Improved Humaneness of Vertebrate Toxic Agents

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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

4.1. 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.

4.2. 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)

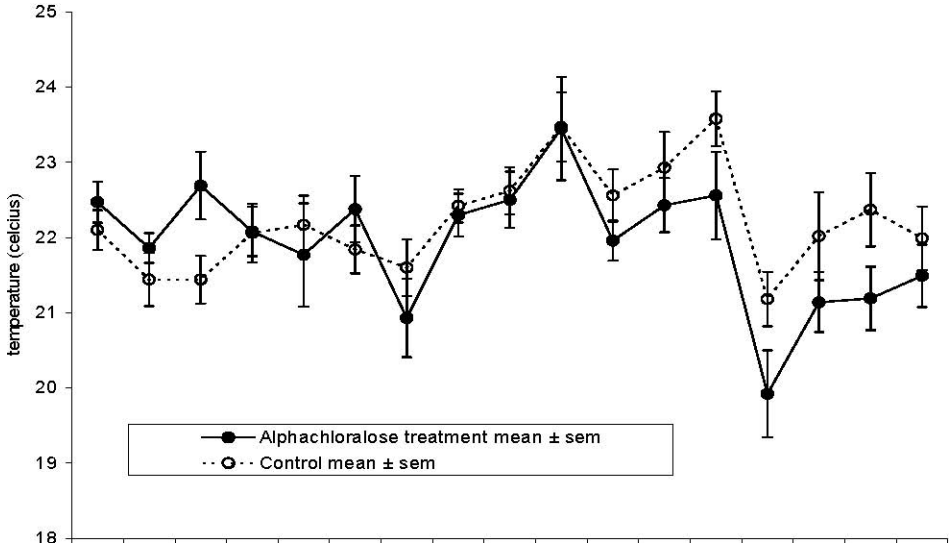
Allocation to treatments was "blind", so that personnel recording scan observations were unaware of the treatment each possum had received. Possums were placed under light fluothane anaesthesia, weighed and given their allocated treatment by gavage (maximum total volume 8 mL/kg) administration. Immediately 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

5.1. 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 percent, 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 percent of their allocation, while none of the alphachloralose possums ate more than 67 percent 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.

Figure 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, t8 = 2.564, p = 0.033),
- weak evidence of a negative effect on the latency to "moderate effect" (slope = -0.03064, SE=0.01444, t5 = 2.122, p = 0.0873),

- weak evidence of negative effect on latency of down but respond (slope = -0.05317, SE=0.02161, t3 = 2.460, p = 0.0908),
- a significant negative effect on latency to "little response" (slope = -0.08448, SE=0.01696, t2 = 4.980, p = 0.038).

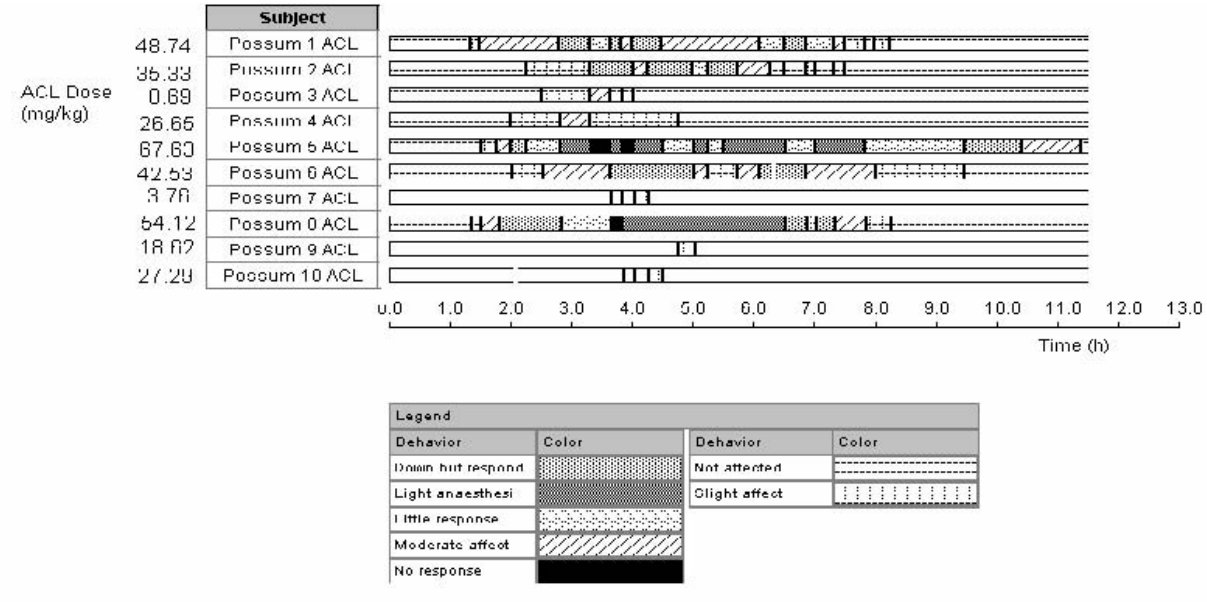
Regression analysis of the effect of the ACL dose ingested on the mean duration spent by a possum in each state of sedation showed a significant negative relationship between mean duration of "no effect" and the ACL dose (slope=-0.04429, SE=0.01646, t8 =2.69, p=0.027) and a highly significant positive relationship between mean duration of "little response" and the ACL dose (slope=0.01356, SE=0.0007127, t2 =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.



5.2. 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.

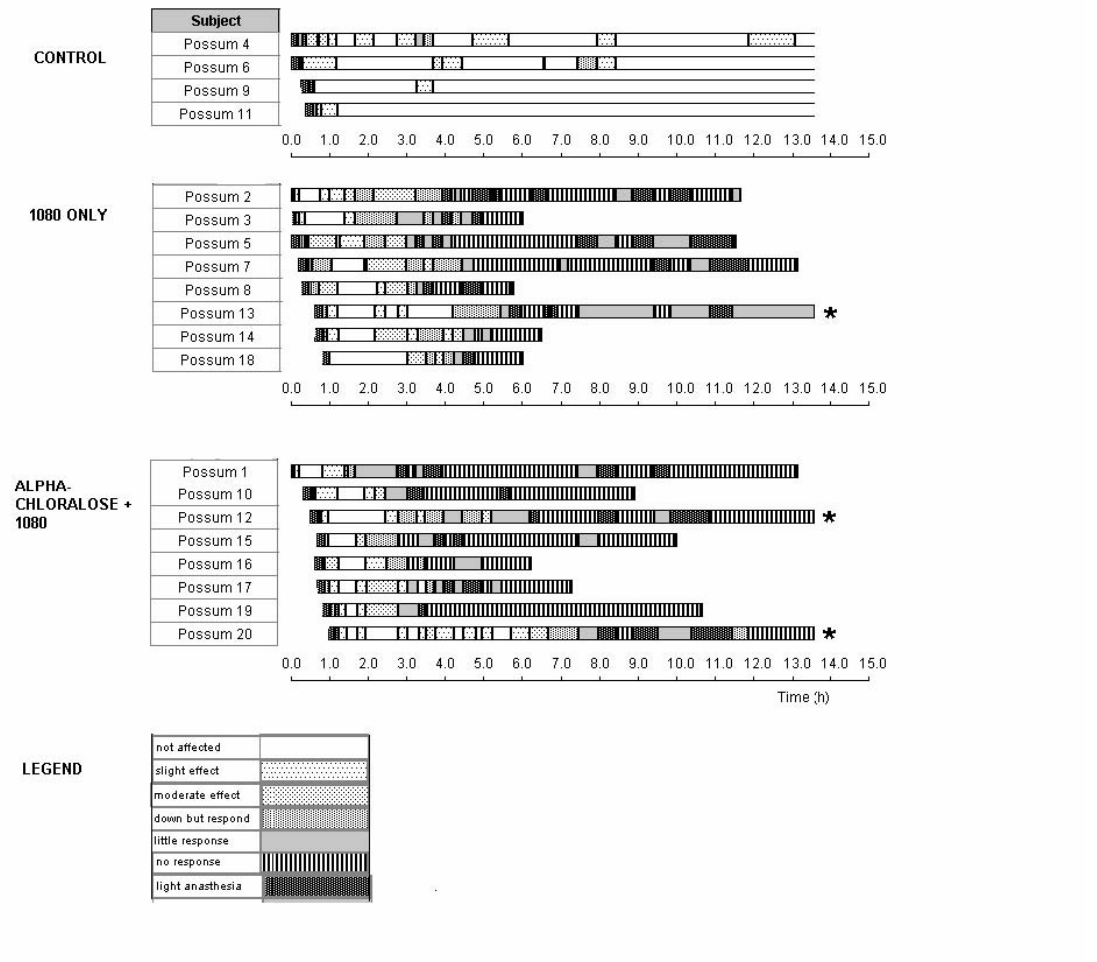
Table 2: Mean (± sem) total durations and percent of total observation time of different states of anaesthesia observed in possums in the ACL+1080, 1080 alone and control treatments.

State	Treatment	Number of possums displaying state	Mean total duration ± sem (hours)	Mean duration as percent 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 percent 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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