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Degradation of Sodium Monofluoroacetate (1080) and Fluorocitrate in Water

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Sodium monofluoroacetate (1080) is a toxicant commonly used in New Zealand for the control of vertebrate pests, principally the brushtail possum (*Trichosurus vulpecula*) and rabbits (*Oryctolagus cuniculus*). The widespread usage of 1080 and its high water solubility have caused community concern about the environmental effects of the compound and its potential for contamination of waterways. This has resulted in research into the persistence of 1080 and its metabolites in water and the environment.

Previous research has shown that 1080 was degraded in stream water, but degradation can be affected by many environmental factors. Parfitt et al. (1994) showed that 1080 was degraded in biologically active water in 2-6 days while Eason et al. (1993) showed that 1080 declined by approximately 70% in 1 d and to below detectable limits (0.0003 ppm) in 4 d in aquaria containing plants and invertebrates. Ogilvie et al. (1995, 1996) showed that temperature significantly enhanced the rate of 1080 degradation, and that this was further enhanced in the presence of aquatic plants and microorganisms. Some microorganisms have been shown to degrade 1080 by cleavage of the C-F bond (Goldman 1965; Goldman et al. 1968; Walker and Bong 1981; Wong et al. 1992) using the adaptive enzyme haloacetate halidohydrolase to produce glycollate (Fig. 1a). In animals, 1080 is thought to be converted by the enzyme citrate synthetase to fluorocitrate (Fig. 1b), which then inhibits the tricarboxylic acid (TCA) cycle via the enzyme aconitase. Fluorocitrate is the proposed toxic metabolite of 1080 in animals, but it is not known whether microorganisms are capable of converting 1080 to fluorocitrate. Therefore, in order to determine whether fluorocitrate is formed in the environment, we have developed a method for the analysis of fluorocitrate in water.

MATERIALS AND METHODS

Stream water and the aquatic plant *Myriophyllum triphyllum* were collected from the Waimakariri River, Canterbury, New Zealand. Eighteen 2-L aquaria were maintained at 21 °C ± 2 with a 15/9-hr light/dark cycle. Aquaria contained either 1.8 L of stream water and 60 g of plant material or 1.8 L of deionised water only (controls). All aquaria were covered with polyurethane film to minimize cross contamination of the sterile controls with microorganisms in aerosols, and were bubbled with air that had passed through a filter of 0.45 µm pore size.

Sodium monofluoroacetate was added to triplicate aquaria at 0.12, 2, and 5 mg/L, and the

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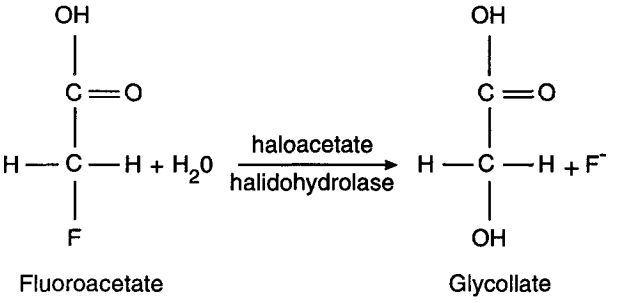

Figure 1a. Pathway for degradation of 1080 by soil microorganisms (Walker, 1994).

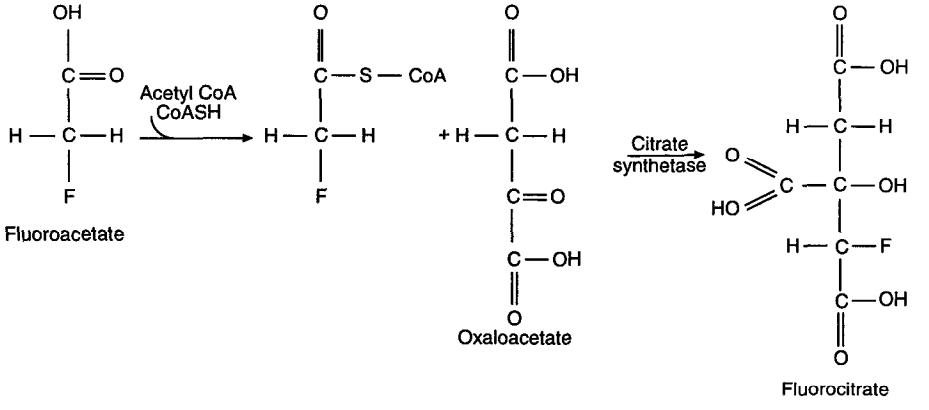

Figure 1b. Proposed biosynthetic pathway for formation of fluorocitrate by the TCA cycle (Walker, 1994).

contents were thoroughly mixed on a magnetic stirrer immediately after the 1080 was added, and again immediately before sampling. The lowest concentration of 1080 added was 35 times higher than the highest concentration measured in field samples (0.0034 ppm) collected after control operations (Booth et al., 1997), therefore the concentrations chosen represent a worst-case scenario. Water samples of 100 mL were taken from all aquaria at 0, 12, and 24 hr, and 2, 4, 8, 14, and 17 d after the addition of 1080. All samples were stored at -20°C and analysed for 1080 and fluorocitrate concentration.

Analysis was conducted with a 50 mL water sample which was acidified and the 1080 was converted to the dichloroaniline derivative by N,N'-dicyclohexylcarbodiimide (DCC) and 2,4-dichloroaniline (DCA). The derivative was chromatographed on a silica cartridge, eluted with toluene, and analysed by gas chromatography with electron capture detection, based on the method of Ozawa and Tsukioka, (1987). This method has a limit of detection of 1 x 10^{-4} mg/L in water.

Fluorocitrate analysis was conducted with a 50 mL water sample which was filtered through a glass filter and evaporated to dryness. Residual fluorocitrate was derivatised with boron trifluoride/trifluoroethanol at 75-80°C for 30 min. The sample was cooled, diluted

with water, extracted with toluene and analysed by gas chromatography using a Hewlett Packard 5890 II gas chromatograph with a split/splitless injector and an electron capture detector. The injector was used in split mode. The column was BP-5 column (30m x 0.33mm). The carrier gas was helium at a flow rate of 3 ml min^{-1}. The injector temperature was 200°C and the detector was 300°C. The column temperature was held at 60°C for one minute, ramped up to 156°C at 4°C/min, then ramped up to 280°C at 25°C/min and held for 10 minutes. Samples can be injected every 30 min. The retention time for fluorocitrate is 15 minutes and the limit of detection is 0.01mg/L.

The rate of degradation of 1080 and formation of fluorocitrate was compared between controls and sample aquaria using repeated-measures analysis of variance with concentration as the categorical variable. The effect of initial 1080 concentration on 1080 degradation and fluorocitrate formation was also determined.

RESULTS AND DISCUSSION

Sodium monofluoroacetate was detected in all aquaria. The concentration of 1080 in the aquaria containing stream water and *Myriophyllum triphyllum* decreased over time to low levels after 17 d (Fig.2). The rate of degradation was dependant on the initial concentration of 1080 in the aquaria, with degradation occurring at a higher rate in aquaria with the highest initial concentration of 1080 ($p=0.021$). In contrast, the control aquaria which contained deionised water, showed no significant decrease in 1080 concentration over time. These findings are consistent with previous reports indicating that microorganisms and plants are responsible for the degradation of 1080 in aquatic environments (Ogilvie et al. 1995, 1996; Parfitt et al. 1994).

Fluorocitrate was detected in all aquaria containing stream water and *M. Triphyllum* after 1 day (Fig. 3). No fluorocitrate was detected in the control aquaria. Fluorocitrate was only detected in aquaria after 1080 degradation had started (Fig. 2), thus providing evidence that fluorocitrate is a product of 1080 degradation. This indicates that microorganisms can degrade 1080 in the same way as animals do, i.e., via the TCA cycle. Walker (1994) suggested that microorganisms degrade 1080 using the enzyme haloacetate halidohydrolase to produce glycollate and fluoride. Our data indicate that microorganisms are also capable of converting 1080 in the environment to fluorocitrate. Fluorocitrate concentrations peaked after 1, 4, and 8 d for the 0.12, 2, and 5 mg/L aquaria respectively and the amount of fluorocitrate produced was dependent on the initial 1080 concentration in the aquaria ($p=0.001$). Therefore, the fluorocitrate in the aquaria can only have been produced by microbial degradation of the 1080 and the presence of plants and this supports the hypothesis that microorganisms are responsible for conversion of 1080 into fluorocitrate.

Further evidence that fluorocitrate is a product of 1080 degradation is provided by the relationship between the amount of 1080 degraded and the production of fluorocitrate over time. Overall the amount of fluorocitrate produced was proportional to the amount of 1080 degraded (Table 1). At the time of peak fluorocitrate concentration 1 mole of 1080 produced 0.6-0.9 moles of fluorocitrate. After this peak, fluorocitrate was rapidly degraded to below the limit of detection for the 0.12 mg/L and 2 mg/L aquaria and to near the limit of detection for the 5 mg/L aquaria.

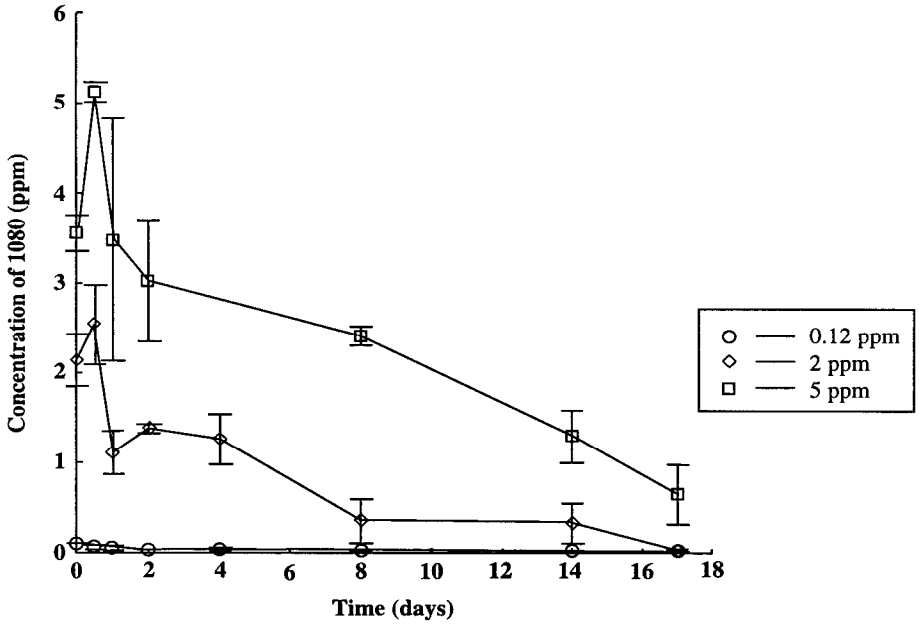

Figure 2. Degradation of 1080 in stream water aquaria over time. Error bars are ± 1 standard error.

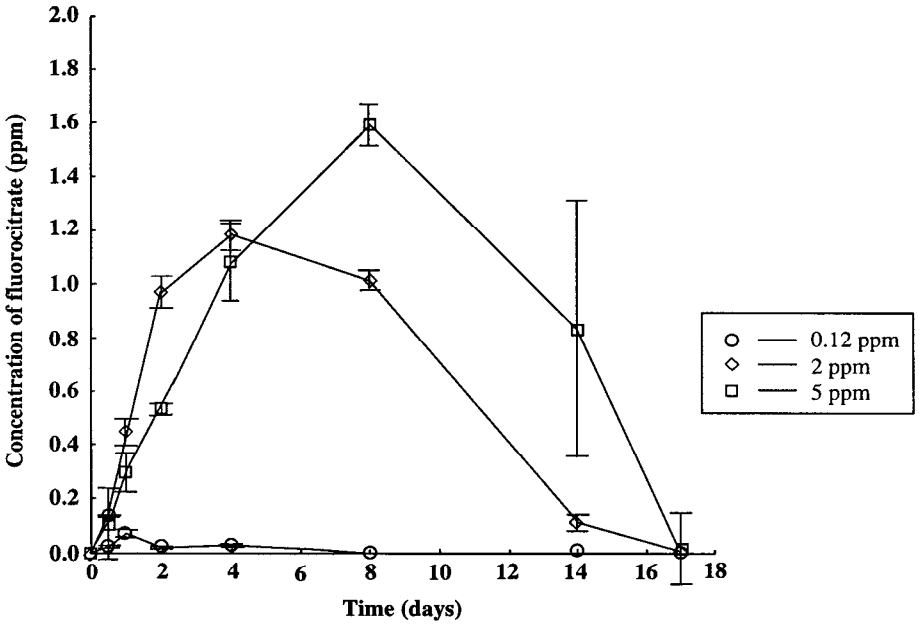

Figure 3. Formation and persistence of fluorocitrate with time in stream water aquaria. Error bars are ± 1 standard error.

Table 1. Mean amount of 1080 (µmol) degraded and fluorocitrate (FC) formed in biologically active aquaria.

	0.12 mg/L aquaria (2.16 µmol 1080)		2 mg/L aquaria (36 µmol 1080)		5 mg/L aquaria (90 µmol 1080)	
Time (days)	1080	FC	1080	FC	1080	FC
0	0	0	0	0	0	0
1	0.8	0.7	19.0	4.1	1.0	2.7
4	1.2	0.2	19.0	11.0		9.9
8	1.4	0	32.7	9.3	21.0	15.0
17	1.6	0	38.6	0	52.0	0.1

This study has provided evidence that 1080 is degraded in stream water and that fluorocitrate is a metabolite of 1080 degradation. Fluorocitrate like 1080 appears to be rapidly degraded in water, so that within 17 d after dosing with 1080 there were either very low levels, or no 1080 or fluorocitrate remaining in the aquaria. The implication of this is that if 1080 is absent from a water sample collected after a 1080 control operation, then fluorocitrate is likely to be absent also. Therefore it is legitimate to measure 1080 only, and not fluorocitrate, when evaluating possible 1080 contamination of waterways after 1080 control operations. In addition, fluorocitrate has lower oral toxicity than 1080 (Peters et al, 1972; Savarie, 1984), due to its large molecular size. Fluorocitrate, therefore is not readily absorbed and distributed throughout the body, in contrast to 1080 which is readily absorbed and converted intracellularly to fluorocitrate (Peters et al, 1972; Savarie, 1984). In conclusion, this research has shown that fluorocitrate is a product of 1080 degradation, but it is rapidly degraded and due to its lower oral toxicity compared with 1080, it is more important to monitor 1080 residues than fluorocitrate after a possum control operation using 1080 bait.

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