1080, Toxic Sugars, Alkaloids, and a Dead Cat: The Search for a Toxicant in Australian *Gastrolobium* Seed

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ABSTRACT: It was hypothesised that reintroduction of Australian native mammals, currently being severely impacted by feral cat predation, would be more successful if these mammals could have a retained toxicity as discussed in historical accounts. Seeds from the Australian genus *Gastrolobium* (Fabaceae: Mirbelieae) were analysed in the search for a toxicant that would explain historical accounts of toxic wildlife. Numerous accounts referring to bronzewing pigeons having toxic bones were specifically noted. Analysis of this seed found no evidence for rapidly toxic alkaloids previously reported as being extracted from the leaves of York Road poison and box poison. However, evidence for the presence of organo-fluorine compounds in addition to the reported fluoroacetate (Compound 1080) was discovered. A limited cat dosing trial found that a highly fluorinated box poison seed caused a cat to cease respiration in 82 minutes, but its chloroform extract produced no adverse physiological response. In addition, citrate accumulation appeared more rapid and acute with increasing seed 'total fluorine.'

KEY WORDS: alkaloids, bone retention, bronzewing pigeon, cat, Compound 1080, fluoroacetate, fluoroacetylated sugar, *Gastrolobium*, organo-fluorine, *Phaps chalcoptera*, *Phaps elegans*, predation, seeds

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INTRODUCTION

The paper by Peacock et al. (2002), presented at the 20th Vertebrate Pest Conference in Reno, Nevada, discussed the issue of catastrophic predation of reintroduced fauna, often carried out by just 1 or 2 feral cats. It was proposed that making the reintroduced fauna toxic would help mitigate this catastrophic predation, and that such a retention of a chemical toxicant was indicated by numerous historical accounts, primarily located in newspapers from the early twentieth century. Two groups of potential toxicants, alkaloids and organo-fluorine compounds, were proposed as most likely to explain these accounts of rapid deaths of cats (*Felis silvestris catus*) and dogs (*Canis lupus familiaris*) and as providing bronzewing pigeons (*Phaps chalcoptera* and *P. elegans*) with toxic bones. This paper continues to detail aspects of this research, focussing on the investigation of these two groups of compounds and including an associated cat dosing pilot study.

Alkaloids

Alkaloids are produced by a variety of organisms, are often toxic, and have been recorded in *Gastrolobium* leaves (Webb 1949, Cannon and Williams 1982). The presence of putative alkaloids in extracts of *Gastrolobium* seeds was described in Peacock et al. (2002). They were considered a toxicant likely to explain rapid death and toxic bone anecdotes. Literature supporting the investigation of alkaloids includes the following:

- A major concentration of the alkaloid colchicine was detected in sheep bone marrow after a single oral dose, having earlier been detected unchanged in the milk (Panariti 1996);
- The alkaloid caffeine was detected in 11 human femoral bone samples (McIntyre et al. 2000);
- New Guinea birds of genera *Pitohui* (5 of 6 species) and *Ifrita* (1 of 1 species) have been found to retain batrachotoxin alkaloids, primarily in their feathers (Dumbacher et al. 2000). These are the same alkaloids sequestered in the skin of the poison-dart frogs (Daly 1995), and which can still be detected at least 2 years after ingestion (J. Daly, pers. commun.);
- The cat is stated to be one of the species most susceptible to alkaloids (Waller and Nowacki 1978).

Organo-Fluorine Compounds

The exploratory work of Hall (1972) suggested the possible presence of a fluorinated carbohydrate or amino acid in the seeds of *G. bilobum*. The extensive analysis of *Gastrolobium* seed within this study has established the presence of multiple unidentified organo-fluorine compounds (see Peacock et al. 2002). The current study used ^{19}F Nuclear Magnetic Resonance (NMR) to observe additional weak triplets downfield of the main fluoroacetate triplet in the methanol extracts of *Gbilobum* (Tambellup) and *G. parviflorum* (Jacup) seeds. As the methanol extracts had a very crystalline, sugar-like

appearance and had 'total fluorine' recorded in multiple fractions, it was proposed that these triplets and 'total fluorine' may be from fluoroacetylated sugars consistent with Hall's speculation, mentioned above.

The presence of fluoroacetylated sugars in *Gastrolobium* seeds could help explain the historical anecdotes. Any inhibition of glycolysis may cause rapid death such as described in the anecdotes, with Taylor (1972) stating that some fluoro-sugars competitively inhibit enzyme activity. In addition, the fluoro-sugar "fluoroacetyl glucosamine" is reported to be incorporated into hyaluronic acid (Kent and Winterbourne 1977, Winterbourne et al. 1979), a polysaccharide component of cartilage and synovial joint fluid. The presence of fluoroacetyl glucosamine, or other fluoroacetyl sugars, and its exhibiting similar chemical properties, may offer an explanation for the toxic bone anecdotes.

Cat Dosing Trial

Cats are the introduced predators for which this project received funding to investigate a control technique. They are reportedly quite unique in their physiology– for example, they have low levels of glucuronyl transferase in the liver (MacDonald et al. 1984), thereby making them less tolerant to drugs/toxins. Domestic cats also have unique dietary factors. They require taurine, arginine, and arachidonate in their diet (MacDonald et al. 1984). However, they can obtain water requirements from their prey (Prentiss et al. 1959).

The primary aim of this experiment was to determine if *Gastrolobium* seed, and/or a chloroform extract, caused any physiological effect on cats. The study objectives were to replicate the historical accounts of rapid deaths, establish a seed solvent extract which also produced rapid death, and to examine a broad spectrum of physiological parameters that could explain the cause of death and the possible toxicant(s) involved.

A replicated study involving significant numbers of feral cats dosed under veterinary-assisted anesthesia, enabling use of additional seed species, seed fractions, and replicated doses was originally proposed. However, the required university ethics approval was only given for a very restricted pilot study, enabling a preliminary examination of the toxicity of 3 species (provenances) of *Gastrolobium* seed and the chloroform fraction of *G. parviflorum* (Jacup) seed to the domestic cat.

METHODS

Gastrolobium seed was purchased from Nindethana Seed Service Pty. Ltd. (Albany, Australia) and before use was hand-cleaned of any contaminating material. Seed was milled in a Cyclotec 1093 sample mill (Tecator) and then successively extracted at room temperature with petroleum spirit (AR grade B.R. 60 - 80°C, BDH), chloroform (AR grade, BDH) and methanol (HPLC grade, BDH). Mixtures were filtered under suction through a Whatman No. 42 ashless filter paper with 2 washes of the seed marc with the appropriate solvent, and the filtrate further filtered through a 0.45µm PTFE hydrophobic syringe filter (Millipore) on a Terumo disposable syringe. Solvents were removed on a Buchi rotary evaporator with the water bath held at 35 ± 1°C. Methanol extracts were transferred to a pre-weighed beaker using distilled water, snap-frozen using liquid nitrogen and freeze-dried. Additional samples of milled *G. parviflorum* (Jacup) seed were extracted with ethanol (AR grade, BDH) or distilled water and *G. bilobum* (Tambellup) also with distilled water. Ethanol extract was treated as above, with water extracts centrifuged (10 min @ 6000 rpm), filtered using 0.45-µm cellulose-nitrate filters (Sartorius) with suction, frozen, and freeze-dried.

Alkaloids

Analytical and Preparative Thin Layer Chromatography (TLC)

Solvent extracts were tested for the presence of alkaloids using Kieselgel 60 F_{254} silica gel (0.2 mm) on an aluminium thin layer chromatography (TLC) plate (Merck) with a 60:40 chloroform:methanol solvent and Dragendorff alkaloid reagent (Sigma).

Dragendorff responsive compounds (putative alkaloids) were purified and extracted using 20-cm × 20-cm Kieselgel 60 F_{254} silica gel (2 mm) on glass preparative TLC plate (Merck) and resolved using a 60:40 chloroform:methanol and 5:2:4 1-butanol:acetic acid:water solvent.

Organo-Fluorine Compounds

Analysis for 'Total Fluorine'

'Total fluorine' content of the intact seed and solvent extracts was determined using the alkali fusion method for total fluorine analysis (Remmert et al. 1953) with 2M NaOH. The Emf (mV) was measured using a Radiometer ISE25F-9 Fluoride Selective Electrode and an Orion 90-02 Double Junction Reference Electrode, then compared to a standard curve. Standard curves of Emf (mV) against log[F-] had an R^2 of ≥0.99.

Synthesis of Fluoroacetylated Sucrose Standards

a) 1 g monofluoroacetic acid (Merck, re-distilled 167°C fraction), 2 g glacial acetic acid (APS Ajax Finechem, AR grade) and 1 g sucrose (Sigma) were mixed in a glass vial. The vial was crimp-capped and heated in a Pierce Reacti-Therm heating module @ 60 ± 1°C for 72 hours. This procedure gave a mixture of mono and poly-monofluoroacetyl sucroses.

b) 10 mg of sucrose (99.5%, Sigma) was mixed with 50 mg sodium monofluoroacetate (SMFA; Pestanal grade, Riedel-deHaën) and 1 ml methanol (HPLC grade, BDH) in a 2-ml glass vial. The vial was capped, sonicated for 30 minutes, shaken for 20 hours, and the methanol removed using a stream of nitrogen and freeze-drying. Material was then acetylated as for the seed extracts. An additional sucrose/SMFA mixture was analysed by ^{19}F NMR without any prior acetylation. This procedure gave mono-monofluoroacetyl sucroses.

Nuclear Magnetic Resonance (NMR) Analysis

NMR analyses were performed using a 600-MHz Oxford magnet with a Varian Unity Inova console using Varian VNMR6.1C software. The probe was a 5-mm pulsed field gradient probe tuned to ^{19}F (Sfrq = 564.350 MHz; Sweep Width = 310, 078 Hz; tof = -8846.1; temp.

= 25°C). Spectra were referenced to an external reference of either trifluoroacetic acid in D_2O (set to 78.0 ppm, in turn referenced to $CFCl_3$ @ 0.0 ppm) or sodium monofluoroacetate in D_2O (set to -63.4 ppm). Deuterated methanol was from Sigma-Aldrich and Pestanal grade monofluoroacetic acid, sodium salt, from Riedel-deHaën.

Cat Dosing Trial

Four 10- to 12-month-old male homozygous normal laboratory-bred cats were purchased from the Institute of Medical and Veterinary Science (IMVS), Gilles Plains Animal Resource Centre (GPARC), South Australia. They had been bred at GPARC, housed in pens which incorporated a room and outside run, and fed daily with IAMS dry biscuits (adult). When required for dosing experimentation, they were transported in an individual cat carry-box directly to the University of Adelaide Medical School.

Dosing experimentation was done in a medical room with a temperature of 21 ± 2°C and with lighting provided by standard fluorescent lights. Cats were fasted for 12 hours prior to dosing experimentation, at which time they were weighed and then placed under light anesthesia with an intravenous injection of 0.7 ml kg^{-1} Saffan (Pitman-Moore). Animals were then intubated and maintained under a light plane of anesthesia with 1.5% isofluorane gas. A 3-mm gastric catheter was then inserted down the oesophagus and through the pyloric sphincter into the cat's stomach for dosing. Electrocardiogram (ECG) and electrocephalogram (EEG) probes were connected and a pulse oximeter was attached to an ear. Two femoral catheters were inserted for blood pressure monitoring and blood sampling. A baseline blood sample and physiological data (e.g., pulse, blood pressure, heart rate) were collected prior to dosing.

The size of the milled seed dose was based on common bronzewing pigeon gastro-intestinal weights (unpubl. data) and the belief that 15 g of seed (5 g in the crop and 10 g in the gastro-intestinal tract) would be the maximum amount in a bronzewing pigeon (Ron Johnstone, Curator of Birds, Western Australian Museum, pers. commun.). Use of the bronzewing pigeon as a guide was based on the anecdote of the insides of a shot bronzewing pigeon causing the death of a retriever dog in 20 minutes (Le Souëf 1907).

Cat #1, a 4.4-kg tabby, was dosed with 3.5 g kg^{-1} (15.4 g) milled *G. bilobum* (Tambellup) seed flour flushed into the stomach with 70 ml water.

Cat #2, a 4.56-kg tabby, was dosed with 3.5 g kg^{-1} (15.96 g) milled *G. parviflorum* (Jacup) seed flour flushed into the stomach with 80 ml water.

Cat #3, a 3.8-kg tabby, was dosed with 3.5 g kg^{-1} (13.3 g) milled *G. calycinum* (Mundaring) seed flour flushed into the stomach with 70 ml water.

Cat #4, a 4.57-kg black and white, was dosed with 0.8633 g chloroform extract from milled *G. parviflorum* (Jacup) seed, adsorbed to 5 g plain wholemeal wheat flour (Bi-Lo) and flushed into the stomach with 30 ml water. From a previous milled seed extraction this equated to the extract from 15.96 g milled seed, guided by the results from Cat #2. The dose was prepared by re-dissolving the chloroform extract in a few millilitres of chloroform, transferring the solution to the flour, adding additional washes of the chloroform flask to the flour, and then ensuring the flour was uniformly mixed. The chloroform was then removed on a Buchi rotary evaporator with the water bath held at 35 ± 1°C.

The doses were prepared as a suspension and transferred to a 60-ml catheter-tip disposable syringe (Terumo). The syringe was inserted into the top of the catheter tube and the suspension dose slowly delivered directly into the stomach. The syringe and catheter tube were then flushed with an additional 5- to 10-ml water wash. Dosed cats were observed and monitored constantly during anesthesia to ensure there was no vomiting, with potential for choking, and to monitor any physiological response.

While under anesthesia the cats were monitored for their pulse and heart rate, blood pressure, respiration rate and depth, heart and brain activity, and blood gases. If dosing caused a lethal response, this occurred while the animal was under anesthesia. If dosing caused no lethal response, the cat was monitored under anesthesia for at least 2 hours post-dosing before being euthanased with Lethabarb (Virbac). Euthanased animals were autopsied with tissue samples extracted for possible histopathological analysis.

Tissue Analysis

Tissue and plasma samples were stored at -18°C to prevent any bacterial defluorinating activity (Soiefer and Kostyniak 1983). Plasma samples were separated using a Clements Orbital 100 centrifuge (Phoenix Scientific Industries Ltd) and immediately frozen.

Plasma Citrate and Biochemistry

Plasma citrate concentrations were determined by the Western Australian Department of Agriculture Animal Health Laboratories. Plasma biochemistry (feline body function) was undertaken by IDEXX Veterinary Pathology Services, Adelaide, with IDEXX veterinary pathologist Martin Copland providing an interpretation of the results provided.

Liver and Kidney Fluoroacetate Analysis

Liver and kidney fluoroacetate concentrations were determined by the Queensland Department of Natural Resources using a method based on Ozawa and Tsukioka (1989).

RESULTS

Alkaloid Analysis

Analytical TLC found no Dragendorff responsive compounds in the petroleum spirit or methanol extracts of *G. bilobum* (Tambellup) seed. Dragendorff responsive compounds were found in the chloroform extract of the seed and remained in this solvent in spite of the addition of HCl and n-hexane. Use of the extraction process described and preparative TLC enabled the extraction of the Dragendorff responsive compounds in sufficient purity to enable analysis by NMR.

Extensive NMR expansions and decoupling experiments identified the putative alkaloids as a mixture of phosphatidyl cholines (lecithins), where the fatty acids

were primarily linolenic, oleic, and stearic acids. Han et al. (1991) describe the NMR analysis of phosphatidyl cholines, which fits with this NMR data, including the reported coupling constants. The principal identifying NMR signal was the most upfield singlet (at delta 3.22), being the quaternary ammonium methyl signals of the phosphoryl choline, the $N(Me)^3$ group, attached to the glycerol. This was evident from the ROESY and the NOESY spectra which showed $N(CH_3)_3$ cross peaks with both the $P\text{-}OCH_2$ and $CH_2\text{-}N(Me)^3$ methylenes. A COSY experiment showed that these two methylenes are coupled with one another.

Fluoroacetylated Sugars ('1080-Sugars')

Synthesis of a fluoroacetylated sucrose standard required heat and time. Several mono- and polyfluoroacetylated sucrose derivatives were synthesised. Replication of the cool methanol extraction process by shaking SMFA with sucrose failed to produce the ^{19}F NMR downfield triplets, with only the SMFA triplet at -63.1 ppm evident. The ^{19}F NMR downfield triplets observed in the methanol extracts of *G. bilobum* (Tambellup) and *G. parviflorum* (Jacup), although still unassigned, are believed to be due to fluoroacetylated sugars. The synthesised fluoroacetylated sucrose standard had a similar shift, with multiple signals between -75.5 to -77.4 ppm.

Cat Dosing Trial

Cats 1 and 4 showed no significant physiological abnormalities at 120 minutes post-dosing and were euthanased. Cat 2 was observed to have ceased respiration at 82 minutes post-dosing with brain and heart activity continuing but deteriorating until dosed with Lethabarb at 120 minutes. Cat 3 was observed to be very close to death on 2 occasions before being euthanased at 180 minutes post dosing.

It was planned to randomly select one of the 4 cats to be a control and receive plain wholemeal flour as a test to ensure the dosing and monitoring procedure was not causing an adverse response. However the survival of Cat 1, and subsequently Cats 3 and 4, suggests the dosing and monitoring methodology were not the cause of the cessation of respiration in Cat 2. Similarly, Cat 4 acts as a control for the ingestion of fluoroacetylated compounds (fluoroacetate and any putative MFA-sugars), as these are absent from the chloroform extract ingested by Cat 4, but present in the milled seed given to Cats 1, 2, and 3.

The analysis of liver and kidney samples from each of the four cats detected fluoroacetate in Cats 1, 2, and 3, with concentrations reflecting the seed 'total fluorine' and therefore potential seed fluoroacetate dosed to each of these cats. The liver and kidney samples from Cat 4, having been dosed with the chloroform fraction of *G. parviflorum* (Jacup) seed, which lacked any ^{19}F NMR triplets, understandably resulted in the detection of no fluoroacetate. Results are presented in Table 1.

DISCUSSION

This study was unable to extract and identify any alkaloids in the *Gastrolobium* seeds analysed. Although previous research had identified alkaloids in *Gastrolobium* leaves, with some research stating compounds of rapid toxicity (Mann 1905, 1906), the Dragendorff responsive compounds extracted in this study were identified as phosphatidyl cholines. It is possible that some alkaloids are present, but very minor, and were thus overlooked for the more obvious phosphatidyl cholines. This is however considered an unlikely scenario, supported by the cat dosing trial indicating that the toxicant(s) within *Gastrolobium* seed is not within the chloroform fraction and most probably within the methanol fraction. It is therefore the conclusion of this study that the anecdotes of toxic bronzewing pigeon bones could not be due to their retention of alkaloids through consumption of *Gastrolobium* seeds.

Evidence of organo-fluorine compounds additional to fluoroacetate was discovered, however the presence of fluoroacetylated sugars in the seed is yet to be verified. ^{19}F NMR analysis supports their presence, however their identification using liquid chromatography wasn't conclusive, as the analytical process with fluoroacetate present could itself fluoroacetylate the sugars.

Although limited to a pilot study only, many questions were raised by the biochemical and physiological responses of the four cats in the cat dosing trial, designed to replicate historical anecdotes. The cat 1080 LD_{50} is stated to be 0.28 mg kg^{-1} (Eason and Frampton 1991). Therefore 1.5 mg would be enough to kill all the cats in this study. Cat 2, which ceased respiration in 82 minutes, was dosed with *G. parviflorum* seed which had a mean 'total fluorine' of 975.5 ppm. Thus in the 15.96-g dose there would have been approximately 16 mg 'total fluorine.' If all of this was incorporated as potassium fluoroacetate (KMFA; potassium found to be the most abundant cation in the seed), there would be a massive total of 93 mg. Half of the dose appeared however to have remained in the stomach at death, but even a 47-mg dose is huge and unparalleled. Cat 3 survived 13.3 g of *G. calycinum* seed with a mean 'total fluorine' of 250 ppm, which equates to approximately 20 mg KMFA, (10 mg if it was only half digested and absorbed). In response to this large dose, the cat was in serious physiological distress, was monitored for an additional hour past the proposed 2-hour limit, but did not die. Cat 1 survived 15.4 g of *G. bilobum* seed with a mean 'total

Table 1. Liver and kidney fluoroacetate concentrations.

Cat #	Liver		Kidney	
	Sample Weight (g)	Fluoroacetate (µg/g)	Sample Weight (g)	Fluoroacetate (µg/g)
1	9.712	0.416	21.395	0.218
2	14.196	4.08	21.351	0.926
3	10.428	1.10	10.183	0.375
4	12.120	ND	48.197	ND

fluorine' of 145 ppm, which equates to approximately 13 mg KMFA. If only half was digested and absorbed, 6.5 mgs is also still a significant dose. This cat did not die within the 2-hour testing time period or exhibit a physiological parameter to suggest it was in trouble.

The biochemical and physiological response of Cat 2 to the 15.96 g of milled *G. parviflorum* seed was broadscale and led to fatal deterioration of function. Biochemistry suggests function at the cell level (Na/K pump) failed, with the kidneys deprived of blood, central nervous system failure, and organ/cell shutdown (Martin Copland, IDEXX veterinary pathologist, pers. commun.). This resulted in the cessation of respiration at 82 minutes post-dosing, presumably due to failure of the brain's respiratory centre. Responses were very similar to those observed by Chenoweth and Gilman (1946) for unanesthetised cats intravenously dosed with methyl fluoroacetate. They found either a cardiac or central nervous system response may arise and 'predominate' in an individual cat, with unanesthetised cats dying primarily due to depression of the respiratory centre, with a heart rate still evident after cessation of breathing. This observation similarly describes the physiological response and subsequent death of Cat 2, anesthetised and dosed with a gastric lavage of milled *G. parviflorum* seed. (Anesthetised cats in the Chenoweth study were found to have minimal depression of their respiration, however all died when injected with at least 0.5 mg kg^{-1} methyl fluoroacetate).

The biochemical and physiological responses of Cats 1, 2, and 3 may have been a response to large doses of fluoroacetate and any other fluoroacetylated compounds present. The detection of high concentrations of fluoroacetate in the liver samples and moderate concentrations in the kidney samples of these cats supports these compounds as being the primary toxicant(s) in these seeds. Physiological distress, biochemical imbalance, tissue fluoroacetate, and the accumulation of plasma citrate (Figure 1) appear directly related to dose amounts. Elevated citrate is considered to indicate the conversion of fluoroacetate to fluorocitrate, with the resulting citrate accumulation. The results suggest Cat 2's quick death was due to a rapid conversion of fluoroacetate, or other fluoroacetylated compounds, to fluorocitrate, with Cats 1 and 3 affected in a similar way, but not as quickly. The rate of citrate accumulation therefore appears directly related to dose level. However, the whole 'lethal synthesis' theory (Peters 1954, 1963) requires a latent period of time to allow for the fluoroacetate to fluorocitrate process and then cell death and organ failure. The time to dose relationship cannot therefore be linear. Eason and Frampton (1991) gave 8 cats (mean weight 3.6 kg) one bait each dosed with 1.6 mg sodium fluoroacetate and had a mean time to death of 15 hours, with one cat dying at 7 hours post-dosing. This was quicker than their smaller doses, thereby supporting a time to death dose response in cats. A study utilising "spinal animals" in a stimulus-response experiment with a maximum intravenous dose of 4 mg kg^{-1} states a mean time to death of 3 hours (Foss 1948). Chenoweth and Gilman (1946) state time to death in rabbits to relate to dose, with intravenous 0.20-0.25 mg kg^{-1} resulting in a delay of up to 24 hours, while 2.0 mg kg^{-1} may cause death in 30-60 minutes. This was not, however, the case in dogs, with the 1- to 2-hour latent period "somewhat, but not markedly, shortened" when using higher doses (Chenoweth and Gilman 1946, p. 98)

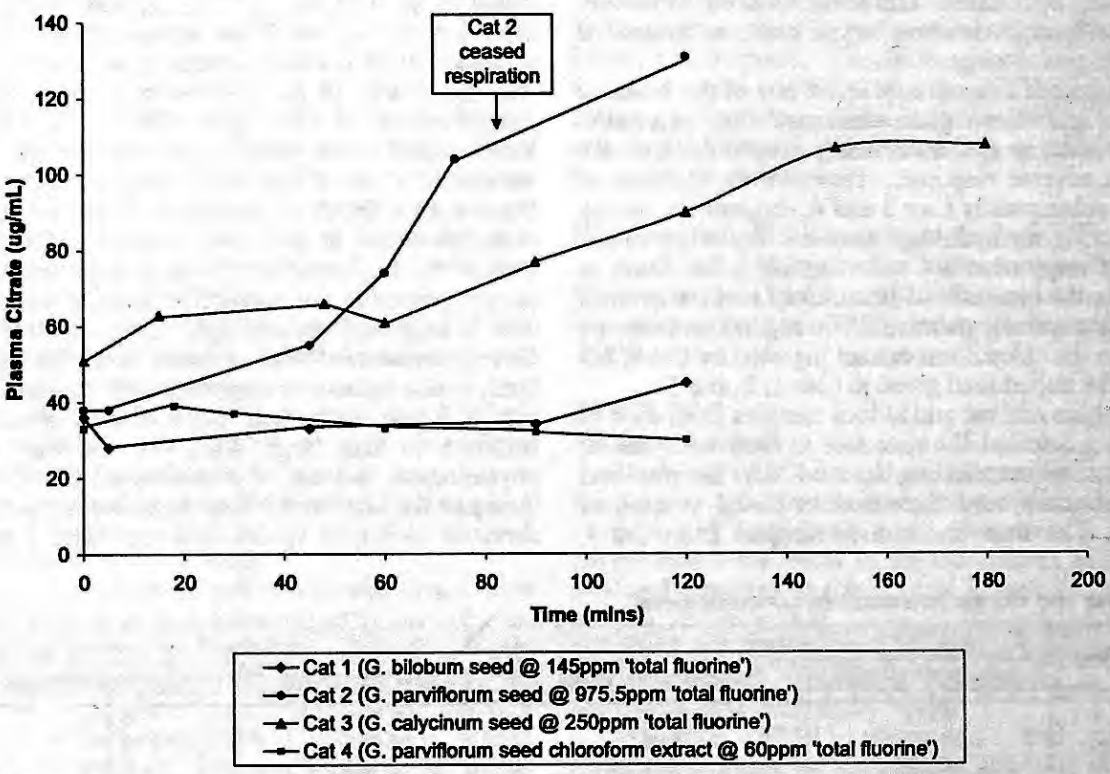

Figure 1. Plasma citrate concentrations in four cats dosed with *Gastrolobium* seed or a chloroform seed extract.

It is possible that the account of 3 cats dead in under an hour from the "insides" of bronzewing pigeons (Knight 1912) could be explained by a massive dose of fluoroacetate, possibly by a larger or more fluorinated dose of *Gastrolobium* seed than used in this experiment. Although anesthesia can slow digestion, thereby slowing the cat deaths, the seed used in this study was milled and in an aqueous suspension, which would significantly quicken extraction of the fluoroacetate. Also, the *G. parviflorum* seed dosed to Cat 2 was one of the most fluorinated recorded. Seeds are hard-coated and likely to be largely intact (except in the gizzard). To achieve the reported deaths in under an hour, the results from this study would suggest ingestion of seed more fluorinated than is recorded in this present study, and/or ingestion of more seed than believed likely within a bronzewing pigeon (R. Johnstone, 2001, pers. commun.).

However, based on the published dog dosing studies, it is considered very unlikely that the anecdotes reporting dog deaths in 20-25 minutes (Webb 1885; Le Souëf 1907) – even with a 100% error in the times to death – are explained by the seed toxicant being solely fluoroacetate. As one of these dog anecdotes also involves the toxicant having been bone retained (Webb 1885), an unrecognised capacity of fluoroacetate, it is suggested that the presence of a toxicant additional to fluoroacetate would better explain these anecdotes. If the approximately 47 mg of fluoroacetate proposed to be digested by Cat 2 was sufficient to cause the 25-minute dog death in the Webb (1885) anecdote, it would equate to the unlikely concentration of almost 12,000 ppm needing to be contained in the pigeon's approximately 4-g breastbone.

If fluoroacetylated sugars are present in the seeds, their presence may cause an inhibition in glycolysis and possibly the more rapid citrate accumulation seen in Cat 2. This may form an alternative explanation for Cat 2's fast death within this pilot study, especially when considered in conjunction with the rapid deaths and bone retention reported in historical anecdotes.

In summary, if the only fluoroacetylated compound within the *Gastrolobium* seed dosed to Cats 1, 2, and 3 is fluoroacetate, the preliminary data from this experiment does then suggest that the larger the dose, the quicker the death of cats. This supports the trend seen in the data of Eason et al. (1992) but is difficult to explain with respect to the 'lethal synthesis' hypothesis, with its required time factor. It is unknown whether this trend is also evident in dogs when the doses are of the magnitude used here. This would be contrary to published studies that report a lengthy latent period before symptoms arise and any subsequent death. A replicated study using a canid model would more effectively test whether the seed doses used in this experiment can cause deaths in times equivalent to the anecdotes.

With confirmation of the presence of fluoroacetylated sugars, other experiments would be beneficial, dosing with MFA-sucrose for example, again seeking to replicate the rapid death and toxic bone anecdotes. The ultimate goal of these additional experiments would continue to be the identification of the toxicant with these properties and its use to assist in predator control and the subsequent survival of sustainable populations of reintroduced native animals.

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