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Tolerance to Sodium Monofluoroacetate in Dasyurids from Western Australia

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Abstract

The tolerances to sodium fluoroacetate (1080) were estimated for *Dasyurus geoffroii* (LD_{50}, ca. 7·5 mg 1080 kg^{-1}), *D. hallucatus* (ca. 7·5 mg kg^{-1}), *Antechinus flavipes* (ca. 11·0 mg kg^{-1}) and *Phascogale calura* (ca. 17·5 mg kg^{-1}) from Western Australia and comparisons were made with *D. viverrinus* (ca. 1·5 mg kg^{-1}) and *A. flavipes* (ca. 3·5 mg kg^{-1}) from south-eastern Australia. The species from Western Australia have had evolutionary exposure to naturally occurring fluoroacetate and were more tolerant to the toxin than dasyurids from south-eastern Australia. Presumably, they have acquired this tolerance through feeding on prey which had fed on plants containing fluoroacetate.

Introduction

Sodium monofluoroacetate (Compound 1080) is highly toxic to most unadapted mammals, particularly to eutherian carnivores (Atzert 1971; McIlroy 1981; McIlroy *et al.* 1986). Fluoroacetate is converted within the animal to fluorocitrate (Peters and Wakelin 1953) which inhibits the tricarboxylic acid cycle at the citrate stage (Morrison and Peters 1954). This results in energy deprivation and large increases in the concentration of citric acid in tissues (Buffa *et al.* 1973; Buffa and Peters 1949; Gal *et al.* 1956; Peters 1957). Elevation of plasma citrate concentration in response to dosing has been shown to reflect these increases and has been used as an index of the sensitivity of animals to fluoroacetate poisoning (King *et al.* 1978, 1981; Mead *et al.* 1985*a*; Oliver *et al.* 1977, 1979; Twigg 1986).

High levels of tolerance to fluoroacetate (1080) have been found in some populations of Western Australian mammals (King *et al.* 1978, 1981; Mead *et al.* 1985*a*; Oliver *et al.* 1977, 1979; Twigg 1986), birds (Twigg 1986), reptiles (McIlroy *et al.* 1985; Twigg 1986) and insects (Twigg 1986). These animals are almost all herbivorous or omnivorous and it has been suggested that their high level of tolerance occurs because they have ingested fluoroacetate from fluoroacetate-bearing plants of the genera *Gastrolobium* and *Oxylobium*. These plants are widespread in south-western Australia (Aplin 1971) and are known to constitute part of the diet of some adapted animals (King *et al.* 1981; Mead *et al.* 1985*b*).

The use of 1080 baiting to control foxes, *Vulpes vulpes*, rabbits, *Oryctolagus cuniculus*, and feral pigs, *Sus scrofa*, is becoming more common within the distributions of many species of dasyurids in Western Australia. Therefore, it is important to determine the tolerance of these dasyurids to fluoroacetate so that baits of adequate size, material and toxic level can be designed to ensure that the pest species is controlled with as little hazard as possible to non-target species. This is particularly so for *Dasyurus geoffroii* and *Phascogale calura* which are currently on the Department of Conservation and Land

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Management's rare and endangered species list. As it is possible that dasyurids in Western Australia may have evolved a secondary tolerance to fluoroacetate, and because of the need to minimise the hazard of accidental poisoning of dasyurid species, a study was made of the tolerance to fluoroacetate of these animals and the hazard posed to them during 1080 baiting programs for fauna management.

Materials and Methods

Housing of Experimental Animals

Dasyurids were housed individually in an animal house maintained at 23 ± 1°C with a 12 : 12 h photoperiod and 70% relative humidity. *Phascogale calura* and *Antechinus flavipes* were held in small weld mesh cages or terrariums and quolls (*Dasyurus viverrinus*, *D. geoffroii* and *D. hallucatus*) were kept in metabolism cages. Nest boxes with shredded paper were provided. Animals were fed insects, tinned dog food, fruit, and a supplement developed for small dasyurids (egg yolk and skim milk powders were the main constituents of the supplement). Quolls were also fed freshly killed laboratory rats or mice. Water was provided *ad libitum*.

Dosing and Bleeding

Before commencing the experiments, all animals were given 1 to 2 weeks to become accustomed to captivity. Only adult animals were used during the dosing trials. Dose groups, which included individuals of both sexes, were randomly selected from the number of animals available for each species (Table 1). However, all individuals of each species were utilised before any were re-dosed.

Commercial grade 1080 (94% sodium monofluoroacetate by HPLC analysis) was administered in aqueous solution to all dasyurids by intraperitoneal injection. Dosing volumes were less than 4% body weight and animals not poisoned received an equivalent amount of deionised water. Following the administration of 1080, animals were inspected at frequent intervals for the first 24 h and then daily for a further 14 days. Mortality and observed clinical symptoms were recorded. As most dasyurids received progressively higher amounts of 1080 over several months, they were given a minimum of 3–4 weeks between doses to allow recovery.

Blood was collected from the orbital sinus of *P. calura* and *A. flavipes* using heparinised haematocrit tubes. Blood samples for the quolls were mostly obtained from the lateral caudal vein using heparinised syringes. Lignocaine was applied before venipuncture. Occasionally when we could not obtain sufficient blood from the caudal vein after 2–4 bleeds, blood was taken from the orbital sinus.

Plasma was collected from whole blood following centrifugation (3000 g for 5 min) and was stored at −20°C until analysis.

Plasma Citrate Determination

Plasma citrate concentrations were determined using 20–60 μL aliquots of plasma as described by Twigg *et al.* (1986). This assay employs the enzymatic method of Mollering and Gruber (1966) as modified by Dagley (1974) except that 0·1 M Tris-HCl buffer, pH 8·2, was used in place of triethanolamine.

Chemicals

Citrate lyase (EC 4.1.3.6), malic dehydrogenase (EC 1.1.1.37) and nicotinamide adenine dinucleotide (reduced) were obtained from Sigma Chemical Co., St Louis, Missouri, U.S.A. Commercial Tenate brand sodium fluoroacetate (1080) was obtained from Rentokil Laboratories, Perth, Western Australia.

Statistical Analysis

Where appropriate, plasma citrate elevations were compared using the maximum increase in citrate concentration for each animal at a given dose. Differences between means for *A. flavipes* were determined using a single factor analysis of variance and the Scheffe test (Keppel 1973). However, as the data for the species of *Dasyurus* were obtained using uneven group sizes, and because the variances were found to be unequal (Levene test; Keppel 1973), the responses of the three *Dasyurus* species were compared using the Mann-Whitney U procedure (Zar 1974).

Results

Comparison of the sensitivity to fluoroacetate (1080) of the congeners and conspecifics was largely based upon their accumulation of citrate in the plasma following administration of 1080. However, to compare their sensitivity to that of other animals, we also approximated a LD_{50} for each population using the mortality of individuals which occurred during the plasma citrate elevation trials (Table 1). To assist with this approximation, within a

Table 1. The number of animals from which individuals were randomly selected, their origin, and the mortality which occurred during the plasma citrate elevation trials

Species	No. available	Source	Dose (mg 1080 kg^{-1}) 0·5	1·0	2·0	3·5	5·0	7·5	10·0	12·5	15·0
Dasyurus viverrinus	6	Captive bred A.C.T. (ex Tas.)	0/3	0/3	5/5						
D. geoffroii	3	Collie and Dwellingup, W.A.			0/3	0/3	0/3	–	1/1		
D. hallucatus	6	Dolphin Island, W.A.			0/3	–	0/4	–	3/3		
Antechinus flavipes											
W.A.	8	Dwellingup and Pemberton			0/3	–	0/4	1/5	1/3	0/2	–
S.A.	4	near Adelaide			0/4	–	3/3				
Phascogale calura	8	Yornaning, W.A.			0/3	–	0/3	0/3	0/6	–	1/3

genus, the increases in plasma citrate concentration and signs of poisoning displayed by those individuals which survived were compared with those of individuals which succumbed.

Changes in plasma citrate concentration for the quolls were determined using basal levels of citrate in plasma samples; these were obtained for each individual immediately before dosing (time zero). The concentrations of citrate in the plasma of undosed *Dasyurus* were; *D. viverrinus*, 94 ± 9 μM ($n = 15$); *D. geoffroii*, 134 ± 9 μM ($n = 13$) and *D. hallucatus*, 133 ± 11 μM ($n = 14$). However, because of the relatively small size of *P. calura* and *A. flavipes*, a blood sampling regime involving several serial bleeds was considered too stressful. Consequently, the mean basal level of citrate in the plasma of undosed individuals of each species was determined 2–3 weeks prior to the dosing experiments. These levels were used as the assumed base level against which to measure increases in plasma citrate concentration following administration of 1080. The mean basal levels of plasma citrate for the Western Australian *A. flavipes*, the South Australian *A. flavipes* and *P. calura* were 121 ± 15 μM ($n = 12$), 101 ± 11 μM ($n = 4$) and 55 ± 11 μM ($n = 8$) respectively.

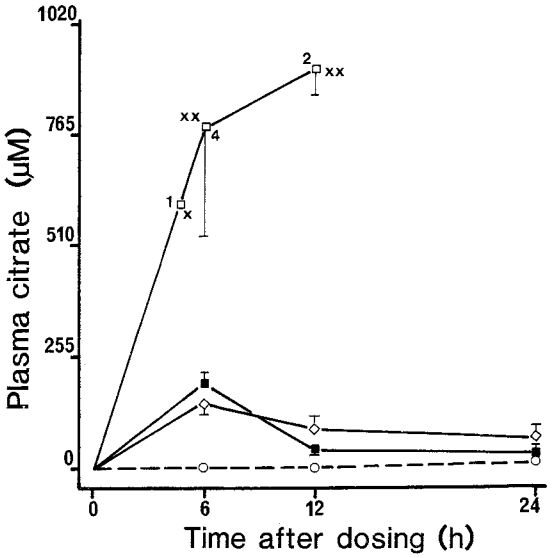

Fig. 1. The mean and standard error of the increase above base-level (time zero) values of plasma citrate concentrations of *D. viverrinus* following administration of sodium fluoroacetate (1080). (○) Undosed ($n = 1$); (◇) 0·5 mg 1080 kg^{-1} ($n = 3$); (■) 1·0 mg 1080 kg^{-1} ($n = 3$); (□) 2·0 mg 1080 kg^{-1} ($n = 5$). Internal numbers refer to the number of individuals where the group size has been reduced due to the death (X) of animals.

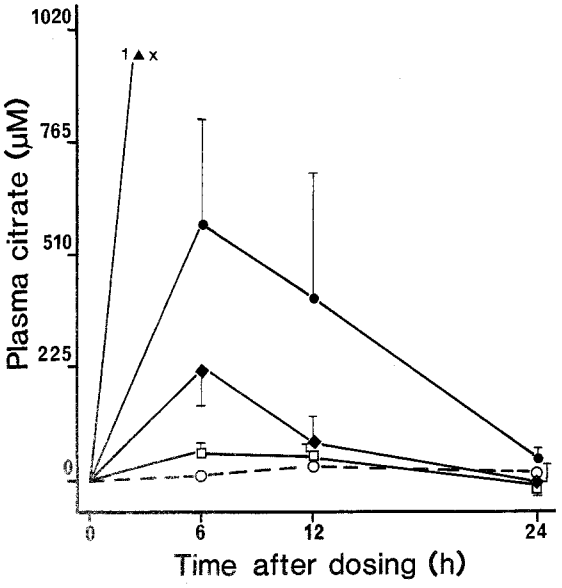

Fig. 2. The mean and standard error of the increase above base-level (time zero) values of plasma citrate concentrations of *D. geoffroii* following administration of sodium fluoroacetate (1080). (○) Undosed ($n = 1$); (□) 2 mg 1080 kg^{-1} ($n = 3$); (◆) 3·5 mg 1080 kg^{-1} ($n = 3$); (●) 5 mg 1080 kg^{-1} ($n = 3$); (▲) 10 mg 1080 kg^{-1} ($n = 1$). X indicates death of an animal.

Dasyurus

Dasyurus viverrinus, whose range does not include areas containing fluoroacetate-bearing vegetation, is more sensitive to fluoroacetate intoxication than are *D. geoffroii* or *D. hallucatus*, whose distributions often coincide with those of the toxic plants. Elevations in plasma citrate concentrations in response to dosing with 1080 are given for each species in Figs 1, 2 and 3.

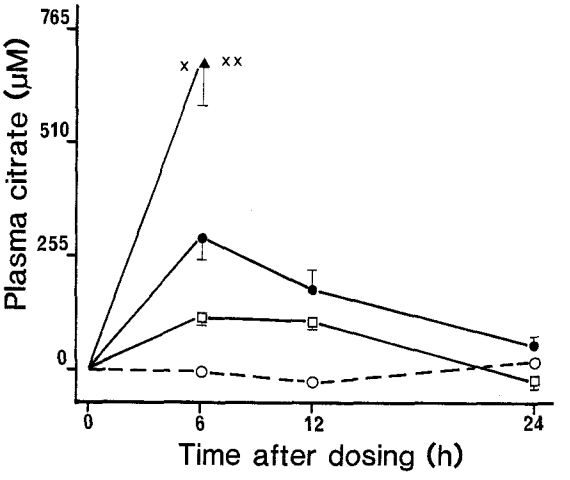

Fig. 3. The mean and standard error of the increase above base-level (time zero) values of plasma citrate concentrations of *D. hallucatus* following administration of sodium fluoroacetate (1080). (○) Undosed ($n=1$); (□) 2 mg 1080 kg^{-1} ($n=3$); (●) 5 mg 1080 kg^{-1} ($n=4$); (▲) 10 mg 1080 kg^{-1} ($n=3$). X indicates death of an animal.

Administration of 1·0 mg 1080 kg^{-1} increased the plasma citrate concentration of *D. viverrinus* considerably, and when given 2 mg 1080 kg^{-1}, all five *D. viverrinus* died within 13 h of dosing (Fig. 1). None of the *D. geoffroii* or *D. hallucatus* administered 2 mg 1080 kg^{-1} succumbed, nor did they display any obvious signs of fluoroacetate intoxication. Furthermore, the concentrations of citrate in the plasma of *D. viverrinus* administered 2 mg 1080 kg^{-1} were significantly greater [$P<0{\cdot}05$; $U=15$, $(\alpha)_2$; 5, 3] than were those of *D. geoffroii* and *D. hallucatus* at this dose level (Figs 1, 2 and 3).

All *D. geoffroii* and *D. hallucatus* administered 3·5 or 5·0 mg 1080 kg^{-1} and 5·0 mg 1080 kg^{-1} respectively, survived (Table 1). However, one of the three *D. geoffroii* and three of the four *D. hallucatus* given 5 mg 1080 kg^{-1} developed signs of fluoroacetate poisoning and the concentration of citrate in the plasma of the poisoned quolls increased considerably (Figs 2 and 3). The onset of the signs of poisoning was obvious within 30 min of dosing in *D. hallucatus*. This was much more rapid than in *D. geoffroii* or *D. viverrinus* which did not display obvious signs of intoxication until 1 or 2 h after dosing. It is possible, therefore, that the concentration of citrate in the plasma of *D. hallucatus* given 5 or 10 mg 1080 kg^{-1} may have been greater at its peak than that indicated in Fig. 3. However, at the 5 mg kg^{-1} dose level, the increases in plasma citrate concentrations exhibited by *D. hallucatus* and *D. geoffroii* 6 h after dosing (Figs 2 and 3) did not differ significantly [$P>0{\cdot}05$; $U=6$ $(\alpha)_2$; 3, 4]. For this reason, and because the three *D. hallucatus* administered 10 mg 1080 kg^{-1} displayed a marked increase in plasma citrate concentration and died within 7 h of dosing (Fig. 3), only one *D. geoffroii* was given 10 mg 1080 kg^{-1}. This individual succumbed 3 h after dosing and also displayed a marked elevation in plasma citrate concentration similar to that found for those *D. viverrinus* which received a lethal dose (Figs 1 and 2).

Thus the mortality which occurred during the plasma citrate determinations, together with the increases in plasma citrate concentration after various doses of 1080, indicate that the approximate LD_{50} values for these dasyurids are: *D. viverrinus*, 1·5 mg 1080 kg^{-1}; *D. geoffroii*, 7·5 mg 1080 kg^{-1} and *D. hallucatus*, 7·5 mg 1080 kg^{-1} (Table 1; Figs 1, 2 and 3).

Antechinus flavipes

Antechinus flavipes in the south-west of Western Australia is exposed to fluoroacetate-

bearing vegetation and is less sensitive to fluoroacetate intoxication than are conspecifics from South Australia not exposed to the toxic plants (Fig. 4). When administered 5 mg 1080 kg^{-1}, all three *A. flavipes* from South Australia succumbed within 6 h of dosing while displaying plasma citrate concentrations significantly greater ($P<0{\cdot}005$; $F=51{\cdot}91$; 1, 5) than those of the Western Australian conspecifics (Fig. 4). None of the Western Australian *A. flavipes* succumbed at this dose level, but one of the five individuals given 7·5 mg 1080 kg^{-1} died 4 h after dosing (Fig. 4). Some of the survivors of the Western Australian *A. flavipes* were dosed at higher levels than those shown in Fig. 4. One of three

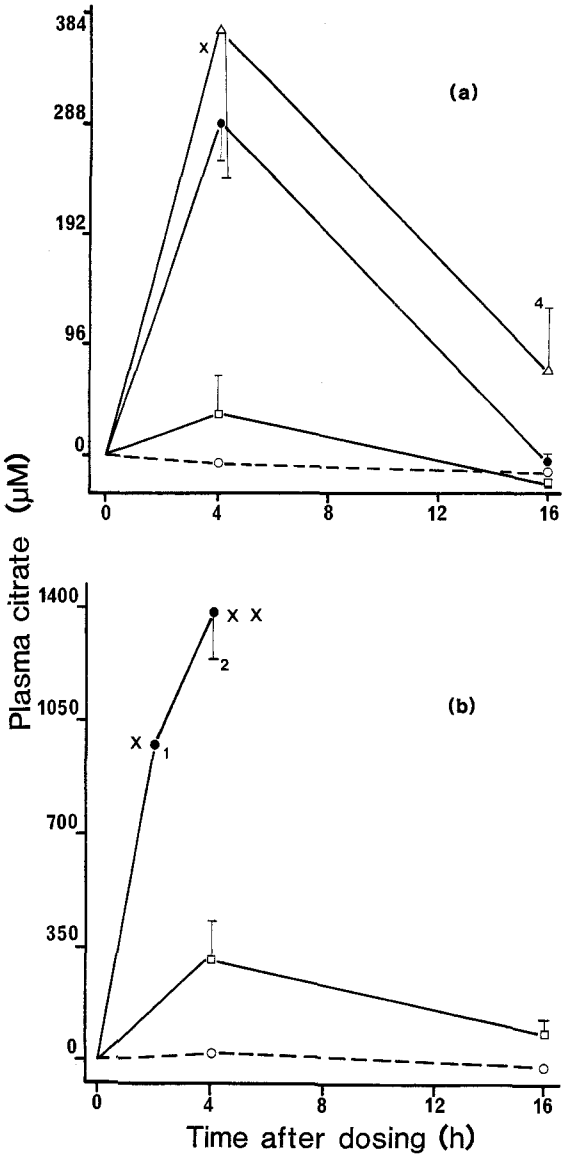

Fig. 4. The mean and standard error of the increase above the mean base-level value of plasma citrate concentrations of *A. flavipes* from (*a*) Western Australia and (*b*) South Australia following administration of sodium fluoroacetate (1080). (○) Undosed ($n=1$); (□) 2 mg 1080 kg^{-1} (W.A. $n=3$; S.A. $n=4$); (●) 5 mg 1080 kg^{-1} (W.A. $n=4$; S.A. $n=3$); (△) 7·5 mg 1080 kg^{-1} ($n=5$). Internal numbers refer to the number of individuals where the group size has been reduced due to the death (X) of animals.

animals given 10 mg 1080 kg^{-1} displayed a marked increase in plasma citrate concentration (568 μM) and died 1·5 h after dosing. The two *A. flavipes* administered 12·5 mg 1080 kg^{-1} survived (Table 1), but one animal exhibited signs of fluoroacetate intoxication. The mean plasma citrate concentration of these individuals 4 h after dosing was 271 μM.

The mortality which occurred during the determination of plasma citrate and the elevations in plasma citrate concentration following different doses of 1080, suggest that

the LD_{50} value for *A. flavipes* from the south-west of Western Australia is in the region of 10–12·5 mg 1080 kg^{-1}, while that of the conspecifics from South Australia is approximately 3·5 mg 1080 kg^{-1} (Table 1; Fig. 4).

Phascogale calura

Phascogale calura, which coexists with fluoroacetate-bearing vegetation, was moderately tolerant to fluoroacetate. Changes in plasma citrate concentration in response to dosing are presented in Fig. 5. Despite the somewhat stressful bleeding regime, only one of three individuals succumbed when given 15 mg 1080 kg^{-1} (Fig. 5). No deaths occurred at doses below this level (Table 1), which, together with the citrate elevation data presented in Fig. 5, suggests that the LD_{50} for this species is between 15 and 20 mg 1080 kg^{-1}. This is higher than the estimated LD_{50} for *A. flavipes* (10–12·5 mg 1080 kg^{-1}) from Western Australia.

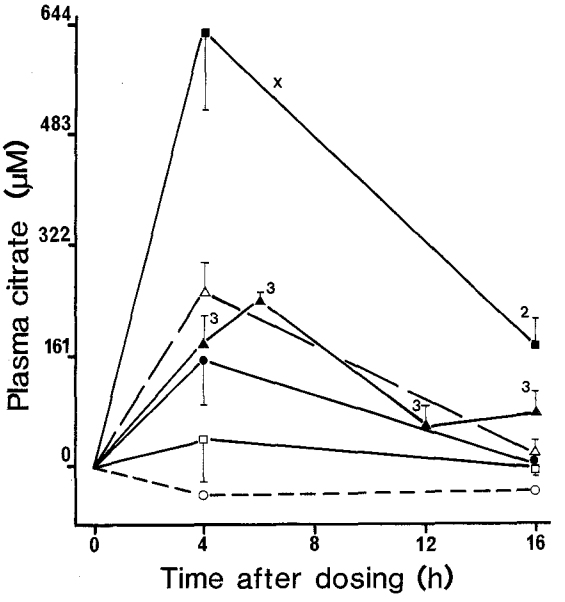

Fig. 5. The mean and standard error of the increase above the mean base-level value of plasma citrate concentrations of *P. calura* following administration of sodium fluoroacetate (1080). (○) Undosed ($n=2$); (□) 2 mg 1080 kg^{-1} ($n=3$); (●) 5 mg 1080 kg^{-1} ($n=3$); (△) 7·5 mg 1080 kg^{-1} ($n=3$); (▲) 10 mg 1080 kg^{-1} ($n=6$); (■) 15 mg 1080 kg^{-1} ($n=3$). Internal numbers refer to the number of individuals and X indicates death of an animal. Of the six animals given 10 mg kg^{-1}, plasma samples were collected from three individuals 4 and 16 h after administration of 1080 and plasma was collected from the other three animals 6 and 12 h after dosing.

At the completion of other dosing, a dosing trial was conducted to determine whether the pooled initial level of plasma citrate adequately indicated the base level concentration of citrate in the plasma of the animals. Plasma citrate concentration was determined for three *P. calura* immediately prior to administration of 7·5 mg 1080 kg^{-1} (time zero) and the elevation in plasma citrate concentration was determined 4 and 16 h after dosing. The response of these individuals was similar to that of the other dosed animals (Fig. 5).

Discussion

As some individuals were dosed more than once, the LD_{50}s presented in this paper do not represent absolute values, but rather, enable a comparison between populations of their sensitivity to fluoroacetate. There are several instances where LD_{50}s have been approximated using small group sizes and where animals have been redosed (McIlroy 1981, 1983*b*; King *et al.* 1981; Mead *et al.* 1985*a*).

The higher sensitivity to fluoroacetate of *D. viverrinus* compared with that of *D. geoffroii* or *D. hallucatus* (Table 2; Figs 1, 2 and 3) is consistent with the differences shown for other species of animals which occur in both the south-east and south-west of Australia (King *et al.* 1978, 1981; Mead *et al.* 1985; McIlroy 1981; McIlroy *et al.* 1986; Oliver *et al.* 1977, 1979; Twigg 1986). The current and fossil distribution of *D. viverrinus* does not include areas where toxic species of *Gastrolobium* and *Oxylobium* occur, whereas the other two species of *Dasyurus* may both inhabit areas where these plants are found. *D. hallucatus* from Nourlangie, Northern Territory, whose distribution coincides with that of toxic

G. grandiflorum, also seem to have developed limited tolerance to fluoroacetate intoxication. Their LD_{50}, assuming a 94% sodium fluoroacetate dosing solution, is 6·02 mg 1080 kg^{-1} (McIlroy 1981) which is not significantly different from the estimated LD_{50} value obtained in this study. The sensitivity of the *D. viverrinus* in our study appears to be greater than that previously reported for this species (LD_{50} 3·97 mg 1080 kg^{-1}, 94% dosing solution; McIlroy 1981). This difference may have resulted from the stress associated with our serial blood sampling regime. The tolerance of fluoroacetate of unadapted dasyurids is, however, greater than that of unadapted eutherians (McIlroy 1981). This is possibly a result of dasyurids generally having lower metabolic rates compared to those of equivalent size eutherian mammals (MacMillen and Nelson 1969).

Table 2. Comparison of the relative susceptibility of five non-target species of dasyurid and four target species to 1080 poison

*In Western Australia, each factory meat bait and 1080-impregnated one-shot oat contains approximately 5–6 mg 1080. 'Conventional' 1080 oats contain approximately 0·34 mg 1080 per oat (Robinson and Wheeler 1983). Reference 1: this paper; 2: McIlroy (1981); 3: King unpublished; 4: McIlroy (1983*a*); 5: Wheeler and Hart (1979). Weight range of adults after Strahan (1983)

Species	LD_{50} (mg 1080 kg^{-1})	Reference	Weight range of adult (kg)	Amount of 1080* equivalent to LD_{50} (mg)
Phascogale calura	*c.* 17·5	1	0·04–0·07	0·7–1·2
Antechinus flavipes				
W.A.	*c.* 11	1	0·02–0·08	0·22–0·88
S.A.	*c.* 3·5	1	0·02–0·08	0·07–0·28
Dasyurus geoffroii	*c.* 7·5	1	0·71–2·10	5·3–15·8
Dasyurus viverrinus	*c.* 1·5	1	0·70–2·00	1·1–3·0
Dasyurus hallucatus				
W.A.	*c.* 7·5	1	0·30–0·90	2·3–6·8
N.T.	*c.* 6·02	2	0·30–0·90	1·8–5·4
Felis catus	0·40	2	2·50–6·40	1·0–1·8
Canis familiaris	0·11	2	9·60–16·00	1·1–1·8
Vulpes vulpes	*c.* 0·13	3	4·70–8·30	0·6–1·1
Oryctolagus cuniculus	*c.* 0·42	4, 5	0·96–2·42	0·4–1·0

A similar situation to that shown by *Dasyurus* from south-eastern and south-western Australia occurs within populations of *A. flavipes* from south-western Australia and from near Adelaide. *Antechinus flavipes* from the Adelaide area are similar in their sensitivity to 1080 to other species of eastern Australian *Antechinus* studied by McIlroy (1981). However, they are considerably more sensitive to fluoroacetate than are conspecifics from the south-west of Western Australia (Table 2; Fig. 4). *Antechinus flavipes* from Western Australia appears to be more heterogeneous for fluoroacetate tolerance than is *P. calura*. In these *Antechinus*, deaths occurred over a broader range of doses (Table 1) and citrate accumulation in the plasma of poisoned animals also displayed greater variability than in *P. calura* (Figs 4 and 5). The lower tolerance and the greater heterogeneity of *A. flavipes* could be explained by lower selection pressure for this trait. The diet of *A. flavipes* consists mostly of insects, but it can include a wide variety of items ranging from flowers and nectar to small mammals and birds (Hindmarsh and Majer 1977; Van Dyck 1983). As well as inhabiting areas where the toxic plants are common, *A. flavipes* is also found in other areas of Western Australia where the plants are absent or infrequent. Thus the more varied diet of *A. flavipes*, together with its greater variety of habitat preferences, could result in lower selection pressure for the development of tolerance to fluoroacetate. This may account for the heterogeneity in the tolerance of this species.

Phascogale calura (Table 2; Fig. 5) is the most tolerant species of dasyurid from south-western Australia with an estimated LD_{50} of 17·5 mg 1080 kg^{-1}. Its current distribution is

largely confined to areas in the south-western wheat belt of Western Australia which have climax vegetation communities with an abundance of toxic species of *Gastrolobium* and *Oxylobium* (Kitchener 1981). *Phascogale calura* is an opportunistic feeder which forages extensively on the ground. Its diet includes a wide array of insects, some small mammals and birds and occasionally carrion. However, the favoured food items are cockroaches and beetles with lepidopteran larvae being included in the spring (Kitchener 1981). The greater tolerance of *P. calura* may therefore be explained by its close association with the toxic species of *Gastrolobium* and *Oxylobium* through the ingestion of insects which feed on these plants. The diets of *D. geoffroii* and *D. hallucatus* are more varied than are those of *A. flavipes* and *P. calura* (Arnold 1983; Begg 1983); the differences in the tolerance to fluoroacetate between these species of dasyurid is likely to be due to the varying degrees of specialisation in feeding habits by each species. The relatively high levels of tolerance to fluoroacetate exhibited by those dasyurids which coexist with fluoroacetate-bearing vegetation suggest that a secondary tolerance to fluoroacetate has evolved in the insectivorous/carnivorous marsupials of Western Australia.

The risk posed to the dasyurid species from 1080 baiting programs for the control of eutherian pests such as foxes, dingoes and rabbits is shown in Table 2. Because of its reasonably large size and moderately high tolerance to 1080, *D. geoffroii* is the only species which appears at little risk from current 1080 baiting programs. The higher tolerance of *A. flavipes* and *P. calura* is somewhat negated by their small size. However, it is not only the sensitivity of individuals to fluoroacetate which determines the hazard to non-target species. Some species of mammals are known to reject food containing fluoroacetate even though they have not been previously challenged by the toxin (Morgan 1982; Sinclair and Bird 1984). Investigations are currently underway to determine the acceptability and consumption of baits in several species of small sized mammals. Furthermore, changes to baiting campaigns for control of pest species found within the distribution of those dasyurids considered rare are being assessed or have been implemented. For example, larger, less concentrated meat baits are now being used in some areas where dasyurids are considered at risk.

Acknowledgments

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