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Persistence of sodium monofluoroacetate in rabbits and risk to non-target species

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1 **Sodium monofluoroacetate (1080), a vertebrate pesticide used in New Zealand, was administered orally to rabbits at two dose levels (sub-lethal and lethal) to determine how long 1080 would persist in plasma, liver, kidney, and muscle so that the risk of consumption of meat from lethally or sub-lethally poisoned rabbits by non-target species could be assessed.**

2 **The plasma elimination half-life in rabbits receiving a sub-lethal dose was 1.1 h. Retention of 1080 in tissue was greater in rabbits dosed with a lethal dose than in those that received a sub-lethal dose. Irrespective of the dose level, concentration of 1080 in muscle, kidney, and liver was substantially lower than in the plasma.**

3 **Poisoning of dogs is possible because of their extreme susceptibility to 1080. Poisoning of birds is less likely. The risk of secondary poisoning is reduced as the concentration of 1080 declines in putrefying carcasses.**

Keywords: sodium monofluoroacetate (1080); rabbit tissues; health risk

Introduction

Sodium monofluoroacetate (1080) is a highly toxic compound used in a number of countries including Australia, Canada, New Zealand, and the USA (where it is restricted to a livestock collar). In New Zealand it has been used since 1954 solely to combat introduced species such as possums, feral cats and rabbits that have become major pests, causing economic and environmental damage.[1]

Despite close monitoring of 1080 baiting programmes the New Zealand public remains concerned about accidental or secondary poisoning of non-target species during large-scale use of 1080. Most rabbits that eat 1080 bait would die, and the carcasses of such animals may pose a risk to other non-target meat-eating species.

Rabbits may receive a sub-lethal dose of 1080, as a result of the decline in potency of 1080 after microbial degradation of poisoned bait,[2] leaching of 1080 from the bait into soil,[3] or by eating only a portion of poisoned bait. Rabbits are hunted for meat, and a sub-lethally poisoned animal would be an easy target for capture/hunting. Consumption of meat of such an animal also poses a risk to other non-target species.

The toxicity of 1080 is based on its conversion to fluorocitrate, which subsequently disrupts the major energy pathway cycle, the citric acid cycle (Krebs cycle), resulting in depression of cellular respiration.[4] Only 2.5% of the 1080 is converted to fluorocitrate.[5] Therefore in this study we focused our attention on the persistence of the parent compound 1080 rather than on the persistence of the fluorocitrate.

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Current understanding of the probable persistence of 1080 in target and non-target mammals is mostly based on metabolism studies in rats many years ago which suggested a retention of 1080 over 1–4 days.[5,6] But a more recent study on mice has shown a rapid clearance of 1080, with elimination half-lives in plasma, muscle, and liver of 1.6–1.7 h.[7] Although this suggests that prolonged persistence of 1080 and risk to humans and meat eating animals is unlikely, the clearance rates for 1080 in rabbits have not been reported.

The aim of this study was to determine how long 1080 would persist in plasma and tissues of the rabbit, including muscle (meat), after oral administration of a sub-lethal or a lethal dose of 1080 and to evaluate the risk of poisoning non-target species eating such a rabbit.

Methods

Experiments

Sodium monofluoroacetate (1080) 95% purity was obtained from the Sigma Chemical Company, USA. Two experiments were carried out in autumn 1991, a sub-lethal dose study and a lethal dose study.

In the sub-lethal dose study (experiment 1), thirty-nine 6 to 8 week-old male New Zealand Large White rabbits (Pitman-Moore, Wellington) weighing between 1.3 kg and 2.4 kg were used. Rabbits were housed in individual cages with free access to specially formulated rabbit pellet feed and water. Thirty six rabbits were orally administered a sub-lethal dose of 1080 at 0.1 mg kg^{-1}. This dose was

based on one-quarter of the published LD_{50} of 0.4 mg kg^{-1} for rabbits.[8] Three other rabbits acted as controls. Test rabbits were killed at various time intervals (0.25, 0.5, 1, 1.5, 2, 3, 6, 9, 12, 24, 48, 72 h) after dosing following anaesthesia ('Saffan', Pitman-Moore, Wellington), with three rabbits killed at each time period. The three control rabbits were killed at 4 h. Blood was collected after anaesthesia via cardiac puncture into heparinised tubes, and liver, kidney, and muscle were placed in plastic vials at post-mortem. Blood samples were centrifuged, and plasma and tissues stored at –20°C until analysis.

In the lethal-dose study (experiment 2), 12 rabbits (weighing 1.5 to 2.3 kg) were orally dosed with 1080 at 0.8 mg kg^{-1}, twice the published LD_{50}. Blood was collected immediately into heparinised tubes at death, and plasma was stored at –20°C for analysis. Tissue samples were taken from three rabbits immediately after death. The other nine rabbits were left at room temperature in the animal house to simulate the natural degradation of a carcass. Autolysis of tissue could be observed after 1–2 weeks, and this was most marked in the soft tissues (liver and kidney). Tissue samples were collected from three rabbits at 1 and 2 weeks. Tissue sampling from the remaining three rabbits was scheduled for 3 weeks after death, but liver and kidney from two animals had undergone complete autolysis and samples could be collected from one animal only. Muscle tissue was collected from all three animals.

Sodium monofluoroacetate analysis

Plasma and tissue residues of 1080 were measured as a dichloroaniline derivative quantified by gas chromatography with electron capture detection, based on procedures developed by Ozawa & Tsukioka.[9,10] The sample extraction and clean-up techniques were modified to improve the recovery of 1080.

Firstly, samples were homogenised in alcohol/water (1/1), centrifuged, and the supernatant collected. The residue was resuspended in alcohol/water, centrifuged, and the supernatants combined. An equal volume of alcohol was added to the extract and protein allowed to precipitate. The sample was centrifuged again and the supernatant diluted with deionised water, filtered, and passed through an anion exchange column. The column was washed with water and the fluoroacetate eluted with sodium chloride solution, yielding a cleaned aqueous extract.

Secondly, the extract was acidified and prepared for derivatisation by addition of dichloroaniline and dicyclohexylcarbodiimide in ethanol solutions, and shaken for 1 h in the presence of ethyl acetate. The solvent layer was removed and the aqueous layer shaken a further 10 min with fresh ethyl acetate. The solvent portions containing the 1080 derivative were combined, washed, and dried with anhydrous sodium sulphate.

Thirdly, the ethyl acetate was evaporated and the residue taken up in cyclohexane/toluene (3/1) and loaded onto a 1-g silica solid-phase extraction column on a vacuum manifold. The column was washed with further solvent before extracting the derivative of interest with toluene. A 1 µl aliquot was injected into the chromatograph equipped with a BP-5 capillary column. The 1080 recovery from biological samples was >90%, and the limit of detection was 0.002 µg g^{-1} in tissues and 0.010 µg ml^{-1} in plasma.

Pharmacokinetic analysis

The following parameters were derived:

(1) Maximum observed plasma and tissue concentration (C_{max}) by inspection of the data.
(2) Time taken to reach C_{max} (T_{max}) by inspection of the data.
(3) Half-lives (t½) estimated from the slope (K_{el}) of the semi-log plots of the terminal phase of the plasma concentration against time curve.

Results

Experiment 1

Clinically, the rabbits appeared normal during the experimental period. Sodium monofluoroacetate was quickly absorbed in the rabbits (Figure 1). Elevation of plasma 1080 concentration was rapid in the first hour (T_{max} = 25 min) and reached a maximum concentration of 0.149 mg l^{-1} (C_{max} = 0.149) (Table 1). Plasma 1080 concentration then declined rapidly (t½ = 1.1 h) at first, and slowly thereafter. Very little 1080 was detected in plasma at 6 h.

The 1080 concentration in tissues was lower than in plasma (Figure 2). Concentration in tissues was in the order muscle > kidney > liver. The concentration of 1080 in muscle remained high from 0.5 to 2 h after dosing, with maximum concentration approximately 16% of plasma C_{max}, and approximately twice that in the kidney. The concentration of 1080 detected in liver was relatively low, with levels in many samples below the quantifiable limit of detection.

Experiment 2

As expected all rabbits in this study died. The first animal died at 66 min after dosing, and all except for two died within 3 h. The signs of toxicity observed were non-specific. Within 0.5 to 2 h post-dosing, the animals appeared lethargic and subdued except for one rabbit that became restless at about 3 h following dosing. This rabbit was moaning at death (280 min post-dosing).

The plasma 1080 concentration was markedly higher (approximately – 3 fold) in these rabbits

Table 1 Plasma and tissue pharmacokinetics of 1080 in rabbits given a sublethal dose of 1080 (0.1 mg 1080 kg^{-1} W). (Range of results is in parenthesis)

Plasma/tissue	C_{max} *(mg kg)*	T_{max} *(min)*	k_{el} *(h)*	*t1/2 (h)*
Plasma	0.149 (0.121–0.167)	25 (15–30)	0.62 (0.48–0.78)	1.1 (0.9–1.4)
Muscle	0.022 (0.019–0.025)	50 (30–60)	2.02 (1.20–2.76)	0.4 (0.3–0.6)
Kidney	0.051 (0.014–0.08)	25 (15–30)	0.9 (0.78–1.140)	0.8 (0.6–0.9)
Liver	0.001 (0.001–0.002)	**15 (15)**	*	*

*Liver results (C_{max} and T_{max}) do not lend to further calculation.

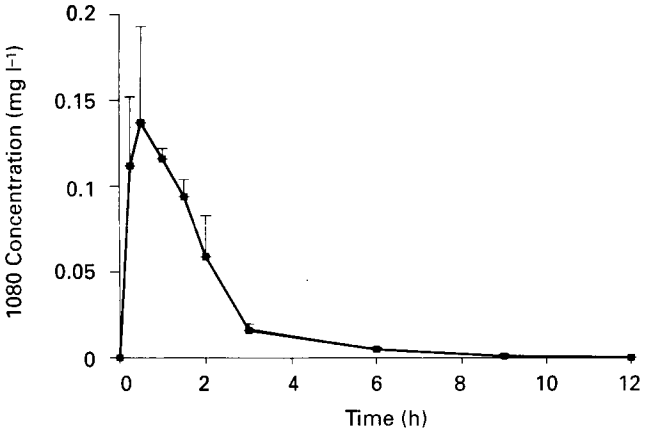

Figure 1 The plasma pharmacokinetic profile (mean ÷ standard deviation for sodium monofluoroacetate (1080) in rabbits given a sublethal oral 1080 dose (0.1 mg kg^{-1}).

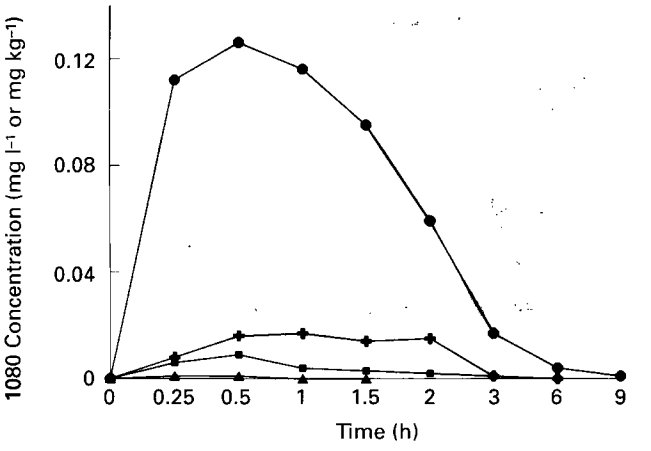

Figure 2 Distribution of sodium monofluoroacetate (1080) in plasma (●——●) and tissues [muscle (+——+); kidney (■——■); liver (▲——▲)] of rabbits sacrificed at varied times after an oral sublethal 1080 dose (0.1 mg kg^{-1}).

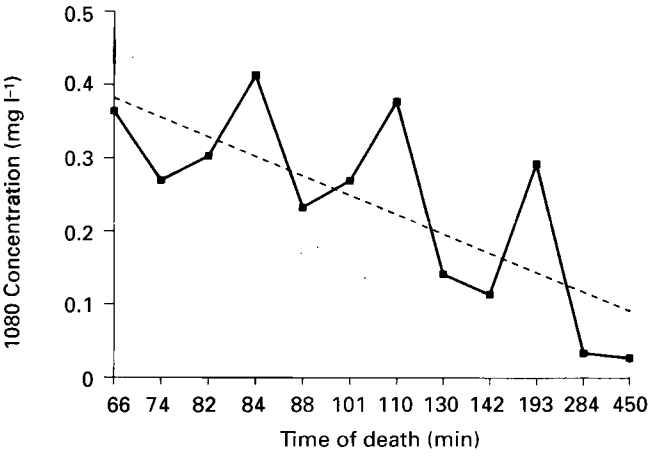

Figure 3 The plasma profile for sodium monofluoroacetate (1080) in rabbits given a lethal dose (0.8 mg kg^{-1}) of 1080. The regression line is shown (y = 0.367 – 0.00086x; r = –0.75).

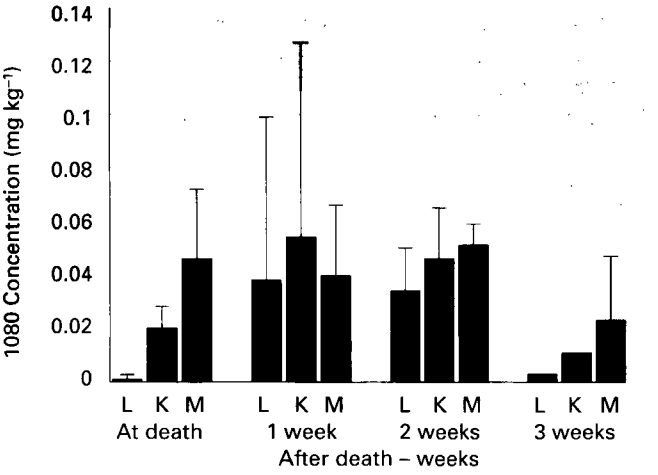

Figure 4 Sodium monofluoroacetate (1080) concentration in tissues [liver (L); kidney (K); muscle (M)] of 12 rabbits sampled, at death, 1 week, 2 weeks and 3 weeks (after death). Results are plotted as mean and standard deviation for groups of three rabbits sampled at each time point except at week 3 when liver and kidney samples were available only from one animal but muscle was collected from all three animals.

(Figure 3) than in those given a sub-lethal dose (Exp. 1). The animals that died early had higher plasma 1080 concentrations at death than in those that died at 280 and 450 min. The concentration of 1080 in tissue liver, kidney, and muscle were highly variable, and concentrations in samples collected at death, 1 week and 2 weeks were generally higher than in samples collected 3 weeks after death.

Discussion

The hazards to non-target species posed by 1080 used to control vertebrate pests have aroused public concern in New Zealand,[11] Australia[12] and elsewhere.[8] In New Zealand, where there is only one indigenous mammal (bats), only a limited range of animal species can be exposed to secondary poisoning. These include livestock, dogs, and carrion-eating birds. Livestock are unlikely to eat dead rabbits, and also the management practices for livestock and dogs in New Zealand usually ensure they do not come in contact with toxic baits or poisoned carcasses. This study has shown that when an oral dose of 1080 is administered to rabbits, 1080 is rapidly absorbed via the gastrointestinal tract and quickly distributed to most tissues, but more importantly is rapidly eliminated from tissues within about 6 h. These findings illustrate that there is minimal risk to non-target species from rabbits exposed to sub-lethal 1080. Unlike many other pesticides, which are comparatively more lipophilic, 1080 is a simple, low molecular weight, water-soluble poison that is easily metabolised and/or excreted from the body.[4] Studies with sheep and goats[13] and rats[5] have shown that 1080 is rapidly excreted in urine. Plasma t1/2 values in rabbits (1.1 h) indicate that clearance of 1080 in rabbits is faster than in goats (3.9 to 6.9 h) and sheep (6.6 to 13.3 h).[13,14]

The rapid excretion of 1080 probably accounts for the rapid disappearance of 1080 from tissues. In both our experiments the concentration of 1080 decreased in the order: plasma > muscle > kidney > liver. In all previous studies with rats and mice,[5,7,15] and ruminants,[13] 1080 was found at a higher concentration in plasma than in other major organs. The lower concentration of 1080 in liver and kidney than in muscle may be related to a glutathione-S transferase or related enzymes predominantly found in liver and kidney that catalyse the breakdown of 1080 both *in vivo*[16] and *in vitro*.[17] In rabbits a sub-lethal dose of 1080 (exp. 1) was cleared from tissues by 3 h. The plasma to muscle ratio of 1080 was 6 at 0.5 h and 3 at 2 h. Therefore, the plasma concentration provides a worst-case profile persistence. A comparison of the findings from this study with clearance of 1080 in sheep and goats[13] and clearance of radiolabelled fluoride (F) from 1080 in mice[7] indicates major species differences, with clearance rates decreasing in the order rabbits > mice > goats > sheep.

The retention of 1080 in tissues at death in rabbits given a lethal dose was in most instances greater than in tissues of rabbits dosed with a sub-lethal dose. This was not surprising since these rabbits received eight times more 1080. The pattern of retention of 1080 in lethally dosed rabbits was similar to that observed in sub-lethally dosed rabbits, but was different to that observed by McIlroy and Gifford.[18] In their study the intake of 1080 in carrot bait by rabbits was high (estimated at 9–16 mg kg^{-1} compared to the 0.8 mg kg^{-1} in our lethal study) and tissue 1080 concentration measured by total F method in the dead rabbits gave values in decreasing order for liver 160 ± 40 mg kg^{-1} dry matter (mean ± s.e.) > kidney (91 ± 25) > muscle (23 ± 4). Even after correction for the higher 1080 intake and the dry matter of tissues examined, these concentrations are at least 100 to 1000 fold higher than those we observed. This is probably related to the analytical method used (via F estimation) by McIlroy and Gifford,[18] which we now believe grossly over-estimates and may have little relevance to the true 1080 concentration.

All rabbits except one in experiment 2, dosed with twice the LD_{50} of 1080 became lethargic, subdued and all died within 8 h. This is in agreement with the reports of Osweiller *et al.*[4] and Egakeze and Oehme,[19] that herbivores in response to lethal doses of 1080 become subdued due to inhibition of the major energy producing cycle (Krebs cycle), heart rate becomes irregular and slow, and die of heart failure.

The exposure of humans to 1080 is rare. It is extremely unlikely that people would eat dead rabbits found in the field, but there is a possibility of a sub-lethally poisoned animal being hunted for meat. However, this possibility is remote since good pest control practices ensure all land owners, including hunters, are notified before 1080 pest management operations. An accurate LD_{50} for humans has, understandably, not been determined, and estimates range from 0.7 to 5.0 mg kg^{-1}. Our present study indicates that 1080 concentration in tissues of poisoned rabbits is so low that an unrealistic quantity of rabbit meat (> 50 kg) would have to be eaten for a human to be poisoned. Another important finding from this study was that the 1080 concentration in tissues of animals given a lethal dose appeared to persist in carcasses, and in most animals the 1080 concentrations were higher in samples collected week 1 and/or week 2 after death. The decomposing rabbit carcasses were in outdoor cages inside locked pens where temperature and rainfall would have fluctuated as would the moisture content of the tissues of dead animals. This apparent increase in tissue 1080 concentration may have been caused by large individual variations between rabbits, and tissues drying at different rates after death rather than an absolute increase in tissue 1080 concentration. The tissue concentrations of 1080 in this study are expressed as mg kg^{-1} fresh weight, and any variation in the water content in tissues would have altered the final result. This tissue drying effect could not be confirmed since the dry matter content of tissues was not measured before the analysis and all the tissues collected were used for analysis of 1080 (reported here) and

subsequently for F and citrate analysis,[14] but prolonged retention of 1080 in desiccated tissues has been previously reported.[18] The lower concentrations of 1080 detected in some liver and kidney samples at week 3 are probably related to tissue autolysis, leaching of 1080 from the tissues, microbial defluorination and removal of 1080 by a variety of invertebrates that inhabit carcasses. It is stressed that the increase/decrease of 1080 concentration in tissues left to decompose at room temperature depends on the degree of drying/autolysis in the carcass. It is well known that soft tissues such as liver and kidney autolyse more easily than muscle tissue.

The risk to individuals or populations of animals to secondary poisoning during or after a rabbit-1080 poisoning campaign depends on a number of factors. These include species sensitivity to 1080, the number of poisoned animals encountered and individual feeding habits, particularly the amount of different tissues or organs from poisoned animals eaten.[18] Poisoning of pets, especially dogs (LD_{50} = 0.11 mg kg^{-1}) is possible because of their extreme susceptibility to 1080.[4] Poisoning of birds is less likely because of their higher LD_{50} values.[18] The risk of secondary poisoning is reduced as the concentration of 1080 declines in decomposing carcasses.

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