

Tissue residue levels in rabbits and rats poisoned with 1080 One-shot bait and the location of poisoned rabbit carcasses

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Abstract. The level of fluoroacetate (1080) found in the carcasses of rats and rabbits poisoned with 1080 ranged from 1.9 to 14.4 $\mu\text{g g}^{-1}$ (mean 5.3 $\mu\text{g g}^{-1}$, $n = 11$) in rats and <0.02 to 0.78 $\mu\text{g g}^{-1}$ (mean 0.353 $\mu\text{g g}^{-1}$, $n = 10$) in rabbits. The concentration of fluoroacetate in the blood and liver of both species was generally higher than in the carcasses, and ranged from <0.02 to 33.6 $\mu\text{g g}^{-1}$. Fifteen of 22 collared rabbits, and 3 freshly killed, uncollared rabbits were recovered during a routine baiting exercise with 1080 One-shot oats. Excluding the 4 collared rabbits taken by predators, only 14% of all carcasses ($n = 14$) were found in the open, with the remaining 86% of carcasses being well concealed in warrens or under thick scrub. The carcasses of both rabbits and rats showed considerable decay within 6 days of poisoning. Except for eutherian carnivores, which are highly sensitive to 1080, there is little potential risk of secondary poisoning of native wildlife as a result of the correct use of 1080 baits in pest-control programs.

Introduction

Sodium monofluoroacetate, also known as 1080, is the mainstay of most vertebrate pest-control programs in Australia and New Zealand (Seawright and Eason 1994; Saunders *et al.* 1995; Williams *et al.* 1995; Choquenot *et al.* 1996; Spurr 1999, 2000). In particular, 1080 baits are used to control European rabbits (*Oryctolagus cuniculus*), foxes (*Vulpes vulpes*), wild dogs (*Canis familiaris*), and feral pigs (*Sus scrofa*) in Australia. 1080 has also been used in the past to control *Rattus villosissimus* in the Ord River Irrigation Area of Western Australia (S. H. Wheeler, unpublished data). In New Zealand, 1080 is used to reduce the impact of introduced brushtail possums (*Trichosurus vulpecula*), European rabbits and mustelids (e.g. stoats, *Mustela erminea*). All of these control programs are aimed at reducing the impacts of vertebrate pests on agricultural production and/or wildlife conservation.

Any responsible pest-control program needs to consider the efficacy, the potential for non-target impacts, the potential for environmental contamination, the humaneness, and the associated cost of a given control strategy. In most of these respects, 1080 has been well studied and has been found to be a cost-effective and environmentally safe pest-control agent (see References for examples). However, although a considerable number of published reports address the potential for non-target impacts (e.g. McIlroy 1982; Twigg and King 1991; McIlroy and Gifford 1991, 1992; Seawright and Eason 1994; Spurr 1999; Martin and Twigg 2002; Martin *et al.* 2002), aside from some canid species

(McIlroy and Gifford 1992; Algar and Kinnear 1996), there are limited data on the potential for non-target animals to be poisoned secondarily through the consumption of prey that contain 1080. The level and persistence of 1080 residues in tissues, the location of the carcasses of animals poisoned by 1080, and the longevity of such carcasses will all influence the potential for secondary poisoning to occur. This paper examines these aspects for rabbits recovered during a routine 1080 baiting program. It also examines 1080 residues in some tissues of rabbits and rats administered/fed known amounts of 1080 in the laboratory. These findings are compared with the 1080 residue levels in tissues reported previously in the literature, and we comment on the potential for secondary poisoning of native animals to occur during routine control programs that utilise 1080.

Materials and Methods

Rat-feeding experiment and 1080 residues

1080 residues in the tissues of rats fed 1080 One-shot oats were determined using 20 adult *Rattus norvegicus*, Sprague Dawley strain laboratory rats (10 male and 10 female, Animal Resource Centre, Perth). Rats were housed individually in wire cages in an animal house maintained at $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a 12:12 h reversed-cycle photoperiod. Except during experimentation, water, rat pellets, and mixed seeds, including plain oats, were provided *ad libitum*. All rats were acclimatised for 12 days prior to experimentation. The One-shot oats used (batch 8/2000) contained 4.28–4.35 mg of 1080 per oat (Gas Chromatography assay).

These trials were run using two batches of 10 rats (5 males and 5 females). On the day prior to experimentation, the rats were weighed and all food was removed from each cage apart from 10 grains of

unpoisoned, plain oats allowed for overnight consumption. Any remaining oats were removed the next morning, and the rats presented with 4 unpoisoned oats and 2 One-shot oats in a glass dish. To assist with the recognition of the oat remains after feeding, the One-shot oats were marked with a ballpoint pen to distinguish them from the plain oats. The amount (%) and part (kernel and/or husk) of the One-shot oats eaten was estimated for each rat. Signs of 1080 intoxication were monitored at least every 30 min for 7.5 h after the oats were offered. All but one rat had consumed some poisoned oats, and developed signs of intoxication, within 5 h of the food being offered. The three rats that developed pronounced signs of poisoning within this time, and the rat that did not consume the oats, were euthanased by cervical fracture. These carcasses, and those of the remaining rats that succumbed 'naturally' to the poison, were stored in individual sealed plastic bags at 4°C for 1–2 h until the tissues from each rat were prepared as described below. Three of the rats (1 female, 2 males) from the second trial were placed outdoors on the soil surface, protected from vertebrate predators, to examine the persistence of rat carcasses and their 1080 content during spring.

1080 residues in free-ranging rabbits collected during a control program

1080 residues in the tissues of rabbits recovered during a routine 1080 One-shot baiting program undertaken in February 2002 were determined. The site was located on a farming property at Boxwood Hill (34°17'S, 118°46'E) in the southern agricultural region of Western Australia. The site was a strip of remnant native sandy heath vegetation situated within two farming paddocks. This bush refuge, where most rabbits resided, was approximately 1 km long and 10–30 m wide, and was surrounded by recently harvested barley stubble on one side, and a paddock with dry pasture on the other. All paddocks were de-stocked during the trial.

In early February 2002, 22 rabbits (8 female, 14 male) were live-trapped using wire cage traps baited with carrot. These rabbits were weighed, sexed, tagged, fitted with radio-collars and then released at point of capture within a few hours of being processed. The frequencies of transmitters used were between 150.015 and 151.257 MHz, which allowed good ground penetration by the signal. Rabbits were then located on subsequent days by means of radio-telemetry (Biotel RX-2 receiver and hand-held Yagi antenna). The poison bait trail was laid once we established that the transmitters were functioning properly, and that the collared rabbits had remained on site.

A 1% (ratio of poisoned to plain oats) One-shot oat bait mix was used where 1 in 100 oats nominally contains 4.5 mg 1080 per oat. Bait was laid on 11 February 2002 in a single trail at 6 kg km⁻¹. The trail was laid within 5 m of the rabbit refuge in the paddocks on both sides of the bush refuge. However, due to light showers (~2 mm) within 2 days of baiting, and to cover the possibility of some leaching of 1080 from the bait, the trail was subsequently re-laid at 3–4 kg km⁻¹ to ensure that sufficient viable bait remained.

Collared rabbits were deemed to have been killed if they had remained at the same precise location for two consecutive days. Such rabbits were then retrieved, usually by digging with shovels. Digging ceased if a rabbit subsequently changed its position. Once a good provisional location was established for each rabbit, the antenna was removed from the receiver to reduce its sensitivity. This enabled more accurate triangulation to determine the precise location of collared rabbits. The location of each recovered carcass was recorded. This included an estimate of the depth, and the distance from the closest pop-hole, for those carcasses located within warrens. The 'state' (e.g. presence and absence of maggots, decay evident) of each carcass was also recorded.

Recovered carcasses were processed in the field. They were skinned, and the head, skin and feet discarded. Where possible, a muscle sample

(5–7 g) was collected from a hind leg, and the liver and the whole gut were removed. Each tissue sample was placed separately into labelled resealable plastic bags. The remains of each carcass (stored in labelled plastic bags), and the tissue samples, were kept frozen (–4°C) until the samples were prepared for analysis as described below.

Residues in rabbits administered known amounts of 1080

Whole blood and muscle samples were collected from a number of rabbits ($n = 31$) that succumbed during the toxicity trials of Twigg *et al.* (2002) undertaken to reassess the sensitivity of rabbits to fluoroacetate (1080). These toxicity trials were conducted under controlled laboratory conditions, and the samples were kept frozen until analysis.

Assay of 1080

Because of financial constraints, and to cross-check between some assays, three assays were used to determine the 1080 concentrations for the various aspects of this study.

The concentrations of 1080 in the rat tissues, and during the initial assay of the rabbit tissues from the field baiting trial, were determined using a bioassay similar to that developed for dried meat, egg, and grain baits (see Kirkpatrick 1999; Twigg *et al.* 2001, 2003). This procedure utilises the growth inhibition of a 1080-sensitive bacterium (*Acinetobacter lwoffii*) in response to known concentrations of 1080 (Wong *et al.* 1995). 1080 muscle standard broths (0.0125–0.1050 mg mL⁻¹) were run with each assay, and the widths of the inhibition zones subsequently generated from these standards were used to establish regression equations. These equations were then used to determine the 1080 concentration in the unknown tissue samples. Thus, with this assay, any binding of fluoroacetate to tissues is allowed for by the use of the meat broth standards, and correction factors are therefore not required. The sensitivity of the bioassay is 6 µg g⁻¹ with recoveries from spiked muscle (1.8 mg 1080 added) and liver (0.9 mg 1080) samples of 80.2% ± 10.4% ($n = 5$) and 86.3% ± 15.0% ($n = 5$), respectively. Samples that gave a nil result for the standard bioassay procedure were concentrated to a known volume using evaporation and then reassayed as above. The resulting values generated from the regression equations for these unknowns were then corrected for the concentrated volume and expressed as micrograms of fluoroacetate per gram of original sample. Hence, some of the final values for the bioassay appear to be less than the limit of detection for this assay.

The samples used in the bioassay, and those assayed by the Australian Government Analytical Laboratories, Perth (AGAL), were prepared as follows. All carcasses assayed excluded the skin, head, feet, gut, liver and gonads but included the heart, lungs and kidneys. Each carcass was minced using a 4.5-mm screen and a Reber 0.4 HP (350 watt) electric mincer. Each sample was minced twice to ensure that a homogeneous mix was obtained. Initially, with rabbits, the resulting carcass homogenate was blended in 250 mL of deionised water and agitated on an orbital shaker (360 rpm) for 16 h to extract the 1080 into the aqueous phase. However, with subsequent assays, a 150–200-g sub-sample of each rabbit homogenate mix was routinely blended in 150–200 mL of deionised water until an adequate slurry was formed. Rat carcasses were blended with 100 mL deionised water and then shaken on an orbital shaker for 60 min at 360 rpm. The resulting supernatants were decanted and stored at –4°C until analysis.

The 1080-residues in the rabbit carcasses recovered from the baiting program, and in some rats, were ultimately determined by the AGAL using a gas chromatography method based on the methods of Collins *et al.* (1981), Okuno *et al.* (1982), and Tomkins (1994). Sub-samples of the tissue homogenates were extracted with an acetone/water mixture. A measured portion of each extract was concentrated, acidified and partitioned with ethyl acetate three times. The ethyl acetate was evaporated to dryness. The residue was then redissolved in acetone and derivatised with pentafluorobenzylbromide. The derivatised sample

was analysed using gas chromatography with electron-capture detection. The limit of detection (L.D.) of sodium fluoroacetate in muscle tissue with this method was $0.02 \mu\text{g g}^{-1}$. Recovery from spiked rabbit muscle samples was 85.7% (s.d. 10.6%, $n = 3$).

The concentration of 1080 in the tissues of those rabbits that died during the toxicity trials of Twigg *et al.* (2002) were assayed by the Alan Fletcher Research Station, Department of Natural Resources and Environment (DNRE), Queensland using the gas chromatography method of Ozawa and Tsukioka (1989). Samples were blended with distilled water and the extracts course-filtered through glass wool and/or centrifuged. Fluoroacetate in the filtrate was then adsorbed onto an anion-exchange resin and eluted with 2% (w/v) sodium chloride solution. The eluents were acidified with hydrochloric acid and a fluoroacetate dichloroanilide derivative prepared by reaction with 2,4-dichloroaniline in the presence of *N,N*-dicyclohexylcarbodiimide. The fluoroacetate dichloroanilide derivative was extracted from the reaction mixture with ethyl acetate, dried, concentrated and then quantified by gas chromatography with mass-spectrometry detection using single-ion monitoring at $m/z = 186$, and confirmed using the electron impact mass spectrum. The limit of detection was: muscle, $0.0005 \mu\text{g g}^{-1}$; blood, $0.01 \mu\text{g g}^{-1}$; and liver, $0.005 \mu\text{g g}^{-1}$, and the mean recovery of 1080 from spiked meat samples was 103.1% (s.d. 17.8%, $n = 23$).

All results obtained with the three assay methods are presented on a wet weight basis for each tissue assayed. These data have also been corrected for the percentage recovery associated with each of the gas chromatography assay methods.

Results

1080 residues in laboratory rats

The amount of 1080 estimated to have been ingested by each rat ranged from 0.86 to 5.18 mg per rat, although Rat 11 did not consume any of the poisoned oats (Table 1). Fluoroacetate (1080) was detected in 81% of the livers, and in 75% of the carcasses, of those rats assayed that had consumed the poisoned oats. Fluoroacetate levels in the remaining samples were below the absolute level of detection (L.D.) for the bioassay ($6 \mu\text{g g}^{-1}$). The concentration of fluoroacetate was greater in the liver than in the carcass, and the maximum recorded, and mean, levels in these tissues were 33.6 and $14.4 \mu\text{g g}^{-1}$, and 14.3 and $5.3 \mu\text{g g}^{-1}$, respectively (Table 1). The concentration of fluoroacetate in the two whole blood samples assayed ranged from <L.D. to $16.9 \mu\text{g mL}^{-1}$. The concentration of fluoroacetate in the muscle of three of these rats was also assayed using duplicate samples and gas-liquid chromatography analysis. These results were: Rat 13, 1.94; Rat 15, 6.31; and Rat 17, 2.43 $\mu\text{g fluoroacetate g}^{-1}$. The results follow the general trends seen for these rats assayed with the bioassay.

Table 1. Fluoroacetate (FAc = 1080) residue levels in the tissues of rats ($n = 17$) fed 1080 One-shot oats in the laboratory
Fluoroacetate determination was by the bioassay, and some samples were concentrated by evaporation prior to final analysis. NA, not assayed

Animal #	Sex	Weight (g)	Estimated mg 1080 ingested	$\mu\text{g FAc g}^{-1}$ liver	$\mu\text{g FAc in liver}^A$	$\mu\text{g FAc g}^{-1}$ carcass ^B	$\mu\text{g FAc whole animal}^C$
Trial 1							
1	F	237	1.73	33.6	260.3	6.7	1598
2	F	212	4.32	23.5	310.5	5.0	1062
3	F	240	1.73	25.7	236.9	4.4	1047
4	F	241	4.32	31.0	245.4	<L.D.	
5	F	234	4.32	27.7	206.9	4.4	1034
11	M	370	0.00	<L.D.		<L.D.	
12	M	386	4.32	<L.D.		0.1	54
13	M	366	3.88	NA		<L.D.	
14	M	366	4.32	NA		<L.D.	
15	M	401	1.73	6.7	102.4	14.4	5774
Trial 2							
6	F	222	0.86	<L.D.		<L.D.	
7	F	243	1.73	21.4	187.0	9.0	2185
8	F	234	1.73	10.3	67.6	4.0	925
10	F	233	1.73	9.8	62.7	5.6	1293
16	M	373	3.45	2.6	40.8	6.3	2336
17	M	377	1.73	<L.D.		0.9	328
18	M	419	5.18	4.7	72.7	3.4	1419
All rats ^D	Mean	303.1	2.77	13.9	130.7	4.7	1396.9
	s.d.	78.1	1.55	11.7	98.4	3.3	1262.5
	n	17	17	15	15	17	17
<L.Ds excluded ($n = 11$)	Mean	299.1	2.81	14.3	137.4	5.3	1588.1
	s.d.	82.4	1.38	11.3	96.6	3.7	1471.3

^ABased upon the total liver weight.

^BMinus the head, skin, feet and gut.

^CBased upon the initial weight of rats.

^D<L.D. values set to $3.0 \mu\text{g g}^{-1}$.

The three rat carcasses placed outdoors (Rats 9, 19 and 20) had rapidly decomposed within 6 days, to such an extent that only muscle samples of poor quality could be collected. No fluoroacetate was detected by the bioassay (L.D., $6 \mu\text{g g}^{-1}$ tissue) in any of these 6–7-g muscle samples, although these rats had consumed 1.73–3.45 mg of 1080. Maximum and minimum temperatures during the 6-day experimental period (30 October to 5 November 2001) were 22–33°C and 9.5–17.0°C, respectively, and 1.0 mm of rain fell on 3 November.

Location and longevity of rabbit carcasses

Of the 22 rabbits fitted with transmitters, 15 carcasses were recovered within 6 days of the One-shot oat bait being laid. Six of the remaining collared rabbits were still alive on site at the end of the 6-day search period (based upon their activity patterns, and on spotlighting). The remaining collared rabbit was hand-caught, and euthanased, due to an ill-fitting collar. A further three untagged, freshly killed rabbit carcasses were also recovered during the carcass-search. Of the 18 recovered rabbits, only 2 were found in the open, 12 were found under thick scrub or in warrens, and 4 were taken/scavenged by predators (Table 2). Three of the predator-related events were attributed to foxes (*V. vulpes*), and the fourth to a wedge-tailed eagle (*Aquila audax*). These carcasses/transmitters, including a cached head with transmitter, were found 50–500 m from the refuge habitat where the rabbits resided. With the predator-related incidents excluded (original location unknown, and predators may take moribund animals), then only 14% of carcasses were found in the open and 86% were concealed, either under thick scrub or in warrens (Table 2).

Some of the rabbit carcasses recovered 3–5 days after baiting showed evidence of obvious decay, as indicated by the condition of the carcass, and the presence of relatively

large maggots. Most carcasses with obvious maggots were collected from the warrens (4 of 5). The mean depth of the 7 carcasses recovered from the warrens was 0.8 m (range 0.1–1.5 m) and, on average, these carcasses were located 1.5 m from the warren entrance (range 0.1–2.5 m). Minimum and maximum temperatures during the 6-day search for carcasses, including the day that bait was laid, were 11.0–16.0°C and 21.0–32.5°C, respectively. Approximately 2 mm of rain also fell as scattered showers on the evening of Day 2.

1080 residues in rabbits

No fluoroacetate (i.e. 1080) was detected in any of the muscle samples collected from the rabbits recovered during the One-shot baiting program ($n = 18$) assayed with the bioassay (L.D., $6 \mu\text{g g}^{-1}$), suggesting that 1080 was either at low concentrations, or was not present. However, 1080 was subsequently found in most rabbit carcasses when 14 of these samples were assayed with the more sensitive gas chromatography technique (L.D., $0.02 \mu\text{g g}^{-1}$) (Table 3). All of these levels were below the level of detection of the bioassay. The mean residue level was $0.26 \mu\text{g fluoroacetate (g tissue)}^{-1}$ when all rabbits were included ($n = 14$) or $0.35 \mu\text{g fluoroacetate (g tissue)}^{-1}$ ($n = 10$) when those rabbits with no detectable fluoroacetate were excluded. The maximum recorded concentration was $0.78 \mu\text{g g}^{-1}$ (Table 3). Assuming that levels were similar throughout the animal, then the greatest fluoroacetate residue level on a whole-animal basis was 1.34 mg.

The levels of fluoroacetate found in the tissues of the rabbits poisoned during the toxicity trials of Twigg *et al.* (2002) were also below the level of detection of the bioassay. Fluoroacetate residue levels, as determined by gas chromatography, were approximately 10-fold higher in blood than in the muscle of these rabbits (Table 4). Only 2 of 31 livers from these rabbits were positive for fluoroacetate: one liver of a rabbit dosed at $0.590 \text{ mg } 1080 \text{ kg}^{-1}$ contained $0.0129 \mu\text{g fluoroacetate g}^{-1}$, the other from a rabbit given $0.864 \text{ mg } 1080 \text{ kg}^{-1}$ had $0.0357 \mu\text{g fluoroacetate g}^{-1}$. At comparable sampling times, orally dosed rabbits also had lower tissue residues than rabbits given equivalent amounts of 1080 via intraperitoneal injection (Table 4). This was likely the result of the increased lag phase associated with fluoroacetate poisoning in animals that are orally dosed (Buffa *et al.* 1973; Twigg and King 1991). The maximum recorded level in the blood and muscle of these rabbits was 0.78 and $0.17 \mu\text{g fluoroacetate g}^{-1}$ respectively. Residue levels were also greater at the higher dose levels (Table 4).

Discussion

About 32% of ingested 1080 is excreted unaltered in urine, with plasma (blood), liver and the muscle of some species generally having the highest tissue 1080 residue levels (Gal *et al.* 1961; Sykes *et al.* 1987; Gooneratne *et al.* 1994). The

Table 2. The location of rabbits recovered during a field control program with 1080 One-shot oats

In all, 22 rabbits were fitted with transmitters and 15 of these rabbits, plus 3 'untagged' rabbits, were recovered 2–6 days after baiting commenced

Location	No.	%
All rabbits ($n = 18$)		
In open	2	11.1
In warren	17	38.9
Under scrub	5	27.8
Taken by predators	4 ^A	22.2
In open v. concealed ^B ($n = 14$)		
In open	2	14.3
Concealed ^B	12	85.7

^AOriginal location unknown, these rabbits may have been moribund or scavenged. Three carcasses were taken by foxes, and one by a wedge-tailed eagle.

^BIn warrens or under scrub.

Table 3. Fluoroacetate (FAc = 1080) residues in the carcasses of rabbits (field control program) poisoned with 1080 One-shot oats

Fluoroacetate assays were undertaken with gas chromatography by the AGAL, Perth. The limit of detection (L.D.) was $0.02 \mu\text{g g}^{-1}$, and values have been corrected for the 85.7% recovery rate

Animal #	Sex	Field weight (g)	$\mu\text{g FAc g}^{-1}$ carcass ^A	$\mu\text{g FAc}$ in carcass ^A	mg FAc in whole animal ^B
Rabbit A	?	?	<0.02	11.51	0.0223 ^C
Rabbit 176	F	1150	0.1774	111.74	0.2040
Rabbit 181	F	1100	0.6153	274.16	0.6768
Rabbit 184	F	1350	0.1443	92.99	0.1947
Rabbit B	F	1700	<0.02	13.41	0.0254 ^C
Rabbit 173	M	1350	0.7778	559.13	1.0500
Rabbit 177	M	1550	0.1177	101.30	0.1824
Rabbit 179	M	1800	0.7439	656.82	1.3391
Rabbit 182	M	1300	<0.02	11.69	0.0188 ^C
Rabbit 185	M	1500	0.1137	85.91	0.1705
Rabbit 187	M	1050	<0.02	11.12	0.0268 ^C
Rabbit 188	M	1350	0.5546	254.71	0.7487
Rabbit 190	M	1450	0.1703	126.15	0.2469
Rabbit 192	M	1850	0.1149	107.46	0.2126
All rabbits:	Mean	1423	0.2572 ^D	172.72	0.3657
s.d.		254	0.2830 ^D	202.53	0.4199
Excluding rabbits with <L.D.:	Mean	1445	0.3530	237.04	0.5026
s.d.		244	0.2828	207.75	0.4264

^AMinus the head, skin, feet and gut. ^BBased upon the field weight of animals. ^CAssumes $0.01 \mu\text{g FAc g}^{-1}$ and/or the animal weighed 1423 g. ^D<L.D. values set to $0.01 \mu\text{g FAc g}^{-1}$.

Table 4. Fluoroacetate (FAc = 1080) residue levels in the tissues of rabbits ($n = 31$) given known amounts of 1080 during the toxicity trials of Twigg *et al.* (2002)

Fluoroacetate was assayed by gas chromatography methods at DNRE, Queensland. Limits of detection were: blood, $0.01 \mu\text{g g}^{-1}$; muscle, $0.0005 \mu\text{g g}^{-1}$. Values have been corrected for the 103% recovery rate. F/M ratio: the ratio of female to male rabbits. E/N ratio: the ratio of the number of rabbits euthanased to those that died 'naturally'. IP, Intraperitoneal administration

Dose (mg kg ⁻¹)	$\mu\text{g FAc}$ per g wet weight				Mean time to sample (h)	F/M ratio	E/N ratio
	Blood		Muscle				
	Mean	s.d.	Mean	s.d.			
0.456 (IP)	0.112	—	0.044	—	2.30	1/0	0/1
0.590 (IP)	0.192	0.095	0.026	0.019	3.38	2/2	3/1
0.590 (Oral)	0.087	0.080	0.011	0.013	3.58	1/3	4/0
0.628 (IP)	0.062	0.059	0.036	0.045	3.00	1/1	0/2
0.864 (IP)	0.313	0.159	0.033	0.018	3.19	4/6	0/10
1.188 (IP)	0.293	0.259	0.029	0.017	3.41	2/3	1/4
1.634 (IP)	0.648	0.131	0.096	0.045	4.84	2/3	0/5
IP only							
Mean	0.316	0.222	0.044	0.036	3.43	12/15	4/23
Minimum	0.020		0.001				
Maximum	0.784		0.168				

half-life of 1080 in plasma, liver, and muscle is ~2–6 h in live sheep administered $1.5\text{--}2.0 \text{ mg } 1080 \text{ kg}^{-1}$ (Rammell 1993; Gooneratne *et al.* 1994). The half-life of plasma-1080 in goats, possums and rabbits is 1.0–5.5 h (Eason 1994). This suggests that 1080 residues in most tissues should quickly diminish to relatively low levels, and therefore represent only a minimal potential risk to native wildlife.

Fluoroacetate (1080) residues have been determined for a variety of tissues from several species (see Table 5). However, because of differences in the administration of 1080 (e.g. oral *v.* intraperitoneal administration, field *v.* laboratory trial), and in the tissues that were examined (e.g. muscle, liver, whole carcass), it is difficult to make direct comparisons between these studies. Nevertheless, some

Table 5. Fluoroacetate (FAc = 1080) residues in the tissues of various animals, both native and exotic to Australia

Source: 1, McIlroy and Gifford (1992), Tables 1 and 3, dry weights converted to wet weight assuming a 65% water content; 2, Meenken and Booth (1997); 3, Casper *et al.* (1984); 4, McIntosh and Staples (1959); 5, Casper *et al.* (1983); 6, Casper *et al.* (1986); 7, Hedgal *et al.* (1986); 8, Hagan *et al.* (1950); 9, Gooneratne *et al.* (1994); 10, present study; 11, Eason *et al.* (1993); 12, Milne *et al.* (2001); 13, Rammell (1993)

Species	Source	Dose (mg kg ⁻¹)	Time after treatment (h)	Tissue	µg FAc (g wet weight) ⁻¹
Brown antechinus (<i>Antechinus stuartii</i>) (mean values)	1	LD ₅₀ Trial (1.2–2.8 ^A)	At death	Muscle	20.0 ± 16.1
				Kidney	1.4 ± 1.4
				Liver	1.1 ± 1.1
Dusky antechinus (<i>Antechinus swainsonii</i>) (mean values)	1	LD ₅₀ Trial (2.1–4.2 ^A)	At death	Muscle	7.4 ± 6.0
				Kidney	19.3 ± 14.0
				Liver	0
Long-nosed bandicoot (<i>Perameles nasuta</i>) (mean values)	1	LD ₅₀ Trial (8.0–10.0 ^A)	At death	Muscle	4.6 ± 0.7
				Kidney	5.6 ± 2.8
				Liver	14.7 ± 1.1
Marsupials (<i>Vombatus ursinus</i> , <i>Trichosurus vulpecula</i> , <i>Macropus rufogriseus</i>)	1	Field baiting trial (higher bait loading than recommended – 0.333 v. 0.200 mg g ⁻¹)	At death ?	Muscle	0–0.7
				Kidney	3.9–38.5
				Liver	3.9–21.0
				Whole carcass	4.9–45.5
Possum (<i>Trichosurus vulpecula</i>)	2	Control program	25–75 days	Stomach & contents	0–35.0
Tasmanian pademelon (<i>Thylogale billardieri</i>)	1	LD ₅₀ Trial (0.3 ^A)	At death	Muscle	0.4 ± 0.4
				Kidney	0
				Liver	0.4 ± 0.4
Southern bush rat (<i>Rattus fuscipes</i>)	1	LD ₅₀ Trial (0.8–3.2 ^A)	At death	Muscle	21.0 ± 10.2
				Kidney	0
				Liver	0
Coyote (<i>Canis latrans</i>)	3	Secondary via 0.5 & 3 mg per squirrel fed	12–120	Kidney	0.003–0.092
				Liver	0.001–0.240
Deer (<i>Cervus dama</i>)	4	Control program	?	Muscle	1.43–9.21
				Kidney	0.94–5.06
				Liver	0.59–10.15
Dog (<i>Canis familiaris</i>)	5	Rat control	?	Kidney	0.055–0.263
				Liver	0.015–0.148
Dog (<i>Canis familiaris</i>)	3	Primary (0.93 ^A)	2 3	Kidney	0.442
				Liver	0.215
Ground squirrel (<i>Spermophilus beecheyi</i>)	6	4.8 ^A	?	Muscle	0.41–0.77
				Kidney	0.28–1.14
				Liver	0.70–1.83
Ground squirrel (<i>Spermophilus beecheyi</i>)	6	0.8 ^A	?	Kidney	0–0.095
				Liver	0–0.027
Ground squirrel (<i>Spermophilus beecheyi</i>)	7	Control program	8–120	Whole carcass	1.6–23.3
				8	1.8–5.8 ^A
Laboratory rat (<i>Rattus sp.</i>)	8	1.8–5.8 ^A	24	Kidney	1.6
				Liver	0.6
				Muscle	0–0.04
Rabbit (<i>Oryctolagus cuniculus</i>)	9	0.8 ^A	7–21 days	Kidney	0–0.12
				Liver	0–0.11
				Muscle	0–0.04
Rabbit (<i>Oryctolagus cuniculus</i>)	1	Field baiting trial (higher bait loading than recommended – 0.333 v. 0.200 mg g ⁻¹)	At death ?	Muscle	2.5–14.0
				Kidney	9.5–92.1
				Liver	20.0–148.1
				Whole carcass	14.2–73.2
Rabbit (<i>Oryctolagus cuniculus</i>)	10	1080 One-shot control program	1–6 days	Carcass (no skin, head or gut)	<0.02–0.778
Rabbit (<i>Oryctolagus cuniculus</i>)	10	LD ₅₀ Trials (0.46–1.63 ^A)	At death	Muscle	0.001–0.168
				Liver	0.013–0.036
				Whole blood	0.020–0.784
Sheep (<i>Ovis aries</i>)	11	0.1 ^A	2.5 & 96	Muscle	0.002–0.042
				Kidney	0.002–0.057
				Liver	0–0.021
				Plasma	0.002–0.098

Table 5. (Continued)

Species	Source	Dose (mg kg ⁻¹)	Time after treatment (h)	Tissue	µg Fac (g wet weight) ⁻¹
Sheep (<i>Ovis aries</i>)	1	LD ₅₀ Trial (0.4–1.0 ^A)	At death	Muscle	0
				Kidney	2.1 ± 1.1
				Liver	0
Sheep (<i>Ovis aries</i>)	12	0.10–0.25 ^A	4–48	Muscle	0.64 ± 0.14
				Liver	0.40 ± 0.05
				Milk	0.0–0.017
				Blood	0.18–0.56
Sheep (<i>Ovis aries</i>)	13	0.15–0.20 ^A	2–32	Muscle	0.014–0.111
				Liver	<0.003–0.038
Ants	7	non-target	?	Pooled sample	1.4

^AAdministered deliberately (IP, IV or Oral).

generalisations can be made. For example, the 1080 residue levels found in our study were consistent with those levels reported previously (see Tables 1, 3, 4 and 5). Further, and for reasons unknown, 1080 residues in rats are generally greater than those in rabbits, and, at comparable dose levels, the concentrations of 1080 in the tissues of rabbits, sheep and dogs are amongst the lowest of any species examined (Table 5). 1080 residues are also often characterised by considerable variation between conspecifics (Casper *et al.* 1986; Hedgal *et al.* 1986; McIlroy and Gifford 1992; Eason *et al.* 1993; Gooneratne *et al.* 1994). The level of 1080 residues seen in tissues generally supports the notion that native Australian fauna should face a relatively low potential risk of secondary poisoning during 1080-based pest-control programs.

The potential risk to non-target species resulting from any retention of 1080 in poisoned animals will, however, depend upon three main factors: the persistence of 1080 in tissues (i.e. tissue residue levels), the location of those individuals that succumb to the toxic bait, and the longevity of carcasses of animals poisoned with 1080.

Tissue residues

The potential hazard posed from any 1080 residues in poisoned animals will depend upon the residue concentration, the persistence of these residues, and the distribution of 1080 throughout the various tissues.

Residues in the carcass of rabbits (minus the head, gut and skin) recovered from our standard 1080 One-shot oat control program were low, and ranged from <0.02 to 0.778 µg fluoroacetate g⁻¹. Mean whole blood and muscle residue levels in those rabbits that succumbed during our formal toxicity trials were also low and ranged from 0.011 to 0.648 µg g⁻¹. Such levels would generally pose little or no hazard to native wildlife. For example, an animal with a LD₅₀ of 2 mg 1080 kg⁻¹ and weighing between 20 g and 5000 g would need to consume 0.2–50 kg of tissue/carcass containing 0.2 µg fluoroacetate g⁻¹ and 0.004–1.0 kg if the food consumed contained 10 µg of fluoroacetate g⁻¹ to receive a LD₅₀ dose (Table 6). This consumption represents approximately 20–1000% of their respective body weights. Moreover, the amount of 1080 that can actually be ingested secondarily via poisoned prey/food is generally less than the amount

Table 6. Secondary poisoning and the amount of food (kg) needing to be consumed by a single animal to receive a LD₅₀ dose of 1080

A range of LD₅₀ values for 1080 are presented, and the level of 1080 in the food ranges from 0.01 to 10 µg g⁻¹

Body weight of non-target animal (g)	Amount of food required to receive an LD ₅₀ dose (kg)							
	LD ₅₀ (mg kg ⁻¹) / tissue residue level (µg g ⁻¹)							
	0.1/0.01	1/0.05	2/0.2	5/0.5	10/0.75	20/1	40/10	2/10
20	0.2	0.4	0.2	0.2	0.3	0.4	0.08	0.004
40	0.4	0.8	0.4	0.4	0.5	0.8	0.16	0.008
80	0.8	1.6	0.8	0.8	1.1	1.6	0.32	0.016
200	2.0	4.0	2.0	2.0	2.7	4.0	0.80	0.040
500	5.0	10.0	5.0	5.0	6.7	10.0	2.00	0.100
1000	10.0	20.0	10.0	10.0	13.3	20.0	4.00	0.200
2000	20.0	40.0	20.0	20.0	26.7	40.0	8.00	0.400
3000	30.0	60.0	30.0	30.0	40.0	60.0	12.00	0.600
4000	40.0	80.0	40.0	40.0	53.3	80.0	16.00	0.800
5000	50.0	100.0	50.0	50.0	66.7	100.0	20.00	1.000

required for individual animals to receive an Approximate Lethal Dose (ALD) for most species of concern (Martin and Twigg 2002). A 2.5–3.5-kg adult wedge-tailed eagle (LD_{50} 9.1 mg 1080 kg⁻¹), for example, would need to consume 2.3–3.2 kg of tissue that contained 10 µg fluoroacetate g⁻¹, and a 4–7-kg adult tiger quoll (*Dasyurus maculatus*) (LD_{50} 1.81 mg 1080 kg⁻¹) would need to consume 0.7–1.3 kg of the same tissue to receive a LD_{50} dose. Even where the bait loading was 1.7 times the recommended concentration, the tissues of poisoned rabbits generally posed only a minimal potential hazard to native wildlife with respect to their daily food requirements and their theoretical ingestion of 1080 (McIlroy and Gifford 1992). Furthermore, because 1080 is readily detoxified and/or excreted by living animals (Twigg and King 1991; Gooneratne *et al.* 1994), prey containing 1080 would need to be consumed over a relatively short time (<2–3 h) for substantial adverse effects to occur.

In contrast to many native species, an adult fox (LD_{50} 0.12 mg 1080 kg⁻¹) weighing 6–8 kg would need to consume only 140–190 g of tissue containing 5 µg fluoroacetate g⁻¹ to ingest an LD_{50} dose. The above findings suggest why there have been so few instances of secondary poisoning of native species as opposed to the recognised poisoning of some canids, felids and mustelids as a result of them consuming 1080-poisoned prey (e.g. McIlroy and Gifford 1991; McIlroy and Gifford 1992; Algar and Kinnear 1996; Heyward and Norbury 1999).

The levels of fluoroacetate (1080) found in the tissues of the rats used in our feeding trials ranged from <L.D. to 34 µg g⁻¹. However, the experimental procedure used, where these rats were provided with two One-shot oats (4.5 mg 1080 oat⁻¹) to ensure that they consumed some of the poisoned oats, meant that this scenario did, in fact, represent more than the likely worst-case-scenario. The LD_{50} for *R. norvegicus* is 1.68 mg 1080 kg⁻¹ (McIlroy 1982) and thus a 300-g rat needs to ingest only ~0.5 mg of 1080 to receive a LD_{50} dose. The rats in our feeding trials ingested 0.86–5.18 mg of 1080. In reality, 1080-poisoned rats are likely to present a low potential risk to native wildlife for the following reasons. (1) Even if the rat carcass contained up to 10 µg fluoroacetate g⁻¹ (our maximum recorded level in a rat carcass was 14.4 µg g⁻¹, and 15 of 17 rats had levels below 7 µg g⁻¹: Table 1), most native animal predators/scavengers would need to consume substantial quantities of 1080-poisoned carcasses relative to their body weight to receive a LD_{50} dose (Table 6). (2) The rate of lay and the method of laying baits would limit the potential exposure of rats to 1080. For example, a 0.5% One-shot oat bait mix has only 1 poison grain in 200 oats, and when laid at 6 kg km⁻¹ there is nominally ~1 poisoned oat for each metre of trail. Dried meat baits used for control of foxes are usually laid at only ~5 baits km² (Saunders *et al.* 1995; Thomson and Algar 2000). (3) Rats are unlikely to be present where many of the baiting programs are carried out, and therefore would not be exposed to 1080 baits.

We recognise that the potential risk of secondary poisoning will be influenced by the type of tissue consumed as the 1080 residue concentration will vary between the different tissue types. For example, the stomachs and their contents of poisoned animals, which often contain remnants of bait, can contain levels of 1080 higher than those reported for the other tissues (up to 35 µg g⁻¹ in New Zealand possums: Meenken and Booth 1997; and 12–56 µg g⁻¹ in ground squirrels, *Spermophilus beecheyi*: Casper *et al.* 1986). Liver also often has higher concentrations than many of the other tissues (Table 5). Although little is known about the residue levels in the tissues of Australian predators poisoned with 1080, the levels reported in coyotes (*Canis latrans*) and domestic dogs (*Canis familiaris*) are relatively low (Table 5), suggesting that the carcasses of 1080-poisoned wild dogs and foxes would present only a low potential risk to most native wildlife. There appears little risk of secondary poisoning to birds as a result of 1080 baiting programs (Hedgal *et al.* 1986; McIlroy and Gifford 1991, 1992; Martin and Twigg 2002). Because 1080 is rapidly leached from biological material by rainfall (Wheeler and Oliver 1978; McIlroy *et al.* 1988; Saunders *et al.* 2000), and as it is readily degraded by at least 20 species of soil microorganisms (Wong *et al.* 1992; Twigg and Socha 2001), it is unlikely that 1080 would persist in carcasses for an extended period. However, the activity of these microorganisms depends upon the provision of adequate soil temperature and moisture levels (Saunders *et al.* 2000; Twigg and Socha 2001).

Depending upon the type of tissue eaten, and because of their relatively small body size and their propensity to consume relatively large amounts of food (Calver *et al.* 1990), the small dasyurids may potentially face a greater risk of secondary poisoning than other native species. However, such fears have not been realised, at least in Western Australia. Red-tailed phascogales (*Phascogale calura*) numbers were monitored with 4.5-mg 1080 dried meat baits and without fox control during 1993–96 on 9 reserves in the Western Australian wheatbelt. After three years of fox control, red-tailed phascogale populations had responded positively and rapidly to the reduction in fox numbers as a result of the 1080 baiting programs. However, the level of response was also greatly influenced by the total annual rainfall at a particular site in the previous year (Friend and Scanlon 1996). The amount of rainfall affects the supply of invertebrate prey. A similar response has also been recorded for brush-tailed phascogales (*P. tapoatafa tapoatafa*). It appears that body size, growth and orphaning rates of these phascogales were similar between the long-baited sites (since 1970s, $n = 3$) and the sites where fox control commenced more recently (June 1993, $n = 3$). Further, the survival of 130 phascogales was also monitored using radio-telemetry as part of this study but no phascogales were reported to have died as a result of the 1080 baiting

programs. As with the red-tailed phascogale, the factors influencing the abundance and distribution of these phascogales are complex, but annual rainfall is probably the single most important factor influencing brush-tailed phascogale populations rather than the effects of the fox-reduction program *per se* in this particular habitat (Rhind 2002; Rhind and Bradley 2002).

Carcass location

It is well recognised that few carcasses of animals poisoned with 1080 during control programs are recovered. This is because most animals killed by the toxin generally die underground, undercover, or in their dens. This is the likely result of the lag phase associated with 1080 intoxication (Buffa *et al.* 1973; Twigg and King 1991) which 'allows' the animals to return 'home' once they begin to feel unwell. In our study, only 14% (i.e. 2 carcasses) were found in the open and 86% (12 carcasses) were recovered from the warrens ($n = 7$) or under thick scrub ($n = 5$). This suggests that only a small percentage of poisoned carcasses would be readily available to scavengers.

Regardless of the concentration of One-shot (0.25–4.0% bait mix) or conventional bait (0.02% A.I.) used during rabbit-control programs, most rabbits are killed within the first 5–8 days after baiting (Robinson and Wheeler 1983; Twigg *et al.* 2002). This, together with the rapid decay of rabbit carcasses (see below), suggests that any potential risk of secondary poisoning to non-target species from 1080-based control programs would only be short-term (i.e. generally <3 weeks).

Carcass degradation

In temperate Australia, depending upon the prevailing climatic conditions, rabbit carcasses generally are completely degraded to dry fur and bones in ~2 weeks (Monzu 1977; J. Bruce and L. Twigg, DAWA, unpublished data). Carcasses in hot arid climates will rapidly desiccate and most likely represent a less preferred or inedible food item to most species. The three exposed poisoned rat carcasses in our study were in an advanced state of decay within 6 days. Thus, this rapid decay suggests that the carcasses of animals poisoned during 1080 baiting programs will generally offer little potential risk to native non-target species. This is supported by the lack of incident reports regarding the secondary poisoning of native species during 1080-based pest-control programs. However, although it can be difficult to separate primary and secondary poisoning events regarding eutherian carnivores such as dogs, foxes and cats, these carnivores have been poisoned by feeding on prey (generally rabbits) that contained 1080 (McIlroy and Gifford 1991, 1992; Algar and Kinnear 1996; Meenken and Booth 1997; Heyward and Norbury 1999). Although the mechanisms are unknown, some native animals are also able to detect 1080 in their food (Sinclair and Bird 1984; Twigg

and King 1991) and this may also contribute to the low potential risk to non-target species associated with 1080 baiting programs.

Although routine cooking procedures appear to have little impact on the level of 1080 in cooked tissue (Milne *et al.* 2001), the level of 1080 residues in target species used for meat consumption are insufficient to pose any risk to human health (e.g. Macintosh and Staples 1959; Eason 1994; Milne *et al.* 2001). For example, given the maximum level of fluoroacetate recorded in our rabbit carcasses ($0.78 \mu\text{g g}^{-1}$), a 70-kg person ($\text{LD}_{50} 2 \text{ mg kg}^{-1}$) would need to consume approximately 180 kg of 1080-poisoned rabbit meat to ingest a LD_{50} dose. Even if food contained $20 \mu\text{g g}^{-1}$ 1080, a 70-kg human would still need to ingest 7 kg of 1080-containing food in a single sitting to receive an LD_{50} dose. Nevertheless, it is routine procedure to prohibit the taking of target species for human consumption during baiting programs.

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