SCHOOL OF BIOMEDICAL SCIENCE

ASSESSMENT OF SODIUM FLUOROACETATE (1080) IN BAITS AND ITS BIODEGRADATION BY MICROORGANISMS

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ABSTRACT

In Western Australia dried meat baits containing 1080 are used extensively by agricultural and conservation organisations to control foxes and dingoes for the protection of agricultural production and native fauna. Field trials were conducted to assess 1080 loss from dried meat baits and this required the analysis of over five hundred baits. Because of this large number of baits it was essential to have a simple and efficient 1080 extraction procedure and method of 1080 analysis. In this study three methods of 1080 extraction and the new bioassay method for 1080 analysis were investigated. A simple and cost-effective 1080 extraction method using water with a 98% 1080 recovery rate was developed and modifications to the bioassay method were made.

Factory-produced 1080 dried meat baits were laid in the field during different seasons at four locations in Western Australia, samples were collected over time and analysed for 1080 content using the bioassay. Rainfall was recorded and temperature data was collected for each site. Baits were exposed to the elements but were placed in mesh or wire cages to restrict invertebrate attack and prevent removal by vertebrates. Some baits were placed on the surface and others were buried. Initially 1080 loss from baits from all 4 sites was minimal, ranging from 0 - 21% at day 7 - 9. Further loss was gradual even when rainfall was recorded. Generally baits had to be exposed to at least 50 mm of rain before 1080 loss increased to 50%. At some sites baits continued to remain toxic to foxes even after long exposure. The mean 1080 content of baits from the Carnarvon site at day 226 was 2.0 mg (55% of the mean 1080 content of baits at day zero) with 137 mm of rainfall recorded for that period. Loss of 1080 from baits buried occurred at a faster rate than from baits placed on the surface during the same time period. By day 14 no 1080 was detected in the buried baits compared to the 68%

detected in the surface baits. Under certain conditions 1080 loss from baits was minimal. Levels of 1080 in baits from Nangeen Hill remained fairly constant during the months of September to December 1995, and again during February to April 1996.

Gastrolobium plant tissue and soil samples from the southwest of Western Australia were investigated for the presence of 1080 degrading microorganisms. Microbes were isolated and individually tested in solution containing 1080 as the sole carbon source. Isolates which showed 1080 degrading ability were further tested for their degrading efficiency in McClung carbon-free solution with added 1080 as the sole source of carbon and in factory 1080 waste solution, at 1080 concentrations of 20 and 200 mM. The effect of temperature on their rate of degradation was also examined. Thirteen isolates (7 fungi and 6 bacteria) showing varying degrees of 1080 degrading ability were obtained. Rates of 1080 degradation varied among isolates but were highest in the factory waste solution at the 20 mM concentration and in the McClung solution, where 1080 was the sole source of carbon, at the higher concentration of 200 mM. The most efficient isolates OSK and 10H (both *Pseudomonas* species) degraded all the 1080 present in sterile factory waste solution up to 20 mM 1080 concentration in 4 days and the isolate 1AF (*Fusarium oxysporum*) degraded 93% of 200 mM 1080 in the McClung solution in 9 days. The optimum temperatures for 1080 degradation were 30°C and fluctuating ambient temperatures of 15 - 28°C.

DECLARATION

I declare that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university, and that to the best of my knowledge and belief it does not contain any material previously published or written by another person where due reference is not made in the text.

W. Kirkpatrick

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CHAPTER 1

REVIEW OF LITERATURE

This chapter reviews the literature on sodium fluoroacetate (1080) and covers a range of topics including; toxic properties of 1080, history of its use in various countries, current uses and applications, natural occurrence of 1080 in plants, factors affecting toxicity loss in 1080 baits, its persistence in the environment, effects of 1080 on flora and fauna and degradation of 1080 by microorganisms. The rationales and objectives of this research study are stated at the end of the chapter.

1.1 1080 : Properties and Mode of Action

Sodium fluoroacetate (FCH_2NaCO_2), commonly known as 1080, is an organofluorine synthetic salt of fluoroacetate or fluoroacetic acid (FCH_2COOH). It is chemically very stable because of the strength of the C - F bond. In appearance pure 1080 is a fine white, fluffy, crystalline powder which has a high solubility in water. It is very hygroscopic but insoluble in organic solvents. 1080 is odourless and virtually tasteless to humans. It becomes unstable above 110°C and decomposes at 200°C, yielding 20% hydrogen fluoride by weight. The commercial grade of 1080 used in the manufacture of 1080 baits has a slight vinegary or acidic odour and often a nigrosine dye is added as a safety measure.

The toxicological profile of 1080 is unusual because as a compound itself 1080 is not toxic. However, once in the body cellular metabolism converts it into one of the most lethal substances known (Peters, 1970). It was first suggested by Kalnitsky and Barron

(1948) that 1080 poisoning was a result of some effect on the tricarboxylic or citric acid cycle (TCA). Evidence of this was established when it was shown in a study by Buffa and Peters (1950) that citrate accumulated in the tissue of rats injected with 1080 (5 - 10 mg 1080 kg^{-1}). These findings were supported by the work of Kandel and Chenoweth (1952). An elevation of citrate concentration in plasma, heart, kidney and brain tissue can be used as an indicator of 1080 poisoning and the amount of accumulation can be used as an index of susceptibility to 1080 (Oliver *et al.*, 1979; Twigg, 1986; King *et al.*, 1996). Fluorocitric acid was isolated as the compound responsible for blocking the TCA cycle by Peters *et al.* (1952) and (-)-*erythro*-fluorocitrate was identified as the active isomer by Dummel and Kun (1969). Fluorocitrate synthesised from 1080 *in vivo* is the toxic agent in 1080 poisoning (Peters, 1954). It causes the TCA enzyme aconitase to be inhibited (Peters, 1952a), resulting in the TCA cycle becoming blocked at the citrate stage due to this inhibition, and citrate accumulates in the tissue (Peters, 1955).

The *in vivo* conversion of the non-toxic compound 1080 to the toxic substance fluorocitrate, via a lethal synthesis (Peters, 1952b), can be summarised as follows. Within the mitochondria in the presence of ATP 1080 combines with acetyl-Co-enzyme A to form fluoroacetyl-Co-enzyme A and during a reaction which involves oxaloacetate, fluorocitrate as (-)-*erythro*-fluorocitrate is produced. The enzyme aconitase is inhibited by fluorocitrate through both direct and time dependent progressive competition (Fanshier *et al.*, 1964). This in turn blocks the TCA cycle at the citrate stage (Morrison and Peters, 1954) and citrate transport into and out of the mitochondria is prevented (Kirsten *et al.*, 1978; Kun *et al.*, 1978). This results in a decline in ATP energy production followed by cell lysis and eventually organ damage. Death becomes inevitable if sufficient 1080 is converted to fluorocitrate (Savarie, 1984; Eason *et al.*, 1994a; Feldwick *et al.*, 1994; Twigg, 1994).

Several other aspects of 1080 metabolism and its toxic effects have also been investigated. Gal *et al.* (1961) found that only small amounts of 1080 were metabolised into fluorocitrate and much of it was eliminated unchanged from the body via the kidneys. Egekeze and Oehme (1979) demonstrated that the liver was a major site for 1080 detoxification. 1080 toxicity is increased when levels of glutathione, a tripeptide involved in oxidative and reductive processes, is depleted in the liver (Kostyniak *et al.*, 1978). 1080 poisoning has also been shown to damage testicular tissue (Mazzanti *et al.*, 1965; Smith *et al.*, 1977; Sullivan *et al.*, 1979; Twigg *et al.*, 1988).

Shapira *et al.* (1980) proposed that the toxic effect of 1080 could be related to a reduction of Ca^{++} because of the chelator effect (enhanced stability) of accumulated citrate from a blocked TCA cycle. Kun (1982) reviewed the metabolic activation of 1080 and detailed the enzymatic condensation of F-acetyl-CoA formation and the metabolic conversion of it and oxalacetate to the toxic factor fluorocitrate (*erythro*-fluorocitric acid). The conversion is a slow process and that may explain the slow onset (latent period) of the symptoms of 1080 poisoning. Other biochemical reactions connected to the formation of fluorocitrate probably add to the toxicity of 1080 (Savarie, 1984; Twigg and King, 1991).

There is no effective antidote available and 1080 is absorbed quickly through the gastrointestinal tract, mucous membranes, pulmonary epithelium and broken skin. There is a 0.5 to 3 hour latent period before any symptoms of poisoning occur (Twigg, 1994). Ambient temperatures can influence survival rates if temperatures are much above or below an animals resting thermonuetral zone (Misustova *et al.*, 1969; Eastland and Beasom, 1986; Oliver and King, 1983). Symptoms of poisoning differ among the major animal groups with carnivores displaying central nervous system (CNS) disorders, herbivores mainly cardiac disorders and omnivores, including

primates, display a combination of CNS and cardiac disorders (Ward and Spencer, 1947; McIlroy, 1986). There is great disparity in sensitivity between and in some cases within the major animal groups as well as sometimes large differences between populations within a species (Ward and Spencer, 1947; McIlroy, 1981a; 1981b; 1982b; McIlroy *et al.*, 1985). 1080 LD_{50}s, a statistical estimation of the dose needed to kill 50% of a sample of animals selected from the species population, have been reported for various animals (Table 1.1).

Table 1.1 Comparison of the LD_{50} of some animals, including WA species with a history of co-evolution with 1080. Some species have separate populations with different LD_{50}s (d) or they have a range of LD_{50}s (r).

SPECIES	LD_{50} mg 1080/kg (WA species)
Dingo *Canis familiaris dingo*	0.11
Fox *Vulpes vulpes*	0.13
Rabbit *Oryctolagus cuniculus*	0.4
Pig *Sus scrofa*	4.1
Goat *Capra hircus*	0.5
Black Rat *Rattus rattus*	0.8
House mouse *Mus musculus*	8.3
Bush Rat *Rattus fuscipes*	1.1 (20 - 80) [r]
Chuditch *Dasyurus geoffroi*	(7.5)
Tammar Wallaby *Macropus eugenii*	0.3 (5, 20) [d]
Southern Brown Bandicoot *Isoodon obesulus*	7 (20)
Brush-tailed Possum *Trichosurus vulpecula*	0.75 (125)
Emu *Dromaius novaehollandiae*	102 - 200 [r]
Common Bronzewing *Phaps chalcoptera*	25 (40)
Rosenberg's Goanna *Varanus roesenbergi*	40 (200 - 300) [r]
Man *Homo sapiens*	1 - 2 *
Rhesus Monkey *Macaca mulatta*	4.0

(Dreisback, 1966; Arena, 1970; Kaye, 1970; Atzert, 1971; King, 1990; King *et al.*, 1978; McIlroy, 1981a; 1981b; 1983; McIlroy and King, 1990; Twigg, 1994; Twigg and King, 1991; Twigg *et al.*, 1988; 1990; Wheeler and Hart, 1979.) * = estimated

1.2 Uses and Application of 1080

Sodium fluoroacetate was first synthesised in 1896 (Pattison, 1959) and first used as a moth proofing agent in 1930 (Peters, 1970), however the full extent of its toxic properties were not recognised until 1934 (Atzert, 1971). During World War II it was used to control rats which were a source of disease for the troops (Anon, 1977; Calver and King, 1986; Fagerstone *et al.*, 1994). The common name 1080 for sodium fluoroacetate refers to the original laboratory number designated to the sample obtained by the Patuxent Laboratory in Maryland USA (Kalmbach, 1945). The potential for misuse of the non-selective poison was recognised by early researchers and many recommended that its use be regulated and strictly controlled (Kalmbach, 1945; Barnett and Spenser, 1949; Leopold, 1964). 1080 was used to control coyotes (*Canis latrans*) in the USA in 1944 (Wade, 1978) and then used as a rodenticide in 1945 (Kalmbach, 1945). Concern about its unregulated use by authorised government agencies causing non-target deaths were expressed by Leopold (1964) and he recommended stronger regulations be implemented. In 1972 the use of 1080 and strychnine were prohibited (Fagerstone *et al.*, 1994), and in 1990 all registered rodenticide uses of 1080 in the US were withdrawn by the Environmental Protection Authority (EPA). Currently the only registered use of 1080 in the US is in the Livestock Protection Collar (LPC) which is used for sheep and goat protection (Fagerstone *et al.*, 1994; Burns and Connolly, 1995).

In Australia 1080 was first tested in 1952 as a pesticide for rabbit (*Oryctolagus cuniculus*) control in Tasmania (Meldrum *et al.*, 1957). In 1953 its use was extended to Victoria and WA (Anon, 1977; King, 1990). Although the release of the myxomatosis virus in the early 1950s was very successful in controlling rabbits, 1080 was used as an additional tool in reducing their numbers (Anon, 1958; 1977). It was

noted by Douglas *et al.* (1958) that 1080 was also an effective control for the introduced fox (*Vulpes vulpes*) and that their numbers had been drastically reduced on many farms due to secondary poisoning by foxes feeding on 1080 poisoned rabbits. In NZ 1080 was initially used in 1953 on rabbits when the biological control using the myxomatosis virus in 1952 failed due to the absence of suitable vectors (Anon, 1977). Its use has been expanded to include other vertebrate pests (Batcheler, 1978) and currently in NZ about 1500 kg of 1080 is used per year (Williams, 1994). Currently in Australia and New Zealand (NZ) 1080 is used to control many vertebrate pest species and it has replaced the use of such pesticides as arsenic, phosphorous and strychnine (Backholer, 1980; Calver and King, 1986) but its use is restricted and governed by legislative control and regulations.

In America 1080 was largely used on endemic species of animals such as the coyote however in Australia and NZ it is used mainly for the control of introduced animal species which have become major pests of agriculture and a threat to endemic fauna and flora. Both Australia and NZ were subject to many animal introductions such as foxes, rabbits, hares (*Lepus europaeus*) and red deer (*Cervus elaphus scoticus*) by Acclimatisation Societies (societies formed by English settlers with the aim of making their new home look more familiar by introducing animals from England) and also by individuals (Anon, 1977; Livingston, 1994). Many of these species successfully adapted to their new habitats because of milder climates and the lack of natural predators. The rabbit was especially successful and quickly became established throughout most of Australia and NZ. Six species of wallaby and the brushtail possum (*Trichosurus vulpecula*) were also introduced into NZ from Australia. Control of the brushtail possum in NZ is now estimated to cost $35 million dollars per year because of damage to forests and agriculture (Eason *et al.*, 1994a).

Currently agricultural protection and conservation agencies in Australia use 1080 baits

to control vertebrate pests such as foxes, rabbits, dingoes (*Canis familiaris dingo*), feral pigs (*Sus scrofa*), and goats (*Capra hircus*) and in NZ, brushtail possums and rock wallabies (*Petrogale penicillata*) (Calver and King, 1986; Eason *et al.*, 1992; Livingstone, 1994). These pests are the cause of major economic loss to primary producers through direct predation of stock, grazing competition, destruction of crops including forestry, and vegetation which can result in land degradation and desertification (Anon, 1977; Calver and King, 1986; Livingstone, 1994; Stewart *et al.*, 1994; Williams, 1994). Vertebrate pest species also displace native fauna and can also act as reservoirs and vectors for diseases such as bovine tuberculosis (Coleman, 1993).

The use of 1080 baits in Western Australia to reduce or limit fox numbers in order to protect native fauna has become an established wildlife management procedure. The fox has become established throughout most of Australia except for Tasmania and the most northern regions where probably a climatic barrier exists. In WA it is only absent from the North Kimberley and offshore islands (King and Smith, 1985). The adverse effect of the fox on populations of small and medium sized mammals has been documented by many researchers (Baynes, 1979; Christensen, 1980; King and Smith, 1985; Morris *et al.*, 1982; Kinnear, 1992). When fox numbers are controlled and reduced native mammal populations and their distributions increase (King and Kinnear, 1991; King *et al.*, 1981; Kinnear *et al.*, 1988; Morris *et al.*, 1982; 1998; Algar and Kinnear, 1996). Feral cats have also had a detrimental effect on ecosystems and populations of native animals on the mainland and many offshore islands in Australian and NZ (Jones and Coman, 1981; Eason and Frampton, 1991). Feral cats from rural and pastoral areas of WA include in their diet native fauna such as frogs, geckos, other small lizards, birds, small dasyurids and rodents (Martin *et al.*, 1996). The experimental use of 1080 baits to control feral cats in WA is currently being assessed but at present there is no 1080 bait registered for use against feral cats.

Malleefowl (*Leipoa ocellata*) protection and endangered fauna re-introduction programmes like the chuditch (*Dasyurus geoffroii*) and numbat (*Myrmecobius fasciatus*) species management plan (Friend, 1990; Wong *et al.*, 1995) depend on the removal or reduction of predators from their habitats. The ultimate success of many of these programmes is determined by the regular use of 1080 baits (King *et al.*, 1981; Kinnear *et al.*, 1988; 1998; Friend, 1990; Twigg, 1994). The Department of Conservation and Land Management (CALM) use large quantities of 1080 meat baits annually during the Western Shield programme aimed at controlling foxes and feral cats on nearly 5 million hectares of conservation land for the protection of native fauna.

1.3 Environmental Occurrence and 1080 Tolerance in Animals

1.3.1 Natural Occurrence of 1080 in the Environment

Sodium fluoroacetate was initially regarded as a synthetically manufactured compound as no naturally occurring organofluorine compounds were known to exist. However in 1943 the toxic feature of Gifblaar (*Dichapetalum cymosum*), an African plant which had been known to poison stock since 1890, was identified as fluoroacetate (1080) by Marais (1943; 1944). Badenhuizen and Slinger (1954) investigated the 1080 concentration of Gifblaar leaves and found that it was very variable between plants. Other species of *Dichapetalum* were identified as containing the toxin in their leaves, seeds and stems (Nwude *et al.*, 1977). Reports of plants poisoning stock were made as early as 1910 in Australia before *Acacia georginae* was identified as being the plant responsible by Bell *et al.* (1955). [In WA it had been reported as early as 1836 that sheep and goats driven towards York and Northam had been poisoned (Twigg and King, 1991)]. Loss in livestock and production due to 1080 was suffered by many

settlers, especially in WA (Cameron, 1977; Oelrichs, 1981) however the poisonous toxin, 1080, was not isolated until 1961 (Oelrichs and McEwan, 1962).

1080 was found to be present in the plant *Gastrolobium grandiflorum* which was known to poison stock in northern Queensland, the Northern Territory and northern WA by McEwan (1964a; 1964b). There are 40 species of *Gastrolobium* that naturally contain 1080 (some were originally included in the genus *Oxylobium*) and 39 of them occur only in the southwest of WA (Aplin, 1971; Hopper, 1991). Plant species containing 1080 vary greatly in 1080 concentration, but it can be as high as 2 600 mg of fluoroacetate kg^{-1} dried weight (Aplin, 1971; Twigg, 1994; Meyer and O'Hagan, 1992).

1.3.2 Non-target Risk and 1080 Tolerance of Native Fauna

Many WA endemic animal species including insects, reptiles, birds and marsupial and placental mammals have an elevated level of tolerance to 1080 (Mead *et al.*, 1985; McIlroy *et al.*, 1985; Twigg and King, 1989). This increase in tolerance has occurred through natural selection during a long history of co-evolution with *Gastrolobium* plants. Animals with a natural tolerance to 1080 would have survived when feeding on the plants and offspring inheriting this tolerance to the toxin have, over time, increased the species tolerance (King, 1990). Elevated tolerance is also evident in insectivorous and carnivorous animals and this has been acquired through natural selection as a result of including prey which forage on 1080 plants in their diet (Twigg, 1986; 1994). The degree of tolerance is mainly influenced by the length of time of exposure to the plants and specificity of diet and habitat (Twigg, 1994). The emu (*Dromaius novaehollandiae*) which belongs to the oldest seed-eating bird genus in Australia has a very high tolerance of 102 mg 1080 kg^{-1} (King, 1990). An increase in tolerance by many WA native fauna to 1080 has proved an advantage for 1080 baiting

programmes. This is because introduced species which have had no evolutionary history with 1080, such as the fox and rabbit, are much more susceptible to it than endemic species (Calver and King, 1986). (See Table 1.1). This large difference in tolerances allows the use of low dosage 1080 baits which are lethal to rabbits and foxes but of reduced risk to many native animals. The size of an animal still needs to be considered however as small native mammals can still be at risk if they consume enough poisoned bait.

Risk assessments on the effects of 1080 poisoning campaigns on non-target animals have been conducted in Australia and NZ and the general conclusion has been that although 1080 baiting programmes do cause the death of individual animals (Anon, 1981; McIlroy, 1981b; 1982a; 1992) the risk to most populations of these animals would be low (McIlroy *et al.*, 1986b; King, 1989; Spurr, 1994a). Nine orders of invertebrates are known to be affected or poisoned by 1080 (Notman, 1989), however Spurr (1994b) found no effect on invertebrate numbers before and after aerial baiting for possum control in NZ. Populations of birds and small mammals were studied during 1080 baiting for wild dogs in NSW by McIlroy *et al.* (1986a) and no significant effect was observed. Populations of WA northern quolls (*Dasyurus hallucatus*) which were radio-telemetry monitored for 2 weeks following dingo baiting with aerially dispersed 6 mg 1080 dried meat baits were assessed as being at minimal risk (King, 1989).

The assessment of secondary poisoning of non-target animals likely to eat 1080 poisoned dead or dying prey animals is difficult due to the number of factors involved including non-target body weight and the required lethal dose, habitat preference and feeding behaviour (Calver *et al.*, 1989; King *et al.*, 1981, 1989; Twigg and King, 1991). The effect of 1080 baiting on carnivorous mammals depends on bait placement and bait density per unit area, 1080 content of baits and the time of year that baiting is

carried out (McIlroy, 1982b). A reduction in the 1080 content of baits would reduce the amount of 1080 consumed by a target animal and this would then reduce the risk of secondary poisoning to non-target species (McIlroy and Gifford, 1992; Savarie *et al.*, 1994).

1.3.3 Persistence Of 1080 in the Environment

Contamination of the environment by the use or misuse of harmful and toxic substances such as pesticides is of major concern to individuals, communities and organisations. Two main issues regarding the use of 1080 as a pesticide have been the subject of debate in the US, NZ and Australia. These are the contamination of the environment by 1080 and the risk of poisoning to humans and non-target animals (Osweiler *et al.* 1985; Eason *et al.*, 1994b; Seawright, 1994). Many studies have been conducted which attempted to address these issues. Peters (1975), using predictive and conceptual models based on mathematical logic, proposed that the use of 1080 carrot baits for possum control in NZ was unlikely to be a source of environmental contamination even though the ultimate destination of any 1080 leaching from baits would be to the soil profile. He also predicted that the risk of baiting to non-target species was minimal and that the risk to humans eating dressed meat from deer killed by 1080 baits would be negligible due to the huge quantities (93 kg) of meat which would need to be consumed. Any 1080 leaching from baits is adsorbed and retained in the soil column where most of it would be defluorinated by microorganisms (Peters, 1975; Pickering, 1985).

Parfitt *et al.* (1994) found that there was no evidence of long or short term contamination of streams, surface water or ground water after 1080 baiting. No 1080 was detected in soil and water samples following possum control operations in NZ with cereal based 1080 baits (Eason *et al.*, 1991; 1992). In another study on aquaria

water with common aquaria plants, treated with 0.1 ppm 1080, Eason *et al.* (1993) could not detect any 1080 after 100 hours.

A study by Ogilvie *et al.* (1995) found that low environmental temperatures slow down the rate of 1080 degradation by microorganisms and that many microorganisms could activate their enzyme systems in the presence of 1080 and defluorinate it. Although some *Gastrolobium* plants contain very high concentrations of 1080 (0.1 - 3875 $\mu g\ g^{-1}$) in their tissue when soil from beneath these plants and water from nearby streams were tested by Twigg *et al.* (1996) only 1 soil sample contained 1080 (0.0039 $\mu g\ g^{-1}$). The results from these studies demonstrate that 1080 does not accumulate in the environment when used as a pesticide or for that matter in the soil under highly toxic vegetation. In summary any 1080 leaching from baits is retained mainly in the soil profile where it is subjected to degradation by soil microbes (Bong *et al.*, 1979; Wong *et al.*, 1992a; Peters, 1975), and in the presence of 1080 many soil and streamwater microbes can activate defluorinating enzymes and become efficient degraders of the poison (Ogilvie *et al.*, 1995).

1.4 Toxicity Loss of 1080 Baits

Meat baits used for fox and dingo control in WA are prepared by injecting 120 g pieces of beef, kangaroo or horse meat with a 1080 solution (volume usually 0.2 mL) and then drying these to an average weight of 40 g (Kok, 1997). 1080 impregnated oats, carrots and cereal-based baits are used for the control of rabbits and brushtail possums (NZ only). Raw eggs with either a 1080 poisoned oat added or injected with a 1080 solution are also used as baits for foxes when the use of meat baits is impractical, such as around swamps or shorelines or when they pose a hazard to non-

target animals (Massam, 1998). During control procedures baits are either placed in the field individually by hand or dropped from an aircraft (Thomson, 1986). To reduce the risk to farm dogs and native animals, baits are sometimes buried or tethered. When baits are placed by hand in rural areas it is a requirement that any remaining at the end of the baiting period be collected and destroyed (Massam, 1998). Baits dropped from aircraft, usually over areas of conservation estate and the pastoral regions of WA, remain in the field until eaten or scavenged or until they disintegrate due to weathering.

Loss of 1080 from baits can be caused by the leaching action of rain and moisture, removal of bait substrate by decomposers such as blowfly larvae, ants and beetles (McIlroy *et al.*, 1988), and by the microbial degradation of 1080. 1080 loss from carrot, oat and cereal-based baits also occurs and has been reported by Griffiths (1959), Corr and Martire (1971) and Wheeler and Oliver (1978). McIlroy *et al.* (1986b) reported rapid 1080 loss from meat baits collected and analysed during a 1080 control campaign in Kosciusko National Park. Poor 1080 recoveries from meat baits due to some metabolic process were obtained by Kramer *et al.* (1987) during an investigation into meat bait preparations. McIlroy *et al.* (1988) found that maggots present on baits had a greater impact on 1080 loss than rainfall although rainfall was also important. Baits which received 5 mm of rain on day 1 contained significantly less 1080 on day 2 than baits not receiving rain. Fleming and Parker (1991) also reported 1080 loss over time in 6 mg 1080 meat baits placed in the field. A significant 1080 loss of 39% was found after only 1 hour and after 200 hours only 8% of the original dose of 1080 was recovered. They concluded that a number of factors such as seepage, 1080 binding, small amounts being metabolised to other organofluoride compounds, leaching by rain, consumption by decomposers, and bacterial degradation could be responsible for 1080 loss. Some 1080 may not be detected because it binds to the bait

matrix and becomes difficult to extract, or due to inefficient 1080 extraction techniques (Livanos and Milham, 1984; Frost *et al.*, 1989; Fleming and Parker, 1991).

To date most of the field trials on 1080 loss from meat baits have been conducted in the Eastern States and there have not been any similar field trials conducted in WA. The loss in toxicity from meat baits and factors affecting loss over a period of time under WA field conditions have yet to be investigated.

1.5 Microbial Degradation of 1080

Microorganisms with the ability to defluorinate or degrade 1080 have been isolated from soil as well as from plants which contain 1080 naturally in their tissue. Kelly (1965), Tonomura *et al.* (1965) and Goldman (1965) all isolated species of soil bacteria which could be induced to metabolise 1080 as a growth medium. Microorganisms (*Fusarium oxysporum* and some species of *Pseudomonas*) which could defluorinate 1080 using haloacetate halidohydrolase enzymes were isolated from NZ soil by Bong *et al.* (1979) and Walker and Bong (1981). Walker (1994) also isolated soil *Pseudomonas* and *Fusarium* species which could grow using 1080 as the sole carbon source and other microflora which could defluorinate 1080 when supplied with an alternate carbon source. David and Gardner (1966) investigated the persistence of 1080 in garden soil and Kettering loam and found that both were decontaminated within a very short time. Rammell and Fleming (1978) found that the rate of 1080 degradation in soil depended on soil conditions and 1080 concentration, with high concentrations persisting for longer. Indigenous microflora, *Pseudomonas acidovorans*, *P. fluorescens*, *Penicillium restrictum*, *P. purpurescens*, *Aspergillus fumigatus* and *Fusarium oxysporum*, isolated from WA soils degraded from 50 - 87% of added 1080 within 5 to 9 days (Wong *et al.*, 1992a). The effects of environmental

factors on these microorganisms were investigated and it was found that inoculum size had an effect on defluorination rates when 1080 was the sole source of carbon and that a fluctuating (11 - 24°C) temperature was optimal for defluorination (Wong *et al.*, 1992b).

The bacterium *Pseudomonas cepacia* isolated from the South African plant *Dichapetalum cymosum* defluorinated 2.69 mg of 1080 x 10^9 cells hour $^{-1}$ and grew in a 1080 enriched solution without any inhibition (Myer *et al.*, 1990). *D. cymosum* is highly poisonous to stock and the concentration of 1080 in fresh leaves was analysed at 231.9 mg kg^{-1}. It was also found that *P. cepacia* isolated from *D. cymosum* could only defluorinate 1080 and did not metabolise it further (Myer, 1994). An investigation into the metabolism of *P. cepacia* isolated from *D. brauni* (another South African 1080 poison plant) showed that 1080 was metabolised to several fluorinated metabolites including fluorocitrate and fluoroglutamate.

Wong *et al.* (1991a) examined meat, oat, egg and crackle (meat composite) bait materials for the presence of 1080 defluorinating microflora. The microorganisms isolated during this study displayed low defluorinating activity but the addition of an alternate carbon source increased the activity of some of the microorganisms.

Soil microflora capable of utilising pesticides as a source of carbon increase in population size in the presence of the pesticide and the rate of degradation increases over time after an initial lag phase during which the microflora population increases (Brown, 1978). It is likely that in the presence of 1080 many microorganisms present in soil and water would adapt similarly and this is a reason why 1080 does not persist for long in the environment. Kelly (1965) first proposed that 1080 degrading bacteria could be useful for detoxifying 1080 contaminated soil and water, however the ability of currently known 1080 degrading microorganisms to detoxify 1080 factory wastes

has not been investigated.

1.6 Extraction and Determination Methods for 1080

There are various methods available for 1080 analysis including fluoride ion-selective electrode, high performance liquid chromatography (HPLC), gas liquid chromatography (GLC), a bioassay and toxicity methods as well as a qualitative detection test. Most methods either measure liberated fluoride ion levels after the C-F bond has been broken, or they measure a derivative of 1080 such as dichloroanilide using HPLC and GLC. Toxicity methods involved using mice and the more recently developed bioassay method by Wong *et al.* (1995) uses 1080 sensitive bacteria.

1080 is extracted from samples with extracting solvents such as water, acetone and water, simulated digest, pancreatin digest and protein precipitation, combined with physical homogenisation of samples using heat or by mechanical means. The particular method of extraction used is often determined by the type of 1080 analysis performed and the rates of 1080 recovered from samples can vary with different extraction methods.

1.6.1 HPLC and GLC Methods

Both HPLC and GLC methods use water to extract 1080 from samples and then organic solvents are used to separate the water from the 1080. Some contamination of the sample with water occurs which can later interfere with the assay so it is usually evaporated off using an inorganic solvent and the pH has to be critically maintained at this stage so as to prevent any loss of 1080. The 1080 can be derivatised to the bromophenacyl ester of fluoroacetic acid or dichloroanilide (Okuno *et al.*, 1982; Ozawa and Tsukioka, 1989; Collins *et al.*, 1981). Recoveries of 1080 have varied

from 83 - 99% for bait samples spiked with 100 and 1000 mg 1080 kg^{-1} (Collins *et al.*, 1981), 70% for soil samples and 81 - 89% for biological samples (Ozawa and Tsukioka, 1989). An alternate GLC method using acetone and water (8:1) to extract 1080 from carrot and beef mince samples followed by derivatisation with pentafluorobenzly bromide with the ester then being analysed by electron capture gas chromatography gave recoveries of 93% (Allender, 1990).

HPLC and GLC methods are very sensitive and can detect 1080 concentrations as low as 0.0015 μg 1080 g^{-1} (Ozawa and Tsukioka, 1989). However these methods require the use of very expensive equipment and skilled technicians which results in a high cost for analysis per sample, approximately $120 per meat bait. This high cost is often prohibitive for carrying out field trials involving hundreds of meat baits and in many cases other cheaper methods such as the fluoride ion-selective electrode method are used.

1.6.2 Fluoride Ion-selective Electrode Method

Ramsey and Clifford (1949) described a method for determining 1080 concentrations in food and biological material. 1080 was extracted from different types of samples using such methods as protein precipitation, boiling in water and pancreatin digest, followed by ether extraction. Any inorganic fluoride was separated from the 1080 by separation chromatography and samples were then ashed and the recovered 1080 defluorinated by hot alkaline conditions. The 1080 concentration was determined from the amount of inorganic fluoride recovered and percentage recoveries ranged from 81 - 105%.

Peters and Baxter (1974) used oxygen combustion to defluorinate 1080 in samples and then measured the liberated F^- ions with a fluoride ion-selective electrode.

Background inorganic fluoride was measured prior to combustion and was then accounted for in the final calculation. Amounts of 1080 recovered in standard solutions ranged from 88.5 - 97.5% and in cereal-based baits from 81 - 92.5%. Livanos and Milham (1984) used the fluoride ion-selective electrode method to measure 1080 in meat baits and standard solutions. Bait samples were first homogenised with an acetone and water (1:1) extracting solvent and then subjected to ultrasonic agitation, steam digested and finally vacuum filtered. The extracted filtrates were subjected to hot alkaline hydrolysis and the C-F bond broken. Liberated fluoride was measured with a fluoride ion-selective electrode and allowances were made for any co-extracted inorganic fluoride. 1080 recoveries were 86% for meat baits and 90% for standard solutions.

The fluoride ion-selective electrode method does not require the use of expensive equipment like the HPLC and GLC methods and the cost of analysis is much less, approximately $20 to $30 per sample. Also samples do not have to undergo intense clean-up procedures which are essential for HPLC and GLC analysis. The main limitation with the fluoride ion method is that it is quite slow to perform as the time taken for the hot-alkaline hydrolysis to break the C-F bond has a limiting effect on the number of samples which can be analysed per day. In addition this method does not distinguish between F^- ions originating from the 1080 or from other organofluorine compounds present in the sample.

1.6.3 Bioassay

The bioassay method of 1080 analysis (Wong *et al.*, 1991b; 1995) measures 1080 concentration directly by measuring its toxic effect on the growth of a 1080 sensitive bacterium, *Acinetobacter lwoffii*. As samples are not subjected to defluorination or derivatisation procedures the method is relatively quick to perform once the 1080 has

been extracted into solution. Zones of inhibition in the growth of *A. lwoffii* are used to determine 1080 concentrations using the same principle as antibiotic sensitivity tests (Hewitt and Vincent, 1989). The growth of the bacteria is inhibited in the presence of 1080 and the inhibition zone size is linearly related to the log concentration of 1080. A major advantage of this method is the large number (64 zones available) of samples which can be analysed on a 30 x 30 cm plate. The minimal 1080 concentration detected was reported to be 0.025 mM (Wong *et al.*, 1995). 1080 was extracted from minced meat using simulated gastric and intestinal digest (Parker and Frost, 1991) and recovery rates were 78.5% for gastric digest and 93% for a combined gastric-intestinal digest. Recovery rates for 1080 oat baits extracted using water were 95 - 107%, and 92 - 114% for soil with 1080 added.

Previously this method has only been used to analyse a small number of meat samples under laboratory conditions. One of the aims of this study was to evaluate the use of the bioassay as a routine method of analysis for a large number of dried meat baits to determine their 1080 content.

1.6.4 Other Methods

Griffiths (1959) used live mice to determine the 1080 concentration of solutions extracted from baits. Two groups of mice were made more sensitive to 1080 during a period of fasting and then 1 group were injected subcutaneously with known 1080 standard solutions and the other group was injected with solutions extracted from 1080 baits. The number of deaths which occurred in mice injected with the bait solutions were compared with the number of deaths in the control group injected with known 1080 standards. Concentrations of unknown bait extracts were extrapolated from the compared death rates. This method is rarely used due to animal ethics and constraints on the use of live animals for toxicity testing.

A qualitative method for detecting the presence of 1080 in liquid samples which could be carried out in the field was used by McGary and Meloan (1982). The test is performed in a test tube and a positive result is obtained with a colour change which is caused by the release of F^- ions during sodium fusion. This method can only be used on liquid samples and is not quantitative in any way.

1.7 The Rationales and Objectives of the Research

1.7.1 Rationales

No field trials have previously been conducted in Western Australia to investigate 1080 loss to determine how long meat baits remain toxic to foxes in the field. It has been assumed that while a bait remains physically intact it is potentially toxic to most animals which may eat it, target and non-target alike, but there is no information available to actually support this. Quality control of the 1080 content of baits is also important and baits should contain sufficient 1080 to kill foxes when first laid and remain lethal for a sufficient time, otherwise the financial cost of baiting could not be justified. Knowledge about how long 1080 dried meat baits remain toxic in the field would help to assess what minimal lethal dosage is required for baits to remain toxic over time. There is also concern that sub-lethal baits may cause bait-shyness (Hickling, 1994) when eaten by the target animal, but it is unknown if this may be a problem with factory-produced meat baits. Information on the variation in initial 1080 content of baits and how long they remain toxic needs to be available so that bait shyness in target animals as well as the poisoning risk to pets, stock and native fauna can be adequately assessed. Just as importantly, information should be available to answer questions concerning 1080 baits which may be asked by individuals, communities, or organisations. Field trial studies and monitoring quality control of manufactured baits has been hampered by the lack of a cost effective analytical procedure for 1080. The recent development of the 1080 bioassay (Wong *et al.*, 1995) was a major advance in relation to this problem but the application of the method to analyse a large number of meat bait samples has to be investigated.

Problems have been encountered with variable recovery rates in many of the investigations which dealt with measuring 1080 concentration in biological samples (see 1.6). Several reasons have been given to explain the cause of these variations including incomplete 1080 extraction due to some form of chemical bonding or due to the particular method of extraction employed. Parker and Frost (1991) developed a simulated gastric-intestinal digestion method for extracting bound and unbound 1080 from meat and they obtained good recovery rates from a small sample of raw ground meat. This method may be applied to analysing large samples of 40 g 1080 dried meat baits but it has not been tested as yet.

During 1080 bait manufacture large volumes of 1080 waste are produced and at present there is no procedure available which could effectively and rapidly detoxify this waste. The waste solution is currently held in large underground storage tanks, diluted over time with water and then released into the sewerage system when the concentration of 1080 is low. No investigation has previously been carried out to examine the possibility of increasing the rate of 1080 degradation in these solutions by the addition of 1080 degrading microorganisms. One potential source of microbial 1080 degraders is from *Gastrolobium* plants and soil surrounding these plants. Meyer *et al.* (1990) isolated a 1080 defluorinating bacterium, *Pseudomonas cepacia*, from the 1080 containing plant *Dichapetalum cymosum* and it is likely that *Gastrolobium* species contain similar bacteria.

1.7.2 Objectives

- To develop a more efficient 1080 extraction procedure for dried meat baits.

- To evaluate the suitability and efficiency of the bioassay method for analysing large numbers of 1080 meat baits.

- To determine how long factory-produced 1080 dried meat baits remain toxic to the target species under different field conditions in WA and factors which affect 1080 loss.

- To isolate microorganisms capable of degrading 1080 and investigate their efficiency in degrading 1080 in factory waste solution.

CHAPTER 2

EXTRACTION AND ANALYSIS OF 1080

This chapter describes two areas of study: (1) the evaluation of 1080 extraction methods, (2) the application of the 1080 bioassay method for 1080 analysis.

2.1 Evaluation of 1080 Extraction Methods

2.1.1 Introduction

There are several methods available for measuring the concentration of 1080 in samples. These include high performance liquid chromatography (Ozawa and Tsukioka, 1989), gas liquid chromatography (Allender, 1990), fluoride ion-selective electrode (Livanos and Milham, 1984) and the bioassay method (Wong *et al.*, 1995). When the 1080 content of bait material is analysed using any of these methods all initial steps involve the extraction of 1080 from the bait material into solution. Water or acetone:water mixtures, (1:1 or 8:1) have been used to extract 1080 from biological materials such as meat baits. Recovery rates of 1080 from meat have varied and for water extraction methods reported rates have been; 60% (Kramer *et al.*, 1987), 82% (Ozawa and Tsukioka, 1989) and 83 & 99% (Collins *et al.*, 1981). Acetone:water extraction methods have reported rates of 86% (Livanos and Milham, 1984), 90 &

94% (Kramer, 1984), 85% (Okuno *et al.*, 1982), 63% (Kramer *et al.*, 1987) and 92.7% (Allender, 1990). The poor recovery rate obtained by Kramer *et al.* (1987) was reported to be due to either inaccurate dosing of baits initially or because of the failure to recover all the 1080 during the extraction process. The variability of 1080 recovery rates was reported to be caused by the inability to extract bound 1080 by Frost *et al.* (1989). A simulated gastric-intestinal digest method by Parker and Frost (1991) was reported to extract both bound and unbound 1080 from ground meat with recoveries of 66 to 83%.

Assessment of minimum lethal dose requirements and studies on 1080 loss from baits placed in the field require the analysis of a large number of meat baits. These types of assessments have been impeded because current methods of 1080 extraction and analysis available are too complex, laborious and expensive (Livanos and Milham, 1984; Wong *et al.*, 1991b; 1995). The development of a simple effective 1080 extraction method for meat baits would be of advantage when used in conjunction with the inexpensive 1080 bioassay method and the analysis of large numbers of meat baits could easily be achieved. This study reports on 1080 recovery rates from meat baits using water, acetone:water (1:1) and simulated gastric digest combined with soaking, shaking and ultrasonic agitation of samples leading ultimately to the development of a simple 1080 extraction method for meat baits.

2.1.2 Materials and Method

Bait Preparation

Both fresh and dried meat baits dosed with 1080 were used during the investigation of 1080 recovery rates and different extraction methods. Fresh meat 3.0 mg 1080 baits

were prepared in the laboratory by injecting 0.5 mL of a 6 mg mL^{-1} analytical grade 1080 solution into the centre of 40 g pieces of beef (called fresh laboratory-prepared in text and tables), baits were then kept at 4°C for 24 hours prior to use. Dried meat baits containing 2.5, 3.0 and 4.5 mg of 1080, toxic levels which have been used for fox control in WA, manufactured at the Agriculture WA Bait Production Unit, Forrestfield, WA (factory-produced baits) were stored frozen prior to use. Nine meat baits were prepared in the laboratory by injecting 0.2 mL of a 23 mg mL^{-1} (4.6 mg of 1080) analytical grade 1080 solution into the centre of 100 g pieces of fresh beef using a 1-mL sterile plastic syringe (called laboratory-prepared in text and table). The baits were dried in a hot room at 35°C to 40% of the wet weight (equal to the dryness of factory-produced baits) and then stored at -20°C.

The number of baits used for each of the experiments varied ranging from 1 to 9 and this was due mainly to constraints of time.

Extraction Procedures

The procedures and solvents used to extract 1080 from the baits were adapted from Parker and Frost (1991), Wong *et al.* (1995) and Livanos and Milham (1984). Gastric digestion fluids were prepared according to United States pharmacopoeia standards (United States Pharmacopoeia Convention 1990).

In the first experiment. Three replicates of two 3.0 mg 1080 fresh meat laboratory-prepared baits and one 3.0 mg 1080 factory-produced bait, each in a 300-mL plastic container (Bipa Products, Australia), had 50 mL of gastric digest, acetone:water (1:1) or just water added (ie. sets of 3 baits were tested in each of the 3 solutions). Baits were held overnight at 4°C to soak. The baits were then cut into 8 - 12 small pieces using 9 inch serrated shears (Fiskars Shop Shears) then returned to their containers and another 50 mL of extraction solution was added. The containers were placed on a

reciprocal shaker at 35°C for 80 minutes and then transferred to an ultrasonic water bath and agitated for 60 minutes. Ten-mL samples were removed from each of the extraction solutions and placed into 10-mL plastic centrifuge tubes. All samples were autoclaved at 112°C for 15 minutes, cooled and then kept at 4°C until analysed for 1080 concentration.

In a second experiment. Four replicates of 4.5 mg 1080 factory-produced baits were each placed into a 300-mL plastic container and either 100 mL of gastric digest or distilled water was added. Bait samples were placed in a Gyrotory water bath shaker (New Brunswick Scientific Co. Inc., Edison, N. J., USA, model G76) for 120 minutes at 35°C and then left overnight at 4°C to soften. The next day baits were cut into small pieces as in the first experiment and placed into double layered plastic bags (12 x 30 cm) with 50 mL of the fluid they had been soaking in. A stomacher was used to macerate the samples for 20 minutes and then samples were returned to their plastic containers. The samples were subjected to ultrasonic agitation for 10 minutes and then kept at 4°C overnight. The next day samples were placed on the shaker for 25 minutes at 35°C, followed by 20 minutes of ultrasonic agitation. Aliquots of 5 mL were removed with a pipette and placed into sterile 10-mL sterile centrifuge tubes. All samples were centrifuged for 10 minutes at 3500 rev/min and the supernatants were then collected in 10-mL sterile centrifuge tubes and stored at 4°C until analysed.

In a third experiment. One 2.5 mg 1080 factory-produced bait was placed into a 300-mL plastic container and 122 mL of distilled water was added. The bait was kept at 4°C overnight and then small volumes were removed at different stages of extraction to measure 1080 levels as follows. A 2 mL aliquot sample was taken before the bait was cut into small pieces. The bait pieces were returned to the container and left to soften overnight at 4°C. The following day another 2 mL aliquot was taken and then the bait was placed in the ultrasonic water bath for 30 minutes. The bait was then

placed on the shaker for 70 minutes at 35°C after which a 10 mL sample was removed. The bait sample was returned to the shaker for a further 120 minutes after which a final 10 mL sample was taken. All samples were kept frozen and then thawed at 4°C and centrifuged at 3500 rev/min for 45 minutes. The supernatants were removed and stored at 4°C until analysed.

Improved Water Extraction Method for 1080

Nine replicates of 4.6 mg 1080 laboratory-prepared baits and 15 replicates of 4.5 mg 1080 factory-produced dried meat baits were each weighed in a pre-weighed 300-mL plastic container. The combined weight of bait and container with lid were recorded. Sufficient distilled water was added to immerse each bait. Baits were allowed to soften and re-hydrate overnight at 4°C in order to minimise microbial growth and any 1080 degradation. The baits were removed from the liquid and cut into small thin (25 mm) pieces using the shears and then they were returned, together with residual solution, to their containers. The containers were placed in a water bath at 70°C for 60 minutes to minimise the occurrence of any microbial 1080 degradation taking place. The containers were then placed on the shaker at ambient temperature (*c.* 20°C) and the samples were gently agitated overnight. The next day the weight of each container and content was recorded. A 10 mL aliquot was collected aseptically from each homogenised bait sample and placed into a sterile 10-mL plastic centrifuge tube. These samples were centrifuged at 3500 rev/min for 40 minutes. The supernatants were removed and heat-treated in a waterbath at 70°C for 45 minutes and then re-centrifuged at 3500 rev/min for a further 30 minutes. The supernatants were removed and sterilised by filtration through 0.45 μm Millipore Millex filter units. Sterile filtrates were collected in 25-mL glass wide-necked bottles and kept at 4°C prior to 1080 analysis.

Determination of 1080 Content by the Bioassay Method

The concentration of 1080 in all bait extract samples were determined using a bioassay (Wong *et al.*, 1995). Diffusion medium containing 1.5% Difco vitamin casamino acids and 1.5% Bitek^{TM} was prepared in 250 mL volumes, autoclaved at 121°C for 15 minutes and then held at 50°C in a waterbath. An inoculum of *Acinetobacter lwoffii* DHW was prepared by emulsifying colonies from an 18 hour culture on Tryptic soy agar (TSA) in 10 mL of sterile saline (*c.* 2.4 x 10^9 cells mL^{-1}). A 2.5 mL volume of inoculum was added to 250 mL of molten (50°C) agar, mixed gently and then poured into a large sterile perspex plate with outer dimensions of 30 x 30 x 1 cm. This plate had an 8 x 8 pattern to accommodate 64 blank filter paper 6 mm discs. The inoculated agar plate was kept at 4°C for 1 hour. 1080 standard solutions with concentrations ranging from 0.06 to 0.9 mM (6 to 90 μg mL^{-1}) were prepared using stock analytical grade 1080 solutions diluted to the required 1080 concentration using liquid sterile non-toxic meat bait extract. Replicates of 3 sterile discs impregnated with 20 μL of 1080 standards and test samples were randomly placed on the surface of the agar. Plates were kept at 4°C for 1.5 hour to allow diffusion of standards and samples into the agar medium and then plates were incubated at 35°C overnight.

The diameters of the growth inhibition zones (y) were measured and regressed against the logarithm of the 1080 standard concentrations (x). Regression statistics derived from the 1080 standards (three zone-width replicates for each concentration) were calculated using Statview for multiple-values of y. Amounts of 1080 recovered from bait samples were obtained by inverse prediction using Excel 4.0 after rearrangement of the regression equations derived from the 1080 standards, and solving for x.

2.1.3 Results

The recovery rates of 1080 extracted from 3.0 and 4.5 mg 1080 meat baits varied among the extracting solutions (Table 2.1). The 3.0 mg 1080 fresh meat laboratory-prepared baits extracted with acetone:water (1:1) gave better recovery rates of 80 and 93% than the gastric digest fluid (53 and 66%) and water alone (50 and 77%). For the 3.0 mg 1080 factory-produced meat bait the rate of 1080 recovered was low (17 - 30%) from all 3 solutions, water (17%), acetone:water (30%) and gastric digest (20%).

In the second experiment, with a prolonged shaking period of the baits in the extraction solutions and with stomacher agitation, higher 1080 recovery rates were obtained from the 4.5 mg 1080 factory-produced baits using water (73 - 93%) than the gastric digest fluid (69 - 89%). Centrifugation of the extracts improved the clarity of the samples and hence the quality of the inhibition zones in the bioassay. The 1080 recovery rates from a 2.5 mg 1080 factory-produced bait at different stages of the extraction procedure using water and with soaking the bait overnight are shown in Table 2.2. Sixty-eight percent of the 1080 was extracted into solution during the overnight re-hydrated period after the bait was macerated. Ultrasonic agitation of the sample did not affect the amount of 1080 recovered. However a prolonged shaking time of 190 minutes instead of 70 minutes increased the recovery rate by 8%. Centrifugation of the sample extract did not increase the percentage of 1080 recovered, however using the supernatant did improve the clarity of the growth inhibition zones.

Using the improved water extraction method for 9 replicate laboratory-prepared 4.6 mg 1080 meat baits gave a mean 1080 recovery rate of 98% (Table 2.3). The 4.5 mg 1080 factory-produced meat baits showed a mean 1080 content of 5.2 mg (s.d. =

0.437) compared to the factory nominated dose of 4.5 mg of 1080 (Table 2.3).

Table 2.1 Amounts of 1080 recovered from 3.0 and 4.5 mg 1080 meat baits using 3 different types of extracting solution, water, acetone:water (1:1) and gastric digest. Results are expressed as a percentage of the nominated 1080 dose.

	Percentage of 1080 Recovered		
Bait Type & 1080 dose	Water	Acetone:Water	Gastric Digest
Experiment 1			
Fresh 3.0 mg	50	93	53
Fresh 3.0 mg	77	80	66
Factory 3.0 mg	17	30	20
Experiment 2			
Factory 4.5 mg	93	-	89
Factory 4.5 mg	73	-	75
Factory 4.5 mg	89	-	69
Factory 4.5 mg	93	-	86

- = not tested

Table 2.2 Amounts of 1080 recovered from a 2.5 mg 1080 factory bait at different stages of the extraction process. Results expressed as percentage of 1080 recovered.

Stage in Extraction Method	% 1080 Recovered
Uncut bait soaked overnight	7
Cut up bait soaked overnight	68
After 30 minutes ultrasonication	68
After 70 minutes shaking at 37°C	68
After a further 120 minutes shaking	76
Final sample after centrifugation	76

Table 2.3 Mean 1080 recovered from 4.5 mg 1080 factory-produced baits and 4.6 mg 1080 laboratory-prepared baits using the improved water extraction method with 95% confidence limits and % of 1080 recovered.

Bait Type (mg 1080)	n	Mean (mg 1080)	S.D. ±	Range mg 1080	95% confidence limits (mg 1080)	% 1080 recovered
Laboratory (4.6)	9	4.5	0.320	3.9 - 4.9	4.2 - 4.7	98%
Factory (nominated 4.5)	15	5.2	0.437	4.5 - 5.9	4.9 - 5.4	101.5%

2.1.4 Discussion

The 1080 recovery rates obtained from the 3.0 mg 1080 factory-produced baits using the three extraction methods; simulated gastric digestion, acetone:water and water in the initial experiment were probably low as a result of the extraction procedure not being sufficiently vigorous enough to release bound 1080. Higher rates of 1080 recovery were obtained using the gastric digest on 3.0 mg 1080 fresh laboratory-prepared baits ranging from 53 - 66% when compared with the 20% recovered from factory-produced baits. These rates are comparable to that obtained by Wong *et al.* (1995) when less 1080 was also recovered from dried meat baits than from fresh meat baits using gastric digest.

However, there are problems associated with the use of simulated digestion methods in that the chemicals are not readily available and they are also expensive when used in the quantities required for the analysis of a large number of meat baits. Although higher rates of 1080 recovery were obtained using acetone:water for both fresh laboratory-prepared (80 - 93%) and factory-produced baits (30%) than using gastric

digest (53 - 66% fresh, and 20% dried) or water (50 - 77%, fresh and 17% dried) there are health hazards associated with the extended use of acetone which would make the use of extraction fume hoods during the entire extraction process necessary.

The problems associated with the use of acetone and simulated digest to extract 1080 from meat baits were overcome by the development of the improved water extraction method. The improved water extraction method gave a mean 1080 recovery rate of 98% for the 4.6 mg 1080 laboratory-prepared baits which is higher than both simulated gastric digestion (20 - 89%) and acetone:water (30 - 93%) methods. The high recovery rate of 101% for the 4.5 mg 1080 factory baits shows that they contained a higher 1080 amount (mean = 5.2) than their nominated dose of 4.5 mg, resulting presumably from errors during production. (This is discussed in section 3.4, pp. 66 - 67).

The prolonged periods of soaking and gentle agitation of the baits improved the rate of 1080 recovery. These results indicate that the volume of water used during extraction should be minimal to avoid over-dilution of 1080 in the sample. The optimised method was inexpensive to use as no chemicals were used and the main cost was consumption of power which was low. Purchase of a water bath / shaker and other equimement used such as plastic containers and the shears would be about two thousand dollars. The method is simple to use as there is minimal handling of the baits but the process requires 36 hours of extraction due to the soaking and agitation times. The method is suitable for processing large numbers of baits within a short time.

2.2 Improvement and Application of the 1080 Bioassay

2.2.1 Introduction

The bioassay method of 1080 analysis, developed by Wong *et al.* (1991b; 1995) is reported to be a quick and reliable method which could be used for monitoring quality control of 1080 baits and for assessing 1080 loss from meat baits in the field. This method measures the actual 1080 and not a derivative or ion. It is a quick, low cost method when compared with HPLC, GLC or fluoride ion-selective electrode methods. The bioassay requires the use of aseptic techniques and basic microbiological equipment.

The technique is an adaptation of the methodology used to assess the sensitivity of bacteria to antibiotics (Hewitt and Vincent, 1989) on agar by a disc diffusion technique. When a bacterium is sensitive to a given antibiotic, or to 1080, a zone of growth inhibition is produced around a disc containing the substance. The bioassay technique for 1080 is based on the fact that the growth of a 1080-sensitive bacterial species, *Acinetobacter lwoffii*, is inhibited on a nutritionally complete agar substrate (Wong *et al.*, 1995). The inhibition zone is visually distinct on the agar plate as a circular, clear area and the diameter of the zone is measured. The inhibition zone diameters (zone-widths, ZW) are linearly related to the log of the 1080 concentration.

This study evaluated the suitability of the bioassay method to analyse the 1080 content of a large sample of factory-produced meat baits. Several parameters were investigated so as to improve the assay. This improved procedure was then used to determine 1080 levels in dried meat bait samples analysed during this research project.

2.2.2 Materials and Method

Extraction of 1080 from Factory Baits

Factory-produced dried meat 1080 baits and baits without 1080 (non-toxic baits) were processed using the improved water extraction method as described in section 2.1. Bait extract samples were purified by centrifugation, heating and filtering before being analysed for 1080 content using the bioassay method.

Bioassay Procedure (Wong et al., 1995)

The bioassay procedure used was as described in section 2.1.2, p. 38.

Procedures for Improving the Bioassay Technique

Inoculum growth medium. Three types of media, Oxoid Tryptic Soy agar (TSA), Oxoid Nutrient agar (NA) and Oxoid Diagnostic Sensitivity agar (DST) were tested for growth of *A. lwoffii* incubated at two temperatures (30°C and 35°C). Two sets of 6 replicate plates for each type of agar medium were inoculated with a loopful of 18 hour *A. lwoffii* culture grown in brain heart infusion broth (BHIB), and 1 set was incubated at each temperature. After overnight incubation the agar medium and incubation temperature which showed the best growth were chosen for subsequent preparations of inoculum.

Diffusion agar. Five types of diffusion agar were investigated for their clarity of growth inhibition zones in the bioassay. Diffusion agar consisted of vitamin assay casamino acids (dehydrated Difco) 1.5% and one of the following agar media: Bacteriological agar (Difco) 1.5%, Bitek^{TM} 1.5%, Mueller Hinton (Gibco BRL) 3.8%, Sensitivity agar (Oxoid) 3.2%, and Davis agar 1.2%. Media were prepared in 100 mL volumes using distilled water. Inoculum grown on DST agar was prepared as

described above and 1 mL of the inoculum was added to each bottle of molten (50°C) agar, mixed thoroughly gently, and then poured into small 9 cm Petri dishes. The inoculated plates were held at 4°C for 30 minutes. Replicates of 2 filter paper discs impregnated with each 1080 standard (concentrations ranging from 0.06 to 1.9 mM) and 2 bait extract samples were randomly placed on the agar surface. There were replicates of 3 plates for each different type of diffusion medium. Plates were kept at 4°C for 1 hour and then incubated at 30°C overnight. Inhibition zone clarity was rated on a 0 - 6 scale (0 = no zone, 1 = zone not clear, 2 = double edged zone, 3 = very faint, 4 = faint, 5 = clear, and 6 = very clear).

Addition of tetrazolium chloride. During bacterial respiration tetrazolium chloride incorporated into growth medium is reduced to a tryphenyl formazan form which is a deep red water insoluble pigment. The addition of tetrazolium to the bioassay medium was examined for its potential to enhance the visualisation of growth inhibition zone edges. A preliminary experiment showed that *A. lwoffii* was sensitive to tetrazolium chloride so it could not be incorporated into the diffusion agar. Consequently two bioassay plates (30 x 30 x 1 cm) were prepared using enriched Mueller Hinton agar (MHA) and inoculum grown on DST agar and the bioassay procedure was performed as before (determination of 1080 by the bioassay method, p. 38). Replicates of 3 filter paper discs impregnated with each 1080 standard (concentrations ranging from 0.06 to 0.19 mM) and bait extract samples were applied to the agar surface. After overnight incubation at 30°C one bioassay plate was flooded with a 0.005% tetrazolium chloride solution and the excess drained off. Both plates were re-incubated for 30 minutes. The clarity of the growth inhibition zones were compared visually on the treated and non-treated plates. This procedure was repeated for the 0.01% and 1.0% concentrations of tetrazolium chloride.

Incubation time. Replicates of 2 bioassay plates (30 x 30 x 1 cm) using enriched MHA and inoculum grown on DST agar were set up as described in the tetrazolium chloride experiment. Incubation times for the inoculated plates were 16 and 18 hours. After incubation the diameters of inhibition zones were measured and assessed visually for clarity.

Cooling time after inoculation. This experiment was set up as before but the inoculated plates, after setting, were cooled at 4°C for 15 and 30 minutes instead of 1 hour as used by Wong *et al.* (1995). After overnight incubation the diameters and clarity of inhibition zones were assessed and compared.

Lid cover tightness. The experiment was set up as described before. The lids of the bioassay plates were either completely sealed with tape or taped down at the corners and the middle. Plates were kept at 4°C for 1 hour and then incubated at 30°C overnight. The diameters and clarity of inhibition zones were assessed and compared.

Application of dry and wet discs. Dried and wet 1080 discs were prepared by impregnating sterile blank 6 mm filter paper discs with 1080 standard concentrations ranging from 0.06 to 0.19 mM and bait extract samples. The impregnation of the discs was carried out aseptically by either pipetting 20 µL of solution onto the disc surface or by dipping the discs into the solution. For dry discs, after impregnation as above the discs were dried at 35°C for 2 hours and then stored in sterile Petri dishes at 4°C. Wet discs were prepared by placing sterile blank discs on the surface of the inoculated agar and then pipetting 20 µL of solution directly onto each one or by dipping each disc into the solution prior to placing the disc on the agar surface. Replicates of 6 bioassay plates (30 x 30 x 1 cm) using enriched MHA and inoculum grown on DST agar were set up as before. There were replicates of 3 discs for each 1080 standard, concentrations ranging from 0.06 to 0.09 mM plus 3 bait extract samples per plate. Plates were kept at 4°C for 1 hour and then incubated at 30°C overnight. The zone

width diameters and the clarity of the inhibition zones were assessed and compared.

The Improved Bioassay Procedure

The following improved procedure was used to analyse the 1080 content of about five hundred factory-produced 1080 dried meat baits during the course of this study. Diffusion agar in 250 mL amounts was prepared with vitamin assay casamino acids dehydrated (Difco) 1.5%, and Mueller Hinton agar (Gibco BRL) 3.8%, sterilised at 121°C for 15 minutes and then held in a 50°C waterbath. Inoculum was prepared by emulsifying 18 hour cultures of *A. lwoffii* grown on 3 DST agar plates in 10 mL of sterile distilled water to give approximately 2.4 x 10^9 viable cells mL^{-1}. The inoculum (2.5 mL) was added to 250 mL of molten (50°C) agar and gently mixed and then poured into a large 30 x 30 x 1 cm perspex plate. The freshly poured plate was allowed to cool and set for 10 minutes then it was held at 4°C for 30 minutes. Discs (3 replicates) impregnated with each 1080 standard (4) and bait extract samples (17) were randomly placed on the agar surface. The lid was taped down at each of the corners and 1 piece of tape was applied to the middle of each side as well. The plate was held at 4°C for 60 minutes to allow diffusion of the solutions before being placed, inverted, into a fan-forced incubator at 30°C for 16 hours. Zone widths were measured using Mitutoyo Digimatic calipers and regression analysis was performed to determine 1080 concentrations as described in determination of 1080 by the bioassay method, p. 38.

Between assays the perspex bioassay plates were washed with detergent and water, disinfected with 70% ethyl alcohol spray, wiped dry and then sterilised under ultra-violet light for 60 minutes.

2.2.3 Results

Of the three types of inoculum growth agar medium tested, DST agar gave the best growth (amount and colony size) of *A. lwoffii* after 18 hours incubation at 30°C. Diffusion agar solidified with Mueller Hinton (Gibco BRL) agar (3.8%) showed the best growth inhibition zone clarity among the five agars tested (Table 2.4). Although flooding the bioassay plate with tetrazolium chloride solution did change the colour of bacterial growth from white to deep pink, there was no improvement in the definition of zone edges. Incubation of the inoculated bioassay plate for 16 hours gave slightly better growth inhibition zone clarity and zone edge definition than an incubation time of 18 hours. Cooling times of 15 and 30 minutes at 4°C, after inoculation, showed no difference in the final quality of the growth inhibition zones. However, the success of growth inhibition zone production was affected by the taping of the bioassay plate and lid. Taping down the corners and the middle of the lid did improve the uniformity of the inhibition zone formation across the plate. Application of filter paper discs impregnated with 1080 using either the dried or wet method showed no significant differences in the inhibition zones formed.

Statistical analysis of the improved bioassay procedure over 35 assays gave the following results. Regression analysis derived from the 1080 standards (4 zone-width replicates for each concentration) for multiple values of y; r^2 values for standard curves gave a mean of 0.973 (s.d. = 0.003). The Co-efficients of variation for the 1080 meat extract standards were: 0.118 mM = 6.8% (n = 16), 0.237 mM = 7.4% (n = 23), 0.38 mM = 4% (n = 19), 0.475 mM = 7.3% (n = 21) and 0.95 mM = 4.3% (n = 19).

Table 2.4 Comparison of inhibition zone clarity on 5 types of agar media for samples of 1080 baits and 1080 standard solutions (mM). Rating 0 = no zone, 1 = not clear, 2 = double edge, 3 = very faint, 4 = faint, 5 = clear, 6 = very clear.

Bait Sample	Bacterio-logical	Bitek™	Mueller-Hinton	Sensitivity	Davis
1	2	1	6	5	1
2	2	1	6	5	1
3	2	3	3	3	3
4	3	1	6	3	1
5	1	1	6	4	1
6	3	3	6	4	4
1080 Standards mM					
0.06	0	4	4	2	3
0.19	4	4	5	4	4
0.38	0	4	6	5	4
0.86	4	1	6	4	5
1.9	0	3	6	5	1

2.2.4 Discussion

The results of this study showed that the 1080 bioassay technique developed by Wong *et al.* (1995) could be improved by growing the *A. lwoffii* inoculum on DST agar at 30°C, using Gibco BRL Mueller Hinton - vitamin assay casamino acids agar for the diffusion agar, taping down the bioassay plate lid and incubating inoculated plates at a lower temperature of 30°C. The incubation of inoculated plates in a fan forced incubator ensured an even air flow and temperature distribution, giving a more reliable and consistent inhibition zone clarity. It was also demonstrated that the cooling time following inoculation could be reduced to 30 minutes and that the diffusion time, after 1080 disc placement, could be reduced to 1 hour without affecting the quality of the inhibition zones. Paper discs impregnated with 1080 could

be applied using either pre-prepared dried discs or wet discs.

A continuous supply of *A. lwoffii* for inoculum preparations was obtained by maintaining the viable stock culture in BHIB at 4°C for several weeks. 1080 standard solutions for analysis of 1080 meat baits should be prepared using non-toxic bait extracts and analytical grade 1080. The minimum 1080 standard giving clear measurable zones of inhibition which could be used for the regression analysis was 0.06 mM. Sometimes zones could not be obtained at this low concentration although Wong *et al.* (1995) stated that the lowest detectable 1080 concentration was 0.025 mM. The recommended range of maximum concentration for 1080 standards is 0.75 - 0.9 mM for readable zone production. Diffusion agar should be freshly prepared prior to use as re-melting pre-prepared agar affected the quality of the growth inhibition zones. This was probably due to some deterioration of the vitamin assay casamino acids caused by the re-heating which affected the growth of *A. lwoffii.*

The zone formation was affected by the tightness of the lid cover. The current lid design does not fit over the edge of the plate so taping down the lid corners and the middle was necessary. A lid design which fitted over the edges of the plate would eliminate this step in the procedure. Visualisation of the inhibition zones was enhanced by the use of a light box which illuminated the bioassay plate from below. Accuracy in measuring the zone widths was improved by using digital vernier calipers. Another factor which determined the inhibition zone formation was the quality of the sample, particularly with meat baits and it was found to be important to use purified extracts of samples. The quality of a bait extract sample was improved by subjecting it to a heat treatment at 65°C for 15 - 30 minutes and centrifugation to obtain the supernatant which was then filtered through a 0.45 μm Millipore Millex filter. One advantage of purifying the samples was that after filtration, samples could be kept at 4°C for several months without any deterioration.

To achieve optimum results using the bioassay method, the established procedure should be followed closely as minimal changes in conditions affect the growth rate of *A. lwoffii* and can lead to the failure of a bioassay. The improved bioassay method as developed was shown to be suitable for analysing large numbers of meat bait samples. Twenty six samples could be analysed on one 30 x 30 x 1 cm plate and three to four plates could be set up in a day. On average, 9 to 12 bioassay plates could be prepared during a normal working week, analysing approximately 300 samples. The bioassay method is a relatively quick and inexpensive method for 1080 analysis and it can be used successfully for monitoring quality control of factory-produced baits and for determining 1080 loss from meat baits. A limitation of the technique was that it is not suitable for analysing samples containing much less than 0.06 mM of 1080.

CHAPTER 3

TOXICITY LOSS OF 1080 MEAT BAITS UNDER WESTERN AUSTRALIAN FIELD CONDITIONS

Abstract

1080 factory-produced dried meat baits were placed in the field at four different locations in Western Australia at various times of the year and exposed for varying periods of time. Sites were representative of different conditions encountered at some of the locations where fox baiting is routinely carried out in WA. Baits containing 3.0 or 4.5 mg of 1080 were placed in wire cages to prevent removal by vertebrates. Most baits were placed on the soil surface, but some 3.0 mg baits were buried. Samples of usually 10 baits were collected randomly from all sites on day zero and thereafter at various time intervals. All bait samples were stored frozen at -20°C until analysed for 1080 content using an improved water extraction method and the modified 1080 bioassay.

Results showed that buried baits lost 1080 more rapidly than baits placed on the surface, but in dry soil they remained lethal to foxes for at least 7 days. In damp soil, buried baits lost 1080 rapidly and became non-lethal within 3 - 5 days. 1080 loss from baits placed on the surface at all sites was negligible up to day 9 and by day 16 average 1080 loss ranged from 17 - 32%. There was an overall moderate correlation (R^2 = 0.64, p = 0.05) between rainfall and 1080 loss from baits at the 4 sites. It was necessary for baits to receive at least 50 mm of rain before a 50% loss of 1080 content occurred. Microbial degradation appeared to be important for 1080 loss when

temperatures were moderate, (from 15 to the low 30s °C). Despite 1080 loss, most baits which received about 90 mm of rainfall during 60 to 80 days exposure in the field remained lethal to foxes but posed less of a risk to native animal species.

3.1 Introduction

Loss in the toxicity of various types of 1080 baits, including fresh and dried meat, oat, carrot and cereal based baits, has been reported by many researchers in Australia and New Zealand over the past 39 years (Griffiths, 1959; Corr and Martire, 1971; Wheeler and Oliver, 1978; McIlroy *et al.*, 1986a; Korn and Livanos, 1986; Kramer *et al.*, 1987; McIlroy *et al.*, 1988; Fleming and Parker, 1991). The main causes of 1080 loss are through the leaching effect of rain and moisture (Griffiths, 1959; Corr and Martire, 1971; Wheeler and Oliver, 1978), removal of bait substrate by decomposers such as blowfly larvae and beetles (McIlroy *et al.*, 1988), seepage of inoculated 1080 solution and also through microbial degradation (Fleming and Parker, 1991). The working party on the national standardisation of 1080 dose rates recommended that 3.0 mg baits be used for fox control and 6.0 mg baits be used for wild dog/dingo control. These dose levels were calculated to take into account the variables which may reduce the toxicity of baits such as, 1080 purity, equipment or operator error, leaching of 1080 and unavailable 1080 due to binding, so that baits would remain lethal to target animals (Thompson, 1993).

Most of the field studies on 1080 loss have been conducted using carrot, oat or cereal based baits which are used for the control of rabbits, possums and wallabies (McIlroy, 1981a; Eason *et al.*, 1994a; Twigg, 1994). Although there have been a few studies on 1080 loss from meat baits used for fox control, fresh meat baits were used in the field

studies and they were all conducted in the eastern states of Australia. In Western Australia factory-produced dried meat baits, which have a firm dry surface skin similar to dehydrated beef strips, such as 'biltong' or 'beef jerky', are used for fox and dingo control. This study investigated the loss of toxicity from factory-produced 1080 meat baits during different seasons to determine how long baits remain toxic to the target animal under various climatic conditions in WA. The effect of rainfall on 1080 loss from baits was also examined as was quality control of the factory-produced baits.

3.2 Materials and Method

Field Trial Sites and Time

In WA most fox baiting campaigns use dried meat baits containing 3.0 or 4.5 mg of 1080 produced by the Agriculture Western Australia Bait Production Unit. Approximately five hundred of these baits were laid in the field at 4 different locations at various times when baiting was normally carried out between 1995 and 1997 for different periods of time. The field site locations included Perth, 31° 55' S, 115° 52' E at Forrestfield (an outer south eastern suburb) during spring in October 1996 for a period of 8 weeks and at Woodvale (an outer northern suburb) during spring in September 1995 for a period of 12 weeks; Nangeen Hill, 31° 50' S, 117° 41' E (located 250 km east of Perth near Kellerberrin) during spring in September 1995 for a period of 12 weeks and also in late summer in 1996 for 9 weeks; Callagiddy Station, 25° 03' S, 114° 02' E (just east of Carnarvon) during late winter in August 1995 for an overall period of 78 weeks and also in late summer, 1997 for 10 weeks (this site is referred to as the Carnarvon site in the text and tables). These sites were representative of climatic conditions encountered at locations where baiting is normally carried out. Other factors considered for site selection were easy accessibility

and as posing no hazard to the public. Rainfall was recorded and temperature data was collected over the duration of the trials for all the sites.

Laying of Baits and Sampling Times

Baits were placed inside wire cages to prevent removal by vertebrates at all sites except at Nangeen Hill where termite-proof mesh cages were used which also restricted invertebrate access. At the Nangeen Hill, Woodvale and Forrestfield sites baits were placed directly on the ground surface and at the Carnarvon site baits were placed on the wire floor of the cage. In addition, at the Forrestfield site 60 baits were also buried approximately 8 cm deep and the location of each was marked. Replicates of 10 baits were randomly sampled at different time intervals for up to 56 - 87 days from Woodvale, Forrestfield and Nangeen Hill. For the Carnarvon site during the August 1995 - February 1997 trial, replicates of 10 baits were randomly sampled at day 16, 38 and 131 and then replicates of 20 and 9 baits were sampled at day 265 and 548 respectively. In the second trial at Carnarvon, during February - April 1997, replicates of 6 baits were sampled at day 21 and 67. At the commencement of each field trial 6 (Carnarvon site 1997) or 10 (all other sites) baits were randomly sampled and kept as the controls. The mean 1080 content of these control baits were used as the day zero 1080 content of baits for each of the sites. All bait samples were kept frozen prior to 1080 extraction and analysis. 1080 was extracted using the newly developed water extraction method described in section 2.1. Purified bait extracts were kept at 4°C before being analysed for 1080 concentration using the modified bioassay method described in section 2.2. 1080 analysis was also performed on soil samples (150 g) collected from where some baits were buried at the Forrestfield site.

3.3 Results

Woodvale Site

Sixty 3.0 mg 1080 factory baits were laid at the Woodvale site and a sample of 10 baits was removed on days 0, 9, 21, 37, 55 and 85 from early September - December 1st 1995. Baits showed from 21 - 82% loss in 1080 content and from 6.8 - 43% reduction in average bait weight (Table 3.1). The 1080 content of the day zero baits ranged from 0 - 5.0 mg (Table 3.7) with a mean content of 2.9 mg (± 1.35). Two day zero baits contained less than 1.5 mg of 1080, the lethal dose required to kill foxes. Baits were dry and firm in appearance with a hard skin. During the first 9 days baits received 39.5 mm of rainfall and 1080 loss at day 9 was 21%. Over the following 12 days with a further 3.5 mm of rainfall the rate of 1080 loss decreased and by day 21 loss was 24%. By day 37, after baits had received a further 9.5 mm of rainfall, mean 1080 loss was 55%. Generally baits at the Woodvale site, during September - December, retained amounts of 1080 lethal to foxes (*c.* 1.5 mg) for between 21 - 37 days (Figure 3.1). Levels of 1080 in baits continued to decline and by day 55 with a further 77 mm of rainfall, mean loss had reached 79% (3.8 times that of day 9). Sixty percent of the 10 baits sampled at day 55 had no detectable levels of 1080 but 30% were potentially lethal to foxes, containing from 1.6 - 2.1 mg of 1080. By day 85 the baits were very dry and small (some were hollow) with a mean reduction in weight of 43 g, however 30% of these were still lethal to foxes. No further rainfall was recorded. The temperature during the trial period ranged from 6 - 32.4°C with an average maximum of 24°C and an average minimum of 12.9°C. Correlation between 1080 loss and time of exposure was strong and regression analysis gave an r^2 value of 0.935, p value of 0.0016.

Table 3.1 Mean 1080 content (mg) and percentage 1080 loss in 10 replicate 3.0 mg 1080 factory meat baits sampled over 85 days and cumulative rainfall at the Woodvale site from early September to December 1st 1995.

Day	Mean Weight (g)	Mean mg 1080 (s.d.)	Mean % 1080 Loss	Rainfall (mm)
0	44.0	2.9 (± 1.35)	0	0
9	41.0	2.3 (± 0.61)	21	39.5
21	34.9	2.2 (± 1.26)	24	43.0
37	35.0	1.3 (± 0.87)	55	52.5
55	33.0	0.6 (± 0.79)	79	129.5
85	25.0	0.5 (± 0.75)	82	129.5

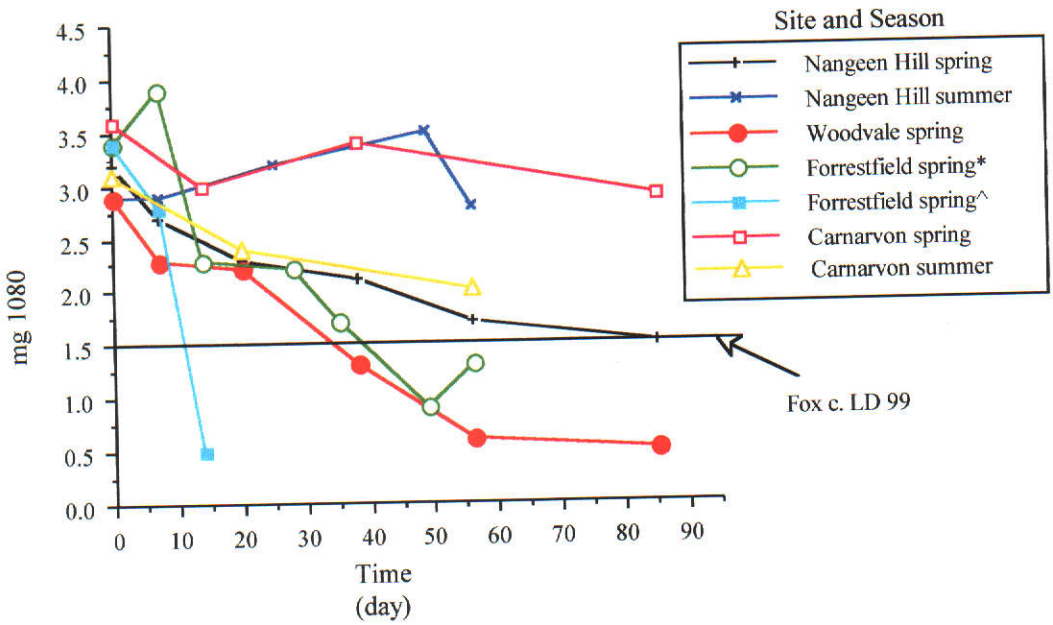

Figure 3.1 1080 loss from 3.0 mg 1080 factory meat baits over 85 days from 4 sites in Western Australia. At the Forrestfield site * = baits placed on the surface and ^ = baits buried 8 cm deep.

Forrestfield Site

1080 loss from 3.0 mg baits sampled from the Forrestfield site over 56 days during early October - December 1996 ranged from 17 - 100% for buried baits and from 0 - 73% for baits on the surface (Table 3.2). The surface baits decreased in weight by 13 - 35% while the buried baits showed an increase in weight of up to 270% for 14 days and then showed a slow decline. The mean 1080 content of the day zero baits ranged from 2.6 - 5.3 (Table 3.7) with a mean of 3.4 mg (± 0.73). In the absence of rain within the first 7 days 1080 loss from buried baits was 17% and 0% from the surface baits. The mean 1080 content of the buried baits was 2.8 mg (± 0.31) and baits had doubled in size (Table 3.2). These baits had no unpleasant odour and no scavenging invertebrates were observed. At day 14 baits had received 49 mm of rainfall and 1080 loss increased to 32% in the surface baits and to 100% in the buried baits. 1080 content in 90% of the day 14 surface baits ranged from 2.0 - 3.3 mg and 10% had no detectable 1080. The buried baits had nearly tripled in weight but they were still odourless and invertebrate free and no 1080 was detected in them. By day 28 there had been a further 11 mm of rainfall but there was no increase in 1080 loss from the surface baits. The buried baits had decreased in weight and were now smelly and colonised by maggots. No 1080 was detected in these baits.

By day 35, and with a further 15 mm of rainfall, 1080 loss from the surface baits had increased to 50%. 1080 was not detected in 2 baits but the mean 1080 content of the 10 baits was 1.7 mg (± 0.9). Of the 10 buried baits sampled at day 35 no 1080 was detected, although 60% of them were still intact pieces of meat with an average weight of 74.8 g. At day 49 the surface baits had lost 73% of their 1080 dose and they had a mean 1080 content of 0.9 mg (± 0.74). Of the 10 baits sampled, no 1080 was detected in 30% of them but 50% contained sufficient 1080 to be lethal to foxes. Most of the buried baits had decomposed at day 49 and only 2 small pieces of meat were

found. These were analysed and no 1080 was detected. No increase in 1080 loss from the surface baits occurred between day 49 and 56, the last sampling day. Of these 10 baits, 80% still contained sufficient 1080 to be lethal to most foxes. Many of these baits were reduced in size due to insect damage, mainly by beetles. No 1080 was detected in soil samples (150 g) collected at day 28, 35, 49 and 56 from locations where baits had been buried. During October - December 1996 the ambient temperatures ranged from 4 - 38.6°C with an average maximum of 25.4°C and an average minimum of 13.5°C. 1080 loss was correlated to time of exposure in the field and regression analysis gave an r^2 value of 0.855, $p = 0.0028$.

Table 3.2 Average weights, 1080 content (mg) and percentage 1080 loss from 10 replicate 3.0 mg factory meat baits, placed on the surface and buried, and sampled over 56 days at the Forrestfield site during spring October to December 1996 with cumulative rainfall.

Time	Mean Weight (g)		Mean (mg) 1080 in Baits		Mean % 1080 Loss		Rainfall
Day	Surface	Buried	Surface (s.d.)	Buried (s.d.)	Surface	Buried	(mm)
0	34.6	34.6	3.4 (± 0.73)	3.4 (± 0.73)	0	0	0
7	30.1	67.4	3.9 (± 1.32)	2.8 (± 0.31)	0	17	0
14	35.1	94.7	2.3 (± 0.84)	0	32	100	49
28	30.7	84.0	2.2 (± 0.41)	0	35	100	60
35	34.6	74.8	1.7 (± 0.90)	0	50	100	75
49	24.0	-	0.9 (± 0.74)	0	73	100	88
56	22.6	-	1.3 (± 0.50)	0	62	100	91

- = baits disintegrated

Nangeen Hill Site

The 3.0 mg baits sampled from the Nangeen Hill site over 87 days during spring in September - December 1995 showed a 15 - 53% loss in 1080 content and a 37.8%

reduction in mean bait weight (Table 3.3). The day zero baits had a mean 1080 content of 3.2 mg (± 0.76) and at day 9, after 5.5 mm of rainfall, mean 1080 loss from baits was 15%. By day 20 loss had increased to 28%, however there had been no additional rainfall. Most (90%) of the baits sampled were lethal to foxes and contained at least 2.0 mg of 1080. Baits had decreased in weight by 32% and were dry and clean in appearance. The baits continued to lose 1080 slowly at an average rate of 0.01 mg per day and by day 38 loss had increased to 34%. A small amount (1.5 mm) of rainfall was recorded. From day 38 - 58, baits received a further 43 mm of rain and 1080 loss increased to 47%. No 1080 was detected in 20% of the day 58 baits however the remaining 80% had sufficient 1080 to be lethal to most foxes (Figure 3.1).

By day 87, the last sampling day, little change had occurred and 80% of the baits sampled were still lethal to foxes. No further rainfall was recorded. During September - December 1995, ambient temperatures ranged from 1.5 - 40.2°C with an average maximum of 26.4°C and an average minimum of 10°C. 1080 loss was strongly correlated to time of exposure and regression analysis gave an r^2 value of 0.943, p value of 0.001.

Table 3.3 Mean weights, 1080 content (mg) and percentage 1080 loss from 10 replicate 3.0 mg factory meat baits sampled over 87 days at the Nangeen Hill site during spring 1995 and cumulative rainfall.

Day	Mean Weight (g)	Mean mg 1080 (s.d.)	Mean % 1080 Loss	Rainfall (mm)
0	44.7	3.2 (± 0.76)	0	0
9	36.6	2.7 (± 0.65)	15	5.5
20	30.3	2.3 (± 0.86)	28	5.5
38	31.8	2.1 (± 0.71)	34	7.0
58	31.5	1.7 (± 1.05)	47	50.0
87	27.8	1.5 (± 0.86)	53	50.0

The 3.0 and 4.5 mg 1080 baits exposed for 60 days in the field during summer from February - April 1996 showed from 0 - 3% and 0 - 14% 1080 loss respectively (Table 3.4). The 3.0 mg day zero baits had a mean 1080 content of 2.9 (± 1.0) with no 1080 detected in 1 bait, and the day 60 baits had a mean content of 2.8 mg 1080 (± 0.47). Results for the 4.5 mg baits were similar although during the 60 days in the field mean 1080 content declined by 0.6 mg, from 4.8 (± 1.17) - 4.2 (± 1.08). No rainfall was recorded until after day 47 when 4 mm fell. These summer 3.0 and 4.5 mg baits darkened in colour and had become very hard, decreasing in weight by 19% (3.0 mg baits) and 28% (4.5 mg baits). All the day 60 baits sampled were still lethal to foxes (Figure 3.1) and 92% of the 4.5 mg baits would have also been lethal to dingoes. During the trial period ambient temperatures ranged from 6.7 - 42°C with an average maximum of 30.5°C and an average minimum of 14°C. 1080 loss was not correlated to time of exposure, $r^2 = 0.055$, $p = 0.703$.

Table 3.4 Mean weights, 1080 content (mg) and percentage 1080 loss from 10 replicate factory meat baits (3.0 & 4.5 mg 1080) sampled over 60 days and cumulative rainfall at the Nangeen Hill site during summer 1996.

		3.0 mg 1080 Baits			4.5 mg 1080 Baits		
Time (Day)	Rain (mm)	Weight (g)	Mean mg 1080 (s.d.)	Mean % 1080 Loss	Weight (g)	Mean mg 1080 (s.d.)	Mean % 1080 Loss
0	0	38.3	2.9 (± 1.0)	0	38.7	4.8 (± 1.17)	0
7	0	31.6	2.9 (± 0.92)	0	30.7	4.9 (± 0.69)	0
25	0	29.6	3.2 (± 0.58)	0	27.9	4.2 (± 0.83)	12
47	0	29.5	3.5 (± 0.63)	0	26.7	4.1 (± 0.88)	14
60	4.0	31.1	2.8 (± 0.47)	3	27.7	4.2 (± 1.08)	12

Carnarvon Site

1080 loss from the 3.0 mg 1080 baits sampled from the Carnarvon site over 548 days from August 1995 - February 1997 ranged from 17 - 92% and the mean reduction in bait weight ranged from 28 - 80% (Table 3.5). The mean 1080 content of the day zero baits ranged from 2.8 - 4.7 mg (mean 3.6 mg ± 0.55) (Table 3.7). There was no rainfall recorded during the first 16 days and 1080 loss was about 17% with a mean 1080 content of 3.0 mg (± 0.46). From days 16 - 38 baits received 8 mm of rain but there was no increase in 1080 loss. Baits were sampled at day 131 in January 1996 and 1080 loss had increased to 47%. These baits had received 75 mm of rainfall within 24 hours on day 111 and 80% of them contained a minimum of 1.7 mg of 1080 and were lethal to foxes. During the following 134 days up to day 265, baits received a further 54 mm of rainfall but there was no increase in 1080 loss (Figure 3.2). By day 548 baits had received a total of 404 mm of rainfall and the mean 1080 content had declined by 92%. Of the 9 baits sampled, no 1080 was detected in 7 baits while 2 baits had 1.0 and 1.7 mg of 1080 respectively (bait weights were 12.7 and 7.5 g). The average maximum and minimum ambient temperatures ranged from 31.8 - 11.7 °C during the period from August 1995 - May 1996. 1080 loss was moderately correlated to time of exposure and regression analysis for 1080 loss over 265 days gave an r^2 value of 0.774, $p = 0.048$.

In the summer February - April 1997 trial at Carnarvon, 1080 loss from the 3.0 mg baits sampled over 67 days ranged from 22 - 35% with a 17 - 27% reduction in average bait weight (Table 3.6). The 1080 content of the day zero baits ranged from 2.2 - 3.6 (mean 3.1 ± 0.47). By day 21 the baits, after receiving 26 mm of rainfall, showed a 22% loss in mean 1080 content. The last samples taken on day 67 had been exposed to a further 53 mm of rainfall and 1080 loss had increased to 35%, but all the baits were still lethal to foxes (Figure 3.1). The average maximum and minimum

ambient temperatures ranged from 34 - 19.2°C during February - April 1997. There was a correlation between 1080 loss and time of exposure over the 67 days with r^2 = 0.907 and p = 0.19.

Table 3.5 Mean 1080 content (mg), percentage 1080 loss and average weights in 10 (9 at day 548) replicate 3.0 mg 1080 factory meat baits sampled over a total of 548 days from August 1995 to February 1997 at the Carnarvon site and cumulative rainfall.

Day	Mean Weight (g)	Mean mg 1080 (s.d.)	Mean % 1080 Loss	Rainfall (mm)
0	43.2	3.6 (± 0.55)	0	0
16	31.1	3.0 (± 0.46)	17	0
38	27.1	3.4 (± 0.55)	6	8.0
131	21.5	1.9 (± 0.58)	47	83.0
265	24.0	1.9 (± 0.68)	47	137.0
548	8.8	0.3 (± 0.61)	92	404.0

Table 3.6 Mean 1080 content (mg), percentage 1080 loss and average weights of 6 replicate 3.0 mg 1080 factory meat baits and cumulative rainfall over 67 days at the Carnarvon site from February to April 1997.

Day	Mean Weight (g)	Mean mg 1080 (s.d.)	Mean % 1080 Loss	Rainfall (mm)
0	32.5	3.1 (± 0.47)	0	0
21	27.0	2.4 (± 0.41)	22	26
67	23.8	2.0 (± 0.44)	35	79

Table 3.7 The mean and range of 1080 content in 3.0 and 4.5 mg (Nangeen Hill* summer 96 only) control baits collected on day zero from all 4 sites.

Site and Season	Range 1080 (mg)	Mean mg 1080 (s.d.)
Woodvale spring 95	0 - 5.0	2.9 (± 1.35)
Forrestfield spring 96	2.6 - 5.3	3.4 (± 0.73)
Nangeen Hill spring 95	1.8 - 4.2	3.2 (± 0.76)
Nangeen Hill summer 96	0 - 3.6	2.9 (± 1.0)
Nangeen Hill* summer 96	1.5 - 5.9*	4.8 *(± 1.17)
Carnarvon spring 95	2.8 - 4.7	3.6 (± 0.55)
Carnarvon summer 97	2.2 - 3.6	3.1 (± 0.47)

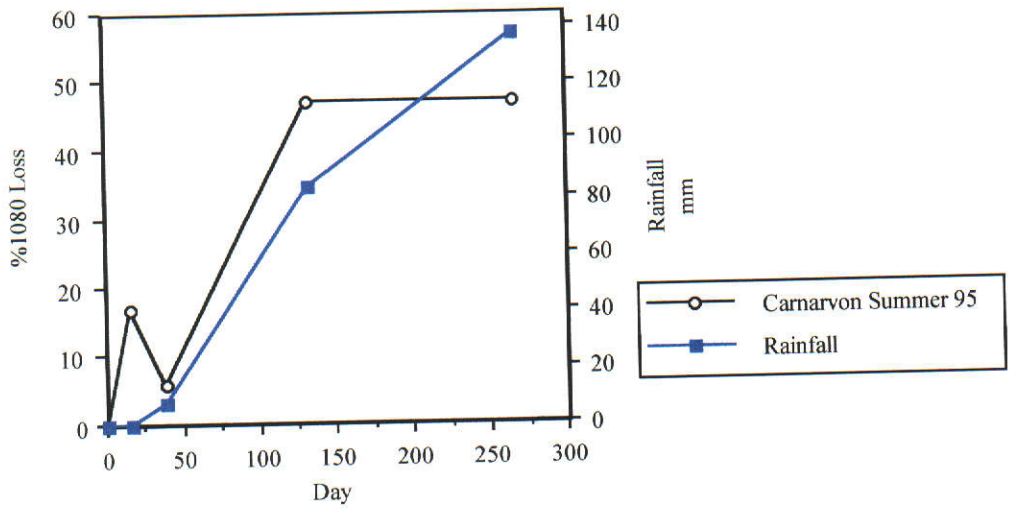

Figure 3.2 Percentage 1080 loss from 3.0 mg 1080 factory meat baits and cumulative rainfall over 265 days at the Carnarvon spring site from August 95 - May 96.

Rainfall

The correlation between rainfall and 1080 loss was analysed by multiple regression using the log of the gradient of 1080 loss over time with rainfall. There was a moderate correlation with $R^2 = 0.635$, SE = 0.006 and $p = 0.057$.

Day Zero Control Baits from All Sites

The 3.0 mg 1080 day zero control baits sampled from the 4 sites had a mean 1080 content of 3.1 mg ± 0.95. The 1080 content of the 55 baits ranged from 0 - 5.3 mg of 1080 (Table 3.7). Ninety-five percent of these baits contained more than 1.5 mg of 1080 and were lethal to foxes. 1080 was not detected in two baits, 1 each from the Woodvale and Nangeen Hill sites. Fifty-seven percent contained from 2.5 - 3.5 mg of 1080 and 30% contained 1080 levels above 3.5 mg. The ten 4.5 mg day zero baits had a mean 1080 content of 4.8 (± 1.17) with a range of 1.5 - 5.9 mg of 1080.

3.4 Discussion

Baits buried 8 cm deep in soil lost 1080 more rapidly than baits placed on the surface under the same conditions. Within 7 days there was a 17% loss in 1080 content from the buried baits whereas baits placed on the surface showed negligible loss. This difference between buried and surface baits was most evident at day 14 when no 1080 was detected in any of the buried baits but the surface baits showed a decline in 1080 content of only 32%. The 17% decline in 1080 content of the buried baits during the initial 7 days was most likely due to microbial degradation with some leaching of 1080 into the soil. These results support the conclusion by King *et al.* (1991) that buried baits would be more rapidly detoxified than baits on the surface.

The sharp increase in 1080 loss from the buried baits from day 7 to day 14, when 49 mm of rainfall was recorded, was most likely due to the physical action of leaching rather than as a result of increased microbial degradation. 1080 loss by microbial degradation would have been secondary to the leaching effect of the damp soil. As the soil became damp baits re-hydrated more quickly by absorbing moisture as was evident from the 270% increase in the average weight of the day 14 buried baits. As baits absorbed water 1080 becomes solubilised and is adsorbed by the surrounding soil profile. In dry soil, from day zero to day 7, the buried baits retained sufficient 1080 to be toxic to foxes, however in damp soil, after rain, 1080 loss was rapid and baits quickly became non-lethal to foxes. Burying baits is an effective way to reduce the poisoning risk to most non-target animals without reducing bait uptake by foxes (Allen *et al.*, 1989). Foxes take most baits during the first few days of them being laid (Marlow *et al.*, 1998) and the results from this study showed that buried baits despite some leaching by moist soil and microbial degradation would remain lethal during that time. No 1080 was detected in the soil samples collected at days 28, 35, 49 and 56 from where baits were buried. Any leached 1080 did not persist for long in the soil but had dispersed or had been degraded to levels below detection by the bioassay. These results support previous conclusions by Peters (1975), Eason *et al.* (1992), Wong *et al.* (1992a), Parfitt *et al.* (1994), Ogilvie *et al.* (1995) and Twigg *et al.* (1996) that 1080 does not persist in soil.

The mean 1080 content of the 55 day zero 3.0 mg baits was acceptable at 3.1 mg although the variation was high at ± 0.95. However, 30% of the baits sampled contained more than 3.5 mg of 1080 and this should be taken into consideration when non-target risk assessments are carried out. Some of the variation may have occurred during the injection of 1080 solution into the baits as seepage can occur at the injection site. When baits come into contact with each other during production and

packaging some of this surface 1080 could be transferred between baits and the toxicity of particular baits would increase. Some variation may have been as a result of variable injection volumes although the delivery of the injection gun is checked and re-calibrated during the injection procedure. Occasionally some baits may be missed or double dosed resulting in high doses or non-toxic baits. Procedures have been changed at the Bait Production Unit since the baits used in these trials were produced, and the recommended practice is to now discard baits if there is doubt about their received dose instead of injecting again.

An important issue concerning 1080 baits is that they must be lethal to foxes and as most baits are taken within the first few days of being laid (Marlow *et al.*, 1998) any baits which are sublethal when laid could result in bait shyness (Hickling, 1994). Based on a fox LD_{99} of *c.* 0.15 mg 1080 kg^{-1} (LD_{50} 0.125 mg 1080 kg^{-1}) (McIlroy and King, 1990) and a weight range for foxes of 4.5 - 8.3 kg (Coman, 1983) a 1080 bait needs to contain at least 1.5 mg of 1080 to be lethal to the largest fox. Of the fifty-five 3.0 mg 1080 baits sampled at day zero 1.8% were sublethal to foxes, containing less than 1.5 mg of 1080.

In contrast with findings from other studies which used fresh meat baits (McIlroy *et al.*, 1988; Fleming and Parker, 1991) 1080 loss from factory-produced dried meat baits placed on the surface at the four sites in this study was negligible up to day 9. This included baits from Nangeen Hill which were exposed to 5.5 mm of rainfall whereas McIlroy *et al.* (1988) found significantly less 1080 in fresh meat baits receiving minimal rain than baits not receiving rain. This shows that the hard dry skin of the factory-produced dried meat bait acts as a barrier to the leaching effects of low rainfall. By day 16 average 1080 loss at all the sites ranged from 17 - 32% and generally, but not consistently, sites which recorded the highest rainfall also showed the greatest decline in 1080 content from baits. Rainfall was a significant factor for

1080 loss, however seasonal differences also had an effect on 1080 loss from baits at some sites. The effects of seasonal differences, probably humidity and temperature, were evident when mean 1080 loss is compared for 2 different seasons at Nangeen Hill. The spring baits at Nangeen Hill received minimal rainfall up to day 38 but 1080 loss was greater from these baits at 34% than from the summer baits which also received minimal rainfall but showed negligible loss (3%).

The higher 1080 loss from the Nangeen Hill baits in spring was most likely due to microbial degradation. During spring, temperature conditions (average maximum and average minimum ranged from 26.4 - 10°C) were more optimal for microbial activity as soil moisture would have been greater than during the hot dry summer conditions where average maximum and minimum temperatures ranged from 30.5 - 14°C. 1080 loss in the Nangeen Hill spring baits increased to 47% when they received 45 mm of rainfall between days 38 and 48, but by day 87 with no further rainfall, there had been no increase in 1080 loss either. These results would indicate that microbial degradation only occurs for a limited time when temperatures are moderate, after which rainfall is the most important factor. After 56 - 87 days in the field during a similar season and when average maximum and minimum temperatures ranged from 25.4 - 13.5°C 1080 loss from the Forrestfield baits in spring was only 9% higher than the maximum loss from the Nangeen Hill baits in spring, although the amount of rainfall at Forrestfield was almost double. This may indicate that microbial degradation was responsible for the 9% greater loss rather than the leaching effect of the rain, as one would expect the increase to be greater if caused by leaching. Results indicate that microbial degradation is an important factor in 1080 loss from meat baits during temperate conditions when temperatures are in the twenty to low thirties degree Centigrade range, with moderate humidity. But in dry, warm to hot conditions such as those at the Carnarvon and Nangeen Hill sites microbial degradation and 1080 loss is minimal even when baits

receive small amounts (4 - 30 mm) of rain.

For baits to lose from 47 - 55% of their original 1080 dose they had to have received from 50 - 137 mm of rainfall and be exposed in the field for periods from 35 - 265 days. Also, baits from the 1997 Carnarvon site showed a mean 1080 loss of only 35% after 67 days exposure and 79 mm of rainfall. These results are similar to those obtained from a study on the affects of artificial rainfall on 1080 loss from meat baits which showed that 1080 loss only approached 50% after baits had received at least 50 mm of rainfall (Kirkpatrick, 1989). After about 60 days in the field the rate of loss declined and 1080 content remained fairly constant even with further rainfall. 1080 microbial degradation ceases or becomes minimal probably because of unsuitable conditions for microbes within the bait such as, low moisture, a change in pH or a decrease in essential nutrients, and microbial activity declines.

A percentage of 1080 resisted further leaching by rain as was evident in the Carnarvon baits where 1080 loss remained constant at 47% for 126 days although baits received a further 54 mm of rainfall. Corr and Martire (1971) found that this also occurred in cereal and oat baits and concluded that it was a result of 1080 adsorption to the bait components. Hilton *et al.* (1969) found that 1080 was adsorbed by cellulosic materials and concluded that 1080 would remain adsorbed to fibrous bait components and would resist being leached by moderate rainfall. After about 80 days in the field further loss in toxicity occurs mainly due to the removal of bait material by invertebrates and the physical breakdown of the meat itself.

Although there was high variation in the 1080 content of baits when first sampled at day zero this variation decreased during the time of the study. 1080 held on or near the surface of baits would be more easily removed by the action of rain and moisture as well as being more prone to microbial degradation. After a short time any variation

in 1080 content would be due to the more strongly held 1080 within the bait and this would most likely vary less between baits. Results which support this were obtained from the summer 4.5 mg baits at the Nangeen Hill site which received only 4 mm of rainfall and the variation in mean 1080 content remained high at around 1 standard deviation over the 60 days of sampling. Variation in the 1080 content of baits from the 1997 Carnarvon site which did receive rain was low initially and remained low throughout 167 days of sampling. This supports the observation that the variation found in the 1080 content of meat baits is mainly due to factors of production.

Most baits from the Forrestfield, Carnarvon and Nangeen Hill sites after 60 to 80 days exposure in the field and after receiving around 80 - 90 mm of rainfall were lethal to foxes (based on baits requiring at least 1.1 - 1.5 mg of 1080) but they would pose less of a risk to any susceptible endemic non-target species in Western Australia.

CHAPTER 4

BIODEGRADATION OF 1080 BY MICROORGANISMS

Abstract

Tissue from various species of *Gastrolobium* plants which naturally contain fluoroacetate (1080), soil samples from beneath these plants and factory waste 1080 solution were investigated for 1080 degrading microorganisms. Thirteen isolates (seven fungi and six bacteria) showed varying degrees of 1080-degrading ability. These isolates were individually tested for their ability to degrade 1080 in non-sterile and sterile factory waste solutions and in McClung carbon-free broth with 200 mM 1080 added as the carbon source. The degradation rates of all thirteen isolates were highest and most rapid in non-sterile 1080 waste solution followed by sterile 1080 waste solution. The most efficient 1080 degraders were two bacterial isolates (10H a *Pseudomonas* sp. and isolate 9H) which gave 100% 1080 degradation at 30°C within 48 hours in sterile 1080 waste solution followed by two fungal isolates (13IF and 1AF) which took about 8.5 days for 100% degradation. Six isolates were tested for affects of temperature on their ability to degrade 1080. The highest rate for most of the isolates was obtained at an incubation temperature of 30°C followed by ambient room temperatures of 15 to 28°C. The ability of three efficient 1080 degrading organisms two *Pseudomonas* isolates, OSK and 10H, and *Fusarium oxysporum*, isolate 1AF were tested in factory waste and McClung broth solutions at 1080 concentrations of 20 and 200 mM. Generally the defluorination rate and time varied with the type of 1080 solution. In 20 mM 1080 factory waste the two *Pseudomonas*

isolates gave 100% degradation within 4 days whereas in 200 mM solutions all three isolates showed lower 1080 degradation rates of 19 to 37% in 15 days.

4.1 Introduction

Soil bacteria capable of utilising 1080 as a carbon source were first isolated by Kelly (1965). He proposed that 1080 degrading bacteria could be used to treat soil and water contaminated by the pesticide. Although other researchers including Tonomura *et al.* (1965), Goldman (1965), Bong *et al.* (1979), Walker and Bong (1981), Myer *et al.* (1990), Wong *et al.* (1991, 1992b) and Walker (1994) have also isolated microorganisms which can degrade 1080 there has been no investigation into their use to treat and detoxify 1080 waste solutions. Populations of soil microflora capable of utilising pesticides as a source of carbon tend to increase in the presence of the pesticide. The rate at which a pesticide is degraded increases over time after an initial lag phase during which populations of microflora utilising the pesticide increase (Brown, 1978). It has been demonstrated that 1080 does not persist in the environment due to the presence of microorganisms which are able to degrade the pesticide completely (David and Gardner, 1966; Rammell and Fleming, 1978; Bong *et al.*, 1979; King *et al.*, 1991; Eason *et al.*, 1992, 1994; Ogilvie *et al.*, 1995; Twigg *et al.*, 1996).

The commercial production of 1080 baits generates large volumes of waste solution contaminated with 1080 and currently the Agriculture WA Bait Production Unit has no satisfactory method of dealing with it apart from long term storage in underground tanks. 1080 waste solutions could be more easily disposed of and handled if they could be rapidly detoxified in a cost effective manner. One possible method would be the use

of efficient 1080 degrading microorganisms to detoxify the solutions.

This study investigated the possibility and the efficiency of microorganisms able to degrade 1080 and occur naturally in the environment to defluorinate or degrade 1080 in factory waste solutions. The investigation involved the isolation of such microorganisms from environmental sources, then tested their efficiency at degrading 1080 and investigated optimum conditions necessary for degradation to occur. The application of the most efficient 1080-degrading microorganisms to detoxify factory 1080 waste solutions was then tested.

4.2 Materials and Method

Isolation of 1080 Degrading Microorganisms

Stem and leaf tissue were collected from 1080 containing plants including *Gastrolobium spinosum* (Prickly poison) and *G. trilobum* (Bullock poison) from the Perth Darling Scarp water catchment area, *G. bilobium* (Heart-leaf poison) from jarrah forest at Mayanup in the southwest of WA and soil and leaf litter from beneath *G. bilobium*. Two methods were used for isolating 1080 degrading microorganisms which were then individually tested for their ability to degrade 1080.

Method 1. 1080 degrading microbes were isolated using the method described by Myer *et al.* (1990). Stem and leaf samples, about 2 cm in diameter, were washed in distilled water, surface sterilised by soaking the pieces in 0.35% sodium hypochlorite (White King) for 15 to 20 minutes and then rinsed in sterile distilled water. The outer skin of the stem was removed with a scalpel and the stem re-treated as before. Several pieces of stem and leaf were placed onto diagnostic sensitivity test agar (DST) and

brain-heart infusion agar (BHIA) in small Petri dishes and incubated at 30°C for 5 days. Any microorganisms which grew were subcultured onto BHIA to obtain pure cultures of each isolate, then they were incubated at 30°C for 3 days.

Method 2. Soil samples, stem and leaf tissue were plated directly onto carbon utilisation agar (Atlas, 1993) with 72 or 30 mM 1080 added as the sole carbon source (see Appendix A). One hundred gram of mixed soil and leaf litter was added to 100 mL of sterile distilled water and shaken on a reciprocal shaker for 30 minutes. Replicates of three 1080 carbon utilising agar plates were either inoculated with 1 mL of the soil suspension or several 2 cm pieces of surface-sterilised stem and leaf tissue were placed on the agar surface. Plates were incubated at 30°C for 5 days. Pure cultures of isolates were obtained as before in method one.

Factory waste 1080 solution (0.5 mL) was also plated out onto carbon utilising agar with 3 mM 1080 added as the carbon source. Plates were incubated at 30°C for 48 hours. One bacterium (OSK) was isolated which was subcultured onto BHIA and then tested for its ability to degrade 1080 in waste solutions at different temperatures and in 20 and 200 mM 1080 solutions.

Ability of Individual Microorganisms to Defluorinate 1080

Eighteen isolates were cultivated from the leaf, stem and soil samples using the two methods above. Individual isolates were screened for their ability to defluorinate 1080 in McClung minimal basal medium (Atlas, 1993), (see Appendix B), hereafter called McClung solution, supplemented with 15 mM 1080 as the sole carbon source. Inocula of bacterial or fungal isolates were prepared by scraping colonies or aerial mycelial growth from 3 day BHIA cultures into 10 mL of sterile distilled water. Replicates of two 30-mL wide-neck McCartney bottles of McClung 1080 solution were inoculated with 3 mL of inoculum and incubated on the shaker at 35°C for 5 days. After

incubation the 1080 concentration of the broth cultures were measured using the 1080 bioassay.

Six bacterial isolates (2D, 8G, 9H, 10H, 11I, 12I) and 7 fungal isolates (1AF, 3DF, 4FF, 5FF, 6FF, 7GF and 13IF) showed defluorinating ability. Their ability to degrade 1080 was further tested in 3 types of solution; non-sterile and sterile factory 1080 waste and 200 mM 1080 McClung solution. Inocula of the 13 isolates was prepared from 48 hour BHIA cultures as before. Two mL of inoculum was added to 150-mL wide necked pyrex bottles of each test solution, the controls had no inoculum added. For each isolate there was 1 bottle of each test solution. The inoculated test solutions were incubated at 30°C and 2 mL samples were aseptically taken at 0, 18, 37, 62 and 109 hours for the non-sterile factory waste solutions; at 0, 26, 48, 96 and 219 hours for the sterile factory waste solutions and at 0, 48, 96, 219 and 502 hours for the 200 mM 1080 McClung solutions. Samples were heated at 80°C for 1 hour to kill the microbe, they were then cooled and kept at -20°C prior to analysis. Isolates showing a high degrading activity were further tested for the influence of temperature on degradation rate.

Effect of Temperature on Microbial Degradation of 1080

Six isolates (1AF, 7GF, 9H, 11I, 13IF and OSK) were tested for their ability to defluorinate 1080 in non-sterile factory waste solution containing 200 mM 1080 at 30, 35°C and fluctuating ambient room temperatures (minimum = 15°C and maximum = 28°C). Inocula were prepared from 24 hour BHIA cultures of each isolate in sterile distilled water as before. For each isolate three 250-mL of 200 mM 1080 factory waste solution, in wide necked pyrex bottles, were each inoculated with 3 mL of inoculum. Control solutions for each of the 3 temperatures had no inoculum added. One bottle of the inoculated 1080 solution for each isolate was incubated at each of

the temperatures. Samples of 2 mL were taken at day 0, 7, 25 and 42 from each culture solution and heat-treated at 80°C in a waterbath for 1 hour. They were then cooled and stored frozen until analysed. Dilutions (1:500) of the samples were made with sterile distilled water for the determination of 1080 concentrations by the modified bioassay method. Fluoride ion levels were also measured in samples using an Orion ion Analyser (EA 940) and Orion fluoride ion and single junction reference electrodes and an Orion automatic temperature compensating probe.

Application of Efficient 1080 Degrading Microbes

Two *Pseudomonas* isolates (OSK and 10H) and one fungal isolate, *Fusarium oxysporum* (1AF) from the previous experiment were tested for their degrading efficiency in McClung solution and factory 1080 waste solution at concentrations of 20 and 200 mM 1080. Three replicates of 150 mL of each test solution in wide necked pyrex bottles were inoculated with 3 mL of 48 hour BHIB culture for OSK and 10H, and 2 cm^2 pieces of 48 hour potato-dextrose agar (PDA) culture for 1AF. Controls for each test solution had no inoculum added. All culture samples were incubated at 30°C in a fan-forced incubator. Samples of 2 mL were taken at days 0, 2, 4, 6, 12 and 16 and stored at -20°C prior to dilution (1:25 and 1:500) with sterile distilled water and 1080 analysis. Fluoride ion levels were also measured as before.

Two soil isolates, *Pseudomonas fluorescens* 1 (S22) and *Comamonas acidovorans* (S13) from Wong *et al.* (1992a) were also tested for 1080 degrading efficiency. Lyophilised cultures of these two isolates were obtained from the DSM (German Collection of Microorganisms and Cell Cultures). Inoculum of S22 was prepared from 24 - 48 hour cultures on BHIA in distilled water as before and tested for their ability to degrade 1080 as a carbon source in the carbon utilisation medium. A 5 mL bacterial suspension (*c.* 1.5 x 10^9 cells mL^{-1}) was added to 95 mL of McClung

solution with 9.5 mg of 1080 added (final 1080 concentration was 95 mM). Controls had no inoculum added. All culture bottles were incubated on a low speed orbital shaker (Gyrotory water bath shaker model G76) at room temperature (minimum = 20°C, maximum = 28°C) for 27 days. Samples of 1 mL were taken at days 0, 2, 12 and 27. The 1080 degrading efficiency of S13 was tested at concentrations of 20 and 200 mM 1080 in enriched mineral-salt medium containing, KH_2PO_4 (2 g L^{-1}), $(NH_4)_2SO_4$ (1 gL^{-1}) and adjusted to pH 6.8 (Bong *et al.* 1979). Two hundred mL of each 1080 test solution, in wide necked pyrex bottles, were inoculated with 5 mL of 48 hour S13 BHIB cultures. Controls had no inoculum added. All culture bottles were incubated at 30°C for 27 days. Samples of 2 mL were taken at days 0, 12 and 27. All samples were heated at 80°C for 1 hour and then kept at -20°C prior to 1080 analysis. Dilutions (1:25 or 1:500) of the samples were made as before for the determination of 1080 concentrations and measurement of F^- levels.

4.3 Results

A total of eighteen microbial isolates were isolated from the leaf, stem and soil samples using the two cultivation methods. Of these isolates thirteen showed varying degrees of 1080 degrading ability in the screening test and all of them were obtained using the carbon utilising medium in method 2. The thirteen isolates consisted of seven fungi (1AF, 3DF, 4F, 5F, 6F, 7GF, and 13IF) and six bacteria (2D, 8G, 9H, 10H, 11I and 12I). Most originated from the plant tissue except for 2D, 6F, 9H and 10H which were isolated from the soil and leaf litter samples. They were differentiated based on colony morphology and some of these isolates might be the same species. Isolate 1AF was identified as *Fusarium oxysporum* and 10H as a *Pseudomonas* species.

When tested individually in three types of solution; non-sterile and sterile factory 1080 waste solution and 200 mM 1080 McClung solution, twelve isolates showed 1080 degrading ability with rates ranging from 5 to 100% (Table 4.1). Rates of degradation by all thirteen isolates were highest and most rapid in non-sterile factory 1080 waste solution followed by sterile factory waste solution. However the non-sterile 1080 waste solution control, which had no inoculum added, also showed a high defluorination rate. A *Pseudomonas* isolate OSK was isolated from this solution on 3 mM 1080 carbon utilisation agar medium. In the non-sterile waste solution, four fungal isolates (3DF, 4FF, 6FF and 7GF) and two bacterial isolates (8G and 9H) were the most efficient degraders with 100% of the 1080 degraded within 37 hours at 30°C.

Two bacterial isolates (10H a *Pseudomonas* sp, and 9H) were the most efficient degraders in the sterile 1080 waste solution with 100% being degraded within 48 hours whereas two fungal isolates, *Fusarium oxysporum* (1AF) and 13IF degraded 100% in 209 hours (*c.* 8.5 days). In McClung solution containing 200 mM of 1080 most isolates showed low rates of defluorination, ranging from 0 to 68% over a period of 3 weeks, except *F. oxysporum* (1AF) and one bacterial isolate (12I) which both gave 100% defluorination.

When tested at three temperatures in 200 mM 1080 factory waste solution, all six isolates (1AF, 7GF, 9H, 11I, 13IF and OSK) showed low rates of degradation, ranging from 0 to 56% (Table 4.2). All the isolates except 7GF (a fungus), were unable to degrade 1080 at 35°C. The rate of 1080 degradation at 30°C and ambient temperatures (minimum = 15°C and maximum = 28°C) varied among isolates and incubation time. Generally by day 42 most isolates showed slightly higher 1080 degradation rates at the fluctuating temperature. Among the six isolates *F. oxysporum* (1AF) gave the highest degradation rate of 56% in 42 days which occurred at 30°C.

Levels of F^- ions increased in solutions over time and percentage increases ranged from 129 - 344% (control solution = 216%) at 37°C; 281 - 876% (control = 274%) at 30°C and 390 - 701% (control = 393%) at fluctuating ambient temperatures.

When tested in factory 1080 waste solution and McClung solution at concentrations of 20 and 200 mM 1080, the 2 *Pseudomonas* species 10H and OSK showed higher rates of degradation than *F. oxysporum* (1AF) (Table 4.3). Both isolates 10H and OSK gave the highest degradation rate of 100% within 4 days in 20 mM 1080 factory waste solution. In the 200 mM 1080 factory waste solution all three isolates showed low degradation rates ranging from 19 - 37% in 16 days. All three isolates showed significantly higher rates of 1080 degradation at day 4, ranging from 10 - 100% in the 20 mM factory waste solution than in the 20 mM 1080 McClung solution (0 - 65%). Rates of 1080 degradation by the three isolates in the 200 mM 1080 concentrated solutions were from 7 - 14% higher at day 16 in McClung solution (range 32 - 44%) than in the factory waste solution (range 19 - 37%). Increases in F^- ion levels were highest in the McCung solutions ranging from 150 - 550 $\mu g\ mL^{-1}$ at 200 mM 1080 concentration (percentage increases of 300 - 900%) and from 20 - 270 $\mu g\ mL^{-1}$ at 20 mM 1080 concentration (percentage increases of 113 - 900%). Increases of F^- in the factory waste solutions ranged from 110 - 310 $\mu g\ mL^{-1}$ at 200 mM 1080 concentration (percentage increases of 122 - 344%) and at the 20 mM 1080 concentration all factory solutions showed a maximum increase of 300 $\mu g\ mL^{-1}$ (percentage increase of 300%).

A known 1080 defluorinating soil bacterium, *C. acidovorans* (S13) from Wong *et al.* (1992) showed 55% 1080 degradation and a 75% (60 $\mu g\ mL^{-1}$) increase in F^- levels after 27 days in 20 mM 1080 enriched mineral-salt medium but no 1080 was degraded at the higher 200 mM concentration (Table 4.4). No 1080 was degraded by the isolate

P. fluorescens (S22) in 95 mM 1080 McClung solution.

Table 4.1 Percentage of 1080 defluorinated by 13 microbial isolates when incubated at 30°C in non-sterile and sterile factory 1080 waste solution and 200 mM 1080 McClung solution at various sampling times.

	Non-sterile 1080 Factory Solution			Sterile 1080 Factory Solution				200 mM McClung Solution			
Time - Hour	37	62	109	26	48	96	219	48	96	219	502
Isolate											
1AF	80	100	100	70	70	80	100	25	25	93	100
2D	83	100	100	0	10	10	10	5	9	9	52
3DF	100	100	100	60	70	70	70	0	5	9	38
4FF	100	100	100	70	80	50	70	0	5	43	50
5FF	83	100	100	70	70	70	70	0	0	0	0
6FF	100	100	100	60	70	70	60	0	5	5	68
7GF	100	100	100	70	70	70	70	0	9	17	48
8G	100	100	100	60	70	70	70	5	14	14	57
9H	100	100	100	90	100	100	100	5	9	9	48
10H	81	100	100	90	100	100	100	5	9	9	62
11I	59	100	100	70	70	70	70	0	0	5	68
12I	83	100	100	70	70	80	80	0	18	31	100
13IF	83	86	100	60	60	80	100	5	25	25	44
Control	40	76	100	0	0	0	0	0	0	0	0

Table 4.2 The effect of temperature on microbial degradation of 200 mM 1080 factory waste solutions over 42 days. The results are expressed as the percentage of 1080 degraded at each temperature from 1 determination.

	Time								
	Day 7			Day 25			Day 42		
°C	35	30	15-28*	35	30	15-28*	35	30	15-28*
Isolate									
1AF	0	5.8	7.3	0	27.8	13.2	0	56.1	43.2
7GF	0	0	5.4	0	0	5.4	8.8	15	17
9H	0	5.3	0	0	5.3	0	0	3	0
11I	0	0	0	0	0	0	0	0	10
13IF	0	0	3.4	0	0	7.8	0	5	7.8
OSK	0	8.8	3.9	0	8.8	10.2	0	12	23
Control	0	0	0	0	0	0	0	0	0

* = fluctuating ambient temperature

Table 4.3 Percentage of 1080 degraded in (1) 200 mM 1080 factory waste solution, (2) 200 mM McClung solution, (3) 20 mM 1080 factory waste solution and (4) 20 mM McClung solution, over 2 to 15 days.

	Isolate											
	10H				OSK				1AF			
Solution	1	2	3	4	1	2	3	4	1	2	3	4
Day												
2	19	8	20	0	9	20	55	45	5	6	5	0
4	30	13	100	10	14	28	100	65	12	9	10	0
6	56	12	100	15	19	32	100	70	15	27	20	0
12	30	35	100	60	22	37	100	80	17	28	50	0
16	37	44	100	-	26	40	-	-	19	32	-	45

- = not tested

Table 4.4 Percentage of 1080 degraded by *C. acidovorans* (S13) in 20 and 200 mM 1080 enriched mineral-salt medium over 4 to 27 days at 30°C.

Solution	20 mM 1080			200 mM 1080		
Day	7	12	27	4	12	25
S13	25	40	55	0	-	0
Control	0	0	0	0	-	0

- = not tested

3.4 Discussion

Several microorganisms capable of degrading 1080 were readily isolated from 1080-containing *Gastrolobium* plant leaf and stem tissues as well as soil and leaf litter from beneath these plants. There were also microorganisms capable of surviving in the factory 1080 waste solution and one *Pseudomonas* species (OSK) was demonstrated to have efficient 1080 degrading ability. These results indicate that microorganisms with the ability to degrade 1080 are prevalent in and on *Gastrolobium* plants as well as in the WA environment and agree with findings by Wong *et al.* (1992a). Similar findings that 1080 degrading microorganisms are common in the environment were reported by other researchers as well, such as Kelly (1965), Bong *et al.* (1979), King *et al.* (1991), Eason *et al.* (1993), Meyer (1994) and Twigg *et al.* (1996). Tonomura (1965) and Walker (1994) found that more 1080 degrading microorganisms were present in soils which had a history of previous exposure to 1080 and results from this study support this and also showed that they can be easily isolated on carbon-utilising medium with 1080 added as the carbon source and using basic microbiological techniques.

The time taken to initiate and complete 1080 degradation varied among the isolates and most showed varying lengths of lag phase which were longest at the 200 mM 1080 concentration. During the lag phase very little or no 1080 was defluorinated and this was probably because time is needed for microorganisms to adapt to alternate metabolic pathways and activate enzymes specific for defluorinating 1080 (Tonomura, 1965). The efficiency of microorganisms to defluorinate 1080 is influenced by 1080 concentration, the availability of alternate sources of carbon and temperature. The optimum maximum temperature for 1080 degradation by most microorganisms was 30°C and a minimum of 15°C. Generally the bacterial isolates could degrade 1080 faster than the fungal isolates, as demonstrated in this study by bacterial isolates 8G and 9H when compared to the fungal isolates 1AF (*F. oxysporum*) and 13IF. This was probably because bacteria grow much faster than fungi and less time is taken by the bacteria to increase in population size. In combination with the pre-existing microbes present in the factory 1080 waste solution, six of the isolates (4 fungi and 2 bacteria) were able to completely defluorinate 20 mM 1080 waste solution within 37 hours at 30°C. However, when acting alone as in the McClung solutions the same two bacteria isolates (8G and 9H) took 11 hours longer to reach 100% 1080 degradation and fungal isolates 1AF and 13IF took about 8.5 days to achieve 100% degradation. The presence of microbes already in the factory waste solution and which were able to degrade low concentrations of 1080 enhanced the degradation of 1080 when temperatures were kept at 30°C. Increasing the temperature of the stored factory waste solutions to around 30°C may be a simple way to improve 1080 degradation by these pre-existing microflora.

The rate of 1080 degradation was faster in 20 mM 1080 factory waste solution than in 20 mM 1080 McClung solution which indicates that microbial 1080 degradation could be enhanced by the presence of other carbon sources. This finding agrees with Wong

et al. (1991). The opposite occurred at the higher 200 mM 1080 concentration when the overall rate of degradation decreased significantly in both factory waste and McCung solutions but it was higher in the McClung solutions where 1080 was the sole carbon source than in the factory waste solutions. This was demonstrated by *F. oxysporum* (1AF) one of the most efficient degraders which at 200 mM 1080 concentration could only degrade 19% of the 1080 in factory waste solution and 32% in McCung solution in 16 days but at 20 mM 1080 concentration 50% of the 1080 in factory waste was degraded in 12 days but in McClung solution only 45% was degraded in 16 days. Reasons for this may have been that the 1080 degrading microbes pre-existing in the factory solution were unable to degrade higher concentrations of 1080 and so no enhancement of 1080 degradation occurred or because the 3 isolates were obligated to defluorinate 1080 in the McClung solutions as no other carbon source was available. The decrease in the overall rate of 1080 degradation at 200 mM concentration could have been caused by the increase in fluorine levels (as shown in this study by as much as 900%) as 1080 was defluorinated and this may have had an inhibitory effect on microbial activity. Similar results were obtained from two of the previously known efficient 1080 defluorinating soil bacteria isolated by Wong *et al.* (1992b), *P. fluorescens* (S22) and *C. acidovorans* (S13). The bacteria were unable to defluorinate solution containing 95 mM and 200 mM 1080 respectively. It is also possible that lyophilization may have affected the 1080 defluorinating ability of these two bacterial species to degrade 1080.

The most efficient 1080 degraders (1AF, 8G, 9H, 10H and OSK) from this study have good application potential to be used to defluorinate and detoxify 1080 waste solution accumulating from 1080 bait production. If the 1080 concentration is high, dilution of the solution may be necessary. Further study is required to determine the inoculum size and the combination of isolates necessary for efficient rapid 1080 defluorination.

The maximum 1080 concentration possible before detrimental effects on the rate of degradation occur should also be investigated. To ensure complete degradation of 1080, random 1080 testing of waste solutions is recommended before they are discharged into the environment or sewerage system. Besides the application to detoxify 1080 solutions, bacterial isolates 8G, 9H, 10K and OSK and fungal isolates *F. oxysporum* (1AF) and 13IF have the potential to be used in 1080 meat baits which require a limited toxic life-span. These microorganisms when inoculated into meat baits may be able to detoxify them under certain environmental conditions, within a particular period of time. This would reduce the risks to non-target animals posed by toxic baits still remaining after 1080 poisoning campaigns.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

The objectives of this project have their origin in the use of 1080 for vertebrate pest control in Western Australia. For many years sodium fluoroacetate has been widely used for the control of vertebrate pests of agriculture such as rabbits, foxes and dingoes. More recently, the use of 1080 for the control of foxes and feral cats in order to protect native wildlife in the CALM Western Shield Program (Bailey, 1996) has led to a marked increase in the use and distribution of 1080 throughout WA. This increased use of 1080 raised issues and questions which required addressing. The first of these being the safe disposal of 1080 contaminated waste, a by-product of the production process. Secondly, problems related to quality control of the bait manufacturing process - how to check and ensure that factory-produced baits contain the prescribed 1080 dose. Thirdly, what factors affected the potency of baits applied during different seasons, given the widespread geographic distribution of baits. This study addressed all of these issues and other matters as well, and in doing so, it provided some answers.

This work would have been difficult to achieve without the prior development of the new 1080 bioassay (Wong *et al.*, 1995), as it provided the technical means for addressing the above issues. Nevertheless, in applying the assay, it became apparent that modifications were needed. This research not only improved the assay, it also led to significant improvements in the methodology used to extract 1080 from bait substrates - a problem that has plagued all studies to date. A more detailed general

discussion follows.

To conduct bait toxicity field trials and monitor quality control of factory-produced 1080 baits a large number of baits need to be analysed. This type of study has previously been hampered due to the available methods of 1080 analysis being too complex and expensive to use, such as HPLC and GLC. The development of the new simple extraction procedure and the modified bioassay method have enhanced the detection of 1080 in meat bait samples and made it possible to carry out such studies.

Results of this study showed that a 98% (± s.d 0.32) recovery rate could be achieved using the newly developed 1080 water extraction method. The method is a beneficial development as it is simple and inexpensive to use requiring the use of only distilled water and equipment such as plastic containers, a variable speed orbital shaker, a water bath, a centrifuge and Millex filter units. Modification of the Wong *et al.* (1995) bioassay method for 1080 analysis was achieved by using Gibco BRL Mueller-Hinton agar as a diffusion agar, growing the *Acinetobacter lwoffii* inoculum on DST agar, shortening the diffusion time by 30 minutes and using an incubation temperature of 30°C. The use of the new extraction procedure with the modified 1080 bioassay method has made processing the large number of meat baits in this project possible without the use of the more expensive and complicated analytical methods. In contrast with these methods an estimated 300 samples can be analysed in a week using the bioassay which requires only basic microbiological skills. The lowest 1080 concentration detectable in this study was 0.06 mM and for accuracy of 1080 determination it is recommended that 1080 standard solutions be made-up using the liquid extract from non-toxic bait material.

Results from the investigation into 1080 loss from factory-produced meat baits at various sites in WA showed that 1080 loss varied with the method of bait laying, and

from site to site, due to different seasons and climatic conditions. However most baits from the Forrestfield, Carnarvon and Nangeen Hill sites remained toxic to foxes for at least 60 - 80 days by continuing to contain at least 1.1 - 1.5 mg of 1080, despite baits having received around 80 - 90 mm of rain. Factors which affected 1080 loss from meat baits evident in this study included rainfall, microbial degradation and invertebrate scavengers. During spring, baits retained a lethal 1080 dose for foxes from between 21 to 37 days at Woodvale, about 35 days at Forrestfield (surface baits) and about 87 days at Nangeen Hill. At the Carnarvon site, baits remained lethal to foxes for from 139 to 265 days during spring through to autumn. During summer at the Nangeen Hill site baits containing 3.0 mg or 4.5 mg of 1080 remained lethal to foxes and dingoes for more than 60 days.

The rate of 1080 loss from baits was affected by rainfall, as demonstrated by the results obtained from all four sites, but 50 to 80 mm was required for baits to lose at least 50% of their initial dose. The results from these field trials provided useful information for a number of regions as the four locations (Perth coastal plain; Woodvale and Forrestfield, Carnarvon; a pastoral region, and Nangeen Hill; a wheatbelt site) were chosen as representative of the climatic conditions of sites in WA where regular fox baiting programs are conducted.

Baits buried in soil lost 1080 more rapidly than baits placed on the soil surface. Under dry conditions buried baits remained toxic to foxes for at least 7 days, but under wet conditions, there was rapid 1080 loss. This method of bait laying is therefore only suitable for sites which have low rainfall or for when the use of baits with a limited toxic life-span is desirable for fox baiting. Under damp conditions baits not found by foxes within the first few days will quickly become less of a hazard to non-target species and in addition any baits cached by foxes beneath soil and leaf litter would also become non-toxic. However more studies are necessary on the toxic-lifespan of buried

baits, particularly during dry conditions. This should also include investigating 1080 loss from egg baits as these baits are also buried.

Loss of 1080 from baits by microbial degradation in summer was probably greater at moderate temperatures of 20 - 30°C, and under damp conditions. In hot, dry environments such as at the Carnarvon site, where summer temperatures are commonly above 30°C and rainfall is low (4 - 10 mm), 1080 meat baits remain toxic for many months. Whether foxes would find these hard dry baits palatable is unknown but foxes do eat a diverse range of food.

Less than 1.5 mg of 1080, the amount required for a lethal fox dose, was found in 3 of the day zero 3.0 mg 1080 factory baits. Two of these baits were non-lethal, not sub-lethal, as they contained no detectable 1080 and therefore would not cause bait shyness in the baited fox population. One bait contained 1.4 mg of 1080 (1.8%) and this shows that factory-produced dried meat baits should not be considered a source of bait shyness in foxes. However 12.7% of the day zero baits contained less than 2.5 mg of 1080 and baits short of the claimed dose (3.0 mg) would be of concern when conditions are optimal for 1080 loss. Baits could quickly become sub-lethal, containing less than 1.5 mg of 1080, and then they would have the potential to induce bait shyness in foxes.

Quality control of factory-produced meat baits should be focused on minimising the variance in the amount of 1080 in baits and this would bring about a lowering of the 1080 dose currently used. An improvement in quality control of meat baits produced by the Bait Production Unit has been in progress since the beginning of this study and a current assessment on the variability of 1080 content in baits would be worthwhile.

Results of this study on the isolation of 1080 degrading microorganisms from

Gastrolobium plants, soil, and 1080 waste solutions support the findings of Bong *et al.* (1979), Wong *et al.* (1992b), Myer (1994), Twigg *et al.* (1996) and other researchers that microorganisms capable of degrading 1080 are prevalent in the environment.

Most microbial isolates were able to degrade 1080 concentrations of up to 20 mM readily. However at the higher 200 mM 1080 concentration the rate of degradation was slow and some isolates showed low or no defluorination activity. The *Fusarium oxysporum* (1AF) isolate and two *Pseudomonas* species (OSK, and 10H) were the most efficient 1080 degraders of both 20 and 200 mM concentrated solutions as well as when 1080 was the sole carbon source. The optimal temperature for 1080 degradation for most isolates was 30°C followed by ambient room temperatures of 15 - 28°C. The most efficient 1080 degrading microorganisms have been shown to be able to degrade 1080 completely in factory waste solution, but the precise inoculum needed for large volumes of solution require further investigation.

These 1080 degrading microorganisms also have the potential to be used for the purpose of producing baits with a limited toxic life-span. Under certain environmental conditions baits containing encapsulated cultures of 1080 degrading microorganisms could become detoxified within a given time period. There is a need for this because during baiting for foxes on farmland, working farm dogs and pets are at risk and have to be tethered or caged until unconsumed baits are collected at the end of the campaign. The use of such a bait would obviate the need to retrieve surplus baits, thus removing the threat to domestic animals and non-target species. However a thorough investigation into the range of conditions necessary for baits to become non-toxic within a given period of time would be essential if recommendations were going to be made concerning the safety of non-target species such as farm dogs.

APPENDIX A

Carbon Utilisation Test Medium

For use in the cultivation and differentiation of Pseudomonas species based on their ability to utilise a specific carbon source.

Components per litre

Ionagar	10 g
NH_4 CL	1 g
$MgSO_4.7H_2O$	0.5 g
Ferric Ammonium citrate	0.05 g
$CaCl_2$	0.5 g
Carbon source	10 mL (1 g/10 mL)

Preparation

Add distilled water to the carbon source, bring volume to 10 mL, mix thoroughly and filter sterilise.

Mix components, except the carbon source, in distilled water and bring the volume to 990 mL. Mix thoroughly, gently heat and bring to boiling. Autoclave for 15 minutes at 121°C. Cool to 45 - 50°C. Aseptically add the carbon source and mix thoroughly. Pour into Petri dishes.

APPENDIX B

McClung Carbon-Free Broth

For use as a basal medium in determining the carbon assimilation capabilities of microorganisms.

Components per litre

$NaNO_3$	2 g
K_2HPO_4	0.8 g
$MgSO_4.7H_2O$	0.5 g
$FeCl_3$	0.01 g
$MnCl_2.4H_2O$	8.0 mg
$ZnSO_4$	2.0 mg

pH 7.2 ± 0.2 @ 25°C.

Preparation

Add components to distilled water and bring volume to 1 L. Mix thoroughly and gently heat without boiling until salts dissolve. Cool to 25°C and adjust pH to 7.2 and then filter sterilise.

REFERENCES

Algar, D., and Kinnear, J. E. (1996). Secondary poisoning of foxes following a routine 1080 rabbit-baiting campaign in the Western Australian wheatbelt. CALMScience. 2(2): 149-52.

Allen, L. R., Fleming, P. J. S., Thompson, J. A., and Strong, K. (1989). Effect of presentation on the attractiveness and palatability to wild dogs and other wildlife of two unpoisoned wild-dog bait types. Australian Wildlife Research. 16: 593-8.

Allender, W. J. (1990). Determination of sodium fluoroacetate (Compound 1080) in biological samples. Journal of Analytical Toxicology. 14: 45-9.

Anon. (1958). Compound 1080. C.S.I.R.O. Leaflet Series No. 22.

Anon. (1977). Use of 1080 poison for agricultural pest control : APDC Agriculture Pests Destruction Council, Wellington.

Anon. (1981). The effect of 1080-poisoning operations on non-target bird populations. What's New in Forest Research, Forest Research Institute, New Zealand. p. 94.

Aplin, T. E. H. (1971). Poison plants of Western Australia: The toxic species of the genera *Gastrolobium* and *Oxylobium*. Western Australian Department of Agriculture Bulletin. 3772: 1-66.

Arena, J. M. (1970). Poisoning-Toxicology-Symptoms-Treatment. Second Edition. Charles C. Thomas Publisher. Springfield, Illinois. p. 715.

Atlas, R. M. (1993). Handbook of Microbiological Media. *ed.* Parks, L. C. CRC Press, USA. pp. 192, 552.

Atzert, S. P, (1971). A review of sodium monofluoroacetate (Compound 1080) its properties, toxicology, and use in predator and rodent control. Special Scientific Report Wildlife. Washington, D. C. No 146.

Backholer, J. R. (1980). Reference information on the toxicity of sodium monofluoroacetate (Compound 1080). Compiled for the Vermin and Noxious Weeds Destruction Board, Department of Crown Lands and Survey, Victoria. p. 34.

Badenhuizen, N. P., and Slinger, J. (1954). Detection of monofluoroacetic acid in Gifblaar, *Dichapetalum cymosum* (Hook.) Engl. :1. South African Journal of Science. 50: 269270.

Bailey, C. (1996). Western shield: bringing wildlife back from the brink of extinction. Landscope. 11(4): 41-8.

Barnett, S. A., and Spenser, M. M. (1949). Sodium fluoroacetate (1080) as a rat poison. The Journal of Hygiene. 4: 426-30.

Batcheler, C. L. (1978). Compound 1080 its properties, effectiveness, dangers, and use. Report to Minister of Forests and Minister of Agriculture and Fisheries, NZ Forest Service, Wellington.

Baynes, A. (1979). Analysis of a late quaternary mammal fauna from Hastings Cave, Jurien, WA. Ph.D. thesis, University of Western Australia.

Bell, A. T., Newton, L. G., Everist, S. L., and Legg, J. (1955). *Acacia georginae* poisoning of cattle and sheep. Australian Veterinary Journal. 31: 249-57.

Bong, C. L., Cole, A. L. J., Walker, J. R. L., and Peters, J. A. (1979). Effect of sodium fluoroacetate ("Compound 1080") on the soil microflora. Soil Biology Biochemistry. 11: 13-18.

Brown, A. W. A. (1978). Ecology of Pesticides, chapter 13 Herbicides and the soil microflora. A Wiley-Interscience Publication, John Wiley and Sons. 390-401

Buffa, P., and Peters, R. A. (1950). The *in vitro* formation of citrate induced by fluoroacetate and its significance. Journal of Physiology. 110: 488-500.

Burns, R. J., and Connolly, G. E. (1995). Toxicity of Compound 1080 livestock protection collars to sheep. Archives Environmental Contamination Toxicology. 28: 141-44.

Calver, M. C., and King, D. R. (1986). Controlling vertebrate pests with fluoroacetate: lessons in wildlife management, bio-ethics, and co-evolution. Journal of Biological Education. 20(4): 257-62.

Calver, M. C., King, D. R., Bradley, J. S., Gardner, J. L., and Martin, G. (1989). An assessment of the potential target specificity of 1080 predator baiting in Western Australia. Australian Wildlife Research. 16: 625-38.

Cameron, J. M. (1977). Poison plants in Western Australia and colonizer problem solving. Journal Royal Society, Western Australia. 59: 71-77.

Christensen, P. E. S. (1980). A sad day for native fauna. Forest Focus. 23: 3-12.

Coleman, J. D. (1993). Occurrence and control of bovine Tb in feral animals. Proceedings of the 23rd sheep and beef cattle seminar. Veterinary Continuing Education Publication No. 50: 59-65.

Collins, D. M., Fawcett, J. P., and Rammell, C. G. (1981). Determination of sodium fluoroacetate (Compound 1080) in poison baits by HPLC. Bulletin of Environmental Contamination & Toxicology. 26: 699-73.

Coman, B. J. (1983). Fox. *In*: Strahan, R. *ed.* The Australian Museum Complete Book of Australian Mammals. Cornstalk Publishing, Collins Angus & Robertson Publishers Australia. 486-7.

Corr, P. V., and Martire, P. (1971). Leaching by rain of sodium fluoroacetate ("1080") from baits used for rabbit control. Australian Journal of Experimental Agriculture and Animal Husbandry. 11: 278-81.

David, W. A. L., and Gardiner, B. O. C. (1966). Persistence of fluoroacetate and fluoroacetamide in soil. Nature. Vol. 5030 (209): 1367-8.

Douglas, G. W., Woodfield, B. R. G., and Tighe, F. G. (1958). 1080 poison a major weapon in rabbit control. The Journal of the Department of Agriculture, Victoria. Vol. 57, part 5.

Dreisback, R. H. (1966). Handbook of Poisoning. Lange, London.

Dummel, R. J., and Kun, E. (1969). Studies with specific enzyme inhibitors. XII. Resolution of DL-*erythro*-fluorocitric acid into optically active isomers. Journal of Biological Chemistry. 244: 2966-69.

Eason, C. T., and Frampton, C. M. (1991). Acute toxicity of sodium fluoroacetate (1080) baits to feral cats. Wildlife Research. 18: 445-9.

Eason, C. T., Batchelor, D., and Wright, G. R. (1991). Environmental impact assessments on 1080 associated with possum control in the Waipoua Forest Sanctuary, Northland. Forest Research Institute Contract Report: FWE 91/8.

Eason, C. T. Wright, G. R., and Fitzgerald, H. (1992). Sodium monofluoroacetate (1080) water-residue analysis after large-scale possum control. Short Communication - New Zealand Journal of Ecology. 16(1): 47-9.

Eason, C. T., Gooneratne, R., Wright, G. R., Pierce, R., and Frampton, C. M. (1993). The fate of sodium monofluoroacetate (1080) in water, mammals, and invertebrates. *In*: Proceedings of the 46th N. Z. Plant Protection Conference. 297-301.

Eason, C. T., Gooneratne, R., and Rammell, C. G. (1994a). A review of the toxicokinetics and toxicodynamics of sodium monofluoroacetate in mammals. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 82-89.

Eason, C. T., Gooneratne, R., Fitzgerald, H., Wright, G. R., and Frampton, C. M. (1994b). Persistence of sodium monofluoroacetate in livestock animals and risk to humans. Human & Experimental Toxicology. 13: 119-22.

Eastland, W. G., and Beasom, S. L. (1986). Effects of ambient temperature on the 1080-LD_{50} of raccoons. Wildlife Bulletin. 14: 234-35.

Egekeze, J. O., and Oehme, F. W. (1979). Inorganic and organic fluoride concentrations in tissues after the oral administration of sodium monofluoroacetate

(Compound 1080) to rats. Toxicology. 15: 43-53.

Fagerstone, K. A., Savarie, P. J., Elias, D,. J., and Schafer Jr. E. W. (1994). Recent regulatory requirements for pesticide registration and the status of Compound 1080 studies conducted to meet EPA requirements. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 33-37.

Fanshier, Z. W., Gottwald, N., and Kun, E. (1964). Studies on specific enzyme inhibitors. VI. Characterization and mechanism of action of the enzyme-inhibitory isomer of monofluorocitrate. Journal of Biological Chemistry. 239: 425-34.

Feldwick, M. G., Mead, R. J., and Kostyniak, P. J. (1994). Biochemical effects of fluoroacetate and related pesticides: the potential of 4-methylpyrazole as an antidote. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 74-81.

Fleming, P. J. S., and Parker, R. W. (1991). Temporal decline of 1080 within meat baits used for control of wild dogs in New South Wales. Wildlife Research. 18: 729-40.

Friend, T. A. (1990). The numbat *Myrmecobius fasciatus* (*Myrmecobiidae*): history of decline and potential for recovery. Proceedings Australian Ecological Society. 16: 369-77.

Frost, R. L., Parker, R. W., and Hanna, J. V. (1989). Detection of the pesticide compound 1080 (sodium monofluoroacetate) using fluorine-19 nuclear magnetic resonance spectroscopy. Analyst. 114: 1245-8.

Gal, E. M., Drewes, P. A., and Taylor, N. F. (1961). Metabolism of fluoroacetic acid-2-C14 in the intact rat. Archives of Biochemistry and Biophysics. 93: 1-14.

Goldman, P. (1965). The enzymatic cleavage of the carbon-fluorine bond in fluoroacetate. The Journal of Biological Chemistry. 240(8): 3434-8.

Griffiths, M. E. (1959). The effect of weathering on the toxicity of baits treated with Sodium Fluoroacetate. C.S.I.R.O. Wildlife Research. 4(1): 93-5.

Hewitt, W., and Vincent, S. (1989). Theory and Application of Microbiology Assay. Academic Press, San Diego.

Hickling, G. J. (1994). Behavioural resistance by vertebrate pests to 1080 toxin: implications for sustainable pest management in New Zealand. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 151-8.

Hilton, H. W., Yuen, Q. H., and Nomura, N. S. (1969). Absorption of monofluoroacetate-2-^{14}C ion and its translocation in sugarcane. Journal of Agriculture Food Chemistry. 17(1): 131-34.

Hopper, S. (1991). Poison peas: deadly protectors. A new classification of *Gastrolobium*, *Oxylobium*, *Callistachys* and *Nemica*. Landscope. 6(4): 45-51.

Jones, E., and Coman, B. J. (1981). Ecology of the feral cat, *Felis catus* (L.), in south-eastern Australia. I. diet. Australian Wildlife Research. 8: 537-47.

Kalmbach. E. R. (1945). "Ten-eighty". A war-produced rodenticide. Science. 102: 232-33.

Kalnitsky, G., and Barron, E. S. G. (1948). The inhibition by fluorocitrate and fluorobutyrate of fatty acid and glucose oxidation produced by kidney homogenates. Arch. Biochem. 19: 75.

Kandel, A., and Chenoweth, M. B. (1952). Metabolic disturbances produced by some fluoro-fatty acids: relation to the pharmacologic activity of these compounds. Journal of Pharmacology and Experimental Therapeutics. 104: 234-47.

Kaye, S. (1970). Handbook of Emergency Toxicology. Third Edition. Charles C. Thomas Publisher. Springfield, Illinois.

Kelly, M. (1965). Isolation of bacteria able to metabolize fluoroacetate or fluoroacetamide. Nature. Vol. 5012 (208): 809-10.

King, D. R. (1989). An assessment of the hazard posed to northern quolls (*Dasyurus hallucatus*) by aerial baiting with 1080 to control dingoes. Australian Wildlife Research. 16: 569-74.

King, D. R. (1990). 1080 and Australian fauna. Agriculture Protection Board, Western Australia. Technical Series. No. 8.

King, D. R., and Kinnear, J. E. (1991). 1080: the toxic paradox. Landscope. 6(4): 15-19.

King, D. R., and Smith, L. A. (1985). The distribution of the European red fox (*Vulpes vulpes*) in Western Australia. Records Western Australian Museum. 12(2): 197-205.

King, D. R., Oliver, A. J., and Mead, R. J. (1978). The adaptation of some Western Australian mammals to food plants containing fluoroacetate. Australian Journal of Zoology. 26: 699-712.

King, D. R., Oliver, A. J., and Mead, R. J. (1981). *Bettongia* and fluoroacetate: a role for 1080 in fauna management. Australian Wildlife Research. 8: 521-36.

King, D. R., Twigg, L. E., and Gardner, J. L. (1989). Tolerance to sodium monofluoroacetate in dasyurids from Western Australia. Australian Wildlife Research. 16: 131-40.

King, D. R., Kirkpatrick, W. E., and McGrath, M. (1996). The tolerance of malleefowl *Leipoa ocellata* to 1080. Emu. 96: 198-202.

King, D., Wong, D., Kirkpatrick, W., and Kinnear, J. (1991). Microorganisms in bait materials and soil which defluorinate 1080. *In*: Proceedings of the Australian Vertebrate Pest Control Conference, Adelaide. Handbook of Working Papers. 282-283.

Kinnear, J. E. (1992). Vexing the vixen. Landscope. 7(4): 92: 18-22.

Kinnear, J. E., Onus, M. L., and Bromilow, R. N. (1988). Fox control and rock-wallaby population dynamics. Australian Wildlife Research. 15: 435-47.

Kirkpatrick, W. E. (1989). Loss of 1080 in fresh meat baits through rainfall. B.Sc. (Biol.) report, Curtin University, Western Australia.

Kirsten, E.; Sharma, M. L. and Kun, E. (1978). Molecular toxicology of (-)-*erythro*-Fluorocitrate: Selective inhibition of citrate transport in mitochondria and the

binding of fluorocitrate to mitochondrial proteins. Molecular Pharmacology. 14: 172-84.

Kok, N. (1997). Using automatic syringes for 1080 baits. Agriculture WA Tech Note. No 2/97 Agdex 674.

Korn, T. J., and Livanos, G. (1986). The effect of dosing technique on the 1080 content of meat baits. Australian Wildlife Research. 13: 455-9.

Kostyniak, P. J., Bosman, H. B., and Smith, F. A. (1978). Defluorination of fluoroacetate in vitro by rat liver subcellular fractions. Toxicology and Applied Pharmacy. 44: 89-97.

Kramer, P. J. (1984). Liquid chromatographic determination of sodium monofluoroacetate (Compound 1080) in baits and formulations. Journal of the Association of Official Analytical Chemists. 16: 1058-61.

Kramer, H. L., Merrell, P. W., and Burren, B. J. (1987). Use of sodium fluoroacetate (Compound 1080) in the control of dingoes I. Meat bait preparation techniques. Australian Wildlife Research. 14: 65-8.

Kun, E. (1982). Monofluoroacetic acid (Compound 1080), its pharmacology and toxicology. *In*: Marsh, R. E. *ed.* Proceedings of the Tenth Vertebrate Pest Conference. University of California, Davis, California. 34-41.

Kun, E., Kirsten, E., and Sharma, M. L. (1978). Catalytic mechanism of citrate transport through the inner mitochondrial membrane: Enzymic synthesis and hydrolysis of glutathione-citric acid thioester. *In*: Azzone, G. F.; Avron, M.; Metcalfe, J. C.; Quagliariello, S.; Siliprandi, N. *eds.* The Proton and Calcium

Pumps. Amsterdam. Elsevier. 285-95.

Leopold, A. S. (1964). Predator and rodent control in the United States. Twenty-ninth North American Wildlife Conference. 27-49.

Livanos, G., and Milham, P. J. (1984). Fluoride ion-selective electrode determination of Sodium Monofluoroacetate in meat baits and formulation. Journal of the Association of Official Analytical Chemists. 67(1): 10-12.

Livingston, P. G., (1994). The use of 1080 in New Zealand. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 1-9.

Marais, J. S. C. (1943). The isolation of the toxic principle "Potassium Cymonate" from "Gifblaar" *Dichapetalum cymosum* (Hook) Engl. Onderstepoort Journal of Veterinary Science and Animal Industry. 18(1 & 2): 203-06.

Marais, J. S. C. (1944). Monofluoroacetic acid, the toxic principle of "Gifblaar" *Dichapetalum cymosum* (Hook) Engl. Onderstepoort Journal of Veterinary Science and Animal Industry. 20: 67-73.

Marlow, N.; Thomson, P., Rose, K., and Kok, N. (1998). Population compensation the red fox: a preliminary analysis. *In*: Proceedings and Programme of the Eleventh Vertebrate Pest Conference. 205-08.

Martin, G. R., Twigg, L. E., and Robinson, D. J. (1996). Comparison of the diet of feral cats from rural and pastoral Western Australia. Wildlife Research. 23: 475-84.

Massam, M. (1998). *ed.* 1080 Operational Procedures Manual Second edition.

Agriculture Western Australia.

Mazzanti, L., Lopez, M., and Berti, M. G. (1965). Atrofia del testiculo produtta dal monofluoroacetato sodico vel ratto albino. Experientia. 21: 446-47.

McEwan, T. (1964a). Isolation and identification of the toxic principle of *Gastrolobium grandiflorum*. Queensland Journal of Agriculture Science. 21: 1-14.

McEwan, T. (1964b). Isolation and identification of the toxic principle of *Gastrolobium grandiflorum*. Nature. Vol. 4921 (201): 827.

McGary, E. D., and Meloan, C. E. (1982). A rapid qualitative method for the detection of monofluoroacetic acid (1080) in liquid baits. Laboratory Information Bulletin. Rodenticides. 2615.

McIlroy, J. C. (1981a). The sensitivity of Australian animals to 1080 poison I. Intraspecific variation and factors affecting acute toxicity. Australian Wildlife Research. 8: 369-83.

McIlroy, J. C. (1981b). The sensitivity of Australian animals to 1080 poison II. Marsupial and Eutherian carnivores. Australian Wildlife Research. 8: 385-99.

McIlroy, J. C. (1982a). The sensitivity of Australian animals to 1080 poison III.* Marsupial and Eutherian herbivores. Australian Wildlife Research. 9: 487-503.

McIlroy, J. C. (1982b). The sensitivity of Australian carnivorous mammals to 1080 poison. *In*: Archer, M. *ed.* Carnivorous Marsupials. Royal Zoological Society. Sydney, Australia. 268-271.

McIlroy, J. C. (1983). The sensitivity of Australian animals to 1080 poison V. The sensitivity of feral pigs, *Sus scrofa*, to 1080, and its implications for poisoning campaigns. Australian Wildlife Research. 10: 139-48.

McIlroy, J. C. (1986). The sensitivity of Australian animals to 1080 poison IX.* Comparisons between major groups of animals, and the potential danger non-target species face from 1080-poisoning campaigns. Australian Wildlife Research. 13: 39-48.

McIlroy, J. C. (1992). The effect of Australian animals of 1080-poisoning campaigns. *In:* Borrecco, J. E.; Marsh R. E. *eds*. Proceedings of the 15th Vertebrate Pest Conference. Published University of California, Davis. 356-359.

McIlroy, J. C., and King, D. R. (1990). Appropriate amounts of 1080 poison in baits to control foxes *Vulpes vulpes*. Australian Wildlife Research. 17: 11-13.

McIlroy, J. C., and Gifford, E. J.(1992). Secondary poisoning hazards associated with 1080-treated carrot-baiting campaigns against rabbits, *Oryctolagus cuniculus*. Australian Wildlife Research. 19: 629-41.

McIlroy, J. C., King, D. R., and Oliver, A. J. (1985). The sensitivity of Australian animals to 1080 poison VIII.* Amphibians and Reptiles. Australian Wildlife Research. 12: 113-18.

McIlroy, J. C., Gifford, E. J., and Cooper, R. J. (1986a). Effects on non-target animal populations of wild dog trail-baiting campaigns with 1080 poison. Australian Wildlife Research. 13: 447-53.

McIlroy, J. C., Cooper, R. J., Gifford, E. J., Green, B. F., and Newgrain, K. W.

(1986b). The effect of wild dogs, *Canis f. familiaris*, of 1080-poisoning campaigns in Kosciusko National Park, N. S. W. Australian Wildlife Research. 13: 535-44.

McIlroy, J. C., Gifford, E. J., and Carpenter, S. M. (1988). The effect of rainfall and blowfly larvae on the toxicity of '1080'-treated meat baits used in poisoning campaigns against wild dogs. Australian Wildlife Research. 15: 473-83.

Mead, R. J., Oliver, A. J., King, D. R., and Hubach, P. H. (1985). The co-evolutionary role of fluoroacetate in plant-animal interactions in Australia. Oikos. 44: 55-60.

Meldrum, G. K., Bignell, J. T., and Rowley, I. (1957). The use of sodium fluoroacetate (Compound 1080) for the control of the rabbit in Tasmania. The Australian Veterinary Journal. August. 186-96.

Meyer, J. J. M. (1994). Fluoroacetate metabolism of *Pseudomonas cepacia*. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 54-7.

Meyer, J. J., and O'Hagan, D. (1992). Conversion of fluoropyruvate to fluoroacetate by *Dichapetalum cymosum*. Phytochemistry. 31: 499-501.

Meyer, J. J. M., Grobbelaar, N., and Steyn, P. L. (1990). Fluoroacetate-metabolizing Pseudomonad isolated from *Dichapetalum cymosum*. Applied and Environmental Microbiology. 56(1): 2152-55.

Misustova, J., Novak, L., and Hosek, B. (1969). Influence of lowered environmental temperature on metabolic and lethal effects of sodium fluoroacetate in mice. Physiology Bohemoslov. 18: 319-24.

Morris, K., Armstrong, R., Orell, P., and Vance, M. (1998). Bouncing back. Landscope. 14 (1): 30-5.

Morris, K., Orell, P., and Brazell, R. (1982). The effect of fox control on native mammals in the jarrah forest, Western Australia. *In*: Marsh, R. E. *ed.* Proceedings of the Tenth Vertebrate Pest Conference. University of California, Davis, California. 177-81.

Morrison, J. F., and Peters, R. A. (1954). Biochemistry of fluoroacetate poisoning. The effect of fluorocitrate in purified aconitase. Biochemical Journal. 58: 473-79.

Notman, P. (1989). A review of invertebrate poisoning by compound 1080. New Zealand Entomologist. 12: 67-71.

Nwude, L. E., Parsons, L. E., and Adaudi, A. O. (1977). Acute toxicity of the leaves and extracts of *Dichapetalum barteri* (Engl.) in mice, rabbits and goats. Toxicology. 7: 23-9.

Oelrichs, P. B. (1981). *Georgina gidyea* poisoning in cattle and sheep. Queensland Agricultural Journal. November - December: 300-02.

Oelrichs, P. B., and McEwan (1962). The toxic principle of *Acacia georginae*. Queensland Journal of Agricultural Science. 19: 1-16.

Ogilvie, S. C., Bowen, L. H., and Eason, C. T. (1995). The effect of the plant *Myriophyllum triphylllum* and temperature on the degradation of sodium monofluoroacetate (1080) in an aquatic ecosystem. *In*: Proceedings of the 48th New Zealand Plant Protection Conference. 260-63.

Morris, K., Armstrong, R., Orell, P., and Vance, M. (1998). Bouncing back. Landscope. 14 (1): 30-5.

Morris, K., Orell, P., and Brazell, R. (1982). The effect of fox control on native mammals in the jarrah forest, Western Australia. *In*: Marsh, R. E. *ed.* Proceedings of the Tenth Vertebrate Pest Conference. University of California, Davis, California. 177-81.

Morrison, J. F., and Peters, R. A. (1954). Biochemistry of fluoroacetate poisoning. The effect of fluorocitrate in purified aconitase. Biochemical Journal. 58: 473-79.

Notman, P. (1989). A review of invertebrate poisoning by compound 1080. New Zealand Entomologist. 12: 67-71.

Nwude, L. E., Parsons, L. E., and Adaudi, A. O. (1977). Acute toxicity of the leaves and extracts of *Dichapetalum barteri* (Engl.) in mice, rabbits and goats. Toxicology. 7: 23-9.

Oelrichs, P. B. (1981). *Georgina gidyea* poisoning in cattle and sheep. Queensland Agricultural Journal. November - December: 300-02.

Oelrichs, P. B., and McEwan (1962). The toxic principle of *Acacia georginae*. Queensland Journal of Agricultural Science. 19: 1-16.

Ogilvie, S. C., Bowen, L. H., and Eason, C. T. (1995). The effect of the plant *Myriophyllum triphylllum* and temperature on the degradation of sodium monofluoroacetate (1080) in an aquatic ecosystem. *In*: Proceedings of the 48th New Zealand Plant Protection Conference. 260-63.

Okuno, I., Meeker, D. L., and Felton, R. R. (1982). Modified gas liquid chromatographic method for determination of Compound 1080 (sodium fluoroacetate). Journal Association of Official Analytical Chemistry. 65(5): 1102-05.

Oliver, A. J., and King, D. R. (1983). The influence of ambient temperatures on the susceptibility of mice, guinea-pigs and possums to Compound 1080. Australian Wildlife Research. 10: 297-301.

Oliver, A. J., King, D. R., and Mead, R. J. (1979). Fluoroacetate tolerance, a genetic marker in some Australian mammals. Australian Journal of Zoology. 27: 363-72.

Osweiler, G. D., Carson, T. L., Buck, T. L., and Van Gelder, G. A. (1985). Fluoroacetate and fluoroacetamide. *In*: Clinical and Diagnostic Veterinary Toxicology. Third edition. Kendall/Hunt Publishing Company, Dubuque, Iowa. 340-4.

Ozawa, H., and Tsukioka, T. (1989). Determination of Sodium Monofluoroacetate in soil and biological samples as the dichoroanilide derivative. Elsevier Science Publishers B. V. 251-59.

Parfitt, R. L., Eason, C. T., Morgan, A. J., Wright, G. R., and Burke, C. M. (1994). The fate of sodium monofluoroacetate (1080) in soil and water. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 159-66.

Parker, R. W., and Frost, R. L. (1991). Free and bound fluoroacetate in 1080 meat baits. *In*: Proceedings of the Australian Vertebrate Pest Conference, Adelaide:

Handbook of Working Papers. 279 -81.

Pattison, F. L. (1959). Toxic aliphatic fluorine compounds. Elsevier Publishing Company, London.

Peters, J. A. (1952a). Lethal synthesis. Proceedings of the Royal Society, London.

Peters, J. A. (1952b). Cronian lecture on the puzzle for therapy in fluoroacetate poisoning. British Medical Journal. 2: 143-170.

Peters, J. A. (1954). Biochemical light upon an ancient poison: a lethal synthesis. Endeavour. 13: 147-54.

Peters, J. A. (1955). Biochemistry of some toxic agents II. Some recent work in the field of fluoroacetate compounds. John Hopkins Medical Journal. 97: 21-41.

Peters, J. A. (1970). Some toxicological aspects of protection forestry - A treatise. Protection Forestry Branch Report No. 93 ODC 156.3, Rangiora.

Peters, J. A. (1975). Contamination of forest ecosystems by sodium fluoroacetate (Compound 1080). Proceedings of the New Zealand Ecological Society. 22: 34-41.

Peters, J. A., and Baxter, K. J. (1974). Analytical determination of Compound 1080 (Sodium Fluoroacetate) residues in biological materials. Bulletin of Environmental Contamination & Toxicology. 11(2): 177183.

Peters, J. A., Wakelin, R. W., Buffa, P., and Thomas, L. C. (1952). Biochemistry of fluoroacetate poisoning. The isolation and some properties of the fluorotricarboxylic acid inhibitor of citrate metabolism. *In*: Proceedings of the Royal

Society London, B. 140: 497-507.

Pickering, W. F. (1985). The mobility of soluble fluoride in soil. Environmental Pollution (Series B). 9: 281-308.

Rammell, C. G., and Fleming, P. A. (1978). Properties and use of sodium monofluoroacetate in New Zealand. Ministry of Agriculture & Fisheries. 49-110.

Ramsey, L. L., and Clifford, P. A. (1949). Determination of monofluoroacetic acid in foods and biological materials. Association of Official Agriculture Chemists. 32(4): 788-97.

Savarie, P. J. (1984). Toxic characteristics of fluorocitrate, the toxic metabolite of compound 1080. *In*: Clark, D. O. *ed.* Proceedings of the Eleventh Vertebrate Pest Conference. University of California, Davis, California. 132-37.

Savarie, P. J., Matschke, G. H., Engeman, R. M., and Fagerstone, K. A. (1994). Susceptibility of prairie dogs to Compound 1080 (sodium monofluoroacetate) baits and secondary poisoning effects in European ferrets under laboratory conditions. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 134-43.

Seawright, A. A. (1994). The environmental toxicology of 1080 - key points revisited. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 166-8.

Shapira, A. R., Taitelman, U., and Bursztein, S. (1980). Evaluation of the role of ionized calcium in sodium fluoroacetate ("1080") poisoning. Toxicology and Applied Pharmacology. 56: 216-220.

Smith, F. A., Gardner, D. E., Yuile, C. L., de Lopez O. H., and Hall, L. L. (1977). Defluorination of fluoroacetate in the rat. Life Sciences. 20: 1131-38.

Spurr, E. B. (1994a). Review of the impacts on non-target species of sodium monofluoroacetate (1080) in baits used for brushtail possum control in New Zealand. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 124-33.

Spurr, E. B. (1994b). Impacts on non-target invertebrate populations of aerial application of sodium monofluoroacetate (1080) for brushtail possum control. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 116-23.

Stewart, H., Jenkin, B., McCarthy, R., and Flinn, D. (1994). Public versus user perceptions of risks and benefits of plantation forestry in Victoria, Australia. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 10-19.

Sullivan, J. L., Smith, F. A., and Garman, R. H. (1979). Effects of fluoroacetate on the testis of the rat. Journal of Reproduction and Fertility. 56: 201-07.

Thompson, J. (1993). Report to the vertebrate pests committee (VPC) on the national standardisation of '1080' dose rates for wild dog and fox baits. Compiled. Working Party on the National Standardisation of 1080 Dose Rates. February: 1-23.

Thomson, P. C. (1986). The effectiveness of aerial baiting for the control of dingoes in north-western Australia. Australian Wildlife Research. 13: 165-76

Tonomura, K., Futai, F., Tanabe, O., and Yamaoka, T. (1965). Defluorination of monofluoroacetate by bacteria. Part 1. Isolation of bacteria and their activity of defluorination. Agriculture Biological Chemistry. 29(2): 124-8.

Twigg, L. E. (1986). The physiological, ecological and evolutionary significance of monofluoroacetic acid in relation to plant animal interaction in Australia. Ph.D. thesis, Murdoch University, Australia.

Twigg, L. E. (1994). Fluoroacetate and Australian plants and animals. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28. 97-113.

Twigg, L. E., and King, D. R. (1989). Tolerance to sodium fluoroacetate in some Australian birds. Australian Wildlife Research. 16: 49-62.

Twigg, L. E., and King, D. R. (1991). The impact of fluoroacetate bearing vegetation on native Australian fauna: a review. Oikos. 61: 412-30.

Twigg, L. E., King D. R., and Bradley, A. J. (1988). The effect of sodium monofluoroacetate on plasma testosterone concentration in *Tiliqua rugosa* (Gray). Comparative Biochemistry Physiology. 91C(2): 343-47.

Twigg, L. E., King, D. R., and Mead, R. J. (1990). Tolerance to fluoroacetate of populations of *Isoodon* spp. and *Macrotis lagotis* and its implications for fauna management. *In*: Seebeck, J. H.; Brown, P. R.; Wallis, R. L.; Kemper, C. M. *eds.* Bandicoots and Bilbies. Surrey Beatty & Sons, Sydney. 185-92.

Twigg, L. E., King, D. R., Davis, H., Saunders, D. and Mead, R. J. (1988). Tolerance to, and metabolism of, fluoroacetate in the emu. Australian Wildlife Research. 15: 239-47.

Twigg, L. E., King D. R., Bowen, L. H., Wright, G. R., and Eason, C. T. (1996). Fluoroacetate content of some species of the toxic Australian plant genus, *Gastrolobium*, and its environmental persistence. Natural Toxins. 4:122-7.

Wade, D. A. (1978). Coyote damage: a survey of its nature and scope, control measures and their application. *In*: Bekoff, M. *ed.* Coyotes: Biology, Behaviour and Management. New York: Academic Press. 347-68.

Walker, J. R. L. (1994). Degradation of sodium monofluoroacetate by soil micro-organisms. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 50-3.

Walker, J. R. L., and Bong, C. L. (1981). Metabolism of fluoroacetate by a soil *Pseudomonas* sp. and *Fusarium solani*. Soil Biology Biochemistry. 13: 23135.

Ward, J. C., and Spencer, D. A. (1947). Notes on the pharmacology of sodium fluoroacetate - Compound 1080. Journal of the American Pharmaceutical Association. 36(2): 59-62.

Wheeler, S. H., and Oliver, A. J. (1978). The effect of rainfall and moisture on the 1080 and pindone content of vacuum-impregnated oats used for control of rabbits, *Oryctolagus cuniculus*. Australian Wildlife Research. 15: 143-9.

Wheeler, S. H. and Hart, D. S. (1979). The toxicity of sodium monofluoroacetate to

wild rabbits, *Oryctolagus cuniculus* (L.), from three sites in Western Australia. Australian Wildlife Research. 6: 57-62.

Williams, J. M. (1994). Food and fibre markets and social trends: implications for pest management. *In*: Seawright, A. A.; Eason, C. T. *eds.* Proceedings of the science workshop on 1080. Royal Society of New Zealand. *Miscellaneous series* 28: 20-32.

Wong, D. H., Kirkpatrick, W. E., Kinnear, J. E., and King, D. R. (1991a). Defluorination of sodium monofluoroacetate (1080) by microorganisms found in bait materials. Wildlife Research. 18: 539-45.

Wong, D. H., Runham, J. E., and DenHollander, L. C. (1991b). A preliminary report on a bacteriological assay for Compound 1080 (Sodium Monofluoroacetate). Letters in Applied Microbiology. 12: 161-3.

Wong, D. H., Kirkpatrick, W. E., King, D. R., and Kinnear, J. E. (1992a). Defluorination of sodium monofluoroacetate (1080) by microorganisms isolated from Western Australian soils. Soil Biology Biochemistry. 24(9): 833-8.

Wong, D. H., Kirkpatrick, W. E., King, D. R., and Kinnear, J. E. (1992b). Environmental factors and microbial inoculum size, and their effect on biodefluorination of sodium monofluoroacetate (1080). Soil Biology Biochemistry. 24(9): 839-43.

Wong, D. H., Kinnear, J. E., and Runham, C. F. (1995). A simple rapid bioassay for Compound 1080 (sodium fluoroacetate) in bait material and soil - its technique and application. Wildlife Research. 22: 561-8.