CHAPTER 2

DEGRADATION OF 1080 IN BAIT AND SOIL

2.1 Introduction

Bait must retain its lethal dose of 1080 for a sufficient period to allow the target animal to find and consume the bait (McIlroy *et al.* 1986). A bait must also degrade so non-target hazards from the 1080 are reduced and long-term environmental persistence is avoided (Calver and King 1986). Considerable effort has often been invested in improving the attractiveness and palatability of bait without regard to 1080 persistence and longevity. Bait with long lasting toxicity will almost certainly provide a potential hazard to farm dogs and other non-target species (Twigg *et al.* 2000). Additionally, public concern about 1080 use for vertebrate pest control in Australia and New Zealand (King *et al.* 1994; Williams 1994; Oogjes 1996) supports the need to investigate, among other things, bait longevity and environmental persistence of 1080.

The longevity of 1080 baits can be considerably reduced by rainfall (Griffiths 1959; Wheeler and Oliver 1978). In eastern Australia, Foxoff® will lose approximately 78% of the initial 3 mg dose after 2.4 weeks in moist soil conditions (Saunders *et al.* 2000) but may remain at lethal levels for greater than 11 weeks when not exposed to rain. Unburied fresh meat bait (6.1 mg nominal dose) may be viable for between 6-12 months in the drier conditions of central Australia (Twigg *et al.* 2000) compared to less than two months in more temperate areas (McIlroy and Gifford 1988; Fleming and Parker 1991). Egg baits injected with 4.5 mg of 1080 remain lethal to foxes for at least 6 weeks, even after considerable (>80 mm) rainfall (Twigg *et al.* 2001). Kirkpatrick (1999) found that buried dried meat baits can remain lethal to foxes after 7 weeks if exposed to little rainfall, but when exposed to moderate rainfall only last about one week. These studies indicate that rainfall and climate are important determinants of bait longevity, and should be included as factors in investigating any bait intended for widespread use in fox control programs.

Chicken wingettes have been used as 1080 fox baits in New South Wales since their introduction in 1998 as a substitute for chicken heads. Preliminary trials observed that chicken wingettes had reduced leakage compared to red meat (T. Abblett, Wentworth Rural Lands Protection Board, pers. comm. 2001), suggesting greater 1080 retention and operator safety. Chicken wingettes are also seen to offer several advantages relative to other bait types including high palatability to foxes and, in turn, a low incidence of caching; reduced longevity and thus reduced withholding periods in high rainfall areas; and low cost in comparison to some other bait types (D. Bate and D. Croft, NSW Department of Primary Industries and Fisheries, pers. comm. 2002). These, however, are only perceptions and require supporting experimental evidence. No studies to date have assessed 1080 longevity in chicken wingettes or any other chicken substrate. Given that their use is becoming increasingly popular (West and Saunders 2003), bait degradation trials are necessary to determine the toxicity of wingettes over time and for different rainfall and climatic conditions.

Microbes found in bait materials are capable of defluorinating 1080 (Wong *et al.* 1991). Primary processing for poultry differs to red meat in ways that increase microbiological contamination and spoilage implications, suggesting that wingettes may have reduced longevity compared to other bait types. Chickens are scalded in hot water (~50^0C) to facilitate feather removal, and each bird contributes micro-organisms to the scald water which may then spread between birds (Frazier and Westhoff 1978; Adams and Moss 1995). Birds are defeathered using mechanically rotating rubber fingers which are liable to microbial contamination which may be passed from one carcass to another (Adams and Moss 1995). This problem is exacerbated since skin, and thus skin-associated organisms, are not removed (Frazier and Westhoff 1978). Additionally, poultry evisceration is normally automated and leads to a high rate of carcass contamination with gut contents (Adams and Moss 1995). These problems can lead to greater spoilage of chicken products and reduced bait longevity.

Apart from those found in the bait medium, micro-organisms capable of 1080 degradation may also be found in the soil environment. Defluorinating soil micro-organisms ensure that 1080 will not persist in the environment once removed from the bait (Twigg *et al.* 2000). Although some studies have determined the presence of defluorinating microbes in areas of

Western and Central Australia (e.g. King *et al.* 1994; Twigg and Socha 2001), no such studies have tested soils in eastern Australia. Given the high usage of 1080 in the eastern states for fox, wild dog, pig and rabbit control, the presence of defluorinating micro-organisms needs to be established to confirm that long-term environmental persistence of 1080 is not occurring in eastern Australia.

In New South Wales, RLPBs are responsible for preparing and distributing 1080 baits to landholders for baiting operations. Automated vaccination inoculators are often used to administer 1080 to meat baits (Korn and Livanos 1986; Fleming and Parker 1991), but 0.1 ml graduated insulin syringes are often used (e.g. Molong, Central Tablelands and Cooma RLPBs) since they require no initial calibration and are disposable. Inoculators deliver an accurate dose (Korn and Livanos 1986) but the accuracy of insulin syringes has not been assessed. This should be done to ensure field-prepared baits are consistently dosed with 1080.

In this chapter, the rate of 1080 degradation in chicken wingettes for two climatic and 3 rainfall regimes are investigated to assess the potential longevity of wingettes for fox control programs in eastern Australia. The degradation of wingettes relative to Foxoff®, another commonly used bait type, is compared for the central tablelands and western slopes of New South Wales, from original and historical data (Saunders *et al.* 2000). Decay models are constructed to examine the relationship between 1080 loss and rainfall, and to provide a predictive model for bait longevity for each bait type under likely climatic conditions. Additionally, the graduated syringe injection technique for bait preparation is examined and 1080 assay method is critically assessed. The presence of defluorinating soil bacteria in eastern Australia is investigated to provide evidence for environmental degradation of 1080. Finally, the likely determinants of 1080 loss from bait and the implications of these findings for baiting programs are discussed.

2.2 Methods

2.2.1 Study sites

The study was undertaken at Trangie Research Station ($31^0$98'61"S, $147^0$94'89"E) and Orange Agricultural Institute ($33^0$32'39"S, $149^0$08'36"E) in New South Wales. Trangie Research Station (TRS) is situated 5 km west of Trangie on the central western slopes and plains and is predominantly composed of native grassland for grazing and small areas of flood irrigation for cotton and lucerne pasture (Robards and Michalk 1975). The dominant soil group at TRS is the red-brown earths (Downes and Sleeman 1955), which is a light textured soil typical of those found in western New South Wales (Murphy and Eldridge 2001). The Orange Agricultural Institute (OAI) is situated on the southern outskirts of Orange on the central tablelands, and comprises mainly improved pastures for sheep and cattle grazing. The dominant soil at the site is a basalt derived deep red krasnozem. Both sites are representative of typical farmland where fox baiting is likely to undertaken by farmers in each region.

2.2.2 Treatments

The OAI trial began on the 5th October and was completed on the 14th December, 2001 and the TRS trial between 11th October and 21st December, 2001. Baits were buried on Day 0 and removed at weekly intervals for a total of 10 weeks. Methods were similar to those of Saunders et al. (2000) to allow for comparison of results.

At OAI, there were 3 treatments undertaken, each consisting of chicken wingettes exposed to a different rainfall regime. The treatments were:

1. Mean weekly rainfall
2. Prevailing rainfall
3. No rainfall

The amount of artificial rainfall applied to the treatment 'Mean weekly rainfall' was calculated from the long-term weekly rainfall averages for the trial period (October-December) for OAI. At OAI, the treatment 'Mean weekly rainfall' required applying a

measured amount of artificial rainfall at weekly intervals. 'Prevailing rainfall' offered no protection to the bait. 'No rainfall' consisted of protecting the bait so no natural or artificial rain was applied during the trial period. At TRS, there were two bait types tested, Foxoff® and chicken wingettes (hereafter called wingettes); both bait types were subjected to the treatment 'Prevailing rainfall' only.

All baits were prepared and/or stored as per the standard for fox control programs in New South Wales. Wingettes were injected with a 0.1 ml dose of 30 mg mL^{-1} 1080 stock solution (92% pure Rentokil Tenate brand 1080) to give a 3 mg nominal dose. Graduated (1 ml) insulin syringes were individually filled to 0.1 ml and injected into the area of muscle between the radius and ulna bones in each wingette. A sample of insulin syringes (n = 3) was calibrated to ensure doses were accurate and repeatable. A 5 g sample of the powder concentrate was stored at room temperature and 10 ml sample of each stock solution was frozen for later analyses. Foxoff® baits are commercially manufactured and do not require injection with 1080. All Foxoff® were from the same manufacturing batch and were shelf-stored until use. Baits were randomly allocated to treatments, and stored in plastic bags.

Level burial sites at TRS and OAI were selected to avoid water-run-off from surrounding areas and fenced to exclude stock. Baits were buried 5 cm below the surface to emulate the recommended bait placement procedures for baiting operations on agricultural lands within New South Wales (Environment Protection Authority 2002). A soil corer was used to ensure that all baits were buried at the same depth. Baits were buried in 300 mm lengths of 100 mm diameter PVC stormwater pipe that had previously been buried to a depth of 200 mm. The PVC pipe sheltered the bait from runoff from surrounding soil but allowed normal micro-organism activity (Saunders *et al.* 2000). All baits were positioned at least 50 cm apart. The top of the each PVC pipe for the treatments 'No rainfall' and 'Mean weekly rainfall' was covered with a transparent 250 mm x 250 mm square of Laserlite 2000® (Laserlite, Cheltenham, Victoria) held in place by a small weight. This sheeting allowed the soil on top of the bait access to light and air, but protected it from natural rainfall. All baits were covered with predator-proof mesh cages to prevent their disturbance or removal by animals and birds. The cages were manufactured from a 750 mm diameter circle of 3/8" steel rod covered with a

1m high cylinder of galvanised chicken mesh, enclosed at one end. At weekly sampling periods, 5 baits from each treatment were removed and placed in individually numbered plastic bags. All retrieved baits remained frozen at -18^{0}C until analyses. Freezing at this temperature stops microbial growth (Adams and Moss 1995) and therefore further defluorination, without affecting 1080 recovery (R. Parker, Department of Natural Resources and Mines, pers. comm. 2002). An 80 g sample of soil was removed from directly underneath each bait, placed in a foil tray, weighed, and oven dried at 80^{0}C for 3 days to calculate soil moisture levels.

Rainfall (mm/day), minimum and maximum ambient temperature (^{0}C) were recorded daily on each site. Soil temperature at 10 cm was recorded daily at OAI and on week days at TRS.

2.2.3 1080 content assays

The 1080 content of bait was assayed by gas chromatography based on the method by Ozawa and Tsukioka (1989) and conducted by the Alan Fletcher Research Station, Queensland Natural Resources and Mines using their routine 1080 analyses procedures (Hannan-Jones 2002; Hannan-Jones 2003): each bait was individually blended with distilled water, and coarse filtered. The extract was divided and half kept for backup. Fluroacetate is then adsorbed onto an anion-exchange resin and eluted with 2% (W/V) sodium chloride solution. The eluent was acidified with hydrochloric acid and treated with 2,4-dichloroaniline and N,N'-dicyclohexylcarbodiimide. A reaction between the mixture and ethyl acetate derived fluoroacetate dichloroanilide, which was quantified with electron-capture detection by gas chromatography and gas chromatography-mass spectrometry. Assays were undertaken in runs with each run corrected for contamination and level of determination using comparisons between negative and positive controls and within positive controls. Each bait was adjusted for 67% recovery within runs as calculated from spiked fresh meat controls (n=6). The detection levels were greater than 0.07 mg. (Ozawa and Tsukioka 1989; Hannan-Jones 2002; Hannan-Jones 2003).

The purity of the stock solution and concentrate powder was tested using the derivation from the high performance liquid chromatography method (HPLC) (Kramer 1984), where the concentration of the bromophenacyl bromide fluroacetate is compared and confirmed with that of library standards or run standards (Hannan-Jones 2002). The determination level was 50 ug mL^{-1}.

2.2.4 Soil micro-organisms

Soils from OAI and TRS sites were collected to investigate the presence and capability of 1080 defluorinating soil micro-organisms. During Week 5 at TRS and Week 6 at OAI, a 30 g soil sample was removed from 5 cm below the surface at three sampling positions in each enclosure. Each sampling position was at least 50 cm from the nearest bait and protective cage. The soil samples were stored in individual 70 ml sterile containers at 7^0C until analyses.

2.2.4.1 Defluorinating activity of soil micro-organisms

The defluorinating activity of soil micro-organisms from each site was monitored after 7 days. Unautoclaved soil from each site (10 g), together with 40 ml of deionised water and 1 ml of 20 mM 1080 solution was kept at 27^0C in an orbital shaker (180 rev min^{-1}) for 7 days. The concentration of fluoride ions (F^-) were then measured using an Orion fluoride electrode (model 94-09-00), an Orion® EA 940 expandable ion analyser, an Orion single junction reference electrode 90-01 and an Orion automatic temperature compensation probe. Fluoride ions will bind to soil particles (Barrow and Shaw 1977). The extent of the binding was calculated by adding a known amount of fluoride ions to sterile soil, which was then allowed to stand for 24 h before measuring F^- concentration. Additionally, background levels of F^- in soil samples, 1080 solution and deionised water were measured. The amount of 1080 defluorinated by each isolate was calculated by assuming that the 20 mM 1080 solution contained 380 ug of F- (Twigg *et al.* 2001). The F^- concentrations resulting from defluorination were determined by subtracting the concentration of F- in a negative control from the measured F- concentration. Final concentrations were adjusted for dilution factors,

binding of F^- to soil (and filter paper) and background F^- levels in soil, deionised water and 1080 solution.

2.2.4.2 Isolation of micro-organisms

Isolation methods were based on Bong *et al.* (1979), Wong (1992), Twigg *et al.* (2000) and Twigg and Socha (2001). An enriched broth using deionised water containing 2 g l^{-1} KH_2PO_4 and 1 g l^{-1} $(NH_4)_2SO_4$ adjusted to pH 6.8 using a few drops of 0.1M NaOH was made for bacterial incubations. For fungal incubations, the broth contained traces of 0.2 mg l^{-1} $CaCl_2$ and 10 mg l^{-1} $FeSO_47H_20$ and adjusted to pH 5.6 with a few drops of 0.1M NaOH. The broths were autoclaved 3 times at 121^0C and 15kPa for 15 minutes and left to cool to $<50^0$C before adding 20 mM of filter sterilised (0.22 um Millipore) 1080 solution. This solution was dispensed in 10 ml aliquots into 120 ml polycarbonate bottles before adding 1 g of air dried soil to each bottle. Three replicates were undertaken for each site. Each bottle was incubated at 27^0C on orbital shaker (180 rev min^{-1}) for 10 days. A 100-fold dilution was made from each of the culture broths and plated onto either nutrient agar (NA) for bacteria, or potato dextrose agar (PDA) for fungi. Bacterial NA plates and fungal PDA plates were incubated at 27^0C. Single colonies were subsequently subcultured onto NA and PDA to ensure purity. Bacterial cultures were stored in Microbank® tubes at -80^0C. Whole-cell fatty acid methyl ester profiles of the bacterial isolates were determined using the Microbial Identification System® (MIS, Microbial ID, Inc. (MIDI), Newark, DE, USA). Bacteria were grown on trypticase soya broth (BBL) with agar (15 g L^{-1}) for 1 day at 27°C. Fatty acids were extracted and analysed using a Hewlett-Packard® 6890 Gas Chromatograph. The extraction process followed the sample preparation procedures described in the Microbial Identification System Handbook. Fatty acid peak areas were identified with the peak-naming component of this system and quantified. The fatty acid profiles of the isolates were compared with known reference strains in the MIS database, which generated a similarity index to express how close the profiles of bacterial isolates were to the mean fatty acid composition of their nearest species match. The fungal species were identified using morphological characteristics as described by Pitt (1988) and Domsch *et al.* (1993).

To ensure isolates were from the soil collected and were not laboratory contaminants, 2 replicates of sterile soil (autoclaved at 121^0C for 15 mins) from each site were incubated and plated onto NA and PDA. No micro-organisms were isolated from sterile soil incubations.

2.2.4.3 Defluorinating activity of microbial isolates

The isolated fungal and bacterial species were determined for individual defluorinating ability when grown in solution of 20 mM of 1080 with trace elements (bacteria: 2 g 1^{-1} KH_2PO_4 and 1 g 1^{-1} $(NH_4)_2SO_4$ adjusted to pH 6.8; fungi: 0.2 mg 1^{-1} $CaCl_2$ and 10 mg 1^{-1} $FeSO_47H_20$ and adjusted to pH 5.6) and with 10 g of sterile soil. Soil was sterilised 3 times by autoclave at 121^0C and 15kPa for 15 minutes. Bacterial suspensions containing 1.5 x 10^9 cells ml^{-1} were prepared from cultures < 72 hours old into 1 ml of 20 mM 1080 and 20 ml of broth. Fungal suspensions were prepared by scraping off aerial mycelium from cultures <72 hours old into 1 ml 20 mM 1080 solution and 20 ml of broth. Two replicates were done for each isolate.

Bacterial and fungal broths were incubated for 28 days at 27^0C in sterile 120 ml polycarbonate bottles. Each bottle was then centrifuged and the suspension filtered using Whatman No. 4 filter paper. A 10 ml sample of the supernatant was added to 40 ml of deionised water for F^- measurement. The amount of 1080 defluorinated was determined by measuring free F^- using a F^- electrode, and corrected for binding and dilution.

2.2.5 Statistical analyses

Regression analysis was used to model the changes in 1080 concentration over time, and to provide a predictive model of the decay rate (Twigg *et al.* 2000). Random regression models were chosen; this regression gives an overall mean response and allows for variable decay rates for different samples as well as random variation within a sample over time (e.g. Thompson and Beacon 1997). This is biologically intuitive, given the random variation in 1080 concentration between baits and within baits over time. Changes in the log 1080 concentration of baits over time were modelled using a random regression model. Included in the model for a given site/year were fixed regression effects for bait type and treatment.

Letting $Y_{ijk}(t)$ denote the log 1080 concentration for the k^{th} bait of type i (i = 1 for foxoff, 2 for wingette) with treatment j (j = 1 for mean weekly rainfall, 2 for prevailing rainfall, 3 for no rain) at time t, the model fitted is:

$$Y_{ijk}(t) = \mu + \alpha t + \beta_i t + \tau_j t + \gamma_{ij} t + b_{ijk} t + e_{ijk}(t)$$

where:

μ is the target log concentration at time zero

α is the overall mean regression

β_i allows differences in regression for different bait types

τ_j allows differences in regression for different treatments

γ_{ij} allows interaction between bait type and treatment with respect to regression

b_{ijk} are random regression deviations

$e_{ijk}(t)$ is random error for bait k of treatment j and type i when sampled at time t.

At time zero, given all baits have the same target concentration, the model is

$$Y_{ijk}(0) = \mu + e_{ijk}(0)$$

It is assumed for the analyses that b_{ijk} are independent $N(0, \sigma)$ random variables, the $e_{ijk}(t)$ are independent $N(0, \sigma)$ random variables and the b_{ijk} and $e_{ijk}(t)$ are independent. This model was used for analyses within a site, but was later extended for comparisons between sites. Differences in the mean regression parameter for the log 1080 concentration for bait types and treatments were examined using ANOVA procedures. The model was fitted to data only up to the time (t) that all subsequent baits had no detectable 1080. As results were recorded as zero, when 1080 was undetectable (ie. <0.07 mg: Hannan-Jones 2002, 2003) the data used in the model corresponds to log (x+0.06) with x the recorded 1080 concentration.

Model parameters were estimated using residual maximum likelihood (REML) to reduce bias associated with estimation of the fixed parameters (Venables and Ripley 2002).

Marginally, for a given *i*, and *j*, $Y_i(t) \sim N\left(\alpha + \beta_i t + \tau_j t + \gamma_{ij} t, t^2\tau^2 + \sigma^2\right)$ hence for a given concentration *c* we can determine that time (*t*0 say) such that

$$P(Y_{ijk}(t) \le c) = p$$

from

$$P(Y_{ijk}(t) \le c) = \Phi\left(\frac{\left[c - \mu + \alpha t + \beta_i t + \tau_j t + \gamma_{ij} t\right]}{\sqrt{t^2\sigma_R^2 + \sigma^2}}\right)$$

where

$$c = (\Phi^{-1}(p) * \sqrt{t^2\sigma_R^2 + \sigma^2}) + \mu + \alpha t + \beta_i t + \tau_j t + \gamma_{ij} t$$

and

$$\left[\left(\Phi^{-1}(p)\right)^2\sigma_R^2 t^2 - \alpha t^2 + \beta_i t^2 + \tau_j t^2 + \gamma_{ij} t^2\right] - \left[\alpha^2 ct - \alpha\beta_i t - \alpha\gamma_{ij} t\right] +$$
$$\left[c^2 - 2c\mu + \mu^2 + \left(\Phi^{-1}(p)\right)^2\sigma^2\right] = 0$$

which corresponds to

$$\therefore a x^2 + bx + c = 0$$

so, the solution is

$$t = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

This expression was used to estimate the 0.05 and 0.95 quantiles, the proportion of baits that are at concentration Y for any given *t* (weeks).

2.3 Results

2.3.1 Weather data

The daily recorded rainfall for TRS and OAI for the period before, during and after the study period is shown in Figure 2.1 and 2.2 respectively. The rainfall that fell in each month of the trial periods and relationship relative to the long-term median rainfall for TRS (1922-2001) and OAI (1966-2001) are shown in Table 2.1.

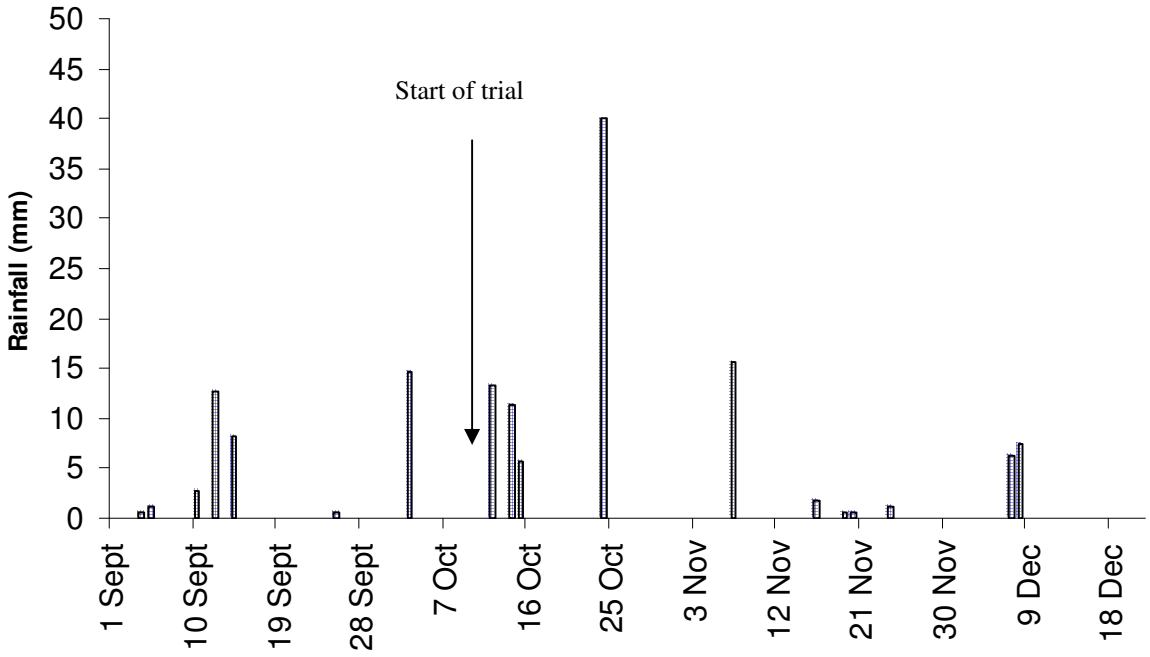

Figure 2.1: The recorded daily rainfall for TRS for the period immediately before and during the study period, specifically the 10th October to 21st December 2001.

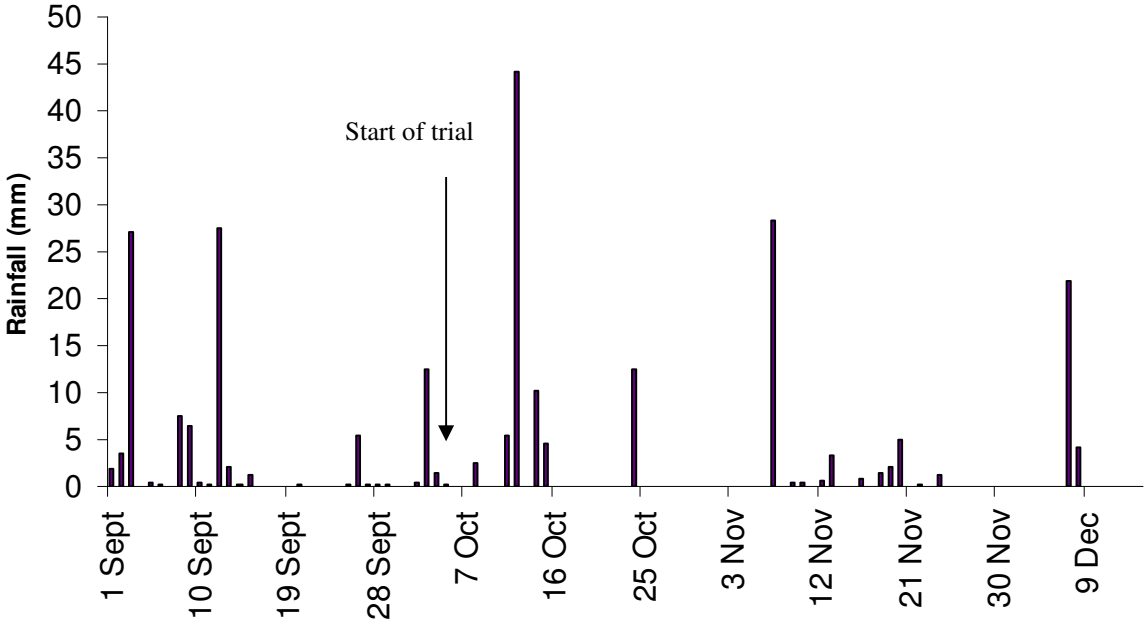

Figure 2.2: The recorded daily rainfall for OAI for the period immediately before and during the study period, specifically the 5th October to 14th December 2001.

The amount of rainfall that fell during the trial period at both OAI (164.9 mm) and TRS (82.1 mm) was less than the long-term median average for both sites (296.6 mm and 155 mm respectively). Rainfall at OAI was identical to the average in the month leading up to the trial (September), slightly higher (+12.2 mm) during October, but was considerably less than average in November (44.0 mm cf. 66.0 mm) and December (26.9 mm cf. 63.0 mm). At TRS the pattern of rainfall was broadly similar to OAI: the amount of rainfall was similar to the long-term mean for September (-2.3 mm), but considerably more fell in October (48.9 mm cf. 34.8 mm). Rainfall for November (19.6 mm) and December (13.6 mm) was less than the average (36.4 mm and 32.2 mm respectively). The 1997 trial at OAI was also lower than average, with only 260 mm falling during the trial period. A higher than average amount (117.3 mm) fell in the month before the trial (September), but less than average fell in October (51.0 mm), November (37.8 mm) and December (61.4 mm).

Table 2.1: The amount of rainfall (mm) that fell in the month prior to and during the trial period for OAI and TRS in 2001. The amount of rainfall (mm) that fell is shown relative to the long-term average (Median); for example +32 is 32 mm greater than the median.

Site	September		October		November		December	
	Rainfall (mm)	± Median	Rainfall (mm)	± Median	Rainfall (mm)	± Median	Rainfall (mm)	± Median
OAI	85.0	0	94.0	+12.2	44.0	-22.0	26.9	-36.1
TRS	26.0	-2.3	48.9	+14.1	19.6	-16.8	13.6	-18.7

The ambient temperature and soil temperature for the trial period is shown for TRS in Figure 2.3 and OAI in Figure 2.4. The average daily maximum (28.7^0C) and minimum (13.1^0C) at TRS were consistently greater than at OAI (19.2^0C and 7.5^0C respectively). Soil temperature at 10 cm was less variable than the ambient air temperature but still reflected the site differences. The average for TRS (21.2^0C), was over 6^0C warmer than OAI (14.6^0C).

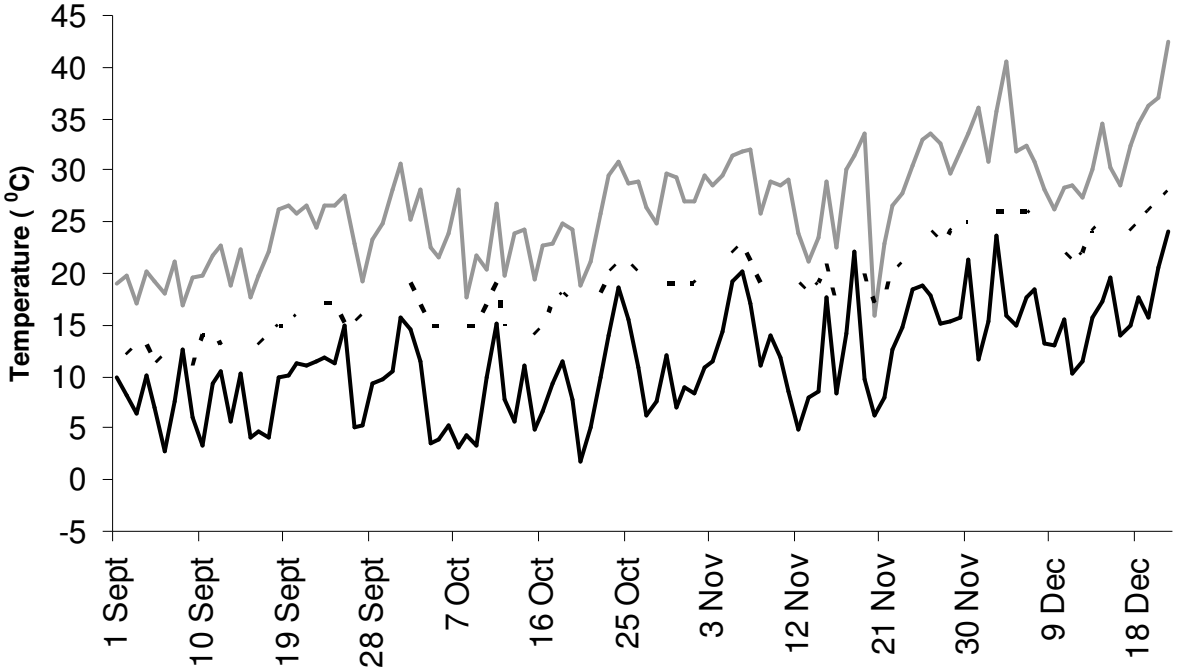

Figure 2.3: Daily ambient and soil temperature at TRS for the period immediately before and during the study period, specifically the 10th October to 21st December 2001. Minimum temperature, solid black line; maximum temperature, solid grey line; and soil temperature, dashed line. Note: Soil temperature only collected during week days.

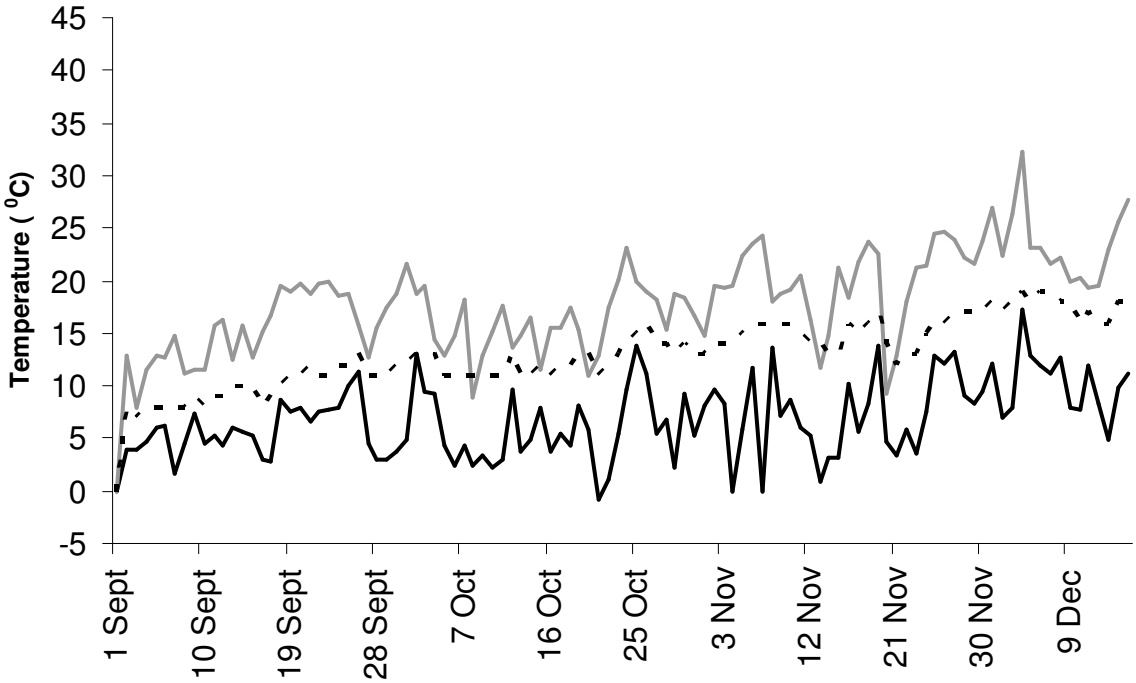

Figure 2.4: Daily ambient and soil temperature at OAI for the period immediately before and during the study period, specifically the 5th October to 14th December 2001. Minimum temperature, solid black line; maximum temperature, solid grey line; and soil temperature, dashed line.

2.3.2 Injection calibration and stock solution

There was little variation in the volume of stock solution delivered by the insulin syringe injecting method. The mean (± SD) amount of stock solution injected for a single dose using the syringe technique was 0.095± 0.003 ml (range = 0.089 - 0.102, n = 30). If the stock solution contained the nominal 30 mg ml^{-1} 1080 concentration, the mean dose injected into each bait would have been 2.85± 0.103 mg (expected range = 2.70 -3.05). Later analyses revealed that a sample of the stock solution contained 36.04 mg ml^{-1} at the time of preparing the OAI baits, and 31.96 mg ml^{-1} when the TRS baits were prepared. Aqueous 1080 solution stored at room temperature will remain stable for at least 12 months (L. Twigg, Department of Agriculture Western Australia, pers. comm. 2001), therefore the variation between the two samples is probably due to measurement error in the assay process (see Discussion). Therefore, a mean of the two solution sub-samples equates to an average dose of 3.23± 0.102 mg 1080 (range = 2.87 – 3.47).

2.3.3 1080 content

There was a large variation in the 1080 concentration of Day 0 baits. The mean (±SD) in Day 0 wingettes at TRS was 3.84± 0.85 mg (n = 8), and at OAI, 3.16± 1.53 mg (n = 3). Both OAI and TRS samples contained baits that had doses greater than 1.5 times the nominal dose of 3 mg. This is quite variable compared to the variation measured in the injection technique. The mean of the Day 0 Foxoff® baits was similar to the 3 mg nominal dose, but was still variable (2.97± 1.08 mg, n = 4).

2.3.4 1080 degradation

Not all replicates collected during this study were analysed for 1080 concentration. For Weeks one to four Statistical tests were only performed using data only up to the time where all subsequent baits had no detectable 1080. At OAI this included data up to and including Week 3; at TRS, up to and including Week 5.

An ANOVA comparing the 1080 concentrations of the treatments "mean weekly rainfall", "prevailing rainfall" and "no rain" for wingettes at OAI and the treatments "mean weekly rainfall" for both Foxoff® and wingettes at TRS indicated significant differences in the factors Time, Type of bait, Treatment and the interaction between Treatment and Time. As a result, a separate ANOVA was undertaken for each site to examine within site differences. Additionally, a Student-Neuman-Keuls (SNK) multiple range test (Snedecor and Cochran 1989) was undertaken to examine the relationship between each factor (Bait type, Site, Treatment) for each week of the trial.

The results from the ANOVA comparing the 1080 concentrations in the "mean weekly rainfall", "prevailing rainfall" and "no rain" treatments for wingettes at OAI indicated that there were significant differences in the factor Time but not Treatment (Table 2.2). There was also no interaction between Treatment and Time. This indicates that there was no significant difference in the decay rate of 1080 between the treatments during the course of the experiment. The treatments were combined given that there was no significant difference in the rate of 1080 degradation between treatments as indicated by the ANOVA and the SNK multiple range test.

Table 2.2: ANOVA of 1080 concentration per bait for wingettes for Treatments "prevailing rainfall", "mean weekly rainfall" and "no rain" at OAI, 2002.

Source of variation	d.f.	s.s.	m.s.	*F*	*P*
Treatment	2	0.404	0.202	0.280	0.758
Time	3	101.097	33.699	140.473	<0.001
Treatment * time	6	0.014	0.002	0.009	0.991
Residuals	22	15.833	0.720		

There was no significant difference in the rate of 1080 decay in wingettes at OAI and TRS for the treatment 'mean weekly rainfall' (Table 2.3). Moreover, both Site and the interaction between Site and Time were not significant. Nevertheless, a separate predictive model of the 1080 degradation of wingettes is presented for both TRS and OAI.

Table 2.3: ANOVA of 1080 concentration per bait for wingettes at OAI and TRS for Treatment "mean weekly rainfall".

Source of variation	d.f.	s.s.	m.s.	*F*	*P*
Site	1	4.366	4.366	2.509	0.127
Time	3	118.309	39.436	67.991	<0.001
Interaction of Site and Time	3	1.251	0.417	0.719	0.405
Residuals	23	40.022	1.740		

An ANOVA comparing the 1080 concentrations in wingettes and Foxoff® at TRS showed a significant difference in the Type of bait and Time, but no significant interaction between the Type of bait and Time (Table 2.4).

Table 2.4: ANOVA of 1080 concentration per bait for wingettes and Foxoff® at TRS, 2002.

Source of variation	d.f.	s.s.	m.s.	*F*	*P*
Type of bait	1	12.541	12.541	10.476	<0.001
Time	4	117.681	29.420	98.308	<0.001
Interaction of Type of bait and Time	4	2.198	0.550	1.836	0.189
Residuals	23	27.532	1.197		

Data from a previous study which measured the 1080 degradation rate of buried Foxoff® at OAI (Saunders *et al.* 2000) was compared with the rate of decay in wingettes collected in this study. An ANOVA was used to compare the rate of 1080 decay in Foxoff® and wingettes for the wet treatments common to both studies, "mean weekly rainfall" and "prevailing rainfall" (Table 2.5). A separate ANOVA was used to compare the dry treatments, "no rain" (Table 2.6). There was a significant difference between the wet treatments for Foxoff® and wingettes in the interaction between Type of bait and Time indicating a difference in degradation rates. The lack of significance in the Type of bait indicates that there was no difference in the amount of 1080 at the start of the experiment (Day 0). For the treatment "no rain", there was a significant difference in the rate of decay for wingettes and Foxoff®,

indicated by a significant interaction between the Type of bait and Time. However, there was a significant difference in the rate of decay of the treatment "mean weekly rainfall" for Foxoff® at TRS and OAI (Table 2.7).

Table 2.5: ANOVA of 1080 concentration per bait for wingettes and Foxoff®, treatments "mean weekly rainfall" and "prevailing rainfall" at OAI.

Source of variation	d.f.	s.s.	m.s.	*F*	*P*
Type of bait	1	0.309	0.309	0.308	0.5803
Time	5	265.065	53.013	264.111	<0.001
Interaction of Type of bait and Time	5	11.874	2.375	11.834	<0.001
Residuals	111	111.385	1.003		

Table 2.6: ANOVA of 1080 concentration per bait for Treatment "no rain" for wingettes and Foxoff® at OAI.

Source of variation	d.f.	s.s.	m.s.	*F*	*P*
Type of bait	1	41.060	41.060	55.980	<0.001
Time	4	50.156	12.539	70.133	<0.001
Interaction of Type of bait and Time	4	10.377	2.590	41.152	<0.001
Residuals	65	45.438	0.699		

Table 2.7: ANOVA of 1080 concentration per bait for Foxoff® at OAI and TRS for Treatment "mean weekly rainfall".

Source of variation	d.f.	s.s.	m.s.	*F*	*P*
Site	1	20.548	20.548	23.722	<0.001
Week	4	138.673	34.668	160.092	<0.001
Interaction of Site and Week	3	160.874	53.625	92.862	<0.001
Residuals	61	52.839	0.866		

A SNK multiple range test was undertaken to compare the mean 1080 content each week between all baits and all treatments undertaken in this trial. There was no significant difference in the amount of 1080 in all treatments at Week 0 ($P>0.05$). At Week 1 and Week 3, two separate groups were apparent; Foxoff® at TRS and the wingette treatments on both sites. At all other times there were no significant differences between the treatments ($P<0.05$). A second multiple range test compared the weekly mean 1080 content in Foxoff® at OAI from an earlier trial (Saunders *et al.* 2000) with Foxoff® and wingettes in this trial for the first 5 weeks. After Week 1, all Foxoff® treatments were significantly different ($P<0.05$) to all wingette treatments, both at OAI and TRS. During Week 2 and 3 results were inconclusive, there were no significant differences ($P>0.05$) between all treatments. At Week 4, three distinct groups were present; 1) the Foxoff® treatment "no rain" at OAI 2) Foxoff® treatments "mean weekly rainfall" and "prevailing rainfall" at OAI; and 3) all wingette treatments from OAI and wingettes and Foxoff® at TRS. At Week 5 only the Foxoff® treatment "no rain" was significantly different ($P<0.05$) to any other treatment.

The parameters in the random regression models for the bait types on each site are shown in Table 2.8. Although wingettes at TRS were not significantly different from OAI wingettes, the parameters are shown for comparison. A decay curve was then fitted to each treatment (Figures 2.5, 2.6 and 2.7).

Table 2.8: Fitted regression coefficients from the random regression models. The lower (5%) and upper (95%) confidence limits for each estimate are shown in parentheses.

Site	Bait type	Treatments	μ	α	b_{ijk}	e_{ijk}
OAI	Wing	1, 2, 3	1.05 (0.80, 1.31)	-1.46 (-1.67, -1.24)	0.42 (0.25, 0. 69)	0.34 (0.21, 0.57)
TRS	Foxoff®	1	1.26 (0.83, 1.70)	-0.79 (-1.06, -0.54)	0.46 (0.23, 0.96)	0.26 (0.12, 0.56)
TRS	Wing	1	1.37 (1.21, 1.53)	-1.46 (-2.09, -1.56)	0.23 (0.13, 0.38)	0.32 (0.17, 0.62)

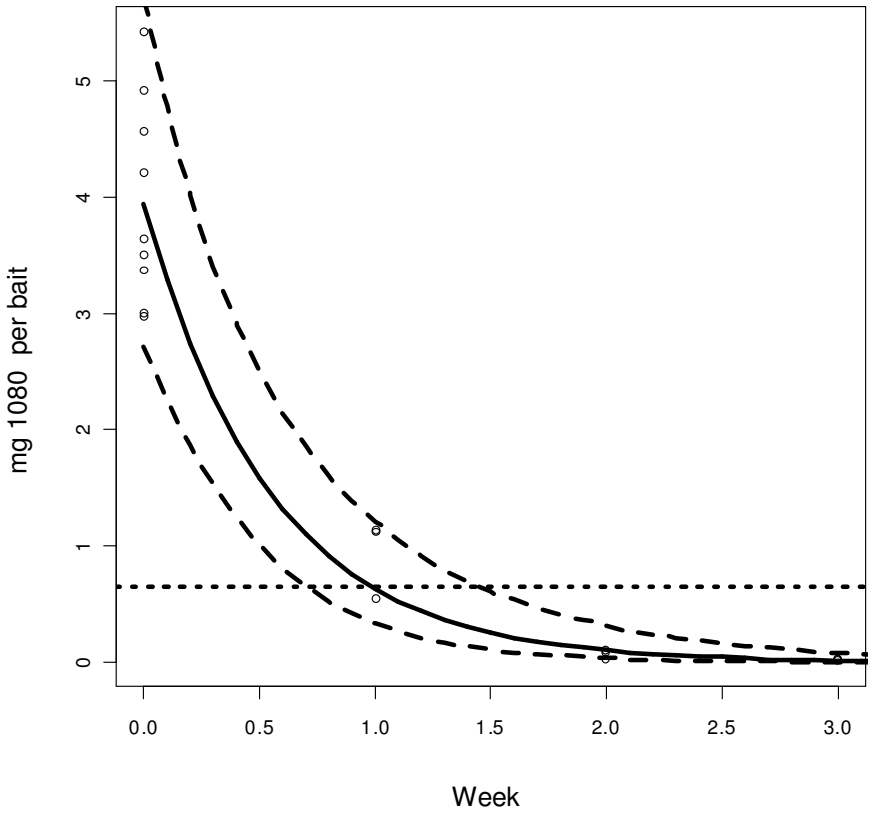

Figure 2.5: Fitted curves for the mean loss of 1080 (solid line) and 0.05 and 0.95 quantiles for wingettes at TRS exposed to 'mean weekly rainfall' up to and including three weeks after initial burial. Dotted line indicates minimum LD_{50} (0.65 mg) for a 5 kg fox.

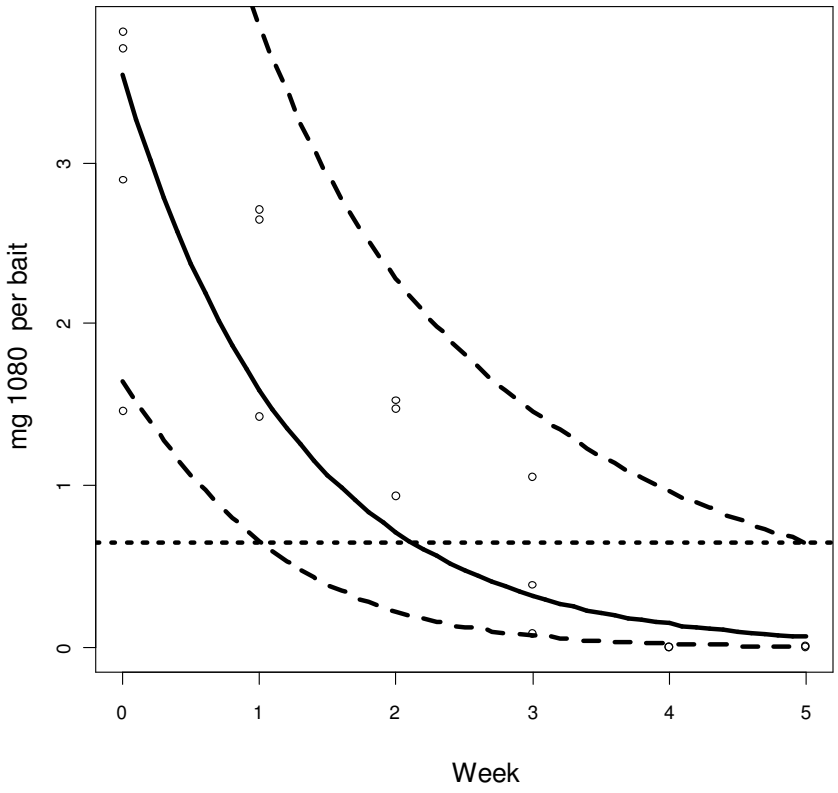

Figure 2.6: Fitted curves for the mean loss of 1080 (solid line) and 0.05 and 0.95 quantiles for Foxoff® at TRS exposed to 'mean weekly rainfall' up to and including five weeks after initial burial. Dotted line indicates minimum LD_{50} (0.65 mg) for a 5 kg fox.

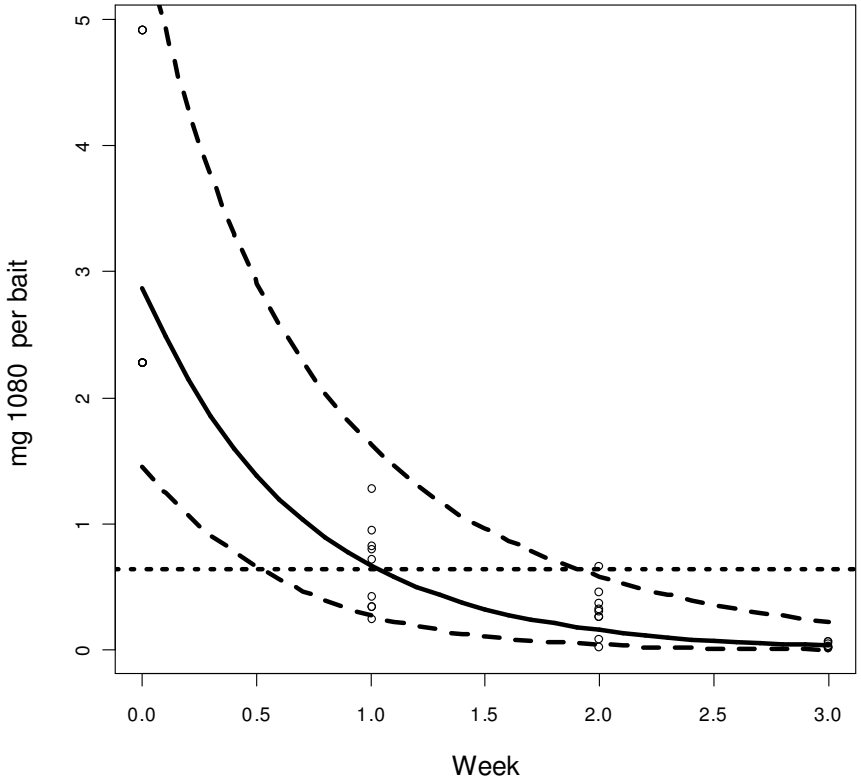

Figure 2.7: Fitted curves for the mean loss of 1080 (solid line) and 0.05 and 0.95 quantiles for wingettes at OAI exposed to 'mean weekly rainfall', 'prevailing rainfall' and 'no rain' up to and including three weeks after initial burial. Dotted line indicates minimum LD_{50} (0.65 mg) for a 5 kg fox.

A two-way fixed factor ANOVA of soil moisture content for all treatments at OAI indicated that there was no significant difference in the amount of moisture in each treatment ($F_{2,44}$ = 1.54, P>0.05) at any week ($F_{2,\ 44}$ = 1.75, P>0.05) or any interaction between week and treatment ($F_{4,44}$ = 0.65, P>0.05). Similarly, there was no significant difference between OAI and TRS wingettes treatment "mean weekly rainfall" for site ($F_{1,23}$ = 0.11, P>0.05), or time ($F_{3,\ 23}$ = 0.817, P>0.05) or interaction between the two ($F_{3,23}$ = 0.56, P>0.05).

2.3.5 Soil micro-organisms

A substantial amount of F^- was unable to be recovered from each soil. The mean recovery rate for adding 220-260 µg of F^- to 10 g of soil was 57.5±2.8%(SD, n=3) for TRS and 32.2±5.9% (SD, n=3) for OAI. The concentration of F^- in deionised water (0.0116±0.003 µg L^{-1} SD, n=3) and 20 mM 1080 solution (0.0374±0.006 µg L^{-1}, SD, n=3) was low but all values were nevertheless adjusted for these concentrations.

The defluorinating ability of unautoclaved soil from both sites was conflicting. TRS showed considerable defluorinating ability, with the micro-organisms defluorinating 47.8±17.7% (n=2) of added 1080 within 7 days. However, the amount of F^- released from the soil at OAI did not exceed the level of the sterile control after 7 days.

A total of thirty-one species of micro-organisms capable of defluorinating 1080 were isolated from the soils (Table 2.9 and 2.10). At TRS, 2 species of fungi, 9 species of bacteria, and 2 actinomycetes were isolated; at OAI, 14 bacteria, 5 fungi and 1 actinomycete were isolated. Two species of bacteria and 2 actinomycete species could not be identified. Actinomycetes are, taxonomically, a bacteria; given their significant differences in morphology and growth to the true bacteria (Bergy *et al.* 1989), they are categorised and presented separately here.

Defluorination by isolates

Ten isolates showed measurable defluorinating ability when grown in a 1080 and sterile soil suspension. Nine of these species were exclusively from the TRS soil, and one from OAI with one common species from both sites. The bacteria defluorinated an average of 27.9±25.2% of added 1080 (range = 0.3-76.5%), the actinomycetes averaged 12.6±5.25% (range = 7.3-17.8), and fungi 5.1±4.1% (range = 1.0-9.1). For each site, the micro-organisms at TRS averaged 18.4±20.3% defluorination and 56.6±14.5% at OAI.

Table 2.9: The mean percentage of 1080 defluorinated (n=2) by fungi isolated from soil at OAI and TRS in a solution containing 20 mM 1080 and 10 g of sterile soil incubated for 28 days at 27^0C. *No measure of variation (SD) available since only one replicate of isolate tested.

Micro-organism	Site	Mean F^- concentration ±SD	% 1080 defluorinated
Aspergillus versicolor	OAI	0	0
Monocillium sp. 1	OAI	0	0
Monocillium sp. 2	OAI	0	0
Penicillium regulosum	TRS	0.0359±0.011	1.02
Penicillium sclerotiorum	OAI	0	0
Penicillium sp. 1	OAI	0	0
Penicillium sp. 2	OAI	0	0
Penicillium sp. 3*	TRS	0.322	9.13
Scopulariopsis brumptii	OAI	0	0

Table 2.10: The mean percentage of 1080 defluorinated (n=2) by bacteria and actinomycetes isolated from soil at OAI and TRS in a solution containing 20 mM 1080 and 10 g of sterile soil incubated for 28 days at 27^0C. *No measure of variation (SD) available since only one replicate of isolate tested.

Micro-organism	Site	Mean F^- concentration ±SD	% 1080 defluorinated
Acaligenes paradoxus	TRS	1.183±0.364	33.5
Alcaligenes paradoxus	TRS	0	0
Alcaligenes paradoxus	TRS	2.698±0.283	76.5
Alcaligenes paradoxus	TRS	1.475±1.581	41.8
Arthrobacter atrocyaneua	OAI	0	0
Arthrobacter oxydans	TRS	0.377±0.015	10.7
Bacillus cereus	OAI	0	0
Bacillus cereus	OAI	0	0
Bacillus flexus	OAI	0	0
Bacillus megaterium	OAI	0	0
Bacillus megaterium	TRS	0.326±0.364	9.2
Bacillus megaterium	TRS	0.011±0.016	0.3
Bacillus megaterium	TRS	0.435±0.229	12.3
Bacillus psychrophilus	OAI	0	0
Bacillus simplex	OAI	0	0
Bacillus thuringiensis kurstakii	TRS	0.199±0.196	5.7
Bordetella parapertussis	OAI	0	0
Burkholderia cepacia	OAI	1.486±0.559	42.1
Burkholderia glathei	OAI	0	0
Kocuria varians	OAI	0	0
*Micromonospora carbonacea**	OAI	2.506	71.0
*Micromonospora carbonacea**	TRS	0.488	13.8
Paenibacillus pabuli	OAI	0	0
Possible *Streptosporangium* sp.	TRS	0.629±0.133	17.8
Pseudomonas huttiensis	OAI	0	0
Streptomyces sp.	OAI	0	0
Streptomyces sp.	OAI	0	0
Streptomyces sp.	OAI	0	0
Streptoverticillium reticulum	OAI	0	0
Unknown	TRS	0	0
Unknown	TRS	0	0
Unknown	TRS	0.257±0.028	7.3

2.4 Discussion

2.4.1 Injection calibration and stock solution

The results of the calibration indicate that injecting baits with a 0.1 ml dose from a graduated 1 ml insulin syringe is an accurate and precise technique. The use of an automatic dispenser is highly accurate (e.g. 0.22±0.003 ml), but requires constant calibration since the volume increases with the number of injections (Fleming and Parker 1991). Insulin syringes are an acceptable substitute, especially for injecting smaller batches of bait where the extra time required to calibrate and clean a dispenser would negate any time savings.

The amount of 1080 in the stock solution for both TRS (31.96 mg ml^{-1}) and OAI (36.04 mg ml^{-1}) was greater than the nominal 30 mg ml^{-1}. This is most likely due to the high purity of powder (1139± 131 g kg^{-1}) used to make the solution. The discrepancy between the purity stated on the label of each commercially manufactured tin (90%) and in the Material Safety Data Sheet (*minimum* of 90%) may result in a 10% error in the concentration of the bait-preparation solutions (Twigg *et al.* 2000). This is supported by the high purity of the 1080 powder used in this trial (100%) as confirmed by the laboratory analyses. As a result, Twigg *et al.* (2000) recommend that, in circumstances where the exact concentration of stock solution is required, such as in research trials, each tin should be assayed before it is used. I did not consider it important to assay the powder concentrate before use; it is irrelevant for consistent dosing (as occurs in the field) if the aqueous solution is up to 10% from the nominal concentration providing the same aqueous solution is used to prepare all baits within a trial and the amount injected is consistent there will be insufficient error to disrupt comparisons between baits and/or treatments. The variation between the two stock solution samples is at first worrying; the identical stock solution was used to inject both OAI and TRS baits, but the TRS baits were injected 5 days later. The relatively large difference in 1080 concentration seen here is unlikely to be caused by degradation of the solution. Two sources of error are possible; the solution may not have been uniformly mixed, resulting in some precipitation in the solution between injection periods or there is error associated with the assay process. The latter is more likely; the overall percentage recovery using spiked solutions was 106% (SD = 9.8%, range = 94.3-124%, n = 6) (Hannan-Jones 2002). The variation

between stock solution samples seen here is within the spiked sample standard deviation (9.8%) of the mean of the two solutions (34 mg ml^{-1}). Given the variation in the technique it is probably safe to assume that the mean is approximately 34 mg ml^{-1} which is greater (13%) than the nominal concentration (30 mg ml^{-1}). This indicates that the mixing of the stock solution, originally calculated for a purity level of 90%, was accurate. To extend the recommendation of Twigg *et al.* (2001) to improve accuracy in baits prepared for field use, at least two replicates would be required to accurately determine the concentration of each tin. This would cost at least $120 per tin ($60+ per sample) which would add considerably to the cost of bait preparation. An accuracy improvement of up to 10% would probably not be worth this additional cost, especially given the measurement errors experienced in this study.

2.4.2 1080 content

The large variation in 1080 in the Week 0 baits has been reported before (e.g. McIlroy *et al.* 1986; Fleming and Parker 1991; Twigg *et al.* 2000) and could be due to a number of possibilities. A time lag between injection and analysis can result in poor recovery of 1080. Kramer (1984) suggested that this low recovery is due to enzyme reactions resulting in chemical transformation to a new organofluorine compound. Liquid chromatography (Kramer 1984) and Fluorine-19 nuclear magnetic resonance spectroscopy (Frost *et al.* 1989) methods measure the specific amount of fluoroacetate from 1080, whereas methods using the fluoride ion electrode are unable to distinguish between fluoride from fluoroacetate and other organofluorine compounds (Fleming and Parker 1991). Therefore, if fluoroacetate were being converted to organofluorine compounds by some metabolic process, the gas chromatography method used in this study would have been unable to detect or correct for such differences. However, Frost *et al.* (1989) were unable to detect any organofluoride compounds, indicating that conversion by metabolic processes was probably not responsible for the 'loss' of recoverable 1080. Frost *et al.* (1989) concluded that the incomplete recovery of 1080 is probably due to the binding of 1080 to the substrate in such a way that removal by aqueous solution is incomplete. Rigorous extraction techniques exchanging an alkaline solvent for water fails to increase the percentage recovery, suggesting that the 1080 is tightly bound to meat (Kramer 1984). This binding is time dependent; earlier studies that measured 1080

content immediately after injection found high recovery rates (90-94%, Livanos and Milham 1984) compared to much lower recovery rates (52 – 88%) when periods of several hours elapsed before assays were undertaken (Kramer 1984; Livanos and Milham 1984; Frost *et al.* 1989; Fleming and Parker 1991). In this study, Week 0 wingettes were injected and placed in the freezer within one hour, leaving a time lag of several hours before baits would have been frozen. This time lag could have been sufficient to facilitate binding, resulting in the low recovery rates of 1080 in wingettes. The 1080 is added to Foxoff® during manufacture and hence would also have considerable time before freezer storage to facilitate binding. Binding is generally not problematic since the use of positive controls (i.e. addition of a known amount of 1080) can be used to calculate the percentage recovery, which in turn is used to correct the measured samples. All baits were adjusted given the mean recovery of 1080 from spiked samples (assay #1, 66%, SD = 5.8%, range = 63-70%, n = 6; assay # 2, 68%, SD = 4.3%, range = 63-70%, n = 6.). However, the positive controls are used to all correct baits within an assay run, and not individual baits *per se*, therefore the variation in the amount of binding between individual baits may result in either under or overcorrection of 1080 content. There may be variability in the amount of binding due to variation in the composition of baits, or the injection site. For example, 1080 in bone tissue is usually 100% recoverable (R. Parker, Department of Natural Resources and Mines, pers. comm. 2003) compared to between 60-75% in meat (Hannan-Jones 2002; Hannan-Jones 2003). The amount of 1080 deposited in each tissue may differ between each injected bait, leading to variable recovery rates and hence under or overcorrecting of 1080 concentration. Conflicting recoveries within samples would cause error in correction estimates.

Despite considerable care being taken to inject the solution into the same position in each wingette, variation between wings probably exacerbates variation in recovery rates. The relative accuracy of the injection technique and the concentration of the stock solution support this conclusion. Variable recovery is less problematic after physical degradation, allowing for a more consistent recovery from the bait substrate. As meat physically degrades, the ability of 1080 to bind decreases, (Kramer and Merrell 1987) and the potential for leaching increases (e.g. McIlroy and Gifford 1988) suggesting more efficient water extraction, reducing variable recovery. Even though Week 0 baits showed large variation in recoverable 1080, the results

from Week 1 onwards, when all baits showed signs of physical deterioration, probably would have been more consistent between baits. The remaining baits that were subject to field conditions were less likely to be affected by variable recovery and thus better reflect the true 1080 concentrations and losses from degradation processes.

Leakage or seepage of the stock solution from baits after injection is probably not responsible for the lower 1080 content of wingettes. Leakage does not affect the recovery of 1080 from meat baits unless a sufficiently large volume is injected to cause the solution to leak out. Only occasional leakage was reported by Kramer and Merrell (1987) during injections of a large amount of stock solution (1 ml) into thinly sliced fresh meat baits; no leakage was observed when the injected dose was halved to 0.5 ml. The small injection volume used in this study (0.1 ml) would mean that leakage would be even less likely. McIlroy and Gifford (1988) and Fleming and Parker (1991) observed little seepage at the time of injection from 0.2 ml injected baits. Additionally, Fleming and Parker (1991) found no trace of 1080 in the rinsings from the bag used to transport the baits to the field site. Seepage may be adsorbed to the surface of the baits (Korn and Livanos 1986) adding a multiplicable source of variation in 1080 content when many baits are transported within the same container since seepage from one bait may adhere to the surface of another (Fleming and Parker 1991). In this study, each bait was individually bagged so any seepage from one bait would have been unable to contaminate another. This indicates that if seepage did occur, it would only account for a loss of 1080 within baits and not contamination of 1080 from one bait to another. All the above arguments suggest that seepage is not an important contributor to the variation in the 1080 content in wingettes in this study.

It is difficult to determine the cause for the low 1080 content of one Foxoff® bait tested at Week 0. This bait may be an anomoly, associated with manufacturing or assay error. Recent batches of Foxoff® baits tested have shown little variation in 1080 concentration (2.74 ± 0.42 mg) (R. Parker, Department of Natural Resources and Mines, pers. comm. 2002). Variation appears more problematic with fresh meat-based baits, as noted in previous studies (Korn and Livanos 1986; Fleming and Parker 1991; Twigg *et al.* 2000). A recent study (A. Claridge, Department of Environment and Conservation, pers. comm. 2003) further illustrates this

variation; fresh meat baits injected using insulin syringes for a 6.0 mg nominal dose averaged 5.97±1.49 mg (range = 4.23-8.60). Although it is impossible to confirm, the relative accuracy of the injection technique compared to the assay results suggest the variation in 1080 content in wingettes is a consequence of binding differences in the bait substrates. The Foxoff® bait matrix may be more uniform due to the manufacturing process, resulting in greater consistency in 1080 recovery. Regardless, the high variability of 1080 in Week 0 bait, especially fresh meat, should be further investigated to ensure that consistent doses are presented in baits prepared for use in the field.

2.4.3 1080 degradation

This study specifically investigated the role of various rainfall treatments on 1080 loss in wingettes and the impact of different climatic conditions on longevity of wingette and Foxoff® baits. The results indicate no difference in the rate of 1080 loss from wingettes in the rainfall treatments 'mean weekly rainfall', 'prevailing rainfall' and 'no rainfall' at OAI. Up to Week 3, 79.6 mm of rain fell on the baits in the 'prevailing rainfall' treatment, considerably more than the 45 mm that was added to the 'mean weekly rainfall' treatment. Surface laid fresh meats baits lose significantly more 1080 from leaching if subjected to rain on the first day (McIlroy *et al.* 1986) before the surface dries to form a crust. This crust offers some protection against leaching until baits begin to physically degrade (Fleming and Parker 1991). The similarities between decay rates in all three treatments and the large differences in the amount and pattern of rainfall received by the 'prevailing rainfall' treatment suggests that leaching of 1080 by rainfall was not an important mechanism for the degradation of wingettes. This is further supported by the insignificant difference between 1080 loss from wingettes at TRS and OAI, despite 13 mm of rain falling on all the baits (on Day 0) at TRS. It is likely that a threshold level of soil moisture is required to initiate the degradation process (e.g. Saunders *et al.* 2000; Twigg *et al.* 2000), a level reached in all treatments in this study (>15%). Soil moisture in each treatment at OAI was not significantly different for any week over the trial period. However, soil moisture was measured immediately after each bait was removed for analysis, leaving a lag period between the time when rain (artificial or natural) fell and the measuring of soil moisture. This was probably sufficient time for the water to

infiltrate through the soil profile, resulting in the lack of measurable differences in soil moisture between treatments.

A comparison between the treatment 'mean weekly rainfall' for wingettes at TRS and OAI, and another between 'mean weekly rainfall' treatments for Foxoff® at TRS and OAI (from Saunders *et al.* 2000) suggest little difference in bait degradation rates on the central tablelands and the central-west slopes of New South Wales. Mean soil temperature at TRS (21^0C) was higher during the trial period than that at OAI (14.6^0C), but the amount of artificial rain added each week was less (11 mm vs 19.5 mm). The amount of evaporation at TRS (258 mm) was also greater than at OAI (165 mm) during the trial period. Warmer conditions favour heightened bacterial and fungal growth, leading to greater (faster) defluorination. Defluorination at 20^0C is 3 times faster than at 10^0C (Parfitt *et al.* 1994), suggesting that if other factors were the same, baits at TRS would last for significantly less time. However, the additional rainfall at OAI may have assisted the leaching of 1080 from the bait following physical degradation. Also, the rain (13 mm) that all baits received at TRS on Day 1 may have assisted to initiate the degradation process, which appeared uninfluenced by the greater evaporative loss during the trial period. Either way, both sites showed remarkably similar degradation rates for both wingettes and Foxoff®.

The similarity of the protocol used in this study with that of Saunders *et al.* (2000) allowed for the direct comparison between Foxoff® and wingettes at OAI. Saunders *et al.* (2000) found that Foxoff® exposed to no rain generally remained toxic for significantly longer periods than Foxoff® exposed to any amount of rain, despite the lack of a statistically significant difference in soil moisture between the treatments. The loss of 1080 from wingettes was independent of rainfall, suggesting that the 'washing through' effect of rainfall (i.e. leaching) has a greater influence on 1080 loss in Foxoff® than for wingettes. Wingettes may resist initial leaching since the skin protects against water infiltration, similar to the protection offered by formation of a skin on fresh meat baits by exposure to the sun and air (e.g. McIlroy *et al.*1986; Fleming and Parker 1991). It appears that 1080 loss from wingettes is more likely to be caused by biological factors.

One possible factor affecting the leaching of 1080 from bait was the application of the artificial 'mean weekly rainfall' treatment. In this treatment, the total volume of the average weekly rainfall was applied on one occasion per week. Obviously, application of artificial rainfall in this manner may vary from the natural pattern of rainfall which may fall on several occasions and at different intensities throughout each week. Such a protocol may have acted to increase the 'washing though' affect of the rain, increasing the leaching of 1080 from the bait. However, the results indicate that it did not influence the rate of wingette degradation compared to natural rainfall conditions given the lack of any leaching effect, and the insignificant differences between the 'mean weekly rainfall' and the 'prevailing rainfall' treatments at OAI. Similarly, it is unlikely to have exaggerated the leaching effect on Foxoff® given the lack of difference between artificial and natural rainfall treatments reported by Saunders *et al.* (2000). Nevertheless, the pattern of rainfall should be considered when assessing rates of 1080 bait degradation.

The degradation rates of wingettes and Foxoff® at TRS were not significantly different, despite the fact that Foxoff® remained viable for considerably longer periods (see Figures 2.5 and 2.6). This is probably due to the large variation in measured 1080 concentration for each bait type, especially wingettes. This is supported by the results of the SNK multiple ranges tests; Foxoff® were significantly different than wingettes only for week one and not the remaining weeks. The large variation, particularly at Week 0, may be a result of either variations in 1080 concentration or measurement variations (see section 2.4.3). The implications of such variation in measured bait concentration for are discussed further in Chapter 8.

The presence and defluorinating ability of micro-organisms is known to vary widely with different bait materials (Wong 1992). Microbial contamination can occur during handling, preparation and storage of bait material. Defluorination rates are generally high in fresh meat baits due to the organic-carbon rich environment for supporting micro-organisms (Wong 1992). Biochemical, enzymic reactions will also physically degrade meat (Adams and Moss 1995). As a result, the 1080 concentration in fresh meat baits will decline under moisture and temperature conditions favourable to micro-organisms and enzymic reactions. However,

when stored in similar conditions the Foxoff® bait matrix appears a poor environment for supporting defluorinating micro-organisms. This is probably due to the combination of low abundance of 1080-detoxifying organisms in the bait and deficient environmental conditions for their growth. The low moisture content of Foxoff® (5.3±1.4%) relative to wingettes (54.4±1.4%) may be responsible, as micro-organisms will only metabolise substrates when sufficient water is available (Parfitt *et al.* 1994). The 1080 content of Foxoff® is highly shelf stable; in temperature and humidity conditions conducive to biological degradation, Foxoff® retain 97% of the initial 1080 for periods greater than six months (Staples *et al.* 1995). Independent of other factors, these arguments suggest that 1080 in wingettes degrade at a faster rate than Foxoff® due solely to biochemical and micro-organism activity. However, in this study, the degradation of wingettes was probably assisted by insect consumption, especially from blowfly maggots (Calliphoridae larvae). Maggots will consume bait material and the contained 1080 (McIlroy and Gifford 1988) resulting in significant losses. Maggots were observed on wingettes as early as Week 1 but were more common during Weeks 2 and 3. No maggots were observed on Foxoff® baits at any time.

2.4.3.1 Loss of 1080 and management implications

Data from this study can be used to estimate the time that bait will present a lethal dose for foxes, given an approximate LD_{50} (see Table 2.11). These estimates are not ubiquitous, but are representative of the situations likely to be encountered in eastern and mid-western New South Wales. The average body weight of an adult fox on the central tablelands is 5 kg (Winstanley *et al.* 1999); foxes in the drier, less productive western slopes region may average slightly less. The LD_{50} for foxes is approximately 0.13 mg kg^{-1} (McIlroy and King 1990), which equates to a lethal dose of 0.65 mg for an adult fox. Applying this to the fitted decay curve for wingettes buried and exposed to various rainfall regimes on the central tablelands and western slopes (Figures 2.5-2.7), wingettes will remain lethal to foxes for a mean of 1.1 week (Table 2.11). The 0.05 and 0.95 quartile confidence limits fitted to the model estimate that 95% of baits will remain lethal for periods up to 0.5 weeks, decreasing rapidly to leave only 5% lethal at 1.8 weeks. Similarly, wingettes at TRS will reach 0.65 mg

of 1080 at 1.0 weeks; 95% are lethal at 0.8 weeks but 95% fall below a lethal dose 1.4 weeks after burial.

Table 2.11: The amount of 1080 required for a LD_{50} and mean time (weeks) wingettes and Foxoff® remain lethal to foxes, sheep and cattle dogs after bait is laid. Figures in parentheses are the estimated 5% and 95% population levels that are ≥ LD_{50} for each species.

	Species		
	Fox	Sheep dog	Cattle dog
Approximate amount 1080 (mg) required for LD_{50}	0.65	0.99	1.32
Wingette – mean time (weeks) ≥ LD_{50}	1.1 (0.5- 1.8)	0.7 (0.2-1.9)	0.5 (0.1-1.6)
TRS Foxoff® – mean time (weeks) ≥ LD_{50}	2.1 (1.0 – 5.0)	1.5 (0.6 -3.9)	1.2 (0.4-3.2)

Foxoff® degrade at a slower rate than wingettes and therefore remain lethal to foxes for considerably longer periods. Under average rainfall conditions on the western slopes of New South Wales, Foxoff® retain 0.65 mg of 1080 for an average of 2.1 weeks after burial, with 95% lethal for at least 1.0 weeks and 95% degrade to below lethal levels by 5.0 weeks. These results confirm previous studies. Saunders *et al.* (2000) found that Foxoff® exposed to various amounts of artificial rainfall on the central tablelands reached LD_{50} levels after 2.4 weeks. The difference between these two results is insignificant (as tested earlier) and probably due to the differences in the fitted models. Staples *et al.* (1995) tested the longevity of 60 g Foxoff® baits containing 3.3 mg of 1080 in both wet and dry soil conditions. Despite the larger bait matrix (60 g vs 30 g) and the 10% higher nominal dose (3.3 mg), after 2 weeks in wet soil the mean 1080 content was 0.69 ± 0.11 mg (n = 2).

With no rain, the degradation of 1080 in Foxoff® is highly variable but considerably slower than wingettes under similar conditions. After 3 weeks, no wingettes from any treatment (n = 16) contained detectable levels (>0.05 mg) of 1080. Many Foxoff® still contained sufficient 1080 to be hazardous to a fox or dog after 11 weeks (Saunders *et al.* 2000). Similarly, the results can be used to estimate potential impacts of baiting campaigns on non-target species

(Table 2.11). On agricultural lands in eastern New South Wales, the main non-target species at risk from 1080 poisoning are domestic and working dogs. Dogs are highly susceptible to 1080, with an LD_{50} of 0.066 mg kg^{-1} (Tourtellotte and Coon 1951). The average weight of a sheep dog is 15 kg and a cattle dog 20 kg (Fleming and Parker 1991), indicating lethal doses of approximately 0.99 mg and 1.32 mg respectively.

These results are important to not only determine periods of lethal effectiveness against foxes, but to calculate safe withholding periods for working dogs. Landholders in New South Wales are legally required to remove and dispose of all untaken baits at the completion of a baiting program (Environment Protection Authority 2002). However, foxes will cache baits (see Chapter 3) resulting in baits that cannot be retrieved and which constitute a threat to working dogs. The results from this study and Saunders *et al.* (2000) suggest that areas baited with Foxoff® require longer withholding periods, especially during dry periods, than areas baited with wingettes. Dry periods would be particularly dangerous since some Foxoff® retained high 1080 concentrations for extended periods. In wet soil conditions, an estimated period greater than 4 weeks after last baits are laid is needed before working dogs should be allowed on properties baited with Foxoff®, twice as long as required when wingettes are used. Consequently, wingettes may be more favourable for use in sensitive areas where long-term hazards from toxic baits are highly undesirable, or periods where shorter withholding periods for working dogs is required to ensure 'safety' regardless of season.

Increased longevity of bait can also be advantageous. Obviously bait must remain toxic for sufficient periods to ensure resident foxes will find and consume a lethal dose. If bait degrades too rapidly for foxes to have this opportunity, it may be detrimental to the efficacy of baiting programs. Reduced longevity would require bait to be replaced more often in continuous baiting programs, reducing cost-effectiveness. Saunders *et al.* (2000) suggest that consumption of a sub-lethal dose of 1080 from degraded bait may lead to bait aversion (see Chapter 5) reducing the likelihood of bait being consumed in subsequent encounters. Research is therefore needed to determine whether the uptake of wingettes by foxes occurs before deterioration to sub-lethal levels. Where baits are not retrieved (e.g. aerially deployed

bait), bait longevity may also add a "time buffer" effect by remaining active to reduce the effect of reinvasion by immigrant foxes (Twigg *et al.* 2000).

2.4.4 Soil micro-organisms

Thirty-one species of micro-organisms capable of defluorinating 1080 were isolated from soils in the central tablelands and western slopes of New South Wales. Many of the species isolated, including *Alcaligenes*, *Arthrobacter*, *Aspergillus*, *Bacillus*, *Penicillium, Pseudomonas* and *Streptomyces,* are known to occur in Australian and New Zealand soils and have been previously reported for their defluorinating ability (Bong *et al.* 1979; Wong 1992; Meyer 1994; Walker 1994; Twigg and Socha 2001). The bacteria *Alcaligines*, *Arthrobacter*, *Bacillus* and *Pseudomonas*, and the fungi *Aspergillus* and *Penicillium* are known for their degradative capacity. These species are often involved in bioremediation, where their degrading capacity is applied for environmental cleanup of chemically contaminated sites (Paul and Clark 1996). However, the fungi *Monocilliuim* and *Scopularis*, the bacteria *Bordetella*, *Burkholderia*, *Kocuria* and *Micromonospora*, and actinomycetes *Streptosporangium* and *Streptoverticillium* have not been reported in earlier studies. The presence of a variety of micro-organisms capable of defluorination is not particularly surprising: the ability to metabolise fluoroacetate is common among soil micro-organisms (Walker 1994). However, the rate of defluorination varies considerably between species.

The isolated bacteria generally showed greater defluorinating ability than actinomycetes and fungi, which supports Wong *et al.* (1992). However, previous studies have found that the common soil fungi *Fusarium*, in particular *Fusarium oxysporum*, appears to be the most efficient microbial defluorinator (Wong *et al.* 1991; Wong 1992; Walker 1994; Kirkpatrick 1999; Twigg and Socha 2001). *Fusarium oxysporum* is ubiquitous in soils in Australia and New Zealand (Burgess *et al.* 1988) and may also be found on bait materials (Wong *et al.* 1991). It was not isolated in this study, possibly due to low abundance in the collected soil samples.

The defluorinating ability of unautoclaved soil was only measurable in the TRS soil, where 47.8$\pm$17.7% of added 1080 was defluorinated within 7 days. This is similar to Parfitt *et al.* (1994) who found that in New Zealand silt loams 50% of added 1080 was defluorinated

within 10 days at 23^0C, and Wong (1992) who found that up to 70% was defluorinated in 9 days (at 28^0C day and 15^0C night). However, this was much greater than the 23% defluorinated in 28 days in soils from central Australia (Twigg and Socha 2001). These differences are probably due to the organic matter composition of each soil. Although not measured, the central Australian soils appear to have less organic matter relative to soils in more temperate climates (Twigg and Socha 2001). The relatively low organic matter would mean lower available carbon and nitrogen for micro-organism growth (Clark 1967), and therefore limit micro-organism defluorinating ability.

The amount of F^- released in the OAI soil did not exceed the sterile soil control. However, there is strong evidence to suggest that soils at OAI contain 1080 degrading micro-organisms. The measurable defluorination rates of two species isolated from OAI soil provide proof that such organisms are present. Secondly, the degradation of wingettes and Foxoff® at OAI strongly suggest that microbes are largely responsible. The relatively rapid degradation of buried Foxoff® compared to the longevity of shelf stored Foxoff® suggests the micro-organisms are likely to be sourced from the soil environment. Despite the inability to detect 1080 degradation in the soil, the circumstantial evidence indicates that the soil at OAI has defluorinating ability.

The identification of micro-organisms capable of defluorination may offer advantages in addition to reassurance of environmental degradation of 1080. If increased longevity is required, meat baits could be dried, or bacteriostats (e.g. mercuric chloride) and fungistats (e.g. paranitrophenol) added to retard microbial growth and hence defluorination (Thomson 1986; Wong 1992). An understanding of the organisms responsible will assist in selecting appropriate retardants. Alternatively, where swift degradation is required, there may be potential to add defluorinating micro-organisms to detoxify a bait after an appropriate period (Wong *et al.* 1991), or improve soil or bait conditions (e.g. moisture, aeration, temperature) to encourage microbial colonisation and growth (Clark 1967). Buried baits may be colonised by defluorinating microbes from the soil at a faster rate than surface laid baits, leading to reduced longevity (Wong 1992). Such strategies may be especially advantageous where Foxoff® is used in sensitive areas, given their greater longevity.

The results show that, using methods similar to Wong (1992) and Twigg and Socha (2001), that defluorinating micro-organisms are present in the soils on the central tablelands and western slopes of New South Wales. However, the defluorinating ability of the OAI soil and the many individual isolates was unable to be confirmed, despite strong circumstantial evidence indicating that defluorination was occurring. Wong *et al.* (1991) and Wong (1992) also reported similar findings, many isolates (48-85%) capable of defluorinating 1080 solution were unable to defluorinate 1080 in the presence of sterile soil. This problem may have been exacerbated in this study by the low 1080 concentration measured to determine defluorination rates. Dilutions were necessary to enable the measurement of fluoride ions, and together with the binding of 1080 to the soil and corrections necessary for background fluoride concentration in 1080 solution and deionised water, meant that the amount of fluoride ions that could be attributed to micro-organism defluorination was very low. Additionally, sterile soil incubations (negative controls) indicated that some defluorination (OAI < 0.07µg L^{-1} and TRS < 0.03 µg L^{-1}) was occurring without micro-organisms. Twigg and Socha (2001) also found that defluorination did occur in sterile soil incubations. Once the data were corrected for this, the small concentration remaining was prone to error. The defluorination level of the isolates identified in this trial should be confirmed in the presence of 1080 without sterile soil, with greater precision to ensure measurable results. Regardless, the results indicate the presence of defluorinating micro-organisms in soils is eastern Australia will ensure no accumulation of 1080 from bait in the environment.

2.5 Conclusion

The results indicate that defluorinating micro-organisms are present in soils in eastern Australia, which will ensure no environmental accumulation of 1080 from baiting operations. The identification of these organisms may assist in selecting appropriate retardants to reduce their activity, or alternatively, where swift degradation is required, there may be potential to add defluorinating micro-organisms or improve soil or bait conditions (e.g. moisture, aeration, temperature) to encourage bait detoxification after an appropriate period. However, where reduced longevity is required, wingettes may be a useful alternative to red meat or

manufactured bait. They degrade at a faster rate than Foxoff®, even when no rainfall is applied. Since rain had no effect on the degradation rate, wingettes may be used to ensure that baits degrade swiftly regardless of rainfall. Areas baited with Foxoff® will require longer withholding periods for working dogs than wingettes, especially during dry periods. Wingettes may also have advantages for use in sensitive areas where long-term hazards to non-target species from toxic baits are highly undesirable. However, these results are reliant upon the threshold of soil moisture being present. The results should be reviewed in dry soil conditions (<18% water content) before being applied to such conditions. For example, Twigg *et al.* (2000) found that fresh red meat in arid areas became highly desiccated within days, restricting breakdown and vastly increasing longevity. In addition, wingettes must be evaluated not only for longevity, but also for attractiveness, palatability and rate of uptake by foxes (Chapter 3), efficacy and cost-effectiveness (Chapter 7). All these issues must be considered before the use of wingettes in any situation can be fully endorsed.