Title
The persistence and secondary poisoning risks of sodium monofluoroacetate (1080), brodifacoum, and cholecalciferol in possums

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shown that (Meinken, pers. comm.), even though the carcasses had contaminated carcasses are well known (Eason and
the secondary poisoning risks would be lethal to dogs for at least two to three months and kidney during the first three weeks after death there is a substantial decrease in poisoning with scavenging poisoned possum carcasses, or from animals (Gooneratne et al. 1994). However, recent analyses of residue in possum carcasses after a control operation have showed that 1080 can persist in tissues in amounts that would be lethal to dogs for at least two to three months (Meinken, pers. comm.), even though the carcasses had substantially decomposed over this period. As for 1080, the secondary poisoning risks from brodifacoum-contaminated carcasses are well known (Eason and Spurr 1995), and toxic amounts of brodifacoum may be retained in a carcass.

The existence of an effective antidote to brodifacoum in the form of vitamin K1 means that dogs that have eaten carcasses containing brodifacoum residues can usually be saved. Unfortunately, there are no consistently effective antidotes for 1080 poisoning.

In contrast, studies of dogs fed rats poisoned with cholecalciferol suggest that the risk of secondary poisoning with this pesticide is low (Marshall 1984). However, these data may not be directly applicable to possums, since higher concentrations of cholecalciferol are used in possum baits (0.8%) compared with rat baits (0.075%) which may lead to higher residue levels in the possum carcasses.

In this paper persistence data is reported which may be used to assess the magnitude and duration of the risks of secondary poisoning of animals preying on live possums which have been sub-lethally dosed with 1080 or brodifacoum. In addition, preliminary tissue residue results are reported in possums, and feeding study data from cats fed possums that have been poisoned with cholecalciferol.

METHODS
Determining the Persistence of Sub-lethal Doses of Brodifacoum and 1080
A stock solution of brodifacoum was obtained from ICI Crop Care, Richmond, New Zealand and 1080 powder from Animal Control Products, Wanganui, New Zealand. Groups of six possums (three male and three female) were orally dosed with 0.1 mg/kg (2 ml/kg of a 0.05 mg/ml solution) of either brodifacoum in propylene glycol or 1080 in distilled water. The animals were allowed free access to food and water before dosing. These doses were equivalent to a possum eating 10-20 g of Talon® cereal baits containing 20 ppm brodifacoum,
or approximately 0.5 g of cereal bait containing 1080 at 0.08%. Earlier studies have demonstrated that 100 to 200 g of Talon® bait or 5 to 10 g of 1080 bait would be sufficient to kill most possums (Eason et al. 1994a). A series of blood samples were taken from the jugular vein of each possum before and after dosing. Samples were taken at 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, 48, and 96 hr after dosing with 1080, and at 4, 8, 24, and 48 hr and 7, 14, 21, 28, and 35 days after administration of brodifacoum.

Previous studies in laboratory animals and sheep demonstrated that concentrations of 1080 in blood exceed those in tissue (Eason et al. 1994b). Thus, tissue samples were not taken for 1080 analysis, since concentrations in the blood provide a worst-case persistence profile. By contrast, experiments in rats and sheep have shown that higher concentrations of brodifacoum in tissues (particularly the liver) exceed blood concentrations (Laas et al. 1985). Hence, a further 32 possums were randomly divided into groups of four animals each (two male and two female). One group was killed before dosing and the other seven groups at 2, 7, 14, 35, 64, 126 or 256 days after dosing with brodifacoum at 0.1 mg/kg. Muscle tissue and liver samples were collected at post-mortem.

All plasma and tissues were stored at -20°C for later analysis. Brodifacoum was analyzed by high-performance chromatography with fluorescence detection using published methods of determining the compound in blood (Kelice and Murphy 1989) and animal tissues (Hunter et al. 1985). A gas-chromatography technique with electron capture detection was used to measure the dichloroaniline derivative of 1080 (Eason et al. 1994b).

The Persistence of Cholecalciferol in Possums

A stock solution of cholecalciferol was obtained from AgrEvo, Pennants Hill, Sydney, Australia. Thirteen possums were randomly divided into two groups and orally dosed with 20 mg/kg (2 ml/kg of a 10 mg/ml solution) of cholecalciferol diluted in corn oil. A dose of 20 mg/kg cholecalciferol would be equivalent to a possum eating approximately 10 g of cereal bait containing cholecalciferol (0.8%) which would be a lethal or near-lethal dose for most animals. Serial blood samples were taken from three cholecalciferol-treated animals immediately before and at 3, 6, 9, 12, 17, 23, and 29 days after dosing. At 3 and 29 days after dosing, the remaining ten possums were killed and samples of heart, kidney, liver, abdominal fat, and femoral muscle tissues were collected.

In order to gain biological activity, cholecalciferol (Vitamin D₃) must undergo metabolic conversion to 25-hydroxy vitamin D (250HD), a major active metabolite (Keiver et al. 1988). Concentrations of 250HD were measured in possums after exposure to cholecalciferol as an indication of vitamin D status. A radioimmunoassay (Amersham International Ltd, Amesham, UK) based on competition between unlabelled and tritium-labelled 250HD for binding to the 25-hydroxy vitamin D binding protein from rickettic rat serum was used to determine 250HD concentrations. As with the 1080 and brodifacoum experiment, all possums were allowed free access to food and water before and after dosing, and plasma and tissue samples were stored at -20°C before analysis.

Persistence of brodifacoum in the liver and muscle differed markedly from persistence in the blood. Measurable concentrations were found in the liver 254 days after dosing. Considerably lower concentrations were also found in the muscle tissue (Table 1).

The Persistence of Cholecalciferol in Possums

Concentrations of 250HD in plasma increased from a mean of 39.6 ng/ml before dosing to a mean of 949 ng/ml six days after exposure, and subsequently declined to approximately 600 ng/ml by 29 days post-dosing (Figure 3).
Figure 2. Concentration of brodifacoum (µg/ml ± SE) in possum plasma after oral administration of 0.1 mg/kg.

Figure 3. Concentrations (µg/ml ± SE) of 25-hydroxyvitamin D (250HD) in possum plasma after oral administration of 20 mg/kg cholecalciferol.

Table 1. Mean brodifacoum concentrations in possum muscle, liver, and plasma after oral administration of 0.1 mg/kg to each group (n=4).

<table>
<thead>
<tr>
<th>Time in days after dosing</th>
<th>Concentration (µg/g or µg/ml ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>2</td>
<td>0.177 (0.011)</td>
</tr>
<tr>
<td>7</td>
<td>0.119 (0.009)</td>
</tr>
<tr>
<td>14</td>
<td>0.100 (0.032)</td>
</tr>
<tr>
<td>35</td>
<td>0.095 (0.023)</td>
</tr>
<tr>
<td>63</td>
<td>0.109 (0.024)</td>
</tr>
<tr>
<td>126</td>
<td>0.075 (0.029)</td>
</tr>
<tr>
<td>254</td>
<td>0.085 (0.009)</td>
</tr>
</tbody>
</table>

Concentrations of 250HD in heart, kidney, liver, and fat ranged from 40 to 60 ng/g 3 days after dosing, and appeared to decrease in all tissues with the exception of fat during the following 4-week period (Figure 4). As with the 1080 and brodifacoum studies, sample size was not adequate to detect any sex difference so the data from both sexes were combined.

Secondary Poisoning Feeding Studies
Each of the 12 cats ate approximately 1 kg of possum carcasses containing residues of cholecalciferol over the five-day period. At the end of five days mean serum calcium concentrations were slightly elevated compared to pre-treatment levels, but remained close to the normal range for cats (2.0–2.7 mmol/L). Calcium concentrations declined to baseline levels by 12 days post-dosing (Table 2). Appetite and body weight of the cats were not affected (Figure 5).

DISCUSSION
After oral administration of sub-lethal amounts of 1080 to possums, 1080 was rapidly absorbed and subsequently eliminated from the plasma. These results in possums are consistent with our early results in livestock (Eason et al. 1994b) and rabbits (Gooneratne et al. 1995), and studies in mice which show rapid elimination of 1080 (Sykes et al. 1987).

For livestock suspected of near or sub-lethal exposure to 1080, it has been suggested that an adequate margin of safety (for avoiding residues in food) would be achieved...
Figure 4. Concentrations (µg/ml ± SE) of 25-hydroxyvitamin D (250HD) in possum tissue 3 (n=7) and 29 days (n=3) after oral administration of 20 mg/kg cholecalciferol.

Table 2. Mean serum Ca++ (± SE) concentrations in cats.

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>After 5 Days</th>
<th>1 Week Later</th>
<th>7 Weeks Later</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.54 (0.06)</td>
<td>2.75 (0.10)</td>
<td>2.72 (0.07)</td>
<td>2.64 (0.03)</td>
</tr>
</tbody>
</table>

by imposing a minimum withholding period of five days. Should a death in a flock or herd be attributed to 1080, the withholding period should be doubled to ten days for the surviving stock and the livestock removed to a 1080-free pasture (Rammell 1993). The results would indicate that a similar safety margin of at least ten days after baits have been removed or decomposed would be appropriate for possum to ensure that residues are not present in meat harvested for human consumption. It is apparent that there is a contrast between the persistence in living animals which have received a sub-lethal dose of 1080 versus the persistence of 1080 in carcasses.

This is not the case with brodifacoum, where persistence in possums is probable in both lethally and sub-lethally poisoned animals. This remarkable persistence highlights the potential secondary and tertiary risks associated with brodifacoum when used for possum control. For example, feral pigs will scavenge possum carcasses and it is apparent from this study that possums dying up to eight months or more after being exposed to brodifacoum may contain residues that could be transferred to pigs. As feral pigs are hunted as a food source in New Zealand, there is at least a theoretical risk of tertiary poisoning of humans long after the use of brodifacoum has been discontinued in a particular area. A sensible precaution would be to recommend that the livers from all game be discarded, since much higher concentrations occur in the liver than in muscle or blood. Even if residues in most animals never reached levels capable of causing serious harm to meat-eaters, the presence of brodifacoum in any meat could be a concern to the public. The presence of brodifacoum in possum carcasses is also likely to pose a hazard to predators such as harrier hawks, as well as farm dogs.

The preliminary results with cholecalciferol indicated that elevated concentrations of 250HD were present in possum carcasses, and that they were likely to persist for several weeks in animals that had received sub-lethal doses. In comparison to other examples in the literature, the clearance of elevated 250HD in poisoned possums appeared to be quite slow. This is perhaps not surprising since it has been shown in other animals that clearance of 250HD is dose dependent (Keiver et al. 1988), and possums in the present study received extremely high near-lethal doses. For example, the plasma elimination half lives of 250HD were 15 to 36 days in humans when vitamin D status was normal, but increased to 25 to 68 days in humans and cows experiencing vitamin D toxicosis (Keiver et al. 1988).

The feeding study of cats appeared to confirm earlier work with dogs (Marshall 1984) which indicated that the risk of secondary poisoning with cholecalciferol is low. This is despite the presence of elevated concentrations of 250HD in possum carcasses. Research in rats has demonstrated that 250HD is active when administered orally (Rambeck et al. 1990), but is partially degraded in the intestinal tract (Frolick and Deluca 1971). Hence, some degradation of 250HD in possum meat by cats...
during digestion probably protects them. Further studies are planned with dogs to confirm the findings with cats, since it is believed that the data suggests a low risk, but not no risk, of secondary poisoning.

Even in the absence of this additional data, it is apparent that the risks of secondary poisoning with cholecalciferol are low when compared with brodifacoum or 1080. Dogs and cats only need to eat a very small portion of a possum carcass poisoned with 1080 to receive a lethal dose, and pets or farm dogs could not feed exclusively on possum carcasses containing 1080 for 5 days without becoming ill or dying. Nevertheless, all pets and farm dogs should be discouraged from eating animals that have been poisoned with cholecalciferol, even if the risk of secondary poisoning is perceived to be low, based on currently available evidence.

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LITERATURE CITED


