Guidelines for Assessing the Welfare Impacts of Vertebrate Poisons

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Contents

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Business/Institution: Landcare Research

Programme Leader: Cheryl O'Connor

Programme Title: Welfare impacts of vertebrate poisons

Goal: To ensure the use of humane methods for vertebrate pest control.

Context of Project:
To eradicate Tb from New Zealand's livestock and feral vectors, and to protect our native fauna and flora, poisons and traps remain essential for vertebrate pest control in New Zealand. We have an ethical duty, however, to use the most humane control methods available and continue to develop more humane methods. The development of draft NAWAC guidelines (based on the ISO standard) has provided an objective process for assessing traps using pathological (e.g. physical injuries) and physiological (e.g. brain stem reflexes) measures. The lack of such guidelines for the assessment of poisons is addressed by this project.

Approach:
Our previous research (FRST C09X0009) had assessed the behavioural, biochemical, and pathological changes in possums following poisoning with cyanide, 1080, phosphorus, cholecalciferol or brodifacoum. We used this research, along with information from the literature, to identify some key welfare assessment principles. From these key principles, we described a 5-step process, created a list of the essential behavioural, physiological and pathological measures required for assessment of the welfare impact of vertebrate poisons and identified how they could be used to assess the humaneness of poisons.

Outcomes:
The welfare impact of vertebrate poisons can be assessed by a five-step process:

- Consider the capacity of the species to suffer
- Anticipate likely effects of the poison
- Determine the type, intensity and duration of effects, and the percentage of animals affected
- Determine the degree of welfare compromise caused by each effect
- Assess the humaneness of the poison

The unpleasant effects caused by a vertebrate poison are determined by closely observing the behaviour and pathology of poisoned animals.

Summary:
In order to use the most humane pest control methods, we need to be able to evaluate their humaneness. This requires an assessment of the potential welfare compromise caused by each poison. From key welfare assessment principles, we created a list of the essential behavioural, physiological and pathological measures required for assessing the welfare impact of

vertebrate poisons. We provide a process for assessing the relative humaneness of poisons by considering the capacity of the target animal to suffer; the mode of action of the poison; and the type, intensity and duration of the main unpleasant effects; then making an assessment on the degree of welfare compromise caused by each effect; before finally comparing the type, degree and duration of welfare compromise between poisons.

Publications:
Warburton, B.; Littin, K.; O'Connor, C. 2002: Animal welfare and vertebrate pest control in New Zealand. Presented at Animal welfare and behaviour: from science to solution conference (to be published in *Applied Animal Behaviour Science*).

1. Introduction

Landcare Research, Lincoln, was contracted by MAF Policy to provide guidelines for assessing the welfare impacts of vertebrate poisons. This work was carried out from August 2001 to June 2002 and was based on a literature review and our previous FRST-funded research, so no new animal research was conducted.

2. Background

To eradicate Tb from New Zealand's livestock and feral vectors, and to protect our native fauna and flora, poisons and traps remain essential for vertebrate pest control in New Zealand. We have an ethical duty, however, to minimise the suffering animals experience during control operations. This means we must use the most humane methods available, improve the humaneness of our current methods, and continue to search for more humane alternatives (Mellor 1999; O'Connor 2000). The growing concern of animal welfare groups and the public about the humaneness of control methods and vertebrate pest welfare both here and overseas provides an additional impetus (Loague 1993; Eason et al. 1997; Oogjes 1999). In order to use the most humane control methods, we need to be able to evaluate their humaneness. This requires an assessment of the potential welfare compromise caused by each poison. The development of draft NAWAC guidelines (based on an ISO standard) has provided an objective process for assessing traps using pathological (e.g., physical injuries) and physiological (e.g., brain stem reflexes) measures (NAWAC 2000). However, there are no such guidelines for the assessment of poisons.

As part of our research on the effects of poisons on possum welfare, we have measured behavioural, physiological and pathological changes in possums following poisoning with cyanide, 1080, phosphorus, cholecalciferol and brodifacoum. Based on our knowledge and experience with rats, stoats, ferrets and, in particular, possums, we provide guidelines for assessing the welfare impacts of vertebrate poisons.

3. Objectives

- To develop a protocol describing the essential behavioural, physiological and pathological measures required for assessing the welfare impact of vertebrate poisons.
- To validate the protocol using data previously collected for the humaneness assessment of possum poisons.

4. Methods

4.1 Developing the protocol

Our research (FRST C09X0009) has assessed the behavioural, physiological and pathological changes in possums following poisoning with cyanide, 1080, phosphorus, cholecalciferol and brodifacoum (e.g., Gregory et al. 1998; Littin et al. 2002; O'Connor 2000). We used this research, along with information from the literature (including observations on other species), to identify some key welfare assessment principles. From these key principles, we described a 5-step process and created a list of the essential behavioural, physiological and pathological measures required for assessment of the welfare impact of vertebrate poisons (examples for possums are shown in Table 1).

4.2 Validating the protocol

To ensure that the guidelines provided the essential data to assess the humaneness of vertebrate poisons, and allowed discrimination between poisons, we calculated a numerical score for each poison (Table 2). We calculated scores by assigning a value to duration, intensity and prevalence of each unpleasant effect caused by each pesticide based on published methods of grading the welfare of laboratory rodents and companion animals where available (e.g. Morton & Griffiths 1985; Sanford et al. 1986; FELASA 1994). A calculation based on all measures was initially made and then the score recalculated with each of the measures removed in turn. This allowed us to determine the key elements required to provide the same overall humaneness assessment as that from our complete data set. We made these calculations as follows:

1. From Table 1, we scored all 'minor' effects as 1, all 'moderates' as 2, and all 'marked' as 3, and calculated total scores (A in Table 2).
2. We adjusted these total scores for the number of effects by dividing them by the number of effects (B in Table 2).
3. As an alternative we then removed from the initial total scores those effects occurring in less than 50% of possums (C in Table 2), and adjusted these scores for the number of effects (D in Table 2).
4. Next, we gave poisons a score for overall duration based on shortest to longest, and multiplied each score (A–D) by the duration factor.

Table 2 shows this working for two possum poisons based on data from Gregory et al. (1998) and Littin et al. (2002). (Note that this is based on summary data for the purposes of explanation, so does not constitute a final assessment of the relative humaneness of any of these poisons).

5. Protocol for Assessing the Humaneness of Vertebrate Pesticides

5.1 Literature review

There are some suggestions in the literature about how to assess the humaneness of vertebrate pesticides. The UK Food and Environmental Protection Act 1985 and EU Directive 91/414/EEC require that vertebrate poisons be assessed for humaneness as part of registration (MAFF 1997). Requirements follow a two-stage approach (PSD 2001). Firstly, based on the assumption that procedures causing pain or distress in humans are likely to do so in other animals, applicants must present a literature review of the experiences of humans poisoned with either the pesticide being registered or similar pesticides, and on the humaneness, efficacy and toxicity of the pesticide for target and similar species. In particular, the following must be provided (PSD 2001):

- Details of the type of compound, dose, method and time of exposure or administration;
- Age, sex and species of the test animal;
- Time at which marked signs of toxicity are first seen, and frequency of observations taken to record this;
- Nature, severity and duration of the signs;
- Time to insensibility;
- Time to death;
- Information on pathology (abnormal structure or function) seen on post-mortem examination.

Secondly, the pesticides must be tested on the target species, presumably to fill in any gaps in existing knowledge. Broom (1999) reports that the UK legal requirements for registering vertebrate poisons require measurements of the following in order to assess the degree of pain and suffering: body weight change, reductions in feed and water intake, changes in appearance and undisturbed behaviour, responses to handling, heart or respiration rate, the influence of analgesics on these effects, and post-mortem examination. The humaneness assessment is then based on the intensity and duration of any suffering, with estimates of severity being guided by the recommendations of the Federation of European Laboratory Animal Science Associations Working Group on Pain and Distress (MAFF 1997), which were based on rodents and lagomorphs (FELASA 1994). This methodology has been used to produce an assessment of the humaneness of several vertebrate pesticides including anticoagulant rodenticides, calciferol, phosphine-generating compounds, hydrogen cyanide and alpha chloralose (MAFF 1997).

Gregory (1998) suggests that the harmful effects of pest control methods can be assessed by noting the overall severity of suffering considered according to the intensity and duration of suffering, the number of animals involved, and the capacity of the species to suffer. This can be done using a table with a list of noxious effects. He further suggests that this suffering must be weighed up against the need for control and the practicality of alternatives.

Kirkwood et al. (1994) suggest that welfare compromise caused to wild animals by humans can be evaluated by 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 suggest the following methodology be complied with to make an evaluation:

1. Describe the cause of harm.
2. Describe the pathological effects based on observations or deduced from knowledge of the effects of the cause of harm.
3. Judge the likely level of suffering in terms of stress, fear, pain and/or suffering.
4. Describe the magnitude of the problem based on the number of animals affected and the duration of harm.

Using the methodology of Kirkwood et al. (1994) to determine the welfare impacts of human interference on wildlife, Sainsbury et al. (1995) graded stress into three categories of severity: 'physiological stress' (small amount of physical resources put into maintaining normal functioning and animal is unaware of it), 'overstress' (animal still unaware but significant level of resources are used) and 'distress' (animal is aware of process and may experience negative side-effects). Fear is categorised only as present or absent, and pain is judged as 'pain' or 'severe pain' on the basis of human experiences of similar pathological effects.

This approach yielded the following information for anticoagulant rodenticides (adapted from Sainsbury et al. 1995):

- Pathological effect – Internal haemorrhage, anaemia, circulatory shock
- Severity of harm (category of maximum stress, fear and/or pain) – Distress, severe pain
- Duration of harm (estimated range) – Hours to days
- No. affected annually (estimated range) – 10–100 million (rodents).

As can be seen, the information lacks some accuracy in terms of duration of harm, and it is still difficult to judge whether this would be worse than, for example, something causing distress, severe pain *and* fear for minutes to hours.

To assess the humaneness of several rodent control methods, Mason & Littin (in press) reviewed the literature on the degree of pain, discomfort or distress, the duration of behavioural change caused by toxicosis, and the effects of sublethal dosing. Degree of pain, discomfort or distress was judged from pathology seen on post-mortem examination and behaviour (validated by comparison with experimentally diseased or injured conspecifics compared to analgesic-treated controls), and human experiences of poisoning or similar clinical conditions.

Broom (1999) suggests the overall severity of welfare compromise (including that of pests subjected to a control method) could be estimated from the area under the curve of the level of welfare plotted against the duration of welfare compromise (Fig. 1). He adds that pathophysiological and behavioural measurements and experiences of poisoned humans and other animals can provide information to determine the level of welfare in terms of pain, fear, anxiety, malaise and other states. However, no estimates of the level of welfare for any pest control methods are provided, to then enable calculation of the areas under the curve.

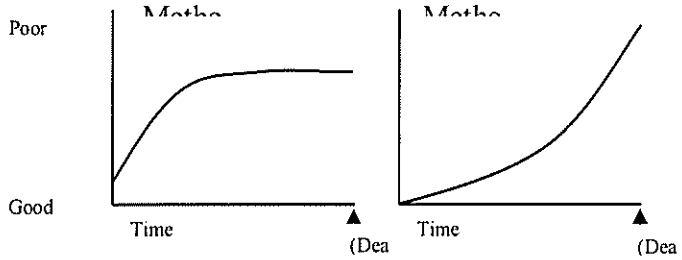

Fig. 1 Method of determining the severity of welfare compromise caused by two hypothetical vertebrate control methods (*a* and *b*) (based on Broom 1999).

Rowsell et al. (1979) assessed the humaneness of several vertebrate poisons including red squill (scilliroside), zinc phosphide and several anticoagulants on the basis of behavioural observations and post-mortem examinations, and the use of electroencephalograms (EEG) to determine the time of unconsciousness. They rated humaneness mainly according to the time to unconsciousness or death, but also on the severity of suffering inferred from the behavioural and post-mortem findings.

5.2 Protocol

Based on a synthesis of the above, we recommend the welfare impacts of vertebrate poisons be determined by the following five steps:

1. Consider the capacity of the species to suffer.
2. Anticipate likely effects of the poison.
3. Determine the type, intensity and duration of effects, and the percentage of animals affected.
4. Determine the degree of welfare compromise caused by each effect.
5. Assess the humaneness of the poison.

The aim is 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), in order to determine the proportion of animals experiencing unpleasant effects, the intensity and duration of those effects, and consequently the welfare impacts of the poison.

Step 1. Consider the capacity of the species to suffer

Are individuals of the species capable of suffering? Are they capable of experiencing particular forms of suffering (e.g. pain compared to anxiety), and are there aspects of the species' natural biology or individual features (e.g., diet, food and water requirements, nocturnal or diurnal, solitary or social, basal metabolic rate, normal pattern of reproduction) that introduce or predispose it to certain welfare consequences (e.g., Spedding 2000; AVMA 2001)? Animals must be conscious (i.e., not anaesthetised or comatose) to be capable of suffering.

It is normally assumed in the animal welfare science literature that, at least, non-human vertebrate animals are capable of feeling pain, and many other emotional states, based on (a) neuroanatomical similarity to humans, (b) similar behavioural responses to pain and distress, and (c) the evolutionary significance of pain and distress (e.g., Bateson 1991, Broom 1998; Kirkwood & Hubrecht 2001, Rutherford 2002). The Animal Welfare Act 1999, presumably on this basis, considers all of the following to be capable of suffering: any mammal, bird, reptile, amphibian, fish, mammalian foetus in the last half of gestation, pre-hatched reptilian or avian young in the last half of development, any marsupial pouch young, and any octopus, squid, or crustacean.

Step 2. Anticipate likely effects of the poison

Prior knowledge of the mode of action, cause of death, and effects in humans and other animals while designing experiments to assess the welfare impacts of a poison means that some of the effects can be anticipated. Likely effects can be anticipated from the literature and/or pilot studies. 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 (e.g., an elevation in plasma calcium).

Step 3. Determine the type, intensity and duration of effect, 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. Data that only records the time to death is insufficient 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 used to determine absolute loss of consciousness. For example, the loss of response to handling indicates when an animal is starting to lose consciousness, whereas the loss of palpebral reflex indicates no brainstem activity and hence total unconsciousness, which is the sign used for testing the welfare impact of kill traps (NAWAC 2000).

Behavioural observations of poisoned animals from poisoning until unconsciousness and pathological findings determined at post-mortem should always be established. Physiological and biochemical measurements can be used to confirm the presence or absence of any unpleasant effects (e.g., ultrasound to assess haemorrhages), consciousness (e.g., through the use of EEG or electrocorticograms) or welfare compromise (e.g., blood-borne compounds or EEG to indicate pain or distress), or suggest further unpleasant effects that might not be seen externally (e.g., hunger, hypovolaemia (decreased volume of circulating blood in the body)).

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)

provide indicators of the onset and duration of illness. It might be helpful to consider the following:

Behaviour
- Appearance
- Posture
- Response to stimuli
- Spontaneous/unprovoked behaviour (including both abnormal behaviour and changes in normal behaviour)

Pathology
- Gross, e.g., 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) and the experience of the observer are both important. For example, rare behaviours might not be seen if behavioural observations are only taken periodically. 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 observations before beginning the experiment will 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 (we graded intensity as 'minor', 'moderate' or 'marked', but other methods of grading have been suggested (see Step 4 below). This helps decide on the degree of welfare compromise. For example, minor breathlessness is likely to be 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. Determine the degree of welfare compromise caused by each effect

The next step is to determine the degree of welfare compromise or level of suffering caused by each effect based on its type, intensity and duration. This evaluation is based on an interpretation of behaviour and pathology in terms of animal welfare, that is accomplished with a thorough knowledge of normal behaviour of the species concerned, the welfare compromise caused by similar effects or poisoning in other animals or humans (being aware of species differences in behaviour and physiology), the responses of animals to known stimuli (e.g., injury, disease, surgery, endotoxin injection) and their amelioration by analgesics (e.g., Sanford et al. 1986; Rutherford 2002).

As an example, Table 1 describes the degree of welfare compromise that is caused by certain clinical effects (e.g., vomiting), or that may be indicated by the expression of certain clinical signs (e.g., body weight loss) of poisoning in possums. The degree incorporates the duration

and intensity of each effect. As noted above, various descriptors could be used to define each degree. We have used 'minor', 'moderate' and 'marked', but it could just as easily be divided into, for example, O, A, B, C, and X as suggested by Mellor & Reid (1994). We have not defined the particular type of suffering in this table, but this can be done in this step, or later in Step 5. We based Table 1 on our experiences with possums and on the guidelines and protocols 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; Mathews 1998; Otto & Short 1998; Flecknell 1999; Hardie 2000; Mellor et al. 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 compromise will be influenced by the capacity of the animal to suffer. 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. 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, environmental conditions, and social environment 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 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. 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 will have a marked effect on results, field studies should be undertaken. The chances of misinterpretation are reduced by ensuring that observations of both behaviour and pathology are made so that each can validate the other, 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 duration of suffering. Anything that influences any of these three features could therefore influence the welfare compromise experienced. Influencing factors could 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, toxicant loading, 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 (e.g., the bait quality guidelines for 1080 in carrot baits; Eason & Wickstrom 2001, p. 117). 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 it is difficult to test the effects 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.

Step 5. Assess the humaneness of the poisons

Once the information suggested above has been collected, results can be compared to assess the humaneness of poisons. Determining the absolute humaneness of any poison would require specification of some cut-off point beyond which a poison is deemed inhumane. The cut-off point could be a grade (e.g., poisons over a certain grade are unacceptable, or those with a certain number of effects of a certain grade are unacceptable, as specified in the trap guidelines (NAWAC 2000, Appendix C), or certain clinical signs could be listed as unacceptable, and any poisons causing these signs would be classed 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.

An alternative would be to decide on a poison's acceptability by comparing each against an 'ideal' or representative poison.

There are considerable problems in determining *absolute* humaneness, particularly with producing a numerical grade for the welfare impacts of poisons, as discussed below. The use of unacceptable signs has some promise, but it would be difficult to list all possible signs that are unacceptable in all contexts. We suggest it would be better to *compare* the humaneness of poisons, rather than assess their absolute humaneness. This is the approach we take to validate our protocol. It is also the approach we suggest for deciding the acceptability of vertebrate poisons used in New Zealand. An approach of judging the relative humaneness of currently used poisons, and only using the most humane, means that we can continue to use these while constantly striving to find more humane poisons and to improve the humaneness of current poisons.

An assessment of the relative humaneness of poisons needs to incorporate the three features of welfare compromise determined in step 3:

1. The number of animals whose welfare is likely to be compromised.
   This is calculated from the percentage of animals experiencing effects (from step 3), whilst also considering the number of animals the poison will be used to control in the wild. The risk and effects of sublethal dosing and non-target poisoning also need to be addressed (e.g., based on literature review). Non-target animals include those that eat baits, poisoned carcasses, and those that are affected by the death of the target animal (e.g., dependent young).
2. The duration of welfare compromise.
3. The degree of welfare compromise.
   We cannot feasibly provide an exhaustive list of the welfare implications of all the possible effects seen after poisoning for all vertebrate poisons. The degrees of welfare compromise need to be determined for each species and each effect, as described in Step 4 above.

Vertebrate poisons have very different effects and durations of effect, so this task is difficult (e.g., we may end up with one poison causing minor to moderate effects for a long time and another causing severe effects for a short time. Which poison is worse?). This is different from some other humaneness assessments where one of these features might not be needed in an assessment. For example, Mellor & Reid (1994) grade the severity of suffering in animals to be used in experiments but do not include the number of animals affected (because 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 (Fig. 1), and hence does not allow for the proportion of animals affected. The New Zealand Guidelines for Assessing Mammalian Restraining and Killing Traps do not incorporate the duration of suffering into any assessments because there is a maximum time to loss of brain-stem reflex after which kill traps are considered unacceptable (NAWAC 2000). Morton & 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 get some idea of duration.

One approach, for example (as suggested by Morton & Griffiths 1985; Kirkwood et al. 1994; Mellor & Reid 1994; Gregory 1998), is to create a single grade or number to compare poisons that takes into account the number affected, and duration and degree of suffering. The overall grade can then be compared between poisons. We tried this approach in order to validate our protocol, as discussed in Section 5.3.

An alternative approach, similar to an idea suggested by Gregory (1998), is to list and compare the appropriate features of each poison. This would allow direct comparison of the important features, but it would not easily solve the issue of whether severe effects for a short time are better than minor-to-moderate effects for a long time. We also tried this approach, as discussed in Section 5.3.

In summary, there are two main approaches that can be used to assess the relative humaneness of different poisons. Both these approaches, creating a numerical grade and comparing a list of the main effects, are evaluated in Section 5.3.

5.3 Validating the protocol: determining overall severity and comparing humaneness

We evaluated two approaches for assessing the relative humaneness of poisons. Firstly, we used a grading system to ensure that addition or removal of key features did not alter the overall position of the poison relative to other poisons. Secondly, we evaluated lists of the main features of each poison.

Approach 1: numerically grading welfare compromise

Calculating a grade is difficult because there are three factors to be included (prevalence, duration, and intensity), and they cannot be related in a simple linear fashion. One solution to the problem is to first combine two of the features to make one number, and then combine that one number with the remaining feature. We did this by incorporating the intensity and duration of each effect into a grade of the degree of welfare compromise in Table 1. An alternative solution is to stipulate limits for one or more of the features. For example, we did this by only including main effects, i.e., those occurring in 50% or more of animals.

We calculated scores in order to compare the humaneness of possum poisons, as described in the methods (Section 4.2). From Table 1, we scored all 'minor' effects as 1, all 'moderates' as 2, and all 'marked' as 3, and calculated total scores as shown in Table 2 (but note that this is based on summary data for the purposes of explanation, so does not constitute a final assessment of the relative humaneness of the poisons). This revealed the following:

- Including the overall duration in the form of a rank score does not affect the overall position of poisons in a relative humaneness ranking (e.g., cyanide first and brodifacoum second if all effects are included (i.e., total A leads to same ranking as A × duration rank). However, this score does not allow for the number of effects seen.
- If the score is adjusted for the number of effects, the ranking is affected, but this can be rectified by including the duration rank in the total score (giving either B or D multiplied by the duration rank). This makes intuitive sense because the score is not unfairly weighted by the number (rather than severity) of effects occurring, and there is allowance for duration, which differs so markedly between the poisons and has a substantial effect on the welfare implications of poisons.
- Including only main effects (>50% prevalence) did not affect the relative humaneness ranking (with or without duration rank). We believe the prevalence of the most prolonged and severe effects (i.e., not necessarily those occurring in most animals) will be of the most interest and have the most weight in a humaneness assessment.

Some of the problems we encountered using this method were:

- It was difficult to know how much weighting to put on each factor (is duration more important than the proportion of animals affected?)
- What mathematical relationship should there be between variables (e.g., should overall duration be multiplied by or added to the total score)?
- If there are more unpleasant effects caused by one poison than another, that poison's overall score increases automatically. This means the grade is not based on the critical feature, the degree of compromise of effects, but on number of effects. Should we therefore divide the final score by the number of effects to compensate for this?
- No allowance can be made for different types of suffering. Pain cannot be given a '1' while distress is a '2', because we expect numbers to be logically related (in which case we would assume that distress is worse than pain). For example, it may be that one poison causes pain but another results in distress due to disorientation. Both of these may be 'marked', and given high scores, but reasoned judgement may decide that pain is worse for animal welfare than distress caused by disorientation (particularly for 'lower' animals). A numerical score would not allow for this level of assessment.
- Some of the signs listed on Table 1 have different implications in different contexts. For example, lying could be due to unconsciousness, weakness, physical impairment, pain or sickness: the welfare implications are different in each situation but would receive the same score.

Similarly, Kirkwood et al. (1994) suggest that assigning a numerical score to the overall severity of welfare compromise (based on the intensity and duration of harm, the number of animals involved and their capacity to suffer) should not be attempted for the following reasons:

- Disagreement about which variables to put into the equation (e.g., should we use a grade for duration, or the absolute value for duration).
- Disagreement about the nature of the variables (should duration be in terms of the lifespan of the animal, or in human terms of minutes, days etc.?).

- Disagreement about the relationship between variables in the equation (should variables be multiplied or added?).
- Scores of intensity of pain, suffering etc. need to have some numerical meaning (e.g., a score of 1 is five times less severe than a score of 5) or they are misleading when used in numerical manipulations to form an overall score.
- Attempts to grade a combination of number of animals affected and severity and duration of harm have been unsuccessful so far.

Rather, they maintain that a clear-enough picture of the level of harm caused to wildlife by human intervention can be gained from following a step-by-step methodology and listing the effects, similar to the second approach discussed below. Likewise, Mellor & Reid (1994) (citing Reid & Mellor 1993) further imply that 'arbitrary numerical thresholds' created from numerical grades should not be used to replace considered judgement.

Nevertheless, using the numerical grade approach, we have shown that our protocol provides information that can discriminate between poisons.

Approach 2: listing and comparing welfare compromise

For this approach, several features can be explicitly compared. The percentage of animals affected and the type, intensity and duration of suffering are always required. The type of effect causing the suffering can be included. For simplicity, only the main effects need to be compared (i.e., those occurring to 50% or more of animals), although a good understanding of the possible range of effects could be gained by also comparing the most severe effects, regardless of prevalence. Table 3 shows the results for two poisons (but note that this is based on summary data for the purposes of explanation, so does not constitute a final assessment of the relative humaneness of any of these poisons).

This simple list method does not provide an objective numerical score that would allow easy comparison between poisons, but it allows consideration of all relevant information by knowledgeable experts. The protocol we have suggested provided all the relevant information for such an assessment.

In summary, because there are several difficulties in assigning an overall numerical score we recommend the approach of listing and through expert opinion comparing the appropriate features of each poison.

6. Conclusions

- The welfare impact of vertebrate poisons can be assessed by a five-step process:
  - Consider the capacity of the species to suffer
  - Anticipate likely effects of the poison
  - Determine the type, intensity and duration of effects, and the percentage of animals affected
  - Determine the degree of welfare compromise caused by each effect
  - Assess the humaneness of the poison
- The unpleasant effects caused by a vertebrate poison are determined by closely observing the behaviour and pathology of poisoned animals.

- The key undesirable effects of pesticides differ greatly in character, intensity and duration.

7. Recommendations

- This protocol should be used to compare the relative humaneness of the vertebrate poisons currently used in New Zealand, by listing and through expert opinion comparing the appropriate features of each poison.

- This protocol should only be used for comparing the humaneness of poisons and **not** used for setting absolute 'cut-off' points.

8. Acknowledgements

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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 wobbly	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.

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

Table 2 Example of use of severity of welfare compromise scores to compare welfare impacts of vertebrate poisons.

Poison	Effect[1]	Category of suffering	Prevalence	Score
Cyanide	Onset of first sign of poisoning: incoordination (followed by lying and prostrate lying)	Moderate	100%	2
	Mild hyperpnoea/dyspnoea[2]	Moderate	50%	2
	Moderate hyperpnoea/dyspnoea	Moderate	5%	2
	Onset of convulsive activity (minor spasms and moderate to marked convulsions)	None (possums unconscious)	75%	-
	Duration of effects = 3:18 (min:s)	A: Total score (all effects)		6
		B: Total / no. of effects		2
		C: Total score (<50% prevalence effects)		4
		D: C / no. of effects		2
		Duration rank		1
		A × duration rank		6
		B × duration rank		2
		C × duration rank		4
		D × duration rank		2
Brodifacoum	Moderate to severe haemorrhages in all possums caused changed appearance, pale mucous membranes, prolonged lying and decreased time spent in normal curled posture, and reduced feed intake	Moderate	72–100%	2
		Moderate		2
		Moderate		2
	Body weight loss of more than 20%	Minor	1/18	1
	Vomited once	Moderate	1/18	2
	Moderate diarrhoea	Moderate	1/18	2
	Minor abnormal breathing	Minor	28%	1
	Minor trembling or shivering	Minor	17%	1
	Minor tremors or spasms	Minor	28%	1
	Minor incoordination	Minor	28%	1
	Duration of effects = 6 days	A: Total score (all effects)		15
		B: Total / no. of effects		1.5
		C: Total score (<50% prevalence effects)		6
		D: C / no. of effects		2
		Duration rank		3
		A × duration rank		45
		B × duration rank		4.5
		C × duration rank		18
		D × duration rank		6

Based on data from Gregory et al. (1998) and Littin et al. 2002

[1] List of all effects seen

2 hyperpnoea = hyperventilation; dyspnoea = laboured breathing

Table 3 Simple list to compare welfare impacts of vertebrate poisons.

Poison	Effect	Time (means)	Prevalence	Notes on welfare implications
Cyanide	Onset of first sign of poisoning: incoordination (followed by lying and prostrate lying)	3:09 (min:s)	100%	Maybe disorientation?
	Mild hyperpnoea/dyspnoea	(duration <15 s)	50%	Minor because short-lived and not severe
	Moderate hyperpnoea/dyspnoea	(duration <15 s)	5%	
	Onset of convulsive activity (minor spasms and moderate to marked convulsions)	6:10	73%	None or very minor because animals were recumbent and soon lost their response to handling, an indicator of unconsciousness
	Onset of loss of response to handling	6:27		
	End of convulsions	6:29		
	Duration of convulsions	0:19		
	Time of death	17:55		
	Duration of effects	3:18 (endpoint = loss of response to handling)		Potentially little more than 3 min of welfare compromise, based on time to loss of response to handling.
Brodifacoum	First severe haemorrhage seen at post-mortem examination	9 days before death	8%	Depends on site, but suggests some form of compromise beginning 9 days before death for this individual.
	Onset of first sign of poisoning: changed appearance, pale mucous membranes, external haemorrhages	7 days before death		Very minor if any compromise, but indicates possums are experiencing effects of blood loss and haemorrhages.
	Onset of prolonged lying and decreased time spent in normal curled posture, and reduced feed intake	6 days before death	72–100%	Suggests pain or weakness or sickness for up to 6 days before death (depends on site and severity of haemorrhages).
	Body weight loss of more than 20%		5.5%	Minor in this context – possum lay in a morbid state and likely did not die through starvation.
	Vomited once		5.5%	Short-lived distress and/or discomfort; maybe nausea. Could indicate gastrointestinal haemorrhage.
	Moderate diarrhoea		5.5%	Moderate discomfort or mild pain. Short-lived.
	Minor abnormal breathing	Intermittent, short-lived within 6 days of death	28%	Likely to be mild since only minor and short-lived.
	Minor trembling or shivering		17%	Could indicate feeling of cold.
	Minor tremors or spasms		28%	Minor (short-lived and minor intensity)
	Minor incoordination		28%	Minor (short-lived and minor intensity)
	Onset of loss of response to touch	2:07 (h:min) before death		Possums remain conscious, therefore able to perceive potential welfare compromises throughout almost the entire sickness period.
	Onset of loss of palpebral reflex	At death		
	Time of death	21 days after first ingestion		
	Duration of effects	6 days		Behaviour and appearance of clinical signs suggest possums can experience any welfare compromise for 6 days before death (but could be 7 days in individuals with haemorrhages occurring earlier).

Based on data from Gregory et al. 1998 and Littin et al. 2002

Relative Humaneness Assessment of Possum Poisons

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Contents

Summary Final Report – Operational Research 2002/03

Project Code: FRM225

Business/Institution: Landcare Research

Programme Leader: Cheryl O'Connor

Programme Title: Welfare impacts of vertebrate poisons

Goal: To assess the relative humaneness of vertebrate pest control poisons

Context of Project:

To meet our goal of developing and using more humane vertebrate poisons, we need to assess the relative humaneness of poisons using a standardised method. The guidelines we developed in Objective 1 (2001/02) described the essential behavioural, physiological and pathological measures required to assess the welfare impact of vertebrate poisons. The guidelines also recommended that the relative humaneness of poisons should be assessed by comparing the type, severity and duration of welfare compromise caused by the main effects of each poison. This project therefore aims to use these guidelines to assess the relative humaneness of the possum poisons currently used in New Zealand.

Approach:

Previous research (FRST C09X0009) provided data on the main behavioural, physiological and pathological effects for all possum poisons used in New Zealand. We used these data and applied the process developed in Objective 1 to rank the currently used possum poisons. The guidelines (Littin & O'Connor 2002) describe a five-step process that considers the type, intensity and duration of the main unpleasant effects to then make an assessment of the degree of welfare compromise caused by each effect. If the principles of the degree of welfare compromise provided in the guidelines (Step 4) are accepted, these data can be used to conduct the fifth step – to compare the type, degree and duration of welfare compromise between possum poisons. This will provide a full assessment of the relative humaneness of current possum poisons.

In addition, this data set provides the opportunity to refine the humane end-points used in toxicity and efficacy testing on possums. These data describe the course of key behavioural effects over time, which can then be related to the time and certainty of death, following such observations.

Outcomes:

In summary, cyanide caused mild abnormal breathing in 52% of the poisoned possums and convulsions occurred in all animals after they had become unconscious. For 1080 there is potential welfare compromise for 9.5 h following poisoning. A small percentage of possums poisoned with 1080 had minor to moderate retching, most became incoordinated and then all had mild to moderate tremors or spasms. The main welfare concern with phosphorus is the congestion of the gastric mucosa, which was linked to the adoption of a crouching posture.

This was probably associated with some mild pain and lasted for 10 h until possums became prostrate. Cholecalciferol caused mineralisation in the organs of 67% of possums and lung damage in 59% of the animals. Seventy-one percent of possums had abnormal breathing for 1.5 days before death. They did not eat for 7 days, on average, and 21% lost more than 30% of their bodyweight. Finally, brodifacoum caused widespread haemorrhages of varying severity in all animals. The welfare consequences depend on the site and severity of the haemorrhages, which makes it difficult to generalise the welfare impact of this poison. Nevertheless, all animals had at least one severe haemorrhage in an area that would cause or contribute to pain, distress or weakness.

In addition, in order to describe a refined humane end-point for efficacy testing, we determined that the behaviour shown by most possums across all poisons was a prolonged period of prostration or lying, on the side, back or belly. We have defined prolonged in this case as a continuous 2 h or more. If this refined end-point, prolonged period of prostration, had been used there would have been a significant reduction, of several hours, in the period of suffering for many animals tested.

Summary:

As with many animal welfare assessments these recommendations are based on our "scientifically informed best judgement". We believe cyanide is the most humane poison for possums and would encourage its use, particularly encapsulated cyanide. 1080 seems acceptable at present, and phosphorus and cholecalciferol could be used with adequate justification. On humaneness grounds, there should be an extremely good practical reason before brodifacoum is used.

Publications:

Nil

1. Introduction

Landcare Research, Lincoln, was contracted by MAF Policy to assess the relative humaneness of the possum poisons currently used in New Zealand. This work was carried out from August 2002 to June 2003 and was based on the guidelines for assessing the welfare impacts of vertebrate poisons (Littin & O'Connor 2002) and our previous FRST-funded research. No additional animal research was conducted.

2. Background

If we are to eradicate bovine tuberculosis from farm stock and wildlife vectors, and protect our native fauna and flora, poisons and traps remain essential requirements for vertebrate pest control in New Zealand. We have an ethical duty, however, to use the most humane control methods available, but in order to do so we need to be able to evaluate their relative humaneness. We have recently developed guidelines (Littin & O'Connor 2002) based on the essential behavioural and pathological measures required to assess the welfare impact of vertebrate poisons. As part of our FRST-funded research on the effects of poisons on possum welfare, we have measured behavioural, physiological and pathological changes in possums following poisoning with each of five vertebrate poisons. The guidelines also recommended that the relative humaneness of poisons should be assessed by comparing the type, severity and duration of welfare compromise caused by the main effects of each poison. This project therefore aims to use these data to assess the relative humaneness of the possum poisons currently used in New Zealand.

In addition, this data set provides the opportunity to refine the humane end-points used in toxicity and efficacy testing of vertebrate poisons on possums. These data describe the course of key behavioural effects over time, which can then be related to the time and certainty of death following such observations.

3. Objectives

- To assess the relative humaneness of vertebrate pest control poisons.
- To refine humane end-points for possum efficacy testing.

4. Methods

Previous research (FRST CO9X0009) provided data on the main behavioural, physiological and pathological effects for cyanide, sodium monofluoroacetate (1080), phosphorus, cholecalciferol and brodifacoum. We used these data and applied the process described in the guidelines for assessing the welfare impacts of vertebrate poisons (Littin & O'Connor 2002). This five-step process considers:
The capacity of the species to suffer.
Anticipates likely effects of the poison.
Determines the type, intensity and duration of effects, and the percentage of animals affected.
Determines the degree of welfare compromise caused by each effect.
Assesses the relative humaneness of the poison.

In addition, these data describe the course of key behavioural effects over time, which can then be related to the time and certainty of death following such observations. Hence they provide an opportunity to refine the humane end-points used in toxicity testing on possums.

5. Assessing the Welfare Impacts of Vertebrate Poisons

5.1 Step 1: Consider the capacity of the species to suffer

The Animal Welfare Act 1999 considers any mammal, bird, reptile, amphibian, fish, mammalian foetus in the last half of gestation, pre-hatched reptilian or avian young in the last half of development, any marsupial pouch young, and any octopus, squid, or crustacean to be capable of suffering. Brushtail possums (*Trichosurus vulpecula)* are obviously included in this list. In addition, they are recognised as being the most adaptable and most widely distributed of the Australian marsupials (Cowan & Tyndale-Biscoe 1997). The ability of possums to adapt to a wide range of conditions has been the primary reason for their ecological success, and subsequent pest status, in New Zealand.

In captivity, possums adapt readily, showing few observable behavioural effects besides a short-term fear response to human caregivers (Day & O'Connor 2000). This suggests that the possum is capable of a relatively high level of cognition, and is therefore at least capable of experiencing pain and distress. Marsupial neuroanatomy is sufficiently complex to suggest, at least, that they are capable of the conscious recognition of pain (e.g. Beck et al. 1996; Catania et al. 2000). Further, possums show behaviour that suggests their welfare is poor in situations where this could be expected (e.g. Eason et al. 1996; Gregory et al. 1998). For example, following sublethal poisoning possums become ill (showing a variety of responses) and on recovery they respond by subsequently avoiding the bait they associate with that illness (e.g. O'Connor & Matthews 1995; Morgan & Milne 2002).

In conclusion we consider possums are as capable of suffering as eutherian mammals, although they may not be as demonstrative in displaying pain or illness as dogs, for example.

5.2 Step 2: Anticipate likely effects of the poisons

Prior knowledge of the mode of action, cause of death, and effects in humans and other animals was considered, as described below, before the earlier FRST experiments to assess the welfare impacts of possum poisons were undertaken.

Cyanide

Cyanide acts as a respiratory stimulant through activation of chemoreceptors in the carotid body (Daly et al. 1978). Experience in humans confirms that dyspnoea (laboured breathing) and convulsions occur during low-dose poisoning with cyanide. In addition, there can be salivation, nausea, vomiting, anxiety and headaches (Salkowski & Penney 1994).

1080

Death from monofluoroacetate poisoning is caused by the inhibition of energy production, which, in turn, results in either cardiac or respiratory failure (Atzert 1971). Animals receiving a lethal dose usually show more severe signs of poisoning, in addition to non-specific clinical signs such as nausea and vomiting, and these include cyanosis, drowsiness, tremors, staggering, and death from ventricular fibrillation or respiratory failure. In general, herbivores experience cardiac failure, whereas carnivores experience central nervous system disturbances and convulsions, then die of respiratory failure (Eason et al. 1994).

Phosphorus

Phosphorus is absorbed from the respiratory and gastrointestinal tract, but the mode of action is still unknown (Clarkson 1991). Phosphorus poisoning symptoms generally include abdominal pain and vomiting, and sometimes haematemesis (vomiting blood), followed by cyanosis, coma and death (Beasley 1997).

Cholecalciferol

In toxic doses, cholecalciferol mobilises stores of calcium from bones into the bloodstream and produces hypercalcaemia and calcification of the blood vessels. Tissue calcification can occur in the cardiovascular system, kidneys, stomach and lungs. Mineralisation and blockage of blood vessels, with death probably from heart failure, appears to be the mode of action of cholecalciferol in rodents (Morrow 2001).

Brodifacoum

Brodifacoum kills by disrupting normal blood clotting. It competitively inhibits recycling of vitamin K. Eventually, circulating vitamin-K-dependent clotting factors break down and are not replaced (Thijssen 1995). As a result any damage to the blood vessels is not adequately repaired, and animals begin to haemorrhage at the injured sites. In addition anticoagulant poisons may cause damage to blood vessels themselves, contributing to the risk of haemorrhage (Kruse & Carlson 1992). If blood loss continues, anaemia and shock due to low blood volume (hypovolaemic shock) develop, and death can ensue by, or as a combination of, cardiac, respiratory or kidney failure (Anderson 1980).

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

A summary of the behavioural and pathological observations of caged and penned possums for each poison is listed in Tables 1–5 below. Data for the time to onset of the first sign of

poisoning, the time of onset and duration of each effect, the time to loss of consciousness, and percentage of animals affected are all provided. Effects specific to the poison (e.g. spasms, vomiting) are also described. These provide information on the intensity and duration of each effect, and the mean overall duration of effects for each poison.

5.4 Step 4: Determine the degree of welfare compromise caused by each effect

The degree of welfare compromise or level of suffering caused by each effect is also listed in Tables 1–5 below. The degree incorporates the duration and intensity of each effect and was predominantly determined from Table 1 in the guidelines (Littin & O'Connor 2002). Notes on welfare implications of each effect are provided, which help decide on the degree of welfare compromise (described here as minor, moderate or marked).

Table 1 Type, intensity, and duration of effects, and degree of welfare compromise observed in captive possums following poisoning with cyanide. (Based on data from Gregory et al. (1998)).

Effect over time	Mean time to onset (minutes:seconds)	Mean duration (seconds)	Prevalence (%)	Notes on welfare implications
Time to first signs of sickness: *Incoordination*	3:09		42/42 (100)	Maybe some disorientation
Breathing (hyperpnoea/dyspnoea): *Mild* *Moderate*		15 15	21/42 (50) 1/42 (2)	Minor because short-lived hyperpnoea, not more laboured dyspnoea
Loss of response to handling	6:27		17/20 (85)	Loss of response to handling is an indicator of unconsciousness
Convulsions		19	31/42 (73)	None or very minor because possums were recumbent and had lost their response to handling.
Mean time to death	17:55			
Duration of effects	**3:18**			Potentially little more than 3 min of welfare compromise, based on time from incoordination to loss of response to handling

Table 2 Type, intensity, and duration of effects, and degree of welfare compromise observed in captive possums following poisoning with 1080.

Effect over time	Mean time to onset (hours:minutes)	Mean duration	Prevalence (%)	Notes on welfare implications
Time to first signs of sickness: *Changed appearance*	1:52		14/27 (52)	Onset of illness, minor welfare implications.
Time to retching and vomiting: *Minor retching* *Moderate retching* *Vomiting*	2:53	 1–2 bouts 2 or more bouts 1	 3/27 (11) 7/27 (26) 1/27 (4)	Minor to moderate distress from abdominal pain after repeat bouts.
Time to onset of moderate incoordination: *Unsteady head movements and walking*	3:37		20/27 (74)	Disorientation, or could indicate weakness.
Minor tremors or spasms	4:05	Few seconds	27/27 (100)	Animals remained conscious or regained consciousness shortly after these. This could be a welfare concern if the spasms caused pain (e.g. as a result of physical trauma, headache, or disorientation). However, they were most often recorded as mild twitches rather than more severe seizures, and would therefore be of minor welfare compromise.
Prolonged lying or prostrate *for periods of longer than 2 hours*	5:38	Until death	21/27 (78)	Could indicate pain, weakness or disorientation, given mode of action.
Time to loss of handling	At death			Possums remain conscious, therefore able to perceive potential welfare compromises throughout the sickness period.
Mean time to death	11:26			
Duration of effects	**9:34**			Up to 9.5 h of potential welfare compromise.

Table 3 Type, intensity, and duration of effects, and degree of welfare compromise observed in captive possums following poisoning with phosphorus.

Effect over time	Mean time to onset (hours:minutes)	Mean duration	Prevalence (%)	Notes on welfare implications
Time to first signs of sickness: *Stopped grooming*	2		Until death	Onset of illness.
Prolonged periods of crouching	8	10 h	4/18 (22)	Suggests pain and weakness. The degree of pain would depend on the degree of congestion of the gastric mucosa, which was typically mild.
Prolonged periods of prostration *for periods of 2 h or longer*	20	Until death	11/18 (61)	
Time to retching and vomiting: *Moderate retching* *Vomiting*	 9 8	 2 or more bouts	 12/18 (67) 8/18 (44)	Minor to moderate distress from nausea or abdominal pain after repeat bouts.
Mild or moderate congestion of the stomach	from 3		10/21 (48)	Some discomfort / minor pain indicated.
Failure of righting response	24:06	Until death	18/18 (100)	Possums remain conscious, therefore able to perceive potential welfare compromises throughout almost the entire sickness period.
Loss of corneal reflex	24:53	Until death	18/18 (100)	
Mean time to death	25:12		18/18 (100)	
Duration of effects	**23**			Up to 23 h of potential welfare compromise.

Table 4 Type, intensity, and duration of effects, and degree of welfare compromise observed in captive possums following poisoning with cholecalciferol.

Effect over time	Mean time to onset (days:hours)	Mean duration	Prevalence (%)	Notes on welfare implications
Time to first signs of sickness: *Reduced exploring (activity)*	2	Until death	23/24 (96)	Onset of illness.
Breathing: *Rapid*	5:12	Until death	17/24 (71)	Moderate welfare implications because prolonged abnormal breathing but not laboured dyspnoea.
Reduced feed intake	2	Until death	23/24 (96)	Moderate welfare implications.
Body weight loss of 20–30% Body weight loss of 30–50%	8:12 20		4/24 (17) 5/24 (21)	Moderate welfare implications. Marked welfare implications.
Prolonged prostration *for periods longer than 2 hours*	6:12	Until death	9/24 (37)	May suggest pain or weakness.
Mineralisation in organs at death: *Mild 1–2 areas* *Moderate 3 or more areas*	 9:5 17:2		 13/24 (54) 3/24 (13)	Minor welfare implications. Moderate–marked welfare implications. Pain and distress are possibilities from mineralisation in some organs.
Lung pathology at death: Mild Moderate	 20 6:19		 3/24 (13) 11/24 (46)	Minor–moderate welfare implications in these possums, which showed lung pathology, ranging from pulmonary emphysema to congestion and oedema. Likely to have caused breathing difficulties and distress.
Time to loss of consciousness	Just prior to death		23/24 (96)	Possums remain conscious, therefore able to perceive potential welfare compromises throughout almost the entire sickness period.
Mean time to death	9		23/24 (96)	
Duration of effects	7			Up to 7 d of potential welfare compromise.

Table 5 Type, intensity, and duration of effects, and degree of welfare compromise observed in captive possums following poisoning with brodifacoum. (Based on data from Littin et al. (2002))

Effect over time	Mean time to onset (days:hours)	Mean duration	Prevalence (%)	Notes on welfare implications
Time to first signs of sickness: *Changed appearance*	14	Until death	1/18 (6)	Onset of illness, minor welfare implications
Reduced feed intake: *Less than 20 g per night*	13	Until death	17/18 (94)	Minor welfare implications
Body weight loss of more than 20%	2	At death	1/18 (6)	Minor in this context – possum lay in a morbid state and likely did not die through starvation.
Vomited			1/18 (6)	Short-lived distress and/or discomfort: maybe nausea. Could indicate gastrointestinal haemorrhage.
Moderate diarrhoea			1/18 (6)	Moderate discomfort or mild pain, short-lived.
Minor abnormal breathing			5/18 (28)	Minor since only short-lived.
Minor trembling or shivering	15	Intermittent, short-lived	3/18 (17)	Could indicate feeling cold.
Minor tremors or spasms			5/18 (28)	Minor (short-lived and minor intensity).
Minor incoordination`			5/18 (28)	Minor (short-lived and minor intensity).
Haemorrhages:				
Mild/moderate	8		4/12 (33)	Minor–moderate welfare implications.
First severe	12		1/12 (8)	Depends on site, but suggests increasing welfare
Severe	20		8/12 (67)	compromise with increasing severity
Onset of prolonged lying: *Over 2 h*	17:12	Until death	6/18 (33)	Suggests pain and weakness
Loss of response to handling	2 h before death	Until death	6/6 (100)	Possums remain conscious, therefore able to
Loss of palpebral reflex	At death		6/6 (100)	perceive potential welfare compromises throughout almost the entire sickness period.
Mean time to death	21			
Duration of effects	**6**			Behaviour and appearance of clinical signs suggest possums can experience a welfare compromise for 6–7 days before death.

5.5 Step 5: Assess the humaneness of the poisons

The guidelines (Littin & O'Connor 2002) recommend judging the relative humaneness of poisons by listing and through expert opinion comparing the effects of each poison. On the basis of duration of effects alone, it is clear that cyanide is the most humane poison, and cholecalciferol and brodifacoum the least humane. Incorporation of a list of several features allows consideration of all relevant information by knowledgeable experts. The percentage of animals affected and the type, intensity and duration of effects causing the suffering can be included. In addition, by fully describing the welfare implications for all the poisons, the most serious areas of welfare compromise are identified.

Briefly, cyanide caused mild abnormal breathing for 52% of the poisoned possums and convulsions occurred in all animals after they had become unconscious. The ability to cause rapid unconsciousness, such as this, is a preferred action for a vertebrate poison. The risk of sublethal dosing should be reduced by careful use. Encapsulated cyanide (Feratox®) might reduce the chances of sublethal effects.

Potential welfare compromise for 9.5 h resulted from 1080 poisoning. A small percentage of the animals had minor to moderate retching, most became incoordinated and then all had mild tremors or spasms and some had a few short-lived, mild-to-moderate seizures. In animals that recover from prolonged or repetitive effects such as these, pain due to physical trauma or headache arising from the seizures could be a welfare issue. The other area of welfare concern would be distress owing to weakness or general sickness in these animals.

The main welfare concern with phosphorus is the congestion of the gastric mucosa, which was linked to the adoption of crouching posture, and retching or vomiting in 67% of the animals. This was likely associated with mild pain and lasted for 10 h until possums became prostrate.

Cholecalciferol caused mineralisation in the organs of 67% of possums and lung damage in 59% the animals (in which lung failure was also considered the primary cause of death). Seventy-one percent of possums had abnormal breathing for 1.5 days before death. They did not eat for 7 days, on average, and 21% lost more than 30% of their bodyweight. Mineralisation is likely to be associated with pain or distress if it occurs in active muscles or certain organs. In cholecalciferol-poisoned possums it largely occurred in the heart and kidneys, which was likely to influence the functioning of these organs, with consequent effects, rather than, or as well as, causing pain per se. Pulmonary emphysema and oedemas in half the animals were likely to have caused the breathing difficulties observed. Lung dysfunction could also potentially have consequences, such as metabolic imbalances, which have implications for animal welfare.

Finally, brodifacoum caused widespread haemorrhages of varying severity in all animals. The welfare consequences of these depend on the site and severity of the haemorrhages, which makes it difficult to generalise the welfare impact. Nevertheless, given that all animals had at least one severe haemorrhage in an area that would cause or contribute to pain, distress or weakness of some kind, this poison is ranked lowest.

We believe the use of cyanide, particularly encapsulated cyanide, should be encouraged. 1080 seems acceptable at present, and phosphorus and cholecalciferol could be used with

adequate justification. In particular, where there are secondary poisoning concerns that the use of phosphorus and cholecalciferol could obviate, animal welfare would be better promoted on balance by the use of these poisons. There should be an extremely good practical reason for recommending the use of brodifacoum. In addition, where it must be used, there should be every endeavour to ensure that animals get as high a dose as possible, in order to reduce the time to death.

6. Humane End-points

Scientists are being increasingly compelled to reduce the potential pain and suffering of experimental animals by including earlier end points in experiments where possible. A humane end-point can be defined as the earliest indicator in an animal experiment of severe pain, severe suffering, or impending death (OECD 2000). Determining such end points can be problematic because many animals do not readily exhibit behaviours that are indicative of pain or distress. Different animal species, and animals at different stages of development, may respond differently to test conditions, and exhibit different indications of distress (OECD 2000).

In addition there is a conflict in efficacy testing, where certainty of death (not illness) is required. The mode of action of the different possum poisons described here also varies. As a result the observed clinical signs vary greatly. For example, the different early indicators of illness were a period of 30 min crouching for phosphorus, and a prolonged period (i.e. 2 h) of rapid breathing (i.e. more than 30 breaths/min) for cholecalciferol. Finding a behaviour that is constant across all poisons was difficult, but the behaviour that most possums (over 33%) showed was a prolonged period of prostration or lying, on the side, back or belly. We have defined prolonged in this case as a continuous 2 h or more. For 1080 and phosphorus a high percentage of animals were prostrate, but less than 50% of animals poisoned with cholecalciferol and brodifacoum became prostrate for more than 2 h (Table 6). There were however, no other behavioural or clinical signs that were any more consistent or prevalent for these poisons (see Tables 4 & 5). In addition, a prolonged period of prostration was seen in more animals, and earlier than bodyweight reductions (the current humane end-point used in efficacy trials) in both cholecalciferol and brodifacoum poisoned possums.

For each poison, the time when each possum first became prostrate for 2 h was determined. The remaining time until death was then calculated (Table 6). This therefore indicates the extra period of suffering which the new humane end-point would alleviate, in future studies. It must be remembered that animals were not necessarily prostrate for this entire period nor does prostration necessarily equate to marked welfare compromise.

If this refined end-point had been used in the trials described here, there would have been a reduced period of several hours of suffering for many animals. This equates to 60%, 24%, 5%, and 6% reduction in the time spent suffering for 1080-, phosphorus-, cholecalciferol- and brodifacoum-poisoned possums respectively (Table 6). Although this is less than 10% of the illness period for cholecalciferol- and brodifacoum-poisoned animals on average, it does reflect a substantial time period for some possums. We believe it is those animals that show a more "lingering death" that become prostrate (for up to 79 h), and hence this refined end-point would greatly reduce suffering in trials that use the poisons.

Table 6 Summary of percentage of animals prostrate, mean times to death, mean time from 2 hours prostrate to death (and range), and percentage of time "suffering" would be reduced.

Poison	Percentage of animals prostrate for 2 hours	Mean time to death	Mean time from 2 hours prostrate to death (and range)	Mean percentage of "suffering" time reduced
Cyanide	-	17:55 (min:s)	-	-
1080	78%	14:10 (h:min)	8:31 (2:23–13:30 h:min)	60%
Phosphorus	61%	27:23 (h:min)	6:30 (0:15–37:05 h:min)	24%
Cholecalciferol	39%	6 (d)	7:50 (0:15–24:30 h:min)	5%
Brodifacoum	33%	18:12 (d:h)	28:08 (3:15–79:22 h:min)	6%

7. Recommendations

- The guidelines should be used to assess the relative humaneness of other vertebrate poisons for other species.
- The refined humane end-point of a prolonged period of prostration should be used in all possum efficacy testing.
- Refined humane end-points for efficacy testing should be determined for all species.

8. Acknowledgements

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Project C0124/2006: Improved Humaneness of Vertebrate Toxic Agents

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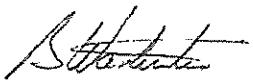	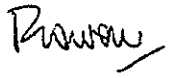
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Contents

Summary Final Report – Operation Research 2006/2007

Project Code: **Biosecurity New Zealand Project C0124**

Business/Institution: Landcare Research

Programme Leader: Penny Fisher

Programme Title: **Improved Humaneness of Vertebrate Toxic Agents**

Context of the project:

Landcare Research, Lincoln, was contracted by Biosecurity New Zealand, Animal Welfare Group, to assess whether the humaneness of possum control using sodium fluoroacetate (1080) poisoning could be improved through the co-administration of the drug alphachloralose. Two laboratory trials with captive possums were carried out in January and May 2007.

Approach:

- Wild-caught possums acclimatised to indoor housing in individual cages were allocated to two treatment groups (each n=10) and offered either alphachloralose in pelleted food or untreated pelleted food. The amount of each food type eaten was measured, and possums were observed over the following 9 hours for effects on their behaviour, body temperature, and responses to stimuli. At 15-minute observation intervals, the degree of responsiveness was estimated from the appearance of each possum and its responses to applied stimuli, using a number scoring system. The Observer® software was used to record scores and observations of posture and illness in real-time.
- In a second trial, wild-caught possums housed and acclimatised as above were allocated to three treatment groups; 5 mg/kg 1080 (n=8), 5 mg/kg 1080 and 60 mg/kg alphachloralose (n=8) or carrier solution only (control, n=4) and doses were administered by oral gavage. Possums were observed over the following 13 hours for illness behaviour, posture, responses to stimuli and time to death, using the same observation system as the previous trial.

Outcomes:

- Possums offered untreated food ate significantly more (mean ± sem 14.84±3.02 g) than those offered alphachloralose-treated food (6.55±1.321 g) (P=0.027). The doses of alphachloralose ingested by possums ranged from 0.69 to 67.63 mg/kg.
- All possums that ingested alphachloralose were affected to some extent but not all reached the 'higher' states of reduced response to stimuli. The most evident effects generally occurred within 2.5 to 8 hours post-consumption, after which recovery towards 'unaffected' state was evident. It was estimated that an intake of at least 55 mg/kg alphachloralose would be sufficient to induce 'high' states of reduced consciousness that could mitigate the painful or stressful effects of 1080 poisoning in possums.
- In the second trial all control possums survived. All possums in both ACL+1080 and 1080 alone treatments died or were euthanased at a 13.5 hour endpoint. There was no significant difference in the mean time to death between the two treatments. The state of "moderate effect" was the only variable significantly affected by ACL – with mean durations of 1.15 hours in the 1080 alone group and 0.47 hours in the ACL + 1080 group. From a count of 'illness events', 4/8 possums in the 1080 alone treatment showed at least one bout of retching while no possums in the ACL+1080 treatment were observed retching. No other statistically significant differences in state latencies,

durations or illness behaviours were attributed to the addition of ACL to an effective lethal dose of 1080.

Recommendations

The addition of ACL to an effective lethal dose of 1080 appeared only to affect the mean duration spent in State 2 (moderate effect) and possibly to reduce the occurrence of retching in poisoned possums. These differences were not considered sufficient to represent an overall improvement of welfare to justify further development of ACL as a welfare-improving agent for 1080 baits.

Summary:

A laboratory investigation was carried out of the potential of alphachloralose as a welfare-improving agent for 1080 poisoning in possums. An initial trial indicated that the onset, duration and nature of the effects produced in possums that ingested alphachloralose in food were suitable for further consideration. However, a second trial that compared the effects in possums given an effective lethal dose of 1080 with those in possums given the same dose plus alphachloralose did not detect any sufficient differences in responses to stimulus, posture or illness behaviour to justify further investigation of alphachloralose as a welfare-improving agent for 1080 baits.

Publications:

Nil.

1. Introduction

Welfare issues in the field of pest animal management are becoming more prominent (e.g., Littin & Mellor 2005). Recent anti-1080 campaigns have targeted the effects of 1080 poisoning as an animal welfare issue, particularly given the large numbers of possums subject to this method of control. Two potential approaches for reducing the negative impacts of poisons on possum welfare have been identified:

- Shorten the duration of illness and other poisoning effects by increasing the speed of action of the poison or by inducing unconsciousness earlier, e.g., by anaesthetics.
- Prevent or reduce those effects of poisoning associated with pain and/or distress by the use of drugs with specific actions, e.g., anti-emetics, anxiolytics (anxiety-reducing agents), analgesics, anticonvulsants.

Recent laboratory trials established 'proof of concept' that the duration and potentially the degree of suffering experienced by poisoned possums could be reduced through the addition of a drug to food (O'Connor et al. 2006). However, the relatively high doses of the drug required to produce these effects in possums would have meant substantial increases in unit cost for a toxic bait formulation that contained the drug as a welfare-improving agent. Further development of this approach to provide a practical and widely adopted humane method of pest control must consider the economic cost in relation to the welfare improvement achieved. Such research can provide information to help develop guidelines for the killing of wild animals under the provisions of the Animal Welfare Act.

2. Background

In this study, we sought to evaluate whether a potentially cheaper oral additive could reduce pain or suffering in 1080-poisoned possums, to commence development of a cost-effective toxic bait formulation with improved humaneness. A review of orally active drugs with anaesthetic or sedative effects identified alphachloralose (CAS # 15879-93-3) as an alternative for investigation. This compound has been used in the past as a veterinary anaesthetic as well as a vertebrate toxic agent for pest birds in New Zealand. It is readily available, relatively cheap, and could provide a practical alternative to the use of drugs that are more restricted by New Zealand regulations. The effect of an oral dose of 200 mg/kg alphachloralose in possums that were also administered an effective lethal dose of 1080, had been previously investigated (K Littin, Landcare Research, unpublished data), and had concluded that there was no significant effect on the time to death, behavioural changes and clinical signs of 1080 toxicosis. However, onset and duration of unconsciousness, or reduced responsiveness to stimuli were not assessed. Although clinical signs ('illness events') such as retching, vomiting and convulsions are an important component of perceived welfare, mitigation of these should only be considered part of the picture. The study noted "there seemed to be a tendency for more 1080+alphachloralose possums to lie on the side or front sooner....and then for more to lie prostrate sooner rather than lying on the belly", which suggested earlier progression and perhaps increased duration of states of reduced responsiveness and unconsciousness – if this was a demonstrably significant effect, an overall improvement of welfare might be achieved despite no apparent changes in time to death and clinical signs.

Another aspect considered worth evaluating was the effect of cool temperature on the progression of 1080 toxicosis in possums with and without co-administered alphachloralose. Ambient temperature is known to influence the susceptibility of mammals to 1080 – the colder it is, the smaller the effective lethal dose required (e.g., Misustova et al. 1969). Highest estimated kill rates of brushtail possums were observed by Veltman et al. (2001) during winter and at southern latitudes, consistent with

previous laboratory studies of 1080 toxicity at warm and cool temperatures. Alphachloralose interferes with thermo-regulation (Hayes & Lawes 1991), and may hasten the onset of unconsciousness and death during 1080 poisoning at cold ambient temperatures.

The first step in the evaluation of alphachloralose was to ensure any potentially mitigating effects of the drug would coincide with effects of 1080 toxicosis that might be painful or stressful in possums. Previous trials of alphachloralose as a toxicant for possums (Eason & Jolly 1992) established that oral doses of 100–400 mg/kg produced death within 2–48 hours, which was preceded in some animals by several hours of unconsciousness. A more complete characterisation of the onset, duration and nature of the effects of alphachloralose on possums was considered necessary to determine whether these were a suitable match to what was known about the progression of 1080 poisoning in possums.

3. Objectives

- Characterise the effect of sublethal oral doses of the drug alphachloralose on brushtail possums and evaluate whether these effects are of suitable nature, degree, time to onset and duration to coincide with the progression of 1080 poisoning in possums
- Compare the effects of an oral dose of alphachloralose co-administered to possums with an effective lethal dose of 1080 on responses to stimuli and times to unconsciousness and death, to possums administered 1080 alone, and evaluate whether this represents an overall improvement in the welfare of poisoned possums.

4. Methods

Acceptance of alphachloralose in food and effect on possums

Twenty wild-caught possums (equal sex ratio) were housed indoors in individual wire cages (350 x 200 x 200 cm) with removable nest boxes (30 x 20 x 20). They were acclimatised for 14 days before the trial to receiving c. 20 g of non-toxic cereal pellets (RS5 base without cinnamon, Animal Control Products) with their normal diet each morning – these pellets were used in the trial to present alphachloralose (ACL) to the possums. Four days before the trial the possums were moved to cages in a room at 12°C ambient temperature. Possums were randomly allocated to two treatment groups with equal sex ratios (each n=10) to be offered either pellet food containing ACL, or untreated pellet food.

The day before the trial, possums were lightly anaesthetised using isoflurane (SOP 5.7), weighed, and a small patch of fur was shaved from the forehead of each to facilitate temperature readings during the trial. Individual rations of pellets were prepared by the Landcare Research toxicology laboratory, according to the bodyweight of each possum allocated to the alphachloralose treatment, to deliver 100 mg/kg of alphachloralose in approximately 20 g of pellets (17.5 mg ACL/g pellet). Control possums received approximately 6 g of untreated pellets per kg of bodyweight. On the morning of the trial nest boxes were removed from the cages, enabling the possums to be easily observed with little disturbance. Possums were offered their weighed allocation of treatment food (without normal rations) at the usual time of morning feeding. We recorded the time it took each possum to begin feeding, the duration of feeding, and the time when pellets were all eaten. Any uneaten food was removed after 6 hours, dried at 37°C overnight and weighed to determine the amount consumed. Behavioural observations began immediately the treatment feeds were offered: each possum was observed by instantaneous scan sampling every 15 minutes, with observations of posture and behaviour recorded. In addition, at each 15-minute scan-observation, the degree of responsiveness to stimuli was estimated from the appearance of each possum and its responses to stimuli, using the

scale:

(0) No effect – normal responses and alertness
(1) Slight effect – slightly ataxic with some in-coordination obvious in movement
(2) Moderate effect – moderately ataxic, severe in-coordination, can stand or sit upright but reluctant to do so
(3) Down but responds – sternally recumbent, unable to stand but easily aroused
(4) Little response – sternally or laterally recumbent, little response to stimulus
(5) Light anaesthesia – laterally recumbent and unable to assume sternal recumbency, or move back to nest box, responds only to painful stimuli
(6) Anaesthetised – no response to painful stimuli

The Observer® Version 4.1 (Noldus Information Technology 2002) software was used to record the above 'behavioural states', posture and/or activity at the time of scan observation, and also illness 'events', e.g., retching, convulsion where they were observed outside of a 'scan'. Scan sampling was conducted until affected possums had recovered to a normal state, returned to a consistent 'slightly affected' behaviour after displaying higher states previously, or had died. Alternative stop points for scan observations were if possums (i) remained unconscious for 6 hours, or (ii) were in evident pain or respiratory distress. Temperature measurements were taken every 30 minutes using a 'surface' laser-reading thermometer (InfraRed Thermometer, Digitech QM-7223) centred on the shaved head patch of each possum. Dose ingested and duration data were analysed using the linear model and t-test procedure in the statistical package 'R' (Version 2.6.1) and the results used to indicate whether it was appropriate to proceed to a second trial of ACL+1080 vs a 1080-alone treatment.

Effects of alphachloralose on possums administered a lethal dose of 1080

To evaluate whether exposure to ACL could mitigate painful or stressful effects of 1080 poisoning in possums, we administered an effective lethal dose of 1080 (5 mg/kg) to possums by gavage, with and without a co-administered dose of ACL. Gavage administration was chosen over voluntary ingestion, as the latter approach was anticipated to produce high variability in the amounts ingested and the timing of the ingestion, precluding a strict comparison between the two treatment groups over time. Twenty wild-caught possums were acclimatised to a 12–14°C room as in the previous trial and randomly allocated to three treatment groups with equal sex ratios;
1) '1080 alone' – an effective lethal dose (5 mg/kg) 1080 by oral gavage (n=8)
2) 'ACL + 1080' – 5 mg/kg 1080 and 60 mg/kg ACL in two gavage doses (n=8)
3) 'Control' – 5 mL of the carrier solution used in the gavage treatments (n=4)

Allocation to treatments was 'blind', so that personnel recording scan observations were unaware of the treatment each possum had received. Possums were placed under light fluothane anaesthesia, weighed and given their allocated treatment by gavage (maximum total volume 8 mL/kg) administration. Immediately after dosing they were replaced in their individual cages and recording of 'scan' behavioural observations began immediately. This continued until possums had recovered to a normal state, returned to a consistent 'slightly affected' behaviour after displaying higher states previously, or died. Alternative stop points for scan observations were: (i) if possums displayed no effect of the treatment for 3 consecutive hours; (ii) possums remained unconscious for 6 hours; or (iii) possums were in evident pain or respiratory distress. Duration and latency data were analysed using the linear model and t-test procedure in the statistical package 'R' (Version 2.6.1).

5. Results

Acceptance of alphachloralose in food and effect on possums

By the end of the 14-day pre-feeding period, 10 of the 20 possums were eating all the pellet food offered in the morning, 6 were eating 50–75%, and the remaining 4 were not accepting the offered food. Possums offered untreated food ate significantly more (mean ± sem 14.84±3.02 g) than those offered alphachloralose-treated food (6.55±1.321 g) (P=0.027). Six of the 10 control possums ate more than 95% of their allocation, while none of the alphachloralose possums ate more than 67% of the treatment offered. The doses of alphachloralose ingested by possums ranged from 0.69 to 67.63 mg/kg (Fig. 2). There were no significant differences in the means and changes in temperature of the control and ACL possums throughout the trial (Fig. 1), although in the last 4 hours of observation the mean temperature of possums in the ACL was consistently lower than the mean temperature of control possums.

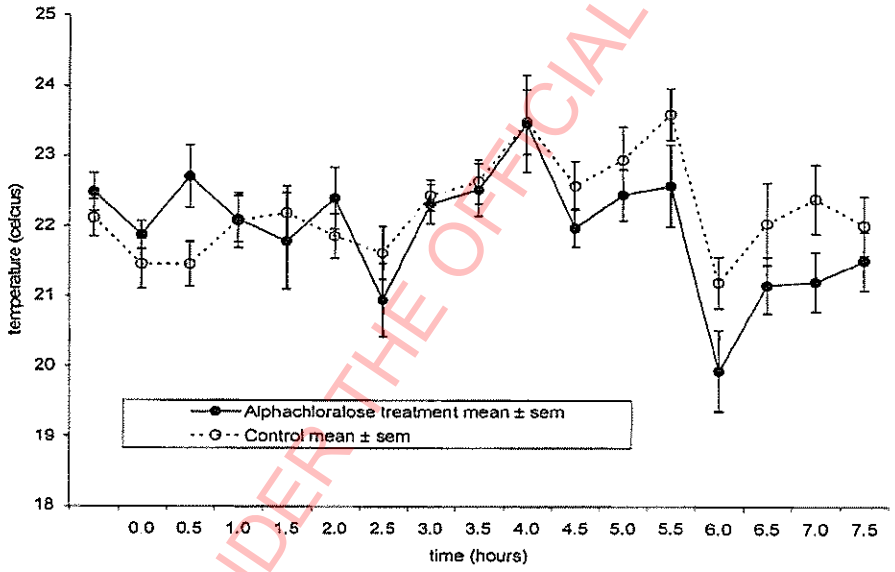

Fig 1. Mean temperatures measured using an infra-red thermometer, from a shaved spot on possums' foreheads after being offered pellets containing alphachloralose or untreated pellets (control group).

All possums that ingested alphachloralose were affected but not all reached the 'higher' states of anaesthesia /reduced response (Table 1). Observations continued for 8.5 hours after dosing, at which point all affected possums were deemed to have recovered to not affected or slightly affected states. On average, states 1–3 were first seen at about 2.5 hours, and states 4–6 (in those possums in which they occurred) seemed to represent a progression, having a similar latency of approximately 3.5 hours. Regression analysis of the effect of the ACL dose ingested on the latency, i.e. the time at which each state was first observed, of the sedation states in order of degree showed:

- a significant negative effect on the latency of 'slight effect' (slope = -0.03675, SE= 0.01434, $t_8 = 2.564$, $p = 0.033$),
- weak evidence of a negative effect on the latency to 'moderate effect' (slope = -0.03064, SE=0.01444, $t_5 = 2.122$, $p = 0.0873$),
- weak evidence of negative effect on latency of 'down but respond (slope = -0.05317, SE=0.02161, $t_3 = 2.460$, $p = 0.0908$),
- a significant negative effect on latency to 'little response' (slope = -0.08448, SE=0.01696, $t_2 = 4.980$, $p = 0.038$).

Regression analysis of the effect of the ACL dose ingested on the mean duration spent by a possum in each state of sedation showed a significant negative relationship between mean duration of 'no effect' and the ACL dose (slope=-0.04429, SE=0.01646, $t_8 = 2.69$, p=0.027) and a highly significant positive relationship between mean duration of 'little response"' and the ACL dose (slope=0.01356, SE=0.0007127, $t_2 = 19.02$, p=0.0028). There was no significant effect of the ACL dose and the mean durations of 'slight effect', 'moderate effect' or 'down but respond'. The two possums that reached the states of 'light anaesthesia' and 'no response' (states 5 and 6 respectively) had ingested the highest doses of ACL (Fig. 2) and hypersensitivity to noise or touch was noted on some scans when these two possums were in these states. Regressions could not be carried out regarding the effect of the ACL dose on latency or duration data for these states, as there were only two data points. Figure 2 shows a 'real time' plot of the most evident effects of alphachloralose on responses to stimuli, which generally occurred between 2.5 and 8 hours, after which recovery progressed towards 'unaffected' state.

Table 1. Mean latencies and durations of the states of anaesthesia observed in possums in the ACL (alphachloralose) and control treatments.

State	Treatment	Number of possums displaying state	Mean latency to state (hours)	Mean duration ± sem (hours)
No effect (0)	Alphachloralose	10/10	-	7.45 ± 1.12
	Control	10/10	-	10.96 ± 0.58
Slight effect (1)	Alphachloralose	10/10	2.53 ± 0.37	1.01 ± 0.26
	Control	0/10	-	-
Moderate effect (2)	Alphachloralose	7/10	2.50 ± 0.36	1.39 ± 0.44
	Control	0/10	-	-
Down but responsive (3)	Alphachloralose	5/10	2.72 ± 0.36	1.70 ± 0.16
	Control	1/10*	1.8*	-
Little response (4)	Alphachloralose	4/10	3.36 ± 0.59	1.47 ± 0.69
	Control	0/10	-	-
Light anaesthesia (5)	Alphachloralose	2/10	3.34 ± 0.51	2.96 ± 0.29
	Control	0/10	-	-
No response (6)	Alphachloralose	2/10	3.49 ± 0.17	0.35 ± 0.16
	Control	0/10	-	-

*observer error on one scan observation, possum was slow to respond but then judged as "No effect"

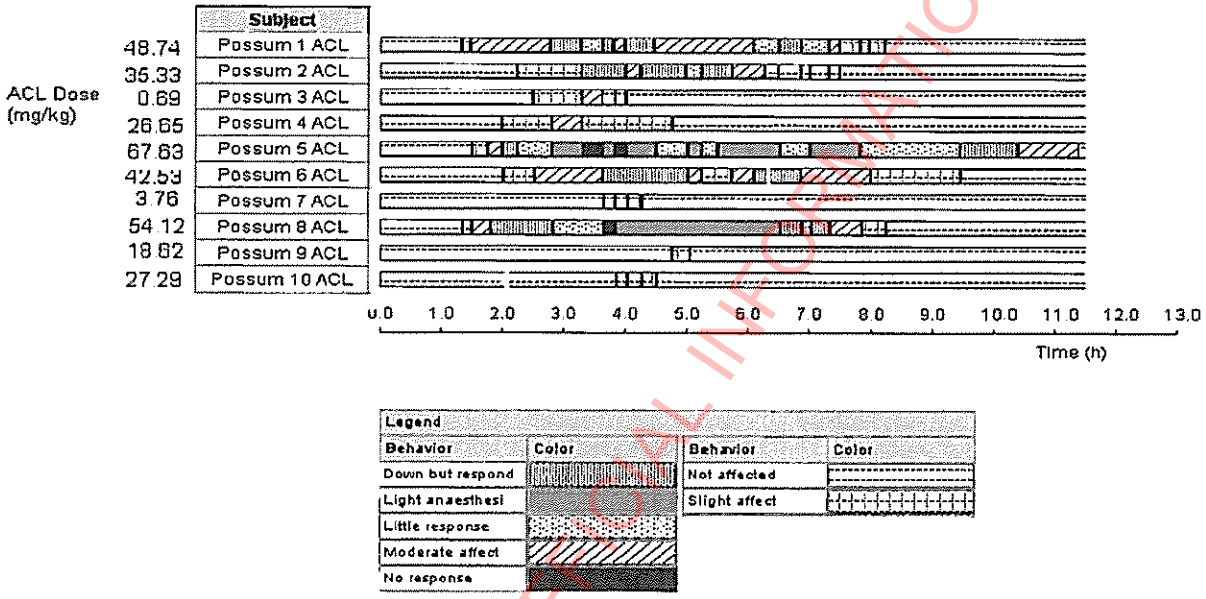

Figure 2. Time plot of sedation states of ACL possums throughout the observation period, with ACL doses ingested (mg/kg) by each possum shown on the left.

Effects of alphachloralose on possums administered a lethal dose of 1080

All possums began the observation in a state of light anaesthesia (score 5) as an unavoidable outcome of the gavage-dosing procedure (Fig. 3). Estimates of times to death and mean duration of light anaesthesia were adjusted accordingly, as it took nearly an hour to complete gavage dosing of all 20 possums. Figure 3 shows the different 'start' times for possums 1–20 as they were dosed and brought into the observation room in numbered order. All control possums survived, and by their contrasting behaviours and states compared with the other possums dosed with 1080, were readily distinguishable by the 4th hour of observation. All possums in the ACL+1080 treatment died (n=6), or were euthanased at the 13.5 hour endpoint (n=2) with a mean time to death of 9 h 43 min. All possums in the 1080 alone treatment died (n=7), with one possum euthanased at the 13.5 hour endpoint with a mean time to death of 8 h 50 min. There was no significant difference in the mean time to death between the two treatments.

Table 2. Mean (± sem) total durations and % of total observation time of different states of anaesthesia observed in possums in the ACL+1080, 1080 alone and control treatments.

State	Treatment	Number of possums displaying state	Mean total duration ± sem (hours)	Mean duration as % of total observation
No effect	ACL+1080	8/8	0.93 ± 1.83	6.87 ± 13.51
(0)	1080 alone	8/8	1.12 ± 2.17	8.24 ± 15.96
	Control	4/4	10.99 ± 3.88	80.78 ± 28.55
Slight effect	ACL+1080	7/8	0.89 ± 1.77	6.59 ± 13.06
(1)	1080 alone	7/8	0.54 ± 0.79	4.00 ± 5.83
	Control	4/4	1.64 ± 3.17	12.05 ± 23.36
Moderate effect	ACL+1080	8/8	0.47 ± 0.77	3.46 ± 5.68
(2)	1080 alone	6/8	1.15 ± 0.62	8.45 ± 4.55
	Control	3/4	0.31 ± 0.36	2.29 ± 2.69
Down but responsive	ACL+1080	8/8	0.66 ± 1.56	4.84 ± 11.50
(3)	1080 alone	8/8	1.02 ± 1.45	7.52 ± 10.67
	Control	4/4	0.26 ± 0.50	1.90 ± 3.70
Little response	ACL+1080	8/8	1.11 ± 1.51	8.17 ± 11.09
(4)	1080 alone	8/8	1.68 ± 4.74	12.35 ± 34.85
	Control	2/4	0.18 ± 0.26	1.36± 1.92
Light anaesthesia	ACL+1080	8/8	1.73 ± 2.27	12.71 ± 16.70
(5)	1080 alone	8/8	1.63 ± 2.28	11.97 ± 16.29
	Control	0/4[1]	-	-
No response	ACL+1080	8/8	4.71 ± 2.66	34.64 ± 48.03
(6)	1080 alone	8/8	2.66 ± 5.43	19.59 ± 39.94
	Control	0/4	-	-

[1]excluding initial anaesthesia for gavage dosing

ANOVA of the state durations expressed as a percentage of the total observation time (Table 2) showed that control possums had a significantly greater duration of the 'not affected' state than either of the other treatments ($F_{2,17}$ = 146.8, p<0.0001), a significantly smaller duration of the 'moderate effect' state ($F_{2,14}$ = 15.62, p=0.0003.), and a significantly smaller duration of the 'light anaesthesia' state ($F_{2,17}$=15.62, p=0.019.) with the latter state in the control possums attributable to recovery from anaesthesia for gavage dosing.. No control possums reached the state of 'no response'.After recovery from anaesthesia for gavage, the control possums spent far greater durations in States 0 (not affected) and 1 (slight effect) than possums in the ACL+1080 and 1080 alone treatments (Fig 3).

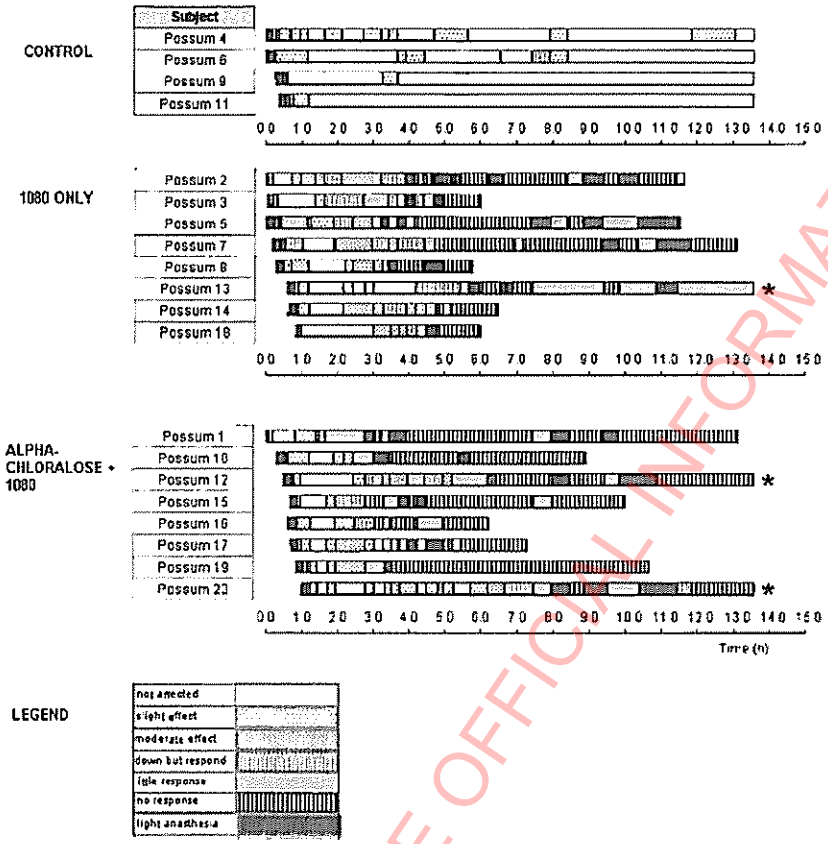

Figure 3. Timeline plot of progression and changes in states of sedation in the three treatments. *indicates possum euthanased at 13.5 hour endpoint.

In two-sample t-tests of the durations of states between the 1080 alone and ACL+1080 treatments, 'moderate effect' was the only variable with a significantly different duration – 1.15 hours in the 1080 alone group and 0.47 hours in the ACL + 1080 group, (t_{12} = 4.73, p = 0.0005). From a count of 'illness events', 4/8 possums in the 1080 alone treatment showed at least one bout of retching, while no possums in the ACL+1080 treatment were observed retching. Some possums in each treatment had at least one bout of convulsions (5/8 in ACL+1080; 4/8 in 1080 alone) and at least one observation of shivering/tremors (6/8 in ACL+1080, 4/8 in 1080 alone). The addition of ACL to an effective lethal dose of 1080 appeared only to affect the mean duration spent in State 2 (moderate effect) and possibly to reduce the occurrence of retching in poisoned possums. There were no observations of hypersensitivity in responses to noise or touch in any of the possums.

6. Conclusions

The results of the first trial indicated that effects of ACL on possums were dose-dependent and that an oral intake at least 55 mg/kg alphachloralose would be required to induce 'high' states of reduced consciousness that could mitigate painful or stressful effects of 1080 poisoning. An earlier assessment of 1080 poisoning in possums (O'Connor et al. 2003) identified potential welfare compromise for 9.5 hours following poisoning – poisoned possums generally showed 'uncomfortable' postures in the first 5 h (crouched, sternally recumbent) and in over 30% of observations progressed to lying from 6 h onwards, generally becoming prostrate again in the later stages before death, which occurred on average 10.5 h after dosing. The onset of the effects of the highest ACL intakes (states 1–3 were first seen at about 2.5 hours, and states 4–6 at about 3.5 hours) generally matched the onset of changes on behaviour observed during 1080 poisoning, although the effects of poisoning on possums appeared of greater duration than the effects of ACL. It was evident from the first trial that ACL was relatively unpalatable to possums, confirming an earlier finding that possums could detect effective lethal concentrations of ACL in food (Eason et al. 1993). Issues of palatability and bait acceptance would become a practical problem for bait delivery if a positive effect of ACL on the welfare of poisoned possums could be demonstrated.

On that basis, we proceeded with the second trial, where possums were administered an effective lethal dose of 1080 by gavage, with or without 60 mg/kg ACL, in order to compare the onset and duration of signs of poisoning in each treatment. Temperature readings were not conducted in this second trial because ACL in the first trial did not appear to affect temperature significantly and because taking the readings substantially increased the time it took to 'scan' 20 possums. The addition of ACL to an effective lethal dose of 1080 in possums did not produce sufficient changes in parameters that might represent an overall improvement in welfare. In particular, time to death and onset and duration of states of reduced response and consciousness were not affected by ACL.

7. Recommendations

The addition of ACL to an effective lethal dose of 1080 appeared only to affect the mean duration spent in State 2 (moderate effect) and possibly to reduce the occurrence of retching in poisoned possums. These differences were not considered sufficient to represent an overall improvement of welfare to justify further development of ACL as a welfare-improving agent for 1080 baits.

8. Acknowledgements

All work was conducted with approval of the Landcare Research Animal Ethics Committee (Project No. 06/12/01). An amendment was also approved by the AEC to use gavage delivery of treatments in the second trial, rather than voluntary bait uptake by possums. Thanks to Ryan Moffat and Karen Washbourne for maintenance of the possums during this trial, to Bruce Warburton and Phil Cowan for review of drafts of this report, Guy Forrester for statistical analyses, Anne Austin for editing, and Wendy Weller for word processing.

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