Analytical Determination of Compound 1080 (Sodium Fluoroacetate) Residues in Biological Materials

by

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Summary

A rapid and accurate method is described to determine toxic organofluorine residues in biological materials. Sample preparation is minimal. The sample is degraded by oxygen combustion, and the liberated inorganic fluoride determined by an ion-specific electrode. The method is specifically designed for the determination of sodium fluoroacetate (Compound 1080), and several of its derivatives with similar physiological action, during vertebrate pest-control programmes.

INTRODUCTION

In New Zealand, and also in several countries abroad, sodium fluoroacetate (Compound 1080) has for many years been the toxin of choice in the lethal control of vertebrate pest populations. The toxin is usually incorporated in various types of bait, composed of pollard, carrot, or other materials of plant or animal origins. In New Zealand, the bait is distributed over often extensive areas of forest or agricultural lands. Detoxication of the bait then proceeds by "weathering" and by microbial action. Thus, among other considerations, careful monitoring of this decline in toxicity is an essential factor in prudent management practice by avoiding premature exposure of farm animals to toxic pasture.

In their concern with the diagnostic determination of toxic organo-monofluorine residues, earlier investigators (for instance, SAWYER et al. 1967, STAPLES 1968, CORR & MARTIRE 1971) employed various modifications of the Willard-Winter procedure (A.O.A.C. 1965). This procedure is complex, laborious, and it requires considerable technical skill. It is therefore unsuitable for investigations that involve multiple organofluorine analyses.

In recent years, the analysis of inorganic fluoride has been considerably simplified by the introduction of the

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fluoride-specific ion electrode (FRANT & ROSS 1966). However, the carbon and fluorine atoms in fluoroacetate and related organo-monofluorine compounds are very firmly bound, and this C-F linkage requires rupture, by a variety of lengthy decomposition procedures (MIROSEVIC-SORGO & SAUNDERS 1959), before the fluorine can be "sensed" by the ion electrode. SELIG (1970) described the decomposition of organic polyfluorine compounds into inorganic fluoride by the oxygen flask combustion procedure (MACDONALD 1961). It occurred to us that this procedure also accomplishes easily the decomposition of sodium fluoroacetate into inorganic fluoride.

This report therefore describes a simple analytical procedure whereby toxic organo-monofluorine residues can be determined rapidly and accurately in a variety of substrates.

METHOD

A suitable aliquot (0.1-0.5 ml) of liquid sample material is adsorbed onto filter paper, briefly dried, and transferred to the platinum basket of an oxygen combustion flask. Solid sample material is homogenised and lyophilised, or dried in an oven at 80°C. A known amount (about 50 mg) is weighed into a gelatin capsule. The combustion flask (500 ml capacity) is made of VYCOR glass (Corning Glass Works, U.S.A.) with wide-mouthed ground-glass joints. It is similar in design to that described by KALBERER & RUTSCHMANN (1961), with provision for the platinum basket and the ignition electrode through the ground-glass stopper. To improve ignition, the sample in the platinum basket is moistened with a drop of n-dodecane, and combustion is carried out by the standard technique (MACDONALD 1961). The combustion apparatus described by MILLER & ALLSOP (1970) is a convenient device for processing multiple combustions during the manipulation of large-scale assays in the laboratory. The combustion products in the flask are adsorbed into 10 ml of 0.05N aqueous sodium hydroxide. The flask contents are quantitatively rinsed into a polythene beaker with the aid of 10 ml CDTA buffer (Orion Research 1970). Fluoride ion activity in this buffered alkaline solution is measured with the Combination Fluoride Electrode (Orion Research, Model 96-09) on the Expanded Scale Meter (Orion Research, Model 407) or on the Digital pH/MV Meter (Orion Research, Model 801). The fluoride ion concentration is calculated either by the addition of suitable aliquots of sodium fluoride standards (Known Addition Method), or by comparison with a suitable standard concentration graph, as described in the Orion Research Technical Bulletins (1970). Inorganic fluorine in the original sample is determined essentially by elimination of the combustion stage. To suitable aliquots of liquid sample material are added sodium hydroxide solution and CDTA buffer, as described above. Solid sample material is homogenised in water and inorganic fluorine is determined directly on suitable aliquots of the homogenate. The value of inorganic fluorine is subtracted from that of the total fluorine in the combusted sample.

RESULTS AND DISCUSSION

To determine the efficiency of the combustion procedure aqueous (deionised) solutions of sodium fluoroacetate were freshly prepared, 0.2 ml of each concentration were combusted and its fluorine composition determined. Table 1 summarises the recovery over a wide range of concentrations. Similar recoveries were obtained with other toxic organofluorine compounds (in solution or in solid form) namely, fluoroethanol, fluoroacetamide, fluoroethyl biphenylacetate, methyl naphthyl fluoroacetamide, α-fluorobutyric acid ethyl ester.

In New Zealand, toxic pellets, composed of pollard, bran, molasses, tallow and sodium fluoroacetate are utilised as bait for the control of opossum populations in forest areas. These pellets are manufactured to a specified composition of 2 lb of sodium fluoroacetate per ton of pellets (890 μg/g pellet). To determine the dispersion and recovery of the toxin during a manufacturing process of toxic pellets, increments of the toxin were added to a toxic pellet and the organofluorine compositions determined by the standard procedure. Table 2 indicates that the accuracy of the procedure is satisfactory.

Chopped carrots impregnated with sodium fluoroacetate are utilised as bait for the control of rabbit populations on pastoral lands. To this purpose, carrots are soaked in solutions of the toxin at the rate of 0.5 lb per ton of carrots (223 μg/g carrot). These control programmes are carried out during the winter months when pasture growth is reduced. Whilst farmers prefer the shortest delay before re-introducing farm stock, premature exposure to toxic pasture cannot be considered prudent management. Therefore, an assessment of the degree of residual toxicity in deteriorating carrot bait by the simple analytical procedure described here can avoid misjudgement. For example, Table 3 outlines the results of such an assessment of field conditions. The toxic carrot bait had been distributed in the field five weeks prior to sample collection. During this period it had been exposed intermittently to several frosts and about three inches of rain. Randomly selected samples were taken and analysed, with no attempt to separate decomposing debris

TABLE 1

Determination and Recovery of Sodium Fluoroacetate (SFA) in Solution

The results of 0.2 ml aliquots are expressed as the mean values ($\pm$SD) from six determinations of each concentration. Reagent blank values were insignificant.

Concentration of SFA solution (mg/ml)	Expected (µg)		Found (µg)		Mean SFA recovery (%)
	SFA	F	F	SFA	
10*	2000	380	342 (14.4)	1800 (72.8)	90.0
5	1000	190	172.5(1.6)	925 (9.9)	92.5
2.5	500	95	85.5(3.7)	451 (19.6)	90.5
1	200	38	36.9(1.0)	195 (6.6)	97.5
0.5	100	19	18.6 (0.4)	97.5 (2.6)	97.5
0.25	50	9.5	8.8 (0.1)	46.3 (0.7)	92.5
0.1	20	3.8	3.7 (0.2)	19.1 (1.0)	95.5
0.05	10	1.9	1.8 (0.09)	9.4 (0.5)	94.3
0.025	5	0.95	0.8 (0.1)	4.4 (0.6)	88.5
0.01	2	0.38	0.36(0.05)	1.9 (0.25)	94.5

* Contained 11.7 µg inorganic fluorine/ml.

TABLE 2.

Sodium Fluoroacetate (SFA) Composition of Pollard Bait Pellets (average weight 4.5 g), and the Recovery of added SFA.

The results are expressed as the mean values ($\pm$SD) from six determinations. Non-toxic pellets contained 15.6 μg F (1.02)/g. Gelatin sample containers contained 0.25 μg F (0.17)/capsule. These contributions have been excluded from the results.

	SFA composition of pellet (mg/g)		SFA Addition (μg)	SFA Recovered (μg)	Mean recovery of added SFA (%)
	Expected	Found			
Toxic Pellet 1	0.89	0.69 (0.02)			
" " 2	0.89	0.82 (0.02)			
" " 3	0.89	0.87 (0.03)			
" " 4	0.89	0.88 (0.04)	1000	925 (40.9)	92.5
			100	87.4 (3.3)	87.4
			10	8.1 (1.5)	81.0

TABLE 3

Organofluorine Residue in deteriorating Toxic Carrot previously impregnated with Sodium Fluoroacetate (SFA)

Inorganic and organic fluorine contents of non-toxic carrot were negligible. Details are described in the text.

	SFA Composition of wet sample (μg/g)	
	Initial concentration	Found
Toxic carrot 1	220	
" " 2		29.8
" " 3		21.6
" " 4		12.8
" " 5		14.8
" " 6		13.2
" " 7		12.7

from apparently healthy carrot tissue. The results (Table 3) indicated that about 13% (29.8 μg/g tissue) of the original amount of toxin had been retained. Assume 12.5 mg of sodium fluoroacetate to be a lethal dose for a 25 kg (55 lb) sheep, (JENSEN <u>et</u> <u>al</u>. 1948). This animal would then require at least 419 g of carrot sample in order to consume a lethal dose. On the basis of these analyses isolation of the toxic pasture was continued.

The above example of the application of the described analytical procedure to field situations places emphasis on a further aspect which takes into account the leaching of sodium fluoroacetate from deteriorating bait materials as distinct from its detoxication by micro organisms. The detoxication and conversion into inorganic fluoride of the toxin by invading micro organisms cannot be evaluated by determination of total fluorine alone. The analytical procedure described here enables inorganic fluorine to be determined in the extracted sample by omission of the combustion stage. Furthermore, earlier investigators in New Zealand (STAPLES 1968) and in Australia (CORR & MARTIRE 1971) reached no unanimity on the extent of leaching by rain of the toxin from carrot baits. It is conceivable that the discrepancy in their observations could be resolved by relating the effects of precipitation to those of temperature fluctuations (freezing, thawing and dehydration). Certainly, with regard to the retention of toxin in the carrot bait the effects of frost are contrary to those of rain. Whilst subsequent thawing facilitates the decomposition process it also facilitates ingress of the toxin, via frost-damaged cellular membranes, from the peripheral tissues to the interior of the bait. Thus, it has been our experience that carrot baits, subjected to repeated freezing-thawing periods in the field, retain higher toxic residues than those subjected to rain. Similarly, dehydration at elevated temperatures facilitates crystallisation of the toxin near the periphery of the carrot bait from which it can be readily leached by rain. This complex process of interactions requires further investigation. Nonetheless, repeated *ad hoc* assays of bait materials by the rapid and reliable analytical procedure described here would obviate premature decisions and exposure.

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