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Toxicity effects of 1080 on pregnant ewes

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ABSTRACT

There are no data on the potential for 1080 used for pest control to cause delayed deaths or impaired productivity in livestock following multiple, sub-lethal doses. Recent losses of late-gestation ewes exposed to weathered 1080 baits has also led to speculation that pregnant ewes may be unusually sensitive to the toxin. To address these data gaps, groups of 20 Perendale ewes, non-pregnant or pregnant with twins, were administered either a single (0.25 mg/kg) or multiple oral doses (0.05 mg/kg over 3 consecutive days) of a 1080 cereal pellet. The highest mortality occurred in the single dose groups (pregnant 45%, non-pregnant 21%) compared to the multiple dose groups (pregnant 35%, non-pregnant 0%). There was no mortality in the control group of pregnant ewes. Log-linear modelling showed highly significant treatment effects (P = 0.0003) and differences (P = 0.045) in acute mortality rates between pregnant (40%) and non-pregnant ewes (10%), which was linked to increased bioavailablity. There were no differences in the incidence of metabolic diseases, lambing percentages, lamb survival, or growth rates between dosed and undosed pregnant ewes. This study demonstrated that extra care should be taken to avoid exposure of pregnant ewes to even small bait fragments, but also provides further evidence that there are no long-term health effects in animals that survive accidental 1080 poisoning.

Keywords: Sodium monofluoroacetate; 1080; sheep; toxicity; toxicokinetics.

INTRODUCTION

The continued use of 1080 is an important component of possum control strategies to protect conservation values and prevent the spread of bovine tuberculosis in New Zealand (Hughes, 1994). However, there are growing concerns over the impacts of our pest control methodologies on non-target species. Misconceptions about 1080 toxicology could threaten future public acceptance of this compound as a tool for pest control. Inadvertent poisoning of non-target species, including livestock, is the most common concern. Rammel and Fleming (1978) reported annual losses of >1000 sheep from 1080 poisoning during the 1960s and '70s, and substantial losses continue to be reported today as 1080 use has increased.

Sheep exposed to a single, sub-lethal dose of 1080 will metabolise and excrete the toxin relatively rapidly, with a plasma elimination half-life of 10.8 hours (Eason *et al.*, 1994). Highest concentrations of 1080 residues occur in the blood immediately after dosing, but by 96 hours, only trace amounts of the toxin can be detected in any tissue (Eason *et al.*, 1994). A recent study demonstrated that ewes that survived a single exposure to 1080 did not experience any adverse long-term effects on health or productivity, as measured by reproductive performance, fleece weight, body weight, and other indices, compared with a matched group of undosed controls, over two lambing cycles (Eason *et al.*, 1999). Pathological abnormalities that may have been induced by 1080 exposure were observed in the heart and brain of some ewes sacrificed at the termination of the study. The clinical significance of these pathological changes was considered to be neglible in light of the lack of adverse clinical effects (Eason *et al.*, 1999).

Although these data have proven valuable in assessing risk to livestock exposed to a single dose of 1080, there are no data on the potential for the toxin to cause delayed deaths or impaired productivity in livestock following multiple, sub-lethal doses. The effects of the toxicant may be cumulative in sheep, with repeated focal damage to heart muscle that may eventually compromise cardiac function (Shultz *et al.*, 1982). Therefore, the results of a single-dose study can not necessarily be extrapolated to cover multiple exposures. Hence the first objective of this study was to improve the understanding of the effects of multiple, sub-lethal doses of 1080 on livestock health and productivity.

In addition, recent losses of late-gestation ewes exposed to weathered bait containing very low concentrations of 1080 has led to speculation that near-term pregnant ewes may be unusually sensitive to the toxin; perhaps due to their vulnerability to disturbances of carbohydrate metabolism (resulting in pregnancy toxaemia or sleepy sickness) or calcium homeostasis (resulting in milk fever). Thus, the second objective of this study was to address data gaps in our understanding of the potential effects of 1080 on late-gestation pregnant ewes and their lambs from parturition to prime lamb.

MATERIALS AND METHODS

A group of 100 unbred Perendale ewes was maintained at pasture until synchronised using vasectomised rams and breeding in mid-April 1998. The ewes were scanned twice by ultrasonography, 45 and 60 days later. Ewes carrying single lambs at the first scan were treated with 1 ml (250 µg/ml) estrumate (a prostaglandin) to induce lysis of the corpus luteum and thus terminate the pregnancy, in order to produce a matched group of non-pregnant (empty) ewes. Ewes were scanned the second time to confirm their

pregnancy status and estimate dates of parturition. The pregnant ewes were allocated into three treatment groups and the empty ewes into two groups (Table 1), balanced for body weight.

TABLE 1: The reproductive state and number of ewes in each treatment group.

Group Number	Treatment	Reproductive state	Number of ewes
1	Undosed Controls	Pregnant	20
2	Single dose	Pregnant	20
3	Single dose	Empty	19
4	Multiple dose (3)	Pregnant	20
5	Multiple dose (3)	Empty	19

The ewes were dosed with 1080 in early September, 2 weeks prior to the mean expected lambing date. Group 1 was a group of pregnant control ewes, Group 2 pregnant and Group 3 empty ewes received a single, near-lethal dose of 1080 of 0.25 mg/kg. Group 4 pregnant and Group 5 empty ewes were originally intended to receive the same total dose of 0.25 mg/kg in divided doses over 5 consecutive days (i.e. 0.05 mg/kg/day). However, due to mortalities in one of the multiple dose groups early on day 4, dosing was stopped after 3 days. Therefore, Groups 4 and 5 received a total dose of 0.15 mg/kg. Dosing was carried out by force feeding a measured amount of standard 1080 cereal bait (assayed as 0.139% 1080 w/w). Animals were monitored for clinical signs of toxicosis following dosing. Treatment groups were compared using log-linear modelling to assess the effect of 1080 exposure (no 1080 cf. single dose cf. multiple dose) on pregnant and non-pregnant ewes. Death was used as the response variable in this analysis.

Serial blood samples were collected by jugular venipuncture from 10 ewes in each group. Five control animals and 10 ewes in each of the single-dose groups (Groups 2 and 3) were bled immediately before dosing, and daily for 4 days. Ten animals in each of the multiple-dose groups (Groups 4 and 5) and the remaining five control animals were bled immediately before the initial dose, prior to the third (final) dose, and then daily for four days to determine if there was any cumulative effect of the multiple-dose regime. Serum was separated and samples held at –20°C. A subset of three samples from each group was analysed to determine serum concentrations of 1080 using gas chromatography procedures (Eason *et al.*, 1994), and by automated analysis (Hitachi 717 analyser) for serum calcium, urea nitrogen, creatinine, ketone bodies, and glucose concentrations, as indicators of the direct effects of the toxin, or metabolic diseases of late gestation (sleepy sickness or milk fever), which may be associated with toxin exposure. The serum concentration data over time were analysed by repeated measures analysis of variance. Standard toxicokinetic data were derived for the single dose groups for maximum observed serum 1080 concentration (C_{max}, µg/ml) and area under the curve (AUC) by the trapezoid rule (µg/ml/day), using Kinetica (Innaphase).

Three ewes and their foetuses from each group, which had either died or were euthanased at 14 days after dosing were subjected to complete post-mortem examination. Samples of heart, lung, kidney, and liver tissue were collected from both the ewes and foetuses, and fixed in 10% formalin. Tissue sections were cut at 5 microns, stained with haematoxylin and eosin, and examined by light microscopy. Histopathological examination of these tissues was conducted to identify potential toxin-induced lesions that may affect long-term fitness.

The ewes were lambed through September, and lambs weighed and tagged at birth. Ewes and lambs were subsequently maintained at pasture in one mob until weaning, and were all weighed monthly. Ewe and lamb weights over time were also analysed by repeated measures analysis of variance. Fishers exact test was used to determine any 1080 exposure effects on lambing percentage and lamb survival data.

RESULTS

No mortality was observed in the control group of pregnant ewes. The highest mortality rate occurred in the single dose groups (pregnant n = 9, 45 %, non-pregnant n = 4, 21%) compared to the multiple dose groups (pregnant n = 7, 35%, non-pregnant 0%). The only model that fitted the acute mortality data well was that which showed the dependence of death on treatment and pregnancy status (χ^2_1 = 3.66, P = 0.056). There was also some evidence that death rate was dependent on pregnancy status (χ^2_1 = 4.01, P = 0.045), with higher death rates occurring in the pregnant ewes (40%) compared to the parallel non-pregnant groups (10%). Interestingly, non-pregnant ewes appeared to suffer lower mortality when given multiple (though smaller) doses of 1080 than when single dosed, while pregnant ewes reacted the same way to both treatments. The final statistical model, however, showed only marginal evidence for this 3-way interaction effect.

There were no delayed deaths in any 1080-treated ewes, nor any signs of pregnancy-associated metabolic disease (sleepy sickness or milk fever) in treated or control ewes. There were also no differences in lambing percentages (P = 0.92), lamb survival (P = 1), or lamb growth rates ($F_{8,188}$ = 0.42, P = 0.81) between 1080-treated and control groups (Table 2).

TABLE 2: The lambing percentage (percentage ewes survived/lambs born), lamb survival (percentage lambs weaned/lambs born), and mean ± S.E.M. lamb live weights (kg) from birth until market (using average age at weighing) for each treatment group.

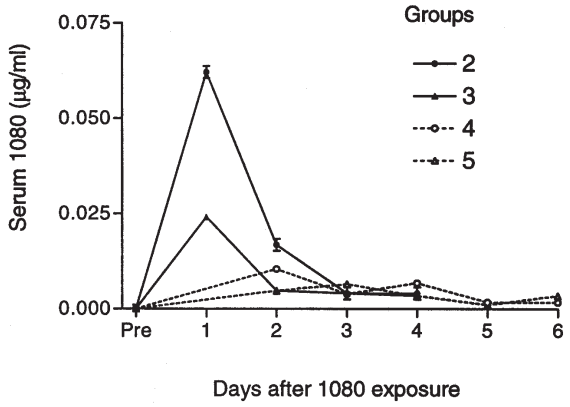
Group	Lambing percentage	Lamb Survival	Live weights (kg) Birth	1 mo.	2 mo.	3 mo.	4 mo.
Control	182%	84%	4.9 ± 0.8	13.8 ± 0.8	25.3 ± 0.8	31.0 ± 0.8	36.6 ± 0.8
Single dose	173%	84%	5.0 ± 1.0	13.1 ± 1.0	24.2 ± 1.2	29.9 ± 1.0	36.0 ± 1.0
Multiple dose	175%	90%	5.2 ± 0.9	13.9 ± 0.9	26.0 ± 0.9	31.3 ± 0.9	36.4 ± 0.9

Pulmonary oedema was found in all of the ewes that died from 1080 poisoning (Groups 2 , 3 and 4). Half the ewes showed moderate diffuse and the others mild multifocal oedema. Two-thirds of the pregnant ewes also showed mild multifocal myocardial necrosis. Such cardiac lesions were also found in one of the non-pregnant ewes that died. There were also some indications of terminal sep-

ticaemia in a third of the ewes that died. In contrast, no significant lesions were observed in any of the ewes which were euthanased (control or Group 5) or any foetal tissue examined.

Serum concentrations of 1080 in the groups receiving the single dose of toxin showed a significant group by day interaction effect ($F_{8, 20}$ = 70.9, P<0.001), with a clear peak on day 1 after dosing, being higher in the pregnant ewes, and beginning to fall on day 2 (Figure 1). By days 3 and 4, serum 1080 concentrations had fallen to levels not statistically different from day 0. The plasma toxicokinetics in the single dose groups showed higher bioavailablity for pregnant ewes (C_{max} = 0.062; AUC = 0.093) compared to non-pregnant ewes (C_{max} = 0.024; AUC = 0.053). In the groups receiving multiple lower doses of toxin, there were differences between pregnant and non-pregnant ewes ($F_{2,6}$ = 6.917, P = 0.028) and this was consistent over time. There was only a slight increase in serum 1080 concentrations on day 2 after the initial dose ($F_{10,30}$ = 2.32, P = 0.059). Although we cannot determine if they would also have peaked on day 1, a much lower C_{max} would be expected because of the low dose given. Further these results do indicate little cumulative effect of the repeated daily dose.

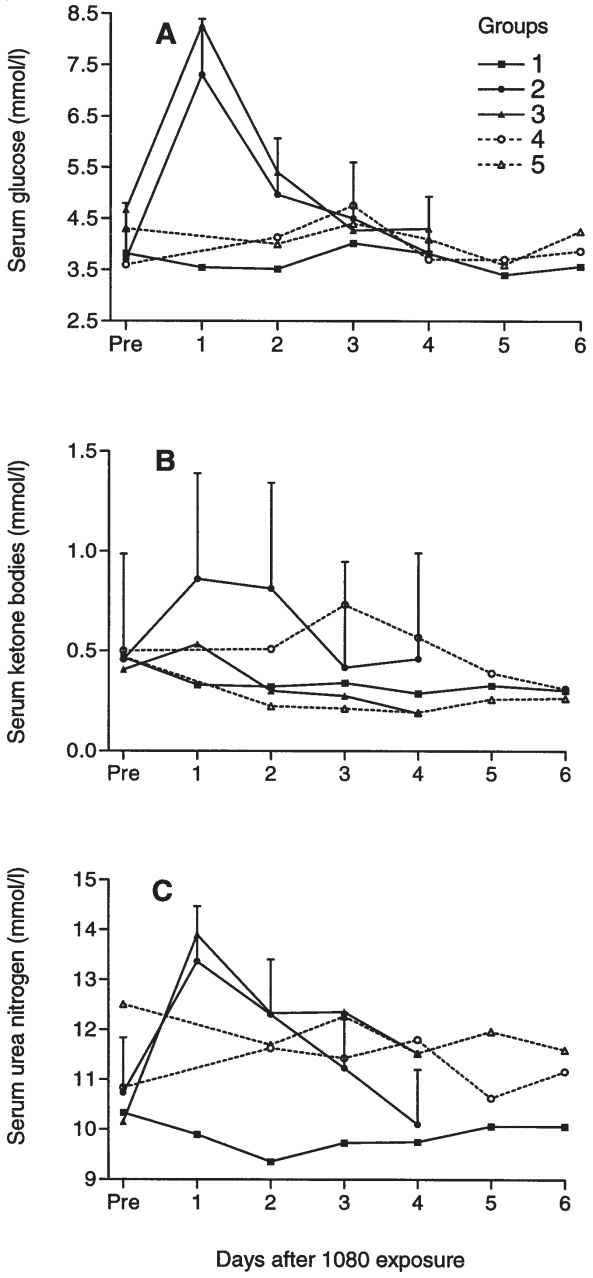
FIGURE 1: Mean serum 1080 concentrations for ewes from the four 1080-dosed treatment groups over time. Representative standard errors are shown for Group 2. (Note: any concentrations less than 0.005 µg/ml are near the limit of sensitivity for the assay).



Blood glucose concentrations peaked on day 1 after dosing in both single dose groups. Values observed on day 1 exceeded laboratory reference values for normal blood glucose concentrations in sheep, and were significantly higher ($F_{20,24}$ = 5.53, P<0.001) than those observed in the multiple dose and control groups (Figure 2). Concentrations of serum ketone bodies in both groups of pregnant ewes receiving 1080 increased compared to parallel groups of non-pregnant ewes, though not significantly. The concentrations were high in the pregnant ewes relative to normal sheep reference values for 2 days after final dosing (i.e. days 1 and 2 for the single dose group and 3 and 4 for the multiple dose group)(Figure 2). Serum urea nitrogen concentrations appeared elevated in all treatment groups compared to controls. In groups receiving the single high dose of 1080, there was a significant change in serum concentration over time ($F_{4,12}$=5.226, P = 0.019), and the concentration dropped significantly after day one ($F_{3,3}$=20.221, P = 0.017). Serum urea nitrogen concentrations exceeded normal reference values for sheep for several days after dosing in both single dose and multiple dose groups (Figure 2). Serum calcium and creatinine concentrations varied over time, but showed no treatment-related pattern.

No treatment-related effects on ewe body weight (e.g. pregnant ewes; $F_{10, 135}$ = 0.42, P = 0.79) were observed at any time after dosing. As expected, non-pregnant ewes (Groups 3 and 5) maintained body weight throughout the spring compared to lactating ewes.

FIGURE 2: Mean serum glucose (A), ketone bodies (B), and urea nitrogen (C) concentrations for ewes from the five treatment groups following 1080 dosing. Representative positive standard errors are shown for Group 2.



DISCUSSION

The acute mortality rate in the non-pregnant ewes receiving the single high dose of 1080 (0.25 mg/kg) was

approximately 20%. This dose equates to 10 g of a standard (0.15% 1080) possum pellet. Although some previous studies have suggested an LD_{50} of 0.25–0.3 mg/kg in sheep (Annison *et al.*, 1960; Eason *et al.*, 1999), toxicity is known to be influenced by route of administration (Jarrett and Packham, 1956), and whether or not sheep were fasted prior to dosing (Eason *et al.*, 1999). In comparison, the LD_{50} for possums is considerably higher at 1.2 mg/kg. Results of the present study are comparable to a recent trial in New Zealand, in which overall mortality in ewes exposed to 1080 at 0.25 mg/kg was 30% (Eason *et al.*, 1999). Although approximately 30% of 1080 is excreted in urine unchanged (Eason *et al.,* 1994) we found no indication of contamination to control ewes that were sharing pasture.

The mortality rate of pregnant ewes in both single and multiple dose groups was higher than that observed in both parallel groups of non-pregnant ewes. These results are consistent with anecdotal evidence indicating that pregnant ewes in late-gestation are more susceptible to 1080 poisoning. Differences between species in tolerance to repeated sub-lethal doses has been identified before (Atzert, 1971) but not, as in this study, between different physiological states within a species. The pregnant ewes appeared to show accumulation of the repeated sub-lethal doses whereas non-pregnant ewes appeared to establish a tolerance. In rats these phenomena are known to be time related (Atzert, 1971) with the 24- hour period between doses used in this study likely to have given greatest chance of tolerance.

At the outset of this study, we hypothesised that increased sensitivity to 1080 in pregnant ewes may be due to the interaction between the toxic mechanism, which involves disruption of carbohydrate utilisation/energy metabolism as well as the potential for citrate-induced hypocalcaemia, and common metabolic diseases (i.e. pregnancy toxaemia/ sleepy sickness and milk fever) of late gestation. However, results of this study demonstrated that toxin-induced perturbations of carbohydrate homeostasis, as evidenced by changes in serum glucose and ketone bodies, were relatively mild and transient, indicating that 1080 exposure does not increase the risk of pregnancy toxaemia in late gestation. Similarly, neither exposure to a single high dose nor multiple lower doses had a significant effect on serum calcium concentrations, indicating that 1080 exposure is not a significant risk factor for triggering milk fever in pregnant or lactating ewes.

The observed increased sensitivity of pregnant ewes in this study compared with non-pregnant ewes appears therefore to be a function of increased bioavailablity resulting in higher serum 1080 concentrations. This increased bioavailablity of 1080 in pregnant animals has not been observed before. The physiological basis for the observed difference in circulating 1080 concentrations is unclear, but could be related to changes in toxin uptake, volume of distribution, or rates of metabolism or excretion.

Histopathologic lesions observed in the heart and lungs of ewes that succumbed to 1080 poisoning are consistent with those reported by previous researchers (Eason *et al.*, 1999; Gregg *et al.*, 1998). It is noteworthy that toxin exposure did not produce pathologic abnormalities in the foetus, even though 1080 would be expected to cross the placental barrier.

Careful monitoring of lambs and ewes from parturition through to sale as prime lambs could detect no differences in indices of ewe health, lambing percentage, lamb survival, and lamb growth rates between single or multiple 1080-dosed groups and controls. The apparent lack of long-term adverse effects on the health of non-pregnant ewes, or pregnant ewes and their lambs that survived acute exposure of either a single high dose or multiple lower doses of 1080 is consistent with the finding of a previous study involving non-pregnant ewes and a single toxin exposure monitored over 2 years (Eason *et al.*, 1999).

This study demonstrated that extra care should be taken to avoid exposure of pregnant ewes to even small fragments of 1080 bait during possum control operations. However, the results also provide further evidence that there would be no significant long-term adverse health effects in sheep that survive accidental 1080 poisoning.

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