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MINI-REVIEW

Minireviews provides an opportunity to summarize existing knowledge of selected ecological areas, with special emphasis on current topics where rapid and significant advances are occurring. Reviews should be concise and not too wide-ranging. All key references should be cited. A summary is required.

The impact of fluoroacetate-bearing vegetation on native Australian fauna: a review

Laurie E. Twigg and Dennis R. King

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Fluoroacetate is a highly toxic compound which is produced by three genera of plants in parts of south western and northern Australia, particularly the south west corner of Western Australia. Native animals in these regions have coexisted with this toxic vegetation for at least several thousand years, and many species have developed a marked tolerance to fluoroacetate. Factors influencing their level of tolerance, the possible causal mechanisms, and the implications to fauna management are discussed.

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Fluoroacetic acid, often referred to as fluoroacetate, is one of the most toxic substances known. Its sodium salt, Compound 1080 (sodium monofluoroacetate), is widely used in many countries for controlling vertebrate pests (e.g. feral pigs, *Sus scrofa;* foxes, *Vulpes vulpes;* coyotes, *Canis latrans;* dingoes, *Canis familiaris dingo;* rabbits, *Oryctolagus cuniculus;* and many species of rodents).

Fluoroacetate was first synthesized in 1896 and its toxicity to insects was recognised in the 1920's when it was patented as a moth-proofing agent. Its potential as a vertebrate pesticide was recognised in the 1940's, and it was first used as a rodenticide in the USA in 1945 (Kalmbach 1945). In the early 1950's, 1080 was introduced into Australia to control rabbits, *O. cuniculus* (Meldrum and Bignell 1957). It is now used in Australia to control a variety of vertebrate pests (McIlroy 1981a).

Many years after 1080 was first synthesized, its toxic principle, fluoroacetate, was found to occur naturally in five genera of plants. Three of these genera occur only in Australia and where this vegetation occurs, native animals have developed varying degrees of tolerance to this potent toxin (Oliver et al. 1979, King et al. 1981, Twigg et al. 1986, King et al. 1989, Twigg and King 1989). Here we review the culmination of approximately 15 yr of research into the role of fluoroacetate-bearing vegetation in plant animal interactions in Australia.

Natural occurrence

Fluoroacetate was first isolated as the toxic principle of the African plant, *Dichapetalum cymosum* (Gifblaar) in 1944. Until that time, no naturally occurring organoflu-

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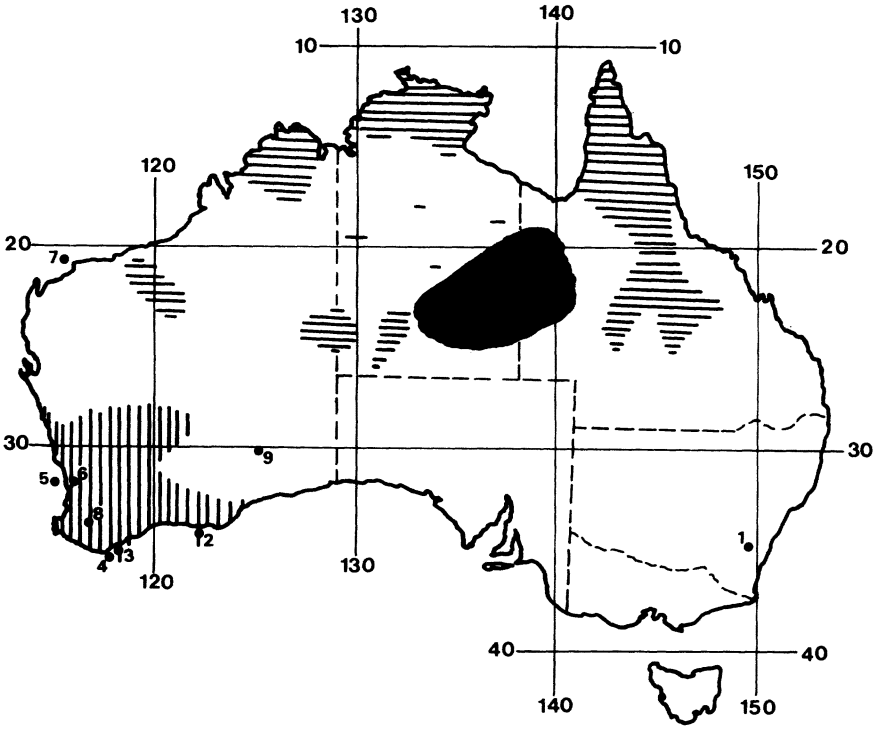


Fig. 1. Distribution of fluoroacetate-bearing vegetation in Australia. Vertical lines: 33 toxic species of *Gastrolobium* and *Oxylobium*. Horizontal lines: *Gastrolobium grandiflorum*. Solid area: *Acacia georginae* (from Twigg 1986). Various locations referred to in the text are: 1= Canberra, 2= Mondrain Island*, 3= Bald Island*, 4= Michaelmas Island, 5= Rottnest Island, 6= Perth, 7= Barrow Island, 8= Kulikup*, 9= Rawlinna. *= has fluoroacetate-bearing vegetation.

orine compounds were known. Since then, several other species of *Dichapetalum* have also been shown to produce fluoroacetate (Vickery et al. 1973, Vickery and Vickery 1975). Fluoroacetate has also been isolated from *Palicourea marcgravii*, a South American species known to be poisonous (de Oliviera 1963).

In Australia, fluoroacetate has been identified as the toxic principle of thirty-five plant species from three genera of Leguminosae (Fig. 1). These plants occur on a variety of soil types but not on deep sandy soils or soils which are calcareous in origin (Aplin 1971). Their abundance in a region varies, but in some areas a single species can constitute 80% of the understorey vegetation.

Thirty-three of the Australian species which produce fluoroacetate belong to the genera *Gastrolobium* or *Oxylobium* and are confined to the south west corner of Western Australia (Fig. 1). Some of these species contain more fluoroacetate than any other fluoroacetate-producing plant known. Air dried leaves of *G. bilobum* (Heart-leaf poison) and *O. parviflorum* (Box poison), for example, can contain up to 2600 mg of fluoroacetate kg^{-1} (Aplin 1971) and their seeds (e.g. *G. bilobum*) can have in excess of 6500 mg of fluoroacetate kg^{-1} (R. Mead pers. comm.). Those species occurring in northern Australia (Fig. 1) are less toxic. Air dried leaves of *Acacia georginae* (Gidyea) can contain up to 25 mg of fluoroacetate kg^{-1} (Oelrichs and McEwan 1961, 1962) while those of *G. grandiflorum* (Wall-flower poison) have up to 185 mg kg^{-1} (McEwan 1964).

However, the fluoroacetate-content of these plants displays both regional and seasonal variation. Fluoroacetate concentration also varies considerably within different parts of the plants, with ephemeral tissues such as flowers, seeds and young leaves containing the greatest concentrations. This is consistent with chemically mediated defence strategies which utilise poisonous compounds (Cates and Rhoades 1977), as the plants are protecting those parts most essential to them.

Significant economic losses of domestic livestock have occurred following the ingestion of fluoroacetate-bearing vegetation both in Africa (Peters et al. 1965, Nwude et al. 1977) and Australia (Bell et al. 1955, Aplin 1971). The poisonous nature of *Gastrolobium* species in Western Australia was recognised as early as 1839 and fears were held that this might discourage settlement of the colony (Cameron 1977). Species of *Oxylobium* also occur in New South Wales and Victoria, but none has been demonstrated to contain fluoroacetate, and there have been no reports implicating them in the poisoning of domestic livestock.

Trace amounts of fluoroacetate or fluorocitrate have been found in a variety of other plants (Lovelace et al. 1968, Peters 1972, Peters and Shorthouse 1972, Vartiainen and Kauranen 1984) but such plants are often associated with soils that have artificially elevated fluoride levels. The ability of plants to produce fluoroacetate appears to be relatively widespread, but in most species, this is usually in insignificant quantities.

Australian soils generally contain low levels of fluo-

Table 1. The sensitivities to fluoroacetate (mg 1080 kg $^{-1}$) of native and introduced Australian animals; (a) which coexist with, or have had past exposure to, fluoroacetate-bearing vegetation; (b) with possible exposure to fluoroacetate-bearing vegetation; and (c) animal populations from outside the known distribution of the toxic plants. LD_{50}'s are from References 1–17 and our unpublished data. A = precise LD_{50}. * = more than one population with differing degrees of exposure tested. In = indigenous to Western Australia; He = herbivore; Om = omnivore; Ca = carnivore

Species	Coexist/ past exposure Approx. LD_{50}	Possible exposure Approx. LD_{50}	Outside known range of the toxic plants Approx. LD_{50}
REPTILES			
Tiliqua rugosa (Om)	500,>800*	–	214[A]
Varanus gouldii (Ca)	?	50	50
V. rosenbergi (Ca)	200–300	–	40
V. varius (Ca)	–	–	100
SEED EATING BIRDS (He)			
Anas superciliosa	15–20	–	18.9[A]
Chenonetta jubata	12.5	–	12.6[A]
Phaps chalcoptera	40	–	25
Ocyphaps lophotes	25	–	25
Geopelia humeralis	16.3	–	?
G. cuneata	35.5	–	?
Polytelis anthopeplus	12.5	–	?
Barnardius zonarius	11.5[A]	–	9
Platycercus icterotis (In)	75	–	–
Purpureicephalus spurius (In)	25	–	–
Platycercus elegans	–	–	0.9
P. eximius	–	–	3.5
Psephotus haematanotus	–	–	5.3
MAMMALS			
DASYURIDS (Ca)			
Phascogale calura (In)	17.5	–	–
Antechinus flavipes	12.5	–	3.5
A. stuartii	–	–	1.9[A]
A. swainsonii	–	–	2.2[A]
Sminthopsis crassicaudata	?	3	2.1[A]
S. macroura	?	–	1.0
S. granulipes	8.5	–	–
S. hirtipes	7.0	–	?
Dasyuroides byrnei	–	–	2.9
Parantechinus apicalis (In)	10.0	–	–
Ningaui timealyi	12.0	–	–
Planigale maculata	4.0	–	?
Dasyurus hallucatus	6–7.5	–	–
D. geoffroii	7.5	–	–
D. viverrinus	–	–	1.5
D. maculatus	–	–	1.9[A]
Sarcophilus harrisi	–	–	4.2[A]
BANDICOOTS (Om)			
Isoodon obesulus	20	–	7
I. auratus	8.9[A]	–	–
I. macrourus	–	?	3.5
Perameles bougainville	9	–	–
P. nasuta	–	–	7.7[A]
P. gunnii	–	–	5.4[A]
Macrotis lagotis	15	–	?
WOMBATS (Om)			
Vombatus ursinus	–	–	0.2[A]
Lasiorhinus latifrons	–	–	0.2[A]
POSSUMS			
Trichosurus vulpecula (He/Om)	125	–	0.75[A]
MACROPODS (He)			
Bettongia penicillata	100	–	–
B. gaimardi	–	?	1.0
B. lesueur	15	–	–
Potorous tridactylus	–	–	0.2
Thylogale billardierii	–	–	0.1[A]
Lagorchestes conspicillatus	5	–	?
Lagostrophus fasciatus (In)	125	–	–
Macropus eugenii	5, 20*	–	0.3
M. fuliginosus	40	–	40
M. giganteus	–	–	2.0
M. rufogriseus	–	–	0.2
M. rufus	–	2–4	?
Setonix brachyurus (In)	10, 40*	–	–
RODENTS (He)			
Zyzomys argurus	3, 5*[A]	–	–
Pseudomys praeconis (In)	4–5	?	–
P. australis	–	–	1.2
P. occidentalis (In)	25	–	–
P. shortridgei	25	–	?
Rattus fuscipes	20–80*[A]	–	1.1[A]
R. lutreolus	–	1.3	?
R. sordidus	5, 20*	–	0.3
Hydromys chrysogaster	–	–	3.0
Notomys mitchelli	–	10, 20, 19.4*[A]	–
N. alexis	–	–	0.2
INTRODUCED (UNADAPTED) SPECIES			
Streptopelia senegalensis	–	5.93[A]	–
S. roseogrisea	–	–	7.5
Columba livia	–	–	3.98[A]
Mus domesticus	–	8.3[A]	–
Rattus rattus	–	0.8	–
Oryctolagus cuniculus	–	0.4[A]	–
Sus scrofa	–	4.1[A]	–
Capra hircus	–	0.5[A]	–
Felis catus	–	0.4[A]	–
Vulpes vulpes	–	0.13	–
Canis familiaris dingo	–	0.11[A]	–
Homo sapiens	–	–	2.0

ride, so plants which produce fluoroacetate must actively concentrate fluoride in their tissues. This results in a metabolic cost to the plant (Peters 1972). While there have been attempts to elucidate the mechanisms involved in fluoroacetate synthesis (Mead and Segal 1972, Peters 1972, Vickery and Vickery 1975), the metabolic pathways involved are not known. However, the plants appear to avoid autointoxication by their inability to readily convert fluoroacetate into fluorocitrate (Eloff and von Sydow 1971) and to a lesser degree, by the relative insensitivity of their aconitate hydratase to fluorocitrate (Treble et al. 1962).

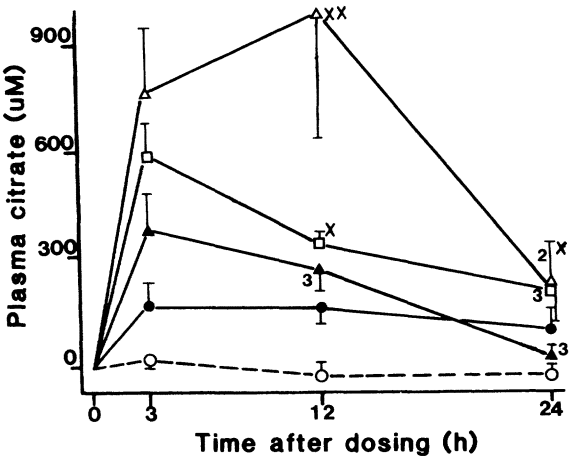


Fig. 2. The mean and standard error for the increase above base-level (time zero) values of plasma citrate concentration following adminstration of fluoroacetate (1080) to *Barnardius zonarius* from Western Australia. Open circles, undosed (n=3); closed circles, 2 mg; closed triangles, 5 mg; open squares, 10 mg; and open triangles, 15 mg 1080 kg^{-1}. For all dosed groups, n=4. Internal numbers refer to group size after death of an animal (X). From Twigg (1986).

Mode of action

Fluoroacetate is highly toxic to a wide range of animals by all the common routes of administration. Most unadapted mammals are fatally poisoned by less than 2 mg kg^{-1} (Atzert 1971). Birds are generally the least sensitive of endothermic vertebrates and ectotherms are relatively insensitive to fluoroacetate (Table 1; Chenoweth 1949, Atzert 1971, McIlroy et al. 1985).

Fluoroacetate is converted within the animal to fluorocitrate (Peters and Wakelin 1953) which competitively inhibits the tricarboxylic acid (TCA) cycle enzyme, aconitate hydratase [EC 4.2.1.3] and blocks the TCA cycle at the citrate stage (Morrison and Peters 1954). This results in accumulation of citrate in the tissues and plasma, energy deprivation, and death (Peters 1957, Buffa et al. 1973, Twigg 1986). Synthesis of fluorocitrate occurs in the mitochondria and the fluorocitrate formed inhibits mitochondrial aconitate hydratase but does not appear to affect the cytoplasmic isozyme in vivo (Buffa et al. 1973). There is evidence to suggest that fluorocitrate may inhibit citrate transport into and out of mitochondria (Kirsten et al. 1978), and fluorocitrate may also have an inhibitory effect on succinate dehydrogenase [EC 1.3.99.1] in intact rat mitochondria (Fanshier et al. 1964). The high levels of citrate concentration occasioned during fluoroacetate intoxication can also have an inhibitory effect on the glycolytic enzyme, phosphofructokinase [EC 2.7.1.11] (Peters 1972).

The actual cause of death from fluoroacetate poisoning is not fully known. Accumulation of citrate results in changes in cellular cation concentration which can cause ion imbalance within cells (Buffa et al. 1973). The resulting ionic and osmotic modifications together with the decline in ATP concentration may be sufficient to induce death. Energy, and ionic, dependent transport mechanisms can also be affected (Buffa et al. 1973).

There is a latent period between the time fluoroacetate is ingested and when signs of poisoning first appear (30 min to 3 h in endotherms), which presumably is the time required for fluoroacetate to be absorbed, to penetrate cells, be converted into fluorocitrate, and then to disrupt cellular processes. Death generally occurs in 24 to 48 h but can occur later. A more detailed account of the toxicology and metabolism of fluoroacetate is given by Buffa et al. (1973) and Peters (1952, 1972).

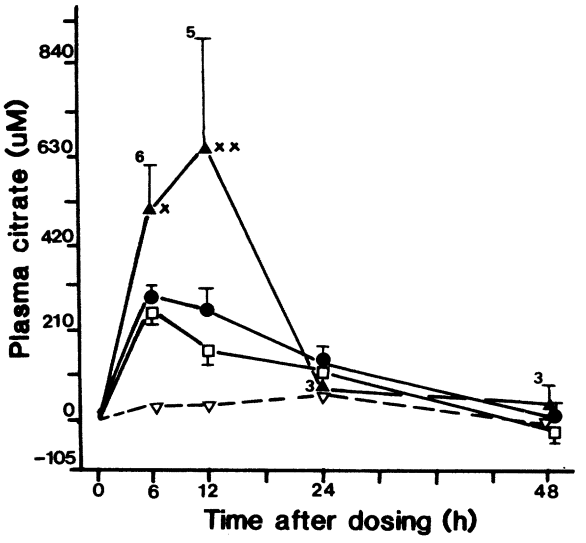
Detoxification and excretion

Detoxification of fluoroacetate by defluorination is known to occur in plants (Preuss and Weinstein 1969, Ward and Huskisson 1972), bacteria (Kelly 1965, Kirk and Goldman 1970, Bong et al. 1979) and a variety of animals (Gal et al. 1961, Smith et al. 1977, King et al. 1978, Mead et al. 1979, Twigg et al. 1986, Twigg et al. 1988a). For example, in moist soils in New Zealand, Japan and England, where the toxin does not occur naturally, several species of bacteria (*Pseudomonas* and *Nocardia*), fungi and algae degrade fluoroacetate (1080) by defluorination (Kelly 1965, Batcheler 1978, Bong et al. 1979). *Pseudomonas*, *Nocardia* and *Fusarium* are found in Australian soils and also defluorinate fluoroacetate (Wong unpubl.).

In rats (*Rattus* sp.) administered sodium fluoroacetate-2-^{14}C, nearly 32% of the label was excreted in the urine within 1–2 d of dosing (Gal et al. 1961). About 3% of the label was found in fluorocitrate and some of the label was incorporated in other fatty acids. In mammals (Mead et al. 1979) and birds (Twigg et al. 1988a), defluorination occurs mainly in the liver via a glutathione-dependent mechanism which is apparently catalysed by a unique glutathione-S-transferase (Soiefer and Kostyniak 1984). Defluorination appears to be a ubiquitous detoxification mechanism (Atzert 1971, Twigg et al. 1986).

Determination of sensitivity to fluoroacetate (1080)

The sensitivities to 1080 of animals with differing degrees of exposure to fluoroacetate-bearing vegetation were determined using four procedures: 1) comparison of the accumulation of plasma citrate in poisoned, closely related animals; 2) estimated LD_{50}'s (Lethal Dose 50 – amount of poison which theoretically kills 50% of test animals) based on the mortality which oc-



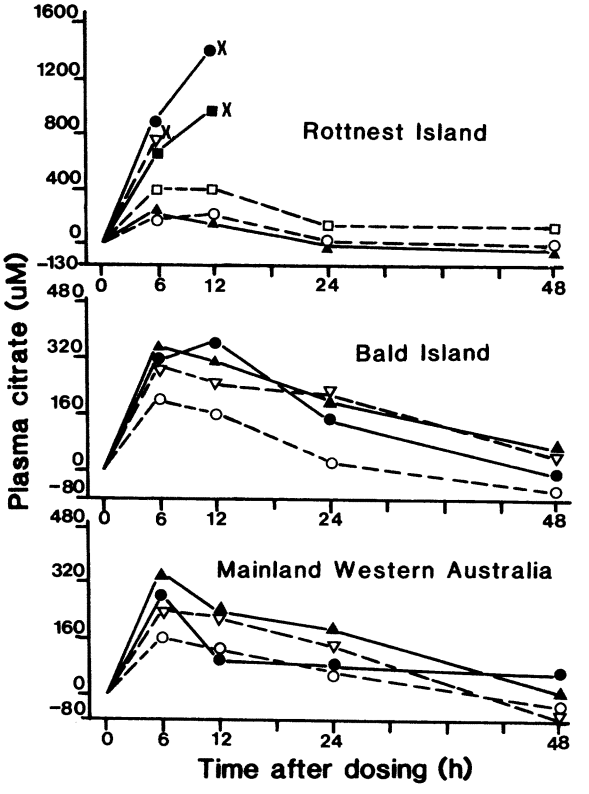


Fig. 3 (top). Mean and standard error for changes in plasma citrate concentration following adminstration of 10 mg 1080 kg^{-1} (fluoroacetate) to *Setonix brachyurus* from: Rottnest Island (n=6), closed triangles; mainland Western Australia (n=4), open squares; Bald Island (n=4), closed circles. An undosed Rottnest Island animal (n=1) is also shown (open triangles). Internal numbers refer to group size after death of an animal (X). From Mead et al. (1985b).
(bottom). Changes in plasma citrate concentration of individual *Setonix brachyurus* from three populations administered 10 mg 1080 kg^{-1} X = death of animal. From Mead et al. (1985b).

curred during plasma citrate determinations or, occasionally, on a small number of dosed animals; 3) precise LD_{50}'s (see Twigg and King 1989), and 4) approximate lethal dose (ALD – the lowest dose at which 10% of a group of test animals die; Calver et al. 1989a). The ALD technique is particularly useful for those species which are too small to allow adequate blood sampling for plasma citrate determination. Most comparisons were made using the plasma citrate accumulation data, where the response of animal populations from south eastern Australia, which are outside the distribution of the toxic plants, was compared with that of populations with past or present exposure to fluoroacetate-bearing vegetation in southwestern Western Australia (Fig. 1).

Within a genus, there is a positive relationship between the dose of 1080 given and the levels of citrate subsequently accumulated in the plasma (Fig. 2; Oliver et al. 1979, Twigg 1986, King et al. 1989, Twigg and King 1989). The least sensitive animals show the smallest increase in plasma citrate concentration (Fig. 3), and at comparable dose levels, those species which have had exposure to fluoroacetate-bearing vegetation accumulate significantly ($p < 0.05$) less citrate in their plasma than do conspecifics or congeners not exposed to the toxic plants (Figs 3–8). Changes in plasma citrate con-

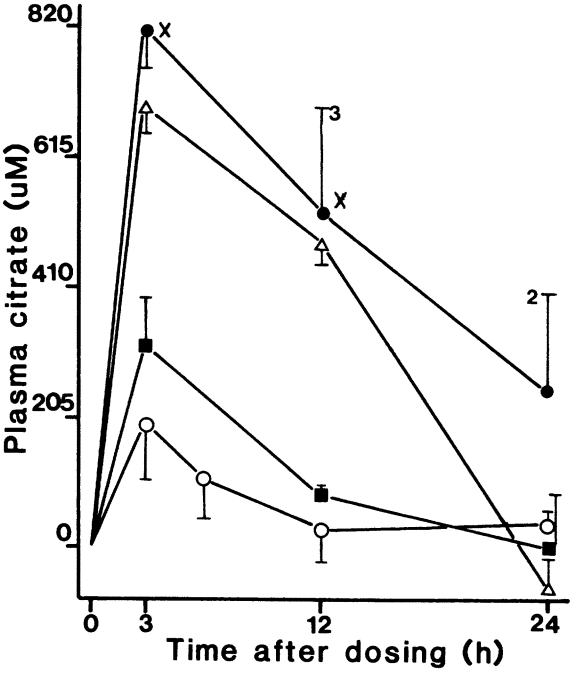


Fig. 4. Comparison of the changes in plasma citrate concentration for Columbiformes from Western Australia administered 5 mg 1080 kg^{-1}. Closed circles, *Streptopelia senegalensis* (n=4); open triangles, Barbary dove (n=3); closed squares, *Ocyphaps lophotes* (n=3); open circles, *Phaps chalcoptera* (n=3). Internal numbers refer to group size after death of an animal (X). From Twigg and King (1989). The LD_{50} for each species is given in Table 1.

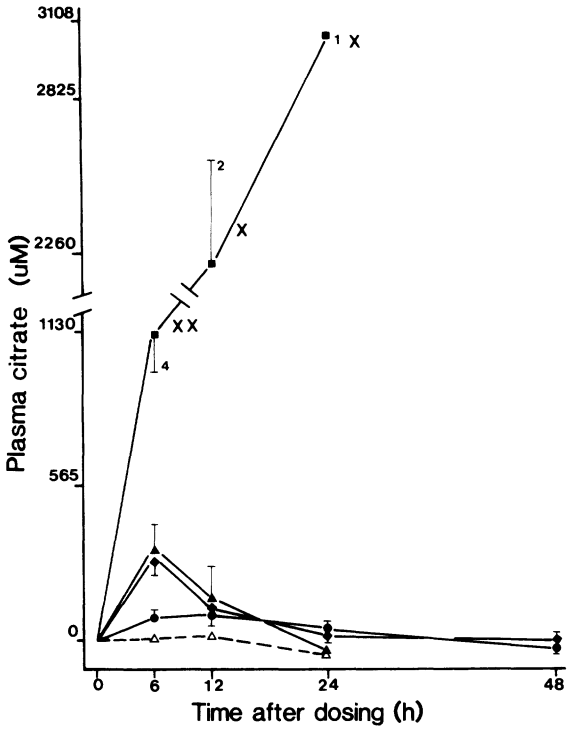


Fig. 5. Mean and standard error for changes in plasma citrate concentration following adminstration of 5 mg 1080 kg^{-1} (fluoroacetate) to species of *Isoodon* (bandicoots). *I. macrourus* (n=4), closed squares; *I. auratus* (n=3), closed triangles; *I. obesulus* from South Australia (n=5), closed diamonds; *I. obesulus* from Western Australia (n=4), closed circles. An undosed *I. auratus* (n=1) is also shown (open triangles). Internal numbers refer to group size after death of an animal (X). From Twigg et al. (1990).

centration in response to dosing are expressed as the mean (μM) and standard error above base-level (time zero) values determined immediately prior to administration of 1080 (Fig. 2).

Determining sensitivity using changes in plasma citrate concentration in response to dosing has several advantages over conventional LD_{50} determinations; 1) considerably less animals need to be killed as individuals can be reused after allowing a 4–8 wk recovery period (Twigg 1986, King et al. 1989, Twigg and King 1989), 2) it allows better determination of the heterogeneity in the response of a population (Fig. 3), and 3) rare species can be tested as animals are able to be reused. As with ALD's (Calver et al. 1989a), the sensitivity of individuals, using changes in plasma citrate concentration, can be determined with as few as three to four individuals.

However, as the degree to which citrate accumulates in tissues and plasma is dependent upon metabolic rate and body size, comparisons can only be made between species of similar size and phylogenetic affinity. For this reason, and to enable comparison of the sensitivities of unrelated species, LD_{50} values were also estimated during the plasma citrate elevation trials. To establish if these estimates were indeed valid, the sensitivity to 1080 of several species including reptiles, birds and mammals, were determined using both precise and estimated LD_{50} values. In all instances, the estimated LD_{50} was always well within the 95% confidence limits of the precise LD_{50} (Table 2). For example, the precise LD_{50} for the parrot *Barnardius zonarius* is 11.46 mg 1080 kg^{-1} (95% C.I.: 9.60–13.67) and the estimated LD_{50} was 12.50 mg kg^{-1} (Fig. 2).

Our precise LD_{50}'s were determined using the mortality of individuals (not reused) at different doses which increased geometrically, and were calculated using the moving average method of Thompson (1947). The sensitivities for some eastern Australian animals are from the studies of Dr John McIlroy, CSIRO, Canberra (see References). LD_{50}'s and ALD's are expressed as mg 1080 (94% pure) per kg body weight.

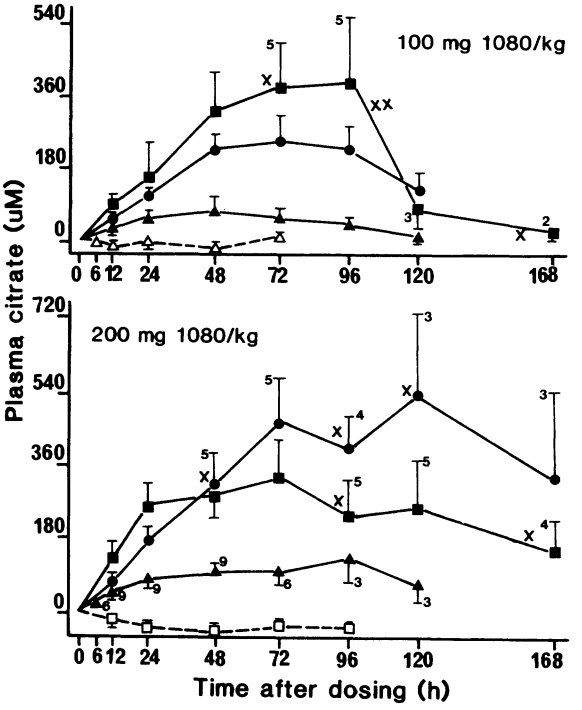


Fig. 6. Changes in plasma citrate concentration (mean and standard error) following adminstration of 100 mg and 200 mg 1080 kg^{-1} to populations of *Tiliqua rugosa* with (Kulikup, WA; closed triangles) and without (Rawlinna, WA; closed squares, and South Australia, closed circles) exposure to fluoroacetate-bearing vegetation. Unless indicated, n=6 for all groups, and open symbols indicate undosed animals for respective populations. Internal numbers refer to group size after death of an animal (X). From Twigg and Mead (1990).

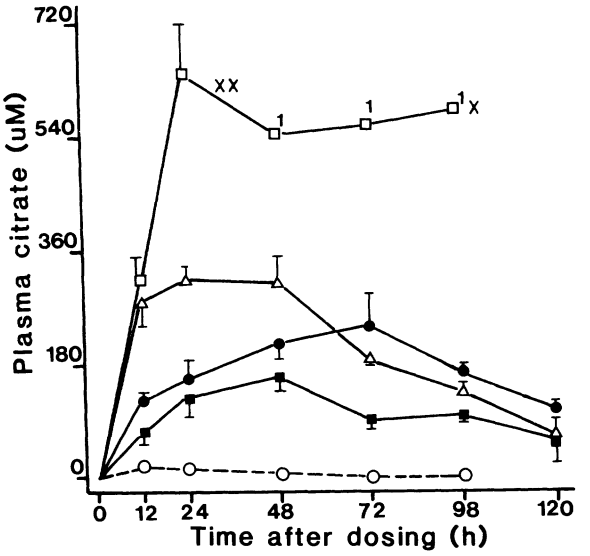


Fig. 7. Changes in plasma citrate concentration (mean and standard error) following adminstration of fluoroacetate (1080) to *Varanus rosenbergi*. Western Australia: closed squares, 50 mg, and closed circles, 100 mg 1080 kg^{-1}. South Australia: open triangles 25 mg; and open squares 50 mg 1080 kg^{-1}. Undosed varanids from SA are also shown (open circles). For all groups, n= 3. Internal numbers refer to group size after death of an animal (X). From Twigg (1986).

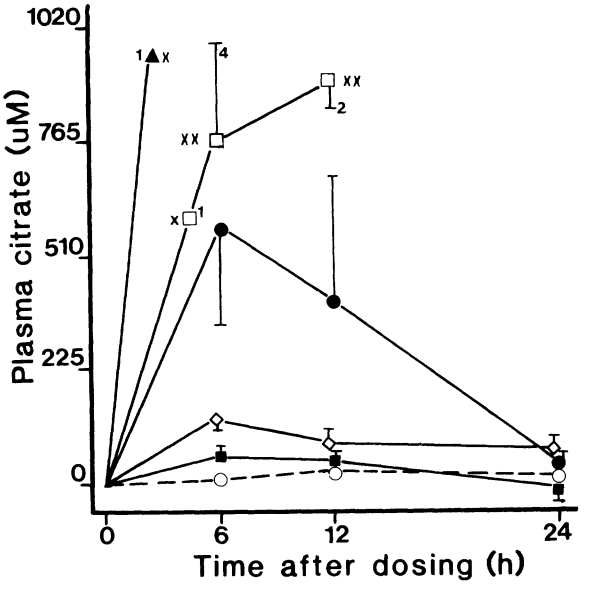


Fig. 8. The mean and standard error for the increase above base-level (time zero) values of plasma citrate concentration following adminstration of fluoroacetate (1080) to *Dasyurus geoffroii* from Western Australia (open circles, undosed, n=1; closed squares, 2 mg; closed circles, 5 mg; closed triangles, 10 mg 1080 kg^{-1}) and *Dasyurus viverrinus* from Tasmania (open diamonds, 0.5 mg; open squares 2 mg 1080 kg^{-1}). For all dosed groups, n=3. Internal numbers refer to group size after death of an animal (X). (Modified from King et al. 1989).

Table 2. Comparison of the sensitivity to 1080 poison (mg 1080 kg^{-1}) using precise LD_{50}'s (with 95% confidence limits) and the estimated LD_{50}'s from the plasma citrate elevation trials.

Species	mg 1080 kg^{-1}	
	Precise LD_{50}	Estimated LD_{50}
Tiliqua rugosa (South Australia)	214.4 (153.0–301.0)	200.0 –
Varanus gouldii	43.6 (27.5–69.2)	50.0 –
Chenonetta jubata	12.60 (10.14–15.67)	12.5 –
Barnardius zonarius	11.46 (19.60–13.67)	12.5 –
Streptopelia senegalensis	5.93 (4.24–8.29)	5.0 –
Dasyurus hallucatus	6.02 (4.16–8.72)	7.5 –

Tolerance to fluoroacetate in Australian animals

The sensitivity to fluoroacetate (1080) of a variety of animals with varying degrees of exposure to fluoroacetate-bearing vegetation is given in Table 1. The changes in plasma citrate concentration in response to dosing for various populations of several species are also given in Figs 3–8. As one would anticipate because of the presence of fluoroacetate in some Australian plants, the degree to which fluoroacetate-tolerance has developed within native animal populations is in the order of: herbivorous > omnivorous > carnivorous species (Table 1). Within a class, tolerance to fluoroacetate is also

Table 3. Sensitivity to fluoroacetate (mg 1080 kg^{-1}), and the accumulation of citrate in individuals administered their respective LD_{50}, for some Western Australian caterpillars collected from areas containing fluoroacetate-bearing vegetation (from Twigg 1990).

Species	LD_{50}	Mean umol citrate per g fresh wt		Food preferences
		Undosed	LD_{50}	
Perga dorsalis (Saw flies)	1.05	3.43 (n=4)	5.60	Eucalypts (exclusively)
Mnesamplea privata	3.88	–	–	Eucalypts and pasture plants
Spiolosoma sp. (Tiger moths)	42.73	2.32 (n=4)	10.01	Cosmopolitan (herbaceous and woody plants)
Ochrogaster lunifer (Bag moths)*	c. 150	–	–	Native legumes (*Acacia*) and some trees

* Collected while they were feeding on toxic *Gastrolobium microcarpum*

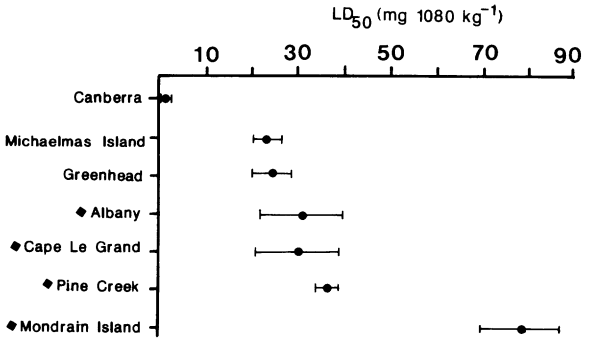


Fig. 9. LD_{50} with 95% confidence limits for various populations of *Rattus fuscipes*. Solid diamonds indicate those populations which coexist with fluoroacetate-bearing vegetation. From Mead et al. (1985b).

most pronounced in those species indigenous to Western Australia (Table 1).

However, the degree to which the tolerance has developed within a species or population also depends upon the length of time they have been exposed to the toxic vegetation, the level of specificity in both their diet and habitat preferences, the size of their home range, and the degree of mobility exhibited by each species. In insects, for example, those species which specialize in feeding on eucalypts (*Eucalyptus* spp. – which do not produce fluoroacetate), are approximately 40 to 150 times more sensitive to the toxin than are species that include fluoroacetate-bearing vegetation in their diet (Table 3).

Herbivores/granivores

Because of the high concentration of fluoroacetate in the seeds of the plants which produce fluoroacetate, seed-eating animals are likely to have had the greatest exposure to the toxin. Of the seed-eating birds in Australia (and elsewhere), the emu *Dromaius novaehollandiae,* which is the oldest seed-eating bird genus in Australia (Rich and Van Tets 1984), has by far the greatest tolerance to fluoroacetate (LD_{50} 102–200 mg 1080 kg^{-1}; Twigg et al. 1988a) of any bird species studied (Table 1). Other seed-eating birds, with past or present exposure to the toxic vegetation (LD_{50}'s range 12.5–75 mg 1080 kg^{-1}; Table 1), are also more tolerant to fluoroacetate than are introduced species, or unadapted species in eastern Australia (Table 1). The LD_{50}'s for other Australian birds outside the range of the toxic vegetation, or whose diets do not include seeds, range from 0.6 mg 1080 kg^{-1} for *Emblema temporalis* (red-browed firetail) to 18.5 mg 1080 kg^{-1} for *Milvus migrans* (forked-tailed kite) with a mean for all species studied of 7.9 ± 0.9 mg 1080 kg^{-1}, $n = 22$ (McIlroy 1984).

The influence of endemism on the development of tolerance to fluoroacetate is illustrated by the sensitivities of the parrots, *Platycercus icterotis* and *Purpureicephalus spurius*, both of which are endemic to Western Australia. Relatively, the LD_{50} values of these parrots are considerably higher than that of any other species of Psittaciformes (Table 1), and they also accumulated the lowest increases in plasma citrate concentration when administered 1080 (Twigg and King 1989). Several species of Columbiformes are also relatively tolerant to fluoroacetate with LD_{50}'s of up to 40 mg 1080 kg^{-1} (Table 1; Twigg and King 1989). Here, the more sensitive unadapted species also accumulate significantly greater ($p < 0.05$) amounts of citrate in their plasma in response to dosing with 1080 (Fig. 4).

The effect of the microhabitat on the selection for fluoroacetate-tolerance is probably best illustrated by comparing the sensitivity to fluoroacetate of various populations of *Rattus fuscipes* (Fig. 9). Eastern Australian (Canberra, ACT) populations of this rat are not exposed to the toxic plants (Fig. 1) and are extremely sensitive to fluoroacetate. However, mainland populations in Western Australia from Albany, Cape Le Grand and Pine Creek all coexist with fluoroacetate-bearing vegetation and are moderate in their levels of tolerance. The heterogeneity is least in the Pine Creek population (Fig. 9) due to a greater presence of the toxic plants in their habitat. The Greenhead and Michaelmas Island populations are outside the current distribution of the fluoroacetate-bearing vegetation. It is postulated that the ancestors of these rats received a genetic contribution from other nearby populations of *R. fuscipes* which coexisted with the toxic vegetation (Oliver et al. 1979). Hence their mid-range level of tolerance. Of greatest interest, however, is the Mondrain Island population. These rats coexist with *G. bilobium,* one of the most toxic of the fluoroacetate-producing plants. The extremely high tolerance (around 80 mg 1080 kg^{-1}; Fig. 9) of this rat population reflects the intense selection pressures which may be evoked when food choices for island populations become limited. These data also support the suggestion that *R. fuscipes* radiated from eastern to western Australia (Oliver et al. 1979, Mead et al. 1985a).

Populations of other rodent species (Muridae) exposed to fluoroacetate-bearing vegetation also display varying degrees of tolerance to fluoroacetate (Table 1). Most rodents in Australia include some seeds in their diet (Watts and Aslin 1981, Strahan 1983).

Within the macropods (kangaroos and wallabies), those species which are endemic to Western Australia, or whose distribution is confined to areas containing fluoroacetate-bearing vegetation (e.g. *Bettongia penicillata*), also possess the highest levels of fluoroacetate-tolerance (Table 1). The western grey kangaroo (*Macropus fuliginosus)* provides an interesting example of how the determination of sensitivity to fluoroacetate can be used as a genetic marker to trace past radiations of Australian animals. Populations of *M. fuliginosus* in eastern Australia are outside the distribution of the

toxic plants, but have tolerances very similar to conspecifics which coexist with fluoroacetate-bearing vegetation. However, the closely related species, *Macropus giganteus* (eastern grey kangaroo), is confined to areas in eastern Australia which are outside the distribution of fluoroacetate-bearing vegetation and this species is very sensitive to fluoroacetate (Table 1). Thus, it is argued that *M. fuliginosus* speciated in Western Australia, before the Holocene, and then radiated eastwards. These data also suggest that: 1) the tolerance of the Western Australian populations of *M. fuliginosus* has not increased measurably over the last 10000 yr, and 2) the coevolutionary equilibrium between these kangaroos and the toxic plants has been stable in the recent past (Oliver et al. 1977, 1979, Mead et al. 1985a).

Another macropod, *Setonix brachyurus* (quokka), provides further insight into the pressures for selection of tolerance to fluoroacetate (Fig. 3). Three *S. brachyurus* populations were studied; a mainland and an island (Bald Island) population which both coexist with the toxic plants, and a population from Rottnest Island which has been isolated from the adjacent mainland and the toxic vegetation for approximately 9500 yr (Fig. 1). The soils on Rottnest Island are unsuitable for fluoroacetate-bearing vegetation (Aplin 1971).

The Bald Island and mainland populations were identical in their tolerances to fluoroacetate (LD_{50}'s approx. 40 mg 1080 kg^{-1}; Mead et al. 1985b). However, the sensitivities of individuals from Rottnest Island displayed marked heterogeneity. The citrate response of some Rottnest Island animals was very similar to that of quokkas from the other two populations, while others were quite sensitive to the poison (Fig. 3). The mainland immediately adjacent to Rottnest Island comprises a coastal sand plain, and as such, is unsuitable for the establishment of fluoroacetate-bearing vegetation (Aplin 1971). Consequently, prior to the isolation of Rottnest Island, there has been a genetic exchange between *S. brachyurus* populations on the coast and those further inland which coexisted with the toxic plants. Sensitive animals dispersing inland would be selected against, and conversely, tolerant individuals dispersing coastwards seem to have faced no selective disadvantage. Hence, in the absence of selection pressure for fluoroacetate-tolerance, there are both sensitive and tolerant individuals in the population presently isolated on Rottnest Island. On Bald Island, there is selection pressure for tolerance to fluoroacetate. However, as both the mainland and Bald Island populations have similar tolerances, there also appears to be an equilibrium which has been reached in the coevolutionary response of the toxic vegetation and the tolerance of these macropods (Mead et al. 1985b).

Omnivores

Of the mammalian omnivores, bandicoots are the main species that have been studied (Twigg et al. 1990). *Isoodon obesulus* from the south west of Western Australia have the highest tolerances and are approximately 3-fold less sensitive than conspecifics from eastern Australia. *Isoodon macrourus* from outside the distribution of the fluoroacetate-bearing vegetation in south eastern Australia is the most sensitive bandicoot species (Fig. 5, Table 1). *Isoodon auratus* on Barrow Island are not exposed to fluoroacetate-bearing vegetation, but the distribution of both the current and ancestral populations on the adjacent mainland include areas containing the toxic plants (Twigg et al. 1990).

Populations of the omnivorous skink, *Tiliqua rugosa,* which coexist with fluoroacetate-bearing vegetation, have exceptional tolerance to fluoroacetate (Table 1, Fig. 6). Individuals from populations with exposure to the toxic plants also accumulate significantly less ($p < 0.05$) citrate in their plasma in response to dosing (Fig. 6). Two populations with exposure to fluoroacetate-bearing vegetation have been examined; one from a coastal area in south western Australia where sandy soils predominate, and which therefore has reduced exposure to the toxic vegetation, and another, inland population which coexists with at least three species of fluoroacetate-producing plants (Twigg and Mead 1990).

For reasons already discussed with regard to *S. brachyurus* from Rottnest Island, the coastal *T. rugosa* population, although 2.5-fold higher in tolerance than unadapted conspecifics, is considerably less tolerant than skinks from the inland population whose habitat includes the three fluoroacetate-producing plant species (Table 1, Fig. 6). The latter individuals are also more homogeneous in their response (Fig. 6).

Carnivores

While carnivores which coexist with fluoroacetate-bearing vegetation are, relatively, the least tolerant of adapted species, they are considerably less sensitive to fluoroacetate than are unadapted carnivores outside the range of the toxic plants (Atzert 1971, Twigg 1986, King et al. 1989). This toxicity differential is clearly demonstrated for the carnivorous/scavenger species of monitor lizard, *Varanus rosenbergi,* where the Western Australian conspecifics are around 6-fold more tolerant to fluoroacetate than their counterparts from eastern Australia (Table 1, Fig. 7). Like herbivorous and omnivorous species, unadapted carnivores sensitive to fluoroacetate also accumulate significantly greater ($p < 0.05$) concentrations of plasma citrate when administered 1080 (Figs 7, 8).

The dasyurid, *Phascogale calura,* is endemic to the south west of Western Australia and has the highest tolerance of the carnivorous marsupials (Table 1; King

Table 4. Retention of tolerance to fluoroacetate (1080) by populations of various species which are not currently exposed to fluoroacetate-bearing vegetation.

Species	Approx. LD_{50} (mg 1080 kg^{-1})	Time of isolation (yr B.P.)	Type of isolation
Bandicoots:			
Isoodon auratus	9	7000	Island
Macropods:			
Setonix brachyurus	10	6000	Island
Lagostrophus fasciatus	125	9500	Island
Bettongia lesueur	15	9500	Island
Macropus eugenii	20	7000	Island
M. fuliginosus (Eastern Australia)	40	10000	Island & Mainland

et al. 1989). The distribution of this species is now confined to areas which have climax vegetation communities with an abundance of toxic species of *Gastrolobium* and *Oxylobium* (Kitchener 1981). Its close association with fluoroacetate-bearing vegetation appears to have resulted in the relatively high tolerance being acquired through ingestion of insects (Kitchener 1981) which feed on the toxic plants. Caterpillars very similar to *Ochrogaster lunifer* (Table 3) have been identified in its diet (Kitchener 1981). This association may also afford protection to *P. calura* from introduced predators in modern times.

Dasyurids are carnivorous marsupials, and to a varying degree, all species rely on insects as food items (Strahan 1983). The toxicity differentials between those dasyurids with exposure to fluoroacetate bearing vegetation can be explained by their habitat and diet preferences. For example, the diets of *Dasyurus geoffroii* and *Dasyurus hallucatus* are more varied than those of *Antechinus flavipes* and *P. calura* (Strahan 1983). The former three species also exhibit less habitat specialisation, and their distribution can include areas where fluoroacetate-bearing plants are infrequent or absent (King et al. 1989). Thus, the selection pressure for the development of tolerance to fluoroacetate acting on these species appears to be less than that acting on *P. calura.*

The high levels of tolerance to fluoroacetate exhibited by those carnivores which coexist with the toxic plants indicates that a secondary tolerance has evolved in insectivorous/carnivorous animals in Western Australia. It also clearly illustrates the degree to which fluoroacetate, or its metabolites, persists in the food chain.

Innate tolerance to fluoroacetate

Many species of amphibians, reptiles and unadapted bandicoots and dasyurids possess an innate tolerance to fluoroacetate which is independent of any prior exposure to the naturally occurring toxin (Chenoweth 1949, Atzert 1971, Twigg 1986; Table 1). This innate tolerance is probably the result of differing metabolic rates between different phylogenetic groups. The skink, *T. rugosa,* for example, has a metabolic rate 10-fold less than that of a similar sized *Rattus norvegicus* (Twigg et al. 1986). Furthermore, it is now accepted that the basal metabolic rates of both dasyurids and bandicoots (Metatheria), are considerably lower than those of equivalent sized eutherians (MacMillen and Nelson 1969). A lower metabolic rate results in fluoroacetate being converted into fluorocitrate more slowly and hence allows greater time for its excretion and detoxification by defluorination (Mead et al. 1979, Twigg 1986).

Retention of tolerance

Several populations of a variety of species have retained a high tolerance to fluoroacetate even though they have been isolated from the selection pressure for 6000 to 10000 yr (Table 4). This implies that this trait carries no selective disadvantage. The rate of its loss in these populations will be influenced by the degree of homozygosity attained by each ancestral population during their exposure to the selection pressure. Its loss will also depend upon the duration of the isolation period. Retention of tolerance by these populations clearly demonstrates the impact fluoroacetate-bearing vegetation has had on the genetic composition of Australian fauna.

Dietary studies

Information on the diets of animals in areas containing fluoroacetate-bearing vegetation is limited as researchers have rarely examined specifically for the inclusion of the toxic plants. A further complicating factor is that present day food choices may not necessarily represent those available in the past. For example, seeds of introduced plants and domestic cultivars currently make a significant contribution to the diets of parrots in south-western Western Australia (Long 1984).

Insect larvae are known to feed on fluoroacetate-bearing vegetation (Table 3; Twigg 1986). When collecting seed from the pods of fluoroacetate-producing plants, it was often found that the entire seed had been consumed by seed weevils (Bruchidae). The larvae of these weevils complete their development and pupate within a single pod (Twigg 1986). Furthermore, in areas containing fluoroacetate-bearing vegetation, the selection of seed preferences by seed harvesting ants does not appear to be influenced by the presence of fluoroacetate in the seed (Twigg et al. 1983). These ants selected seed on the basis of size rather than the presence or absence of fluoroacetate (Fig. 10).

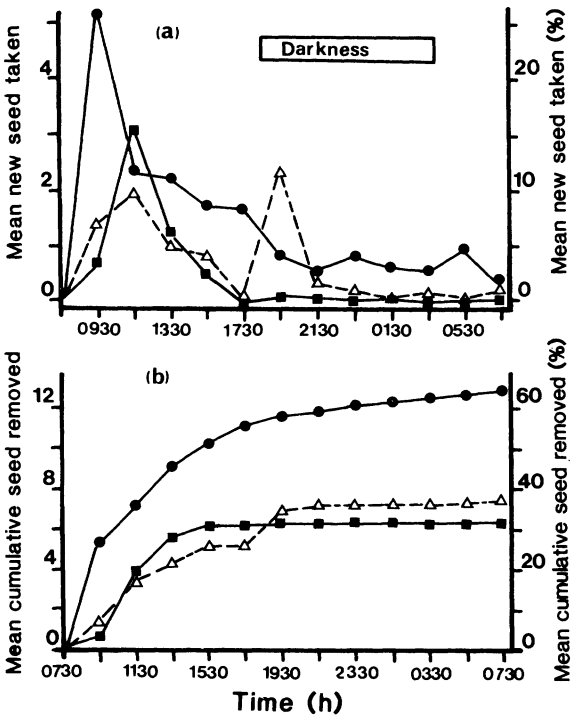


Fig. 10. Removal of seed containing fluoroacetate by seed harvesting ants. Seed mass: *Acacia pulchella* (squares) > *Gastrolobium microcarpum* (triangles) > *Bossiaea eriocarpa* (circles). All three species are legumes but only *G. microcarpum* contains fluoroacetate (from Twigg et al. 1983).

In laboratory studies, pigeons have been offered a choice between seed with and without fluoroacetate. *Phaps chalcoptera* (LD_{50} c.40 mg 1080 kg^{-1}; Table 1) ate the toxic seed with apparent impunity regardless of whether they were given an alternative food choice. *Ocyphaps lophotes* (LD_{50} c.25 mg 1080 kg^{-1}; Table 1), however, rarely consumed the toxic seed on two consecutive days and one of these pigeons died when it consumed toxic seed (no choice given) over 3 d (L.E. Twigg unpubl.). However, these data should be taken as circumstantial evidence only, as they do not necessarily imply that pigeons consume the seed under natural conditions.

The degree to which fluoroacetate-bearing vegetation is incorporated in the diet of *M. fuliginosus* has been determined by the analysis of naturally occurring faecal pellets from several regions in the southwest of Western Australia (Mead 1980, Mead et al. 1985a). Up to 25% of the diet of these kangaroos consists of the toxic plants. More interesting, however, is that despite 1080 supposedly being odourless and tasteless (Atzert 1971), these kangaroos appear to be able to discriminate between the highly toxic and less toxic plant species. They consume considerably less of those plants (eg. *G. bilobum*) with high concentrations of fluoroacetate (Fig. 11). Faecal pellets of *S. brachyurus* from Bald Island also contain cuticle particles similar to those of *G. bilobum* (L.E. Twigg unpubl.), which naturally occurs on this island.

The breeding season of many animals which coexist with fluoroacetate-bearing vegetation (eg. *T. rugosa, R. fuscipes, P. chalcoptera*) usually coincides with the flowering and seed set of the toxic plants. Consequently, during this period, the browsing pressure on the plants

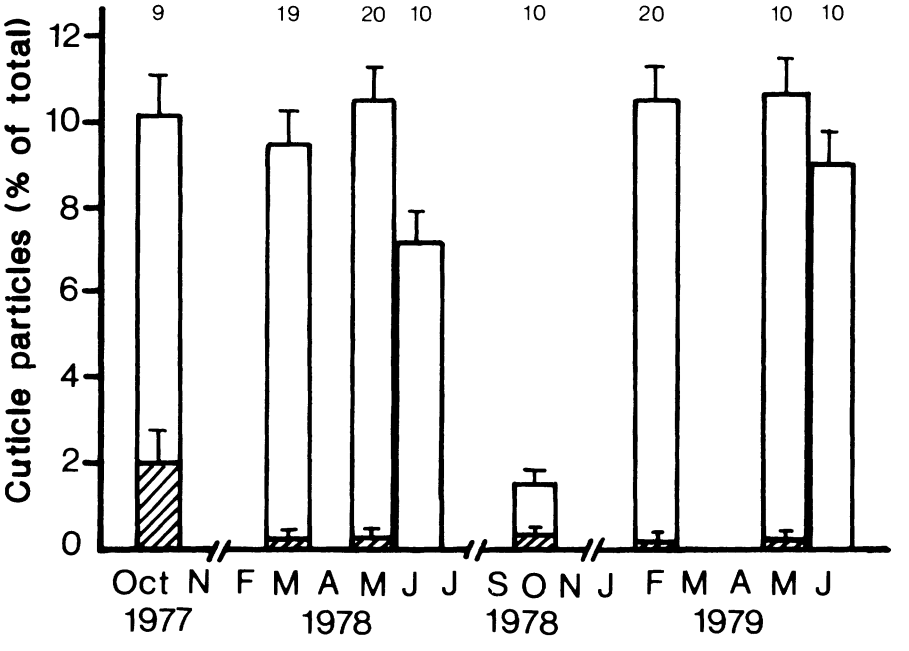


Fig. 11. Percentage (with 95% confidence limits) of two toxic species of *Gastrolobium* in faecal pellets of *Macropus fuliginosus*. Faecal pellets were analysed for both plant species from two localities in the lower south west of Western Australia (from Mead et al. 1985a). Open histograms, *G. spinosum* (400 mg fluoroacetate kg^{-1}; Aplin 1971); stippled histogram, *G. bilobum* (2600 mg fluoroacetate kg^{-1}; Aplin 1971).

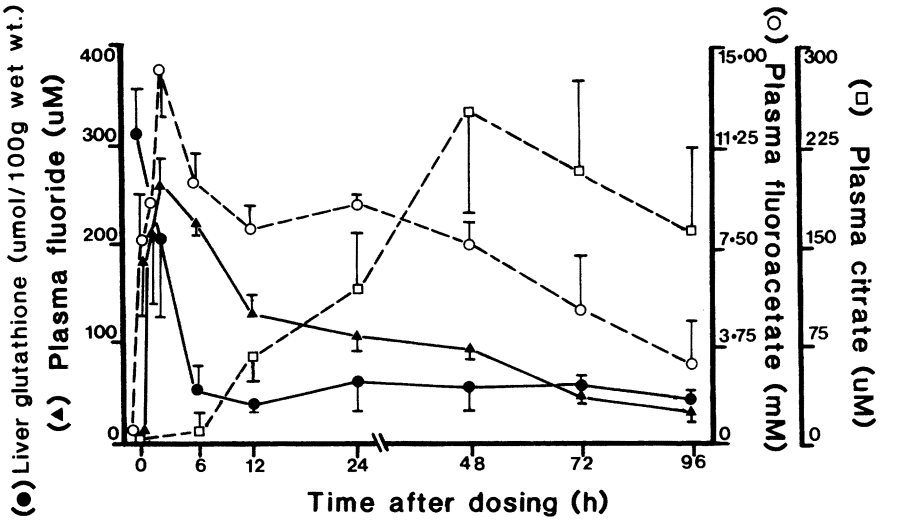


Fig. 12. Plasma fluoroacetate (open circles, n=3), plasma fluoride (triangles, n=3), plasma citrate (squares, n=6) and liver glutathione (closed circles, n=3) concentrations of *Tiliqua rugosa* following intraperitoneal administration of 500 mg 1080 kg^{-1}. From Twigg et al. 1986.

is likely to increase. The seeds, young shoots, and seedlings of the fluoroacetate-producing plants would be an attractive and nutritious food source, and it appears that the high level of fluoroacetate in these tissues (Aplin 1971) provides protection against heavy browsing pressure. Any animal which feeds on the toxic vegetation must therefore obtain a balance between adequate nutrition and avoidance of being poisoned, particularly during the breeding season. Conversely, plants which produce fluoroacetate appear to have achieved a balance between producing protective chemicals and vegetative growth and seed. Thus we have a "biological arms race" between the toxic plants and their predators.

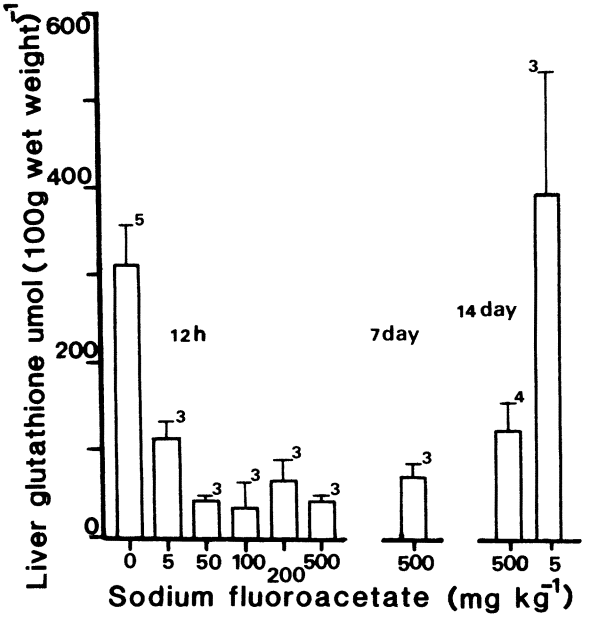


Fig. 13. Effect of fluoroacetate on reduced glutathione concentration (mean and standard error) in the liver of *Tiliqua rugosa* 12 h, 7 d, and 14 d after intraperitoneal administration of 1080. From Twigg 1986.

Possible mechanisms for the tolerance

Biochemical

There are four obvious areas in which the metabolism of fluoroacetate may vary between sensitive and tolerant animals; 1) the rate of conversion of fluoroacetate to fluorocitrate may differ, 2) there may be differences in the sensitivity of their aconitate hydratase to fluorocitrate, 3) fluorocitrate may differentially affect the citrate transport system in mitochondria, and 4) they may have differing abilities to detoxify fluoroacetate by defluorination.

Firstly, to illustrate the major metabolic changes evoked during fluoroacetate intoxication, changes in fluoroacetate, fluoride and citrate concentrations in the plasma, and reduced glutathione concentration in the liver, in response to administration of fluoroacetate to the skink, *T. rugosa,* are presented in Fig. 12. After absorption, fluoroacetate is rapidly defluorinated by an enzymic glutathione-dependent mechanism which results in rapid elevation of plasma fluoride concentration accompanied by a rapid depletion of reduced glutathione in the liver (Twigg et al. 1986). Once glutathione levels are low, fluoroacetate is no longer effectively detoxified and the inhibition of aconitate hydratase and/or citrate transport in mitochondria increases. This increased inhibition is indicated by the elevated plasma citrate concentration, which is only evident after both plasma fluoride and liver glutathione levels become minimal (Fig. 12; Twigg et al. 1986). Despite extensive defluorination, low levels of fluoroacetate remain present in the plasma of these skinks for at least 96 h and

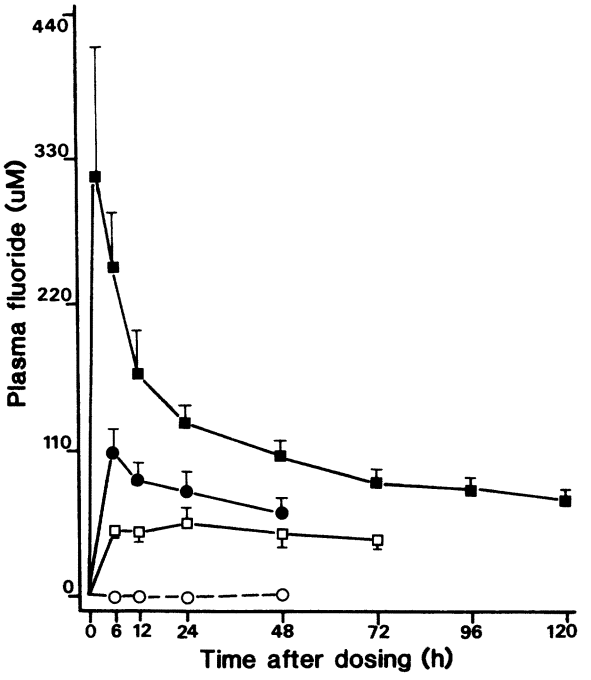


Fig. 14. Plasma fluoride concentration of *Tiliqua rugosa* following intraperitoneal administration of fluoroacetate (1080). Open circles, undosed (n=1); open squares, 100 mg 1080 kg^{-1}; closed circles, 200 mg 1080 kg^{-1}; closed squares, 800 mg 1080 kg^{-1} (n=3 for all dosed groups). Modified from Twigg 1986.

liver glutathione levels were depressed for up to 14 d (Fig. 13; Twigg 1986). The ability of *T. rugosa* to defluorinate varying amounts of fluoroacetate, in vivo, is also given in Fig. 14. While the magnitude and time scale involved may vary, these metabolic changes are indicative of those which also occur in mammals (Mead et al. 1979, Twigg et al. 1986) and birds (Twigg 1986, Twigg et al. 1988a) in response to administration of fluoroacetate.

When comparing the metabolism of fluoroacetate between ectothermic (e.g. *T. rugosa*) and endothermic (eg. *R. norvegicus)* animals and between those species with exceptional tolerance (eg. *D. novaehollandiae, T. rugosa)* and those that are relatively sensitive to the toxin (eg. *B. zonarius, R. norvegicus)*, there are significant ($p<0.05$) differences in the metabolism of fluoroacetate (Table 5). Fluoroacetate had greater effect in vitro on the TCA cycle of the sensitive species; their aconitate hydratase has greater sensitivity to fluorocitrate, and they may be less able to convert fluoroacetate to fluorocitrate. The sensitive species also have less ability to detoxify fluoroacetate by defluorination (Table 5). The toxicity differential between such species may be at least partially attributed to these metabolic differences. However, the effect of fluorocitrate on citrate transport is also an important consequence of fluoroacetate toxicity and its possible role in bringing about the toxicity differential should not be discounted. The effect of fluorocitrate on citrate transport in mitochondria is yet to be compared between sensitive and tolerant conspecifics (see "Some Paradoxes" below).

When comparing the metabolic effects of fluoroacetate in tolerant and sensitive conspecifics of both *T. rugosa* and *T. vulpecula,* there were no significant differences ($p>0.05$) in the metabolism of fluoroacetate (Table 5). Conspecifics of both species are similar in their abilities to convert fluoroacetate to fluorocitrate, sensitivities of aconitate hydratase to fluorocitrate, and capabilities to defluorinate fluoroacetate (Table 5). This is despite a 4- and 150-fold difference in the sensitivity of the conspecifics of both species, respectively. The more sensitive conspecifics do, however, accumulate significantly ($p < 0.05$) greater amounts of plasma citrate in response to dosing than the more tolerant conspecifics (Mead et al. 1979, Twigg and Mead 1990). This suggests that the effect of fluorocitrate on citrate transport in mitochondria could vary significantly between sensitive and tolerant conspecifics. Clearly, the biochemical mechanisms responsible for the development of tolerance to fluoroacetate are yet to be fully understood (see "Some Paradoxes" below).

All the in vitro experiments mentioned above employed liver acetone powder preparations as the enzyme source with incubation mixtures containing 1–7 mg of protein. Fluorocitrate synthesis was determined by incubating the enzyme source (aconitate hydratase) in the presence and absence of fluoroacetate (5 mM) and measuring the rate of citrate synthesis from added isocitrate (5 mM). Inhibition of aconitate hydratase (Lineweaver-Burk plots) was established by incubating aliquots of enzyme with and without fluorocitrate for 15 min and then adding isocitrate and measuring the amount of citrate synthesis over a further 30 min incubation. Defluorination of fluoroacetate (5 mM) was determined from incubation of liver acetone powder extracts in the presence and absence of reduced glutathione (5 mM). Incubation was for 1, 3 and 5 h and the rate of defluorination was determined by free fluoride measurement. No defluorination occurred in the absence of enzyme or where enzyme integrity had been deliberately destroyed. Detailed procedures can be found in Mead et al. (1979), Twigg et al. (1986) and Twigg (1986).

During fluoroacetate poisoning it is possible that fluoroacetate, or the high concentration of some metabolites produced during intoxication, may differentially affect anaerobic metabolism of different species. This was examined in vitro using erythrocytes of *T. rugosa* and *R. norvegicus* (Twigg et al. 1986). Suspensions of washed erythrocytes were incubated with glucose in the presence and absence of fluoroacetate, citrate, and fluoride and the rate of conversion of glucose to lactate monitored (Table 6). Compared to normal glycolytic activity, high levels of either fluoroacetate or citrate did not significantly ($p>0.05$) affect the rate of glycolysis in either species. However, high levels of either fluoride or

Table 5. Comparative metabolism of fluoroacetate in mammals, birds and reptiles, and in sensitive and tolerant conspecifics of *Tiliqua rugosa* and *Trichosurus vulpecula*. * = $p<0.05$; underscores indicate statistical comparisons between populations; A = in vitro study using liver acetone powder preparations as enzyme source; FAC = sodium monofluorocetate (5 mM); FCIT = (-) erythro-fluorocitrate (0.02 mM); B = 0.13 mM L-fluorocitrate used; C = 12 h after administration of 50 mg 1080 kg^{-1}; D = 3 h after adminstration of 50 mg 1080 kg^{-1}. Modified from; (E) Twigg et al. 1986, (F) Twigg and Mead 1990, (G) Mead et al. 1979, (H) Twigg et al. 1988a.

	Rattus norvegicus	*Tiliqua rugosa*			*Trichosurus vulpecula*		*Dromaius novae-hollandiae*	*Barnardius zonarius*
		WA	WA	SA	WA	SA		
Coexist with fluoroacetate-bearing vegetation:	No	Yes	Yes	No	Yes	No	Yes	Yes
LD_{50} (mg 1080 kg^{-1}):	2–3	>800	>800	214	125	0.7	102	11.5
Conversion of FAC to FCIT:[A] (As indicated by inhibition of aconitate hydratase activity)	87±4%* (n=3)	57±4%* (n=3)	51±6% (n=3)	48±5% (n=3)	74±12% (n=3)	72±13% (n=3)	33±6%* (n=3)	63±3%* (n=3)
Inhibition of aconitate hydratase by FCIT (0.02 mM):[A] Ki (mM) =	0.026 *	0.065 *	0.065	0.055	0.044[B]	0.032[B]	–	–
Defluorination of fluoroacetate: 1) In vitro (nmol fluoride mg $protein^{-1}$ h^{-1}):[A]	9±4* (n=3)	72±20* (n=5)	82±24 (n=4)	158±21 (n=3)	7.3±1.2 (n=4)	6.8±1.1 (n=4)	204±37* (n=3)	59±2* (n=3)
2) In vivo (plasma fluoride uM):	–	–	56±17[C] (n=3)	58±22[C] (n=3)	–	–	584±103[D] (n=3)	–
3) By erythrocytes (in vitro) (nmol fluoride g Hb^{-1}):	90±25* (n=3)	631±110* (n=3)	–	–	–	–	–	–
Incubation temperature for in vitro studies (°C):	37	37	30	30	37	37	37	37
Source:	E		F		G		H	

Table 6. Effect, in vitro, of fluoroacetate (FAC), citrate and fluoride on the glycolytic rate of erythrocytes of *Rattus norvegicus* and *Tiliqua rugosa* (Means ± SE) A = Typical basal level in mammals, B= maximum level for fluoroacetate intoxicated mammals and C= maximum level for fluoroacetate intoxicated *T. rugosa*. * indicates $P<0.01$ when compared with the respective control. From Twigg et al. 1986.

Treatment	Lactic acid production (µmol g Hb^{-1} h^{-1})	
	Rattus norvegicus	*Tiliqua rugosa*
Control:	24.4±1.1 (n=4)	6.1±0.6 (n=8)
FAC (15 mM):	22.8±0.7 (n=4)	6.4±0.8 (n=4)
Citrate (µM):	24.1±0.6	6.6±1.0
A 212	(n=4)	(n=4)
B 1058	23.8±0.8 (n=4)	8.8±1.8 (n=3)
Fluoride (µM):	21.9±2.0	7.2±1.3
B 263	(n=4)	(n=4)
C 526	18.1±2.1* (n=3)	7.3±0.5 (n=3)
1315	6.2±0.5* (n=3)	4.7±1.0 (n=3)
15 mM FAC + 1058 µM citrate + 263 µM fluoride	16.3±2.0* (n=4)	6.4±0.4 (n=4)

fluoride in combination with citrate and fluoroacetate, significantly ($p<0.01$) reduced the glycolytic activity of *R. norvegicus* erythrocytes but had little affect on that of erythrocytes of *T. rugosa* (Table 6). Thus, at levels approaching their LD_{50} for fluoroacetate, the production of energy from glycolysis, and more importantly, the amount of phosphoenol pyruvate available for generation of pyruvate for aerobic respiration, in mammals, also appears to be considerably reduced. In reptiles, a serious disruption of the TCA cycle would be expected to induce a greater reliance in anaerobic metabolism with a concomitant increase in lactic acid production. The mean blood lactate levels of *T. rugosa* (1.32 mM; SE = 0.18; n = 27) showed no significant ($p>0.05$) changes within 120 h in skinks administered from 0 to 800 mg 1080 kg^{-1} (Twigg 1986).

Physiological

Given the high level of innate tolerance in those *T. rugosa* populations without exposure to fluoroacetate-bearing vegetation, it is difficult to appreciate the ecological necessity for the development of tolerance to

fluoroacetate in conspecifics which coexist with the toxic plants unless factors other than straight mortality are involved. A 350 g *T. rugosa* consumes approximately 14.4 g of food a day, and even if this consisted entirely of one of the highly toxic plant species, then this omnivorous skink could only ingest 37 mg of fluoroacetate per day. A 350 g skink with an LD_{50} of 800 mg 1080 kg^{-1} would need to ingest more than 280 mg of fluoroacetate in a relatively short period to succumb to the toxin (Twigg and Mead 1990). Provided these skinks were able to thermoregulate and did not become more prone to disease or predation, its discontiguous activity pattern (Satrawaha and Bull 1981) would enable sufficient time for ingested fluoroacetate to be detoxified by defluorination.

Selection pressure for tolerance to fluoroacetate is likely to be operating at a lower level than that indicated above; that is, it is unlikely to be a straight-forward mortality response. Chronic effects of fluoroacetate, such as long-term reduction in glutathione levels (Fig. 13) and reduced fertility, may be an equally important selection pressure. Animals require adequate levels of glutathione for defluorination of fluoroacetate (Mead et al. 1979, Twigg et al. 1988a). Thus, those individuals with low "natural" levels of glutathione, or who are unable to rapidly replace diminished glutathione, may face a selective disadvantage once challenged by fluoroacetate. Sublethal doses of fluoroacetate well below their respective LD_{50}'s are known to affect the fecundity of a variety of mammals (Mazzanti et al. 1965, Sullivan et al. 1979, Hornshaw et al. 1986) and birds (Balcomb et al. 1983).

Low levels of fluoroacetate (12.5% of LD_{50}) administered either as a single or multiple (over 15 d at 3 d intervals) dose causes a reduction in plasma testosterone concentration and may affect spermatogenesis in male *T. rugosa* (Twigg et al. 1988b). Whether these skinks remained fertile could not be ascertained. A similar phenomenon has been observed in *R. fuscipes* where sublethal doses (23% of LD_{50} for each of 4 consecutive days) also caused a marked regression of the germinal epithelium in the testis (D. King and A. Oliver unpubl.). Relatively, reproductive tissues have high energy demands and thus require an adequate supply of ATP. They are also very low in glutathione concentration and hence would have little protection against fluoroacetate intoxication. Consequently, in some animal populations, like those of *T. rugosa,* it appears that the main selection pressure for tolerance to fluoroacetate has resulted from selection against depressed fertility (fecundity) rather than for avoidance of the acute effects of the toxin.

Is fluoroacetate-tolerance unique to Australian animals?

There is a very limited amount of information on the sensitivity to fluoroacetate of animals indigenous to the other continents where plants produce significant amounts of fluoroacetate.

In South West Africa, the LD_{50} values for two species of antelopes (*Taurotragus oryx* and *Trageluphus strepsiceros)* which coexist with toxic species of *Dichapetalum* are approximately 5–8 mg fluoroacetate kg^{-1} (Basson et al. 1982). Spider monkeys (*Ateles geoffroyi)* and Virginia opposums (*Didelphis marsupialis),* which often coexist with *Palicourea marcgravii* in South America, have LD_{50}'s of 15 mg (Chenoweth and Gilman 1946) and 60 mg 1080 kg^{-1} (cited in Atzert 1971) respectively. These sensitivities are considerably less than those of most unadapted mammals (LD_{50}'s < 2 mg 1080 kg^{-1}; Atzert 1971). Thus, it appears that tolerance to fluoroacetate has evolved on all three continents where some native plants contain significant amounts of the toxin.

Some paradoxes

1) As mentioned previously, the degree to which fluoroacetate-tolerance has developed in some animal populations (eg. *T. rugosa, D. novaehollandiae)* appears unnecessarily high to be protecting them solely from the acute effects (mortality) of the toxin. However, it should be remembered that LD_{50} determinations are an anthropomorphic evaluation of the sensitivities of animals to toxins, and as such, may not necessarily reflect the level of evolutionary selection pressure for a particular trait. Selection pressures are therefore likely to be operating at a level below that suggested by LD_{50} determinations. Furthermore, animals are not able to "consciously" regulate their evolutionary response to phytotoxins; that is, selection for tolerance to toxins is a "passive" process dependent upon the characteristics of a particular genome, its degree of homozygosity, and its plasticity or ability to undergo evolutionary change.

When considering fluoroacetate toxicity, the chronic effects of fluoroacetate on animals have been largely ignored to date. However, we believe that both the chronic and acute effects of fluoroacetate are equally important factors in the selection for the development of tolerance to fluoroacetate. For example, depletion of liver glutathione may predispose animals to liver damage from other metabolites or toxins, which in turn, may result in increased incidence of disease or predation. Animals may also lose their ability to thermoregulate. In some instances (eg. *T. rugosa)*, it appears that selection pressure for fluoroacetate-tolerance is acting on the fecundity of individuals. Supporting evidence for this is that reproductive tissues have high energy de-

mands but possess very low levels of glutathione to protect their aconitate hydratase from fluorocitrate. Animals must be able to, at least, partially overcome the toxic effects of fluoroacetate and produce sufficient numbers of viable gametes to enable reproductive success. Thus, like many others (Culvenor 1970, Leopold et al. 1976, Harborne 1977, Labov 1977, Swain 1977) we also argue that the effects of many plant toxins or secondary compounds are often subtle in their action rather than direct.

2) Reduced glutathione plays a significant role during fluoroacetate intoxication, being involved both in the defluorination mechanism and in providing partial protection to aconitate hydratase from the effects of fluorocitrate (Mead et al. 1979, Mead et al. 1985c, Twigg et al. 1986, Twigg 1986). The concentration of reduced glutathione in erythrocytes of various species of Australian marsupials has been determined (Agar and Stephens 1975). There is no correlation between erythrocyte glutathione concentration and the sensitivity to fluoroacetate of these marsupials. It should be remembered, however, that the liver is the organ with the greatest amount of glutathione, and it is also the main site for defluorination of fluoroacetate. However, despite there being only limited information, the concentration of reduced glutathione in the liver appears similar in tolerant and sensitive animals, but endothermic species (c. 900 μmol per 100 g wet wt of liver; Mead 1980, Twigg et al. 1988a) have approximately 2-fold greater amounts than that found in the ectotherm, *T. rugosa* (Twigg et al. 1986).

3) There were no obvious differences in the metabolism of fluoroacetate by conspecifics with a considerable toxicity differential between populations with and without exposure to fluoroacetate-bearing vegetation. If inhibition of aconitate hydratase by fluorocitrate is the cause of this toxicity differential, it is difficult to interpret the differences in citrate accumulation between the tolerant and sensitive conspecifics. The competitive nature of the inhibition should ensure that toxicity is diminished as citrate accumulates. This does not appear to happen in vivo. However, it is likely that other metabolic effects of fluoroacetate are involved in bringing about the toxicity differential. For example, fluorocitrate can inhibit succinate deydrogenase [EC 1.3.99.1] (Fanshier et al. 1964) and this enzyme may be less sensitive to fluorocitrate in tolerant individuals. The effects of fluorocitrate on the mitochondrial citrate transport system could also vary between sensitive and tolerant individuals.

Inhibition by fluorocitrate of citrate transport into and out of mitochondria was recently suggested as an additional mode of toxic action for fluorocitrate (Kun et al. 1977, Kirsten et al. 1978). However, transport of isocitrate from mitochondria, and cytoplasmic aconitate hydratase (Buffa et al. 1973), are unaffected by fluorocitrate. The concentration of fluorocitrate (0.1 μM) in the mitochondria of lethally poisoned rats (*R. norvegicus* – very sensitive to fluoroacetate) is less than their Ki for aconitate hydratase (Table 5), but this concentration is sufficient to inhibit citrate transport enzymes in the mitochondrial membrane of these rats (Kun et al. 1977, Kirsten et al. 1978).

In the tolerant Australian animals, mitochondrial citrate transport may only be minimally affected by fluorocitrate, which would allow unrestricted distribution of citrate. This, together with a reduced inhibition of aconitate hydratase by fluorocitrate, could account for the differences in the accumulation of plasma citrate between tolerant and sensitive animals. The citrate transport system in sensitive animals may be inhibited by fluorocitrate resulting in accumulation of citrate in the mitochondrion. This citrate would ultimately be converted to isocitrate by aconitate hydratase. Isocitrate could then readily exit the mitochondria via its own transport system, and be reconverted to citrate in the cytoplasm by the cytoplasmic aconitate hydratase. The citrate thus formed could not re-enter the mitochondria due to the inhibition of the citrate transport system, and would be redistributed to the plasma.

However, these proposals are speculative because: 1) the concentration of fluorocitrate in the mitochondria of lethally poisoned Australian animals is not known. Sensitive Australian species may metabolise greater concentrations of fluorocitrate in the mitochondria than has been reported for *R. norvegicus*, and 2) the effect of fluorocitrate on the mitochondrial citrate transport system, and on succinate dehydrogenase, has not been studied in Australian animals.

Fluoroacetate-tolerance and Australian fauna management

Clearly, the tolerance to fluoroacetate (1080) possessed by many Australian animals, which results in a marked toxicity differential between target animals (mostly introduced species) and non-target species (Table 1), considerably increases the target specificity of 1080 poison. However, it is not only the sensitivity of non-target species which determines the potential hazard to them during 1080 baiting programs. Equally important in evaluating primary and secondary poisoning hazards to non-target species is their body size relative to the target-species, whether they are exposed to the toxic baits, and if so, whether the baits are palatable, and the timing of baiting programs. For example, because of the possible effects of 1080 on fecundity, baiting programs are best avoided during the breeding season of some dasyurids where total post-mating male dieoff occurs (eg. *P. calura;* King et al. 1989). Nevertheless, tolerance to fluoroacetate does enhance target specificity during many 1080 baiting programs and this is discussed more fully in King et al. 1981, 1989, Twigg 1986, Calver et al.

1989a, b, King 1989, Twigg and King 1989 and Twigg et al. 1990.

In parts of Western Australia, some of the more rare species of mammals (eg. *B. penicillata, Myrmecobius fasciatus)* are being successfully reintroduced into areas of their former range (Johnson et al. 1989, Friend 1990). As these areas can contain fluoroacetate-bearing vegetation, knowledge of their sensitivity to fluoroacetate, and its likely effects upon them, is essential for successful introductions. Reduction of fox (*V. vulpes)* numbers is an integral part of the management of many native species as fox predation can have a devastating effect on small native animal populations (Christensen 1980, Kinnear et al. 1988, King et al.1989, J. Kinnear pers. comm.). This is most often achieved by 1080-baiting (Kinnear et al. 1988, Calver et al. 1989b, King 1989).

Summary

Animal populations in parts of Australia, particularly those in the southwest of Western Australia, have coexisted with fluoroacetate-bearing vegetation for at least several thousand yr. Consequently, they have developed varying degrees of tolerance to this potent toxin which depends upon their dietary and habitat specialisation, the size of their home range, the degree of mobility exhibited by each species, and the length of evolutionary exposure to the toxic vegetation. Once developed, this tolerance is retained by animal populations even when they become isolated from the toxic vegetation for 7000 to 10000 yr. Fluoroacetate-bearing vegetation does not occur in southeastern Australia.

However, the biochemical mechanisms responsible for the large toxicity differential between conspecifics with and without exposure to fluoroacetate-bearing vegetation are poorly understood. Despite conspecifics with exposure to fluoroacetate-bearing vegetation accumulating significantly less ($p < 0.05$) citrate in their plasma in response to fluoroacetate administration, and being up to 150 times more tolerant to the toxin than their unadapted counterparts, both conspecifics appear to be similar in their metabolism of fluoroacetate. There are no significant differences ($p > 0.05$) in their abilities to convert fluoroacetate into fluorocitrate, their aconitate hydratase is similarly inhibited by fluorocitrate, and they have similar capabilities to detoxify fluoroacetate by defluorination. In contrast, however, the exceptional tolerance of the skink, *T. rugosa,* and the emu, *D. novaehollandiae,* is at least in part due to a greater capacity to defluorinate fluoroacetate. In vitro, the ability of these species to convert fluoroacetate into fluorocitrate, and the inhibition of their aconitate hydratase, is much less than that which occurs in most other animals.

Fluoroacetate can cause a reduction in animal fertility, and in levels of reduced glutathione in the liver. Thus, it is argued that both the acute and chronic effects of fluoroacetate poisoning are equally important selection pressures for the development of tolerance to fluoroacetate. Dietary studies indicate that animals are able to discern fluoroacetate, and discriminate between the highly toxic and less toxic plant species, consuming less of the former.

The development of tolerance to fluoroacetate (1080) by native Australian animals enhances the target-specificity of 1080 poison which is used in vertebrate pest control programs directed against those pest species which are introduced to Australia. Baiting with 1080 also plays an important role in protecting our native fauna from the effects of predation and competition from introduced predators and herbivores.

Tolerance to fluoroacetate is present in insects, reptiles, mammals and birds and is in the order of herbivores > omnivores > carnivores. Those species indigenous to Western Australia have the greatest level of tolerance, and presently, a coevolutionary equilibrium appears to have developed between the toxic plants and their predators. The development of tolerance to fluoroacetate by animals has evolved on at least three continents where indigenous plants are known to produce fluoroacetate which acts as a chemically mediated defence strategy against herbivory.

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