Investigation of biological samples for monofluoroacetate and *Dichapetalum cymosum* poisoning in southern Africa

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ABSTRACT

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A need has existed for the accurate identification of monofluoroacetate (MFA) poisoning in southern Africa. The development of a new method for the determination of MFA has made the analysis of a variety of biological samples (n = 50) feasible. The method has been used in the laboratory over 24 months. Monofluoroacetate was present in 66 % of samples from cases of suspected poisoning, reflecting the extent of the problem. Stability of MFA in samples was also determined so as to have a time-bound baseline for the acceptance of samples submitted. It was found that there was a decrease in the level of MFA and, after 14 days at room temperature, only 50 % of the spiked dose could be identified. It is suggested that samples be analyzed within 7 days of mortality if they not kept frozen.

Keywords: *Dichapetalum cymosum*, gifblaar, liquid chromatography, monofluoroacetate poisoning

INTRODUCTION

Synthetically-produced monofluoroacetate (MFA or Compound 1080; CFH_2COO^-) is a banned substance in South Africa in terms of the Hazardous Substance Act (Act 15 of 1973). This is mainly due to its physical, chemical and toxicological properties, i.e. extreme toxicity, water solubility, colourlessness, tastelessness, difficulty of detection, and a latent period between ingestion and development of clinical signs. The potential of secondary poisoning also exists. In countries such as New Zealand, compound 1080 is still used extensively as a predacide and rodenticide, and for the control of problem animals (Rammel & Flemming 1978).

Despite being banned in South Africa, compound 1080 is used for the malicious poisoning of dogs, cats and other animals. Typical clinical signs of poisoning include severe convulsions and muscular fibrillations (Osweiler, Buck & Van Gelder 1985). In South Africa, the substance is possibly obtained from neighbouring countries where MFA is not banned, from expired stock purchased prior to it having been banned, or from possible illegal production of the compound.

Dichapetalum cymosum, known colloquially as "gifblaar" (which means poison leaf), is an indigenous shrub which contains monofluoroacetate. *Gifblaar* poisoning often results in acute death of ruminants, particularly cattle, in southern Africa. Mortalities in ruminants have been reported from Gauteng, Mpumalanga, North West, and Northern Provinces in South Africa. There are also reports of mortalities from Zimbabwe, Botswana and Namibia (Kellerman, Naudé & Fourie 1996). *Gifblaar* poisoning is considered the fourth most important plant poisoning syndrome in South Africa (Kellerman *et al.* 1996). Mortality as a result of ingestion of the leaves of this plant occurs mainly during the months of August to November, as

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well as in March (Steyn 1949, Kellerman, Coetzer & Naudé 1988).

The duration of the clinical signs of *gifblaar* poisoning in cattle is extremely short (Steyn 1949; Egyed & Shlosberg 1973; Kellerman *et al.* 1988). Veterinary practitioners usually do not observe the animal alive. In dogs, the clinical signs of MFA are of somewhat longer duration and resemble those manifested in strychnine or convulsive pesticide poisoning. Unless fragments of the *gifblaar* plant are found in the rumen, the diagnosis can only be based on clinical signs and circumstantial evidence.

The aim of this investigation was to apply a modified high-performance liquid chromatographic (HPLC) method for the determination of MFA (Minnaar, McCrindle, Naudé, Botha, Swan & De Beer 2000) to specimens received for diagnostic purposes by a veterinary laboratory, and to estimate the lability of MFA in them over a period of time. The specimens originated from a variety of animal species, chiefly cattle, as well as two from humans, animal baits and water.

MATERIALS AND METHODS

The method used for determining MFA (Minnaar *et al.* 2000) was modified and used over a period of 24 months (1997/98) in 50 cases of suspected *gifblaar* or synthetic MFA poisoning. The reported method was modified by changing the eluent composition from 0,02 M H_3PO_4 to a solution comprised of 0,04 M H_3PO_4 and 5 % acetonitrile. The flow-rate was also decreased from 0,8–0,4 mℓ/min. The limit of detection for MFA was established at 12 µg/ℓ. Analysis was done on submitted samples, which consisted mainly of rumen or stomach contents, kidney or liver, originating from cheetah (n=1), bird (n=1), horse (n=1), human (n=2), sheep (n=2), cat (n=7), dog (n=13) and cattle (n=17). In addition, five specimens were of bait and one comprised water. The samples were extracted in an aqueous solution and analyzed directly without any chemical clean-up. Rumen or stomach contents from the animals or humans were initially analyzed, but if found to be negative, the liver (if available) was also analyzed for the presence of MFA.

To determine the stability of MFA in biological samples, fresh rumen content and liver samples from a bovine were collected from an abattoir and sufficient MFA was added to make the concentration of MFA in the rumen sample 7 µg/mℓ and in the liver sample 5 µg/mℓ. These two spiked samples were then homogenized and each was divided into ten equal aliquots. One aliquot each of rumen content and liver were immediately analyzed. Another aliquot of each was placed in a freezer at –23 °C. All the remaining aliquots were left at room temperature (approximately 10–27 °C day-time temperature) and analyzed periodically during the following 20 weeks (Table 2). The MFA concentrations of the frozen aliquots were determined after 14 d.

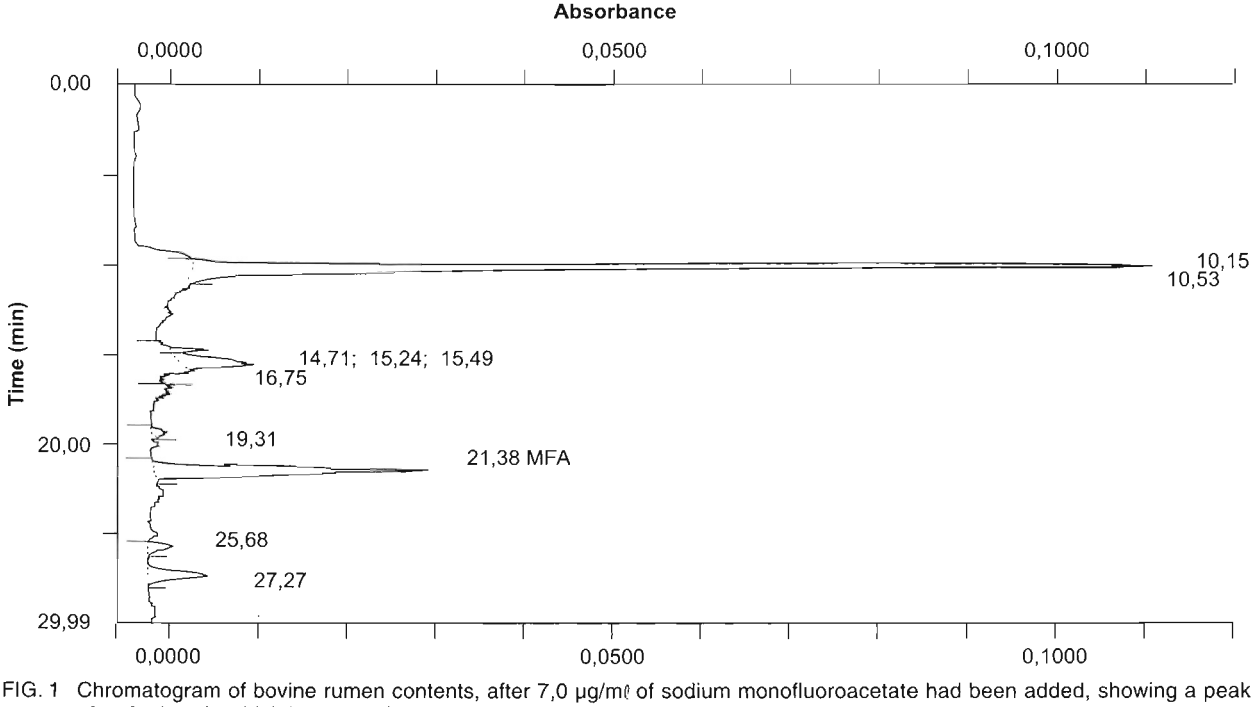

FIG. 1 Chromatogram of bovine rumen contents, after 7,0 µg/mℓ of sodium monofluoroacetate had been added, showing a peak after 21,38 min which indicates the presence of MFA

TABLE 1 Results of suspected MFA poisoning in different species

Species or sample type	Number of samples positive samples	Number of samples (µg/mℓ)	MFA concentration in positive
Bait	Bait (n = 5)	4	16,2–432,0, $\bar{x}$ = 253,0
Bird[c]	Liver (n = 1)	1	336,0
Cat[a]	Stomach (n = 7)	6	74,2–210,0, $\bar{x}$ = 122,8
Cattle[b]	Rumen (n = 17)	10	15,4–966,0, $\bar{x}$ = 408,9
Cheetah	Stomach (n = 1)	–	–
Dog[a]	Stomach (n = 13	8	38,2–744,0, $\bar{x}$ = 226,9
Horse[c]	Stomach (n = 1)	1	34,2
Human[a]	Liver (n = 1)	2	280,0, 500,0, $\bar{x}$ = 440,0
	Stomach (n = 1)	–	
Sheep[b]	Rumen (n = 2)	1	61,2
Water	Water (n = 1)	–	–
Total	50	33	$\bar{x}$ = 235,3 µg/mℓ

[a] Deliberate poisoning
[b] Natural poisoning
[c] Accidental/secondary poisoning

TABLE 2 Stability of MFA in spiked rumen and liver samples

Time (d)	Recovered MFA conc. in rumen samples (µg/mℓ)	Recovered MFA conc. in liver samples (µg/mℓ)
6	6,6	4,4
13	6,1	4,0
20	5,8	3,6
27	5,8	3,4
34	5,8	2,8
56	5,7	2,7
83	5,6	2,6
104	5,6	2,3

RESULTS

Results of submitted specimens

The modified HPLC method was found to be most effective in separating the important components in the samples analyzed as is exemplified by the chromatogram of a spiked rumen sample shown in Fig. 1.

Sixty-six per cent of the samples submitted were positive for the presence of MFA. Of the 50 cases investigated (Table 1), 20 were submitted for confirmation of *gifblaar* poisoning. All the cattle and sheep were poisoned by ingestion of the actual plant. It was found that if the rumen samples were not 'fresh', MFA detection was unreliable and the lability of specimens was investigated further. Thirty of the specimens submitted were from cases that were suspected of having been poisoned with MFA, and eight dogs and seven cats were found to be positive. This implies that these companion animals, in general, may be considered victims of deliberate poisoning. The MFA concentration in the samples from dogs, consisting mainly of stomach content, liver, and kidney, were found to be high, with a mean MFA concentration of 227,0 µg/mℓ, whereas the mean from the cat samples were 100,6 µg/mℓ. Most of the incidents of suspected deliberate poisoning occurred in the Eastern Cape Province. All 50 samples were submitted 'fresh' frozen or 'fresh' on ice.

Results of spiked specimens

The concentration of MFA in the spiked rumen and liver samples is given in Table 2. The concentration of MFA in the samples frozen immediately and analyzed after 14 d were within 2 % of those analyzed immediately after the addition of MFA. After 83 d, the concentration of MFA in the rumen samples held at room temperature, stabilized at 85 %, while that of the liver samples decreased to 52 % after 104 d. Further time lapse revealed no additional changes in the MFA concentration in either the rumen or liver samples.

DISCUSSION

During the two year period from 1997–1998, MFA has been one of the compounds used to poison dogs and cats maliciously in South Africa. No less than 14 cases have been considered deliberately poisoned with this substance during this period. Non-target animals are usually incidently poisoned by ingestion of poisoned carcasses. It is suspected that the bird, a Blue Crane, died as a result of secondary poisoning after it had fed on a donkey carcase which is thought to have contained MFA although this was never proved. The horse died after eating fodder which is presumed to have been contaminated with *gifblaar*.

The results obtained during the two year period by the modified HPLC method developed by Minnaar *et al.* (2000) for the determination of MFA in animal tissues and rumen or stomach contents has been

employed indicate that MFA poisoning, either natural or intended, are of great concern in South Africa.

Although compound 1080 is banned in South Africa it would appear, as there is, to our knowledge, no other feasible source of MFA, that there is a supply of it particularly in the southern Eastern Cape Province and that this substance is being used for the malicious poisoning of animals.

The South African Police Services were assisted in their investigations by analyzing two of the samples included in this survey.

In order to obtain the best quality material for analysis by the modified HPLC method, it is recommended that specimens from cases of suspected MFA poisoning should comprise, if possible, 100 g each of liver and kidney tissue, and the same amount of stomach or rumen contents. These should be placed separately in scrupulously clean containers, preferably glass or plastic, as soon after the death of the animal as possible, and immediately frozen. These should be submitted in the frozen state to a laboratory equipped to perform the analysis preferably within 14 d.

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