Appendix C: Ecotoxicity and Environmental Fate of 1080

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Key points

- Hazard classifications (see Table C1).
  - The Agency agrees with the applicants' classifications for 1080 technical grade active.
  - The Agency disagrees with the applicants on several of the classifications of substances containing 1080. These are discussed in detail in the relevant sections below.
  - Where there are differences in classification between the applicants and the Agency, the Agency considers its classifications should be adopted.

Table C1: Agency's and applicants' hazard classifications

Hazard sub-class	Tech. grade 1080	Pellets 0.4–0.8 g/kg 1080	Pellets 1.0 g/kg 1080	Pellets 1.5–2.0 g/kg 1080	Paste 0.6–0.8 g/kg 1080	Paste 1.5 g/kg 1080	Paste 10 g/kg 1080	Gel 1.5 g/kg 1080	Gel 50 g/kg 1080	Gel 100 g/kg 1080	Soluble conc 200 g/L 1080
9.1 Aquatic ecotoxicity											
Agency's classification	9.1A	9.1D	9.1D	9.1D	9.1D	9.1D	9.1D	9.1D	9.1A	9.1A	9.1A
Applicants' classification	9.1A	9.1C	9.1C	9.1C	9.1C	9.1C	9.1C	9.1D	9.1C	9.1A	9.1A
9.2 Soil ecotoxicity											
Agency's classification	9.2B	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.2D	9.2D
Applicants' classification	9.2B	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.2C	Does not trigger	9.2C	9.2C	9.2C
9.3 Terrestrial vertebrate ecotoxicity											
Agency's and applicants' classification	9.3A	9.3B	9.3B	9.3A	9.3B	9.3A	9.3A	9.3A	9.3A	9.3A	9.3A
9.4 Terrrestrial invertebrate ecotoxicity											
Agency's classification	9.4A	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.4A	Does not trigger	9.4A	9.4A	9.4A
Applicants' classification	9.4A	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.4B	Does not trigger	9.4B	9.4B	9.4B

- Data quality, gaps and uncertainty
  - Many of the older data are of poor quality, with insufficient information presented in the literature to allow full evaluation of the results reported. Some of the more recent studies, as presented in the published literature, also lack details which would be expected in a full regulatory report.
  - Data gaps include definitive (that is, in accordance with current international guidelines) studies as shown in Table C2.

Table C2: Comments on data gaps

Study	Mitigating factors
Toxicity to algae	Low concentrations in water from normal operating practices, but could be relevant to spills
Toxicity to additional aquatic invertebrate species given the high toxicity to mosquito larvae indicated in an old screening test	Low concentrations in water from normal operating practices, but could be relevant to spills
Chronic aquatic toxicity data	Rapid dilution in natural water will result in low likelihood of chronic exposure
Biodegradation in aquatic systems and in soils at varying pH, soil type and temperature	Low application rates limiting amount of active in environment, but could be relevant to spills
Adsorption/desorption in a range of soils	Low application rates limiting amount of active available for leaching
Reproductive toxicity to birds	Lack of observed effects in monitoring although monitoring information is limited by factors such as short pre-operational observation period, seasonal fluctuations
Toxicity to honeybees	Low attractiveness of current bait formulations to bees

Study	Mitigating factors
Toxicity to other terrestrial invertebrates	Lack of observed effects in monitoring although monitoring information is limited by factors such as short pre-operational observation period, seasonal fluctuations

- The Agency sought an independent expert review of the degradation of 1080 in water and soil, with the conclusion that the substance meets the criteria in the Hazardous Substances and New Organisms Act 1996 (HSNO Act) for rapid degradation in both soil and water. The expert review is included as Attachment 1 to this appendix. The Agency still has concerns regarding the applicability of the degradation studies using aquatic plants to environments which may receive 1080 baits.
- Collectively, the information available allows for a weight of evidence approach to assessing the ecotoxicity and environmental fate of 1080.
- Loss of 1080 from soil stored at -20°C was identified in a report by Landcare Research on the toxicity of 1080 to earthworms and snails (O'Halloran and Jones 2003). The authors of that report indicated that further work was needed to investigate the reasons for the loss. The Agency sought clarification from Landcare Research via the applicants as to whether any further investigation had been undertaken, and what they considered were the possible implications for other analyses of environmental samples stored under similar conditions.

  Their response highlights the uncertainty around the loss of 1080 from stored samples and suggests that concentrations of 1080 in such samples may have been under-reported by Landcare Research prior to changes in its internal procedures since the issue was identified in 2003. Samples are now stored at -80°C for a maximum of six weeks prior to analysis.

  The accuracy of results from other laboratories may be similarly uncertain, with details on sample collection and storage frequently absent from the published literature.

  Landcare Research's full response to the query is included as Attachment 2 to this appendix.
- Persistence of Wanganui No 7 baits in a simulated stream was assessed and showed leaching of 1080 within 5 h and disintegration of the baits within 84 h. (Suren and Lambert 2004, Suren 2006). Wanganui No 7 baits are considered more persistent than other bait types.
- Persistence of 1080 baits on soil is dependent on rainfall, bait type and size. Baits can remain intact for several weeks.
- No standard bioaccumulation studies have been undertaken with 1080. Studies conducted with New Zealand long-fin eels (Lyver et al 2004, 2005, 2006) and koura/freshwater crayfish (Suren and Bonnett 2004 and 2006) showed that while residues can occur in these species shortly after exposure to 1080, those residues decline significantly over a short period of time (8-9 days) indicating a lack of bioaccumulation.

- 1080 is very toxic to algae, but of low toxicity to fish and aquatic invertebrates, with the exception of one old study indicating high toxicity to mosquito larvae (Deonier et al 1946).
- 1080 residues are persistent in animal carcasses for prolonged periods under winter conditions, an issue which is discussed further in Appendix N in terms of risks of secondary poisoning.
- Very little 1080 is taken up by plants.
- The few soil organism toxicity data available indicate low toxicity.
- 1080 is very toxic to vertebrates. In general, mammals are more sensitive than birds, with an acute toxicity to dogs of 0.06 mg/kg bw. Birds generally show acute LD_{50} values in the range 1-10 mg/kg bw. Reptiles and amphibians appear to be more tolerant than birds and mammals, but time to onset of symptoms is slow and data on New Zealand species, which are taxonomically distinct, is sparse.
- 1080 is highly toxic to terrestrial invertebrates based on acute oral honeybee and ant data.

C1 General

C1.1 Hazard classification

The following hazard classifications are based on a review of the information supplied by the applicants, and a search for other readily available information which may affect the classifications.

In this report, classifications are derived first for technical grade 1080, and then the ecotoxicity classifications of the different substances containing 1080 are derived using standard mixture rules consistent with those of the Globally Harmonised System for Hazard Classification of Chemicals (GHS) (OECD 1998) and set out in the HSNO Act *User Guide to Thresholds and Classifications* (ERMA 2001).

The identity of the non-1080 components of the formulated baits is proprietary to the manufacturers. The Agency has reviewed the information available on the potential hazards of these components and concludes that they are all non-hazardous when assessed against the HSNO Act thresholds for ecotoxicity.

C1.2 Data quality

The applicants provided an extensive list of studies on the ecotoxicity of 1080, mostly from the published scientific literature, supplemented by some recent studies undertaken specifically to address perceived data gaps in the area of soil ecotoxicity. Fewer studies were presented on the environmental fate of 1080 in the aquatic and terrestrial environments. Summary data were also provided from extensive monitoring of the use of 1080 over the past 30–40 years in New Zealand; these are included in Appendices E and F to this report.

Much of the older data are of poor quality, with insufficient information presented in the literature to allow full evaluation of the results reported. Some of the more recent studies, as presented in the published literature, also lack details which could be expected in a full regulatory report.

Wherever possible, the Agency has sourced and reviewed the primary data sources in an attempt to reduce some uncertainties inherent in relying on secondary or tertiary sources.

Collectively, the information available allows for a weight of evidence approach to assessing the ecotoxicity and environmental fate of 1080, despite some of the limitations noted in the relevant summary tables below. Where relevant, the Agency has identified where significant uncertainty has arisen because of the lack of data from high quality studies.

C1.3 Relevant physico-chemical properties of 1080

Physical and chemical properties of 1080 relevant to the interpretation of ecotoxicity tests, environmental fate and exposure assessment are summarised in Table C3.

Table C3: Physical and chemical properties of 1080

Property	1080	Test method (reference)
Water solubility	1110 g/L	Unknown
Log Kow	-0.06	Estimated using CLOGP (Ellington and Stancil 1988)
pH	10.3	Unknown (USEPA 1995)
pKa	2.72 at 25ºC	Unknown (Serjeant and Dempsey 1979)

C2 Sub-class 9.1: Aquatic ecotoxicity, fate and degradation of 1080

Classification under this sub-class requires consideration of the acute and chronic aquatic toxicity and the properties of bioaccumulation and persistence of the substance (or in the case of mixtures, for components of the substance).

C2.1 Aquatic fate and degradation of 1080

No standard guideline studies were submitted by the applicants on the aquatic fate and degradation of 1080 and none were located by the Agency other than on the hydrolysis of 1080 under United States Environmental Protection Agency (USEPA) test conditions.

A summary of the information available is in Table C4.

C2.1.1 Abiotic degradation of 1080

As noted in Table C4, 1080 is stable to hydrolysis in the absence of micro-organisms. There are no data on photolytic degradation of 1080 in water.

C2.1.2 Biodegradation of 1080

In 1994 the Auckland Regional Council commissioned analysis of eight water samples for the presence of micro-organisms capable of degrading 1080 (Lloyd-Jones et al 1994). The source of the water samples was not identified in the report. Two of the eight samples had the capacity to de-fluorinate 1080 (ie, detoxify the molecule, yielding glycollate and fluoride ions (Liu et al 1988)), but only in the presence of an alternative carbon source. No defluorinating activity was found over a period of four weeks in the remaining samples using enrichment techniques, indicating that a microbial population capable of defluorination were absent from those samples. The authors made several comments regarding the results: sampling water without any sediment present is problematic as the greatest proportion of the microbial community will be located in the sediment and in biofilms; the very low concentrations of 1080 which may occur in the environment may be too low to favour microbial degradation or induce the necessary enzyme systems; detection of micro-organisms capable in defluorination in the laboratory does not confirm that degradation will occur *in situ* unless conditions are favourable.

(See section C3.1.1 for further information about biodegradation of 1080.)

C2.1.3 Uptake of 1080 by aquatic plants and influence on degradation

In a study with the aquatic plant *Elodea canadensis*, formation (and degradation) of fluorocitrate from 1080 in the water was observed (Booth et al 1999b). Presumably, micro-organisms present in the test system were able to synthesise and release fluorocitrate into the water column. In the other studies summarised in Table C4, no monitoring of fluorocitrate was reported.

Table C4: Aquatic fate and degradation of 1080 under laboratory conditions

Study type	Test results	Test method (reference)
Abiotic degradation		
Hydrolysis	Stable at pH 7 and 25ºC	USEPA internal method (Ellington et al 1987)
Photolysis	Not submitted	
Biodegradation		
Ready degradability in water	Not submitted	
Water/sediment	Not submitted	

Study type	Test results	Test method (reference)
Aquatic plant uptake/dissipation		
Water + aquatic plants + invertebrates	A brief summary only of the test results was included in the paper, indicating a 70% decline in 1080 in the water after 24 hours in the aquaria containing plants and invertebrates, falling to <LOD of 0.0003 mg/L after 100 hours. In the control aquarium, the concentration of 1080 remained relatively stable. 1080 residues were detected in the plants reaching a maximum of 0.051 mg/kg after one hour, falling to 0.027 mg/kg after 2 hours, and <LOD of 0.002 mg/kg after 330 hours. No information was provided on effects on, or residues in, the aquatic invertebrates in the test aquaria.	Non-standard method; non-GLP. Aquaria (80 litre) containing stream water (source unspecified); aquatic plants (quantity and source not specified) *Myriophyllum triphyllum, Potamogeton cheesemanii, Elodea canadensis,* and *Nitella* spp.; aquatic invertebrates including *Potamopyrgus* spp, *Hydra* spp. and *Trichoptera* spp. (numbers and source not specified). Test concentration 0.1 mg 1080/L; distilled water control. Weight basis for concentrations in plants not specified; assumed to be fresh weight. Plant sample assumed to be inclusive of all species present as this was not stated in the paper. 20ºC otherwise test conditions not specified; no statistical analyses reported. (Eason et al 1993)
Water + aquatic plants + micro-organisms	**Study 1** 1080 below limits of detection in water after 48 hours (LOD 0.0003 mg/L = 0.3 µg/L) Degradation attributed to presence of *Pseudomonas* spp., which has been shown to be able to degrade 1080 by cleaving the C-F bond. **Study 2** 1080 below limits of detection after 141 hours, the longer time to degrade was attributed to the absence of *Pseudomonas* spp. In both studies the fluoride ion concentration was monitored,; 0.043–0.052 mg/L at time zero and reaching a maximum of 0.071–0.75 mg/L, stated as being closely correlated with degradation of 1080 but data not provided. No assessment made of plant uptake of 1080, or any monitoring for fluorocitrate.	Non-standard method; non-GLP. **Study 1** Aquaria (80 litre) containing stream water (source unspecified) and 450 g *Elodea canadensis* an invasive submersed aquatic plant (source unspecified); and four spp. of bacteria; and three spp. of fungi (source and size of inoculum not specified) Test concentration 0.1 mg 1080/L (2 aquaria); distilled water only (control) 21ºC otherwise test conditions not specified **Study 2** Aquaria (80 litre) containing stream water (source unspecified) and 100 g *Elodea canadensis* (source unspecified); and seven spp. of bacteria; and four spp. of fungi (source and size of inoculum not specified). Test concentration 0.1 mg 1080/L (two aquaria); distilled water only (control). 21ºC otherwise test conditions not specified. In both studies, degradation results presented graphically only. (Parfitt et al 1994)

Study type	Test results	Test method (reference)
Water + aquatic plant	1080 concentration data were presented in graphs only making it difficult to determine measured values. Those stated below are approximate only. At 23ºC the concentration of 1080 in water reduced more quickly in the test system containing plant material than stream water alone. Day 1 after treatment (DAT) the concentration was approx. 3.3 µg/L with the plants; 33 µg/L without plants and 126 µg/L in the control. Seventeen 17 DAT stream water without plants declined to 12 µg/L and 75 µg/L in the control. At 7ºC slower declines were observed to those in the warmer test systems. Three DAT stream water with plants the conc. declined to approximately 3.3 µg/L. Seventeen DAT stream water without plants the concentration declined to approximately 27 µg/L and in the control to approximately 80 µg/L Temperature and presence of plant material clearly had an influence of the rate of disappearance of 1080 from the water in the stream water test systems Plant uptake of 1080 peaked at day 1 at both 7ºC and 23ºC (approx, 23 µg/kg) falling below the detection limit of 1.5 µg/kg 3 DAT at 23ºC and 1 3DAT at 7ºC. **Metabolites** The fluoride ion concentration was measured in the test systems containing plant material immediately after addition of 1080 with a mean of 62 µg/L and mean final concentration of 83 µg/L (assumed to be 17 DAT; not stated in the study). Fluoride ion concentration in the control was 1–2 µg/L. On the basis of the increasing fluoride ion concentration with time, the author concluded that defluorination of 1080 by cleavage of the C-F bond by micro-organisms is a key degradation pathway. No analysis was undertaken for potential formation of fluorocitrate. It is possible that fluorocitrate was formed and degraded during the test (see Booth et al 1999, below).	Non-standard test method; non-GLP Aquaria (2 L) containing stream water; stream water plus 60 g plant material (endemic New Zealand *Myriophyllum triphyllum*); or deionised water (sterile control) dosed with 120 µg/L (nominal) and held at either 7 or 23ºC for 17 days 12 hours light: 12 hours dark Type of plant material added was not stated, assumed to be shoots only; source of stream water not stated; pH not stated; monitoring of temperature not reported. Weight-basis of concentrations in plants not reported (ie wet or dry weight); assumed to be wet weight. (Ogilvie et al 1995)

Study type	Test results	Test method (reference)
Water + aquatic plant	25% decline in stream water concentration during the first 24 hours at both temperatures. Between 24 and 48 hours the concentration in the warmer water had declined significantly compared to that at 11ºC. No 1080 could be detected in the warmer water after 141 hours, but approximately 30% of the initial dose was still present in the cooler water. The 1080 concentration in the controls remained relatively stable over the test duration at both temperatures. The amount of fluoride ion measured increased proportionately with the decline in 1080 in the stream water at both test temperatures, indicating biodegradation of the C-F bond (Meyer et al 1990). 1080 reached a maximum in plants after approximately 24 hours at both test temperatures (~0.08 µg/kg at 21ºC and ~0.055 µg/kg at 11ºC) declining faster at 21ºC than at 11ºC. After 191 hours, the concentration was <LOD at 21ºC and only traces remained at 11ºC.	Non-standard method; non-GLP Aquaria (80 litre) containing stream water plus 1.5 kg plant material (*Elodea canadensis*) or deionised water (sterile control) dosed with 0.12 mg 1080/L and held at either 11±0.5ºC or for 21 ±0.5 ºC 10 days; ; pH 6.98. Light regime not stated; two aquaria plus one control at each temperature. Stream water and plant material sourced from the Waimakariri River, Canterbury, New Zealand. Samples taken for 1080 analysis stored at -20ºC; LOD 0.0001 µg/L for water and 0.0015 µg/kg for plant tissue (assumed to be fresh weight as no mention of drying plants). Fluoride ion concentration in water measured with ion-specific electrode. (Ogilve et al 1996)
Water + aquatic plant	Control showed no significant decline in 1080 concentration over time (data not presented). Concentrations of 1080 and fluorocitrate in plants not reported. *Concentrations of 1080 and fluorocitrate in stream water* 1 DAT fluorocitrate was measured in the test aquaria containing 1080: none was present in the control aquaria. Peak fluorocitrate concentrations were reached: 1 DAT ~0.4 mg/L at the lowest test conc. of 1080. 4 DAT ~1.2 mg/L at the middle test concentration of 1080 8 DAT ~1.6 mg/L at the highest test concentration of 1080 At 17 DAT, no 1080 was measured in the 0.12 or 2 mg/L treatments. In the 5 mg/L aquaria, the 1080 concentration was ~0.75 mg/L. No fluorocitrate was measured in any of the control aquaria (limit of detection 10 µg/L)	Non-standard method; non-GLP Aquaria (2 L) containing stream water plus 60 g plant material (endemic New Zealand aquatic plant *Myriophyllum triphyllum*); or deionised water (sterile control) dosed with 0.12, 2 and 5 mg 1080/L and held at 21ºC±2 for 17 days 15 hours light : 9 hours dark; pH not stated. Stream water and plant material sourced from the Waimakariri River, Canterbury, New Zealand; aquaria covered to minimise airborne microbial contamination. Study aim to investigate whether fluorocitrate is formed by microbial action in water. Issues with data manipulation (refer Attachment 1 to this appendix). (Booth et al 1999)

Study type	Test results	Test method (reference)
Water + aquatic plant	**Test 1** Results obtained from two repeats of the test were inconsistent, with time to loss of half ($t_{1/2}$) the 1080 from the water varying markedly after 48 hours exposure (eg, at 0.33g plant/L $t_{1/2}$ was 17.9 hours in one test and 65.6 hours in the other). However, there was a consistent trend for shorter $t_{1/2}$ with increasing quantity of plant material. **Test 2** Little difference between rates of decrease of 1080 under light or dark conditions. Greater loss from test systems at ≥17ºC. **Test 3** The concentration of 1080 in water remained relatively constant for the first 6 hours (5.1 ±0.2 µg/L), declining to 1.2 ±0.3 µg/L after 16 hours and <LOD at 24 hours. In the plant tissue, the concentration reached 2.5 µg/kg after 2 hours and remained relatively stable up to 16 hours (2.2 ±0.3 µg/kg) and declined to <LOD by 24 hours.	Non-standard; non-GLP Single concentration of 1080 tested 5 µg/L; small test flasks (150 mL); deionised water used in all tests (and as controls) to eliminate likelihood of microbial contamination but plants not described as being from axenic culture; plant material chopped into 10 mm pieces. Monitoring of test conditions not reported (ie, temperature, light, pH). No analyses presented for control samples. LOD 0.1 µg/L; <2 µg/kg plant tissue – stated as less then two due to absence of impurities and extraneous organic matter but not how much less. Source of plant material (*Elodea canadensis*) not stated. **Test 1** Assessment of quantity of aquatic plant (0.33–3.3 g/L) on 1080 uptake from deionised water for 48 hours at 16ºC continuous light (intensity not stated) **Test 2** Assessment of temperature (approx. 5, 11, 17, and 23ºC) on uptake of 1080 from water after 15 hours in the dark and then test repeated under artificial light; 3.3 g plant/L. **Test 3** Plant uptake of 1080 from water over a 72-hour period under lights at 15ºC; 0.4 g plant/L. (Wright et al 2003)

C2.2 Additional information on the aquatic fate of 1080

C2.2.1 Fate of baits in water

(See Appendix E for degradation of baits in the field under operational conditions.)

The information available on the disintegration and weathering of cereal baits has been almost invariably reported for one of two bait types; either Wanganui No 7 or RS5; with the former being more moisture resistant and taking longer to breakdown than the latter. The manufacturer of cereal bait pellets has advised that these names are largely historical and with one exception, are no longer manufactured. As such, the information presented below can only be considered as indicative for the current cereal bait formulations.

In the studies summarised in Table C5, leaching from other bait types (including RS5 cereal baits) was not assessed as Wanganui No 7 baits are considered to be more water resistant based on results from testing under simulated rainfall (see Table C5) and therefore provide a more conservative indication of likely disintegration/loss of 1080. The studies

do not assess degradation of 1080 in the baits or which has been leached from into the water.

Table C5: Disintegration and leaching of cereal baits in water under laboratory conditions

Bait type and size	Test results	Test system (Reference)
0.15% 1080 'DOC' (no further information on type) Mean 11.1 g 0.15% 1080 Wanganui No 7 cereal Mean 6.4 g	**Test 1: Bait disintegration** Significant decrease in mass of bait over time, with similar pattern for both bait types. Greatest weight loss occurred in the first 72 hours. Baits remained relatively intact for the first 48 hours and slowly lost their green colouring. After 72 hours, baits were swollen and started to fragment. After 84 hours, baits had lost integrity and disintegrated. **Test 2** Wanganui No 7 baits had lost 50% 1080 after 5 hours. At 24 hours, baits contained only 0.019% 1080 (<90% of the original). No 1080 was detected after 36 hours in any baits.	Flume tank, with cobbles buried in the channel to 7 cm; water velocity 20 cm/s; water temp 11 ±2ºC. **Test 1** Three baits removed for sampling at 8 and 24 hours; 6 baits at 48, 72 and 84 hours. Baits dried (60ºC) and weighed to assess weight loss as % of original. **Test 2** Leaching of 1080 from Wanganui No 7 baits. Six baits removed at 2 and 4 hours to assess short-term loss and four baits at 6, 12, 24 and 36 hours, frozen at -18ºC for later analysis. LOD 0.5 mg/kg (Suren and Lambert 2004; Suren 2006)

C2.3 Assessment of aquatic degradation data against HSNO Act criteria

In many publications 1080 is referred to as being 'rapidly degradable' but no criteria are provided as to how such an assessment has been made. The term 'rapidly degradable' is defined in clause 1 of Schedule 6 of the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 as:

> **rapidly degradable**, in relation to a substance in water, means that—
>
> (a) 28 days after a solution containing the substance is inoculated with micro-organisms, there is at least—
>
> (i) a 70% reduction in dissolved organic carbon in the solution; or
>
> (ii) a 60% depletion of oxygen in the solution, when compared with the maximum depletion of oxygen that would occur if the substance were completely degraded; or
>
> (iii) a 60% generation of carbon dioxide in the solution, when compared with the maximum generation of carbon dioxide that would occur if the substance were completely degraded; or

(b) if only COD and BOD5 data is available, the ratio of BOD5 to COD is greater than or equal to 0.5:1; or

(c) at least 70% of the substance can be degraded biotically or abiotically, in the aquatic environment within 28 days

The lack of data from a standard test method (eg, OECD guidelines 301A-F, 308 and 309 (OECD 1992, 2002a and 2004)) makes the assessment of the rate of degradation more complex in the context of the HSNO Act criteria for a rapidly degradable substance. Test results from OECD guideline 301A–F align with paragraph (a). Results from OECD guidelines 308 and 309 can be used directly for assessment against paragraph (c).

The OECD (1998) guidance on assessment of biodegradation data notes that in the absence of data from a 301 guideline, in general, only data from an aquatic simulation study (such as OECD guideline 308 or 309) is considered directly applicable. A half-life of <16 days under such test conditions is considered to meet the criteria set out in paragraph (c).

The Agency sought an independent expert opinion on the degradation of 1080 in water (see Attachment 1 to this appendix). The expert review concluded that 1080 is rapidly degradable in water according the HSNO Act criteria, with the main route of degradation being microbial defluorination to glycolate and fluoride ions. The half-lives of 1080 in laboratory studies were in the range <<1 to 8 days.

Overall, the relevance of the aquatic plant/water studies to the degradation of 1080 in water in New Zealand is not clear. Streams which may receive 1080 baits during aerial applications are likely to be first order or small second order streams. These streams are likely to be heavily shaded by surrounding vegetation and not support significant aquatic macrophyte growth.

C2.4 Bioaccumulation

No standard bioaccumulation studies have been undertaken with 1080. Studies conducted with New Zealand long-fin eels (Lyver et al 2004, 2005, 2006) and koura/freshwater crayfish (Suren and Bonnett 2004 and 2006) showed that while residues can occur in these species shortly after exposure to 1080, those residues decline significantly over a short period of time (8–9 days), indicating a lack of bioaccumulation (see section C2.5.1 for further details of these studies).

Given the very high water solubility, and estimated log Kow of -0.06, **the Agency considers that 1080 does not meet the HSNO Act criteria for a bioaccumulative substance** (log Kow ≥4 or bioconcentration factor of ≥500) (ie, is not bioaccumulative).

C2.5 Toxicity of 1080 to aquatic organisms

The aquatic toxicity data for 1080 are summarised in Table C6. The available set of high quality acute data is small. No chronic studies have been undertaken on aquatic organisms.

The applicants did not provide, and the Agency was not able to locate, any data on the aquatic toxicity of the metabolite fluorocitrate in water or soil.

On the basis of the information available, some algae are more significantly more sensitive to 1080 than fish or invertebrates. The small data set does not provide much indication of the sensitivities of different aquatic species, in particular, the screening result for mosquito larvae suggests that some species and/or life stages may be much more sensitive to 1080, but this cannot be confirmed.

The Agency notes that there is significant uncertainty regarding the aquatic classification of 1080 due to the quality of the data available. None of the aquatic plant studies has been conducted to current international standards. The level of uncertainty could be reduced if high quality data were available on the toxicity of 1080 to algae (eg, OECD test guideline 201; OECD 2006).

In the absence of high quality data, the Agency has classified 1080 as **9.1A highly toxic to the aquatic environment** due to the toxicity to aquatic plants at <1 mg/L.

Table C6: Summary of aquatic toxicity laboratory data for 1080

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Fish			
Rainbow trout, *Onchorynchus mykiss*	96-hour static	LC_{50} 54 mg/L (95% CI 39–74 mg/L) 10% mortality at 23 mg/L LOEC 23 mg/L sub-lethal effects on survivors—not specified NOEC 13 mg/L	Stated as conducted to USEPA Guideline 72-1; no indication whether GLP compliant or not. The applicants cited Fagerstone et al 1994 (a conference paper) as the reference for this study. The study is also summarised in USEPA 1995, which cites the original study as Collins 1993a. The USEPA document provided less detail than Fagerstone et al, only stating the LC_{50}. Without access to the full text of the original study, the Agency is not able to fully verify the summary information available. (Collins, 1993a, cited in Fagerstone et al 1994 and in USEPA 1995)
Rainbow trout	24 hours	No 'ill effects' at 580 mg/L	No further information available. (Bentley et al, 1958 cited in Batcheler 1978)
Bluegill sunfish, *Lepomis macrochirus*	96-hour static renewal	LC_{50} >970 mg/L NOEC 970 mg/L (highest test concentration	Stated as conducted to USEPA Guideline 72-1; no indication whether GLP compliant or not. Comments as for Collins 1993a (above) (Collins, 1993b, cited in Fagerstone et al 1994 and in USEPA 1995)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Fingerling bream and bass (species not identified)	Unknown	No signs of toxicity at 370 mg/L	No further information available. (King and Penfound, 1946)
Fingerling trout (species not identified)	Unknown	No 'ill effects' at 500 and 1000 mg/L	No further information available (unpublished New Zealand Wildlife Service report cited in Batcheler 1978)
Adult trout (stated as brown and rainbow)	Force-fed, duration not specified	Force-feeding of one or two bait pellets at 0.08% 1080 (equivalent to approx. 5 mg/kg bw) No effects reported	No further information available (unpublished New Zealand Wildlife Service report cited in Batcheler 1978)
Invertebrates			
Daphnia magna.	48-hour static	EC_{50} 350 mg/L LOEC 220 mg/L (5% immobilisation) NOEC 130 mg/L	Stated as conducted to USEPA Guideline 72-2; no indication whether GLP compliant or not Comments as for Collins 1993a (above) (Collins, 1993c, cited in Fagerstone et al 1994 and in USEPA 1995)
		EC_{50} 100 mg/L NOEC 10 mg/L (24hr EC_{50} 170 mg/L 72hr EC_{50} 50 mg/L)	OECD 202; GLP status unknown (Zurita et al 2007)
Mosquito larva – fourth instar, *Anopheles quadrimaculatus*	48 hour static	15% mortality at 0.025 mg/L 40% mortality at 0.05 mg/L 65% mortality at 0.1 mg/L	Screening test only—test protocol not fully specified; no controls; test concentrations 0.025, 0.05 and 0.1 mg/L. (Deonier et al 1946).
Aquatic plants			
Green alga, *Pseudokirchneriella subcapitata* (syn. *Selenastrum capricornutum*)	72 hour	E_rC_{50} 12–120 µg/L	Minimal information on test protocol used. Test concentrations 0.12, 1.2, 12 and 120 µg/L. Test stated as not intended to provide definitive NOEC or EC_{50} Two replicates per test concentration (Berends, 1994; Berends et al, 1994; Groeneveld and Berends, 1994—all cited in Berends et al 1999; Boutonnet et al 1999)
Green alga, *Scenedesmus subspicatus*	72 hour	E_rC_{50} 12–120 µg/L	
Green alga, *Chlorella vulgaris*	72 hour	E_rC_{50} >120 mg/L	Minimal information on test protocol used. Test concentrations 0.12, 1.2, 12 and 120 mg/L. Test stated as not intended to provide definitive NOEC or EC_{50}. Test concentrations and test duration only. Single vessel per test concentration (Berends, 1994; Berends et al, 1994; Groeneveld and Berends, 1994—all cited in Bernends et al 1999; Boutonnet et al 1999)
		E_rC_{50} 60 mg/L NOEC 100 mg/L (24hr E_rC_{50} 1200 mg/L; 48hr E_rC_{50} 120 mg/L)	Microplate assay—GLP status unknown 96-well culture plates with 200 µL per well of $1x10^6$ cells/mL; algal culture in exponential growth phase. (Zurita et al 2007)
Blue-green algae, *Microcystis aeruginosa*	Unknown	toxicity threshold 0.4 µg/L	No information on test protocol used (Bringmann and Kühn, 1976 cited in Berends et al 1999)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Duckweed, *Spirodelia oligorrhiza*	?18 days (read from graph)	73% reduction in frond growth rate at 0.5 mg/L	Non-standard test species but common in New Zealand. Test concentrations 5 and 50 µM (= 0.5 and 5 mg/L respectively) Test protocol poorly documented, initial number of fronds not specified, no analytical monitoring of test substance, pH or temperature, test duration not clearly specified; light intensity not reported. Only parameter reported was frond number. (Bong et al 1979)
Duckweed, *Spirodelia oligorrhiza*	9 days (read from graph)	Complete growth inhibition at 50 mg/L	Test protocol not fully documented. Test concentrations read from graph 1 µM, 5 µM, 10 µM, 50 µM, 100 µM, 0.5 mM, 1 mM (= 0.1, 0.5, 1.0, 5.0, 10.0, 50 and 100 mg/L respectively). *Spirodelia* spp non-standard. Initial no. of fronds per test vessel 6 (9–12 current standard guideline OECD 221); no. of replicates not specified temperature 20ºC (std 24±2 20ºC); PAR higher than std; no analytical monitoring of test substance, pH or temperature, test duration not clearly specified (Bong et al 1980)
Duckweed, *Spirodelia polyrrhiza*		Complete growth inhibition at 100 mg/L	
Duckweed, *Lemna minor*		Complete growth inhibition at 100 mg/L	

Notes

1 Nominal concentrations unless otherwise stated; (test guidelines allow for reporting of nominal concentrations where measured concentrations indicate test substance within 20% of nominal).

2 Ninety-five percent confidence intervals are stated where available.

3 Unless otherwise mentioned, the tests were conducted according to the named guideline.

C2.5.1 Effects of baits on native fish and invertebrates

Native eels under laboratory conditions

Trials have been undertaken to assess both primary and secondary poisoning in wild-caught long-fin eels (*Anguilla dieffenbachia*).

Eels were exposed either directly or indirectly to baits (RS5) containing 0.16% 1080 under flow-through laboratory conditions (Lyver et al 2004 and 2005). Each eel either had direct access to one bait in the same tank compartment, or indirectly (bait wrapped in mesh) for 96 hours.

Five hours after baits were placed in the tanks, the concentrations in water were 57 µg/L and 25 µg/L for the direct and indirect placements respectively. Baits had disintegrated completely after five hours. Water concentrations declined to <0.1 µg/L (LOD) and 0.38 µg/L after 48 hours. No 1080 was detected in eel tissue from either exposure type (LOD 2 µg/kg tissue). Eels did not consume available cereal baits.

The potential for secondary poisoning was assessed by feeding eels over an 11-day period either pieces (1–1.5g) of muscle containing 1.4 mg 1080/kg or guts containing 8.3 mg 1080/kg from possums which had died from 1080 poisoning. There were 12 eels in each treatment, with two

control treatments consisting of eels fed either uncontaminated possum muscle or gut.

No 1080 was measured in water from tanks where eels were fed 1080-contaminated possum tissue. Low concentrations of 1080 were measured in five of eight eels which consumed muscle tissue (0.003–0.32 mg/kg, mean 0.0174 ± 0.0104 mg/kg), with four eels not consuming any contaminated food. Three of nine eels which consumed gut tissue had low concentrations (0.016–0.056 mg/kg, mean 0.0306 ± 0.022 mg/kg), with three eels not consuming any contaminated food. The difference in concentrations in eels between the treatments was not significant.

Nine days after the last feed of contaminated tissue, low residues (~0.015 mg/kg) were measured in one eel of the three eels which had consumed contaminated gut, but were <LOD in those which consumed contaminated muscle tissue, sampled two days after the last feed.

No mortality, weight loss or unusual behaviour was observed in eels exposed to baits containing 1080 (Lyver et al 2004, and Lyver et al 2006 erratum to Lyver et al 2005).

Native freshwater crayfish under laboratory conditions

In a laboratory study, wild-caught native freshwater crayfish, koura (*Paranephrops planifrons*) were presented with one 6 g (nominal) cereal bait containing 0.15% 1080 alongside their normal diet of detritus and stream invertebrates (Suren and Bonnett 2004, 2006). The test system used was a stream simulator with both pools and riffles; caged crayfish were placed in both types of habitat.

Crayfish were observed moving or consuming the baits, however there were no mortalities during the eight-day study. Residues in crayfish viscera and tail muscle were measured. The highest concentration was 3.3 mg/kg in viscera and 5 mg/kg in tail muscle, both on a wet weight basis (LOD 0.005 mg/ kg for viscera and 0.01 mg/kg for muscle; wet weight). Concentrations in muscle decreased significantly between days 4 and 8 (from mean ~1.2 mg/kg to ~0.2 mg/kg, data presented graphically only), indicating the ability to metabolise and or excrete 1080. The highest total concentrations (viscera + muscle) of 7.7 mg/kg came from a small crayfish (bodyweight = 17.4 g) one day after exposure to bait. A relatively high total concentration of 7.1 mg/kg was also measured in a larger bodied crayfish (bodyweight = 59.4g) four days after initial exposure.

The greatest amount of 1080 in one individual (0.422 mg in a crayfish weighing 59.4 g) indicated that only a small amount of the available 1080 in the bait was taken up over time, based on an average concentration of 9.6 mg 1080/bait. The concentration of 1080 was monitored in the water and reached a maximum of 1.1 µg/L after two days, and <0.1 µg/L (LOD) after eight days.

Control animals were presented with one non-toxic cereal bait, but exposed to the same circulating water as the animals exposed to toxic baits. No residues were measured in the tissues of the control animals even though they were exposed to low levels of 1080 in the water, suggesting that uptake occurs primarily from direct ingestion, not uptake from the water, or alternatively that uptake from the water occurred at a low level, with rapid metabolism/excretion of 1080.

Controlled field study: native fish and invertebrates

In an attempt to assess the effect of 1080 of native fish and invertebrates, two trials were undertaken in four small streams (<3 m wide) in Mawhera State Forest, Grey Valley, placing baits in-stream and monitoring caged fish and invertebrate community indices (Suren and Lambert 2004).

In the caged fish trial, eight individuals of three species (long-fin eels (*Anguilla dieffenbachii*), koaro (*Galaxias brevipinnis*) and upland bullies (*Gobiomorphus breviceps*)) were placed in separate cages at four sites in each stream. Two sites were above the point at which 1080 baits would be added (10 m and 100 m) and two below (10 m and 100 m). The baits were placed in mesh bags across each stream. The type of bait used in these trials was not clearly specified, but was most likely 6 g Wanganui No 7 at 0.15% 1080 based on other information in the report. The number of baits chosen for placement in each stream was estimated to be 10 times higher than determined in a separate study which counted the numbers of baits deposited in streams during four 1080 operations (Suren and Lambert 2004; Suren 2006).

During the fish study, the flows in the streams fluctuated markedly, with a number of mortalities occurring as a result of high flows. Three cages in one stream were stolen during the study. No mortalities in any cage could be attributed to 1080. No sampling of tissues was undertaken to assess whether residues were present in the fish. The fish did not have direct access to the baits. Bullies and koaro are primarily insectivorous (Kusabs and Swales 1991; FishBase); it is unlikely that they would consume baits if presented with an opportunity to do so. In a study where eels were able to access cereal baits, none were observed to do so (Lyver et al 2004). The release of 1080 from baits used in this trial was assessed at the same locations but in a separate study, with ~50% decline in 1080 in the baits after eight hours (analytical method and LOD not reported).

The same four streams were used to assess the potential impact of 1080 on aquatic invertebrate taxonomic richness and density. As for the fish study, bagged baits were placed in-stream and assessments of the invertebrates made above and below the baits. No 1080 was detected in the stream water at day 1 or day 4 after deployment (analytical method and LOD not reported). The authors calculated that the four-hour 1080 concentration in water would potentially have been between 0.83 µg/L and 1.67 µg/L if all 1080 had leached during that time-frame. There were no significant differences between the sites above and below the baits for any of the

invertebrate community parameters assessed during the study (Suren and Lambert 2004).

C2.6 Aquatic hazard classifications of substances containing 1080

During the transfer of substances containing 1080 to the HSNO Act, the algal date were not available to the Agency, resulting in the differences in classifications as noted on pp 93 and 94 of the application.

The Agency has used the additivity formula for estimating the ecotoxicity of mixtures (set out below) to estimate the toxicity of these substances (ERMA 2001). The toxicity value used by the Agency is the algal EC_{50} 0.012 mg/L (Berends et al 1999).

Table C7 sets out the results of the calculations and the classifications derived by the Agency and the applicants.

$$L(E)C_{50\,mixture} = 100 \Big/ \sum_{\eta} \frac{Ci}{L(E)C_{50\,i}}$$

where:

C_i=concentration of component i (weight percentage)

$L(E)C_{50i}$= LC_{50} or EC_{50} for component I (mg/L)

η = number of components

The Agency has used the additivity method for estimating the toxicity of the substances containing 1080 as the resulting classifications are more conservative than those derived from the summation approach which is consistent with the approach recommended in the GHS guidance and current practice within the Agency.

The applicants used the same algal value of 0.012 mg/L and the summation approach, with a multiplier of 10, to classify formulations containing 1080. The applicants were also of the view that 1080 is rapidly degradable in the aquatic environment. If the substance is considered rapidly degradable, then the C classifications (toxicity in the range 10–100 mg/L) should become D classifications. For a substance to be assigned a 9.1C (or 9.1B) classification, the substance must also be considered bioaccumulative (which 1080 is not) and/or not rapidly degradable according the HSNO Act criteria.

However, there are is no apparent reason for the discrepancy in the classification for gel containing 50 g/kg 1080 between the Agency and the applicants. The summation approach would also result in classification of this substance as 9.1A highly toxic to the aquatic environment.

As noted in section C2.5, the aquatic hazard classification of technical grade 1080 is very uncertain, and such uncertainty also applies to the classifications of the substances containing 1080.

Table C7: Aquatic toxicity classifications of substances containing 1080

Name of substance	Tech. grade 1080	Pellets 0.4–0.8 g/kg 1080	Pellets 1.0 g/kg 1080	Pellets 1.5–2.0 g/kg 1080	Paste 0.6–0.8 g/kg 1080	Paste 1.5 g/kg 1080	Paste 10 g/kg 1080	Gel 1.5 g/kg 1080	Gel 50 g/kg 1080	Gel 100 g/kg 1080	Soluble conc 200 g/L 1080
% 1080	95–98%	0.08	0.1	0.2	0.08	0.15	1	0.15	5	10	20
Estimated EC_{50} (mg/L)	-	15	12	6	15	8	1.2	8	0.24	0.12	0.06
Agency's classification	9.1A	9.1D	9.1D	9.1D	9.1D	9.1D	9.1D	9.1D	9.1A	9.1A	9.1A
Applicants' classification	9.1A	9.1C	9.1C	9.1C	9.1C	9.1C	9.1C	9.1D	9.1C	9.1A	9.1A

C3 Sub-class 9.2: Soil ecotoxicity and terrestrial fate of 1080

Classification under sub-class 9.2 requires consideration of the persistence of the components of the substance in soil (Tables C8 and C10) and the toxicity of the substance to soil-dwelling invertebrates (eg, earthworms), soil microbial function, and plants (Table C12).

C3.1 Terrestrial fate of 1080

C3.1.1 Abiotic degradation in soil

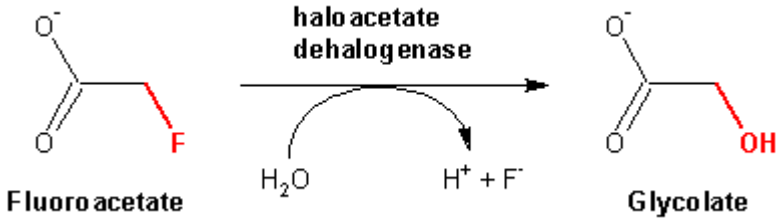
No data were submitted on the photolysis of 1080 in soil.

Under sterile conditions, 1080 is stable as indicated by the lack of degradation of 1080 in autoclaved soil (Parfitt et al 1994).

C3.1.2 Biodegradation of 1080 in soil

Studies have demonstrated that there are a wide variety of soil micro-organisms which are able to defluorinate (biodegrade) 1080 by cleavage of the carbon-fluorine bond to yield fluoride ions and glycolate (eg, Goldman 1965; Liu et al 1998) as indicated in the pathway in Figure C1.

Figure C1: Cleavage of carbon-fluorine bond to yield fluoride ions and glycolate



Source: Liu et al 1998.

In most of these studies, the micro-organisms have been isolated through the use of enrichment culture of soil organisms, that is, culturing the micro-organisms on 1080-enriched media in the laboratory (eg, Tonomura et al 1965; Kelly 1965; Walker 1994) rather than using soil as in standard soil biodegradation studies.

Soil organisms identified as being able to degrade 1080 include bacteria (eg, *Pseudomonas acidovorans, Pseudomonas fluorescens*) and fungi (eg, *Fusarium oxysporum, Aspergillus fumigatus*) (Wong et al 1992b) isolated from sites with or without prior exposure to 1080 in Australia (King et al 1994). In New Zealand, there are reports of similar organisms being isolated from soil (Parfitt et al 1994; Walker 1994; Bong et al 1979; Bong and Walker 1981) and vegetation (Henry unpublished data in Parfitt et al 1994).

In 1994 the Auckland Regional Council commissioned analyses of eight soil samples for the presence of micro-organisms capable of degrading

1080 (Lloyd-Jones et al 1994). The source of the soil samples was not identified in the report. Three of the eight samples had the capacity to de-fluorinate 1080. Bacteria were isolated from these samples with the ability to utilise 1080 as the sole carbon source. No defluorinating activity was found over a period of four weeks in the remaining samples using enrichment techniques, indicating that a microbial population capable of defluorination were absent from those samples. The authors noted that due to the highly heterogeneous nature of the soil environment a more comprehensive sampling would be required to adequately characterise the microbial population. The very low concentrations of 1080 which may occur in the environment may be too low to favour microbial degradation or induce the necessary enzyme systems; detection of micro-organisms capable of defluorination in the laboratory does not confirm that degradation will occur *in situ* unless conditions are favourable.

In Australia, micro-organisms found on bait materials (meat-based and oats) have also been shown to be able to defluorinate 1080 (Wong et al 1991). The micro-organisms isolated from the oats had a higher ability to defluorinate 1080 than several of those from the animal protein baits and particularly *F. oxysporum,* noted as being ubiquitous in soils.

Bong et al (1979) reported no adverse effects on soil microflora (unicellular algae *Chlorella* spp. and *Chlamydomonas* spp. isolated from New Zealand soils previously exposed to 1080) when grown in test solutions of up to 5 mg 1080/L.

Table C8: Summary of degradation and fate studies on 1080 in soil in the laboratory

Test type	Test results	Test method (reference number)
Abiotic degradation		
Photolysis	Not submitted	
Biodegradation		
Aerobic conditions	Kaitoke silt loam from native forest near Upper Hutt, included decomposing litter Incubated at 5, 10 and 23ºC for 120 days Approximate DT_{50} values: 10 days at 23ºC 30 days at 10ºC 80 days at 5ºC Four additional samples at field moisture capacity were sterilised by autoclaving and after 27 days at 23ºC there was no change in the 1080 concentration. Conroy sandy loam central Otago, location not specified Incubated at 21ºC and either 9%, 20% or 36% moisture content for 100 days. Approximate DT_{50} values: >40 days at 9% soil moisture 35 days at 20% soil moisture 15 days at 36% soil moisture	Non-standard; non-GLP Two soils each with 6.1 mg of 1080 added by dripping onto surface of soil dissolved in 1 mL of distilled water (to simulate leaching from a single bait onto the soil surface) Soil samples used only 14 g; pH, %OC, microbial biomass and pesticide history of sites not specified Soil moisture levels not specified for Kaitoke soil. No determination of degradation products or non-extractable residues (Parfitt et al 1994)

Test type	Test results	Test method (reference number)
Degradation in leaf litter	Degradation was more rapid at the warmer temperature. At 15ºC 'low levels' were reached by day 8 (assumed from graphed data to be at or near LOD) At 5ºC an equivalent 'low level' was reached at day 32. DT_{50} eight days	Non-standard; non-GLP 200 g samples of field-moist forest leaf litter with 18 mg 1080 added in 10 mL of deionised water. Held at either 5ºC or 15ºC for 32 days. Sprinkled with 5 mm of simulated rainfall each day. Samples analysed at days 4, 8, 16 and 32. LOD 5 µg/kg (assumed to be fresh weight; no mention of drying litter to determine moisture content). It is not clear from the results reported what effects the additional simulated rain may have had (ie, potential leaching compared with loss via degradation). (Wright et al 2002)

C3.1.3 Degradation of 1080 in animal carcasses

There is limited information available on the degradation of 1080 in animal carcasses. One study reported on 1080 residues in rabbit carcasses under laboratory conditions (Gooneratne et al 1995) and several on residues in possums under field conditions (Meenken and Booth 1997; Wright 2004; Eason et al 1991a).

Two Australian studies have examined the loss of 1080 from meat baits used to poison wild dogs in eastern Australia (McIlroy et al 1988; Fleming and Parker 1991). The presence of blowfly (Calliphoridae) maggots increased the rate of loss of 1080 under experimental conditions, independent of the effects of rainfall (McIlroy et al 1988). Other invertebrate decomposers such as ants were noted as being present at various stages of the experiments. The authors noted that under Australian conditions, blowfly maggots are present in summer but not in winter.

It is clear that 1080 residues remain in the guts of dead animals for prolonged periods (at least 75 days under cool winter conditions (Meenken and Booth 1997; Wright 2004)) and only degrade slowly.

The reasons for the slow rate of degradation are not clear, but the Agency considers it may be a function of low pH in the gut, absence of bacteria able to degrade 1080, toxicity to gut bacteria, low levels of decomposing invertebrate activity, accompanied by cool temperatures occurring at the time of 1080 bait distribution.

Table C9: Degradation/loss of 1080 in carcasses

Bait type and application method	Location and date of application	Assessment method	Weather conditions/ temperature during assessment period	Results 1080 content of carcass samples	Reference
Possums					
0.08% apple paste (~5 g) on earth spits at 30–50 baits/ha	Wairarapa, May/June 1994	Thirty-two dead possums located and covered with wire cage to prevent access by mammalian scavengers. Carcasses not moved and left in contact with soil. Stomach contents from selected carcasses assessed for 1080 residues at 25, 30, 39, 52, 71 and 75 days after estimated date of death.	Not reported	Carcasses remained relatively intact at 39 days; between days 40 and 75 most fleshy parts decomposed and fur came away from skin. At day 75 stomach and contents of 6 of 12 remaining carcasses still able to be sampled. Stomach and intestines were last soft tissue (excluding skin) to degrade. Average weight of stomach including contents was 200 g. All stomachs/contents analysed contained 1080 residues: Day 25 mean 30.06 mg/kg (n=6) Day 75 mean 4.9 mg/kg (n=6) LOD 15 µg/kg	Meenken and Booth 1997
0.15% Wanganui No 7 (11 g) cereal pellets 2 kg/ha	Hutt water supply catchment July 2003	Carcasses assessed at two sites within the operational area. Number of carcasses sampled was not reported. **Site 1** Dryer/warmer; Hutt catchment access road; hard/mountain beech with 4 m canopy. **Site 2** Cooler/wetter; Phillips stream rain gauge; mixed hardwood/podocarp valley floor 10 m canopy.	Cumulative rainfall reported but not temperature (though noted as for measurement during future operational monitoring)	**Site 1 (days after operation)** Day 29 bait 0.005% (30 mm rain) Day 36 bait 0.0018% (71 mm rain); fly eggs present, no maggots Day 40 bait <LOD (88 mm rain) bait still green and largely intact Day 52 black beetles present on carcass, no maggots Day 73 stomach contents 6 mg/kg (231 mm rain) **Site 2 (days after operation)** Day 29 bait 0.004% (70 mm rain) Day 36 bait 0.0002% (162 mm rain) Day 40 bait <LOD (186 mm rain) Day 73 stomach contents 13 mg/kg (458 mm rain); maggots noted as present Day 88 maggots observed inside possum stomach (718 mm rain) Day 178 stomach contents <LOD At nearly 6 months (178 days – Jan 2004) the remaining carcass still had green dyed bait in its stomach but <LOD (LOD not stated) Following a significant flood event in September 2003 three	Wright 2004

Bait type and application method	Location and date of application	Assessment method	Weather conditions/ temperature during assessment period	Results 1080 content of carcass samples	Reference
				possum carcasses were washed down-river well beyond the boundaries of the operational area with 1080 measured in the stomach contents: ~6km Kaitoke 7 mg/kg ~15km Whakatikei 6 mg/kg ~50km Petone <LOD Another possum carcass and a stag carcass were found in October 2003 following another (larger) flood event having been washed down-river from the treatment area. ~40km Kennedy Good Bridge stag 0.5 mg/kg ~50km Petone possum 1.7 mg/kg Non-toxic possum carcasses frequently appear on Petone beach all year round.	
Aerial 0.08% Wanganui No 7 cereal bait at 12 kg/ha	Rangitoto Island November 1990	Three possum + one wallaby sampled carcasses one day after operation Four possum carcasses 13 days after operation	Not reported	Day 1 mean concentrations: stomach 16.7 mg/kg: liver 3.4 mg/kg; leg muscle 1.3 mg/kg Day 13 mean concentrations: stomach 2.6 mg/kg; liver 5.0 mg/kg; leg muscle 0.25 mg/kg After 28 days, carcasses had significantly decomposed and comprised largely pelts and bone so not sampled further.	Eason et al 1991a
Rabbits					
Direct administration of single lethal oral dose of technical grade 1080 at 0.8 mg/kg bw	Not applicable	Nine rabbit carcasses left within animal research facility; no mention of contact with soil, or whether cages had artificial floors preventing contact Liver, kidney and muscle tissues sampled 1 and 2 weeks after death At week 3, liver and kidneys in two of three remaining carcasses had degraded; so only one sample of each was available for analysis; muscle tissue taken from all three carcasses	Stated as "room temperature" in one part of the report and "outdoor cages inside locked pens where temperature and rainfall would have fluctuated" in another part of the same report. No further information	Breakdown of soft tissues observed after one–two weeks, particularly liver and kidney. Concentrations (fresh weight) in all samples were highly variable and results presented in a graph only. LOD 0.002 mg/kg fresh weight. At time of death, mean concentrations (n=3) were: <0.01 mg/kg (liver); 0.02 ±0.01 mg/kg (kidney); ~0.05 ±0.02 mg/kg (muscle) One week after death (n=3): ~0.04 ±0.06 mg/kg (liver); ~0.05 ±0.07 mg/kg (kidney); ~0.04 ±0.0.02 mg/kg (muscle) Two weeks after death (n=3):	Gooneratne et al 1995

Bait type and application method	Location and date of application	Assessment method	Weather conditions/ temperature during assessment period	Results 1080 content of carcass samples	Reference
				~0.035 ±0.0.1 mg/kg (liver); ~0.045 ±0.01 mg/kg (kidney); ~0.05 ±0.005 mg/kg (muscle) Three weeks after death: (n=3 muscle; n=1 for liver and kidney) <0.005 mg/kg (liver); ~0.01 mg/kg (kidney); ~0.02 ±0.02 mg/kg (muscle) Note: Samples not taken from the same carcass over time, each set of analyses was from three different carcasses. The authors note that the apparent increase in concentration from that at time of death relative to that at one week after death may be due to some dehydration of the carcasses over time; with results being reported on a fresh weight basis. No measurement of tissue moisture content was undertaken prior to analysis.	

Effects of environmental variables on degradation of 1080

The effects of pH, temperature and soil moisture on the rate of defluorination of 1080 by Western Australian soil bacteria have been investigated (Wong et al 1992). The optimum pH for the soil bacteria (*Pseudomonas acidovorans* and *P. fluorescens*) was neutral to alkaline and pH 5 for the fungi (*Fusarium oxysporum* and *Penicillium restrictum*). The greatest defluorination in soil occurred at fluctuating temperatures (11–24º C) and soil moisture content of 8–15%. The least defluorination occurred at 30% soil moisture, but significant defluorination (54–60%) was observed at 40% and 50% soil moisture. The optimal conditions observed in this study may be linked to the Western Australian environment from which the micro-organisms were sourced. Their relevance to New Zealand conditions and micro-organisms is not known.

As highlighted by Parfitt et al (1994), many vertebrate pest control operations are conducted in New Zealand during winter or early spring when ground temperatures are well below 20ºC, microbial activity is slower and soils are wet. Under dry conditions, microbial activity is also less than optimum, resulting in slower degradation times even when soil temperatures are higher.

C3.1.4 Assessment of 1080 soil degradation data against HSNO Act criteria

No studies have been conducted using standard international guidelines to assess the route and rate of degradation of 1080 in soil. While studies have indicated the ability of some soil organisms to biodegrade 1080, the rate of such degradation under New Zealand conditions is uncertain. Likewise, no robust studies on the adsorption/leaching of 1080 were available to the Agency for review.

The criterion of whether a substance is considered to be degradable in soil is less well defined under the HSNO Act than that for rapid degradability in water. Schedule 6 of the Hazardous Substances (Classification) Regulations 2001 refers to a soil half-life (DT_{50}) as being "the half-life in soil, which is the time required to reduce the original concentration of the substance in the soil by 50%". Where the DT_{50} is >30 days, a substance is assigned a higher hazard classification than if the DT_{50} is <30 days.

The GHS (OECD 1998) does not include soil hazard classification and in the absence of guidance from that source, the Agency has interpreted the DT_{50} as being a result of biodegradation, consistent with that for assigning aquatic degradation 'classification', rather than dissipation which refers to any loss from the soil whether by degradation, leaching or some other process.

The Agency sought an independent expert review of the available information on the degradation of 1080 and soil (refer Attachment 1 to this appendix). The reviewer concluded that 1080 has representative half-lives in soil of 10 days at 23°C and 30 days at 10°C.

C3.1.5 Adsorption, desorption and mobility of 1080 in soil

No standard guideline studies on the adsorption, desorption or leaching of 1080 were submitted by the applicants or located by the Agency.

Fluoroacetate occurs predominantly as an anion at environmentally relevant pH (pKa 2.72 at 25ºC), and is not subject to binding at cation exchange sites on soil particles. It may be weakly bound on anion-exchange sites, such as on hydrous oxides, and the clay mineral allophane (Parfitt et al 1995). The high water solubility of 1080 also indicates a potential for leaching. Parfitt et al (1995) examined the adsorption and leaching potential of three New Zealand soils stated as being high in allophane and hydrous iron oxides. The Agency only had access to the study as published in the open scientific literature and a large number of deficiencies are noted in Table C10.

Table C10: Summary data on the adsorption, desorption and mobility of 1080 in soil

Study type	Soil type	Test results	Test method (reference)
Adsorption	Stratford silt loam (Andisol[1]); pH 5–6 0–100 mm and 200–300 mm soil depth	"Small amounts" of 1080 were adsorbed on the soil sample taken from 200–300 mm depth after 16 hours (results not reported for the 0–100 mm soil sample) Amounts adsorbed were reported as being similar to nitrate but less than sulfate, but neither of these analytes is mentioned in the 'materials and methods' section for the adsorption study.	Non-standard; non-GLP Test conditions and results not well documented. Not reported: source locations of test soils; number of replicates; controls; pH of aqueous phase; temperature; %OC; clay content; CEC; moisture holding capacity; storage of samples prior to analysis. Standard method states soil from top 0–200 mm should be used and sieved ≤2 mm (OECD 106); test soils were sieved to 6 mm. Soil mass (3 g) and solute volume (5 mL) small compared with standard test method (2–50 g in 50 mL). Test system allowed to equilibrate for 4 hours, 24 hours is recommended (OECD 106) Equilibrium of test substance soil not demonstrated; toluene added to prevent microbial activity. Preparation of supernatant not specified. Analysis by anion chromatography; limits of detection not stated (Parfitt et al 1995)
Column leaching	Stratford silt loam (as above) Westmere silt loam (Inceptisol[2]) pH 5–6 0–100 mm soil depth	Stratford silt loam (200–300 mm soil depth) 1080 first detected after addition of 15mL water at 50 mg/L and maximum concentration of 250 mg/L after addition of 25mL water. 1080, nitrate and chloride stated as being fully recovered from the column at the end of the study. Westmere silt loam 1080, nitrate, and chloride and stated as being fully recovered from the column after addition of 40mL water to the column, having reached a peak after 20mL.	Non-standard; non-GLP Test conditions and results not well documented. 4 mg 1080 dissolved in 5 mL of water added to top of column; and leached with 10 x 5 mL pulses of water over eight hours, equivalent to 160 mm rain. Repeated using nitrate, chloride and sulfate, but preparation of these test solutions was not specified. Test columns (20 mm diam., 150 mm long) smaller than standard (400 mm x 350 mm). Columns leached of chloride and nitrate before adding 1080. Similar issues as for adsorption study regarding test soils, analytical method/LOD etc (Parfitt et al 1995)

Study type	Soil type	Test results	Test method (reference)
		Sulfate fully recovered at end of study. More rapid leaching of 1080 from Westmere soil attributed to that soil containing less hydrous oxides than in the Stratford soil.	
Leaching from intact soil cores	Holmes Bay (inceptisol) pH 5–6 0.140 mm soil depth	First core 1080 completely leached after the equivalent of 180 mm rain Second core 1.4 mg of the original 3 mg 1080 recovered in the eluate, and 0.3 mg recovered from the soil. The remainder may have degraded within the soil after the end of the test and before analysis of the soil.	Non-standard; non-GLP Test conditions and results not well documented. Soil cores 100 mm diam. x 140 mm deep, 3 mg 1080 in 1 mL water added to top of core. Two replicates; no control. Column leached with 0.001 M chloride at 12 mm/hr for 15 hours. Suction equivalent to 100 mm rain applied to base of column and leachate collected in 50mL aliquots. Similar issues as for adsorption study regarding test soils, details of test protocol, analytical method/LOD etc (Parfitt et al 1995)

Notes

1 Andisols are soils formed in volcanic ash and defined in terms of the dominance of glass and poorly crystalline colloidal materials such as allophane, imogolite, and ferrihydrite.

2 Inceptisols are recent soils which do not have clearly developed horizons.

C3.1.6 Leaching and degradation of baits

As noted in the section on the aquatic fate of baits containing 1080, the information available on the degradation and weathering of cereal baits has been almost invariably reported for one of two bait types; either Wanganui No 7 or RS5; with former being more moisture resistant and taking longer to breakdown than the latter. The manufacturer of cereal bait pellets has advised that these names are largely historical and with one exception, are no longer manufactured. As such, the information presented below can only be considered as indicative.

Degradation and leaching of cereal bait in a landfill under anaerobic conditions

Southland Regional Council disposed of approximately 12,000 kg of toxic bait to landfill in August 1996 due to deterioration in storage. The waste bait comprised 11,000 kg of 6 g Wanganui No 7 baits at 0.15% 1080 and 40 two-litre pails (opened) of apple paste bait at 0.08% 1080 (Bowman 1999). The waste bait was buried in a biologically active section of the Winton landfill (temperature 30–45ºC; anaerobic conditions). The natural water table was approximately 1–1.5 m below the base of the disposal pit. Groundwater bores at 5 m (bore 1) and 13 m (bore 2) from the disposal point in the direction of groundwater flow were monitored for 1080 over a period of 13 months. Samples were taken weekly for the first five weeks and then monthly. Degradation of the bait was monitored *in situ* in October 1996 and again in October 1997.

At bore 1, 1080 concentrations were measured in three of the 14 groundwater samples taken (LOD 0.1 µg/L), with the highest 24 µg/L five

weeks after disposal. The other two samples were 7 µg/L in January 1997 and 0.4 µg/L in April 1997. At bore 2, two samples were measured at 6 µg/L and 1 µg/L in January and February 1997 respectively. No 1080 was measured in groundwater during the last six months of monitoring.

Sampling of the buried bait 10 weeks after burial indicated that degradation was occurring from the top of the waste mass downwards, and had reached 30% of the original concentration, and remained the same at the bottom of the pit. A year later, 20% of the original remained at the top, and 7% at the bottom. There were insufficient data to calculate a degradation rate.

Leaching of 1080 from baits under simulated rainfall

An early Australian study (Griffiths 1959) assessed the toxicity of oats and carrot baits (0.03% 1080) placed on turf and left exposed to the weather. The first rainfall (~38 mm) occurred after nine days of sunny weather, followed by two further rainfall events (~23 mm each) at four and six weeks after baits were placed in the field. While the bioassay methods used do not represent current practice, both carrot and oat baits remained lethal to mice for the first nine days. The carrot disintegrated after the first rainfall, with little detectable 1080. The oats remained toxic to rabbits for the duration of the trial (seven weeks). Analysis of the oats indicated that the 1080 was present on the husk of the oats, with none detectable in the grain. Griffiths (1959) also cited unpublished studies where vacuum impregnated oats subject to sprinkler irrigation 24 hours after treatment with 1080 lost all the 1080 after two hours (~6–76 mm rain). Where oats are used in New Zealand, they are boiled and a 1080 stock solution applied (additional information from the applicants), so the relevance of the loss of 1080 from oats under Australian conditions to those in New Zealand is unknown.

Several New Zealand studies have assessed the leaching of 1080 from formulated baits and decomposition of the bait matrix. These are summarised in Table C11.

A study by Staples (1967) found that in all cases thicker carrot baits retained 1080 for longer than thinner, smaller baits regardless of initial trial concentration. Unfortunately this study did not report the time elapsed between treating the carrot bait with 1080 and placement in the field. A more recent study (Thomas et al 2004) indicates that a delay of 48 hours after treatment and before first rainfall results in the carrot bait retaining 1080 for longer than those deployed immediately after treatment. Work by Staples (1969) indicates that peeled carrot absorbs 1080 to a greater extent (58–138%) than does unpeeled carrot; with absorption inversely related to the thickness (ie, small baits take up a greater amount of 1080 than larger baits) resulting in a greater toxic loading per small bait.

Cereal baits lose 1080 and degrade/disintegrate faster than carrot, with RS5 cereal baits disintegrating more rapidly than Wanganui No 7 cereal baits under comparable conditions (Thomas et al 2004; Bowen et al 1995; Booth et al 1999).

In-field leaching of 1080 from cereal and carrot bait

The rate of 1080 breakdown in baits in the field was assessed by Thomas et al (2004) based on data provided by two regional councils and two possum control contractors. Analysis of the data provided was complicated by: inconsistent recording of bait size; 1080 loading varying 0.08–0.15%; rainfall and habitat not recorded in a way which allowed meaningful interpretation; and bait type not always being recorded. The available data indicated that in the field RS5 baits leached more rapidly than Wanganui No 7, with most 1080 removed after 150–200 mm of natural rainfall—consistent with results for simulated rainfall. Small sample size (n=8 records) for carrot baits limited the conclusions which could be drawn from the data. The authors did conclude that most 1080 is leached from carrot bait after 200 mm natural rainfall, also consistent with the simulated trials, and noted that this was not consistent with the findings of Bowen et al (1995) where there was no decrease in 1080 under 200 mm simulated rainfall. Carrot baits were generally in the field for longer than the 48 hours in the simulation trial, resulting in some decay of the baits and the 1080 present.

Effect of bait size on leaching

The effect of cereal bait size on leaching was also considered by Thomas et al (2004) by comparing the results from their studies with large baits (11–12g) with those of Bowen et al (1994) with smaller baits (6g). The larger cereal baits retained a greater amount of 1080 than the smaller baits at <100 mm rain. Both sizes of cereal bait had lost almost all 1080 after 100 mm simulated rainfall; with the exception of the small Wanganui No 7 bait which still retained ~35% of its original concentration.

The 1080 content of the large carrot baits gradually declined during exposure to 200 mm rain (Thomas et al 2004), whereas no significant loss was observed from the smaller carrot baits (Bowen et al 1995). There is no apparent reason for the observed differences but a number of environmental parameters were unreported for both studies (eg, temperature and sunlight) and time from preparation of the carrot bait to deployment of baits in the trial was not described by Bowen et al (1995).

Leaching of 1080 from pastes or gels

Gels 5% and 10% 1080

The degradation of a carbopol polymer-based gel containing 10% 1080 was assessed on Stewart Island (Batcheler and Challies 1988) and results indicated a loss of 90% 1080 after 207 mm rain at a sheltered site and

81 mm rain at an exposed site. Gel was spread on leaves of cut branches placed on the ground (see Table C11).

Additional work on the loss of 1080 from gels was undertaken to investigate the rainfastness of different gel formulations by testing blends of carbopol and petrolatum on broadleaf, *Griselinia littoralis*, on leaves *in situ* (Challies and Thomson 1988). Under these trial conditions, rates of loss decreased proportionally with increasing petrolatum content. The rate of loss from carbopol alone was less than that for the previous study (Batcheler and Challies 1988) and attributed to differences from testing cut branches where cut branches are placed so that the treated leaf surfaces are more exposed than they would be *in situ*.

In another study by Parkes (1991), carbopol, petrolatum and blends of the two carriers containing 1080 caused some leaf abscission. The results of loss of 1080 from the various carriers were consistent with the earlier studies. Loss of 1080 from fallen leaves was not linked to cumulative rainfall, but to loss of the leaf cuticle. Given the high toxic loading from 10% 1080, the author concluded that the fallen leaves could remain toxic to ground-feeding organisms for up to 300 days (at approximately 2 mg 1080/leaf from an initial 25 mg 1080/leaf).

Gel block 0.15% 1080

No data are available on the degradation of the long-life gel block. However, given that it is placed in bait stations only, it is not exposed to the weather and expected to remain toxic for a prolonged period. It is possible that the gel block may dehydrate over time, but the Agency has no information to support this.

Fruit-based pastes

The effect of dehydration on fruit paste baits has been assessed together with stability under field conditions (Morgan 2000). If baits dehydrate, the concentration of 1080 increases, potentially increasing risks to non-target species. The current fruit-paste formulation has been developed to resist dehydration in the field (additional information from manufacturer via applicants 13 February 2007).

Peanut-based paste

One currently registered paste is peanut based (Pestoff Exterminator 0.15%) and there is no information available on loss of 1080 from, or degradation of, the paste. This product is applied in bait stations only (clarified in additional information from applicants 13 February 2007) so is likely to remain toxic for an extended period.

Fish-based pastes

Fish-based baits used for wasp control are reported to putrefy within 1–2 days of being laid or may dehydrate under dry weather conditions. Efforts have been made to improve the formulation to reduce dehydration (Spurr et al 1996).

No data are available on the degradation of the fish-based paste formulation. Given the composition and physical form of these substances it is expected that they would be 'washed-out' relatively quickly by rain if left exposed.

Table C11: New Zealand studies on the leaching of 1080 from baits under trial conditions

Bait type (size)	Toxic loading (nominal)	Test results	Conditions of trial (Reference)
Carrot	0.25% or 0.5% ('moisture free' basis; ~0.02% and ~0.04% freshweight).	**Trial A** Half-inch cubes disintegrated by end of week 7 (118 mm rain). One-inch cubes outer layers decayed after week 7. All baits except 1-inch cubes at high rate had ≤10% original 1080 by end week 7. All baits <LOD by end of week 9. Overall greater 1080 retention by the large bait at both concentrations	Study undertaken to assess effect of bait size and thickness on weathering/loss of 1080. Time between preparation of bait and placement outdoors not stated, assumed to be on same day. Trial A: Carrot cubes ½ or 1 inch placed on grass outdoors in April for nine weeks. First 10 days fine. Trial B: Carrot cubes ½- or 1-inch carrot slices ¼ and ⅛ inch thick placed on grass outdoors in July for four weeks (very wet weather). Trial C: As for B, commenced in February for six weeks. Four periods of rain at two-week intervals. Trial D: Quarter-inch carrot slices at low rate 1080, one batch exposed to weather as for A, B and C, the other batch was placed under cover to prevent wetting by rain, cover removed in fine weather, in the last week the 'covered' baits were exposed to the weather for six weeks. (Staples 1967)
		Trial B advanced decomposition of all baits at lower concentration After 15 days and 100 mm rain, ¼ inch slices 10% original conc. and by week 6 all baits were <LOD. Overall greater 1080 retention by the large bait at both concentrations.	
		Trial C heavy rain in the first week (98 mm): greatest loss ~25% from 1-inch cubes at high concentration. At end of trial, all baits ≤10% original 1080.	
		Trial D 'covered' baits showed little sign of degradation, but some desiccation at end of trial and retained the original concentration of 1080. Exposed baits degraded in a similar manner to those in the other trials.	
Carrot (6 ± 0.5 g)	0.08% and 0.15%	No decrease in 1080 after 200 mm rain.	Baits placed on turf and sprinkler irrigated 20 mm/hr for 48 hours and sampled after 5, 10, 20, 50, 100, 150 and 200 mm rain. Temperature and LOD not reported. RS5 pellets noted as being more crumbly than the Wanganui No 7 baits prior to commencement of the trial. (Bowen et al 1995)
Cereal RS5 (6 g)	0.08% and 0.15%	Baits started disintegrating after 5 mm rain. Rapid decline in 1080 concentration after 5 mm; <LOD at 150 mm.	
Cereal Wanganui No 7 (5 g)	0.08%	Shape retained for the duration of the trial. Rapid decline in 1080 concentration, but slower than for the RS5 baits. Concentrations were 0.007% in the 0.08% bait and 0.02% in the 0.15% bait after 200 mm rain.	
Cereal Wanganui No 7 (6 g)	0.15%		

Bait type (size)	Toxic loading (nominal)	Test results	Conditions of trial (Reference)
Cereal Wanganui No 7 (6 g)	0.15%	Initial moisture content of bait 12%, reaching up to 65% after 250 mm rain. Shape retained for the duration of the trial. Rapid decline in 1080 measured in the bait after 10 mm rain. Concentrations were ~0.005% after 100 mm rain and 0.00085% after 250 mm rain. 1080 was detected in soil under the baits after 20 mm rain, reaching a maximum after 100 mm rain and close to the LOD after 250 mm.	Baits placed on turf, in winter under full sun and sprinkler irrigated 20 mm/hr and sampled after 5, 10, 20, 50, 150 and 250 mm of rain. Bait LOD 2 mg/kg. 20 g soil samples taken from the top 5 cm of soil under baits at time of bait sampling and both frozen at -20ºC for later analysis but 1080 concentrations measured in soil were not reported in the paper. Soil LOD 0.8 µg/kg. (Booth et al 1999)
Carrot (12 g)	0.12–0.15%	Treatment 1: Loss of 74% 1080 after 10 mm rain. Treatment 2: Loss of <40% 1080 after 500 mm rain, indicating binding of 1080 to carrot.	Baits placed on hessian sacks under sprinkler irrigation 20 mm/hr and sampled after 5, 10, 20, 50, 100, 200, 300, 400 and 500 mm rain. Carrot baits had two different treatments: Treatment 1: Baits exposed as above one hour after application of 1080 solution. Treatment 2: Baits exposed as above 48 hours after application of 1080 solution (stated as more representative of field use as operations planned for three consecutive days of fine weather). Bait LOD 0.5 mg/kg; storage at -10ºC prior to analysis. (Thomas et al 2004)
Cereal RS5 (11 g)	0.15%	Bait disintegrated rapidly Initial 1080 concentration retained for the first 50 mm rain, followed by rapid loss to ~LOD during the second 50 mm	
Cereal Wanganui No 7 (11 g)	0.15%	Shape retained for the duration of the trial Steady decline in !080 concentration to ~LOD after 100 mm rain	
Carbopol gel on broadleaf (*Griselina littoralis*) foliage	10%	**Trial 1** Rain fell on days 1–6 and day 31. Rapid decline in concentration following first two days of rain, then observed to increase slightly up to day 16. Some degradation of 1080 in stored samples in field. Overall 1080 loss of 90% after 207 mm rain	Leaves on cut branches spread with gel and placed with treated side down in two locations on Stewart Island. Initial amount of gel per leaf 0.15–0.25 g per leaf (15–25 mg 1080/leaf), with 20 leaves per ‘bait’ branch. **Trial 1** Sheltered site under intact forest canopy.

Bait type (size)	Toxic loading (nominal)	Test results	Conditions of trial (Reference)
		Trial 2 Gradual decline in concentration until day 14; first significant rain occurred days 13–20, then on days 25, 33–35. 90% loss of 1080 from gel after 81 mm rain **Trial 3** 70% decline of 1080 on intact leaves during first 15 days of exposure. Overall loss of 91% in 45 days; 285 mm fell over the bait laying and observational period.	**Trial 2** Exposed ridge, open canopy, no understorey Leaves sampled every two days, kept at ambient temperature until able to send to Christchurch for analysis where stored at -14ºC until analysis by fluoride ion specific electrode. **Trial 3** Assessment of bait weathering during a 1080 operation Baits sampled at 0, 15, 30 and 45 days. Initial amount of 1080 per leaf 30.2 mg. Leaves treated over a one-month period. (Batchelor and Challies 1988)
Carbopol/ petrolatum gel blends on broadleaf *Griselina* littoralis) foliage	10%	**Carbopol alone**: 75% loss after 465 mm rain 1 : 1 mix: 75% loss after 875 mm rain 1 : 1.5 mix: 75% loss after 1175 mm rain 1 : 2 mix: 75% loss after 1390 mm rain 1 : 2.5 mix: 75 % loss after 1580 mm rain 1 : 3 mix: 75% loss after 1820 mm rain **Petrolatum alone**: very little leaching after 2500 mm rain	Trials undertaken in Westland *in situ* with the undersides of leaves treated with 10% 1080 gels comprised of different ratios of carbopol and petrolatum (1 : 0, 1 : 1, 1 : 1.5, 1 : 2, 1 : 2.5, 1 : 3, 0 : 1). Leaves sampled and frozen within 24 hours of collection at -18ºC until analysis by fluoride ion specific electrode. (Challies and Thomson 1988)
Carbopol/ petrolatum gel blends on mahoe (*Melicytus ramiflorus*) foliage	10%	**Leaf abscission** Both carriers and the toxic blends caused leaf abscission in mahoe, minimal days 1–10; more rapid days 10–20 **1080 in leaves in situ** After 64 days and ~208 mm rain: Carbopol alone—most (95.2%) 1080 leached All blends—~60% 1080 leached Petrolatum—little change (78.8% remained) **1080 in abscised leaves** Linear reduction in 1080 concentration over 300 days; attributed to loss of leaf cuticle rather than cumulative rainfall	Abscission of leaves assessed after application of non-toxic carriers and toxic blends. Initial amount of 1080 per leaf 25 mg. Persistence of 1080 assessed on leaves *in situ* and on abscised leaves in blends of a carbopol/petrolatum as in Challies and Thomson (1988), above. Analysis by fluoride ion specific electrode. (Parkes 1991)

C3.1.7 Plant uptake of 1080

As noted in the application, the responses of plants to 1080 are highly variable. Some synthesise and hyperaccumulate the substance without any adverse effect, for example up to 2600 mg/kg in the leaves of the Western Australian plant *Gastrolobium bilobum* (O'Hagan and Harper, 1999), others are able to defluorinate 1080 (Preuss et al 1958). Twenty-one samples of commercial tea, *Camellia sinensis,* analysed for 1080 contained 0.06–0.48 mg 1080/kg dw, mean 0.19 mg/kg (Vartiainen and Kauranen 1984).

Systemic translocation of 1080 was demonstrated in a number of studies investigating the use of 1080 as an agricultural insecticide for example, in broad bean plants grown in soil (David and Gardiner 1966) and sunflowers grown in sand (Cooke 1976). Recent studies undertaken in New Zealand on the uptake of 1080 by plants are summarised in Table C12.

The toxicity of 1080 to plants in standard laboratory tests has been summarised in section C.3.1.7.

Table C12: Uptake of 1080 by plants

Test species	Test type and duration	Test results	Test method (Reference)
Broadleaf, *Griselinia littoralis* Perennial ryegrass, *Lolium perenne*	Uptake and persistence in plants Potted plants, 38 days	**Ryegrass** Mean maximum concentration 0.08 mg/kg 3 DAT, declining close to LOD by 7 DAT **Broadleaf** Mean maximum concentration 0.06 mg/kg 10 DAT, declining to LOD by 38 DAT Leaching of 1080 from RS5 baits is relatively rapid (Bowen et al 1995) and may have accounted for loss of 1080 from the test pots. However, no analysis of the soil was undertaken, so it is not possible to draw definitive conclusions on the fate of the 1080 in the study. Some degradation may also have occurred in the soil during the study.	Broadleaf seedlings nursery-sourced. Ryegrass grown from seed and allowed to establish in pots for one month and trimmed to 80 mm immediately prior to testing to simulate pasture. A single 6 g RS5 cereal bait containing nominal 0.15% 1080 (= 9 mg 1080/bait) placed on the soil in each plant container. Controls used a non-toxic bait. Samples of four test plants plus one control taken for analysis at 0, 3, 7, 10, 18, 24 and 38 days; aboveground tissues only (whole seedling or trimmed grass). Frozen at -20ºC for later analysis. LOD 1.5 µg/kg (assumed to be fresh weight as no indication given of moisture determination). Ryegrass grown in Templeton silt loam; broadleaf assumed to be grown in potting mix (not reported). Plants placed outdoors for the duration of the study. Fifty mm rain in 2.5 days; other environmental conditions during test not reported; size of broadleaf seedlings not reported. (Ogilvie et al 1998)

Test species	Test type and duration	Test results	Test method (Reference)
Pikopiko, *Asplenium bulbiferum* Karamuramu, *Coprosma robusta*	Uptake and persistence in plants In-forest trial near Lake Waikaremoana 56 days	Leaf/frond samples taken at days 0, 3, 7, 14, 28 and 56 and analysed for 1080 from three randomly selected plants of each species exposed to 1080 and one control plant. Temperature during study: Air -1–12ºC; Ground 2–11ºC Rainfall: 120 mm over 56 days Baits were analysed for 1080 at day 56 and found to contain <1% of the original amount of 1080, though baits were still intact. Pikopiko No 1080 was detected in any of the samples at any time during the study. (LOD 2 µg/kg) Karamuramu No 1080 measured in the control plants at any time during the study. 1080 was measured in only one of three test plants, with a maximum of 5 µg/kg at day 7, declining to 2.5 µg/kg at day 14 and <LOC at day 28.	Single 6 g Wanganui No 7 cereal baits containing nominal 0.15% 1080 (= 9 mg 1080/bait) placed at the base (on the ground) of seven plants of each species. Three plants of each species were used as controls. Trial plants were caged to exclude wildlife. Concentrations in plants assumed to be on a fresh-weight basis, though not reported in the study. Small sample size. (Ogilvie et al 2006)

C3.2 Toxicity of 1080 to soil organisms

A summary of the toxicity of 1080 to soil-dwelling invertebrates, soil microbial function and terrestrial plants is in Table C13.

C3.2.1 Soil-dwelling invertebrates

Until very recently, no data were available on the toxicity of 1080 to soil-dwelling invertebrates. The applicants have submitted three studies on two earthworm species (O'Halloran and Jones 2003) and one on the garden snail *Helix aspersa* (O'Halloran et al 2003).

In the 14-day and 28-day toxicity of 1080 to the earthworm *Aporrectodea caliginosa* are deficient, only nominal concentrations reported in the test soils. Likewise, there were significant issues with the analysis of the test concentrations in soil in the 28-day test with garden snails attributed to degradation of 1080 in the samples in storage. The Agency considers that there is significant uncertainty around the test results as the actual exposure concentrations are not known. Springtails (Collembola) have been observed on 1080 baits (see Appendix F), and as such, a study on the toxicity of 1080 to these organisms would be useful as a representative of soil insects. Collembola are recommended test organisms under European Union Directive 91/414/EEC due to their high abundance in soil ecosystems (ISO 1999).

C3.2.2 Plants

The applicants submitted a guideline study on the effects of 1080 on seedling emergence and early growth of one monocotyledous plant (oats, *Avena sativa)* and one dicotyledonous plant (lettuce, *Latuca sativa*) (O'Halloran et al 2005). A standard regulatory assessment would normally include a greater number of species in order to assess the variability in responses (ie, six species from four dicot families and four monocots from at least two families). On the basis of the information available, the most sensitive end point is early seedling growth in lettuce, with a 14-day EC_{50} of 10 mg 1080/kg soil and LOEC of 7 mg/kg soil.

C3.2.3 Soil microbial function

Only one study was submitted on the toxicity of 1080 on soil microbial function (ie, effects on soil nitrogen transformation), indicating no adverse effects up to the maximum concentration tested of 1,000 mg/kg (O'Halloran et al 2005), rather than two studies as would normally be expected in a full regulatory data package, that is, a study of the effects on soil respiration has not been conducted.

An additional study on the effects of possum urine containing 1080 was provided in O'Halloran et al (2003). The results indicated a possible 15% reduction in nitrate production compared with control soils. Due to the very small sample size (one replicate of one treatment) the differences could not be compared statistically.

Studies have used 1080 as a tool to elucidate various metabolic pathways in a range of micro-organisms and are difficult to interpret in terms of potential responses in the environment. For example, Kalnitsky and Barron (1947) found that fluoroacetate inhibited respiration in yeast, *Corynebacterium creatinovorans* and *Escherichia coli* cultured in the presence of acetate for one hour at 100 mg/L. In another study, Mager et al (1955) found that 1080 at 100 and 400 mg/L strongly inhibited growth of *E. coli* for 15–24 hours and then recovery was observed at 48 hours; permanent growth suppression was not achieved at 10 g/L. Forced aeration of medium increased the inhibitory effect and anaerobic conditions decreased the effect. Inhibition was not affected by the size of the innoculum. The authors concluded that fluoroacetate slows down the synthesis of metabolites essential for initiating growth of *E. coli* rather than detoxifying the substance given the high concentration relative to the innoculum size.

Table C13: Summary of toxicity of 1080 to soil-dwelling organisms in the laboratory

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Soil-dwelling invertebrates			
Earthworm, *Aporrectodea caliginosa*	14 day	Nominal test concentrations 0, 10, 25, 50, 100, 250, 500 and 1000 mg/kg dw soil LC_{50} 296 mg/kg dw soil (95% CI 228–401) NOEC 25 mg/kg dw soil LOEC 50 mg/kg dw soil: reduction in bodyweight	OECD 207; GLP Non-standard test species (std – *Eisenia fetida*), and test conditions (soil moisture, light regime, natural soil used – Templeton silt loam, pH 6.8) modified to support maintenance of *A. caliginosa* in the laboratory based on prior experience. Insufficient numbers of laboratory reared earthworms available so additional earthworms sourced from pasture with no history of pesticide use. Analytical confirmation of test concentrations in soil not reported, while not required by the test guideline, would have been useful. Results of analytical QA/QC not reported (ie blanks, spiked samples). (O'Halloran and Jones, 2003)
	28-day reproduction	**Study 1** Nominal test concentrations 0, 10, 25, 50, 100, 250 mg/kg dw soil $NOEC_{growth}$ 100 mg/kg dw soil $NOEC_{cocoon}$ production 50 mg/kg dw soil $LOEC_{cocoon}$ production 100 mg/kg dw soil Total number of cocoons produced in the control did not meet the test criteria. 97.5% mortality at 250 mg/kg dw soil **Study 2** Nominal test concentrations 0, 1, 2.5, 5, 10, 25, 50, 100 mg/kg dw soil No reliable values were obtained, with reductions in bodyweight and mortalities in all treatments which were not dose dependent. Poor cocoon production in all test containers including controls. Reduction in soil volume (0.5 kg cf 1 kg), lack of energy reserves and possible lack of sexual maturity were identified as possible causes for the poor results obtained in this study. Residues in earthworms were at or below the LOD of 0.002 mg/kg.	ISO 11268-2; GLP Cocoon hatching was observed for eight weeks (four weeks specified in test guideline). As for the acute study, a number of test conditions were modified to suit *A. caliginosa.* Earthworms fed twice during the study at days 0 and 14 with 14 g grassmeal/kg soil. Soil volume reduced from 1 kg to 500 g per test container Two experiments undertaken, Study 1 in June 2002; Study 2 in November 2002. Analytical confirmation of test concentrations in soil not reported. Results of analytical QA/QC not reported (ie, blanks, spiked samples) (O'Halloran and Jones, 2003)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Earthworm, *Eisenia fetida*	28-day reproductio n	Measured test concentrations at day 0: 0, 10, 25, 50, 100, 225, 445, 865 mg/kg dw soil Measured test concentrations at day 28: 0,0.004, 0.009, 0.008,0.022,0.013, 0.018, 0.026 mg/kg dw soil No mortalities at any test concentration. Residues in earthworms were at or below the LOD of 0.01 mg/kg fw. Growth: NOEC 865 mg/kg soil Juvenile production: EC_{50} 160 mg/kg (95%CI 70–380) NOEC 50 mg/kg LOEC 100 mg/kg Cocoon production: EC_{50} 90 (95%CI 50–160) NOEC 50 mg/kg LOEC 100 mg/kg	ISO 11268-2; GLP Conducted in natural soil – Templeton silt loam. Large confidence intervals on the EC_{50} values Results of analytical QA/QC not reported (ie, blanks, spiked samples) (O'Halloran et al 2003)
Garden snail, *Helix aspersa*	28 days	Nominal test concentrations 0, 500, 1000, 1500 mg/kg dw soil Measured test concentrations at day 0 (analysed after storage at -20ºC for 8 months) <LOD, 238 mg/kg, 476 mg/kg, 775 mg/kg 28-day NOEC 1500 mg/kg dw soil 7-day LOEC 500 mg/kg dw soil (avoidance behaviour) Residues in snails at end of study after overnight depuration were reported as : 1.9 mg/kg after exposure at 500 mg/kg dw soil 23 mg/kg after exposure at 1000 mg/kg dw soil 61 mg/kg after exposure at 1500 mg/kg dw soil These values were based on duplicate analyses of a single sample at each concentration.	Adapted OECD 207; GLP Snails collected from 'spray-free gardens" and maintained in test containers on a layer of soil. Concentrations in soil at day 0 were measured at 64% of nominal after eight months' storage in a freezer at -20ºC. Analysis of spiked soils which had not been in storage for more than several weeks suggested loss of 1080 may occur during long-term storage at this temperature The report did not state whether the concentrations in the snails were reported on a wet- or dry-weight basis; or whole animal or only soft tissue. Results of analytical QA/QC not reported (ie, blanks, spiked samples). Wet to dry weight concentrations in soil based on assumed moisture content of 23%, not measured moisture content. (O'Halloran and Jones, 2003)
Terrestrial plants			
Lettuce, *Latuca sativa* Oats, *Avena sativa*	14 day Seedling emergence and early growth	Measured test concentrations at day 0: 0, 0.01, 0.35, 2.1, 7.0, 22, 92 mg/kg dw soil, which at day 14 had declined to <LOD in the three lowest treatments and to 0.06, 3.5 and 39 mg/kg dw soil in the three highest treatments. *Lettuce* Percentage germination: EC_{50} 45 mg/kg dw soil (95%CI 33–62); NOEC 22 mg/kg dw soil; LOEC 92 mg/kg dw soil	OECD 208; GLP Used natural soil, Templeton silt loam, 1.9%OC, pH 6.8. Samples were stored frozen at -80°C prior to analysis. (O'Halloran et al 2005)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
		Time to emergence NOEC 2.1 mg/kg dw soil LOEC 7 mg/kg dw soil Shoot growth EC_{50} 10 mg/kg dw soil (95%CI 8–12) NOEC 2.1 mg/kg dw soil LOEC 7 mg/kg dw soil Root growth EC_{50} 11 mg/kg dw soil (95%CI 6–20) NOEC 7 mg/kg dw soil LOEC 22 mg/kg dw soil *Oats* % germination EC_{50} >100 mg/kg dw soil NOEC 22 mg/kg dw soil; LOEC 92 mg/kg dw soil Time to emergence NOEC 22 mg/kg dw soil LOEC 92 mg/kg dw soil Shoot growth EC_{50} 42 mg/kg dw soil (95%CI 30–58) NOEC 7 mg/kg dw soil LOEC 22 mg/kg dw soil Root growth NOEC 22 mg/kg dw soil LOEC 92 mg/kg dw soil	
Wheat, *Triticum aestivum*	Seed germination 14 days	69% germination relative to controls	Screening method; non-GLP Seeds treated with 2% aqueous solution; assessed at 6 and 14 days after treatment (Schafer and Bowles 2004)
Soil microbial function			
Natural soil microflora	28-day Nitrogen transformation	No adverse effect on nitrogen mineralisation at any test concentration	OECD 216; GLP Templeton silt loam, 6.7%OC (cf OECD 0.5–1.5%) , pH 6.8, 42% sand (cf OECD 50–75% sand) Nominal test concentrations at day 0 0, 1, 3.2, 10, 32, 100, 320, 1000 mg/kg dw soil Samples were stored frozen at -80°C prior to analysis. (O'Halloran et al 2005)

Notes

1 Nominal concentrations unless otherwise stated; (test guidelines allow for reporting of nominal concentrations where measured concentrations indicate test substance within 20% of nominal).

2 95% confidence intervals are stated where available.

3 Unless otherwise mentioned, the tests were conducted according to the named guideline.

C3.3 Soil hazard classifications of substances containing 1080

As there is no direct toxicity data for substances containing 1080, the additivity formula for estimating the ecotoxicity of mixtures (set out below) is used to estimate the toxicity of these substances (ERMA 2001). The toxicity value used by the Agency in the mixture calculations is the

lettuce shoot growth EC_{50} 10 mg/kg soil. This study was not available to the Agency at the time substances containing 1080 were transferred to HSNO Act, resulting in the differences in classifications indicated on pp 93 and 94 of the application.

The results of the calculations based on the new data for technical grade 1080, and the soil hazard classifications derived by the Agency and the applicants for substances containing 1080 are set out in Table C14.

$$L(E)C_{50\,mixture} = 100 \Big/ \sum_{\eta} \frac{Ci}{L(E)C_{50\,i}}$$

where:

C_i = concentration of component i (weight percentage)

$L(E)C_{50i}$ = LC_{50} or EC_{50} for component I (mg/kg soil)

η = number of components

The rationale for the applicants' classification of paste containing 10 g/kg 1080 and gel containing 50 g/kg 1080 is not clear in the application. They have used the same EC_{50} value as a starting point but have not indicated whether they used the additivity or summation approach. The threshold for classification under sub-class 9.2 is 100 mg/kg soil. Greater than this value, a substance does not classify as hazardous to the soil environment under the HSNO Act.

In order to classify as 9.2C, the toxicity of the substance must be in the range of 10–100 mg/kg soil, and contain a component which is bioaccumulative (which 1080 is not) and/or a component which has a half-life in soil of >30 days under standard test conditions. Based on the independent experts' opinion, 1080 meets the criteria of having a DT_{50} of <30 days and the Agency has assigned soil hazard classifications accordingly.

Table C14: Soil toxicity classifications of substances containing 1080

Name of substance	Tech. grade 1080	Pellets 0.4–0.8 g/kg 1080	Pellets 1.0 g/kg 1080	Pellets 1.5–2.0 g/kg 1080	Paste 0.6–0.8 g/kg 1080	Paste 1.5 g/kg 1080	Paste 10 g/kg 1080	Gel 1.5 g/kg 1080	Gel 50 g/kg 1080	Gel 100 g/kg 1080	Soluble conc 200 g/L 1080
% 1080	95–98%	0.08	0.1	0.2	0.08	0.15	1	0.15	5	10	20
Estimated EC_{50} (mg/kg soil)		12500	10000	5000	12500	6670	1000	6670	200	100	50
Agency's classification	9.2B	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.2D	9.2D
Applicants' classification	9.2B	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.2C	Does not trigger	9.2C	9.2C	9.2C

C4 Sub-class 9.3: Terrestrial vertebrate ecotoxicity of 1080

There is a very large data set for the toxicity of 1080 to terrestrial vertebrates, much of which is very old (1940s and 1950s) and not conducted according to current standard test guidelines. The most recent data, and those considered by the Agency to be most relevant to the New Zealand situation have been summarised in Tables C15-21 (ie, species present in New Zealand and/or closely related).

In general, mammals are more sensitive to 1080 than birds. Reptiles and amphibians are less sensitive than either of these groups. Differences in sensitivity are likely to be related to differences in metabolic rates and physiology. Australian species which have co-evolved with fluoroacetate-bearing vegetation are more tolerant than those which have no evolutionary exposure to the substance.

Across all species tested, there is a wide variation in the latency period (ie, time until symptoms are observed (0.1 hour to >7 days), time to death (0.1 hours to >21 days) or signs of recovery in the case of sub-lethal exposure (2 hours to 18 days)).

As noted by McIlroy (1986), predicting the toxicity of 1080 to an untested species from data even on closely related species is difficult given the variations in response across and within taxonomic groupings. For indicative purposes, common New Zealand native land birds have been listed in Tables C19 and C20.

The vulnerability or susceptibility of an animal to 1080 in the field is dependent on a number of factors including; food preferences, body weight, food ingestion rates, metabolic rates, as well as its sensitivity to the substance (ie, LD_{50} or other measure of toxicity). These factors are addressed in Appendix N.

Based on the toxicity to the dog (LD_{50} 0.06 mg/kg bw, Toutellotte and Coon 1951), technical grade classifies as 9.3A highly toxic to terrestrial vertebrates.

C4.1 Mammals

Only a subset of the available data on toxicity of 1080 to mammals has been reviewed and included here and summarised in Table C15.

The mammalian toxicity of 1080 as it applies to the assessment of potential human health effects has been addressed in detail under sub-class 6. Likewise, an extensive discussion of the metabolism and mode of action of 1080 is discussed in Sections B15 & B16, Appendix B. A study on the reproductive effects of 1080 on ferret and mink (Hornshaw et al 1986) has been evaluated under sub-class 6.8 (see Appendix B).

Data for native Australian rodents has been excluded, though it is of note that many of these animals are less sensitive to 1080 than the true rats

listed, probably due to their co-evolution with fluoroacetate-producing plants in Western Australia, Queensland and the Northern Territory and development of tolerance over time (McIlroy 1982a). Data for carnivorous Australian marsupials have also been excluded from the summary due to lack of relevance to species present in New Zealand.

C4.1.1 Native New Zealand bats

The New Zealand native short-tailed bat is taxonomically distinct (Family Mystacinidae) from the one species for which there is a toxicity value, the American big brown bat (see Table C15). The native long-tailed bat is in the same family as this American bat. The New Zealand bats feed on arthropods, the short-tailed bat being a ground feeder and the long-tailed bat an aerial feeder.

The sensitivity of the New Zealand bats to 1080 is uncertain, but likely to be within the range of that for other mammals. (See Appendix N for further discussion.)

C4.1.2 Effect of ambient temperature on sensitivity to 1080

The effects of ambient temperature of the toxicity of 1080 to mice (*Mus musculus*), guinea pigs (*Cavies porcellus*) and possums (*Trichosurus vulpecula*) was evaluated under laboratory conditions by Oliver and King (1983). Mice were more sensitive to 1080 at 12.2ºC than at 24ºC (LD_{50} 2.6 mg/kg bw compared to 12.8 mg/kg bw). At the higher temperature of 33ºC the LD_{50} was 4.5 mg/kg bw. A similar variation in toxicity was observed with guinea pigs over the temperature rang 4–32ºC. The LD_{50} for possums was 16.8 mg/kg bw at 10.5ºC and 41.2 mg/kg bw at 23.5ºC. The authors considered that the observed variation in sensitivity with temperature may also be a reason for the variations in reported toxicity to various species in the published literature.

As part of a modelling study to assess the variables which may contribute to the success or otherwise of an aerial possum control operations, temperature was identified as a significant variable (Veltman and Pinder 2001). Based on the assessment of 48 operations between 1994 and 1999, kill rates were correlated with low temperature at both local and regional scales. The authors did note that other factors such as a decrease in 'trappability' of possums in winter may have an influence in assessing the operational outcomes.

Table C15: Toxicity of 1080 to mammals under laboratory conditions

Test species (age/mean weight)	Test type and duration	Test results[1]	Latency period (LP) and time to death (TTD)	Test method (reference)
Rodents				
House mouse, *Mus musculus* Adult/14 g	Acute oral	LD_{50} 8.33 mg/kg bw (95%CI 6.32–11.0)	LP 1.3–2.8 hours TTD 2.2–68.3 hours	Single oral dose; five animals per dose, ambient temperature 20°C Non-GLP Test substance analytical grade 1080. Note: Eight species of indigenous Australian rodents also tested, but not reported here (McIlroy 1982a)
Bush rat, *Rattus fuscipes* Adult/147 g		LD_{50} 1.13 mg/kg bw (95%CI 0.85–1.51)	LP 0.6–5.1 hours TTD 0.7–24.8 hours	
Swamp rat, *R. lutreolus* Adult/154 g		LD_{50} 1.71 mg/kg bw (95%CI 1.39–2.11)	LP 1.7–2.3 hours TTD 2.3–14.4 hours	
Norway rat, *R. norvegicus* Adult/428 g		LD_{50} 1.71 mg/kg bw (95%CI 1.22–2.41)	LP 0.4–2.3 hours TTD 2.5–112 hours	
Ship rat, *R. rattus* Adult/229 g		LD_{50} 0.76 mg/kg bw (95%CI 0.37–1.04)	LP 0.8–27.8 TTD 2.4–36.5	
Kiore, *R. exulans* adult/40–80g				
Marsupial herbivores				
Brushtail possum, *Trichosurus vulpecula* (immature/1.3 kg)	Acute oral	LD_{50} 0.86 mg/kg bw (95%CI 0.67–1.09)	LP 1.0–19.8 hours TTD 5.0–97.0 hours	Single oral dose: Test substance analytical grade 1080. (McIlroy1982b)
Brushtail possum (adult 2.6 kg)		LD_{50} 0.47–0.79 mg/kg bw (95%CI 0.34–1.03) (data from a series of tests) Normal behaviour and feeding during first 1–29 hours after dosing, followed by hypersensitivity to noise or movement; lethargy; shallow respiration, poor coordination and balance, brief convulsions, squeaking		Single oral dose: Test substance analytical grade 1080. (McIlroy1982b)
Brushtail possum (Western Australia)		LD_{50} >125 mg/kg bw		(King et al 1978)

Test species (age/mean weight)	Test type and duration	Test results[1]	Latency period (LP) and time to death (TTD)	Test method (reference)
Tammar/Dama wallaby, *Macropus eugenii* Pouch young/0.9 kg		LD_{50} 0.15 mg/kg bw (95%CI 0.12–0.20)	TTD 11.5–80.5 hours	Single oral dose: Test substance analytical grade 1080. (McIlroy1982b)
Tammar/Dama wallaby adult/7.0 kg		LD_{50} 0.27 mg/kg bw (95%CI 0.23–0.31)	TTD 13.8–37.1 hours	Single oral dose: Test substance analytical grade 1080. (McIlroy1982b)
Tammar/Dama wallaby (Western Australian)		LD_{50} >2.0–10.0 mg/kg bw (95%CI)		(Oliver et al 1979)
Bennett's wallaby, *M. rufogriseus* Adult/13.1 kg		LD_{50} >0.21 mg/kg bw Hunched posture; non-alert, shivering/shaking forelimbs and poor balance; convulsions.. Death from gradual cardiac failure on post-mortem exam.	LP <16.9–23.2 hours TTD 8.9–38.9 hours	Single oral dose: Test substance analytical grade 1080. (McIlroy1982b)
Eutherian herbivores				
Horse, *Equus caballus*	Acute oral	LD_{50} 0.32–0.50 mg/kg bw		Single oral dose (Tucker and Crabtree 1970 cited in McIlroy 1982b)
Cattle, *Bos taurus*	Acute oral	Calves (104–213 kg) n=24 LD_{50} 0.22 mg/kg bw (95%CI 0.15–0.33) Adult cows (469–607 kg) n=10 LD_{50} 0.39 mg/kg bw (95%CI 0.25–0.63) Consistent symptoms in all animals, with absence of gross signs until just before death. Slight lethargy in some animals immediately after dosing, with normal eating and drinking until approx. 10 minutes prior to onset of symptoms. Fatal symptoms lasted 3–20 minutes; urination, staggering, falling down, slight spasms, in-place running, death. LLD[2] 0.156 mg/kg bw (one ♂ calf) Highest dose survived 0.312 mg/kg bw (one cow; one ♂ calf). No post-mortem examinations made	LP 1.5–29 hours TTD varied inversely with dose	Single oral dose (gelatin capsule) at 0.078, 0.156, 0.312 and 0.624 mg/kg bw (Robison 1970)

Test species (age/mean weight)	Test type and duration	Test results[1]	Latency period (LP) and time to death (TTD)	Test method (reference)
Goat, *Capra hircus*	Acute intra-muscular injection	<0.6 LD_{50} mg/kg bw 3 of 4 animals died at 0.6 mg/kg (lowest dose tested);	TTD 4–24 hours	Tested as methyl fluoroacetate (Chenoweth and Gilman 1946)
Pigs 'domestic' young/2–10kg	Acute IP injection	LD_{50} ~0.5 mg/kg		Tested as methyl fluoroacetate (Chenoweth and Gilman 1946)
Sheep, *Ovis aries* Adult wethers/36.4 kg	Acute oral	LD_{50} 0.5 mg/kg bw (95%CI 0.42–0.64) Very weak appearance, slow and noisy breathing, traces of froth from mouth and nose. Survivors had at least one convulsion before resuming grazing, with some problems balancing prior to full recovery. All animals which died showed convulsions; copious white froth from mouth and nose.	LP 6.2–37.6 hours TTD 9.6–61.6 hours Recovery of survivors 45.5 to 120 hours after dosing	Single oral dose Test substance analytical grade 1080. (McIlroy1982b)
	Acute	Fifty-two adult ewes dosed with 0.25–0.3 mg/kg bw. Twenty-one died within four days of dosing and no further deaths occurred. Lesions in lungs and cardiac tissues of animals which died from 1080 poisoning. Surviving animals were monitored for 2 years; no differences observed between dosed and control animals in any of the following: incidence of infection chronic organ damage mortality general condition/liveweight fleece weight lambing percentage lamb birth weight lamb survival and growth rate		(Wickstrom et al 1997)

Test species (age/mean weight)	Test type and duration	Test results[1]	Latency period (LP) and time to death (TTD)	Test method (reference)
		Scattered foci of fibrous tissue observed in cardiac muscle at necropsy of animals after two years. The authors concluded that the significance of these lesions was uncertain given the absence of biological effects observed in the live animals. The lesions are non-specific and could be attributed to any event (toxic or non-toxic) resulting in damage to the heart muscle but were not present in the control animals.		
Adult perendale ewes	Acute oral	Acute 45% mortality in pregnant ewes 20% mortality in non-pregnant ewes		Pregnant and non-pregnant ewes fed 0.25 mg 1080/kg (as a standard cereal bait) (O'Connor et al 1999)
	3-day repeat dose	Three-day repeat 35% mortality in pregnant ewes no mortality in non-pregnant ewes Lesions in lungs and cardiac tissues of animals which died from 1080 poisoning. No differences in incidence of metabolic diseases, lambing percentages; lamb survival or growths rates between surviving dosed animals and controls.		Pregnant and non-pregnant ewes fed 0.05 mg 1080/kg (as a standard cereal bait) on three consecutive days. (O'Connor et al 1999)
Mule deer, *Odocoileus hemionus* 8–11 months		0.27–0.9 LD_{50} mg/kg bw		(Tucker and Crabtree 1970 cited McIlroy 1982b)
Fallow deer, *Dama dama*		Estimated to be similar to that of sheep (ie, LD_{50} 0.45 mg/kg bw)	TTD 2.0–30 hours	(Daniel 1966)
European rabbit, *Oryctolagus cuniculus*	Acute oral	Immature/0.8 kg LD_{50} 0.35–0.37 mg/kg bw (95%CI 0.30–0.42) (data from a series of tests) Adult/1.5 kg LD_{50} 0.34–0.50 mg/kg bw (95%CI 0.26–0.58) (data from a series of tests) Increased sensitivity to noise or disturbance, followed by recovery or onset of further symptoms: convulsions with coughing or squeaking and death	LP 1.1–10.1 hours TTD 3.0–44.3 hours Recovery of survivors 5–23.2 hours after dosing	Single oral dose: Test substance analytical grade 1080. (McIlroy1982b)

Test species (age/mean weight)	Test type and duration	Test results[1]	Latency period (LP) and time to death (TTD)	Test method (reference)
Carnivores				
Dog, *Canis familiaris*	Acute oral	LD_{50} 0.06 mg/kg bw LD_{100} 0.08 mg/kg bw LLD 0.05 mg/kg (1/6 dogs) Sudden onset of overexcitement, generalised muscular twitching and chronic convulsions	Mean TTD not dose dependent 4 hours at 0.12 mg/kg bw 5 hours at 0.05, 0.07 mg/kg bw 6 hours at 0.08 mg/kg bw 9 hours at 0.06 mg/kg bw	Single oral dose in water after 16 hour fast Test doses: 0.05, 0.06, 0.07, 0.08, 0.12 mg/kg bw (Tourtellotte and Coon 1951)
Dingo, *Canis familiaris dingo*, (adult)	Acute oral	LD_{50} 0.11 mg/kg bw (95%CI 0.09.0.15)	LP 4.8–14.6 hours TTD 20.7– 21 hours recovery time <24–28 hours	Single oral dosing, but in some cases, survivors were given a second, higher dose after an eight-day recovery period due to shortages of some test animals (dingoes, cats) Test substance analytical grade 1080. Note: Ten carnivorous marsupial species also tested, but not found in New Zealand and have not been included here (McIlroy 1981)
Cat, *Felis catus* immature/1 kg	Acute oral	LD_{50} 0.40 mg/kg bw (95%CI 0.31–0.52)		
Cat, *Felis catus* Wild-caught mixed-age feral cats; bodyweight range 0.6–4.8 kg	Single bait	LD_{50} 0.28 mg/kg bw (95%CI 0.07–0.49) LD_{90} 0.35 mg/kg bw (95%CI 0.14–0.56) Onset of symptoms was dose-dependent and included; disorientation, lethargy, with immobility for several hours before death	TTD varied but all died <24 hours in the three highest test groups	Non-standard; non-GLP Animals acclimatised for two months and then fed a single 1 g bait containing 1080 at varying concentrations. Test concentrations in one gram bait 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16% Purpose of trial to determine most appropriate bait concentration for feral cat control (Eason and Frampton 1991)

Test species (age/mean weight)	Test type and duration	Test results[1]	Latency period (LP) and time to death (TTD)	Test method (reference)
European ferret, *Mustelo putorius furo*	Acute oral	LD_{50} 1.41 mg/kg bw (yearling)		(Tucker and Crabtree 1970)
	28-day dietary	**Adult** LC_{50} 9.4 mg/kg diet (95%CI 6.1–14.5 mg/kg diet) Reduced feed consumption in all groups by day 3; adults: food avoidance, lack of coordination, paralysis of hindquarters and unconsciousness. Reduced haematocrit at 8.56 mg/kg diet. Decreased weight of liver and spleen. **Young** No young ferrets died during the 28-day exposure period. Significant reductions in red and white blood cell counts in all young ferrets and in haemoglobin and haemotcrit in the highest 3 concentration groups. Decreased weight of liver, kidney and thymus. Testes weight significantly reduced at 1.94 and 3.50 mg/kg diet. No gross lesions observed at post-mortem	LP adults three days; young five days	Protocol developed for toxicity testing of carnivores; non-GLP Test concentrations 2.5-month-old animals 0, 1.08, 1.94 3.5 mg/kg diet >1 year old animals 0, 4.76, 8.56, 15.4 mg/kg diet (Hornshaw et al 1986)
Mink, *M. vison*	Acute oral	Approximate lethal dose 0.25 mg/kg bw		Range-finding test to determine suitable dosing for further testing. Single oral dose by gavage. Protocol developed for toxicity testing of carnivores; non-GLP (Hornshaw et al 1986)
	28-day dietary	Test concentrations 0, 0.50, 0.90, 1.62, 2.90, 5.25 mg/kg diet LC_{50} 3.2 mg/kg diet (95%CI 2.4–4.5 mg/kg diet) Reduced haemoglobin levels at ≥1.62 mg/kg diet; significant reduction in bodyweight at 0.9 mg/kg diet (♂) and 2.9 mg/kg diet (♀); reduction in feed consumption at 0.9 mg/kg diet by day 3; no gross lesions observed at post-mortem.		Protocol developed for toxicity testing of carnivores; non-GLP Animals six months old at time of test initiation (Hornshaw et al 1986)

Test species (age/mean weight)	Test type and duration	Test results[1]	Latency period (LP) and time to death (TTD)	Test method (reference)
Bats				
Family Vespertilionidae American big brown bat, *Eptesicus fuscus*	Acute	LD_{50} 0.15–0.21 mg/kg bw		Very limited information available on the test protocol: was either acute oral or IP injection. Two LD_{50} values were listed, but no clarification as to the basis of these (eg, different sexes or ages) and have been expressed here as a range. (Schafer and Bowles 2004)
Long-tailed bat, *Chalinolobus tuberculata*				
Family Mystacinadae Short-tailed bat, *Mystacina tuberculata*				

Notes

1 95% confidence intervals are stated where available.

2 LLD = lowest lethal dose.

C4.2 Birds

C4.2.1 Acute toxicity

Detailed regulatory-type studies are summarised in Table C17 and include dietary exposure, food and water avoidance studies. The Agency only had access to the results of the studies as presented in the open literature, and not to the full reports, including the two dietary studies submitted to the USEPA (Campbell 1984a, 1984b) for which only the end points are available (USEPA 1995).

There is a large 'non-regulatory' data set on the toxicity of 1080 to birds, much of the more recent studies having been undertaken in Australia (McIlroy 1984). Some of these data are summarised briefly in two 'compilation' tables (Table C19 for non-passerine birds and Table C20 for passerine birds). The data are largely restricted to acute dosing by either oral or subcutaneous exposure. In many cases, sub-cutaneous injection was probably the route of choice due to the difficulties in administering an oral dose. As noted in the section on human toxicology, the route of exposure makes little apparent difference in response and therefore the data from the different routes are considered to be comparable for the purposes of assessing relative toxicity.

Assessment of biochemical and histopathological changes in the serum and muscles of adult mallard ducks dosed with 8 mg 1080/kg bw indicates that skeletal muscle damage may occur in birds exposed to 1080 (Ataria et al 2000). The study was conducted for 24 hours only, with birds sacrificed at 0, 2, 4, 6, 12 and 24 hours after dosing. The source and history of the test animals was not reported. Mortalities which occurred during the study were also not reported. The dosing was at or above the LD_{50} values for mallard (see Table C17). Peak concentrations of 1080 in serum and heart tissue largely coincided with the onset of clinical signs of toxicity. Residues of 1080 were substantially eliminated within 24 hours of dosing.

Differences in sensitivity

Statistically significant differences in the sensitivity of different groups of Australian birds were observed by McIlroy (1983). Of 120 different comparisons made, 41 were significant. Australian parrots are significantly more sensitive than other groups of Australian non-passerines. Australian doves and waterfowl are more tolerant than their introduced counterparts possibly as a result of co-evolution with vegetation containing 1080 and/or differences in metabolic rates.

Sparrows were the most sensitive of the six bird species tested by Tucker and Haegele (1971), with overall sensitivity sparrow>chukar>pigeon>pheasant>mallard>coturnix quail.

The more common New Zealand native birds have been listed in the compilation tables in the taxonomic groups for those species where toxicity data are available, but their sensitivities relative to those of the

introduced species is not known. In the tables, native species are indicated by shading. Bodyweights of New Zealand native birds were sourced from Robertson and Heather (1999). In the study by McIlroy (1984) all birds were adults and were dosed by sub-cutaneous injection.

The Order Passeriformes is under fairly constant revision. As far as possible, the species listings in the following tables are based on those of the New Zealand Ornithological Society's list of recognised bird names (OSNZ 2003).

Effect of age on sensitivity to 1080

In a study to investigate whether young birds are more susceptible to 1080 than more mature birds, mallard ducks of varying ages were given a single oral dose (by gavage) (Hudson et al 1972). There was no consistent pattern of sensitivity across the different age groups of birds; the youngest birds appeared to be less sensitive than older birds (see Table C16 below).

Table C16: Susceptibility to 1080 across different age groups of birds

Age of test birds	LD_{50} (95%CI) mg/kg bw
36 ± 3 hours	9.78 (6.31–15.2)
7 ± 1 day	3.71 (2.50–5.48)
30 ± 3 days	3.71 (-)
6 months ± 3 days	4.81 (2.57–8.99)

Source: Hudson et al 1972.

Table C17: Acute toxicity of 1080 to birds under standard laboratory conditions

Test species	Test type and duration	Test results	Test method (reference)
Bobwhite quail, *Colinus virgianus*	Five-day dietary exposure + three-day post-exposure monitoring	LC_{50} 486 ppm diet (95%CI 339–696)	USEPA Guideline; GLP End point data only (Campbell et al 1984a in USEPA 1995)
		LC_{50} 385 mg/kg diet (95%CI 0–485) Significant reductions in food consumption and bodyweight at ≥95 mg/kg diet, with increases in both during the 3-day post-exposure period. Lowest lethal concentration 142 mg/kg diet	Stated as standard USEPA method; GLP status unknown Test concentrations 0, 95, 142, 213, 320,480, 720 mg/kg diet (Kononen et al 1991)
	Five-day drinking water exposure + three-day post-exposure monitoring	LC_{50} 31 mg/L Feed consumption and bodyweight declined significantly at ≥29 mg/L All birds died in the three highest dose groups	Drinking water LC_{50}; non-GLP Test concentrations of 0, 18, 29, 51, 93, 167, 300 mg/L (Kononen et al 1991)
	Water avoidance Five-day exposure + three-day post-exposure monitoring	At 9 mg/L, treated water accounted for 44% of total water consumption, indicating that the avoidance threshold for this species is <9mg/L	Birds offered choice of contaminated and uncontaminated drinking water and normal food rations for five days. Test concentrations of 0, 9, 16, 29, 51, 93 mg/L (Kononen et al 1991)
	Food avoidance Five-day exposure + three-day post-exposure monitoring	Birds did not discriminate between treated and untreated food until day 2 of exposure. At 95 mg/kg treatment consumption accounted for 29% of total. Nine of 10 birds survived in all treatments.	Birds offered choice of contaminated and uncontaminated feed for five days. Test concentrations 0. 95, 142, 213, 320, 480 mg/kg diet (Kononen et al 1991)

Test species	Test type and duration	Test results	Test method (reference)
Mallard duck, *Anas platyrhynchos*	Five-day dietary exposure + three-day post-exposure monitoring	LC_{50} 231 ppm diet (95%CI 150–338)	USEPA Guideline; GLP End point data only (Campbell et al 1984b in USEPA 1995)
		LC_{50} 527 mg/kg diet (95%CI 373–3522) Significant reductions in food consumption and bodyweight at ≥236 mg/kg diet with increases in both during the 3-day post-exposure period. Lowest lethal concentration 236 mg/kg diet	Test concentrations 0, 236, 295, 369, 461, 576 and 720 mg/kg diet Standard USEPA method; GLP status unknown (Kononen et al 1991)
	Five-day drinking water exposure + three-day post-exposure monitoring	LC_{50} 18 mg/L Significant reduction in water, feed consumption and bodyweight at >13 mg/L; Only one bird survived exposure to 24 mg/L, and one died at 13 mg/L	Drinking water LC_{50} and water avoidance study; non-GLP Test concentrations of 0, 13, 24, 43, 77,138, 250 mg/L (Kononen et al 1991)
	Water avoidance Five-day exposure + three-day post-exposure monitoring	At 7 and 13 mg/L birds consumed equal amounts of treated and untreated water, indicating an avoidance threshold of 13–24 mg/L. Significant reduction in water and feed consumption an bodyweight at >7 mg/L	Birds offered choice of contaminated and uncontaminated drinking water and normal food rations for five days. Test concentrations of 0, 7, 13, 24, 43,77 mg/L (Kononen et al 1991)
	Food avoidance Five-day exposure + three-day post-exposure monitoring	Birds able to discriminate between treated and untreated food from day 1 of exposure. At 236 mg/kg treatment consumption accounted for 11% of total intake.	Birds offered choice of contaminated and uncontaminated feed for five days. Test concentrations 0, 236, 295, 369,461, 565 mg/kg diet (Kononen et al 1991)

Test species	Test type and duration	Test results	Test method (reference)
Magpie (US), *Pica pica*	Acute oral	Indoors LD_{50} 1.78 mg/kg bw (95%CI 1.62–1.98) Outdoors LD_{50} 1.91 mg/kg bw (95%CI 1.66–2.20) summer LD_{50} 2.30 mg/kg bw (95%CI 2.06–2.56) winter Average for all conditions 2.00 mg/kg bw progressive symptoms nervousness, lethargy, ataxia, recumbency, seizures	USDA method; non-GLP Wild-caught adult birds Acute oral test conducted under three different regimes; indoors, and outdoors in summer (6–33ºC) and winter (-18–4ºC) Acute dietary exposure—indoors (Burns and Connolly 1992)
	Acute dietary	Test 1 concentrations 0, 2.5, 5.0, 10.0 and 20 ppm diet Test 2 concentrations 0, 40 and 80 ppm diet Results were combined to calculate LD_{50} 20 ppm diet (96.9%CI 10–40); weight loss was significant at ≥5 ppm, but survivors regained weight. No consistent effect on feed consumption up to 20 ppm. At 40 and 80 ppm feed consumption decreased and birds died rapidly	

Acute toxicity of 1080 to New Zealand native birds

The weka (*Gallirallus australis*) and tauhou/silvereye (*Zosterops lateralis*) are the only native New Zealand birds for which toxicity data are available. The weka study was undertaken in 1966 (McIntosh et al 1966) and measurement of 1080 concentrations was made using an old analytical method, while the study with silvereye was part of large study of the sensitivity of Australian birds to 1080 (McIlroy 1984). Summary information is included in Table C20.

Acute toxicity of 1080 to other birds

Data are available on a number of introduced birds which are found in New Zealand, and these are included in the compilation tables (Tables C19 and C20).

Table C18: Laboratory testing of New Zealand native birds

Test species	Test type and duration	Test results	Latency period (LP) and time to death (TTD)	Test method (reference)
North Island weka, *Gallirallus australis greyi*	'Single' oral dose	Approx. LD_{50} 8.1 mg/kg based one death at 6.75 mg/kg, 3 deaths at 8.1 mg/kg and all birds dying at the highest two doses. Birds dosed at 2.25 and 4.5 mg/kg, and one at 6.75 mg/kg showed no symptoms of poisoning NOEL mortality 4.5 mg/kg bw The birds were not fasted prior to dosing, and there was wide variation in the bodyweight of the birds tested, with the smaller birds (576–942 g) given the highest doses (9 and 18 mg/kg)	TTD Variable 4 to 18 hours Survivors of all dosing regimes showed a range of symptoms including: loss of balance; periodic adoption of threat or defence posture; increased respiration; circling on the spot; coma.	Non-standard; non-GLP Wild-caught weka Single oral dose Birds each given a dose of either 2.25 mg/kg (n=1), 4.5 mg/kg (n=1) 6.75 mg/kg (n=4), 8.1 mg/kg (n=6 of which 4 had previously survived an earlier dosing 15 days before); 9.0 mg/kg (n=2), 18 mg/kg (n=1) Analysis of residues based on organic fluorine (McIntosh et al 1966)
	Repeat oral dose 14-day	The birds at the three lowest daily doses showed no symptoms after 14 days; the bird receiving the highest daily dose died after 5 doses Birds dosed at 0.9 and 1.8 mg/kg maintained normal bodyweight throughout the dosing period. The bird dosed at 2.7 mg/kg lost 100g in the first three days of dosing and then regained weight.		Four survivors from the single oral dose study were rested for 11 days and then dosed daily with 0.9, 1.8, 2.7 and 3.6 mg/kg bw/day for 14 days (McIntosh et al 1966)
Silvereye, *Zosterops lateralis*	Acute sub-cutaneous injection in back of neck	Approximate lethal dose 9.25 mg/kg bw	LP 1.1–1.8 hours TTD 13.9–14.8 hours	Non-standard; non-GLP Wild-caught birds One bird at each of seven dose levels (McIlroy 1984)

Table C19: Acute toxicity of 1080 to non-passerine birds: data compilation

Taxonomic Order Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time (hours)	Reference
Struthioniformes								
Emu, *Dromaius novaehollandiae*	2680	1800–3500	-	278	1.5–5.8	124	29.4	McIlroy 1984
Anseriformes (ducks, geese swans)								
Pacific black duck, *Anas superciliosa*	950	690–1040	♂ 18.91 (16.33–21.89) ♀ breeding 10.01 (7.43–13.48) non-breeding 23.8 (15.3–37.03)	-	1.0–5.7	1.4–75.7	7.0–168	McIlroy 1984
Mallard, *A. platyrynchos*		1090–1260	5.61–8.56 (3.99–13.72)		2.0–5.9	1.9–36.7	12.5	Tucker and Crabtree 1970 cited in McIntosh 1984
Maned duck, *Chenonetta jubata*	730	610–860	12.6 (10.14–15.67)					McIlroy 1984
Whio/blue duck, *Hymenolaimus malacorynchos*		♂ 900 ♀ 750						
Falconiformes (hawks, vultures)								
Black kite, *Milvus migrans*	580	490–690	18.51 (14.97–23.17)		1.0–3.9	3.2–47	10.6–44	McIlroy 1984
Wedge-tailed eagle, *Aquila audax*	3180	2260–4250	9.49 (7.2–12.51)		1.0–60	8.0–158.5	50.4–166.0	McIlroy 1984
Marsh hawk, *Circus cyaneus hudsonii*				~10 (vomited after dosing)				(Ward and Spencer 1947)
Kahu/Australasian harrier, *Circus approximans*		♂ 650 ♀ 850						
Karearea/New Zealand falcon, *Falco novaeseelandiae*		♂ 300 ♀ 500						
Galliformes (domestic fowl, gamebirds)								

Taxonomic Order Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time (hours)	Reference
Common turkey, *Meleagris gallopavo*		4310–8080	3.76 (1.13–12.50)					Tucker and Crabtree 1970 cited in McIntosh 1984
Californian quail, *Lophortyx californica*		140–180	1.0–5.0					California Fish and Game 1962; Sayama and Brunetti 1952 cited in McIntosh 1984
Domestic fowl, *Gallus gallus*		2270–3400	7–8 mg/kg (Cottral et al 1947 noted slightly greater sensitivity in laying hens LD_{50} 6–7 mg/kg compared with non-laying hens LD_{50} 9–10 mg/kg; maximum tolerated dose 4–5 mg/kg bw)					Cottral et al 1947
Ring-necked pheasant, *Phasianus colchicus*		970–1340	6.07 (3.62–10.15)					Tucker and Crabtree 1970 cited in McIntosh 1984
Gruiformes (Ralliformes)								
Weka, *Gallirallus australis*		♂ 1000 ♀ 700		8.1		4–18		McIntosh 1966
Pukeko, *Pophyrio porphyrio*		♂ 1050 ♀ 850						
Columbiformes (doves, pigeons)								
Bar-shouldered dove, *Geopelia humeralis*	130	110–150	16.25 (14.51–18.20)		2.4–4.2	2.1–25.1	49.2–49.3	McIlroy 1984
Diamond dove, *G. cuneata*	30	25–35	35.50 (27.17–46.36)		1.2–7.2	1.1–38.9	38.7–168	McIlroy 1984

Taxonomic Order Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time (hours)	Reference
Feral pigeon, *Columba livia*	328	190–340	3.98 (3.16–5.02)					Tucker and Crabtree 1970 cited in McIntosh 1984
Laughing dove, *Streptopelia senegalensis*		100–110	5.46 (3.90–7.63)					D. King personal communication, in McIlroy 1984
Kereru/kukupa/New Zealand wood pigeon, *Hemiphaga novaeseelandiae*		650 (mainland)						
Psittaciformes (parrots)								
Galah, *Cacatua roseicapilla*	280	240–410	4.67 (3.10–7.04)		1.7–31	3.4–108	35.7–56.9	McIlroy 1984
Sulphur-crested cockatoo, *C. galeria*	820	760–910	3.46 (2.90–4.14)		9.9–17.7	9.0–73.7	-	McIlroy 1984
Yellow-tailed black cockatoo, *Calyptorynchus funereus*		540–790		1.84				D. King personal communication, in McIlroy 1984
Budgerigar, *Melopsittacus undulatus*	30	30–50		2.13	1.1–3.3	14.3–39.1	-	McIlroy 1984
Crimson rosella, *Platycercus elegans*	150	130–170		0.88	3.4	12.9–45.9	-	McIlroy 1984
Eastern rosella, *P. eximis*	100	90–110		3.45	-	10.3–13.3		McIlroy 1984
Port Lincoln ringneck, *Barnardius zonarius*		120–180		>9.20				D. King personal communication, in McIlroy 1984
Red-rumped parrot, *Psephotus haematonotus*	60	50–60		5.25	3.1	14.4–14.8	-	McIlroy 1984
Kaka, *Nestor meridionalis*		♂ 525 ♀ 475						
Kea, *N. notabilis*		♂ 1000 ♀ 800						
Kakariki/red-crowned parakeet, *Cyanoramphus novaezelandiae*		♂ 80 ♀ 70						

Taxonomic Order Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time (hours)	Reference
Kakariki/yellow-crowned parakeet, *C. auriceps*		♂ 50 ♀ 40						
Cuculiformes (cuckoos)								
Fan-tailed cuckoo, *Cuculus pyrrhophanus*	40	40–50		>6.0				McIlroy 1984
Pipiwharauroa/shining cuckoo, *Chrysococcyx lucidas*		25						
Koekoea/long-tailed cuckoo, *Eudynanys taitensis*		125						
Strigiformes (owls)								
Great horned owl. *Bubo virginianus pallescens*				~10 (vomited after dosing)				Ward and Spencer 1947
Ruru/morepork, *Ninox novaeseelandiae*		175						
Coraciiformes (kingfishers)								
Laughing kookaburra, *Dacelo novaeguineae*	280	190–360		>6.0				McIlroy 1984
Kotare/New Zealand kingfisher, *Halcyon sancta*		65						

Notes

1 Bodyweight of birds tested.

2 Average bodyweight for the species.

3 ALD = approximate lethal dose; calculated as the mean of the lowest dose level at which an individual died and the highest level at which and individual survived (one animal per dose, reduces the number of animals required for testing).

Table C20: Acute toxicity of 1080 to passerine birds: data compilation

Taxonomic family Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time (hours)	Reference
Acanthisittidae (New Zealand wrens)								
Titipounamu/rifleman, *Acanthisitta chloris*		♂ 6 ♀ 7						
Muscicapidae (flycatchers, thrushes etc)								
White's thrush, *Zoothera dauma*	90	80–120		>12.0				McIlroy 1984
Grey shrike-thrush, *Colluricincla harmonica*	70	60–70		>12.0	1.2	-	2.7	McIlroy 1984
Blackbird, *Turdus merula*	80	70–100		9.5	5.8	10.8–30.5	-	McIlroy 1984
Pachycephalidae (whistlers and allies)								
Golden whistler, *Pachycephala pectoralis*	30	20–30		>18.0	4.1–6.1	-	18.0–24.9	McIlroy 1984
Popokatea/whitehead, *Mohoua albicilla*		♂ 18.5 ♀ 14.5						
Mohua/yellowhead, *M. ochrocephala*		♂ 30 ♀ 25						
Pipipi/brown creeper, *M. novaeseelandiae*		♂ 13.5 ♀ 11						
Acanthizidae (Australasian warblers)								
White-browed scrub wren, *Sericornis frontalis*	10	11–12		4.5	-	5.7	-	McIlroy 1984
Riroriro/grey warbler, *Gerygone igata*		6.5						
Monarchidae (monarch flycatchers)								
Piwakawaka/fantail, *Rhipidura fuliginosa*		8						
Eopsaltridae (Australasian robins)								
Eastern yellow robin, *Eopsaltria australis*	20	20–30		11.65	9.0	28.8	-	McIlroy 1984
Miromiro/tomtit *Petroica macrocephala*		11						
Toutouwai/New Zealand robin *P. australis*		35						

Taxonomic family Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time (hours)	Reference
Zosteropidae (white-eyes)								
Tauhou/silvereye, *Zosterops lateralis*	10	12–17		9.25	1.1–1.8	13.9–14.8	-	McIlroy 1984
Maluridae (fairy wrens)								
Superb fairy wren, *Malurus cyaneus*	10	9–11		3.38	1.8	40.5	-	McIlroy 1984
Meliphagidae (honeyeaters)								
Little wattlebird, *Anthochaera chrysoptera*	70	60–70		7.75	3.5	24.5	-	McIlroy 1984
New Holland honeyeater, *Phylidonyris novaehollandiae*	20	19–23		7.99	1.0–15.1	60.3	14.7–27.5	McIlroy 1984
Yellow-faced honeyeater, *Lichenostomus chrysops*	10	14–19		8.00	2.1–3.2	3.9	-	McIlroy 1984
Yellow-tufted honeyeater, *L. melanops*	20	19–23		<7.5	1.1	4.6	-	McIlroy 1984
Korimako/bellbird *Anthornis melanura*		♂ 34 ♀ 26						
Tui, *Prosthemadera novaeseelandiae*		♂ 120 ♀ 90						
Fringillidae (finches)								
European goldfinch, *Carduelis carduelis*	10	13–14		3.50	-	6.1	-	McIlroy 1984
Zebra finch, *Poephila guttata*	10	10–13		3.13	1.8-5.3	14.3–17.0	-	McIlroy 1984
Ploecidae (weaverbirds; Passerinae sparrows)								
Red-browed firetail, *Emblema temporalis*	10	8–11	0.63 (0.41–0.96)		2.2–28.2	12.4–26.2	72.2	McIlroy 1984
House sparrow, *Passer domesticus*		27–30	2.82 (2.24–3.55)					Tucker and Crabtree 1970 cited in McIntosh 1984
Sturnidae (starlings)								
Common starling, *Sturnus vulgaris*	70	60–80		4.75	1.1–5.2	3.6–13.2	-	McIlroy 1984
Callaeidae (New Zealand wattlebirds)								

Taxonomic family Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time (hours)	Reference
Kokako, *Callaeas cinerea*		230						
Tieke/saddleback, *Philesturnus carunculatus*		♂ 80 ♀ 70						
Corcoracidae (white-winged chough and apostlebird)								
White-winged chough, *Corcorax melanoramphus*	310	220–390		1.75	14.0–24.1	13.3–61.6	-	McIlroy 1984
Grallinidae (magpie-larks)								
Australian magpie-lark, *Grallina cyanoleuca*	100	70–120	8.83 (3.96–13.47)		4.1–4.8	6.7–120.9	14.8	McIlroy 1984
Cracticidae (bell magpies)								
Australian magpie, *Gymnorhina tibicen*	330	250–380	9.93 (7.59–12.92)		3.6–10.7	5.7–59.5	34.3–34.5	McIlroy 1984
Pied currawong, *Streptera graculina*	290	250–360	13.09 (10.90–15.72)		1.9–11.4	4.0–262.3	14.4–24.0	McIlroy 1984
Corvidae (crows, ravens and jays)								
Australian raven, *Corvus coronoides*	590	460–750		5.1	3.7–29.8	5.7–37.5	-	McIlroy 1984
Little raven, *C. mellori*	570	460–650	3.10 (2.68-3.59)		7.3–11.0	9.9–86.5	-	McIlroy 1984
Little crow, *C. bennetti*	390	320–460	13.37 (11.73–15.24)		1.1–7.1	4.1–10.9	5.9	McIlroy 1984

Notes

1 Bodyweight of birds tested.

2 Average bodyweight for the species.

3 ALD = approximate lethal dose; calculated as the mean of the lowest dose level at which an individual died and the highest level at which and individual survived (one animal per dose, reduces the number of animals required for testing).

C4.2.2 Longer term studies

Brief results of a sub-acute toxicity study with mallard ducks indicate a 30-day minimum lethal dose of 0.5 mg/kg bw/day for 1080 (Tucker and Crabtree 1970). Groups of six birds were administered a gelatine capsule containing 1080 at geometrically spaced doses each day. The lowest daily dose resulting in one or two deaths at the end of the 30-day period was determined to be the minimum lethal dose. No details were provided of the signs of toxicity or whether any investigation of organ damage/histopathological examination was undertaken.

Only a single study on potential long term effects of sub-lethal exposure to 1080 in birds was submitted by the applicants (Balcomb et al 1983) and mentioned briefly in the section on reproductive toxicity (p 101 of the application) and the environmental effects register (p 301 of the application). In the reproductive classification section of the application, it is noted that there were effects on testes in starling, when the results of the study are stated by the study authors as not being statistically significant.

Adult male starlings were fed a diet containing 15 mg 1080/kg over a four-week period following a five-day range-finding study to determine a suitable sub-lethal test concentration in the diet (Balcomb et al 1983). The concentration chosen for the four-week study was half the no effect concentration in the five-day study. There were no significant differences in the bodyweights of test and control birds at the beginning of the study, but they were significantly different at the end of the study, though not assessed as being clearly treatment related by the study authors. Histopathological examinations of the testes indicated no significant differences in sperm abundance or development, nor were any lesions observed. Starlings exposed to 1080 had approximately 14% less testis weight development than control birds, but this was not statistically significant. The biological relevance of the results is unknown.

The Agency has not been able to locate any data from standard tests on the reproductive toxicity of 1080 to birds. Given that the application of 1080 at some locations may overlap with the breeding season of some birds (Atkinson et al 1995), a full reproductive study (eg, OECD guideline 206, (OECD 1984) or the revised draft OECD avian reproduction protocol (OECD 2000) would assist in assessing whether short-term sub-lethal exposure may impact on reproduction (Mineau et al 1999).

C4.3 Reptiles and amphibians

An Australian study investigated the acute toxicity of 1080 to Australian native frogs and reptiles following oral administration of analytical grade 1080 (McIlroy et al 1985). While generally more tolerant to 1080 than birds and mammals, the time to onset of symptoms and time to death are long. Signs of toxicity included lethargy or lack of movement associated with thermoregulation (eg, not moving into or out of shade). Of all animals tested, 82% which showed signs of poisoning died, and 18% survived. Shingle-back lizards sourced from Western Australia where the

vegetation has a naturally high 1080 content were less sensitive compared with those sourced from South Australia.

Data from the older studies (eg, Chenoweth and Gilman 1946; Quin and Clark 1947) are all of very limited reliability due to the lack of information available in the published literature.

The effects of 1080 on plasma testosterone have been examined in the Australian shingle-backed lizard *Tiliqua rugosa* from south-west Western Australia (Twigg et al 1988). A single dose (100 or 250 mg/kg bw) caused a decrease in plasma testosterone in males by 52% at day 3, with no effect observed at 25 mg/kg bw. By day 12, the plasma testosterone levels in the 250 mg/kg dose group had reached 81% of the initial concentration, indicating recovery from exposure. When the dose was distributed over 12 days, at 3 day intervals, a lesser effect was observed (and not statistically different to that of the controls), with a steady decline in plasma testosterone over 15 days, but still within bounds for normal reproductive function. Some degeneration of seminiferous tubules was observed in the high dose groups, but did not occur consistently in all animals (ie, was not clearly dose-dependent).

C4.3.1 Native New Zealand frogs and reptiles

New Zealand native frogs are distinctly different taxonomically from any of the species tested and there is significant uncertainty as to their sensitivity to 1080 relative to that of other frogs, despite some preliminary investigations.

The toxicity of 1080 to New Zealand two species of native frogs (Archey's frog *Leiopelma archeyi* and Hochstetter's frog, *L. hochstetteri*) has been assessed via laboratory exposure to contaminated water and prey and to baits containing 1080 (Perfect and Bell 2005). The trials were preliminary, rather than providing standard end point data.

In the first trial, to assess exposure to 1080-contaminated water, 12 adult frogs of each species were maintained under laboratory conditions and were dehydrated under controlled conditions prior to placement in treated water, presumably to attain a 'worst-case' state prior to exposure. The water was prepared by soaking a 0.17% Wanganui No 7 bait (6g) in 40 mL of distilled (bait dyed green and cinnamon lured). Control solutions were prepared from non-toxic, undyed and unlured baits, which had a measured 1080 concentration of 0.2 mg/kg. Frogs were then placed in the jars, which still contained the soaked bait, for two hours, then removed, blotted to remove excess water and reweighed. Two frogs of each species were killed for subsequent analysis. The remaining frogs were kept under observation in individual terraria for 24 hours. Measured mean concentrations in the test solutions were 230 mg/L (Archey's) and 205 mg/L (Hochstetter's) and residues in Archey's frogs were 0.6–4.1 mg/kg (mean 2.65 mg/kg) and in Hochstetter's 0.1–1.3 mg/kg (mean 0.98 mg/kg). Residues were also measured in the control frogs at <0.1–0.4 mg/kg and in the control solutions at (<0.1–0.2 mg/L) and were

attributed to some contamination which occurred during handling of the frogs. Archey's frogs were noted as generally immobile in the post-immersion observation period, with occasional attempts at climbing, while Hochstetter's frogs were more active. All of the latter frogs had been in direct contact with the bait during the trial, but fewer Archey's frogs were observed in contact. In a pilot study for trial 1, one Hochstetter's frog exhibited symptoms which were noted as possibly being attributed to 1080 poisoning.

In the second trial, frogs were maintained in individual terraria containing a single 6 g toxic or control bait (as above). Frogs were observed for 38 days in total, mouldy baits were replaced at day 14. Live house flies were provided as food, but flies were prevented from accessing the baits to remove the possibility of exposure from secondary poisoning (ie, uptake of 1080 by the flies prior to consumption by the frogs). Of the two individual frogs sampled for 1080 residues, the Archey's frog contained 2.4 mg/kg and the Hochstetter's frog contained 3.8 mg/kg. A number of frogs died during the study, including one of the two control Archey's frogs which contained 1.4 mg 1080/kg. As noted by the study authors the small numbers of animals used and the complexities in monitoring frogs (cryptic and variable behaviour during the study) make it difficult to draw any conclusions regarding causes of death in relation to 1080 exposure.

In the third trial, frogs were fed houseflies which had been maintained in either close (confined in a small space with the bait for one hour prior to release) or casual (in terraria with bait but no otherwise confined) contact with dry and moist baits prior to having access to the frog enclosures. Frogs were fasted for 10 days prior to feeding then given access to the flies for 24 hours. Only one frog of each species was used for each treatment combination. Measured residues in flies in casual contact with baits were 31 and 33 mg/kg and in close contact were 26 and 91 mg/kg. Corresponding residues in the frogs feeding on these flies were 1.3, 0.41, 2.6 and 0.66 mg/kg. Weight gains in frogs exposed to treated flies was positive, but nil or slightly negative in the controls. As noted in the previous trials, small numbers of test animals limit any conclusions which can be drawn from the study.

C4.3.2 Native New Zealand lizards, skinks, tuatara

No data are available on the toxicity of 1080 to native New Zealand reptiles (geckoes, skinks and tuatara). Tuatara are endemic to New Zealand and not closely related to any other living reptile species. However, tuatara are now only found in the wild on predator-free offshore islands or in mainland 'island sanctuaries' where they would not be exposed to 1080.

Table C21: Acute toxicity of 1080 to reptiles and amphibians under laboratory conditions

Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time	Reference
Amphibia								
Spotted grass frog, *Limnodynastes tasmaniensis*	4.6			60[4]	12.9–77.5	36.8-98.3	-	McIlroy et al 1985
American bullfrog, *Rana catesbiana*			51 (24–108)					Tucker and Crabree 1970 cited in McIlroy et al 1985
Leopard frog, *R. pipiens*			Approx. 300 mg/kg (tested as methyl fluoroacetate; sub-cutaneous)					Chenoweth and Gilman 1946
African clawed toad, *Xenopus laevis*			>500 (two toads tested; no deaths; sub-cutaneous; tested as potassium monofluoroacetate)					Quin and Clarik 1947
Archey's frog, *Leiopelma archeyi*		1.5						(Perfect and Bell 2005)
Hochstetter's frog *L. hochstetteri*								
Reptilia								
Bearded dragon, *Pogona barbatus*	267			<110	15.2	14.9–24.2		McIlroy et al 1985
Gould's monitor, *Varanus gouldii*	732		43.6 (27.5–69.2)	-	24.2–141.2	66.5–292.5	7.5 and 18 days	McIlroy et al 1985
Lace monitor, *V. various*	3647			<119	26.6–141.3	73.6–145.4		McIlroy et al 1985
Blotched blue-tongued lizard, *Tiliqua nigrolutea*	434		336.4 (232.4–487.1)	-	13.3–160.9	14.4–522.5	17.7 days	McIlroy et al 1985
Shingle-back lizard, *T. rugosa* (South Australia)	468		205.9 (147.2–289.1)	-	-	21–134		McIlroy et al 1985

Species	Mean bodyweight[1] (g)	Average bodyweight[2] (g)	LD_{50} (95%CI) (mg/kg bw)	ALD[3] (mg/kg bw)	Latency period (hours)	Time to death (hours)	Recovery time	Reference
T. rugosa (SW Western Australia – indoor cage)	351		507.7 (447–577.1)	-	-	22–363		McIlroy et al 1985
T. rugosa (SW Western Australia – outdoor cage)	349		543.2 (500.5–589.5)	-	-	24–432		McIlroy et al 1985

Notes

1 Mean bodyweight of animals tested.

2 Average bodyweight for the species.

3 ALD = approximate lethal dose; calculated as the mean of the lowest dose level at which an individual died and the highest level at which an individual survived (one animal per dose, reduces the number of animals required for testing).

4 Intraperitoneal injection, all other species, oral gavage unless otherwise stated.

C4.4 Terrestrial vertebrate hazard classifications of substances containing 1080

As there is no direct toxicity data for substances containing 1080, the Agency has used the additivity formula for estimating the ecotoxicity of mixtures (set out below) to estimate the toxicity of these substances (ERMA 2001). The toxicity value used by the Agency is the acute oral toxicity of technical grade 1080 to the dog LD_{50} 0.06 mg/kg/bw (Tourtelotte and Coon 1951). Table C22 sets out the results of the calculations and the classifications derived by the Agency and the applicants.

$$L(E)C_{50\,mixture} = 100 \Big/ \sum_{\eta} \frac{Ci}{L(E)C_{50\,i}}$$

where:

C_i=concentration of component i (weight percentage)

$L(E)C_{50i}$= LC_{50} or EC_{50} for component I (mg/kg bw)

η = number of components

The applicants used the same toxicity value and reached the same classifications for all substances containing 1080 as the Agency.

Table C22: Vertebrate toxicity classifications for substances containing 1080

Name of substance	Tech. grade 1080	Pellets 0.4–0.8 g/kg 1080	Pellets 1.0 g/kg 1080	Pellets 1.5–2.0 g/kg 1080	Paste 0.6–0.8 g/kg 1080	Paste 1.5 g/kg 1080	Paste 10 g/kg 1080	Gel 1.5 g/kg 1080	Gel 50 g/kg 1080	Gel 100 g/kg 1080	Sol. conc 200 g/L 1080
% 1080	95–98%	0.08	0.1	0.2	0.08	0.15	1	0.15	5	10	20
Estimated LD_{50} (mg/kg bw)	-	75	60	30	75	40	6	40	1.2	0.6	0.3
Agency's classification	9.3A	9.3B	9.3B	9.3A	9.3B	9.3A	9.3A	9.3A	9.3A	9.3A	9.3A
Applicants' classification	9.3A	B	B	A	B	A	A	A	A	A	A

C5 Sub-class 9.4: Terrestrial invertebrate ecotoxicity

The toxicity of 1080 to terrestrial invertebrates has not been assessed using standard international guidelines (eg OECD 213 Acute oral toxicity or OECD 214 Acute contact toxicity). The data presented in the application are either very old (eg, David 1950, Palmer-Jones 1958) or have been conducted more recently to directly assess risks of formulated baits to specific organism of interest. The studies undertaken by David and co-workers (David 1950, David and Gardiner 1954) were aimed at assessing the efficacy of 1080 as an insecticide for commercial horticulture.

A summary of the data which are available on the toxicity of 1080 to honeybees and other terrestrial invertebrates under laboratory or glasshouse conditions is provided in Table C23. New Zealand native species are indicated by shading in the table.

There is considerable uncertainty regarding the toxicity for bees as derived by Palmer-Jones (1958) due to the quality of the study and the lack of information on how the LD_{50} was derived, and even the units for the LD_{50}. Given the non-standard nature of the testing and issues with data quality, the relative toxicity between species is also highly uncertain.

Despite the uncertainty in the data, the Agency considers that **technical grade 1080 can be classified as 9.4A highly toxic to terrestrial invertebrates (48hr LD_{50} <2 μg/invertebrate) based on the acute oral honeybee and ant data.** The uncertainty in this classification could be remedied by the testing of 1080 in a standard regulatory study (such as OECD 213).

Table C23: Toxicity of 1080 to terrestrial invertebrates under laboratory conditions

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Honeybee, *Apis mellifera*	24 hr oral	LD_{50} 0.8 µg/bee (~8 mg/kg)	Non-standard; non-GLP Current standard test duration is 48 hours Study poorly documented and intended for screening only; eg exposure time not stated, derivation of LD_{50} not specified; dose levels not stated; (Palmer-Jones 1958)
	Semi-field study	A single load of 1080 bait picked up by foraging bees killed 62% of foragers; remainder recovered with no apparent adverse effects. Some 'house bees' died several hours after the start of the trials, having received toxic bait from the foragers. A delayed action was observed (2 hours after feeding) with vigorous shaking, inability to hold onto comb and death. In the foraging trial – foragers made repeat trips from the bait to the hive, recruiting more foragers. 55% of foragers made >1 trip ; 2 individuals made 4 trips	Non-standard; non-GLP 'Jam' baits containing 1080 were placed near foraging hives and survival of bees monitored for 24 hours; number of foraging trips to a 1080 jam bait was also assessed in a separate trial. Concentration of 1080 in baits used in the trials was not stated. (Goodwin and Ten Houten 1991)
Common wasp, *Vespula vulgaris* German wasp, *V. vulgaris*	Field study	0.001% 1080 causes some individual mortality; Average reduction in wasp numbers after 6 hours 17% at 0.1% 1080 78% at 0.5% 1080 89% at 1.0% 1080 (100% at some nests) At 0.5% and 1%, rapid reduction in wasp numbers was observed two hours after baits deployed and occurred up to 300m from the bait stations. Reductions were attributed to mortality rather than avoidance of bait as baits required replenishing during that time.	Study undertaken to determine efficacy of varying concentrations of 1080 (in fish bait) in controlling wasp populations in the field. Baits placed in bait stations in areas of known high wasp populations (predominantly common wasp (97%), but also some German wasps present). Duration of exposure to bait was 6 hours. (Spurr 1991)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Striated ant, *Huberia striata*	48 hr oral	48hr LC_{50} 0.036% 1080 average consumption per ant was 0.24 mg ~LD_{50} 42 mg/kg (95%CI 34–49) = 0.08 µg/ant (500,000 ants per kg at 2 mg bw/ant) 24hr LD_{50} 72 mg/kg ~0.14 µg/ant) Mortality of control ants was not reported. Mean 1080 residues in ants At 0.03% 1080 (sub-lethal concentration) 5.51 mg/kg ants 1 DAT with rapid decline in week following exposure but still measurable after 7 days (0.27 mg/kg ants) At 0.15%1080 (lethal concentration) 56 mg/kg ants	Non-standard; non-GLP Wild-collected worker ants from 3 colonies in the Port Hills, Banks Peninsula, New Zealand; maintained in laboratory on local soil placed in aquaria; kept at 15°C in the dark. Species choice based on difficulty in maintaining the forest ant *Huberia brouni* under laboratory conditions and smaller body size {~0.5 mg) compared with *H. striata*) Ants fasted for 24 hours prior to exposure to 1080 in 10% sucrose solution in shallow petri dishes. Test concentrations 0, 0.03, 0.06, 0.09 and 0.15% 1080 in sucrose. Surviving ants were moved to fresh aquaria with food and water and monitored for additional 4 days. Dead ants frozen at -20°C for later analysis The duration of exposure to 1080 was not stated except as "*6 hours the usual length of time that ants were observed to feed on sugar water*"; no analytical confirmation of test concentrations; source and grade of 1080 not specified; full results not reported; analytical methods and LOD not stated. Control mortality not reported. (Booth and Wickstrom 1999)
		No difference in mortality between the two groups offered 1080 bait At 24 hours 7% mortality At 48 hours 12% mortality 1080 residues in pooled sample of dead ants were measured at 4.78 mg/kg ants Bait fragments observed spread around the aquaria.	Non-standard; non-GLP Ants divided into three groups and presented with either 0.15%Wanganui cereal bait + water Same bait + canned pet food + water Pet food + water (controls) Husbandry and other information as for the study with sucrose solution (above) (Booth and Wickstrom 1999)
Wellington tree weta, *Hemideina crassidens*	Oral	LD_{50} 91 mg/kg bw (sex unknown) Based on mean bodyweight of *H. crassidens* (Kelly 2004) approximate LD_{50} per weta ♂ 360 µg/weta (3.95 g weta) ♀ 430 µg/weta (4.75 g weta)	No information on test protocol used; doses or number of weta tested. Note – Table 4 of the application states an estimated LD_{50} of 60 µg/weta which is not correct, the text following that table has the LD_{50} of 365 µg/weta based on an average bodyweight of 4 g. (Landcare Research, unpublished data; cited in Booth and Wickstrom 1999)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Tree weta (species not stated)	Oral	1080 residues in individual weta were (elapsed time after dosing) 5.8 mg/kg at 4 and 12 hours 5.0 mg/kg at 24 hours 2.8 mg/kg at 48 hours 4.5 mg/kg at 96 hours 0.2 mg/kg at 144 hours 0.033 mg/kg at 240 hours	Non-standard; non-GLP Weta dosed individually by micro-syringe at 15 mg/kg and then killed at various times after dosing. (if a bodyweight of 4 g is assumed this dose equates to 60 µg/weta); no other information on test protocol used or analytical methods. Each residue value appears to be based on a single weta. No observations were reported on the behaviour or mortality of dosed weta therefore it is not possible to draw conclusions regarding effect levels from the data, as has been done in Table 4 of the application where 60 µg/weta is stated as a 'no effect level' (Eason et al 1993)
Steel blue sawfly larvae *Perga dorsalis*	Abdominal injection 5-day	LD_{50} 1.05 mg/kg (95%CI 0.86-1.28) (mean bw 1.386 g; ~LD_{50} 1.45 µg/larva)	Non-standard; non-GLP Wild-collected larvae from Western Australia. Species feeds mainly on eucalypts (non-fluoroacetate bearing plants) Commercial grade 1080 used, administered by micro-syringe into body cavity mortality monitored for 5 days. (Twigg 1990)
Caterpillar larvae Autumn gum moth, *Mnesampela privata* Tiger moths, *Spilosoma sp.* Processionary caterpillar, *Ochrogaster lunifer*	Abdominal injection 5-day	*M privata* LD_{50} 3.88 mg/kg (mean bw 0.421 g; ~LD_{50} 1.45 µg/larva) *Spilosoma* sp. LD_{50} 42.73 mg/kg (mean bw 0.888 g; ~LD_{50} 38 µg/larva) *O. lunifer* LD_{50} between 100 and 200 mg/kg (mean bw 0.989 g; ~LD_{50} 148 µg/larva) Elevated citrate levels measured in larvae dosed with 1080 indicates disruption of TCA cycle as occurs in vertebrates. Authors suggest the differences in sensitivity to 1080 reflect the co-evolution of the more tolerant species with 1080-bearing vegetation.	Non-standard; non-GLP Wild-collected caterpillars from Western Australia *M.privata* sourced from eucalypts; *Spilosoma* sp. from a variety of garden plants but also known to feed on 1080-bearing vegetation; *O. lunifer* from toxic *Gastrolobium microcarpum* (1080-bearing) Other details as for Twigg 1990 above (Twigg 1990)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
Native cockroaches (adult), *Celatoblatta undulivitta,* *C. vulgaris* *C. subcorticaria*	Dietary 2 weeks	Impaired behavioural effects observed (starting days 1–3) orientation and escape responses affected and general decline in responsiveness to stimuli. Most exposed cockroaches died during the 14 day period, though a few recovered but were not monitored further. As a rough assessment of 1080 residues the author calculated the amount of bait an adult cockroach could accommodate in its crop as ~0.2 g (*C. undulivitta*) and ~0.1 g (*C. vulgaris*) which would equate to approx. 0.16 and 0.09 mg 1080 respectively but takes no account of 1080 residues which may be present in the body tissues of the cockroaches.	Non-standard; non-GLP Caged wild-collected cockroaches fed 0.5g cereal bait containing 0.08% 1080, aniseed lure, dyed green and observed for behavioural effects (self-righting ability) and mortality at 24 hour intervals for two weeks (McIntrye 1987)
American cockroach (adult), *Periplaneta americana*	Abdominal injection 24 hours	LD_{50} 43 mg/kg Citrate accumulation indicates inhibition of aconitase.	Non-standard; non-GLP Doses administered by abdominal injection but actual doses not specified. To assess citrate accumulation, a separate trial was conducted using a single dose of 30 mg/kg (Matsumara and O'Brien 1963)
House fly (adult) *Musca domestica* "Wilson susceptible" strain	Thoracic injection 24 hours	LD_{50} 21 mg/kg	(Matsumara and O'Brien 1963)
Mustard beetle, *Phaedon cochleariae*	Acute contact 5 days	Larvae: No mortality observed at the end of the study Adults: 5% mortality at end of study	Non-standard; non-GLP Adults and second-stage larvae dipped into 0.1% 1080 solution and mortality assessed at 2 and 5 days after treatment. (David and Gardiner 1954)
	Feeding/contact with treated foliage 5 days	Trial 1 50% mortality of larvae at 0.1% 1080; no mortality at the lower concentration. Trial 2: Larvae 48-hour 5% mortality at 0.01% 1080; 40% mortality at 0.1%1080 120hr 25% mortality at 0.01 1080;100% at 0.1% Adults: 15% mortality at 0.1% after 120 hours (no mortality in the other treatment/times)	Non-standard; non-GLP Trial 1: Turnip leaves bearing beetle eggs were dipped in either 0.01 or 0.1% 1080 solution and maintained for 5 days after larval hatching Trial 2: Adults and larvae reared on leaves which had been dipped in 0.01 or 0.1% 1080 solution. (David and Gardiner 1954)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
	Feeding on treated plants 5 or 20 days	Trial 1 (solution) At 0.01, and 0.0.5% 1080 in solution, significant damage occurred to plant leaves, no phytotoxicity at 0.005% 1080. newly hatched larvae 100% mortality at 0.05%1080; none in other treatments Second-stage larvae at 0.1% 1080 20% mortality at day 5; none in other treatments Adults at 0.05%1080 35% mortality at day5 at 0.1% 1080 20% mortality at day 2, 100% at day 5 no mortality in other treatments Trial 2 (soil application) newly hatched larvae at 0.1% 1080 80% mortality at day 5 at 0.5% 1080 100% mortality at day 5 Second-stage larvae and adults no mortality in any treatments Trial 3 (systemic exposure) No mortality at day 5 Trial 4 (20 day, soil application) no mortality recorded up to day 10, plants were then too wilted for the trial to continue	Non-standard; non-GLP Three life-stages assessed; hatching of eggs placed on turnip leaves prior to treatment of plants; second-stage larvae and adults were fed on potted turnip plants either grown in solution containing 0, 0.005, 0.01, 0.05 or 0.1%1080 (Trial 1) or in soil treated with 20 mL of 0, 0.001, 0.01 or 0.05% 1080 solution (Trial 2) In Trial 3 , leaves were painted with 0.2% 1080 and larvae exposed to non-treated leaves of same plant (not able to directly access treated leaves) Trial 4 was similar to Trial 2, except that the soil was treated with 20 mL of 0.5% 1080 2 days prior to adults were exposed (for 20 days) (David and Gardiner 1954)
Aphid, *Aphis faba*	Acute contact	100% mortality within 2 days at 0.001% or 1 day at 0.1%1080 At 0.1% 1080, the plants remained toxic to aphids for 2 days; and were no longer toxic after 5 days.	Non-standard; non-GLP Aphids placed on broad bean plants of which the foliage had been dipped in 1080 solution (David 1950)

Test species	Test type and duration	Test results[1,2]	Test method[3] (reference)
	Feeding on treated plants	Whole plant treatment 1.0 mg 1080 in 400 g soil 100% mortality in 5 days; 0.1 mg 1080 in 400 g sand 100% mortality, plants remained toxic for at least 10 days 0.00005% 1080 culture solution 100% mortality (=0.05 mg per 10–15 g plant)	Non-standard; non-GLP Broad bean plants grown in either sand or soil or solution treated with 1080 (David 1950)
Grain moth parasitoid wasp, *Bracon hebetor*	Reproductive abdominal injection or oral exposure 15 days	Both routes of exposure decreased egg production for the life of the females. Of the eggs laid, significantly lower hatchability occurred only in the eggs laid in the first two days after injection or first 4 days and days 11–15 after oral exposure. There were no significant differences in egg viability between controls and treatment groups except on day 2 in the oral exposure group.. Injection caused a greater reduction in egg production than dietary exposure, with injected wasps reaching equivalent production levels a week later. Examination of dissected ovarioles 1–2 DAT no mature eggs in egg sacs; contained misshapen and collapsed oocytes; oocytes in the proximal vitellarium were yolk-deficient 3 DAT normal oocytes had replaced the impaired structures 5 DAT ovariole contents appeared normal	Non-standard; non-GLP Single dose administered to female adults either by abdominal injection (0.025 mg/kg bw) or fed in 10% sucrose solution (0.063 mg/kg bw). In a second trial, female wasps were fed 1080 (assumed to be the same amount as in the first trial), wasps were dissected on days 1–7 after treatment and ovarioles examined (Smith and Grosch 1976)

C5.1 Terrestrial invertebrate hazard classifications of substances containing 1080

As there is no direct toxicity data for substances containing 1080, the Agency has used the additivity formula for estimating the ecotoxicity of mixtures (set out below) to estimate the toxicity of these substances (ERMA 2001). The toxicity value used by the Agency is the acute oral toxicity to the honeybee LD_{50} 0.8 µg/bee (Palmer-Jones 1958). Table C24 sets out the results of the calculations and the classifications derived by the Agency and the applicants.

$$L(E)C_{50\,mixture} = 100 \Big/ \sum_{\eta} \frac{Ci}{L(E)C_{50\,i}}$$

where:

C_i=concentration of component i (weight percentage)

$L(E)C_{50i}$= LC_{50} or EC_{50} for component I (µg/invertebrate)

η = number of components

The applicants used the additivity formula and the toxicity of 1080 to the striated ant LC_{50} 0.08 µg/ant to estimate the toxicity of substances containing 1080 to terrestrial invertebrates. However, they did not present their calculations in the application. The rationale for classifying paste containing 10 g/kg 1080 as 9.4B was stated as being that the substance is used to control wasps. If the bee toxicity value is used, this substance does not trigger the threshold, if the ant value is used, then it would classify as 9.4A highly toxic to terrestrial invertebrates. Likewise, the Agency is not aware of how the applicants concluded the 5 and 10% gels and 20% solution classify as 9.1B toxic to terrestrial invertebrates if they used the ant toxicity value as stated.

Table C24: Invertebrate toxicity classifications for substances containing 1080

Name of substance	Tech. grade 1080	Pellets 0.4–0.8 g/kg 1080	Pellets 1.0 g/kg 1080	Pellets 1.5–2.0 g/kg 1080	Paste 0.6–0.8 g/kg 1080	Paste 1.5 g/kg 1080	Paste 10 g/kg 1080	Gel 1.5 g/kg 1080	Gel 50 g/kg 1080	Gel 100 g/kg 1080	Soluble conc 200 g/L 1080
% 1080	95–98	0.08	0.1	0.2	0.08	0.15	1	0.15	5	10	20
Estimated LC_{50} µg/invert (using bee data)		1000	800	400	1000	530	80	530	16	8	4
Estimated LC_{50} µg/invert (using ant data)		100	80	40	100	53	8	53	1.6	0.8	0.4
Agency's classification based on ant data	9.4A	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.4A	Does not trigger	9.4A	9.4A	9.4A
Applicants' classification	9.4A	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	B	Does not trigger	B	B	B

C6 Ecotoxicity classification summary for 1080 and substances containing 1080

The ecotoxicity hazard classifications derived by the Agency for all substances containing 10808 are set out below. Details of how the Agency's classifications were derived are set out in the relevant sections above.

Where there are differences in classification between the applicants and the Agency, the Agency considers its classifications should be adopted.

Table C25: Ecotoxicity hazard classifications

Hazard sub-class	Tech. grade 1080	Pellets 0.4–0.8 g/kg 1080	Pellets 1.0 g/kg 1080	Pellets 1.5–2.0 g/kg 1080	Paste 0.6–0.8 g/kg 1080	Paste 1.5 g/kg 1080	Paste 10 g/kg 1080	Gel 1.5 g/kg 1080	Gel 50 g/kg 1080	Gel 100 g/kg 1080	Soluble conc 200 g/L 1080
9.1 Aquatic ecotoxicity											
Agency's classification	9.1A	9.1D	9.1D	9.1D	9.1D	9.1D	9.1D	9.1D	9.1A	9.1A	9.1A
Applicants' classification	9.1A	9.1C	9.1C	9.1C	9.1C	9.1C	9.1C	9.1D	9.1C	9.1A	9.1A
9.2 Soil ecotoxicity											
Agency's classification	9.2B	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.2D	9.2D
Applicants' classification	9.2B	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.2C	Does not trigger	9.2C	9.2C	9.2C
9.3 Terrestrial vertebrate ecotoxicity											
Agency's and applicants' classification	9.3A	9.3B	9.3B	9.3A	9.3B	9.3A	9.3A	9.3A	9.3A	9.3A	9.3A
9.4 Terrestrial invertebrate ecotoxicity											
Agency's classification	9.4A	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.4A	Does not trigger	9.4A	9.4A	9.4A
Applicants' classification	9.4A	Does not trigger	Does not trigger	Does not trigger	Does not trigger	Does not trigger	9.4B	Does not trigger	9.4B	9.4B	9.4B

Attachment 1: Independent expert review of environmental fate of 1080

PETER FISK ASSOCIATES

CHEMICALS ◆ ENVIRONMENTAL ◆ REGULATORY

REVIEW OF PAPERS CONCERNING THE FATE OF THE MONOFLUOROACETATE ION IN THE ENVIRONMENT OF NEW ZEALAND

Authors: P.J. Jewess, P.R. Fisk

Date: 23rd March 2007

Record of report finalisation

Conclusions drawn and recommendations made in this report represent the unbiased view of Peter Fisk Associates on the basis of the data presented and obtained.

Signed on behalf of Peter Fisk Associates

…….

Dr. Peter Fisk

Date …….

Review of papers concerning the fate of the monofluoroacetate ion in the environment of New Zealand

Introduction

Background

On March 8th 2007 Peter Fisk Associates (PFA) was asked by Dr. Robin Toy of ERMA New Zealand to provide a review of a number of sources describing the behaviour of the monofluoroacetate ion in the environment. Monofluoroacetate can reach the aquatic and terrestrial environments resulting from the use of sodium monofluroacetate (also known by the commercial name "1080") as a mammalian pest control agent, the main target being the possum. The purpose of this review, reported herein, is to assess whether available published evidence is sufficient to assess the persistence of monofluoroacetate.

The sources were provided in electronic form by ERMA. No prior review other than the published sources was provided, and PFA does not know the full purpose to which this work will be applied. Therefore the conclusions of this report can be considered as a true and unbiased interpretation of the data.

PFA has relevant experience to enable the assessment of biodegradation of monofluoroacetate to be made.

The chemistry of sodium monofluoroacetate does not need to be reviewed in detail. It is sufficient to know that it is highly water soluble, involatile, and stable to hydrolysis.

The biochemical aspects of the mechanism of toxicity are well known in the literature and need not be reviewed in depth. As well as the activation mechanism that yields the respiration inhibitor fluorocitrate via reaction of fluoroacetate with oxaloacetate catalysed by citrate synthase, loss of fluoride via "haloacetate hydrolase" to give glycolate has been proposed in micro-organisms and possibly plants (see below). In mammals and reptiles the major detoxification mechanism is via direct conjugation with glutathione, which ultimately yields the sulfoxide and sulfone of mercapturic acid. Conjugation of fluoroacetate with glutathione has also been shown in plants.

Definition of persistence in the environment

The present authors consider that persistence in the environment concerns degradative processes. That is, irreversible transformation of the fluoroacetate ion into some other chemical species. Therefore, removal processes from water into some other environmental compartment would not be considered as degradation. For example, adsorption onto sediment from the water column would simply change the focus onto the behaviour of the substance in the sediment compartment. Uptake by an organism might well be important in determining the overall fate of monofluroacetate in the environment, and could result in subsequent degradation (metabolism) by that organism. The pathway and rate would need to be considered in order to determine whether such a process is important in determining the assessment of overall persistence.

Once basic pathways and rates are delineated, it is possible to use mathematical environmental models to establish whether monofluoroacetate is persistent in the total environment or individual compartments of it.

The authors have also been provided with documentation on the views of ERMA concerning degradation and its definition within regulatory contexts:

> Degradability in aquatic systems
> The term 'rapidly degradable' is defined in Schedule 6 of the Hazardous Substances (Minimum Degrees of Hazard) Regulations 2001 as:
>
> > "*rapidly degradable, in relation to a substance in water, means that—*
> >
> > *(a) 28 days after a solution containing the substance is inoculated with micro-organisms, there is at least—*
> >
> > *(i) a 70% reduction in dissolved organic carbon in the solution; or*
> >
> > *(ii) a 60% depletion of oxygen in the solution, when compared with the maximum depletion of oxygen that would occur if the substance were completely degraded; or*
> >
> > *(iii) a 60% generation of carbon dioxide in the solution, when compared with the maximum generation of carbon dioxide that would occur if the substance were completely degraded; or*
> >
> > *(b) if only COD and BOD_5 data is available, the ratio of BOD_5 to COD is greater than or equal to 0.5:1; or*
> >
> > *(c) at least 70% of the substance can be degraded biotically or abiotically, in the aquatic environment within 28 days*"

Degradation in soil

The regulatory criterion for assessing the half-life in soil is:
Schedule 6 of the Hazardous Substances (Classification) Regulations 2001 refers to a soil half-life (DT_{50}) as being "*the half-life in soil, which is the time required to reduce the original concentration of the substance in the soil by 50%*" and would usually be assessed using a standard test guideline (e.g. OECD 307).

ERMA interprets the DT_{50} as being a result of biodegradation, consistent with that for assigning aquatic degradation 'classification', rather than dissipation which refers to any loss from the soil whether by degradation, leaching or some other process.

Scientific papers provided by ERMA and reviewed by PFA

The papers are referenced in Section 2. This list is believed to have been developed following extensive literature searching by ERMA, and therefore no additional searching was carried out by PFA.

Review of sources

Source: Booth et al., 1999

Ref. 1

Method:

Degradation studies were carried out in 18 L aquaria containing either deionised water (control) or stream water containing the submerged aquatic plant *Myriophyllum triphyllum* at 21°C. Sodium fluoroacetate was applied at rates of 0.12, 2 and 5 mg/L. The water was analysed for fluoroacetate and fluorocitrate by a derivatisation GLC technique using the 2,4-dichloroaniline adducts (fluoroacetate) or TFA/BF_3 (fluorocitrate). Assays were performed up to 17 days after treatment commenced.

Results:

Fluoroacetate was stable in the control aquaria and no fluorocitrate was detected. In the tanks containing plants and micro-organisms, fluoroacetate disappeared quite rapidly. DT_{50} values are not quoted but from the graphs the present authors assess it to be ca. 8 days at the 5 mg/L dosage, 4–5 days at 2 mg/L and 1–2 days at 0.12 mg/L. This is at variance with the assertion of "degradation occurring at a higher rate in aquaria with the highest initial concentration of 1080 (P=0.21)". Fluorocitrate peaked at 1, 4 and 8 days for 1.2, 2 and 5 mg/L fluoroacetate

concentrations respectively. The maximum fluorocitrate peak was 0.4, 1.2 and 1.6 mg/L at these same concentrations. Confusingly, the data are also presented as a table in the form of μmol fluorocitrate produced or fluoroacetate degraded. These data are all incorrectly calculated, being 10-fold lower than actual amounts, although this does not negate the general conclusions. In general the data manipulation in this paper is very poor and should not have passed the referees' scrutiny. Notwithstanding this, the following conclusions appear to hold:

- Fluoroacetate is degraded in stream water and fluorocitrate is a metabolite.
- Higher levels of fluorocitrate were detected in aquaria dosed with higher amounts of fluoroacetate.
- Both fluoroacetate and fluorocitrate were rapidly degraded so that after 17 days, levels were almost undetectable.
- It was not known which organisms are responsible for this degradation, micro-organisms, plants (or both).

This is poorly written paper but the basic conclusions appear to be supported. It is, however, the only one in which the toxic metabolite of fluoroacetate, fluorocitrate is reported with stream water and plants, rather than a defluorinating mechanism yielding glycolate and F^-.

Source: Eason et al., 1993

Ref. 2

This paper discusses the degradation of sodium fluoroacetate in water, invertebrates and mammals. Only the water degradation studies are assessed in this abstract. The article is a summary of the work carried out, as are most conference proceedings papers and not a full detailed paper, consequently a number of experimental details are lacking.

Methods.

Three 80 L aquaria, two containing submerged aquatic plants and small invertebrates and the third, a control tank containing distilled water were dosed with sodium fluoroacetate (0.1 mg/L). The assay method for fluoroacetate was the same as in ref. 1. It was stated that recoveries were >90% and the limit of detection was 0.3 μg/L. Only fluoroacetate was measured. Water temperature was 20^{o}C. It was not stated whether the tanks were aerated. Assays for fluoroacetate were performed 5 min, 1, 24, 100, 170, 330 h (13.75 days) after dosing.

Results:

Fluoroacetate disappeared rapidly from both biologically active aquaria with DT_{50} values of less that 24 h. None was detectable after 100 h. There was little degradation in the distilled water aquarium. Fluoroacetate residues were detectable in plants, the highest amount in the 1 h samples; however, none was detectable after 330 h.

Conclusions:

- Sodium fluoroacetate was rapidly degraded in stream water containing plants, invertebrates and (presumably) micro-organisms.
- Information on the metabolic fate could not be assessed, as potential metabolites were not tested for.

Source: Ellington et al., 1987

Ref 3.

Methods:

Pseudo first-order hydrolysis rates (L. $mol.^{-1}$ hr^{-1}) were measured for a number of potentially polluting chemicals at 25°C. Hydrolysis was carried out in neutral, acidic and basic media for some of the materials. For some, data were measured at different temperatures, Arrhenius plots performed and activation parameters (ΔG^*) calculated.

The detailed information for sodium fluoroacetate (p 39) and includes all the relevant purity (by IR and MS), concentration and analysis method data. The raw hydrolysis data were used to calculate neutral hydrolysis rates at 25°C assuming a value of ΔG^* of 20 Kcal/mol, which results in the rate constant increasing by a factor of 10 per 20°C increase in temperature. The analytical method used to measure the hydrolysis rates for sodium fluoroacetate was ion exchange HPLC with analysis of the fluoroacetate ion by conductivity monitor. Initial concentration was 4.8 mg/L. The duration of the experiment was not stated. The buffers used to control the pH were not stated.

Results:

The data reported are:

pH	Temp.	k_1 (hr^{-1})	$t_{1/2}$(days)	r^2
3.14	87.0	2.4E-04	120	0.425
7.25	68.7	4.0E-04	72	0.912
9.99	68.7	8.0E-05	365	0.395

The data reported in table 5 for the neutral pseudo-first order hydrolysis (k_1) rate constant was <1.7E-06 hr^{-1}.

Conclusions:

The data and data manipulation are presented in a very confusing manner. Clearly, fluoroacetate is hydrolysed in aqueous solution in a pH dependent manner with a maximum rate at near neutrality, which tends to rule out a simple SN_2 reaction with OH^- to give F^- and glycolate. The experiments at the high and low pH give poor correlation coefficients so that only the pH 7.25 value should be accepted. Using the activation parameters given, the k_1 value at 25°C would be ca. 1.8E-05 hr^{-1} or a half life of 1600 days (4.38 years).

- Sodium fluoroacetate is hydrolysed very slowly in water at neutral pH in the absence of biota.
- At ambient temperatures the half-life would be expected to be at least 4 years.

Source: Goldman, 1965

Ref. 4.

Methods:

A bacterial inoculum identified as a *Pseudomonas* species was isolated from river sediment by a process of an enrichment culture technique that could utilise fluoroacetate as the sole carbon source. An enzyme was extracted and purified 30-fold that was capable of catalysing the hydrolysis of fluoroacetate to glycolate and fluoride:

$$FCH_2CO_2^- + OH^- \rightarrow HOCH_2CO_2^- + F^-$$

Fluoroacetate and glycolate were identified and quantified by anion-exchange chromatography, paper chromatography and various colorimetric assays.

Results:

It was convincingly shown that the reaction shown above was catalysed in a strict stoichiometric ratio so that 1 mol fluoroacetate gave 1 mol glycolate and 1 mol fluoride. The enzyme also catalysed chloroacetate and iodoacetate hydrolysis, but at a much slower rate. It was inhibited competitively by small organic acids and in a time dependent manner by a number of thiol regents. The authors hypothesise that the mechanism proceeds via the formation of a carboxymethylated thiol (cysteine) at the active site, followed by hydrolysis of this thioether to release glycolate. However, iodoacetate is a much better alkylating agent of thiols than is fluoroacetate, so the access to the active site must be restricted to only allow fluoroacetate to easily access the site. This proposed mechanism is probably wrong (see Liu *et al*. ref. 7). However, it is an excellent and convincing paper.

Conclusions:

- Some soil bacteria contain enzymes capable of hydrolysing fluoroacetate to glycolate and fluoride.
- It is not possible to say how important this is for the degradation of fluoroacetate in soils and sediments as it is not an eco-toxicological paper, but it does demonstrate the process can happen.

Source: Kelly, 1965

Ref. 5.

Methods:

A gram-negative bacterium, isolated from garden soil from SE England, was capable of growing on fluoroacetate as a sole carbon source. The bacterium was grown in flask culture and fluoroacetate, fluoroacetamide and fluoride were determined by a referenced method that was not described.

Results:

The bacteria grew slowly under conditions where fluoroacetate or fluoroacetamide were the sole carbon sources but much more quickly on complete medium. They grew more quickly at 30^{o}C than at 37^{o}C. Bacteria conditioned to grow on only fluoroacetate would not utilise fluoroacetamide, but those adapted to fluoroacetamide would utilise both. When fluoroacetamide or fluoroacetate were utilised in a flask culture of the bacteria, fluoride was produced in tandem with the disappearance of the substrate. This is a sound paper but rather lacking in detail.

Conclusions:

- Bacteria can be isolated from soil that are capable of catalysing the degradation of fluoroacetate via a defluorination mechanism.
- It is not possible to say how important this is to the rate of fluoroacetate degradation in soil but it does demonstrate that the process occurs.

Source: King et al., 1994

Ref. 6

This paper examines the ability of soil microbes isolated from five sites from temperate western Australia. A large number of Australian plant species synthesise fluoroacetate and a number of vertebrate species have been shown to

develop increased tolerance to this toxin. Similarly, soil micro-organisms isolated from areas where fluoroacetate occurs naturally might be expected to show increased ability to metabolise this compound. This is a competent paper but it is clearly a summary and a few more details would be useful.

Methods:

Both bacterial and fungal species were isolated and examined for their ability to metabolise fluoroacetate added to either autoclaved soil or aqueous solution. The extent of fluoroacetate breakdown was assessed by measuring fluoride with a fluoride electrode. The detailed method is not described but is referenced (Wong 1992, two papers, abstracted herein).

Results:

Micro-organisms capable of degrading fluoroacetate were only isolatable from temperate soils, but not from semi-arid tropical regions. Activity was optimal at temperatures of 11–28^{o}C and soil moisture of 8-15% and for bacteria in neutral to alkaline soils, although fungi were most active in acid soils. The main bacterial genera were *Pseudomonas* and the fungi were mostly *Fusarium* and *Penicillium*. In the degradation experiments in water and autoclaved soil, the maximum amount of fluoroacetate degraded was 65–87% and occurred in 5–49 days. Bacteria were generally better at degrading fluoroacetate than fungi. All micro-organisms degraded the material much slower at 37^{o}C than at lower temperatures. There was almost no breakdown in the autoclaved soil experiment where the soil moisture content was only 2.6%. Inoculum size had little effect on the rate, except when fluoroacetate was the sole carbon source, when a large inoculum was required to effect a significant breakdown.

Conclusions:

- Temperate Australian soils contain a large spectrum of micro-organisms capable of detoxifying fluoroacetate.
- Degradation rates obtained from this area might be expected to show faster detoxification rates as the soil biota are likely to be adapted to the natural occurrence of fluoroacetate.
- Soil pH, moisture and temperature had marked effects on the rate of fluoroacetate decomposition, with high temperature, low moisture and acidic pH all being deleterious to rapid metabolism.

Source: Liu et al., 1998

Ref. 7

This is a purely mechanistic paper exploring the reaction mechanism of fluoroacetate dehalogenase from the bacterium *Moraxella* sp. B.

It was anticipated from protein homology that the nucleophile that attacks the alpha-carbon of the fluoroacetate (and other haloacetates) was Asp105 to give an intermediate ester. The hydrolysis of this intermediate is then catalysed via a histidine(272)-activated water molecule acting as the second nucleophile. Accordingly, if H272 is mutated to another amino acid, the carboxymethylated intermediate on Asp105 would be stable.

Methods:

Both native and H272N (asparagine) recombinant proteins were expressed in *E. coli*. The proteins were purified and analysed for ability to catalyse the hydrolysis of chloroacetate (a poorer substrate than fluoroacetate but chloride is easy to assay). The recombinant H272N protein that had been reacted with fluoroacetate and $H^{18}OH$ was subjected to tryptic digest and the peptides subjected to MS/MS analysis.

Results:

H272N mutant fluoroacetate dehalogenase was devoid of catalytic activity.
The peptide that had an aspartate group esterified by carboxymethyl was identified by MS. There was incorporation of ^{18}O into the tryptic peptide incorporating Asp210.

Conclusions:

- The mechanism of fluoroacetate dehalogenase in this organism involves nucleophilic attack of an aspartic acid group on the alpha carbon of the substrate and the formation of a carboxymethylated ester intermediate.
- A histidine group acting as a general base activates a water molecule as a second nucleophile, effecting the hydrolysis of the ester intermediate.
- The alternative mechanism (occurring in a *Pseudomonas* species) whereby there is direct nucleophilic attack of a base-activated water molecule was ruled out by the lack of incorporation of ^{18}O).
- There is homology of the enzyme with a haloakane dehalogenase and an epoxide hydrolase.

Comment:
Although this is an excellent mechanistic paper it does little to address the question of how quickly fluoroacetate is hydrolysed by bacteria in the soil.

Source: Lloyd-Jones et al., 1994

Ref. 8.

This report describes an attempt to isolate fluoroacetate-utilising micro-organisms from eight soil and eight water samples.

Methods:

Samples of soil and water were taken from eight localities in (presumably) New Zealand. There are no data in the report on the type or localities of these samples and nothing on their prior history. Nor does it state whether the water samples are from streams, rivers, ponds or lakes. The water samples were apparently devoid of sediment. Micro-organisms were cultured using a batch enrichment system by growing the cultures in liquid minimal medium containing sodium phosphate, potassium phosphate, ammonium sulphate and mineral salts with the addition of sodium fluoroacetate (10 mM) as the sole carbon source. The cultures were grown at 28^{o}C with aeration. After 3 weeks the cultures were supplemented with an additional carbon source (yeast extract, YE). Culture supernatants were also monitored for free fluoride ions (method not defined). Serial dilutions were plated out onto minimal salts/agar containing 10 mM sodium fluorophosphates after 3, 21and 28 days or minimal agar medium containing sodium fluoroacetate. Only those cultures showing increased colony growth in the presence of fluoroacetate were considered to be fluoroacetate-utilising.

Results:

In three (out of eight) and two (out of eight) water samples it was possible to culture micro-organisms (bacteria) that could use fluoroacetate as the sole carbon source. The soil-derived cultures produce stoichiometric amounts of F^- from fluoroacetate. However, the water-derived cultures would only demonstrate defluorinating activity in the presence of YE supplement and then the conversion of fluoroacetate was not stoichiometric with only 2–5 mM F^- being produced from 10 mM fluoroacetate. It was not possible to isolate colonies capable of using fluoroacetate as sole carbon source from these cultures.

Conclusions:

- It was possible to isolate bacteria capable of utilising fluoroacetate as a sole carbon source in 3 out of 8 soil samples but none of the water samples.
- Fluoroacetate-utilising bacteria were either absent in the other samples or were not viable under the conditions used for enrichment.

Comments:

There are a number of flaws and omissions present in this report, especially the lack of information concerning sample identity. The lack of any sediment, bio-films or other organisms in the water samples means that the experiments did not reflect a real-life situation where fluoroacetate would be degraded in fresh water (admitted by the authors). Sampling soil also presents similar problem of heterogeneity.

Source: Ogilvie et al., 1995

Ref. 9.

This paper describes the degradation of sodium fluoroacetate in tanks of stream water (source stated) containing the submerged aquatic plant *Myriophyllum triphyllum*.

Methods:

The following treatments were used: all studies were carried out in 2 L aquaria containing (a) 1.8 L of biologically-active stream water (b) 1.8 L of biologically-active stream water plus 60 g fresh weight *Myriophyllum triphyllum* (b) 1.8 L of deionised water. All treatments were carried out in quadruplicate and at 7±1^{o}C or 23±2^{o}C (total of 24 tanks). The tanks were aerated, stirred before testing and subjected to a 12:12 L:D regime. Fluoroacetate and fluoride were measured as described in Ref. 1 above. Analysis was carried out at 0, 1, 3, 7, 13, 17 days (water) and 0, 1, 3, 7, 13 days (plants) after dosing. Initial dosing was 0.12 mg/L (120 ppb) sodium fluoroacetate, which was stated to be 40x the highest environmental water level found in the field. All data were treated statistically (chi^{2}).

Results:

(a) Plants and stream water: at 23^{o}C the degradation was rapid with barely detectable levels of fluoroacetate on day 1 (DT_{50}<1day). At 7^{o}C the rate was slower, but by day 3 the concentrations of fluoroacetate were hardly detectable (DT_{50} ca. 2 days).

(b) Biologically-active stream water only. At both temperatures there was a marked lag phase, probably indicating the induction of a population of fluoroacetate utilising micro-organisms. At 23^{o}C the DT_{50} was ca. 6 days and at 7^{o}C ca. 10 days.

(c) Deionised control: unlike ref. 1 there was significant loss of fluoroacetate in the control tank after a lag phase, but much less than the biologically active treatments. The authors hypothesise this was due to either a described (with reference) decarboxylation of fluoroacetate to bicarbonate and fluoromethane or (more likely) contamination of the control tanks with micro-organisms.

(d) The concentration of fluoroacetate in the plant tissue peaked at 23 ppb on day 1 and thence rapidly declined to barely detectable levels on day 3 (23^{o}C) or day 13 (7^{o}C).

(e) The concentration of fluoride in the tanks containing plant material rose to 83 ppb. There was no concomitant increase of fluoride in the deionised water control tanks.

Conclusions:

- Sodium fluoroacetate is rapidly degraded via a defluorination mechanism in biologically active stream water.
- The degradation can be carried out by aquatic plants, which take it up and rapidly degrade it or by micro-organisms in the water.

- At lower temperatures it is more persistent, but at 7°C it reaches very low levels after 17 days.
- Loss of fluoroacetate from the control tanks probably indicated contamination by micro-organisms.

Comments:

This is an important paper that demonstrates that fluoroacetate is degraded quite rapidly by plants and micro-organisms under laboratory conditions.

Source: Ogilvie et al., 1996

Ref. 10.

This paper examines the degradation of fluoroacetate in large aquaria containing stream water and the introduced submerged aquatic plant *Elodea canadensis*.

Method:

Sodium fluoroacetate (initial concentration 0.12 mg/L) were added to 80L tanks containing stream water and 1.2Kg *Elodea canadensis*. One tank was maintained at 11°C, the other at 21°C. The experiment was carried out in duplicate at both temperatures. Analyses for fluoroacetate and fluoride (F^- electrode) were carried out by the same methods used for refs. 1 and 9. These were carried out 0, 2, 24, 48, 72, 101, 141, 192 (water samples) and 0, 2, 8, 32, 77, 192, 240 hr (plants) after dosing. pH values and dissolved oxygen concentrations were measured. Control tanks contained deionised water.

Results:

At 11°C the concentration of fluoroacetate declined with a DT_{50} of ca. 80–120 hours. At 192 hours there was still 23% of the fluoroacetate remaining. At 21°C the degradation was much more rapid. The DT_{50} was ca. 45 hours and the concentration of fluoroacetate was barely detectable at 101 hours. There was little degradation in the deionised water tanks (cf. ref. 9). There was a strict stoichiometry between the concentration of fluoroacetate degraded and the amount of fluoride formed. In the plants, the concentration of fluoroacetate peaked at 24 hr (both temperatures) but declined at a faster rate at the higher temperature such that it was undetectable at 191 hr; at 11°C there were still detectable levels at 240 hr.

Conclusions:

- Sodium fluoroacetate is rapidly metabolised via a defluorination route by submerged aquatic plants and (probably) by micro-organisms present in stream water.
- The degradation is slower at lower temperatures so fluoroacetate may persist longer when applied in the winter.

Comment:
A similar paper to 1 and 9 with similar conclusions.

Source: Parfitt et al., 1994

Ref. 11

One part of this paper describes laboratory studies in which fluoroacetate was added to tanks containing stream water, plants, micro-organisms and small invertebrates in a study very similar to references 1, 9 and 10 above, with near identical results and conclusions, consequently, this part of the paper was not abstracted nor criticised. The other parts describes laboratory studies in which degradation and leaching of fluoroacetate in soils were measured and field studies in which water samples were taken from stream in which 1080 baits were spread by aerial application to control opossums or rabbits in stated NZ localities.

Methods:

Soil degradation studies:

Three types of soil from different localities were sieved. 14 g soil plus 16 ml water were incubated with sodium fluoroacetate (6.1 mg). For analysis, soil samples were centrifuged and the sodium fluoride measured by GLC. In general, the limit of detection of fluoroacate by GLC was 0.3–1 ppb. Samples were analysed up to 100 days after dosing (maximum).

Leaching studies:

A laboratory lysimeter experiment was set up using 3 soil types pH 5–6 held in 10mm (diam.) x 120 mm tubes. Sodium fluoride (4 mg) was applied to the column and 10 x 5 ml pulses of water applied to the column equivalent to 160 mm rain. The eluate was analysed by GLC.

Field analysis of water after aerial baiting for possum control:

Four aerial baiting programmes in various NZ localities for possum control in 1990 – 1993 were studied. Application rates were 5 – 14 kg/ha of 0.018% 1080 bait. Water samples totalling 288 from stream, river, surface water and ground water were taken and assayed for fluoroacetate by GLC (limit of detection 0.3–1 ppb). Sampling was spread from prior to application to 120 days after baiting. In another study in 1992, baited carrots were applied over 1050 ha for rabbit control (16-60 kg/ha of 0.023% bait). Sampling of ponds, streams, groundwater was spread up to 120 days after application. In all of these studies, rainfall was monitored and recorded.

Results:

Soil degradation:

The DT_{50} of fluoroacetate was 10 days at 23°C, 30 days and 10°C and 80 days at 5°C. Autoclaved soil samples showed no degradation of fluoroacetate. An experiment using soil of only 9% moisture showed a much slower degradation rate of fluoroacetate.

Leaching studies:

Sodium fluoroacetate was recovered totally from the columns, showing there was no decomposition during the course of the experiment. It was minimally absorbed on to the soil and moved through the columns very similarly to nitrate or chloride, eluting at 1–2 column volumes.

Field studies: watercourse contamination.

In three of the four possum baiting experiments fluoroacetate was below the limit of detection in all of the samples. Extremely low (<<0.3 ppb) were found in some of the samples in one trial. It was hypothesised that this was due to contamination, as control samples also tested positive. In the rabbit control experiment, very low levels of fluoroacetate were detected in three of the four sites. This was at a level of 0.3 to 0.6 ppb. One of the positive samples was collected before the aerial dosing began the other on the same day as dosing. Later samples showed no traces of fluoroacetate contamination.

Conclusions:

- Sodium fluoride was rapidly degraded in the laboratory soil sample, at 23°C with a DT_{50} of 10 days; however at lower temperatures it is more stable. There was a direct relation between soil moisture and degradation rate.
- fluoroacetate was not adsorbed to soil and was leached at a similar rate to other monovalent anions such as nitrate and chloride.
- There was little contamination of watercourses, groundwater supplies and ponds due to aerial baiting of 1080, even under conditions of high rainfall.

Comments:

A sound and important paper that shows sodium fluoroacetate is degraded rapidly in soils at higher temperatures but is more stable at lower ones. It is not adsorbed to soil and leaches at a similar rate to chloride or nitrate. There was little evidence of water contamination after aerial baiting. The one criticism is that soils and water used in the degradation studies could have been subject to prior exposure to fluoroacetate.

Source: Tonomura et al., 1965

Ref. 12.

This paper shows that enzymes capable of catalysing the hydrolysis of fluoroacetate to fluoride and glycolate from soil bacteria.

Methods:

Bacteria were grown from various soil samples (localities not specified) and cultured via and enrichment technique on fluoroacetate-containing media. Bacteria were disrupted and the enzyme purified by standard biochemical methods. Fluoroacetate, fluoride and glycolate were measured by colorimetric methods (referenced but not described). Glycolate was also characterised by its behaviour on ion exchange chromatography.

Results/conclusions:

- Bacteria, tentatively identified as *Pseudomonas* spp. capable of utilising fluoroacetate as a source of carbon were grown from soil samples.
- The enzyme that catalysed defluorination of fluoroacetate to glycolate was induced by growing them in media containing fluoroacetate as the sole carbon source.
- The products glycolate and fluoride were unequivocally identified.
- Fluoroacetamide was a poor substrate for the enzyme.

Comments:

This is a sound, basic biochemistry paper. It is noteworthy that the enzyme was induced by fluoroacetate. This enzyme is probably the similar to the one described in ref. 7.

Source: Walker, 1994

Ref. 13.

This paper uses enrichment culture techniques to isolate micro-organisms capable of detoxifying fluoroacetate from various soil samples.

Methods:

The methods used for enrichment culture are not specified in the paper; however, a fluoride electrode was used to monitor F^- release. They state that soil samples were taken from many and diverse sites in NZ. Enzymology methods used to study the enzyme were delineated.

Results/Conclusions:

- Micro-organisms capable of defluorinating fluoroacetate were isolated from many and diverse samples of NZ soils.

- *Pseudomonas* and *Fusarium* were capable of growing on fluoroacetate as the sole carbon source.
- Other organisms demonstrated defluorinating abilities only if supplied with a supplementary carbon source.
- Soils with a prior history of fluoroacetate exposure showed a higher incidence of fluoride degrading micro-organisms.
- Several very common soil fungi (*Fusarium, Aspergillus, Mucor* and Penicillium also demonstrated defluorinating ability.
- Ability to defluorinate fluoroacetate is a common attribute in NZ soils.

Comments:

This is a summary paper with little experimental detail; however, it does have some very important conclusions that appear to be supported that the ability to detoxify fluoroacetate is a common attribute in soil bacteria and fungi and is easily inducible. There was no information on rates of fluoroacetate degradation.

Source: Walker and Bong, 1981

Ref 14

This is a standard biochemistry paper that describes the purification and properties of enzymes capable of defluorinating fluoroacetate to fluoride and glycolate from a bacterium and a fungus.

Method:

The two micro-organisms were isolated by an enrichment technique. Purification of the enzymes involved standard biochemical methods and analysis techniques described in other papers listed in this report.

Results/conclusions

- Active enzymes capable of defluorinating fluoroacetate to fluoride and glycolate were isolated from both a soil *Pseudomonas* sp. and the fungus *Fusarium solani*.
- There are no rate data in this paper.

Source: Bong et al., 1992a

Ref. 15.

This paper explores whether micro-organisms can be isolated from a western Australian soil that have been exposed or not exposed to fluoroacetate and the differences between them.

Methods:

Micro-organisms were isolated from four sites in temperate Western Australia, two of which had had exposure to fluoroacetate via fluoroacetate synthesising plants and two of which had no exposure. Micro-organisms were isolated using minimal broth media supplemented with fluoroacetate. Conditions were optimised for bacterial (pH 6.8) of fungal (pH 5.6) isolation. Pure cultures were isolated by plating out. The ability of bacterial and fungal cultures was assessed by their ability to defluorinate 20 mM sodium fluoroacetate in liquid culture and in autoclaved soils. This was assessed by measuring fluoride with a fluoride electrode. Concentrations of fluoride were corrected using experimentally measured adsorption coefficient on soil. Samples were cultured for up to 57 days at 28^{o}C (day) 15^{o}C (night).

Results/conclusions

- Most samples from all four sites showed defluorinating activity.
- Sites 1 (no fluoroacetate exposure) and sites 3 and 4 (fluoroacetate exposure) had a soil microflora population that rapidly defluorinated fluoroacetate.
- The main species responsible were *Pseudomonas acidivorans, P. fluorescens*, and an unidentified *Pseudomonas* sp. (bacteria), Fusarium *oxysporum*, *Penicillium purpurescens* and *P. restrictum* (fungi).
- Site 2 (no fluoroacetate exposure had a lower defluorinating activity than the other three sites.
- Different species varied in their ability to defluorinate fluoroacetate and from 2 - 87% was degraded in 57 days. The common soil bacterium *P. fluorescens* was particularly efficient, with 87% degradation after 1 day and showed no lag (induction) phase like some other species, particularly fungi.
- Some indigenous microflora populations were able to defluorinate 50–80% of fluoroacetate in soils of 10% moisture over 5 – 9 days under the conditions used.
- Soils from arid tropical areas (~0% moisture) showed no ability to defluorinate fluoroacetate. They also had very low microflora populations.

Comments:

A competent paper that shows the presence of micro-organisms able to detoxify fluoroacetate is widespread and does not rely on prior exposure to fluoroacetate. However, there might be problems with persistence in very hot dry areas.

Source: Bong et al., 1992b

Ref. 16

The paper is a follow-up report to the previous one by the same authors (Bong *et al*., 1992a). In this paper they examine the influences of various conditions, viz. inoculum size, soil pH, temperature, moisture and temperature on fluoroacetate detoxification rates by soil micro-organisms were assessed in more detail.

Methods:

The six species of fluoroacetate-defluorinating organisms isolated in the previous paper were used for this study (thee bacteria, thee fungi). Additionally a culture of *Pseudomonas fluorescens* was isolated from kangaroo meat baits.

(a) Effect of pH: Solutions of sterile 20mM sodium fluoroacetate were adjusted to various pH values between 5 and 8 were inoculated with the same level of cells of the various cultures. The nature of the buffer (if any) was not stated. Cultures were grown for 14 days at 28^{o}C. The pH was monitored and the fluoride concentration measured by a fluoride electrode. Growth was measured by plating and colony counting.

(b) Effect of temperature: Samples were prepared in either 10ml 20mM sodium fluoroacetate of 1% peptone or 12g sterile soil. Temperatures from 25 to 37°C were used or a fluctuating temperature 11–24^{o}C. Micro-organism growth and defluorinating ability was tested as above. The culture purity at the end of the 14 day incubation period was also checked.

(c) Effect of soil moisture: Sterile deionised water was added to 12g sterile oven dried soil to produce soil in range 8.3–50%. Each sample was inoculated with 1 ml of 20mM sodium fluoroacetate containing a bacterial of fungal inoculum of the optimum inoculum size. After 14 days the amount of fluoride released from the fluoroacetate was measured using a fluoride electrode.

(d) Effect of inoculum size: 12 g sterile soil (pH6.3, 9% moisture content) or 10ml 20mM sodium fluoroacetate were incubated with 1 ml. Of ten-fold dilutions of cell inocula (original cell densities were 1.5x10^{9} cells/ml). Culture were incubated for 14 days at the organism's optimum temperature (assessed as above) and the amount of defluorination assessed by the concentration measured by an fluoride-specific electrode.

Results/conclusions:

- The optimum pH values for defluorination by the bacteria were at neutral to slightly alkaline values. The fungi worked better at slightly acidic values. The *P. fluorescens* culture isolated from meat had no defluorinating ability at all.
- All cultures were able to defluorinate fluoroacetate at a wide range of temperatures up to 30^{o}C and at fluctuating temperatures. Different species showed very different temperature optima. Rates at 37^{o}C were much slower.
- All the micro-organisms were capable of defluorinating fluoroacetate at a wide range of soil moisture contents from 8.3 to 50%. The highest rates (70 to 81% degradation occurred at soil moisture levels of 8.3 to 15%. The lowest rate occurred at 30% moisture (?).
- The optimum inoculum size varied with the micro-organism. When fluoroacetate was the sole carbon source the defluorination rate decreased with inoculum size. In the soil experiments there was still significant defluorination at lower inoculum levels.

Comments:

This paper is reasonably competent and shows that even the presence low numbers of micro-organisms capable of defluorinating fluoroacetate result in effective degradation. They are also able to work in a wide range of conditions. The only criticism is that defluorination levels at very low moisture contents and temperature were not assessed and other workers have shown that fluoroacetate is considerably more stable under these conditions.

Source: Wright et al., 2003

Ref 17

This report describes experiments designed to establish whether aquatic plant can eliminate sodium fluoride from water at low concentrations near to the provisional maximum acceptable level (PMAV) for NZ drinking water, 3.5 ppb; consequently, in all experiments described, the original dosing levels were 5 ppb. In an attempt to eliminate microbial detoxification all experiments were carried out in deionised water using washed plant material. The effects of plant mass (the submerged aquatic plant *Elodea canadensis*), temperature and absence or presence of light were assessed.

Methods:

The report describes laboratory experiments where cut sections of plants were washed and added to deionised water (150 ml) held in glass flasks, which were mechanically agitated. Sodium fluoroacetate (5 ppb) was added and the water of plant material assayed for fluoroacetate by a referenced GLC method.

(a) The effect of plant mass was assessed by varying the ratio of fresh weight to water between 0.33 and 133 mg/ml. The temperature was 16^{o}C and the duration 48hr.
(b) The effect of temperature was assessed by adding plant material at a rate of 3.3 mg/ml water and running the experiment at 5, 11, 17 and 23^{o}C.
(c) The effect of light/dark was assessed by incubating the plant material in the light or dark and assaying the water for fluoroacetate at 1, 2, 4, 6, 16, 24, 48, and 72 hr. The concentration of fluoroacetate in the plant material was also measured.

Replicates were used in all tests and subjected to statistical analysis. Elimination of fluoroacetate was fitted to a first order equation and the $t_{1/2}$ values calculated. Potential products of fluoroacetate degradation (fluoride, glycolate, fluorocitrate) were not measured.

Results:

- There was no elimination of fluoroacetate in the absence of plant material.
- There was a positive relationship between the rate of fluoroacetate loss and the mass of plant material. The $t_{1/2}$ varied between 18.9hr at 1.3

mg/ml and 2.5 hr at 133 mg/ml. The authors fitted these data to a regression line. One trial gave faster rates of elimination (microbial contamination?) and was ignored.

- The rate of elimination was positively correlated with temperature and was significantly lower at 5^{o}C and 11^{o}C.
- There was no significant effect of light on the rate of degradation.
- The concentration of fluoride in the plant material peaked at 1 hr and then rapidly fell so that only very low levels were detectable at 48 hr.

Conclusions:

- The elimination of fluoride from aquatic systems is influenced by the amount of plant material. Loss is faster with more plant material.
- Elimination is not just due to plant adsorption but the material is rapidly metabolised in plants.
- There are no data as to whether the metabolic products are fluorocitrate (toxic) or glycolate (non-toxic) or any other potential product of metabolism.
- The rate of plant metabolism is not influenced by artificial light so is not influenced by photosynthesis.
- There is a vanishingly low theoretical risk of acute secondary poisoning by consuming aquatic plants that have been in contact with low levels of fluoroacetate in water.

Comments

This report is not clearly or concisely written and examination of the results shows that some of the actual conditions used in the experiments differ from those stated in the methods section. There is also a methods summary section that is different from the main methods section. A repeat series of experiments where the products of metabolism are identified and measured would be advantageous.

Review and conclusions

Biochemistry of monofluoroacetate

Of the 17 papers reviewed, one (Ref. 1, Booth *et al*., 1999) showed that sodium fluoroacetate can be metabolised to the toxic metabolite fluorocitrate in experimental tanks containing both plants and micro-organisms; however, the fluorocitrate was also rapidly lost from the aqueous phase. It is likely that this product of metabolism was due to the plants rather than the micro-organisms. This mechanism should not release fluoride, at least initially. In all other papers where the metabolites were assessed, fluoroacetate was metabolised by bacteria and fungi via a defluorinating hydrolytic mechanism to inorganic fluoride and glycolate; usually the concomitant release of inorganic fluoride was demonstrated

directly using a fluoride selective electrode. The mechanism of the bacterial enzyme catalysing this reaction has been elegantly delineated by Liu *et al.* (1998).

Interpretation of the source reports concerning persistence

- MAIN CONCLUSION: monofluoroacetate is very mobile and rapidly biodegradable in natural waters and soils, although this is very temperature-dependent. Degradation is also much slower in soils with lower moisture content. However, in naturally arid soils, the soil microflora have been shown to degrade fluoroacetate quite rapidly.
- It is possible that adaptation of plants and micro-organisms has occurred due to the presence of the ion in the environment from natural and anthropogenic sources - but if so, adaptation must be widespread, and therefore the main conclusion is still valid.
- The main mechanism of degradation in the environment is expected to be defluorination to glycolate and fluoride. Enzymes that catalyse this reaction have been isolated and characterised. These enzymes are present in soil bacteria and fungi. Their induction by fluoroacetate appears to be facile.
- Uptake by plants is a removal mechanism but degradation can take place within those plants. It is possible that plants may metabolise it to fluorocitrate and not by defluorination to fluoride and glycolate.
- The scientific quality of the sources is variable but the weight of accumulated evidence suggests that the main conclusion is not compromised.

The overall conclusions with respect to persistence as expressed as DT_{50} values in soil and water ecosystems (laboratory studies) are as follows:

- In soil there are no reports where degradation studies have been performed using a standard test guideline such as OECD 307.
- Where reported, DT_{50} values are highly dependent on temperature (and also soil moisture). The values quoted by Parfitt *et al*. (1994) (ref. 11) viz. **23^{o}C 10 days, 10^{o}C 30 days,** 5^{o}C 80 days are fairly representative of other papers studied. *Note that OECD 307 recommends a test temperature of 20^{o}C for substances likely to be applied to soils in temperate climates and 10^{o}C if substances are likely to be applied in winter.*
- Laboratory studies mimicking water ecosystems have mostly used a mixture of micro-organisms (and also small invertebrates) and plants; the one exception being Wright *et al*. (2003) (ref. 17), which used only plants. Consequently, the reported DT_{50} values vary greatly depending on the conditions. The DT_{50} values quoted in the various papers generally encompass the range <<**1 to 8 days**.

Therefore it can be concluded that monofluoroacetate is not persistent in the sense of the ERMA definitions.

Recommendation

There are some references within the sources provided which could be obtained and reviewed, although it is unlikely that they will change the overall conclusions.

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Attachment 2: Response from Landcare, loss of 1080 from stored soil samples

"Since O'Halloran & Jones (2003) where the potential for a decrease in fluoroacetate in spiked soil samples stored at -20°C was noted, Landcare Research studies involving 1080 in soil have attempted to minimise the duration (minimum 10 days, maximum 6 weeks) of sample storage at -80°C, on the assumption that this would minimise any microbial activity likely to degrade fluoroacetate, if indeed that was occurring at -20°C (O'Halloran, Jones & Fisher 2003; O'Halloran, Jones & Booth 2003). As previously communicated to ERMA, no further work has been undertaken to more fully investigate factors potentially affecting degradation of 1080 in soil during frozen sample storage e.g. soil type, water content, microbial population , and from there identify the most valid procedures for storage and processing samples of this nature.

Many previously-published assessments of the degradation of 1080 in soil did not utilise frozen storage and/or the same analytical methods as O'Halloran & Jones (2003):

- David & Gardner (1966) – no details of analytical method or sample storage conditions given
- King et al. (1994) used detection of F^- by electrodes to estimate 1080 concentrations in soil treated with 1080, with no described storage time.
- Parfitt et al (1994) – soil samples tested for biodegradation were not stored, but analysed as needed throughout the experiment
- Tomkins (1994) – reported 40% recovery of 1080 from soil samples using a GC-electorn capture detection method. Soil samples were spiked with 1080 and analysed straight away without significant storage time.

Thus we can only respond in the context of Landcare Research studies carried out using storage of soil samples at -20°C and the current analytical method for 1080 in soil conducted by the Landcare Research toxicology laboratory. This includes Wright et al (2002), where soil samples were taken before and up to 30 days after aerial application of 1080 baits at three sites. These samples were stored at -20°C over varying periods before analysis (1997 Rangataua operation samples stored for 6-12 weeks, 1997 Titirangi operation samples stored for 2–6 weeks and the 1998 Whitecliffs operation samples were stored for over a year before analysis). Six of 118 samples tested had detectable, but relatively low (maximum 0.024 µg/g) residues, with no residues detected in samples taken more than 5 days after bait application. Five of the six samples with detectable 1080 were stored for a year prior to analysis. In the light of the later findings by O'Halloran & Jones (2003), fluoroacetate present in some soil samples in the Wright et al (2002) study, especially those stored for the longer periods, may have been degraded during storage at -20°C to present undetectable concentrations at analysis. Further experimentation would be needed to test this hypothesis.

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Sent via email, 8 March 2007, From Sara Clarke, URS, on behalf of applicants"