follows:

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Littin, K.E.; O'Connor, C.E. (2001) Welfare of brodifacoum-poisoned rats and possums. *Conservation Science* **40/41**, 4-5.

Littin, K.E.; O'Connor, C.E. (2001) Welfare of brodifacoum-poisoned rats and possums – report of a talk given at the 13th AWMS meeting, 2000, Queenstown. *Australasian Wildlife Management Society (AWMS) Newsletter.*

Table of contents

Abstract

Brodifacoum is a second-generation anticoagulant rodenticide used widely for controlling brushtail possums (*Trichosurus vulpecula*) in New Zealand. The toxic effects on possums were studied in order to make inferences about the welfare of brodifacoum-poisoned possums. Caged possums were fed a lethal dose of brodifacoum in cereal baits then either bled and killed 4, 8, 12, 16 or 20 days later to establish the effects on blood-clotting, or observed for behavioural changes until death. Blood-clotting time was prolonged 8 days after initial brodifacoum ingestion and time to death was 20.7 ± 1.7 days (mean ± SEM). Clinical signs of poisoning, including changed appearance, a pale nose and external bleeding, appeared from 14 days after initial poisoning (7 days before death). Possums gradually became inactive and lethargic, typically crouching and lying in abnormal postures for 6 days before death. Feed intake reduced concurrently, resulting in significant body weight loss of 5.9 ± 2.1%. All possums had widespread, usually severe, haemorrhaging. Internal haemorrhages were first observed in all possums 8 days after initial ingestion. These haemorrhages, and consequent blood loss, may cause distress, pain, weakness or sickness, and this suggestion is supported by evidence from humans and other animals. Reduced feed intake, inactivity, lethargy and the occurrence of abnormal postures suggest that possums probably experience distress for at least 6 days before death by brodifacoum poisoning.

Introduction

Brodifacoum is a synthetic coumarin-type compound originally developed as a second-generation anticoagulant poison to control warfarin-resistant rodents (Redfern *et al.* 1976; Dubock & Kaukeinen 1978). It has since been used to control a variety of agricultural and forest pests throughout the world (Merson *et al.* 1984; Eason *et al.* 1999; Kaukeinen *et al.* 2000). In New Zealand it is used largely for the control of commensal and wild rodents and possums: it was registered for use on possums in New Zealand in 1991 (Eason *et al.* 1996*b*) and has now become an important tool for their control.

The main aim of brodifacoum use for possums is to keep populations at a low level once initial numbers have been reduced by the use of 1080 or other fast-acting

poisons (Eason *et al.* 2000). Commensal rodents are controlled with a variety of proprietary baits. Possums and wild rodents are typically controlled using dyed cereal bait pellets generally containing 20 or 50 ppm brodifacoum, respectively, and a scent lure. These are offered in bait stations. Novel baits, such as one based on peanut paste, have also been developed.

Brodifacoum has three main advantages compared to 1080, as follows:

- it can be bought and used without a licence (Eason & Wickstrom 2001);
- accidental poisoning can be treated before damage or death occurs – although treatment can be prolonged and expensive (Eason & Wickstrom 2001);
- illness occurs long after brodifacoum ingestion, meaning that bait shyness does not occur and it can be used against animals that are bait shy after previous exposure to another poison (Eason *et al.* 2000).

Its main disadvantages are:

- it is relatively expensive (Eason & Wickstrom 2001);
- it persists in carcasses (Eason *et al.* 1996*b*; Meenken *et al.* 1999), baits (Booth *et al.* 1999) and the environment (Eason & Wickstrom 2001), so secondary poisoning can be a problem (e.g., Rammell *et al.* 1984; Eason & Spurr 1995; Dowding *et al.* 1999; Eason *et al.* 1999; Empson & Miskelly 1999; Harrington & Macdonald 2002), although, as discussed previously with regard to 1080 (Chapter 2), the benefits of the poisoning for population growth in valued non-target wildlife species may outweigh the mortality of individuals by secondary poisoning (e.g., Robertson & Colbourne 2001). The significance of low anticoagulant residues found in non-target animals has been disputed (Kaukeinen *et al.* 2000), although there is concern that residues could accumulate in carrion-eating game animals such as pigs, thereby posing a risk to humans (Eason *et al.* 1996*a*,*b*, 1999).

Toxicity

Brodifacoum has the potential to affect any animal using vitamin K-dependent blood-clotting factors in its coagulation system – while vertebrates are susceptible to differing

degrees, invertebrates are apparently not susceptible (see Eason & Wickstrom 2001). A single dose that is relatively low in comparison to that needed for other anticoagulants, can kill: the LD_{50} is 0.17 mg/kg for possums and 0.27 mg/kg for rats (Eason & Wickstrom 2001).

Toxicokinetics

As with 1080, the absorption, distribution and elimination of brodifacoum has been well-studied in several species and there are several published reviews (e.g., Parmar *et al.* 1987; Eason & Spurr 1995; Eason & Wickstrom 2001). Brodifacoum is absorbed through the gastrointestinal tract and, to a lesser degree, the skin. It rapidly accumulates in the liver and other internal organs. High levels remain in the plasma for several hours and in the liver for several months. For example, Eason *et al.* (1996*a*) found the half-life of brodifacoum in the plasma of possums to be 20–30 days and the liver half-life to be greater than 252 days.

Toxicodynamics

In common with other anticoagulant pesticides, brodifacoum kills by disrupting blood clotting or coagulation. It competitively inhibits the action of vitamin K epoxide reductase and vitamin K reductase, enzymes required for the recycling of vitamin K (Thijssen 1995). The conversion of precursor vitamin K-dependent blood-clotting factors II, VII, IX and X to active factors in the liver is linked to this cycle, and is therefore halted by brodifacoum (Thijssen 1995). Eventually, circulating vitamin K-dependent clotting factors break down and are not replaced. As a result, any damage to blood vessels is not adequately repaired, and animals begin to haemorrhage at the injured sites (Osweiler *et al.* 1985; Thijssen 1995). In addition, anticoagulant poisons may cause damage to blood vessels themselves (Kruse & Carlson 1992), thereby contributing to the risk of haemorrhage. If blood loss continues, anaemia and shock due to low blood volume (hypovolaemic shock) develop, and death can ensue by one, or a combination, of cardiac, respiratory and kidney failure (Anderson 1980; Radostits *et al.* 1999).

Pathophysiological changes

Brodifacoum causes widespread haemorrhages throughout the body in humans and other animals, with further effects dependent on the speed of blood loss and site of

haemorrhages (Osweiler *et al.* 1985; Helmuth *et al.* 1989; Kruse & Carlson 1992; Omstein *et al.* 1999). The haemorrhages themselves may have pathophysiological implications, for instance where they interfere with normal cardiac function through cell damage or necrosis. Additionally, pathophysiological changes related to blood loss and its sequelae can occur. These changes are reflected by biochemical changes including changes in blood clotting factors II, VII, IX and X and consequently in blood clotting times.

Clinical signs

Clinical signs – relating also to the effects of haemorrhages themselves or to the effects of blood loss and it consequences – occur after a lag phase. An example of an effect related to haemorrhage is breathlessness due to pleural haemorrhages; an effect of a consequence of blood loss is the weakness caused by anaemia. Clinical signs in anticoagulant-poisoned animals include reduced activity, anorexia and poor body condition, bloody droppings, weakness, staggering, pale gums and mucous membranes, and external bleeding (Osweiler *et al.* 1985; Radostits *et al.* 1999; Mason & Littin 2003).

Humans are most often admitted after accidental or intentional brodifacoum poisoning with some combination of abdominal and flank pain, haematoma, bruising, external bleeding (typically through the nose and gums), visible haemorrhaging in the skin (ecchymoses and petechiae), haematuria (blood in urine) and haematochezia (blood in faeces). Other signs include haemarthrosis (blood in joints), haematemesis (vomiting blood), lethargy, malaise, anorexia, confusion, dizziness, fainting, seizures and coma (Helmuth *et al.* 1989; Katona & Watson 1989; Kruse & Carlson 1992; Hollinger & Pastoor 1993; Rauch *et al.* 1994; La Rosa *et al.* 1997; Ornstein *et al.* 1999). Symptoms seem unrelated to dose, but are affected by the position and severity of haemorrhages. For example, intracerebral haemorrhages are linked with neurological effects such as seizures (Helmuth *et al.* 1989; Kruse & Carlson 1992; Ornstein *et al.* 1999) and headache (Helmuth *et al.* 1989; Ornstein *et al.* 1999; Schoenen & Sándor 1999).

Pathological changes seen at necropsy

Widespread internal haemorrhaging and external bleeding are characteristic of anticoagulant poisoning or overdose in humans (e.g., Lilly & Lee 1949; Macon *et al.*

1970; Stanton *et al.* 1974; Palmer *et al.* 1999) and other animals (Osweiler *et al.* 1985; Radostits *et al.* 1999; Eason & Wickstrom 2001; Mason & Littin 2003). Other pathological changes may be related to the consequences of blood loss, such as changes relating to anaemia or shock (Eason & Wickstrom 2001; Mason & Littin 2003).

Humaneness concerns and aims of this study

Anticoagulant poisons typically cause multiple, widespread haemorrhages – including in locations that are likely to cause pain or distress, and that do cause pain and distress in humans. They also cause behaviour suggestive of weakness, pain or distress, and a long period from poison ingestion to death. For these reasons, anticoagulant poisons have been criticised as inhumane for rats (Scott 1969; Kirkwood *et al.* 1994; MAFF 1997; Broom 1999; Mason & Littin 2003). By contrast, Rowsell *et al.* (1979) concluded that anticoagulant poisons were humane for rats on the grounds that electroencephalograms and behaviour provided no evidence of pain or distress, an opinion also held by others (Hadler & Buckle 1992).

There apparently has been no work on the impacts of brodifacoum on possum welfare to allow a similar assessment. Therefore, the purposes of this study were to investigate behaviour, coagulopathy and pathology in brushtail possums given brodifacoum in order to make inferences about its impacts on possum welfare. A refinement of the hands-off/ hands-on methodology previously used for assessing 1080 (Chapter 2) was used: all possums were regularly observed prior to testing, and were then tested with a series of stimuli after they had been lying continuously for 1 h or had become prostrate.

Materials and methods

This study was conducted with prior approval from the Landcare Research Animal Ethics Committee (project 98/6/1).

Animals and housing

Male and female brushtail possums (*Trichosurus vulpecula*) trapped from tuberculosis-free areas in the South Island were used in this study. Female possums were not lactating or carrying pouch young. Possums of mixed age were housed in individual wire cages (350 x 200 x 200 cm) with removable nest boxes (30 x 20 x 20 cm) in

temperature-controlled rooms (19 ± 5°C) under natural daylength fluorescent lighting, and were acclimated for 12 weeks prior to experiments. All possums had free access to water, and fresh apples and carrots were fed once a day throughout experiments. Standard cereal feed pellets (Weston Milling, Christchurch, New Zealand) were also freely available, except during brodifacoum exposure.

Brodifacoum exposure

As with the study on 1080 (Chapter 2), possums were fed brodifacoum in baits rather than orally dosed by gastric lavage. This was so as to better represent the field situation, and in particular to ensure that bait effects on toxicodynamics and toxicokinetics would not reduce the applicability of the results to the field. In two experiments, possums were fed brodifacoum in RS5 cereal-bait pellets containing green dye and cinnamon containing a nominal concentration of 20 ppm (20 μg/g) brodifacoum (PESTOFF®, Animal Control Products, Wanganui, New Zealand). The concentration of brodifacoum in the baits was later determined using high-performance liquid chromatography followed by fluorescence detection (Hunter 1983) to be 16.6 ± 1.3 μg/g. The dose (1 mg/kg) was calculated to be high enough to kill all exposed animals (the LD_{50} in possums is 0.17 mg/kg; Eason & Wickstrom 2001). The total dose was offered over 3 nights (days 0–2), with no standard feed pellets available. Bait was removed and intake recorded each morning, and fresh bait fed each afternoon, to simulate exposure in the wild: typical control operations use bait stations that remain in place and are refilled as necessary. Possums can then feed from baits over several nights, and being nocturnal, are unlikely to feed on baits during the day. Unlike in a typical control operation, the duration of exposure was limited in the current study in order to limit the dose of brodifacoum.

Assessment of coagulopathy

Twenty-eight possums were fed brodifacoum as described above, then randomly allocated to one of five sampling groups, but balanced for average poison intakes and body weights. Seven possums died from brodifacoum-poisoning prior to the time at which they were scheduled to be sampled. The times to death of these possums were not included in data described below because it was considered that they would skew the mean time to death owing to the fact that no possums were allowed to survive

beyond particular times up to a maximum of 20 days after dosing in this experiment. Remaining possums in each group were bled and killed as follows, at 4 (n = 3), 8 (n = 3), 12 (n = 5), 16 (n = 5) or 20 (n = 5) days after first exposure to brodifacoum. Four untreated possums were bled on each sampling day as controls. Possums were anaesthetised with CO_2/ O_2 (50% CO_2 and 50% O_2 according to Landcare Research Standard Laboratory Practice No. 12), and then blood samples (3 – 5 ml) were taken from the median tail vein. Poisoned possums were then euthanased by cardiac injection of sodium pentabarbitone while under anaesthesia, and weighed. Untreated possums were weighed then allowed to recover in their cages. Blood samples were analysed for activated partial thromboplastin time (APTT) and one-stage prothrombin time (OSPT). Each of these indicates the state of a different part of the blood-clotting system – an abnormally prolonged APTT indicates a deficiency in blood clotting factors VIII, IX, X, XI or XII and an abnormally prolonged OSPT indicates a deficiency in factors I, II, V, VII or X. Using platelet-poor plasma in a fibroanalyser, activated thrombotax-optimised reagent including bovine-brain phospholipids and ellagic acid was used to analyse APTT, and rabbit-brain thromboplastin with calcium was used to determine OSPT, as described in Eason *et al.* (1996*a*).

Assessment of behaviour

Thirty-six possums were assigned to two groups of 18 possums (11 male, 7 female), each made up of three sets of six adjacently caged possums. One group was fed brodifacoum as described, while the other group remained on a diet of standard cereal pellets to act as controls. Both groups were observed for changes in behaviour for 20 days following initial ingestion of brodifacoum by treated possums. To facilitate observations, nest boxes were removed and rooms kept in continuous light, beginning 5 days before exposure (day –5). Possums were observed once every 15 min for two periods each day (between 0800 – 0845 h, and 1900 – 2045 h) from day –5 until day 20. They were further checked at around 1200, 1600 and 2200 h to note times to death. During days 17-20, which was the anticipated sickness period, possums were observed once every 15 min throughout the entire 96-h period, to monitor behavioural changes more closely. At every 15-min observation, the behaviour and any clinical signs of sickness were noted for each possum. Standard-feed-pellet intake and changes in colour and consistency of droppings were recorded at 0900 h every morning from day –5, and possums were weighed on days –5, 10 and 20, or at death.

To determine the time to loss of consciousness, all possums were tested for responses to a variety of stimuli approximately once every 30 min after they had been lying continuously for at least 1 h, or became prostrate. A distinct physical reaction was recorded as a positive response. Stimuli were: an air blow (a quick blow of air onto the possum's face); threatening gesture (an open hand held with palm toward and above the possums face and moved toward the head); touch (tested by laying a hand on the back); ear, tail, and toe pinches (a brief pinch with large forceps or fingernails on the pinna, tail tip, and web of a back foot); and palpebral and corneal reflexes (tested with a piece of straw). These possums were not handled, in contrast to those in the study on 1080 (Chapter 2), in order to avoid producing haemorrhages.

Death was defined as the loss of palpebral and corneal reflexes, combined with the absence of a heartbeat on external palpation and/or relaxation of the iris causing mydriasis. It was confirmed by drying out and wrinkling of the cornea, which usually occurred within about 15 min of death.

Necropsies

The bodies of all possums from the coagulopathy experiment and 12 from the behaviour experiment were chilled at 4°C immediately after death and necropsied within 8 h. Haemorrhages were grouped according to the region in which they occurred (head, thorax, abdomen, pelvic cavity, limbs) and whether they occurred within organs or other tissues in each region. They were then qualitatively graded according to severity: mild (through one layer of tissue of the same type, e.g., through the subcutaneous fat); moderate (two layers of tissue, e.g., through the subcutaneous fat and into the connective tissue covering muscle, or moderate depth in an organ); severe (three or more layers of tissue, e.g., through the subcutaneous fat, connective tissue, muscle and into the periosteum, or through the entire depth of an organ), and also according to distribution: focal or multifocal (within a discrete boundary, e.g., petechial or ecchymotic haemorrhages); patchy (no marked boundary); locally extensive or diffuse (large areas of bleeding through most or all of an organ or limb). Both hip and stifle joints were checked for intra-articular haemorrhage. Any other pathological changes were noted if observed, but were not systematically recorded. Livers were removed from the possums used for the assessment of behaviour and stored immediately at –-

18°C. The concentration of brodifacoum in each liver was later assessed by high-performance liquid chromatography followed by fluorescence detection (Hunter 1983).

Statistics

All times were calculated from the time at which possums were first offered brodifacoum baits. This is because while brodifacoum was offered over three nights in this study, it begins acting shortly after it is first consumed (Thijssen 1995) and blood clotting time in possums begins decreasing at some time within two days of exposure (Eason *et al.* 1996*a*). The use of any other time as a fixed starting point over the 3-day exposure period would be relatively arbitrary.

The relationship between dose of brodifacoum consumed and the time until death was investigated using a Spearman Rank Correlation. The proportion of observations during which possums were seen exhibiting each behaviour was calculated for each day, and data were arcsin (square root) transformed. The time spent crouching or curled, and the feed intake, were analysed by fitting linear mixed-effect models by maximum likelihood. Treatment and time were taken as fixed effects while possum was considered to be random. The serial correlation between successive observations on each possum was modelled as an autoregressive process of order one. Feed intake modelling was followed by post-hoc *t*-tests with Bonferroni adjustments. APTT and OSPT data were natural log transformed then analysed by two-way ANOVA followed by post-hoc *t*-tests with Bonferroni adjustments. The difference between pretreatment body weight, and body weight at death was investigated with a paired *t*-test. The difference between times to death of male and female possums was tested using a *t*-test. Analyses were performed using Systat 7.0 (SPSS Inc.), GraphPad Prism 2.01 (GraphPad Software Inc.) and S-plus 4.5 (MathSoft Inc.).

Results

Individual possums consumed varying doses of brodifacoum in the two experiments, and all but one possum required three nights of exposure to consume a lethal dose of brodifacoum. Possums used in the assessment of coagulopathy consumed 0.86 ± 0.04 mg brodifacoum/ kg body weight (mean ± SEM) by eating 165.1 ± 9.6 g pellet baits over 3 nights. Possums used in the assessment of behaviour consumed 0.88 ± 0.04 mg brodifacoum/kg body weight by eating 182.3 ± 13.1 g pellet baits over 3 nights.

Poisoned possums first showed changes in blood-clotting times and then pathological changes. After that they displayed clinical signs of poisoning followed by overt changes in behaviour and feed intake.

Coagulopathy

Activated partial thromboplastin times (APTT) of untreated possums remained similar to pretreatment levels of poisoned possums throughout the experiment (Fig. 1).

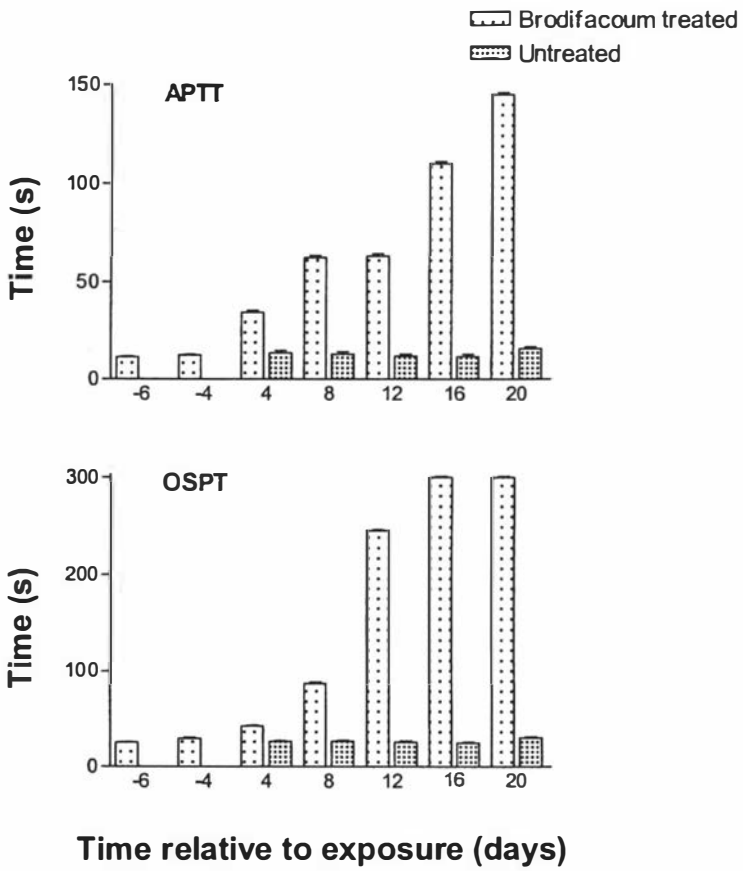

Fig. 1. Mean (± SEM) activated partial thromboplastin time (APTT) and one-stage prothrombin time (OSPT) of brushtail possums exposed to brodifacoum in cereal baits ('brodifacoum treated') or untreated cereal baits ('untreated') over three days (day 0–2). Brodifacoum-treated possums were bled 4 and 6 days before exposure (days -6 and -4), then bled and killed 4, 8, 12, 16 or 20 days after exposure. Untreated possums were bled 4, 8, 12, 16 or 20 days after exposure. *N* varies: untreated, $n = 4$; brodifacoum treated, days -6 and -4, $n = 48$; days 4 and 8, $n = 3$; days 12, 16 and 20, $n = 5$.

This contrasted significantly with APTT of treated possums ($F_{4,23}$=5.37, $P < 0.01$), which rose steadily after brodifacoum exposure to reach 144.9 ± 1.2 s on day 20 (Fig. 1). APTT differed significantly from controls from day 8 ($P < 0.05$), and differed from previous values from day 12 ($P < 0.05$).

Similarly, one-stage prothrombin times (OSPT) of untreated possums did not change over time, whereas values for treated possums showed a sharp increase after brodifacoum ingestion, rising to a peak at the maximum testable limit of 300 s on day 16 ($F_{4,23} = 22.46$, $P < 0.05$). OSPT of treated possums also differed from both untreated animals ($P < 0.05$) and previous values from day 8 ($P < 0.05$) (Fig. 1).

Pathology and liver concentrations of brodifacoum

Haemorrhages were found in all possums that died or were killed later than 4 days after initial exposure to brodifacoum in both experiments. The mean concentration of brodifacoum in liver of possums that were used for behavioural observations (and had consumed 0.50 – 1.07 mg brodifacoum/ kg body weight), was 0.53 ± 0.05 μg/g, but it varied from 0.17 to 1.04 μg/g.

Based on necropsies of possums killed at a range of times after exposure, haemorrhages became increasingly more severe, more extensive within each region and occurred in more possums as time after initial ingestion of brodifacoum increased (Table 1).

At 4 days after initial exposure to brodifacoum, only one possum had a mild haemorrhage (a small focal haemorrhage in the gastric mucosa), whereas all possums had haemorrhages of varying severity from day 8 onwards, with the first severe haemorrhage occurring on day 12, and at day 20 all but one had severe haemorrhages. Haemorrhages also became more widespread and prevalent in each possum. At 4 days after initial exposure, one possum had one haemorrhage, whereas at 8 days after exposure, all possums had at least one haemorrhage in one or two areas of the body. All possums had at least one haemorrhage in one to three areas at day 12, and in up to five areas thereafter (Table 2).

Possums allowed to die from brodifacoum had a similar pattern of haemorrhages. Those dying earlier had fewer severe haemorrhages than those dying later, which had similar characteristics to the possums killed on day 20. Nearly all had

mild haemorrhages, and 8 of the 12 possums necropsied (with liver brodifacoum concentrations from 0.33–1.04 μg/g) had severe haemorrhages (Table 1).

Table 1. Total number of caged possums with haemorrhages of different severity and distribution* at 4, 8, 12, 16 or 20 days after initial exposure to brodifacoum, or at death.

Day	Group size	Severity of haemorrhage			Distribution of haemorrhage		
		Mild	Moderate	Severe	Focal/ multifocal	Patchy	Locally extensive/ diffuse
4	3	1	0	0	1	0	0
8	3	3	0	0	1	2	1
12	5	4	1	1	4	2	0
16	5	5	2	2	5	4	2
20	5	5	4	4	5	5	3
Death	12	10	5	8	11	10	4

*Note that the same possum could have a number of haemorrhages of the same severity or distribution, so this table does not accurately represent the number of haemorrhages in each possum. All possums had haemorrhages from day 8 onward, and at death (see text for details).

Overall, most haemorrhaging was in subcutaneous or deep tissues within the limbs, abdomen, and lumbar and sacral regions of the back (included in the pelvic cavity data in Table 2), and in the lungs, heart and gastrointestinal tract.

Haemorrhages commonly included focal or multifocal and patchy haemorrhages in the lungs; petechial and patchy haemorrhages in the heart; petechial haemorrhaging, focal and multifocal haemorrhaging and congestion in the gastric mucosa or serosa; focal and patchy intramural haemorrhaging in the intestinal tract; petechial haemorrhaging and congestion within the urinary bladder mucosa; haemorrhaging in or between one or more subcutaneous layers of fat, connective tissue (including periosteum) and muscle in the thoracic region, abdomen, lumbar and sacral regions of the back, pelvic cavity, limbs, scrotum or testes (including free blood and petechial haemorrhaging between the seminiferous tubules, and free blood in connective tissue outside the tunica albuginea). Moderate and severe haemorrhaging in these areas often caused swelling of associated tissues. Blood in the hip joint capsule, blood within the orbit behind an eye (causing the eye to protrude), and blood under the cornea and the

lachrymal caruncle (causing it to swell) were seen in one possum each. Oedema and congestion in some organs, haemorrhage and congested blood vessels in the brain, enlarged spleens, gastric ulceration and enlarged adrenal glands were also noted in some possums. NB: The overall prevalences are not given here because it was either irregularly looked for during necropsies (e.g., enlarged adrenal glands), it was difficult to distinguish from post mortem changes in every case (congestion), or it was seen in possums used in the coagulopathy experiment: because these were killed at certain time points rather than allowed to die, it is possible that more possums could have developed these pathologies before death, and the number of these possums afflicted is therefore not a true representation of prevalence.

Table 2. Number of haemorrhages of different severity (mild, moderate or severe) and extents (focal or multifocal, patchy, diffuse or extensive) in the organs or remaining tissues other than bone in different areas of the body occurring in penned brushtail possums poisoned with brodifacoum, and number of possums with haemorrhages in different regions of the body and of different severity and extents.

Body region		No. of possums affected	Severity			Extent		
			Mild	Mod	Severe	Focal	Patchy	Extensive
Thorax	Tissue	9	1	0	2	0	1	3
	Organs		6	0	5	14	5	1
Abdomen	Tissue	7	1	0	1	1	1	1
	Organs		6	1	0	10	6	1
Pelvic cavity	Tissue	11	1	2	6	1	5	3
	Organs		5	1	0	5	3	0
Limbs	Tissue	5	4	1	3	6	3	5
No. of possums affected (n = 12)		12	10	5	8	11	10	4
Total number of lesions		46	24	5	17	37	24	14

Clinical signs

Clinical signs of poisoning (Table 3) were observed slightly earlier than overt behavioural changes, beginning on average 14 days after initial exposure (around 7 days before death). Most of the 18 possums had, in order of appearance, black tarry or bloody droppings (13 possums), pale noses, ears or foot pads (14), a changed appearance typical of a sick possum, with sunken and staring eyes, drooping faces, and lowered ears (16) and external haemorrhages (14). Abnormal breathing (including apnoea, dyspnoea, tachypnoea and bradypnoea) (5), trembling or shivering (3), myoclonic spasms (sudden jerking movements of limb or body) (5), intermittent tremors (repetitive twitching of limb or body) (2), and incoordination when moving (5) were also seen infrequently, and also one possum vomited and one developed diarrhoea. In addition, the ears, tail and paws often felt cold when possums were manipulated during response tests.

Table 3. Time after dosing at which clinical signs of poisoning appeared in caged brushtail possums fed a lethal dose of brodifacoum, compared to the time to death. Times are the mean or mean ± SEM, range is in parentheses, and total *n* = 18 for each sign.

Sign	Number affected	Day of appearance after baits offered*
Dark or bloody droppings	13	14# (11 – 19)
Pale extremities	14	14 (13 – 18)
Noted sick	16	15 (13 – 20)
Abnormal postures	18	16 (12 – 20)
External bleeding	14	16 (13 – 19)
Time to death	16	20.7 ± 1.7 (15 – 45)

*Baits were first offered on day 0 and were removed on day 3.

#Times are given as whole numbers as clinical signs were only checked twice each day until 17 days after baits were offered and are only accurate to this degree of significance.

Feed intake decreased gradually after poisoning, and was significantly reduced 15 days after initial exposure to brodifacoum (around 6 days before death) ($P < 0.01$), differing significantly from that of untreated possums ($\Pi^2_{17} = 29.31$, $P < 0.05$), in which intake remained relatively constant throughout the experiment (Fig. 2). Body weight of poisoned possums declined significantly after they stopped eating ($t_{14} = 2.879$; $P <$

0.05), and at death was 5.9 ± 2.1% lower on average than pretreatment body weight, with one possum losing 23.6%.

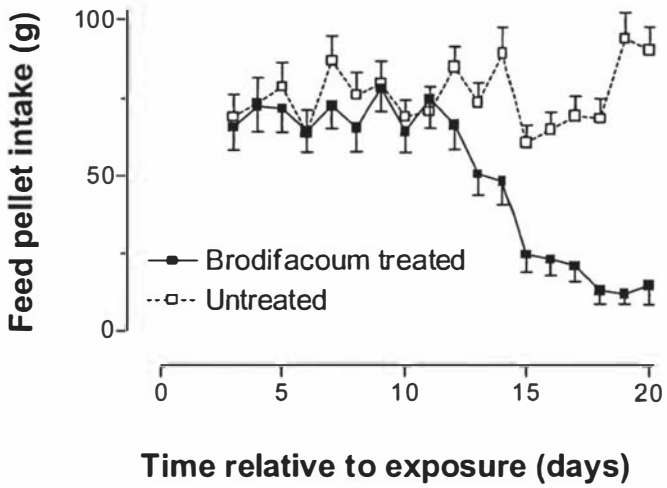

Fig. 2. Mean (±SEM) daily feed pellet intake of brushtail possums after exposure to brodifacoum in cereal baits ('brodifacoum treated', $n = 18$) or untreated cereal baits ('untreated', $n = 18$) over three days (day 0–2).

Behaviour and responses to stimuli

Behaviour of untreated possums did not change significantly throughout the experiment (Fig. 3). They were mainly seen sitting or lying in a curled posture (curled in a ball lying on one side, or curled resting upright on the lumbar or sacral regions of back with the tail, forelegs and usually the head tucked into the chest or abdomen). They were occasionally seen crouching (sitting or standing in a hunched posture, often with the head down), and the proportion of time this was observed generally appeared to be higher in the second half of the experiment (Fig. 3). They were occasionally seen grooming, eating, drinking, sitting, standing, walking, climbing, urinating, defecating, and very rarely lying.

In the first stage of poisoning, the behaviour of brodifacoum-poisoned possums was indistinguishable from that of untreated animals. Then the proportion of time spent crouching increased significantly, and beginning at 15 days after initial exposure (approximately 6 days before death), poisoned possums were seen crouching significantly more frequently than untreated animals (Fig. 3) ($\Pi^2_4 = 21.78$, $P < 0.001$).

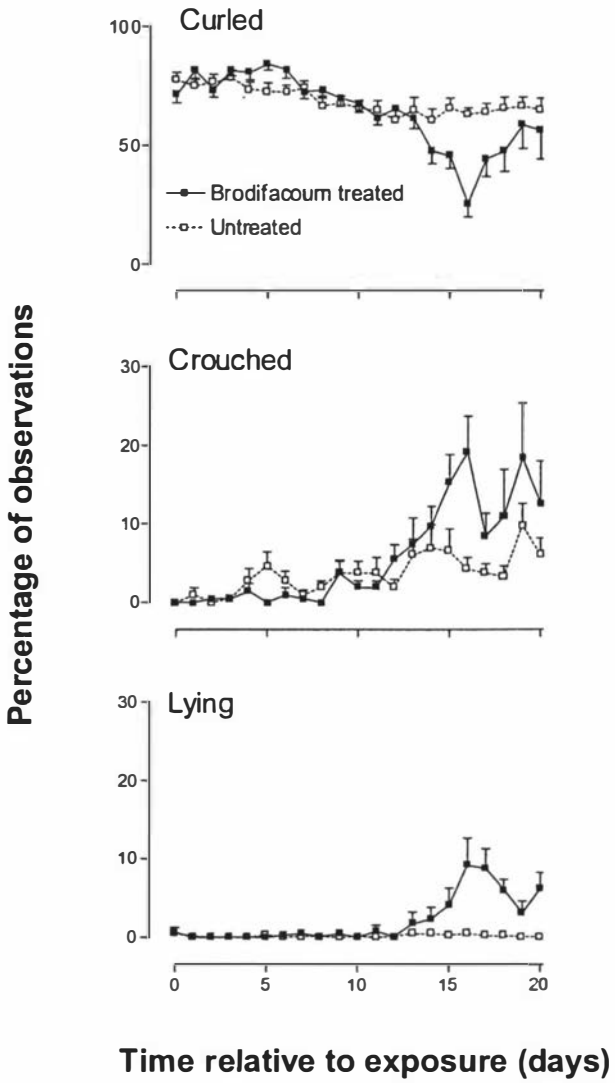

Fig. 3. Mean (±SEM) percentage of observations in which brushtail possums were observed sitting or lying in a curled posture ('curled'), crouching with hunched back ('crouched') or lying on the side, back or front ('lying'). Possums were fed brodifacoum in cereal baits ('brodifacoum treated', $n = 18$) or untreated cereal baits ('untreated', $n = 18$) over three days (day 0–2). Note different scales on y-axis.

The proportion of time spent lying also increased substantially from around 15 days after exposure. Correspondingly, the proportion of time spent sitting or lying in a curled posture progressively dropped, and beginning at 15 days after initial exposure to brodifacoum, the frequency of this behaviour was significantly lower than that seen in treated possums before exposure, and in untreated possums ($\Pi^2_4 = 30.87$, $P < 0.001$).

During this period, possums often appeared to be lethargic or listless. They were still occasionally seen grooming, drinking, sitting, standing, urinating and defecating, but were often in abnormal postures that were not seen in untreated possums, or previously in the same possums.

Finally, seven possums were seen lying prostrate for an average of 2.3 ± 0.8 h before death. A complete record of loss of response to stimuli until death was only achieved for six possums. The pattern of loss of response to stimuli was not consistent between possums, and not all of these possums could be tested each time. However, it was clear that although they had lost some responses to stimuli from more than 3 h before they became prostrate (Table 4), they remained sensitive to a touch on the back and a quick blow of air onto the face during this time, and corneal and palpebral reflexes were not lost until death.

Table 4. Time before death (mean ± SEM) at which brushtail possums lost overt responses to stimuli compared to the time at which possums became prostrate, and number of values contributing to each mean. (See materials and methods for details of stimuli.)

Stimulus	*n*	Time before death
		Mean ± SEM (h:min)
Threatening gesture	5	5:54 ± 3:02
Ear pinch	6	3:23 ± 0:56
Tail pinch	5	3:01 ± 1:14
Toe pinch	5	2:31 ± 0:50
Prostrate	7	2:20 ± 0:50
Touch	6	2:04 ± 0:43
Air blow	6	1:12 ± 0:15
Palpebral stimulation	6	(range: 0:00–0:02)
Corneal stimulation	6	(range: 0:00–0:02)

Two brodifacoum-treated possums did not die and were euthanased on day 50. The mean time to death of the remaining treated possums was 20.7 ± 1.7 days, which

included one possum that died 45 days after initial exposure. The two possums that did not die had consumed the lowest and third-lowest doses of brodifacoum, but there was no correlation between dose consumed and time to death overall ($r^2 = 0.092$; $P = 0.273$). There was no significant difference between the times to death of male possums (21.1 ± 2.7 days) and female possums (20.0 ± 1.0 days) ($t_{14} = 0.292$, $P = 0.775$).

Discussion

Possums poisoned with brodifacoum showed clear increases in blood-clotting times, followed by the development of haemorrhages, display of clinical signs of poisoning and then changes in behaviour.

Times to death

Possums died around 21 days after they were initially fed brodifacoum, but the range was 15 to 45 days. Such wide variations in times to death of brushtail possums killed with brodifacoum have been reported previously (Eason & Jolly 1992). This similarity suggests that there is no major effect of handling on the time to death in the possums. It is possible, though, that the variation masked any effect of handling. Future studies could use a sample or group of possums for handling tests, leaving an unhandled group by which the time to death can be established. Other experiments described in this thesis follow this suggestion (Chapters 3 and 5).

Individual variation in times to death may be caused by individual differences in a number of features such as poison metabolism, age and dose (Brown 1980). It is clear, however, that dose was not responsible for the variation in this study as no significant relationship was found between dose and time to death. This has also been established in previous studies with anticoagulants in possums (Eason & Jolly 1992, 1993).

Time to death in this experiment could be affected by experimental or environmental conditions, and hence may differ from that observed in wild possums. For example, adverse weather conditions combined with blood loss could lead to hypothermia and accelerate the onset of haemorrhage-induced heart failure, or injury could cause more rapid blood loss. Similarly, some aspect of social interaction (such as fighting-induced injury) or available space (such as increased activity) may hasten death, as Cox and Smith (1992) found that colony-housed rats died sooner following

anticoagulant ingestion than rats housed singly in cages. The influence of environmental variables is explored further in a later chapter (Chapter 5).

Coagulopathy

Blood clotting was impaired within 8 days of initial exposure to brodifacoum, implying that haemorrhages could develop at any time after 8 days of initial poisoning. Indeed, haemorrhages were first found in all possums 8 days after initial exposure, as discussed below. Eason *et al.* (1996*a*) reported significantly elevated APTT and OSPT at 4 days after oral dosing and values that were higher than those in the present study. Dose rates were lower than those in this experiment (0.1 mg/kg cf. an average of 0.88 mg/kg), but it is possible that oral dosing allowed more rapid or greater uptake of brodifacoum than that from the cereal bait pellets used here (Brown 1980), which more than compensated for the lower dose. It is worthwhile mentioning that dietary supply of vitamin K, which may vary between possums in the wild and the captive possums in this study, can influence the toxicity of anticoagulants (Osweiler *et al.* 1985; Thijssen 1995), so that a ready dietary supply of vitamin K could feasibly delay the onset of coagulopathy.

Pathology

Possums had many, mainly severe haemorrhages following brodifacoum poisoning. It has been suggested that animals with liver concentrations of brodifacoum of 0.7 μg/g or less do not show significant haemorrhagic changes after brodifacoum ingestion (Kaukeinen *et al.* 2000), and hence would not die. However, there were possums with lower liver brodifacoum concentrations in the present study, and even those with concentrations as low as 0.33 μg/g developed severe haemorrhages and died. This implies that possums which eat a low dose of brodifacoum still develop haemorrhages, and if they do not die, could experience consequential noxious effects of the haemorrhages.

Haemorrhages were largely in subcutaneous or deep tissues in the lumbar and sacral region of the back, abdomen or limbs, and in the lungs, heart and gastrointestinal tract, but also occurred elsewhere throughout the body. This has also been described in rats (MAFF 1997) and other animals (Osweiler *et al.* 1985). Likewise in humans, accidental or intentional overdose of anticoagulants has led to haemorrhages at a range of sites (Lilly & Lee 1949; Macon *et al.* 1970; Stanton *et al.* 1974; Helmuth *et al.* 1989;

Weitzel *et al.* 1990; Kruse & Carlson 1992; Hollinger & Pastoor 1993; Ornstein *et al.* 1999).

Haemorrhages and other pathological changes first occurred in all possums after 8 days, then haemorrhages became more widespread, severe and prevalent over time. The first severe haemorrhage occurred 12 days after initial brodifacoum exposure. Since possums died about 21 days after initial poisoning, this suggests that possums may have haemorrhages and other pathological changes for up to about 13 days before death, and severe haemorrhages for 9 days. It was anticipated that possums with severe haemorrhages would die earlier, since it seems likely that blood loss from a large or severe haemorrhage may be rapid enough to cause death before further haemorrhages develop. However, it appears that haemorrhages in possums become increasingly severe over time until they eventually cause death some time after poisoning. Even possums that had extensive blood loss through internal bleeding within the abdominal cavity or external bleeding from the nose, ears or a laceration, died at the same times as possums that were not affected in these ways. It may be that the relatively big splenic reserve of red blood cells in possums (Dawson & Denny 1968) and their considerable ability to conserve water (Hume 1982) mean that they are able to compensate, to a large extent, for the anoxia and fluid loss that is caused by haemorrhage.

The time of onset, location and severity of pathological changes may also be affected by environmental and experimental conditions. For example, temperature, behaviours performed, degree of activity and other events such as social interactions or injury could all influence haemorrhaging in possums in the wild. These features were likely controlled or constrained by the indoor cages in the present study, and so could have affected the observed pathological changes.

Clinical signs and behavioural changes

Poisoned possums first showed overt clinical signs of poisoning from 14 days after initial exposure to brodifacoum. The principal signs were pale noses, bloody or black tarry droppings, changed appearance and external bleeding. However, abnormal breathing, diarrhoea, shivering and trembling, and spasms and tremors were also seen in some possums. The clinical signs seen in our study were similar to the well-recognised signs of anticoagulant poisoning and subsequent haemorrhage reported in other animals (Osweiler *et al.* 1985; Radostits *et al.* 1999). To a certain extent, these signs apparently

depend on the location and severity of haemorrhages (Osweiler *et al.*1985). For example, while a pale nose can be a reflection of general blood loss and its consequences (Radostits *et al.* 1999), haemorrhages in the lungs could be expected to cause abnormal breathing (Osweiler *et al.* 1985). Because haemorrhages varied so widely in location and severity in the present study, it seems likely that it cannot necessarily be predicted which specific clinical signs a particular brodifacoum-poisoned possum would exhibit.

Possums gradually became inactive and appeared lethargic after brodifacoum poisoning. Inactivity was marked about 15 days after initial exposure, or about 6 days before death. During this period possums often adopted abnormal postures while lying or crouching. At the same time, feed intake was significantly lower or had ceased, so that nearly all possums lost weight.

Time to loss of consciousness

Nearly half the possums were prostrate for around 2 h before death. They lost responses to a pinch on the ear, toe or tail before this time, and to touch shortly afterwards. However, they remained responsive to an air blow onto the face for a further hour and palpebral and corneal reflexes were not lost until death. Importantly, this implies that possums would be capable of experiencing noxious effects of brodifacoum until shortly before death. Rowsell *et al.* (1979) came to a similar conclusion for rats, on the basis that electroencephalograms only became flattened (indicating unconsciousness) when rats became prostrate just before death.

As was discussed in Chapter 2 however, consciousness can be considered as a continuum (e.g., Piggins & Phillips 1998; Sommerville & Broom 1998; Tassi & Muzet 2001). Brodifacoum-poisoned possums were not fully unconscious until close to death, but it is likely that the prolonged cachexia, in addition to or apart from the loss of blood, induced a state of reduced awareness long before then. It is likely that this would reduce the severity of any suffering, but it would be conjecture to say by what degree. With regard to the duration of this reduced awareness, it could be argued that lethargy is an indicator. Possums were noticeably lethargic from six days before death when they began lying. Feed intake was significantly lower at that time (although it tended to decrease from five days earlier), and possums would have had haemorrhages for at least seven days prior to this. Together these observations suggest that the level of awareness

may have been reduced for at least six days before death. Assuming that severe haemorrhages cause more distress than moderate haemorrhages, this further implies that possums may experience the poorest welfare for the three days (from nine to six days before death) between the onset of severe haemorrhages and the onset of reduced awareness. However, this was not supported by the changes in behaviour in this study, except for feed intake which tended to drop from 11 days prior to death.

Implications for animal welfare

The pathophysiological changes in possums poisoned with brodifacoum may have produced specific forms of distress (such as respiratory distress), or distress due to pain, weakness or sickness (malaise). For instance, bleeding into the lungs can cause breathlessness, which could correlate with the abnormal breathing seen in possums. The widespread haemorrhages seen in brodifacoum-poisoned possums may have caused pain, weakness or sickness, dependent on the location and severity of haemorrhages. For example, while haemorrhages are not inherently painful if they occur into a relatively open space, they may lead to pain if they cause tissue swelling or pressure within an enclosed area such as joint cavities or limb compartments (MacLain & Weinstein 1999).

With regard to pain, there is evidence that brodifacoum ingestion by humans causes pain (Helmuth *et al.* 1989; Smolinske *et al.* 1989; Weitzel *et al.* 1990; Kruse & Carlson 1992; Hollinger & Pastoor 1993; Rauch *et al.* 1994; La Rosa *et al.* 1997; Ornstein *et al.* 1999). In addition, haemorrhages caused by anticoagulants or diseases such as haemophilia, in the gastrointestinal tract, kidney, adrenal glands, liver, ovaries, lungs, heart, pericardial cavity, eye, joints, skin, muscle and soft tissues, and throughout the head and brain, cause varying degrees of pain in humans (Lilly & Lee 1949; Macon *et al.* 1970; Stanton *et al.* 1974; MacLain & Weinstein 1999; Procacci *et al.* 1999; Schoenen & Sándor 1999). Such haemorrhages were all found in possums in the present study. It has also been suggested that haemoperitoneum can cause pain due to algesic substances contacting the peritoneum (Rapkin 1999). Assuming that non-human mammals are able to experience similar pain to humans (e.g., Bateson 1991), although intensity and threshold may be uncertain, it is reasonable to suggest that haemorrhages such as those caused by brodifacoum, and some associated changes, may lead to pain in possums.

Furthermore, behavioural evidence from our study affirms that possums may be experiencing pain. The inactivity and abnormal postures could feasibly be a manifestation of possums' attempts to alleviate discomfort or pain, or limit movement of a damaged area. For example, crouching with a hunched back and pressing the head onto the floor, both seen in possums in this study, are commonly considered to indicate abdominal pain or headache, respectively, in dogs (Hellyer & Gaynor 1998). Inappetence and inactivity or lethargy are also considered to be indicators of distress and pain in a variety of animals (Morton & Griffiths 1985; Sanford *et al.* 1986; Johnston 1996).

Certain consequences of blood loss may also contribute to distress. Following major blood loss, animals often appear weak and cold (Osweiler *et al.* 1985; Radostits *et al.* 1999), suggesting that the inactivity, and possibly the abnormal postures, seen in possums in our study, could also be caused by weakness. In addition, the shivering seen may have been caused by mild hypothermia, which could be accompanied by a feeling of being cold. This is supported by our observation that the possums' extremities felt cold when they were handled, but would need to be confirmed by appropriate monitoring of body temperature, for example by rectal thermometer. Such monitoring would also be needed to indicate the degree of cold that the animal was likely to be feeling, in order to assess the degree of welfare compromise, if any. Shivering may also have been caused by the increased amount of adrenaline which is released into the blood in response to haemorrhage (Anderson 1980). Hypoxia and dehydration can also cause headache in humans (Schoenen & Sándor 1999) and so could presumably do so in possums. This could perhaps account for the head-pressing seen in possums in this study. If the intestine is compromised by haemorrhagic damage, a further consequence of hypovolaemic shock may be the release of bacterial endotoxin into the blood (Niedringhaus 1983; Radostits *et al.* 1999), leading to the release of inflammatory mediators (Radostits *et al.* 1999), and eventually sickness (Gregory 1998). Gregory (1998) further suggests that listlessness, fatigue and inappetence are indicators of sickness, implying that the similar behaviours seen in the present study could have indicated sickness in the possums.

In summary, regardless of the exact cause, it seems reasonable to suggest that widespread haemorrhages and blood loss caused by brodifacoum would produce distress in possums. Behavioural evidence from possums in the present study supports

this, despite the fact that it was not possible to discriminate between pain, sickness or weakness. The occurrence and severity of distress depend on the site and severity of haemorrhages. However, because haemorrhages were widespread and of varying severity in possums, it would be difficult to predict the nature and severity of distress caused by brodifacoum to any one possum. The duration of distress also depends on time of onset of haemorrhage (which was 13 days before death in this study) and time until death. Anything that affects the tendency to bleed, and the speed of bleeding, may hasten both the onset of clinical signs and death. These features in turn may depend on the environmental or experimental conditions, as discussed earlier.

Humaneness of brodifacoum

The period between the appearance of changes and death caused by brodifacoum is longer than that caused by other poisons used for possum control in New Zealand including cyanide (Gregory *et al.* 1998) and 1080 (Morgan 1990; Chapter 2). Coupled with the potential distress resulting from widespread and severe haemorrhages, this suggests that brodifacoum is relatively inhumane for possums, compared to these other poisons. Rats show similar pathophysiological and behavioural changes after anticoagulant poisoning (Rowsell *et al.* 1979; Desheesh 1983; Cox & Smith 1992; MAFF 1997), and a similar conclusion about humaneness has been reached, with at least three reviews suggesting that anticoagulant poisons are markedly inhumane for rats (Kirkwood *et al.* 1994; MAFF 1997; Mason & Littin 2003). Nevertheless, humaneness issues must be balanced against other considerations, such as practicality, efficacy, safety to non-target animals, user safety, environmental contamination and cost, when deciding which possum control tool to use as noted in Chapter 1.

Improving the humaneness of anticoagulant poisons

It would be advantageous to determine whether brodifacoum could be made more humane by reducing the time to death through adding synergistic compounds, or made less noxious by adding ameliorative agents to baits. In this way, brodifacoum could continue to be used as a practical control tool that is safe for humans, without the disadvantage of causing poor animal welfare.

Poisons can be made more humane by amelioration of unpleasant effects (either by targeting specific effects, or by sedation or anaesthesia) or by reducing the duration

of sickness through hastening the onset of unconsciousness or death. Due to the prolonged time until onset of toxicosis following anticoagulant poisoning and the prolonged time until death, finding ameliorative drugs with an appropriate duration of action could be difficult. In this case, it may be more practical to improve the humaneness of brodifacoum by shortening the duration of sickness. Two ways of doing this are to affect the toxicokinetics or toxicodynamics, or to increase the tendency to bleed. There are several ways by which drugs or other compounds might do this (Koch-Weser & Sellers 1971*a*,*b*; Lewis *et al.* 1974; Bachmann & Sullivan 1983; Sridhara & Krishnamurthy 1992). They may:

a) Affect anticoagulant toxicokinetics or toxicodynamics by influencing:

   i) vitamin K availability – examples include the use of drugs that bind bile salts, alter intestinal motility (e.g., laxatives, anticholinergics), induce steatorrhea, or interfere with mucosal function (e.g., antibiotics, colchicine), or compounds which bind vitamin K (e.g., charcoal);

   ii) anticoagulant absorption – such as by drugs that increase gastric pH (e.g., antacids), reduce gastrointestinal motility, interfere with mucosal function (e.g., antibiotics, colchicine), or form complexes with anticoagulants (e.g., cholestyramine), and non-absorbable oils which dissolve anticoagulant (mineral oils);

   iii) anticoagulant-albumin binding through the use of highly protein-bound drugs which are given in high doses and which accumulate in plasma (e.g., phenylbutazone);

   iv) anticoagulant biotransformation through the use of drugs which alter hepatic function or the action of mixed-function oxidases;

   v) hepatic receptor affinity for anticoagulants.

b) Increase the tendency to bleed by affecting:

   vi) the synthesis of the prothrombin complex – such as by drugs affecting vitamin K, clotting factor synthesis, protein synthesis, or hepatic function;

   vii) prothrombin complex catabolism – such as by drugs causing hypermetabolic states including thyroid drugs;

   viii) haemostasis – such as by drugs affecting platelet function or fibrinolysis;

ix) the vasoconstriction mediated by the sympathetic nervous system which occurs in response to major haemorrhage through the use of vasodilators;

x) tissue stability – such as by drugs increasing likelihood of tissue damage (e.g., ulcerogenic drugs).

Most drugs have more than one action, and actions are often linked, for example, affecting vitamin K availability also affects prothrombin complex synthesis. There has apparently been one study with an alternative aim but that nevertheless showed that two non-steroidal anti-inflammatory drugs reduced the time to death of anticoagulant-poisoned rats (Sridhara & Krishnamurthy 1992) and similar studies investigating the effects of various compounds on warfarin toxicity that would also have the effect of reducing the time to death, if successful (Krishnakumari & Muralidhara 1977; Muktha Bai *et al.* 1978; Muktha Bai 1979). This offers some hope that it would be possible to improve the humaneness of brodifacoum for possums. However, this possibility was not pursued further in this thesis as the long time to death of possums poisoned with brodifacoum meant that time constraints prevented further work.

Summary

In summary, caged possums showed overt pathology, coagulopathy and changes in behaviour after brodifacoum poisoning. The timing and character of the responses suggest that possums experience a degree of distress due to pain, weakness, sickness or other causes for at least 6 days before death, and possibly for up to 13 days. The nature, severity and duration of distress are apparently affected by the time of onset, location and severity of haemorrhages, which could make them difficult to predict in individual animals.

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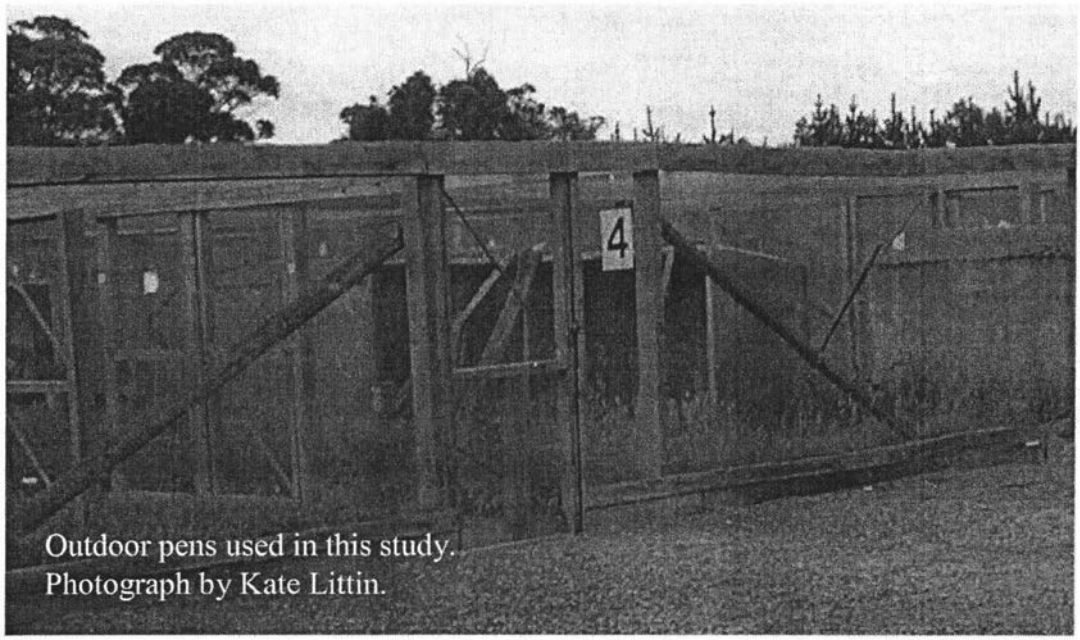
Chapter 5
Behaviour, pathology and pathophysiology of penned possums poisoned with 1080 or brodifacoum.



Outdoor pens used in this study.
Photograph by Kate Littin.

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Abstract

Caged possums have been used for all previous studies described in this thesis. The air temperature, weather, diet, nature and level of social interaction, and nature and level of physical activity could all differ between possums caged indoors and possums living in the wild. The aim of this study was to determine whether the behaviour and pathology of possums poisoned with 1080 or brodifacoum kept in a simulated wild environment differed from that in previous studies using caged possums. This would enable a determination of the applicability of the previous studies using caged possums to a wild environment. Possums were kept in outdoor pens with logs to stimulate physical activity and with limited social contact. They were offered 1080 in carrot baits or brodifacoum in cereal pellets (PESTOFF®). Nine possums that had consumed a lethal dose of 1080 (3.26 ± 0.37 mg/kg (mean±SEM), compared to a LD_{50}[5] of 1.2 mg/kg) and nine that had consumed a lethal dose of brodifacoum (0.86 ± 0.22 mg/kg, compared to a LD_{50} of 0.17 mg/kg) were observed regularly until death. Individual times to death following 1080 poisoning varied widely: the average time to death was 10 h 19 min ± 2 h 56 min after baits were consumed. From 2 h 3 min ± 13 min on average, animals had a changed appearance typical of sick possums. No possums were observed or heard retching or vomiting. Lying became predominant from 3 h 33 min ± 45 min, with most possums showing abnormal breathing during this time. Two were observed having up to four bouts of spasms and three having up to four bouts of seizures of around 2–12 s each and within 1 h 10 min ± 1 h prior to death. The corneal reflex was usually retained during these seizures or regained immediately thereafter in those possums tested. The pattern and character of behavioural changes, duration of toxicosis and the times to death are similar to those in previous studies using caged possums (Chapter 2) suggesting that those studies are applicable to the wild. Following brodifacoum poisoning, one possum was euthanased 52 days after the first day of exposure and the remaining possums died 22.2 ± 1.4 days after first exposure. Pale noses were seen within four days of the bait first being offered, reduced feed pellet intake at 15 days and reduced apple intake at 18 days, external bleeding within 18 days, a changed appearance typical of sick possums within 20 days, and inactivity within 21 days followed by

[5] The median dose that kills 50% of the exposed animals.

prolonged lying until death. Possums lost up to 14.1% of their pre-exposure body weight. All eight possums that died had mild or moderate haemorrhages and four had severe haemorrhages, largely in the subcutaneous or deep tissues in the thorax, and in the lungs and gastrointestinal tract. The pattern and character of behavioural changes was similar to those in caged, brodifacoum-poisoned possums described in Chapter 4 but the appearance of most signs of toxicosis was delayed by about 2–4 days, suggesting that the duration of sickness was shorter. The reasons for this are not clear.

Introduction

Previous studies described in this thesis assessed the behavioural, pathophysiological and pathological effects of 1080 and brodifacoum on brushtail possums in individual, indoor cages. The implications of these changes for possum welfare were discussed. These studies were unique since no such studies had apparently been conducted on possums, and important since they could influence policy and practice in possum control in New Zealand. However, as discussed in the preceding chapters, environmental factors encountered by possums in the wild could alter these effects and thereby the animal welfare impacts of these poisons used for possum control. Indeed, several environmental factors, such as circadian rhythm, social environment, air temperature and diet are known to influence the action of drugs and poisons on animals (Clarke & Clarke 1967; Ellis 1967; Brown 1980). This would mean that the results and animal welfare implications already described in this thesis might not be directly applicable to possums in the wild.

Differences between indoor cages and outdoor pens

There are five main differences between conditions experienced by free-living possums in the wild and possums that are caged individually indoors which might influence the behavioural, pathophysiological and pathological changes described previously in this thesis. These conditions may interact together or work individually to affect the speed with which poisons act and the effects they cause. The conditions are (1) air temperature; (2) weather; (3) diet; (4) social interactions and (5) level and type of activity. The first four of these are influenced by the experimental set-up of individual caging indoors and feeding on a laboratory diet. The last is a product of the proximity to both conspecific neighbours and human observers, and the size of the cages.

Potential impacts of environmental factors on 1080 and brodifacoum toxicosis

Some environmental factors are of particular relevance to the animal welfare impacts of 1080 and brodifacoum toxicosis. For 1080, air temperature, weather, and level of activity might be important, whereas social interactions and diet would be less so. For brodifacoum, air temperature, weather, diet, social interactions, and level of activity might all be important, as follows.

1080

As outlined before (Chapter 2), 1080 causes death by inhibiting essential enzymes in the TCA cycle causing its cessation and, consequently, energy deprivation (Clarke 1991). It is has been established that a low ambient temperature reduces the dose of 1080 required to kill (Misustova *et al.* 1969; Oliver & King 1983). Presumably, at least part of the explanation for this is some interaction between the requirement for energy by thermogenesis and the reduced energy available for other essential processes following 1080 ingestion. Air temperatures below about 7–10 °C induce thermogenesis in brushtail possums (van den Oord *et al.* 1995), suggesting that 1080 would be more toxic at these temperatures. This further suggests that the same dose would be more effective at lower temperatures, and indeed, Veltman and Pinder (2001) showed that mortality was increased following 1080 possum control operations in New Zealand at colder air temperatures, regardless of the amount of bait spread over an area (influenced by the sowing rate) or bait concentration. Given that a higher dose of 1080 can cause the more rapid appearance of clinical signs of toxicosis (Chenoweth 1949; McIlroy 1982, 1983; Morgan 1990) and can kill more quickly (Morgan 1990), it also seems possible that the time to death would be reduced at lower temperatures because any given dose of 1080 would be more effective – a potential advantage for animal welfare given that the duration of toxicosis and possible suffering could be shortened. A possible explanation for this is that the actions of 1080 and any other process requiring substantial energy would together lead to energy depletion and its sequelae more rapidly than either process acting on its own.

Similarly, adverse weather might also affect the toxicity and time to death of 1080 if it is linked to low air temperatures. For example, wet and windy conditions could be expected to increase evaporative heat loss and promote thermogenesis and

could thereby decrease the time to death in animals poisoned with 1080. Further, if 1080-poisoned possums became hypothermic as a result of the inability to produce heat due to the lack of energy to do so combined with the cold conditions, this could also act to reduce the time to death.

Generally speaking, stomach contents influence the toxicokinetics and potentially the toxicodynamics of poisons (e.g., Brown 1980; Ross *et al.* 2000). The type and amount of feed already in the gut at the time of dosing, particularly with regard to available energy, might also influence the toxicity of 1080. However, the type and amount of feed consumed *after* dosing are not likely to alter a possum's response to 1080 since possums stop or substantially reduce feed intake following 1080 poisoning (Morgan 1990; Chapter 2).

The effect of cages on the level and type of activity might also be particularly pertinent in 1080 poisoning for two main reasons. Firstly, the 'manic running' induced by 1080 in carnivores (e.g., foxes; Marks *et al.* 2000), and occasionally in other animals (e.g., sheep; Schultz *et al.* 1982) could lead to physical injury and thereby animal welfare compromise. It is also of concern aesthetically, probably contributing to the general opinion that 1080 is inhumane for canids. Such manic running or a similar extreme increase in activity might be seen in 1080-poisoned possums if they were not confined by small cages, as were used in previous studies in this thesis. Secondly, the speed of action of 1080 might be enhanced by an increased amount of physical activity after dosing for the same reason put forward for thermogenesis above.

Brodifacoum

Brodifacoum is a coumarin anticoagulant poison that impairs the cycling of inactive vitamin K-dependent blood clotting factors to active factors so that blood clotting itself is markedly reduced and widespread haemorrhaging occurs (Osweiler *et al.* 1985). Death is caused by the consequences of hypovolaemia (Anderson 1980; Radostits *et al.* 1999).

Air temperature and weather may have implications for the welfare of brodifacoum-poisoned possums: cold temperatures might be of concern because hypovolaemia predisposes animals to hypothermia, and the cold environment could exacerbate this. While there may be a feeling of being chilled or cold at the onset of hypothermia which could be unpleasant, significant hypothermia could be a benefit to

possum welfare if it dulls consciousness (Mellor & Stafford 2003) or reduces the time to death. It seems possible that this feeling could be exacerbated by air temperatures below the critical low level for possums of about 7 to 10 °C (van den Oord *et al.* 1995).

Diet is also important following poisoning by coumarin anticoagulants, largely because the level of vitamin K in the diet influences the likelihood of anticoagulation (Thijssen 1995) and therefore could influence the onset of sickness or the time to death.

So too, the action of brodifacoum might be influenced by the level of activity and anything else that increases the risk of injury, including aggressive social encounters. In fact, there is evidence that colony housed and free-living rats die more quickly after brodifacoum poisoning than caged rats (Redfern *et al.* 1976; Cox and Smith 1992). Additionally, brodifacoum caused intra-articular haemorrhaging in one possum in the study described in Chapter 4. This could be expected to cause pain and lameness owing to stimulation of intra-articular nociceptors by the pressure caused by accumulated blood, and indeed causes pain in humans (MacLain & Weinstein 1999). However, lameness cannot be easily observed if possums are not able to move about freely, as is the case in small cages.

Assessing environmental impacts on toxicosis

One way of determining whether environmental conditions may influence the results described in this thesis would be to conduct studies on wild, free-living possums. However, detailed observation and evaluation of behavioural and pathophysiological changes is needed in order to compare the outcome with that of caged studies. Such close observation is not possible in the wild because possums are free to move away after consuming poisons. In addition

- there could be practical difficulties with ensuring that all possums received a similar and lethal dose;
- it has sometimes been necessary in studies described in this thesis to restrict food intake immediately prior to presenting possums with baits in order to increase bait intake, and food intake could not be as easily restricted in the wild as it can be in captive possums;
- the health and history, such as breeding history and status, and prior exposure to toxins and pesticides, might not be well-known.

Another way of determining whether environmental factors affect the animal welfare impacts of 1080 and brodifacoum would be by studying possums in outdoor pens in conditions simulating those experienced in the wild. There have apparently been no published studies on penned or free-living possums that are detailed enough to provide sufficient information for an evaluation of the welfare impacts of the poisons used for killing possums. It was therefore decided that the animal welfare impacts of poisons in penned possums should be investigated.

The environmental factors discussed above were provided or managed in outdoor pens in a manner which was designed to ensure that pen conditions as closely as possible resembled the natural environment of free-living wild possums in New Zealand.

- Air temperature and weather – possums were outside and exposed to natural variations in temperature and weather during Canterbury spring-summer.
- Diet – wild possums in New Zealand consume a wide variety of plant- and animal-based foods (Nugent *et al.* 2000), and it was not feasible to provide captive possums with a similar variety and amount of feed. In addition, too much manipulation of feed could bias results owing to the variable nutritional composition of different types of feed. However, the pens used here contained grass which possums eat and penned possums were allowed free access to cereal pellets and fresh fruit and vegetables as described below.
- Social interactions – possums are not naturally social animals but will interact during breeding or at bait stations (Day *et al.* 2000). It was thus thought that keeping possums in groups when in pens would falsely raise the chance of social interactions above that encountered in the wild, since captive possums are likely to compete for the same feeder and drinker, and for space within the enclosed area of a pen. Possums were consequently penned individually rather than in groups.
- Level of activity – possums were provided with logs to stimulate climbing, and enclosures were large and complex enough to allow possums to climb, jump and run freely, which they were seen to do (see below).

Within these limits, therefore, the main aim of this study was to establish the duration and character of the principal behavioural effects and the time to death in penned

possums poisoned with 1080 or brodifacoum, in order to determine whether there were differences between caged and penned possums in these features.

Methods

All experiments were conducted with prior approval from the Landcare Research Animal Ethics Committee (project 00/9/3). Penned possums were fed a lethal dose of 1080 or brodifacoum equivalent to that fed to caged possums in previous studies (Chapters 2 and 4), then observed for behavioural changes and signs of sickness until death. Brodifacoum-poisoned possums were necropsied in order to determine pathophysiological changes.

Animals and housing

Wild-caught male and female brushtail possums (*Trichosurus vulpecula*) of mixed age were used in two experiments. They had been trapped from tuberculosis-free areas of the South Island in New Zealand. Possums were initially kept in individual wire cages (350 x 200 x 200 cm) with removable nestboxes (30 x 20 x 20 cm) in temperature-controlled indoor rooms (19 ± 5 °C) under natural daylength fluorescent light. Females were not lactating or carrying pouch young. Possums had free access to water and cereal feed pellets (Weston Milling, Christchurch, New Zealand) and were fed fresh apples or carrots once a day. After 6–15 weeks in these conditions, and after possums had started to gain weight, they were transferred to outdoor pens.

The outdoor pens measured approximately 4 x 4 x 4 m (l x w x h) and were constructed from timber framing with a covering of wire mesh (Fig. 1, and see Title page of this chapter). They were in rows of eight, with each internal row in the compound abutting onto another row behind it, meaning that each possum had visual, auditory and olfactory contact with several others, and potential tactile contact with at least one other possum. Pens had logs leaning against the walls and an open-fronted shed as a shelter with solid corrugated iron on three sides and a roof, and containing a feed hopper and a hessian sack positioned in the corner as a nest (Fig. 1). The bottom of each pen was covered in pasture. The exact composition of pasture was not determined, but included clover and rye grass species. During behavioural observations, an open-sided, plastic apple-crate (approximately 70 x 50 x 50 cm) was substituted for sacks as a nest box. Water was freely available, fresh apples or carrots

were fed once each day, and cereal pellets were freely available throughout the experiment except immediately prior to and during dosing, as detailed below. Possums were kept in these pens for 7 weeks (1080) and 21 weeks (brodifacoum) prior to each experiment.

Possums were divided into two groups of nine as follows. First, the average body weight of all possums was calculated. Next, males and females were assigned to below average, approximately average and above average body weight categories. Three possums from each of these categories were then randomly assigned to each treatment so that the sex ratio and average body weight were similar for both groups. One group was offered 1080 in one experiment and the other group was offered brodifacoum in a second experiment, as described below. Three possums from each group were further randomly chosen for focal behavioural observations, as further described below. The timing of the experiments was reliant upon possum availability. They took place during Canterbury spring–summer: the 1080 experiment during October, and brodifacoum experiment during January. Air temperatures are noted in the results below.

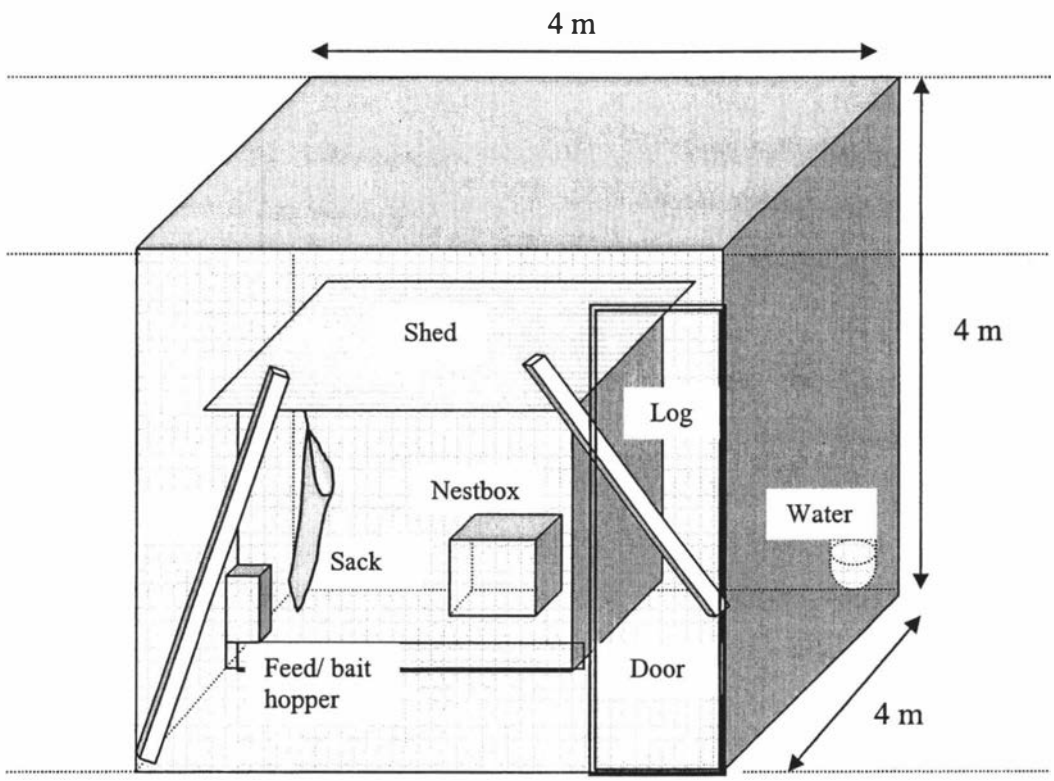

Fig. 1. Layout of pens for study on effects of 1080 or brodifacoum on penned possums.

1080 bait manufacture and exposure

Nine possums (5 male, 4 female) were fed 1080 in carrot baits, rather than orally dosed by gastric lavage, so as to better represent a field situation. Baits remained in the pens for one night (day 0) during which time possums also had continuous access to normal cereal feed pellets. As with the previous 1080 study described in Chapter 2, baits were manufactured in a process simulating normal field production of 0.15% 1080 carrot baits (Morgan & Hickling 2000), as follows. A solution of 20% 1080 in water, blue dye and cinnamon oil were added to fresh, unpeeled carrots diced into approximately 10-g pieces and shaken in a zip lock bag until the baits were coated. Baits were immediately stored at less than 4 °C until use within 12 h. A sample of bait was kept at -20 °C for determination of the concentration of 1080 using gas chromatography (DWRC 1989).

Baits were fed at 18:15h in October (New Zealand spring), and the time at which possums started and finished eating baits and the amount eaten were recorded. Given the nominal bait concentration of 0.15%, possums were offered 3.0 g carrot bait per kg body weight to provide about 4.5 mg per kg body weight, compared to the oral LD_{50} of 1.2 mg/kg for possums (Eason & Wickstrom 2001). Residual bait was removed and weighed in order to determine the amount consumed.

Brodifacoum exposure

Nine possums (5 male, 4 female) were exposed to brodifacoum in a commercially available form, as could be used in a normal pest control field operation. Standard cereal feed pellets were removed and feed hoppers filled with 1 mg brodifacoum per kg body weight in cereal pellets (containing a nominal concentration of 20 mg brodifacoum per kg RS5 cereal pellets; PestOff™, Animal Control Products, Wanganui) at 11:00–11:30 h in January (New Zealand summer). Bait remained in pens for three nights (days 0–2) in order to simulate exposure in a field situation. Remaining bait was removed, weighed and replaced in pens between around 11:00–11:30 h each morning in order to determine the amount consumed each night.

Weather and air temperature

The weather and air temperature were recorded at each behaviour observation session. Weather was assessed subjectively with respect to wind, rain and cloud cover, and temperature by means of a maximum-minimum thermometer placed inside a shed in an empty pen. Recordings were taken from day –5 to day 1 for the 1080 experiment, and from day –5 to day 29 for the brodifacoum experiment. Maximum and minimum air temperatures were recorded at 0900h on each of these days and the thermometer reset. These local measurements were used in preference to regional meteorological data as the pens are located in a sheltered site and it was thought that regional data would not accurately reflect conditions in the pens.

Behavioural observations

Five days prior to exposure in both experiments, sacks were removed from pens and replaced with nestboxes – open-sided, plastic apple-crates (approximately 70 x 50 x 50 cm, l x w x h). A 100W incandescent bulb in every second pen provided low 24-h illumination. The bulb was coloured red in the brodifacoum experiment where it was anticipated that observations would occur over several weeks and that the exposure to constant light could influence behaviour. These measures were to facilitate behavioural observations.

Behavioural observations were recorded from instantaneous scan sampling and *ad libitum* focal animal sampling. For instantaneous scan sampling, possums were sequentially observed and the position within the pen, and behaviour or posture of the animal were recorded instantly. Postures and behaviours recorded were sitting or curled in a resting posture; standing or hanging onto the side or top of the pen or log; walking; running, climbing or jumping; grooming; crouching; lying on the belly, back or side; and lying prostrate – completely flat – on the side, back or belly. Externally apparent clinical signs of poisoning such as incoordination or a changed appearance typical of a sick possum as described in earlier chapters, were recorded at the same time.

For *ad libitum* focal sampling, three animals were randomly chosen in each experiment. They were separately observed for a 5-min period and every behaviour was recorded as it occurred. All observations were undertaken from before dosing until death; death was confirmed by the loss of corneal and palpebral reflexes, and the cessation of breathing and a palpable heartbeat.

1080

From 5 days prior to dosing with 1080, instantaneous scan samples were performed once every 15 min for 30 min–1 h in the morning and for 1–2h at night. This was to habituate possums to an observer and to determine the time at which the experimental possums were active at night. Between scan samples, focal observations were made once each hour in a random order. From 22 h prior to exposure (–22 h), instantaneous scan samples were performed once every 15 min until death in all possums but one. This possum was observed every 15 min until it had been prostrate for 4 h and then once every 2 h until death. The observations were taken from –22 h so that pre-treatment data from the same group of possums, rather than different data from an untreated control group of possums, could be used as a baseline indication of activity levels and health status (i.e., possums acted as their own controls).

Brodifacoum

As with the 1080 experiment, observations were started prior to dosing in order to habituate possums to an observer and so that possums could act as their own controls. Beginning five days before exposure to brodifacoum (day –5), instantaneous scan samples were performed once every 15 min from 1000–1100 h, when they were usually inactive, and from 2200–2300 h, when they were usually active, each day until death. This gave eight observations every day. Between scan samples, focal observations were made once each hour in a random order. In addition, an extra instantaneous scan sample was taken once an hour, at the same time every hour, and signs of poisoning, such as external bleeding or a changed appearance indicating sickness, were recorded.

Responses to stimuli

If focal animals became prostrate or lay continuously for more than one hour, they were tested for their responses to stimuli in order to determine the time until loss of consciousness. Tests were conducted once every hour, or at more or less frequent intervals if it appeared that the possum had changed its state of consciousness, for example by becoming obviously aware of the observer. The stimuli were presented in the same order each time, as follows: (1) quick blow of air into the face; (2) if they did not respond, they were picked up in order to determine their response to handling; (3) they were then laid down on their back in order to determine the functionality of their

righting reflex; (4) if they did not roll over, their palpebral and corneal reflexes were tested with a piece of straw. Any animals that were observed having a seizure had their corneal reflex tested immediately on resolution of the seizure.

Necropsies

The bodies of all possums in the brodifacoum experiment were necropsied within 1 h after death or frozen immediately at –20 °C for later necropsy. Haemorrhages were grouped according to the region in which they occurred (head, thorax, abdomen, pelvic cavity, limbs) and whether they occurred within organs or other tissues in each region. They were then qualitatively graded according to severity: mild (through one layer of tissue of the same type, e.g., through the subcutaneous fat); moderate (two layers of tissue, e.g., through the subcutaneous fat and into the connective tissue covering muscle, or moderate depth in an organ); severe (three or more layers of tissue, e.g., through the subcutaneous fat, connective tissue, muscle and into the periosteum, or through the entire depth of an organ), and also according to distribution: focal or multifocal (within a discrete boundary, e.g., petechial or ecchymotic haemorrhages); patchy (no marked boundary); locally extensive or diffuse (large areas of bleeding through most or all of an organ or limb). Both hip and stifle joints were checked for intra-articular haemorrhage. Any other pathological changes were noted if observed, but were not systematically recorded.

Feed pellet and fruit intake

In the brodifacoum experiment, cereal feed pellets and remaining fruit and vegetables were removed from each pen at 1100h each morning. Pellets were weighed in order to determine intake, and the amount of fruit or vegetables remaining was recorded to the nearest quarter of a portion. Feed intake was not assessed in the 1080 experiment.

Body weight

All possums were weighed on placement into pens, again 6 days prior to exposure to 1080 or 11 days prior to brodifacoum exposure, and again at death.

Statistics

All results are reported as mean ± SEM (standard error of the mean) unless otherwise stated. In the 1080 experiment, as with the previous experiment on 1080 (Chapter 2),

times to death and times to onset of clinical signs and the loss of responses to stimuli were calculated from the time at which possums had finished consuming baits. In the brodifacoum experiment, as with the previous study on brodifacoum (Chapter 4), times were calculated from the time at which possums were first offered brodifacoum baits. This is because while brodifacoum was offered over three nights in this study, it begins acting shortly after it is first consumed (Thijssen 1995) and blood clotting time in possums begins decreasing at some time within two days of exposure (Eason *et al.* 1996). The use of any other time as a fixed starting point over the 3-day exposure period would be relatively arbitrary.

The intake of cereal feed pellets before brodifacoum exposure was compared to that after brodifacoum exposure using one-way repeated measures ANOVA followed by post-hoc *t*-tests with Bonferroni adjustments on selected columns. The pre- and post-dosing intakes of apples were similarly compared. A one-way ANOVA showed no between-day differences in feed pellet intake on days –5 to –1 ($F = 1.762$, $P = 0.161$). Apple intake likewise did not differ across these pre-treatment days ($F_4 = 1.103$, $P = 0.372$). Therefore day –5 was chosen as representative of pre-treatment data for post-hoc *t*-tests. Data from day –5 were compared to days 15, 16 and 17 for feed pellet intake, and days 16, 17 and 18 for apple intake.

The difference between times to death of male and female possums, and between focal and non-focal possums, was analysed with *t*-tests with Welch's correction for unequal variances (1080) or standard *t*-tests (brodifacoum).

Pen and cage comparisons were assessed with *t*-tests except for the comparison of 1080 doses (Welch's corrected *t*-test) and 1080 times to death (Mann-Whitney *U*-test).

Results

1080

Possums showed clinical signs of poisoning shortly after consuming 1080 baits and then showed overt changes in behaviour, reduced activity and eventually lying, some with intermittent seizures, until death. Data are described below.

From –22 h until death, the air temperature fluctuated between 4°C and 22°C and the weather was mild, with little or no rain or wind.

Time to death

The concentration of 1080 in baits was found to be 1.06 mg per g bait (0.106 % w/v). Possums started to eat baits at up to 3 h 6 min after baits were first offered and had consumed all of the 1080 bait offered within 22 min of starting to eat. This was equivalent to a dose of 3.26 ± 0.37 mg/kg in 9.59 ± 1.09 g carrot baits; the LD_{50} for possums is 1.2 mg/kg (Eason & Wickstrom 2001). Individual times to death varied widely: possums died between 3 h 28 min and 33 h 2 min after consuming 1080 – an average of 10 h 19 min ± 2 h 56 min. Three possums that had consumed a similar amount to the 'low dose' possums in the 1080 study described in Chapter 2 took the longest time to die: doses were 1.61 mg/kg, 1.94 mg/kg and 2.01 mg/kg and times to death were 33 h 2 min, 12 h, and 9 h 20 min, respectively. The average time to death of the possums that had consumed an equivalent dose to the 'high dose' possums in the previous 1080 study was 6 h 25 ± 38 min. There was a significant negative correlation between dose and time to death ($r^2 = 0.513$, $P = 0.03$) for all possums combined. The time to death of focal possums did not differ significantly from that of other possums ($t_5 = 1.582$; $P = 0.1746$); the average times to death were 5 h 46 min ± 1 h 9 min and 12 h 36 min ± 4 h 10 min respectively. Likewise there was no significant difference between the times to death of males at 6 h 49 min ± 58 min compared to females at 14 h 43 min ± 6 h 13 min ($t_3 = 1.254$; $P = 0.2986$).

Caged possums fed a lethal dose of 1080 in the hands-off experiment described in Chapter 2 consumed 3.29 ± 0.16 mg/kg 1080 and died 11 h 26 min ± 1 h 55 min later. Neither the dose ($t_{10} = 0.089$; $P = 0.931$) nor the time to death ($U = 29.00$; $P = 0.541$) differed significantly from the dose or time to death of all possums in the present study.

Clinical signs

Beginning 2 h 3 min ± 13 min after dosing, seven animals had a changed appearance typical of sick possums as described in previous chapters: they were less alert, had lowered ears, and eyes were not fully open (Table 1). No possums were observed or heard retching or vomiting. One appeared incoordinated 1 h 42 min after consuming 1080.

Possums then began lying, as described below. During this period of prolonged lying, seven possums showed abnormal breathing. This included hyperpnoea and hypopnoea, and in two possums, laboured breathing. Following this, from 6 h 29 min ± 48 min after possums had consumed the 1080, or 1 h 24 min ± 44 min before death, some possums were observed in spasms or seizures. Two of nine possums were seen exhibiting repeated spasms in up to four bouts of around 2 s each. These spasms took the form of occasional leg paddling. Three possums, including one of those exhibiting spasms, were seen in seizures occurring in up to four bouts of around 2–12 s each and within 1 h 10 min ± 1 h prior to death. As with previous experiments described in this thesis, seizures consisted of more than one of the following: tail wagging, circling or flailing, body rolling, body rigidity, back arching, limb paddling, and the possum sometimes propelled itself forward or up in the air. One possum was heard vocalising abnormally (grunting) during a seizure.

Table 1. Time after dosing at which clinical signs of poisoning were observed in penned brushtail possums fed a lethal dose of 1080, compared to the time to death. Times are the mean±SEM and total *n* = 9 for each sign.

Sign	Time after dosing (h:min)	Number affected
Onset of change in appearance	2:03 ± 0:13	7
Onset of retching or vomiting	-	0
Onset of incoordination	1:42	1
Onset of prolonged lying	3:33 ± 0:45	9
Onset of spasms, tremors and seizures	6:29 ± 0:48	4
Time to death	10:19 ± 2:56	9

Of the instances when the corneal reflex was tested immediately on cessation of seizures, possums usually retained or regained the corneal reflex: one possum had a corneal reflex immediately after two consecutive bouts of seizures that lasted 3 s and 2 s and which began 15 min before death, and at the end of seizures in two consecutive bouts of 12 s and 2 s beginning 4 min before death. One possum had no corneal reflex

after two consecutive bouts of 2 s and 6 s that occurred 6 min prior to death. One possum retained a corneal reflex after a 2-s seizure that occurred 1 h 15 min before death.

Behaviour

Figure 2 shows the patterns of behaviour before and after dosing with 1080. Possums were normally active between around 2000h and 0600h. During this time, corresponding to the period between –22h and –12h before poisoning on Figure 2, they were usually observed in different parts of the pen sitting, standing, hanging, climbing, feeding, drinking, eating (including grazing grass), grooming, walking, running, marking the pen or furniture with the chin or chest, and vocalising. For the remainder of the time before poisoning they were typically inactive, staying in the normal curled posture in their nestboxes or sheds, very occasionally lying on the side or belly, and never lying on the back or prostrate.

Following poisoning, they showed a brief transient rise in all normal active behaviours which finished within a few hours of dosing (Fig. 2). They stopped moving throughout the pen at the same time and instead remained in nestboxes, sheds or pens. The time spent lying increased correspondingly so that all possums were observed lying for more than four consecutive observations (i.e., for more than one hour) by an average of 3 h 33 min ± 45 min after doses were finished.

Possums remained lying or later lying prostrate, with intermittent spasms or seizures as described above, until death 6 h 45 min ± 2 h 29 min later. During this period of prolonged lying, the possums' locations in the pen sometimes changed between scan samples.

The focal sampling was discontinued when possums became sick because it was not possible to perform scan samples and observe sickness in all possums as well as conduct focal samples in the time allowed.

Responses to stimuli

One of the possums tested had lost the response to a blow of air onto the face and handling, and its righting response at the last time it was tested, 1 h 12 min before death: it still had its palpebral and corneal reflexes at this time. The second had lost the response to a blow of air onto the face by 2 h before death, but retained a strong

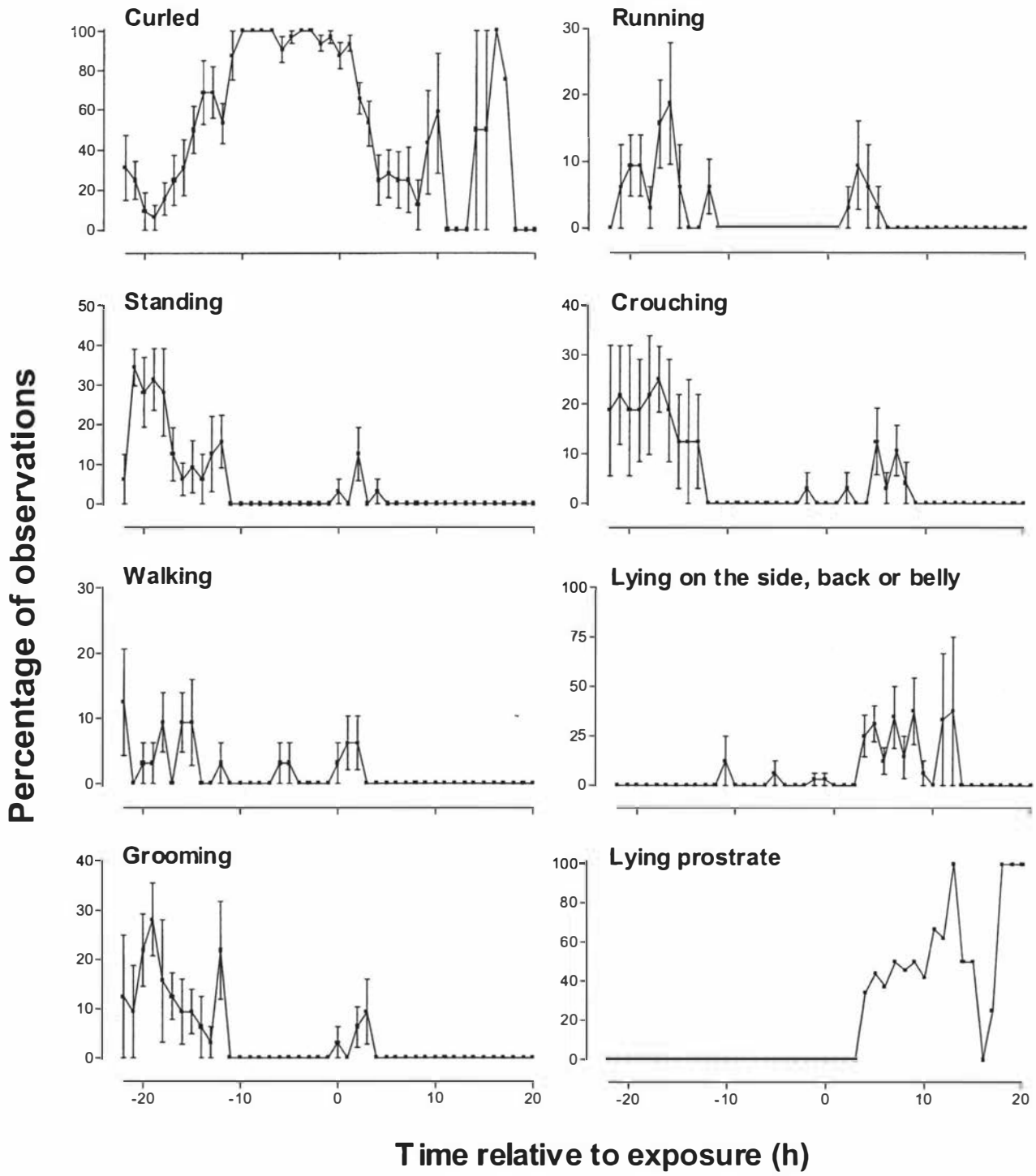

Fig. 2. Mean (±SEM) percentage of observations in which penned 1080-poisoned brushtail possums were observed running ('running') or walking ('walking') about the pen, standing still ('standing'), sitting or lying in a curled posture ('curled'), crouching with hunched back ('crouched'), lying on the side, back or belly ('lying'), or lying prostrate. Possums consumed 1080 in carrot baits between hours 0–3. Note different scales on y-axis.

response to handling 20 min before death. Other responses were not tested at this time, owing to the strong response to handling. The third possum had not lost its response to a blow of air onto the face or handling at 9 min before death, and retained its righting response and corneal reflex. None of these possums were tested again before death.

Bodyweight

All possums lost weight so that body weight was significantly lower at death than at 6 days prior to 1080 exposure ($t_8 = 6.335$, $P = 0.0002$), being 3.15 ± 0.18 kg before dosing and 2.97 ± 0.18 kg at death. Individuals lost 5.7 ± 0.7 % of their body weight on average (a maximum of 9.9%). The possum that died after 33 h 2 min lost 5.6% of its body weight.

Brodifacoum

Possums poisoned with brodifacoum showed clinical signs of poisoning and changes in feed intake before obvious changes in behaviour.

Weather

The air temperature ranged from an average of 9.5 ± 0.5°C to 26.9 ± 0.9°C each day, with 3°C being the lowest temperature reached and 40°C the highest. The weather was generally fine and mild. It was equally overcast or clear during night-time observations, and overcast or clear and sunny during the day with occasional moderate cloud. There was usually a light to moderate breeze or light wind during the day, and occasionally a light to moderate wind at night. There was a strong wind twice only during the day-time observation periods and twice at night and it only rained lightly over three days.

Time to death

One possum that had consumed 0.18 mg brodifacoum per kg body weight was euthanased 52 days after the first day of exposure to brodifacoum. The remaining eight possums consumed 0.86 ± 0.22 mg/kg brodifacoum in 188.85 ± 14.48 g cereal pellets over three nights, and died 22.2 ± 1.4 days after they had first began consuming brodifacoum. There was no significant relationship between dose and time to death ($r^2 = 0.072$; $P = 0.522$; $n = 8$). Likewise, there was no significant difference between male and female possums with average times to death of 22.5 ± 1.1 days and 22.0 ± 2.7 days,

respectively ($t_6 = 0.182$; $P = 0.861$). Neither was there a significant difference between focal possums at 22.4 ± 2.7 days to death and other possums at 22.2 ± 1.7 days to death ($t_6 = 0.050$; $P = 0.962$).

The caged possums that died after being fed brodifacoum in the experiment described in Chapter 4 consumed 0.92 ± 0.04 mg/kg. This was not significantly different from the dose consumed by possums that died in the present study ($t_{22} = 0.754$; $P = 0.459$). Possums in the previous study died 20.7 ± 1.7 days after baits were first offered: again the difference between the two studies was not significant ($t_{22} = 0.566$, $P = 0.577$).

Behaviour and clinical signs of poisoning

Prior to dosing, possums were normally active at the night time observations between 2200 and 2300 h and inactive between 1000 and 1100 h. During the active period possums generally used all of the pen and pen furniture; normal behaviour over this time included sitting, standing, hanging and climbing on the pen or furniture, drinking, eating (including grazing grass), grooming, walking, running (including running at a set pace repetitively following the same route), marking with the chin and chest on pen furniture, banging the tail on the shed roof, and vocalising. During the inactive period possums were normally in the typical curled posture in their sheds or nestboxes. They were seldom seen lying on the side or belly before poisoning, never on the back and were never seen prostrate.

Within four days of the bait being offered, possums developed pale noses, and two weeks later most showed signs of external bleeding from the nose (Table 2). One possum also bled from an ear. Shortly afterward, all possums except the one that had consumed the lowest dose had adopted abnormal postures and exhibited lethargy and the typical changed appearance of a sick possum, including lowered ears and staring eyes. They then became less active at night and the amount of time spent lying on the side, front or belly consequently increased. Nocturnal active behaviour was no longer observed from nearly 21 days after dosing. By the next day, four possums were observed lying on the side, back or belly in their nestboxes, sheds or pens rather than curled. They remained lying for a maximum of one day until death. The remaining four possums were not seen lying before they were found dead.

Table 2. Time after dosing at which clinical signs of poisoning appeared in penned brushtail possums fed a lethal dose of brodifacoum, compared to the time to death. Times are the mean±SEM and total $n = 8$ for each sign.

Sign	Number affected	Time after dosing (days)
Pale nose	8	3.8 ± 0.6
External bleeding	7	18.2 ± 1.5
Noted sick	7	19.7 ± 1.5
Abnormal postures	6	19.7 ± 1.1
Inactivity at night	7	20.7 ± 1.7
Prolonged lying	4	22.4 ± 2.5
Time to death	8	22.2 ± 1.4

Feed intake

Feed pellet intake declined significantly over time (Fig. 3; $F_{22} = 6.745$, $P < 0.0001$). At 15 days after initial exposure, it was significantly lower than at 5 days prior to exposure, with a mean difference of 18 g ($P < 0.05$). In a similar way, the intake of apples decreased significantly over time ($F_{23} = 5.190$, $P < 0.0001$), being lower at 18 days after exposure to brodifacoum than at day –5 ($P < 0.01$).

Body weight

Body weight was significantly lower at death than at 11 days prior to death ($t_8 = 3.280$, $P = 0.011$), dropping from 3.67 ± 0.15 kg to 3.42 ± 0.13 kg. This represented a range from a 7.3% increase in body weight in one possum to a 14.1% loss in another possum.

Responses to stimuli

One possum had lost the response to a blow of air onto the face when it was first tested 10 h 45 min before death. The remaining two possums that were tested for their responses to stimuli retained their response to a blow of air at 1 h 1 min and 3 h 15 min prior to death. All three possums responded to handling when last tested 1 h 1 min, 1 h 30 min and 3 h 15 min before death, respectively. The one possum that was tested retained its righting response, and palpebral and corneal reflexes 1 h 1 min before death.

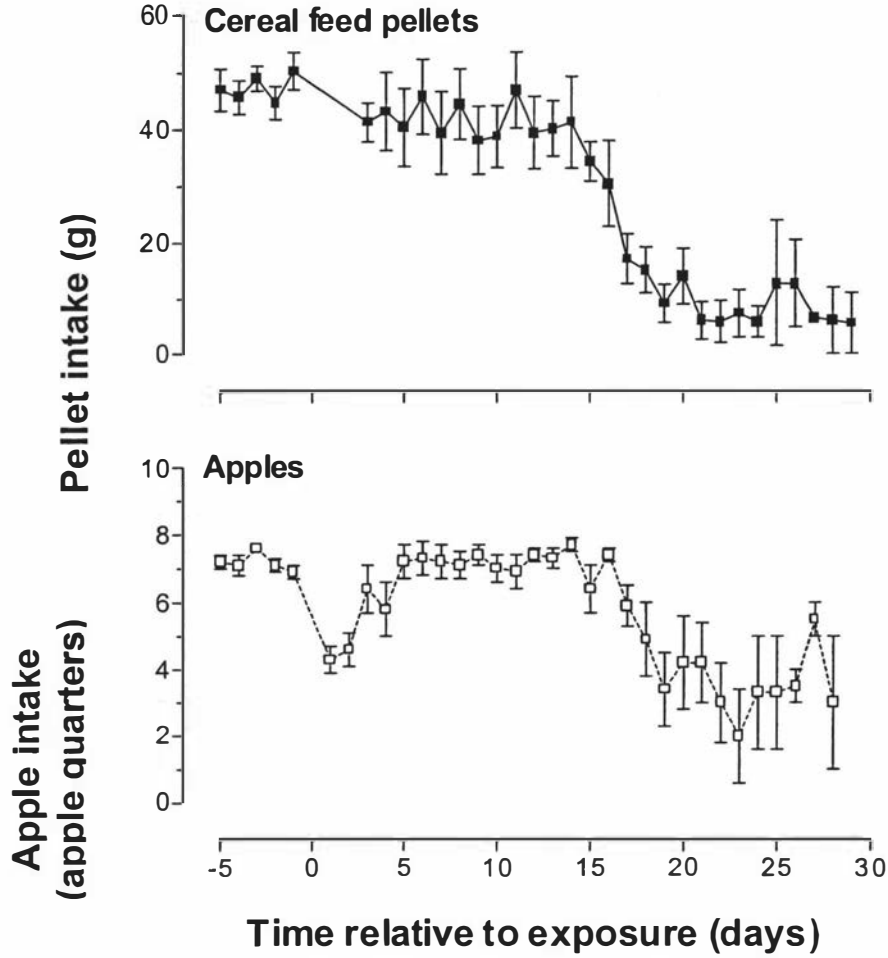

Fig. 3. Mean (±SEM) daily intake of cereal feed pellets and apples by brushtail possums after exposure to brodifacoum in cereal baits over three days (day 0–2).

Necropsies

All eight possums that died had haemorrhages: four of these had 1–4 severe haemorrhages (Table 3). Most haemorrhages, though, were mild or moderate, and occurred in subcutaneous or deep tissues in the thorax, and in the lungs and gastrointestinal tract.

Haemorrhages commonly included focal or multifocal and patchy haemorrhages in the lungs; focal and patchy intramural haemorrhaging in the intestinal tract; and extensive haemorrhaging in or between one or more subcutaneous layers of fat, connective tissue (including periosteum) and muscle in the thoracic region, and less often in the abdomen, or limbs.

Table 3. Number of haemorrhages of different severity (mild, moderate or severe) and extents (focal or multifocal, patchy, diffuse or extensive) in the organs or remaining tissues other than bone in different areas of the body occurring in penned brushtail possums poisoned with brodifacoum, and number of possums with haemorrhages in different regions of the body and of different severity and extents.

Body region		No. of possums affected	Severity			Extent		
			Mild	Mod	Severe	Focal	Patchy	Extensive
Thorax	Tissue	7	7	4	1	2	0	10
	Organs		1	3	2	2	2	2
Abdomen	Tissue	6	2	0	2	1	1	2
	Organs		2	5	3	7	1	2
Pelvic cavity	Tissue	5	2	2	0	1	0	3
	Organs		3	1	1	3	0	2
Limbs	Tissue	5	4	2	0	0	1	5
No. of possums affected (n = 9)		8	8	7	4	8	5	7
Total lesions		47	21	17	9	16	5	26

Two possums had intramural intestinal haemorrhages that caused one or two haematomas of approximately 2 x 2 x 1cm in size (l x w x h), one had extensive haemorrhaging in the facial muscles causing them to swell, one had a congested, enlarged and protruding penis, one had petechial haemorrhages between the seminiferous tubules, one had a moderate diffuse haemorrhage in the prostate gland, and one had some mild focal haemorrhages in the urinary bladder. None had intra-articular blood in the stifle or hip joints.

There was no clear association between the time to death and the number of regions affected by haemorrhage, the number of extensive haemorrhages or the total number of haemorrhages (Table 4).

Table 4: Time to death of individual penned brushtail possums poisoned with brodifacoum compared to haemorrhage characteristics determined at necropsy.

Possum No.	Time to death (days)	Number of regions in which haemorrhages occurred	Number of extensive haemorrhages	Total number of haemorrhages
7	18.94	1	0	1
5	19.15	2	1	2
10	19.69	4	2	6
4	19.71	4	9	11
2	21.98	4	5	10
9	23.31	4	3	6
11	25.02	2	3	5
12	30.10	3	3	6

Discussion

Penned possums succumbed to a lethal dose of 1080 or brodifacoum at similar times to caged possums in previous studies in this thesis. Gross behavioural changes were also similar.

1080

Following a lethal dose of 1080, *penned* possums on average appeared to be sick 2 h after baits were consumed, began lying after 3.5 h and remained so until death 6 h 45 min later at just over 10 h after bait consumption. Three possums were seen having seizures within 1 h 10 min of death, on average. Two of the three possums tested still had a response to handling at up to 20 min before death, and all three retained the palpebral and corneal reflexes until death. This suggests that penned possums experienced the effects of 1080 for about 8 h before death.

In comparison, *caged* possums in a previous experiment fed a similar dose of 1080 (Chapter 2) had the typical appearance of a sick possum almost 2 h after baits were consumed, began retching and vomiting for short period from one hour later,

became incoordinated after around 3.5 h, and began lying shortly thereafter for around 7.5 h until death after almost 11.5 h. Five animals had seizures, with these occurring within an average of 1 h 52 min of death. Possums retained the response to handling, or had lost it up to 1 h 15 min before death. Similarly, the righting response was not lost, or was lost up to 2 h before death, and the corneal and palpebral responses mostly remained until death. It may be inferred from these observations that caged possums experienced the effects of 1080 for about 9.5 h before death.

It is evident that the duration of apparent toxicosis, and the times to death are similar in penned and caged possums. Accordingly, the different environmental conditions in pens and cages did not apparently affect the response of possums to 1080.

Two exceptions to this similarity in responses to 1080 were the occurrence of retching and vomiting, and incoordination: the former was not noted at all in the pen study, and the latter was noted in one possum. This could be an effect of the method of observation: whereas with cages most or all of a small group of possums could be observed simultaneously or very close to it, with the pens the observer has to actively move along the row of pens to observe all possums. With rare events such as retching or vomiting, and the appearance of incoordination – which was normally detected from the observation of only a few incoordinated movements – it is possible that these events occurred but were not observed. Although no vomitus was found in the pens, the likely volume would be small enough that it could have been absorbed into the ground by the time the pens were cleaned around 24 h after baits were offered.

The absence or rarity of incoordination and retching or vomiting is not unprecedented however: in the mitigation study described in Chapter 3, these events were not observed in all caged possums that were watched continuously from dosing until death.

The similarity in results between penned and caged possums was somewhat surprising, because at the time that this study was planned it was anticipated that the opportunity for increased activity in pens compared to cages would influence 1080 toxicosis, and it was also expected that physiological responses to air temperatures as low as 4 °C could influence toxicity and therefore the time to death. However, it is now evident that because possums quickly reduce their activity following 1080 ingestion,

activity would not have been increased at a time when it might have influenced toxicosis.

With regard to air temperature, Misustova *et al.* (1969) and Oliver and King (1983), amongst others, have shown that it affects the toxicity of 1080. In planning the current study, it was anticipated that the exposure of penned possums to air temperatures below the 19 °C level present in cages might mean a reduction in the time to death in penned possums. The results of the current study do not bear this out. Despite dropping to a minimum temperature of 4°C, the air temperature may have remained at a thermoneutral level for possums for much of the time of this experiment and the air temperature may not have been cold enough shortly after dosing in order to affect time to death; there are not enough data to support or refute this suggestion.

Brodifacoum

Possums fed a lethal dose of brodifacoum in pens had a reduced feed intake about 15 days after bait was offered, and were first seen bleeding externally after around 18 days. They were noted as sick about 1.5 days later, and became inactive after nearly 21 days. One day later, half of the possums began lying until they died shortly after 22 days. Of three possums tested, all still had a response to handling when last tested between 1 h 1 min and 3 h 15 min before death. Using reduced feed intake as an index, the duration of sickness of before death was about seven days. By comparison, *caged* possums examined in a previous experiment (Chapter 4) had pale extremities, a reduced feed intake and the typical changed appearance of a sick possum about 15 days after baits were offered. They adopted abnormal postures and showed external bleeding after about 16 days, and nearly all were seen lying for a short period before death after nearly 21 days. These criteria suggest a duration of sickness of about six days.

While the overall duration of sickness was similar in pens and cages, due to the similar times at which feed intake was reduced, the appearance of most signs of toxicosis was delayed by about 2–4 days in penned possums compared to caged possums. The reasons for this are not clear, although it is possible that possums grazed enough pasture to supplement the diet with enough vitamin K to influence the onset of coagulopathy and thereby the onset of toxicosis. This could be proven by investigating the time at which coagulopathy began by measuring the activated partial thromboplastin time or one-stage prothrombin time at different stages after dosing. As shown by Eason

et al. (1996) and in Chapter 4, these times are changed four days after dosing. The time to death in the current pen study was not significantly different from that of caged possums. If the time to onset of coagulopathy was delayed, this would mean that the time between the onset of coagulopathy and death would need to have been shortened by some aspect of penning.

Haemorrhages differed in location, incidence and extent between penned and caged possums. *Penned* possums had most haemorrhages in the thoracic tissue excluding bone, such as the musculature around the ribs, and the next highest number of haemorrhages in the gastrointestinal tract. Most extensive haemorrhages were in the thoracic tissue. By contrast, most haemorrhages in *caged* possums were in thoracic organs, with the next highest number in the tissue of the pelvic cavity and lumbar-sacral region and the limbs. Most of the extensive haemorrhages were in the limbs. Of note is that penned possums had a higher incidence of extensive haemorrhages, with 7/9 possums compared to 4/12 possums being affected. The incidence of lesions was also higher overall, being about six haemorrhages in each of the eight penned possums that had haemorrhages compared to four in each of the 12 caged possums that were necropsied.

It seems likely that the incidence of haemorrhaging in tissues was influenced by physical trauma and its effects in the tissue. Certainly, humans with haemophilia or suffering from an overdose of anticoagulants can experience more extensive bruising or haematomas at sites of physical trauma. Likewise, anticoagulated animals bruise or bleed more freely when knocked (Osweiler *et al.* 1985). Consistent with this is the observation that most haemorrhaging in the tissues (rather than organs) of caged possums occurred in the lumbar-sacral region on which they spent much of the time resting on the hard cage floor. In contrast, penned possums could be expected to experience physical trauma on the torso, as was indeed the case in the present study, when running and climbing about the pen. The higher incidence of haemorrhages overall in penned possums in the present study could be similarly explained as a side-effect of their greater physical activity, at least initially.

Relevance of studies in this thesis to the wild

The conditions in which possums were kept in outdoor pens in this study were intended to closely simulate conditions that would be experienced by possums in the wild in New

Zealand. This was so that the results could be extrapolated to wild possums. Additionally, if there were no differences between the present study and previous experiments on caged possums, it would mean that previous results from caged possums were probably also applicable to wild possums in New Zealand.

Possums kept in pens responded similarly to caged possums when poisoned with 1080, although there was a suggestion that the time to death may have been slightly reduced in penned animals. Further, penned possums were not seen retching or vomiting, another advantage for animal welfare. Accordingly, this suggests that 1080-poisoned possums exposed to environmental conditions in the wild that are similar to those experienced by possums in this pen study could be expected to react similarly to 1080 and that their welfare may be slightly better than predicted by the studies using caged possums.

With regard to brodifacoum, there was a suggestion that the duration of sickness was shorter in penned possums than caged possums. In addition, penned possums were not seen to be lame and none had intra-articular haemorrhages. This suggests that possums in the wild may not experience the lameness and pain associated with intra-articular bleeding, an obvious advantage for possum welfare. Likewise, there was no paresis or paralysis in penned possums: this was seen in a previous experiment on the effects of brodifacoum in rats conducted by the author (Littin *et al.* 2000), and it was postulated that the paralysis was due to haemorrhaging around the spine brought about by activity. It seems possible that it could also be due to compartment syndrome induced by muscular activity, and consequent nerve entrapment or pressure on nerves.

However, two other lesions, the severe intramural haematomas and numerous gastric erosions, found in the penned possums at necropsy might be noteworthy for animal welfare.

Intramural haematomas were not seen in caged possums exposed to brodifacoum but were seen in two of nine poisoned possums in the present study, meaning that a low proportion of wild possums could experience pain related to similar lesions following brodifacoum poisoning.

Intramural haematomas might have been caused by direct external force on the gut, for example by sharp contact with the pen furniture when jumping from one area to another. Alternatively, they could have been due to shearing forces caused by such

activity acting on the gut, although there would presumably need to have been significant force for such haemorrhaging to occur in the gut situated deep in the abdominal cavity. In line with this suggestion, animals can cause gut torsion by rolling on the ground – torsion could feasibly create such shearing forces. Intramural haematomas on the scale seen in this study are likely to be painful as they would presumably trigger mucosal stretch receptors.

Erosions and ulcers were seen in four of nine penned possums, or nearly half of the animals. They were also seen in an uncertain number of caged possums in the previous study. If they were caused by brodifacoum poisoning, this could be a further concern for the welfare of a high proportion wild possums poisoned with brodifacoum. The gastric erosions and ulcers may be a result of long-term captivity rather than brodifacoum *per se*, but this would need to be confirmed by conducting necropsies on possums that have been caged or penned for a long period but have not been treated with brodifacoum. Gastric ulcers are common in captive wildlife in zoos (Schmidt & Hubbard 1987) and can be a sequel to chronic stress in humans and other animals (Breazile 1988). Such ulcers would therefore have been present before dosing with brodifacoum. However, disruption in local blood flow and consequent ischaemic necrosis can contribute to the formation of gastric ulcers, with both hypotension and shock capable of disrupting local blood flow (Twedt 1992). It therefore seems possible that hypotension and hypovolaemic shock induced by haemorrhage could have at least promoted gastric ulceration. An analysis of the age of the erosions and ulcers would be needed in order to distinguish these different aetiologies: this was not done in the current experiment.

Erosions are macroscopically distinct from ulcers: they specifically occur in the superficial layers of the mucosa whereas ulcers continue to deeper layers. While erosions might not be painful, ulcers may cause pain: they can certainly be associated with pain in humans (e.g., Chernin & Osadchii 2003; Gubler *et al.* 2003), horses (Gelberg 2001), dogs and cats (Twedt 1992; Gelberg 2001), suggesting that they may cause pain in possums.

An additional animal welfare issue is suggested by the presence of fly eggs on the fur: although fly larvae were not found on any possums, the occurrence of fly eggs suggests at least a risk of fly strike in moribund possums. Fly strike could be expected to cause some discomfort or pain in animals that are still conscious.

A final comment is needed on the applicability of these studies with regard to weather and air temperature. The average monthly winter air temperature between 1971 and 2000 ranged from 2.2 °C to 15 °C for 14 areas in the North Island of New Zealand and from –2.6 °C to 13.1 °C for 16 areas in the South Island (NIWA 2003). Average summer temperatures ranged from 11.1 °C to 24.9 °C for the North Island, and 11.8 °C to 23.8 °C for the South Island (NIWA 2003). As the temperature range in the present experiment on 1080-poisoned possums was 4 °C to 22°C, this suggests that the results of the 1080 experiment are applicable to summer conditions throughout New Zealand, and to winter in most areas of the North Island. However, it could be recommended that a further pen study on the effects of 1080 be undertaken in conditions that are more representative of those in winter in the South Island. Likewise, it was suggested in the introduction that low air temperatures might induce hypothermia in anticoagulated possums. Air temperatures in the current study on brodifacoum-poisoned possums did not drop below the critical level for possums of about 7–10 °C (van den Oord *et al.* 1995) for prolonged periods so it was not possible to investigate this question. It would be valuable to study this question further in conditions representative of South Island's winter.

Summary

Penned possums that had consumed a lethal dose of 1080 in carrot baits experienced around 8 h of sickness during which the signs of toxicosis occurred, before death 10 h 20 min after exposure. Caged possums in a previous study described in Chapter 2 died 11.5 hours after exposure to 1080 in carrot baits following a 9.5-h period of sickness. The character and time of onset of the clinical signs of sickness and behavioural changes was similar, except that retching and vomiting and incoordination were not seen in penned possums. This suggested that results from caged possums in previous studies are applicable to wild possums that have been poisoned with 1080.

Penned possums that had consumed a lethal dose of brodifacoum in cereal pellets experienced the first clinical sign of toxicosis around seven days before death at 22 days after first exposure. The onset of appearance of most other signs of toxicosis was delayed by 2–4 days in comparison to caged possums in a previous study (Chapter 4) which showed signs of toxicosis for six days before death 21 days after first exposure to brodifacoum. This suggested that the duration of sickness was shorter in penned

possums than in caged possums. Penned possums also had more haemorrhages and the main location of haemorrhages differed between studies. This was probably a reflection of the increased amount of physical activity in pens, and the difference in sites of physical trauma. Together this suggests that studies using pens may more accurately demonstrate the effects of brodifacoum on wild possums.

Author's note and acknowledgements

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Chapter 6
General Discussion: applying assessments of the welfare impacts of poisons used for killing possums to the practical task of evaluating humaneness

Author's note: Sections of this chapter have been produced in part in unpublished reports:

Littin, K. E. & O'Connor, C. E. (2002). 'Guidelines for assessing the welfare impacts of vertebrate poisons.' Landcare Research Contract Report LC0102/006.

O'Connor, C. E., Airey, A. T., & Littin, K. E. (2003). 'Relative humaneness of possum poisons.' LCR Contract Report LC0203/158.

Brushtail possum in a typical curled resting posture. Photograph by Kate Littin.

Table of contents

Summary of this thesis

It cannot be denied that brushtail possums are serious pests. Nevertheless, they are sentient and capable of suffering and as such we are obliged to be concerned about their welfare when subjected to control methods, as laid out in Chapter 1. This ethical obligation is underpinned by legislative requirements and public concern. Any method used must be the most humane one suitable for the circumstances. This requires research into the animal welfare impacts of control methods and an interpretation of their humaneness. A description of the effects of 1080 or brodifacoum on possums and the implications for possum welfare has been presented in this thesis, in order to make such an assessment.

In summary, most possums experienced infrequent retching, vomiting and seizures following 1080 poisoning (Chapter 2). Spasms and apparent malaise were common, the latter lasting for 10 h on average. They apparently did not fully lose consciousness until death, although they were likely to have been in a reduced stated of awareness for about 2 h beforehand.

The experiments on 1080 suggested that it had some unpleasant effects, and raised the possibility that some of the effects may be ameliorated, for example, by the use of compounds that specifically target them (such as analgesics to reduce pain) or by inducing unconsciousness. A pilot study revealed that alpha-chloralose and paracetamol had little or no effect on the behaviour of 1080-poisoned possums (Chapter 3), but suggestions for furthering this work were made.

Brodifacoum commonly induced a prolonged period of malaise, and was likely to have caused pain in all possums (Chapter 4). Possums did not lose consciousness until death, but again, were likely to have been in a reduced state of awareness for up to six days before death.

As the overall context for these experiments was to determine the impacts of poisons used for possum control on the welfare of wild, free-living possums, a further question needed to be addressed. Were these results applicable to the wild situation? The question of whether or not environmental conditions in the wild would influence possum welfare was addressed experimentally using possums kept in outdoor pens designed to simulate environmental conditions that would be encountered by wild possums (Chapter 5). The experiment on 1080 yielded similar results to prior

experiments on 1080 suggesting that studies using caged possums were representative of the situation that may occur in the wild. However, the experiment on brodifacoum showed that studies using pens may be more representative of the wild situation. It was also suggested that these experiments be repeated in conditions typical of winter in the South Island.

Methodological considerations

Before discussing the application of the results from the present experiments, some methodological difficulties and the implications of these need to be outlined.

Behavioural sampling techniques

Firstly, the behavioural sampling techniques used constrained the data that could be collected. This has been discussed in the individual chapters. Essentially, the problem is that instantaneous scan sampling may only allow an accurate assessment of states rather than discrete events. The accuracy depends on the frequency of observations. This is constrained by the number of possums that must be observed and the detail required when recording observations. Continuous focal sampling can give a complete record of all behaviours and behavioural states, but only one possum can be observed at one time. It could be possible to repeatedly observe single animals from poisoning until death, but this would be impractical with long-acting poisons such as brodifacoum. Ultimately, the method used must be a compromise between the aim of getting a true record of the prevalence and duration of events, and practical considerations.

Other problems with the behavioural observations undertaken in experiments described in this thesis have been described in the individual chapters. It is worth noting, specifically in the context of the 1080 studies described herein, that the methodology initially used to observe the behaviour and the time to loss of consciousness in 1080-poisoned possums (Chapter 2) was altered during the course of the project in order to gain more meaningful results from poisoned possums in subsequent experiments (Chapters 3 and 5).

Interpretation of behaviour

Behavioural and other observations, and their interpretation in terms of animal welfare have been presented in the preceding chapters. There are considerations underlying

these interpretations that have not been discussed deeply, and, I would suggest, they ought to be. The central issue is: Can we ever know the real experiences of animals? For example, does head-pressing show that a possum has a headache? Does crouching testify to abdominal pain? It can be argued that we cannot know the true experiences of animals, just as we can never know the true experiences of another person. If this is so, how can we proceed with an interpretation of behaviour in terms of animal welfare?

In some cases, interpretation can be based on analogy with other species where there is confirmation, or at least a firm basis for belief, that a particular behaviour indicates a particular state or experience. For instance, it is well established in a clinical setting that crouching with a hunched back is an indicator of pain in dogs (e.g., Hellyer & Gaynor 1998). Interpretation can also be based on the parallel appearance of pathological changes. For example, an inflamed gastric mucosa coupled with crouching suggests that the crouching was caused by gastric pain due to the inflammation. Anthropomorphism is also helpful to a certain extent: human experiences of the same situation may give an indication of the possible experiences of the animal. Another potential way of determining what a behaviour means is to treat an animal in a way that could be expected to produce particular signs and experiences. For example, lipopolysaccharide could be expected to produce an experience of sickness (Gregory 1998*a*). This approach was not adopted in the present studies because combining behavioural indicators with necropsy findings, coupled with an investigation of human experiences, was considered likely to be informative. Further, it was considered at the time that the disadvantages for animal welfare of the administration of lipopolysaccharide would outweigh the advantage: the potential elucidation of the interpretation of sickness behaviour. Another way in which the administration of substances may help interpretation of behaviour is to give compounds that ameliorate the experience that the behaviour is thought to represent, or to remove its cause. For example, if a preparation known to reduce nausea is given to a possum that is not eating, and the possum resumes eating, it may be concluded that reduced feed intake in that situation was probably caused by nausea. This leads to an important point: a behaviour can mean different things in different situations and in different individuals. For instance, reduced feed intake could result from nausea or from pain in the jaw or teeth, or other factors.

Assessment of loss of consciousness

Issues to do with the assessment of the loss of consciousness have been described in individual chapters. Again though, some factors deserve further discussion. Essentially, awareness or consciousness is a continuum from total awareness to total unconsciousness (Piggins & Phillips 1998; Sommerville & Broom 1998; Tassi & Muzet 2001). Techniques commonly used in a veterinary clinical setting to determine neurological function were adapted for use in the experiments described in this thesis. They were primarily chosen on the basis of the ease-of-use in a laboratory setting balanced with the amount of information they would provide. The techniques required interpretation of responses to various stimuli; the existence or loss of a response must be evaluated with caution. A positive response may mean that the animal is conscious to some degree, but care must be taken as reflexes can be elicited in animals with no cerebral activity. A loss of response can, but does not necessarily, indicate loss of consciousness, because conscious animals may be physically unable to respond, for instance, if they are paralysed.

Alternative means of assessing consciousness are available, such as various brain imaging techniques, electroencephalogram (EEG) and electrocorticogram (ECoG). However, these can have interpretative and practical difficulties too. For example, the EEG is not likely to have been measured in possums before and would thus require extensive experimentation to determine baseline patterns. This was the primary reason that it was not used in the present studies.

The use of wild-caught possums

The individual characteristics of the possums used in the experiments described in this thesis may have influenced the results. The possums were wild-caught which means that their age and previous health and reproductive status were largely unknown. All of these features can influence the toxicodynamics and toxicokinetics of poisons (e.g., Clarke & Clarke 1967; Osweiler *et al.* 1985) and thus could feasibly have influenced the results of the current experiments. This possibility was reduced as follows.

Age can be estimated by size, body weight and physical condition. The studies described in this thesis used possums of an even range of body weights, with possums smaller than 2 kg being avoided. This was to greaten the chance that animals were mature, and the chance of achieving a relatively broad spread of adult ages, as would be

encountered in the wild. Examination of the structure of the teeth to determine age, or the use of captive-bred or hand-reared possums could be used to ensure the age of possums in future studies, if this were required.

Previous health status was more difficult to ascertain. *Current* health status was assessed when possums were first brought to the animal facility: they were inspected for obvious external indicators of ill-health and were not used unless they appeared healthy and were gaining weight prior to the start of experiments. Many of the possums had been in captivity for some time (ranging from several weeks to months) and their health was monitored throughout. This would have largely avoided the effects of previous poor health on the results of the present studies.

With regard to reproductive status, some females were carrying young when caught, and these were removed as soon as animals entered the research facility. According to a set protocol (Standard Laboratory Practice No. 10, Landcare Research, Lincoln), pouch young up to 30 mm in length from the crown to the rump were euthanased by cervical dislocation and larger young were euthanased by intraperitoneal injection of sodium pentabarbitone. The reproductive status of possums that were in captivity for prolonged periods would have changed after capture as they were kept under lighting that followed a natural daylength (it was activated by a light-sensitive sensor positioned outdoors), so that their reproductive cycles may have been affected by changes in day length. It may therefore have been preferable to conduct all experiments at the same time of year, but time constraints meant that this was not possible.

Application of these results

When it was initiated, the programme of research of which the experiments described in this thesis were a part, was unique in several ways. Firstly, it represented the first systematic attempt to describe the animal welfare impacts of the poisons used for possum control. Indeed, even now there are apparently very few published reports of experiments that have explicitly aimed to assess the animal welfare impacts of any vertebrate poisons, as covered in the general introduction (Chapter 1): the reviews by Rowsell *et al.* (1979), the UK Pesticides Safety Directorate (MAFF 1997) and Mason and Littin (2003) are some exceptions. The studies described in this thesis are particularly useful because they use the same methodology and experimental conditions

for both 1080 and brodifacoum. It could be hoped that such work would provide an impetus for further studies in this area.

Secondly, these studies raised stimulating ethical and animal welfare questions, and provided a unique opportunity to explore some issues that would not otherwise have been explored. This is because possums were wild-caught, were deliberately subjected to suffering – it was anticipated that the poisons would elicit at least some degree of suffering – and the experimental endpoint was death rather than a humane endpoint prior to death. On the other hand, we are obliged to conduct this research in order to ameliorate suffering in wild possums. Is it justifiable to deliberately cause suffering to an experimental possum in the interests of improving the welfare of another possum? This research also raised questions on the nature of suffering. For instance, what is the implication of the duration of suffering to an animal that has no recollection of the beginning of a period of suffering and no anticipation of the end? Further questions are explored in Littin (1999).

Thirdly, the results of this project allowed the sickness behaviour of possums to be more clearly evaluated and described quantitatively. Previous descriptions (Spielman 1994; Duckworth & Meikle 1995; Gregory *et al.* 1996) are helpful but brief or qualitative. This has led to an initiative to describe some humane endpoints for research on poisons and other control techniques for possums (O'Connor *et al.* 2003).

A fourth and important application was that this project led to the development of a sampling methodology that can be used and improved upon for future studies of this kind. This methodology is described below and by Littin and O'Connor (2002).

Finally, and most importantly, the studies described in this thesis can be directly applied to the assessment of the humaneness of poisons used for killing possums in New Zealand. As has been explained, these studies were part of an overall programme aimed at assessing the animal welfare impacts of the poisons most commonly used for possum control in New Zealand. It is now clear that all of the poisons have some impact on animal welfare (O'Connor *et al.* 2003). The different poisons also have different advantages and disadvantages in terms of their impacts on the environment and people. The choice of any one poison is therefore a balance between the advantages and disadvantages in these different areas.

The obligation to ensure that the most humane poison that is suitable for each situation is used requires an assessment of the humaneness of poisons used to kill possums.

A methodology for assessing the impacts of poisons on possum welfare

On the basis of the studies described in this thesis and on other studies in the Landcare Research programme of which these studies were a part (see Chapter 1), a scheme was developed for the assessment of the animal welfare impacts of vertebrate poisons (Littin & O'Connor 2002). The scheme took account of the methodological difficulties just described and was designed to provide the basis for practical guidelines for assessment, as follows.

The specific aims of the scheme are to develop a list of potentially unpleasant effects caused by a vertebrate poison by close observation of poisoned animals in cages or pens (at least in the first instance), and to determine the proportion of animals experiencing unpleasant effects, the intensity and duration of those effects, and consequently the welfare impacts of the poison. Four steps can provide the information to achieve these aims, as follows.

Step 1. Ascertain capacity to suffer

This means the capacity of the species to suffer at all (with the general acceptance that all vertebrates can suffer, as noted earlier), and the capacity of the species to suffer in particular ways (e.g., pain compared to anxiety). The latter requires consideration of whether individual or species traits introduce or predispose it to certain welfare consequences (e.g., Spedding 2000; Beaver *et al.* 2001). Such traits might include normal dietary, food and water requirements, basal metabolic rate, normal pattern of reproduction and whether the animals are nocturnal or diurnal, solitary or social. Finally, animals must be conscious (i.e., not anaesthetised or comatose) to be capable of suffering.

Step 2. Anticipate likely effects of poisoning

Prior knowledge of the mode of action, cause of death and effects in humans and other animals allows some of the effects to be anticipated. Published literature and/or pilot studies are means of getting this information. This knowledge may also suggest

appropriate behavioural sampling strategies for the next step, and can suggest whether further physiological measurements will be necessary to show the presence of effects that cannot be seen externally.

Step 3. Determine the type, intensity and duration of effects, and the percentage of animals affected

Experimental observations of caged or penned animals should be used to determine these. It is essential to record in each animal:

- the time of onset of the first sign of poisoning,
- the time of onset and duration of each sign of poisoning, and
- the time to loss of consciousness.

This provides information on the intensity and duration of each effect, and the overall duration of effects in each animal.

The time to loss of consciousness is more important than the time to death because an animal cannot suffer when it is unconscious. Recording only the time to death is not sufficient for an assessment of humaneness, particularly if the animal is unconscious for a substantial period of time before death. Because consciousness is a continuum, there is a need to predetermine the sign(s) used to indicate absolute loss of consciousness. For instance, the loss of response to handling might indicate when an animal is starting to lose consciousness, whereas the loss of palpebral reflex indicates no brainstem activity and hence total unconsciousness; this is the sign used for testing the welfare impact of kill traps (NAWAC 2000).

Both the behaviour of poisoned animals from dosing until unconsciousness and the pathological changes determined at post-mortem should always be established. Physiological and biochemical measurements can also be used to help to confirm the presence or absence of:

- potentially unpleasant effects, such as haemorrhages assessed by ultrasound;
- consciousness, assessed through the use of EEG or ECoG;
- compromised welfare, indicated by blood-borne compounds such as stress hormones or power analysis of EEG traces to indicate pain or distress;

- further unpleasant effects that might not be seen externally, such as hunger or hypovolaemia.

A thorough understanding of the normal behaviour, physiology and pathology of the species is required before observing poisoned animals. It is important to consider signs specific to the poison (e.g., seizures, vomiting), but general signs of sickness (e.g., altered appearance) also provide indicators of the onset and duration of illness. Accordingly, it might be helpful to consider the following:

Behaviour

- Appearance
- Posture
- Response to stimuli
- Spontaneous behaviour, including both abnormal behaviour and changes in normal behaviour.

Pathology

- Gross pathology, for instance, according to organs, or divided into regions of the body (head, thorax, abdomen, pelvic cavity, limbs), or according to function (cardiovascular, musculoskeletal, neural, digestive, respiratory)
- Histopathology to confirm gross pathology observations.

The method of behavioural observation and recording (e.g., instantaneous scan sampling compared to continuous focal animal sampling), the experience of the observer and the frequency of the behaviour are all important. For example, rare behaviours might not be noted if behavioural observations are only made intermittently, as discussed earlier in this chapter. It is also important to remember that some factors will influence behavioural observations, and might influence both perception of pain, distress or suffering and the expression of those behaviours in an experimental context. For example, the position or availability of cage furniture could constrain the degree or type of activity displayed by the animal, and the presence of observers might limit the expression of some behaviours. Using control groups and allowing the animals ample time to become acclimatised to the experimental apparatus and to observation before

beginning the experiment will help to reduce or obviate these effects. As suggested above, pilot studies and/or reviewing the literature on the effects of the poison on humans and other animals before starting can indicate the best sampling strategies.

Once this information is collected, effects can be graded according to intensity. Grades of 'minor', 'moderate' or 'marked' may be used (Table 1), but other methods of grading have been suggested (see Step 4 below). This helps decisions on the degree of welfare compromise. For example, minor breathlessness could be considered less distressing than marked breathlessness.

In summary, behavioural and pathological observations of poisoned animals should always be conducted to determine the type, intensity and duration of effects.

Step 4. Assessing welfare compromise

As an example, Table 1 suggests the degree of welfare compromise that is caused by certain clinical effects such as vomiting, or that may be indicated by the expression of certain clinical signs of poisoning in possums (shown in shaded areas in Table 1), such as body weight loss. The degree incorporates the duration and intensity of each effect. As noted above, various descriptors could be used to define each degree. 'Minor', 'moderate' and 'marked' have been used here, but the range could just as easily have been divided into, for example, O, A, B, C, and X as suggested by Mellor & Reid (1994). Table 1 was based on direct experience with possums during the present and related studies and on the guidelines and protocols developed by Morton & Griffiths (1985), Sanford *et al.* (1986) and FELASA (1994). Similar tables, and catalogues of pain- and distress-related behaviour have been produced for many animals (Sanford et al. 1986; Spinelli & Markowitz 1987; Otto & Short 1998; Flecknell 1999; Hardie 2000; Rutherford 2002), including laboratory animals and rodents (Morton & Griffiths 1985; FELASA 1994; Mellor & Reid 1994; Carstens & Moberg 2000), and possums (Spielman 1994). These publications could be used to aid in determining the severity of welfare compromise of effects caused by poisons used on vertebrates other than possums.

The degree of welfare compromise will be influenced by the capacity of the animal to suffer, which is the reason for including step 1 in this scheme, above. For example, species with high basal metabolic rates (and therefore high energy requirements) may suffer more due to food deprivation than those with lower rates.

Table 1. Degree of welfare compromise caused by (□) or indicated by ■) several clinical signs of poisoning observed in possums.

Feature	Minor	Moderate	Marked
Convulsions/ seizures[1]		Recovery from intermittent/ short tonic or tonic-clonic convulsions[1]	Recovery from regular/ prolonged tonic or tonic/clonic convulsions
Tremors/ spasms	Occasional twitching (clonic spasm)	Prolonged twitching	
Vomiting/ retching	Occasional (e.g., 1–2 bouts) of retching	Vomiting or high frequency of bouts with many in each bout, with or without vomiting	
Pathology[2]	Lesions/changes in 1–2 areas, or causing/indicating short-term minor–moderate pain/discomfort or long-term minor discomfort	Lesions/changes in 3–4 areas, or causing/indicating short-term severe pain, or long-term discomfort	Lesions/changes in 5 areas, or causing/indicating long-term moderate–severe pain
Incoordination	Able to move freely but may be unstable	Not able to move freely; may fall over	
Breathing	Occasional abnormal breathing pattern	Prolonged abnormal breathing, or short–medium periods of laboured breathing (dyspnoea)	Prolonged laboured breathing
Inactivity/lethargy/listlessness	Mostly inactive with reduced awareness	Mostly prostrate or lying with reduced awareness	
Feed/water intake	Prolonged reduction to 50% or less of normal (for 72 h or more in possums)	Zero for prolonged time (72 h or more in possums) – note: this could differ according to species tolerance	
Body weight	Weight loss of < 20% (severity would differ with species)	Weight loss of 20–30%	Weight loss of greater than 30%
Appearance	Small–moderate change, e.g., a few of: Drooping ears Hanging head Half-closed eyes Staring, glazed eyes Piloerection Sunken eyes Discharges Ungroomed (loose hairs/dirty coat)	Many of: Drooping ears Hanging head Half-closed eyes Staring, glazed eyes Piloerection Sunken eyes Discharges Ungroomed (loose hairs/ dirty coat)	
Voiding	Minor permanent change in faecal/ urine output (e.g., altered consistency), or substantial short-lived change	Substantial or prolonged moderate change (e.g., cessation, blood, diarrhoea)	Extreme prolonged diarrhoea
Abnormal posture	Occasional abnormal posture	Mostly abnormal posture, e.g., crouching, head-pressing	
Normal behaviour	Loss of normal behaviour, e.g., grooming		
Vocalisation	Occasional vocalisation	Prolonged vocalisation	

[1] There is no effect on welfare if consciousness is never regained after seizures. Hence these categories only occur if the possum recovers from these types of seizures.

[2] Pathology areas are head, thorax, abdomen, pelvic cavity, limbs.

- It is assumed that suffering increases with increasing magnitude of injury or change.
- If an animal is permanently unconscious, no effect is recorded because it cannot perceive a welfare compromise while unconscious. If animals regain consciousness, they could suffer welfare compromise due to events occurring during unconsciousness, e.g., physical trauma due to grand mal epilepsy.
- Note that this table allows comparison across as well as between features.

The perception of experiences leading to suffering and the expression of behaviour related to suffering can also vary between and within individuals because of many factors, as mentioned for behavioural observations in step 3 above. Individual, strain and species genetics, age, sex, body weight, previous history and experience, social environment and position in a hierarchy, health and environmental conditions all impinge on an animal's perception and expression of pain (e.g., Morton & Griffiths 1985; Sanford *et al.* 1986; Hardie 2000; Spedding 2000; Rutherford 2002). This can affect our interpretation of the internal state of the animal. For example, animals can exhibit behaviour in response to a painful stimulus without actually perceiving pain, or can suffer but not show any external signs: a lack of detectable behavioural change does not necessarily mean the animal is not suffering.

This also means that experimental conditions can influence the results of any assessment and their relationship to what actually occurs in the wild environment, as already discussed in previous chapters. However, welfare assessment requires close observation of poisoned animals, so cages or pens must be used initially. If there is reason to think that environmental conditions that would be encountered in the wild will have a marked effect on results, field studies should be undertaken.

The chances of misinterpreting the internal state of the animal can be reduced by ensuring that behavioural and pathological observations are made so that each can validate the other, re-enforced by a thorough knowledge of the normal behaviour of the target animal and by sound experimental design.

The mode of action, the dose of pesticide consumed, and the way the pesticide is absorbed, distributed, metabolised and excreted (its toxicokinetics) all influence the unpleasant effects experienced as a result of poisoning, and hence the intensity and/or the duration of suffering. Anything that influences any of these three features could therefore influence the welfare compromise experienced. Potentially influential factors include age, species, diet and health (e.g., Clarke & Clarke 1967; Brown 1980), and characteristics of the bait and usage including pre-feeding, physical and chemical properties, concentration of the poison in baits, handling and storage, attractiveness, and the placement density of baits or bait stations. Good quality-control during bait manufacture and bait use in control operations can ensure that standards of bait quality, storage and use are maintained (reviewed by Morgan & Hickling 2000). For example, bait quality guidelines for 1080 in carrot baits are shown by Eason and Wickstrom

(2001). It is accepted that to maximise welfare, and efficacy, as high a dose as possible needs to be consumed by pests. Although sublethal dosing may have negative impacts on pest welfare, as suggested by the experiments on 1080 (Chapter 2) and brodifacoum (Chapter 4) described herein, it is difficult to test the effects in the laboratory because an extensive range of doses could be consumed in the wild and it would be necessary to test the welfare effects of this range.

In summary, the degree of welfare compromise or level of suffering should be defined, such as in Table 1. New data from different poisons may require the addition of new features to the table, as will the determination of effects for different species.

Assessing the acceptability of poisons

The primary purpose of assessing the animal welfare impacts of pest control methods is to make decisions about their humaneness (O'Connor *et al.* 2003), or acceptability in terms of their impacts on animal welfare. The terms are used interchangeably here, although it can be argued that the term 'humane' also has connotations of compassion, civility and kindness which are outside the meaning intended in this discussion.

Before assessing the humaneness of a poison, the question is raised: what reference point should be used to judge humaneness? One option is "zero suffering", which would rule out all but a few current methods. Alternatively, negative welfare impacts may be judged against the suffering that pest animals normally experience prior to death in the wild, and which commonly involves predation, starvation, dehydration, hypothermia and the like (Warburton & Choquenot 1999). Such a reference point might allow the inclusion of some methods that, on other grounds (e.g., that more humane alternatives are available), we would seek to exclude (Warburton *et al.* in press). Nevertheless, because pest control involves deliberate human intervention to disrupt animals or cause their death, we are ethically obliged to minimise any suffering that such intervention might cause, as discussed in Chapter 1.

Determining whether a poison is absolutely acceptable in terms of animal welfare, or the absolute humaneness of any poison (i.e., to state absolutely that a poison is humane or inhumane), would require specification of some cut-off point beyond which a poison is deemed to be inhumane. The cut-off point could be derived as follows:

1) As a grade, where, for example, poisons over a certain grade are unacceptable, or poisons with a certain number of effects of a certain grade are unacceptable, as specified for traps in the New Zealand guidelines for humane trapping (NAWAC 2000, Appendix C); or

2) By listing certain clinical signs as unacceptable, and classing any poisons causing these signs as inhumane. For example, Gregory *et al.* (1996) suggest that the following effects of poisons are detrimental for animal welfare and should be avoided:

    - Prolonged partial or total paralysis whilst conscious;
    - Hyperexcitability or aggression;
    - Seizures while the animal remains fully conscious;
    - Intermittent seizures where the animal regains consciousness between episodes;
    - Persistent vomiting or retching;
    - Self-mutilation.

3) An alternative would be to decide on a poison's acceptability by comparing its effects against those of an 'ideal' or representative poison. A good candidate for this could be either cyanide, as it lies on one extreme (Gregory *et al.* 1998), or 1080, as it is the most commonly used poison and is intermediate in duration and severity of action (Chapter 2; O'Connor *et al.* 2003).

There are however considerable problems in determining *absolute* humaneness, particularly with producing a numerical grade for the welfare impacts of poisons, as suggested in 1) above. This is discussed further below. The use of unacceptable clinical signs (option 2 above) has some promise, but it would be difficult to list all possible signs that are unacceptable in all contexts. It may also be hard to rank qualitatively different effects. For example, it is difficult enough to compare breathlessness with trauma-induced pain, let alone to include the severity (mild,

moderate, marked or severe) of these noxious effects in the calculation, and even harder to include different durations of these effects.

The *relative* acceptability can be compared, in some respects at least, within a class of control method (e.g., traps or poisons) and also between classes (e.g., traps compared to poisons or shooting). Comparing methods within a class, for instance, kill-traps with very short times to unconsciousness, capture-traps which cause minor injuries and poisons that rapidly cause death with minimal signs of suffering would all be preferred to those methods within each class that have the opposite effects. Likewise, comparing between classes, a kill-trap with a short time (minutes) to unconsciousness may be preferred to a poison which causes signs of suffering for hours or days, a poison that causes death within minutes may be preferred to a capture-trap which causes severe injuries and pain for hours, and shooting that causes death instantaneously may be preferred to all of these. It is evident that judgements about the acceptability of different methods within and between classes are easier to make at the extremes, allowing us to favour the best and rule out the worst methods or approaches.

Assessing relative humaneness

An assessment of the relative humaneness of poisons needs to incorporate the three features discussed above:

1. The number of animals for which welfare is likely to be compromised (i.e., the proportion of target animals experiencing the effects of the poison as determined experimentally, but also including a consideration of the number of animals that the poison will be used to control in the wild, and the risk and effects of sublethal dosing and non-target poisoning).
2. The duration of welfare compromise.
3. The degree of welfare compromise for each effect.

Vertebrate poisons have very different effects and durations of effect, so this task is difficult. For instance, one poison may cause minor to moderate effects for a long time and another causes severe effects for a short time. Which poison is worse? Such cases are different from some other humaneness assessments where one of these features (number affected, duration, intensity) might not be needed in an assessment. For

example, Mellor and Reid (1994) grade the severity of suffering in animals to be used in experiments but do not include the number of animals affected because, in their assessments, the number of animals is later limited according to the expected severity of suffering. Likewise, Broom (1999) suggests calculating severity as the area under a curve of intensity plotted against duration, and hence does not allow for the proportion of animals affected. The New Zealand Guidelines for Assessing Mammalian Restraining and Killing Traps (NAWAC 2000) do not incorporate the duration of suffering into any assessments of killing traps because there is a maximum time to loss of brainstem reflex after which such traps are considered unacceptable. Morton and Griffiths (1985) state that they found it difficult to include duration in their grading system of laboratory animal welfare, and therefore excluded it. Rather, they suggest making repeated assessments over time in order to include duration in the assessment.

One approach suggested by Morton and Griffiths (1985), Kirkwood *et al.* (1994), Mellor and Reid (1994) and Gregory (1998*b*) is to create a single grade or number to compare poisons that takes into account the number affected, and the duration and degree of suffering. The overall grade can then be compared between poisons.

An alternative approach, similar to an idea suggested by Gregory (1998*b*), is to list and compare the appropriate features of each poison, allowing direct comparison of the important features. It does not, however, make the judgement on poison humaneness easier and would not easily solve such issues as whether severe effects for a short time are better than minor-to-moderate effects for a long time. Such judgements are subjective, and as such, could be made by consensus of an expert panel or committee charged with making such decisions.

Nevertheless, an overall assessment of the humaneness of the possum poisons that currently used in New Zealand can and has been made on the basis of such a list. This assessment has been described in full by O'Connor *et al.* (2003) and noted briefly at the end of the preceding chapters describing studies on 1080 (Chapter 2) and brodifacoum (Chapter 4). This assessment was based on the use of poisons to kill brushtail possums in the forms in which they are most commonly used and commercially available in New Zealand. The assessment cannot be applied to other forms of poison or to other species, given the impacts of bait type and species on toxicodynamics and toxicokinetics already discussed. According to this assessment,

cyanide could be considered the most humane poison for killing possums, 1080 the second-most humane poison for possum control along with cholecalciferol, and brodifacoum the least humane (along with phosphorus). The challenge remains to consistently use the most humane poison applicable for each control operation, to strive to improve the humaneness of existing poisons, and to develop more humane poisons for possum control.

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