Detection of the Pesticide Compound 1080 (Sodium Monofluoroacetate) Using Fluorine-19 Nuclear Magnetic Resonance Spectroscopy

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Fluorine-19 nuclear magnetic resonance (^{19}F NMR) spectroscopic measurements were used to determine the chemical nature and amounts of organofluorine in dosed meat baits. Earlier work implied that sodium monofluoroacetate (compound 1080) in meat baits was broken down into other organofluorine compounds such as fluorocitrate. No chemical evidence was found for such compounds. Only monofluoroacetate was detected in the prepared 1080 bait samples. Once the baits have aged, aqueous extraction fails to recover all the added 1080. Analysis using ^{19}F NMR confirmed that the 1080 present in the aqueous extracts of the bait is recovered by Kramer's liquid chromatography method. It was shown here that the aqueous extracts do not recover all the 1080 in the meat bait.

Keywords: *Compound 1080; meat bait; recovery; liquid chromatography; nuclear magnetic resonance spectroscopy*

Sodium monofluoroacetate or compound 1080 (hereafter referred to as 1080) is a pesticide used in Australia for the control of vertebrate pests such as the dingo/feral dog (*Canis familiaris*), the feral pig (*Sus scrofa*) and the rabbit (*Oryctolagus cuniculus*). The toxicity of 1080 and the lack of an effective antidote necessitate a high level of operator safety and environmental consideration.

Extensive testing has been carried out on bait preparation quality control.[1,2] Kramer *et al.*[1] reported losses of up to 49% post dosing and inferred that the loss was due to "biochemical reaction rather than physical loss." Using a fluoride ion technique for the analysis of 1080,[3] no such loss was found in similar work on field prepared baits,[2] with samples of injected baits showing a retention of 90–97% of the nominal dose. However fluoride ion techniques detect all the organofluorine present as 1080.

Any loss of 1080 has important implications in baiting strategies as the loss would need to be compensated for to maintain toxicity. Using ^{19}F NMR spectroscopy the type and concentration of organofluorine compounds in the bait can be studied. This paper describes experiments conducted to establish the whereabouts of the "missing" 1080 and to identify any breakdown products.

Experimental

Sample Preparation

To obtain a reproducible method of bait preparation and homogeneous 1080 distribution, ground meat was used in place of the meat pieces used in actual bait preparation. Pet food grade kangaroo meat was ground and weighed into plastic bags (Stomacher 400 Blender Bags) and a known amount of aqueous 1080 solution was added. Baits for meat slurry NMR were re-ground using a laboratory processor (Omni Mixer 17106, OCI Instruments) before addition of 1080. This further grinding was carried out to give a more homogeneous sample so as to obtain the best possible ^{19}F NMR spectra. The bag contents were blended (Stomacher Lab-Blender 400, Seward Medical) for a period of 60 s to promote uniform mixing of 1080 throughout the meat. The bag was sealed and stored at room temperature for a period of 2–4 d before analysis.

Homogeneity of BIT Sample Preparations

Aged 1080 ground meat baits were prepared as described above. A sample of each bait was removed and placed in another blender bag with the mass of each fraction being noted. The 1080 content of both fractions was subsequently determined using high-performance liquid chromatography (HPLC).[4]

Comparison of Aqueous Extract 1080 Contents Using ^{19}F NMR and HPLC

Baits were prepared as described above and extracted with water, as in the first stage of the HPLC 1080 analysis technique

Table 1. 1080 spike recovery

Sample	Spike 1080 content/mg	HPLC 1080 content/mg	Recovery, %
1	598	330	55
2	685	356	52
3	557	240	43
4	625	322	52
5	794	305	38
6	429	200	47
7	583	241	41
8	763	384	50
9	516	220	43

Table 2. Testing of homogeneity of bait sample preparations

Sample	Fraction [1080]/ mg g^{-1}
1A	1.91
1B	1.59
2A	1.53
2B	1.85
3A	2.01
3B	1.71

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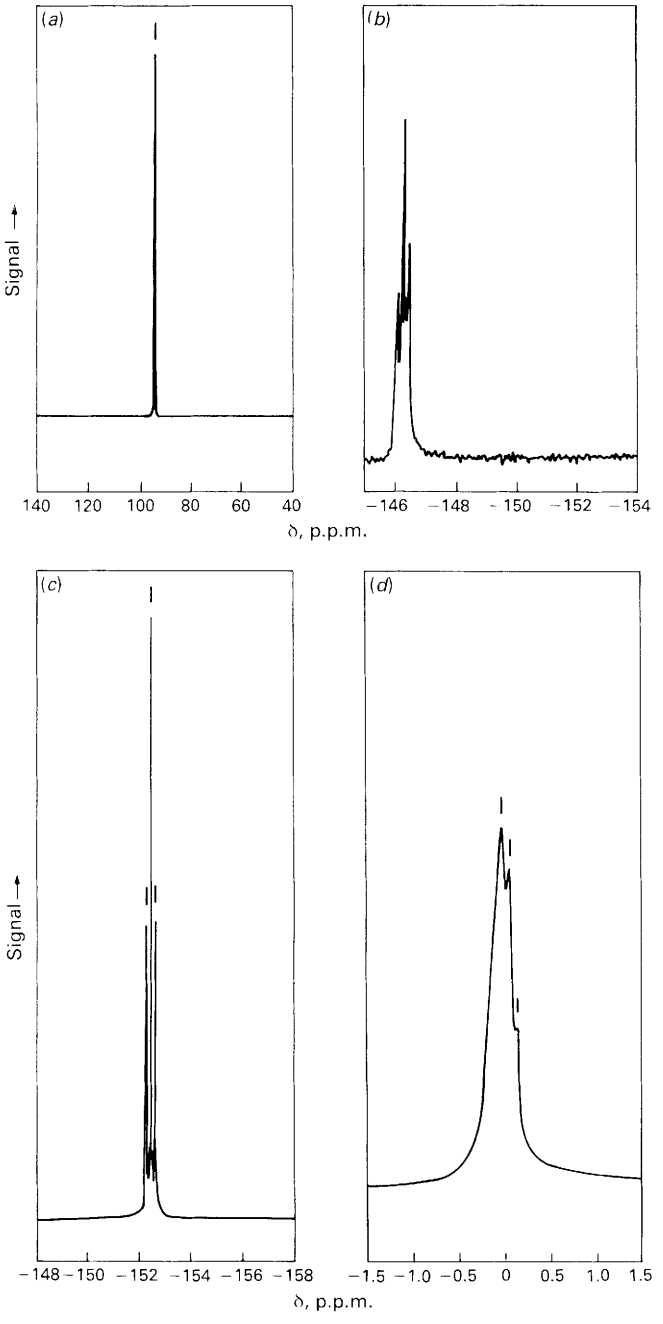

Fig. 1. ^{19}F NMR spectra of aqueous solutions of (*a*) sodium fluoride; (*b*) fluoroacetic acid; (*c*) sodium monofluoroacetate; and (*d*) sodium monofluoroacetate dosed meat

Table 3. Peak position information for the samples studied

Solution	Position,* p.p.m.
1080†	−138.85
Fluoroacetic acid†	−149.76
Sodium fluoride	−44.08
Meat slurry†	−139.55

* Relative to an external trifluoroacetate reference compound (δ_{TFA} = 0 p.p.m.). The positions have an error of 0.1 p.p.m.
† The chemical shift is to the centre of the triplet.

of Kramer,[4] with the 1080 content of the unused aqueous extract being determined by ^{19}F NMR spectroscopy.

Fluid and Slurry 1080 Contents

Aged 1080 ground meat baits were blended for 60 s and two sub-samples taken. The 1080 content of one sub-sample was determined by HPLC,[4] whereas the other sub-sample was centrifuged for 5 min at 650 *g*. Centrifugration separated the sample into a liquid supernatant and a compacted meat slurry; 1 cm^3 of the supernatant was removed, diluted with 1 cm^3 of deuterium oxide (D_2O) (Merck) and the 1080 content determined by ^{19}F NMR spectroscopy. A sample (*ca.* 1 g) of the compacted meat slurry was also diluted with D_2O (*ca.* 5 cm^3) and the ^{19}F NMR spectrum obtained.

Table 4. Comparison of 1080 contents of aqueous extracts of samples analysed using ^{19}F NMR and HPLC

Sample	Spike 1080 content/mg	1080 content by ^{19}F NMR/mg	1080 content by HPLC/mg
1	598	383	330 (86%*)
2	625	335	322 (96%*)
3	583	410	241 (59%*)

* Value of 1080 content determined by HPLC expressed as a percentage of the 1080 content determined by ^{19}F NMR.

Analysis of 1080

High-performance liquid chromatography

The high-performance liquid chromatographic analysis of 1080 was carried out using the method of Kramer.[4] A Millipore-Waters high-performance liquid chromatograph equipped with a μ-Bondapak C_{18} reversed-phase column was used, with UV detection at 260 nm. The column temperature was maintained at 35 °C. The mobile phase was tetrahydrofuran (Millipore-Waters) - de-ionised, distilled water (38 + 62). All HPLC data for 1080 were corrected for an over-all recovery of 90%, established by Kramer.[4] Samples and standards [fluoroacetic acid (Merck)] were assayed following derivatisation with 2,4′-dibromoacetophenone (Aldrich) in an acetonitrile (Mallinkrodt) 18-crown-6 ether (Aldrich) solution.

The 1080 solutions were prepared by dissolving commercial grade 1080 (Rentokil Pty.) in distilled water; the concentrations were verified by HPLC.[4]

Fluorine-19 NMR spectroscopy

All ^{19}F NMR spectra of 1080 were accumulated on a Bruker CXP300 NMR spectrometer operating at 282.32 MHz for the ^{19}F resonances of interest. Each spectrum occupied blocks of 8k data points, which were zero filled to 16k and multiplied with an exponential line-broadening function (line-broadening constant of 1 Hz), prior to Fourier transformation. A spectral width of 10 000 Hz led to an acquisition time of 4.096 s. Each spectrum was recorded at a probe temperature of 297 K. Sample solutions and meat slurries were diluted with D_2O, with a range of dilutions from 1 + 1 to 1 + 6 (sample - solvent) being used. Peak heights and peak areas were calculated from the ^{19}F NMR spectra, and with no significant differences between the two methods, peak areas were used for all calculations. Concentrations were determined from the peak-height ratios of the sample to external standard.

Results

Table 1 presents the results obtained from nine aged 1080 ground meat samples analysed using HPLC. Table 2 gives the results obtained when three ground meat bait samples were separated into two fractions and analysed separately for their 1080 content using HPLC. The corresponding fractions are designated as A and B.

Fig. 1 shows the ^{19}F NMR spectra obtained for aqueous solutions of 1080, fluoroacetic acid, sodium fluoride and 1080

Table 5. ^{19}F NMR fluid 1080 and slurry 1080 contents

Sample	1080 spike content/mg	Fluid 1080 content/mg	Slurry 1080 content/mg	Theoretical* HPLC content/mg
1	620	434	239	339 (78%†)
2	616	533	261	321 (60%†)
3	647	386	207	260 (67%†)

* The original samples used for this experiment were mixed and split into two fractions prior to analysis. One fraction was analysed for its 1080 content using HPLC; the remaining fraction was used in this experiment. The theoretical HPLC content is based on the HPLC 1080 concentration obtained for the corresponding fraction.

† HPLC 1080 content expressed as a percentage of the ^{19}F NMR 1080 fluid content.

Table 6. Comparison of 1080 content spikes and the sum of the corresponding 1080 fluid and slurry contents

Sample	1080 spike content/mg	Sum of fluid and slurry 1080 contents/mg	Recovery, %
1	620	673	109
3	616	794	129
5	647	593	92

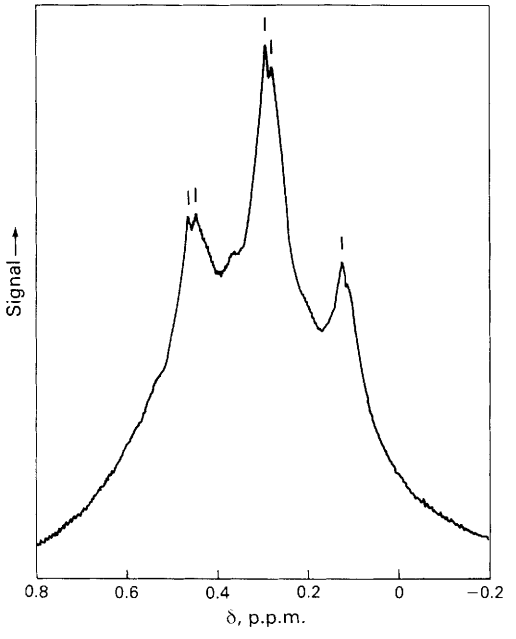

Fig. 2. ^{19}F NMR spectrum of a sodium monofluoroacetate dosed meat sample showing the separation of triplets thought to be due to two different fluoroacetate environments

impregnated ground meat baits. The peak positions obtained using these and other spectra are given in Table 3.

The aqueous extracts prepared for HPLC analysis were also analysed by ^{19}F NMR spectroscopy for their 1080 content. Table 4 presents the results obtained for both forms of analysis.

Table 5 gives the 1080 content for both slurry and fluid components of three ground meat samples spiked with an aqueous solution of 1080.

Overall, the results reported have an error of 2% for the spikes, 5% for ^{19}F NMR and 10% for HPLC.

Discussion

The results presented in Table 1 confirm the findings of Kramer *et al.*[1] They show a mean recovery of 47% (standard deviation = 5.8) and demonstrate that the 1080 loss is reproducible using this bait preparation method. The results given in Table 2 show that the bait preparation method used here gives a homogeneous distribution of 1080 in the bait, which is crucial for the work being undertaken. A paired comparison between corresponding samples shows no significant difference ($p > 0.05$, $t = 0.609$, no. of degrees of freedom = 4); hence representative sub-samples of the baits can be taken.

Fig. 1 shows typical ^{19}F NMR spectra of aqueous solutions of 1080, fluoroacetic acid, sodium fluoride and 1080 dosed meat. Both the fluoroacetic acid and 1080 ^{19}F NMR spectra are triplets. This is due to spin - spin coupling between the fluorine atom and the protons in the fluoromethyl group of fluoroacetate. For the ^{19}F NMR spectra obtained on 1080 dosed meat, the only fluorine containing species detected was 1080. This was in spite of the fact that the meat had been impregnated with large amounts of 1080 (*ca.* 1 g). With a 47% "loss" any organofluorine breakdown products should be detected. Hence there is no ^{19}F NMR evidence for any organofluorine breakdown compounds.

In Table 4, the ^{19}F NMR 1080 contents indicate good recovery using the HPLC method[4] of all the 1080 in the aqueous extract, but also shows that the aqueous extraction stage does not recover all the 1080 from the bait. Hence either some of the 1080 is more tightly bound or the aqueous extraction is not an efficient method for the recovery of 1080. The latter is in contrast to the work of Kramer *et al.*,[1] who reported that even when more vigorous extraction procedures were used, the recovery of 1080 did not increase.

To investigate this bound 1080, another experiment was carried out to separate the two forms of 1080. In this instance the fluid and solid portions of the meat were separated by centrifugation and the two components analysed for their 1080 content by ^{19}F NMR (see Table 5). Table 6 presents a comparison of the 1080 spike values with the corresponding 1080 values obtained by summing the values obtained for the slurry and fluid samples. From this table the mean recovery is 110%. However, the slurry 1080 content will be inflated due to residual fluid 1080 resulting in an exaggerated recovery. This in turn helps to verify the existence of a bound form that is not recoverable by aqueous extraction.

On several occasions the ^{19}F NMR spectra of the 1080 dosed meat split into two triplets; an example of this splitting is shown in Fig. 2. Such a separation is consistent with two forms of 1080 in the bait and appears to be a function of the sample to solvent (D_2O) ratio, with its occurrence being more prevalent at higher dilutions. The peak separation is very small (*ca.* 0.7 p.p.m.), indicating similar molecular environments. If the fluoroacetate is chemically bound it will be through the carboxyl group where the influence on the chemical shift of the fluorine atom will be least.

Conclusions

The phenomenon of "missing 1080" appears to be due to incomplete recovery of 1080 from aged meat baits rather than the formation of an organofluorine breakdown product. The original HPLC method[4] gave recoveries of 90–94%. The work described here substantiates this claim, provided that the baits are analysed within a short time of dosing. Other work at this laboratory involving the analysis of 1080 on formulations and grain baits indicates that the "missing 1080" phenomenon does not extend to these bait substrates. The loss is not attributable to chemical breakdown of 1080; ^{19}F NMR studies carried out here showed that no other significant fluorine species was

present. The incomplete recovery is caused by binding of a proportion of the 1080 in such a way that the aqueous extraction is unable to remove it. The ^{19}F NMR spectrum of 1080 in meat indicates that the binding leaves the monofluoroacetate intact. Work on the exact nature of the binding is continuing.

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