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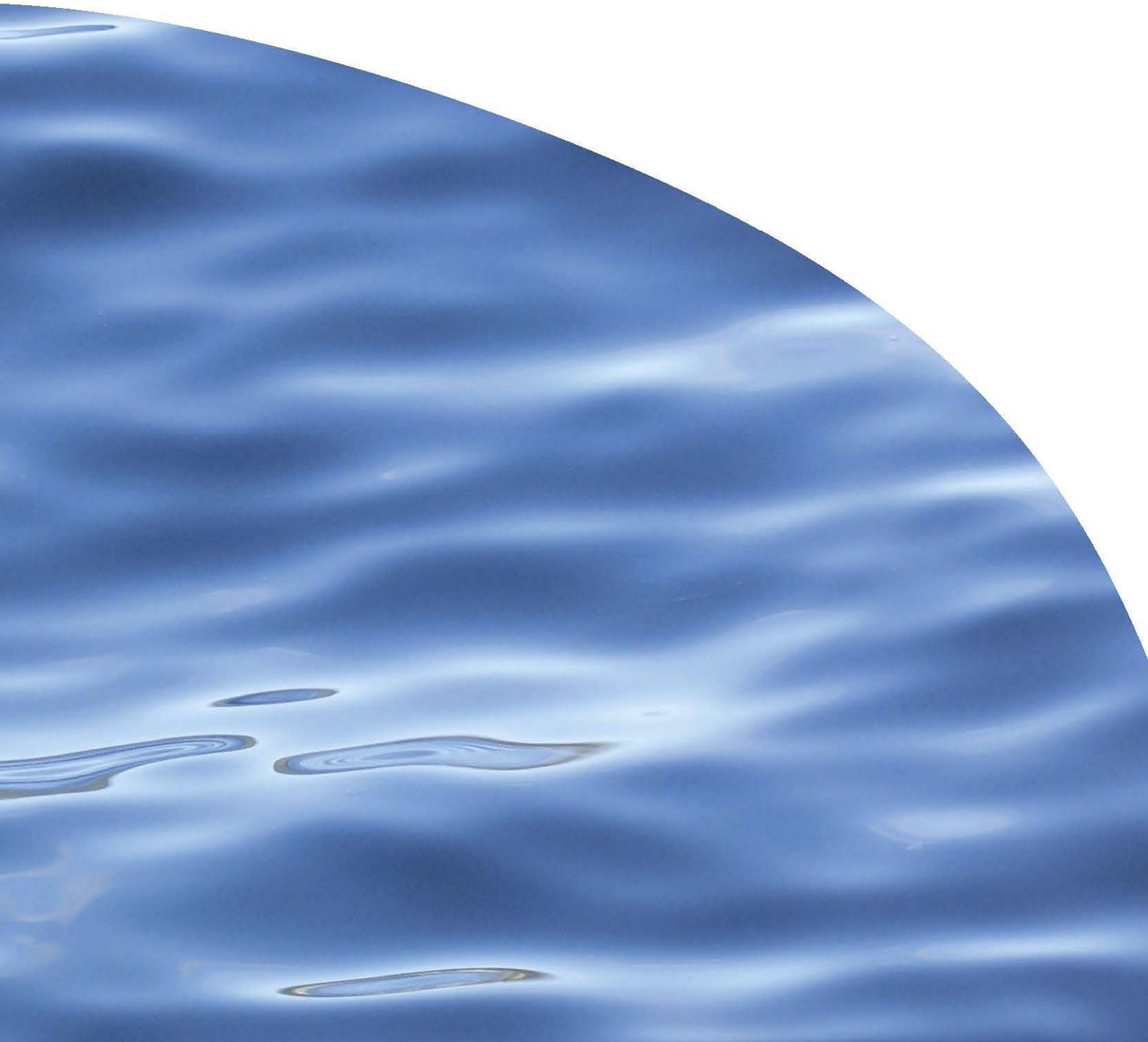
REPORT NO. 2611

1080 UPTAKE AND ELIMINATION IN RAINBOW TROUT



1080 UPTAKE AND ELIMINATION IN RAINBOW TROUT

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Prepared for Department of Conservation

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ISSUE DATE: 30 October 2014

RECOMMENDED CITATION: Champeau O, Knight B, Tremblay LA 2014. 1080 uptake and elimination in rainbow trout. Prepared for the Department of Conservation. Cawthron Report No. 2611. 15 p. plus appendices.

EXECUTIVE SUMMARY

The Department of Conservation (DOC) conducts aerial sodium fluoroacetate (compound 1080) operations to control possum, rat and stoat numbers. Trout are known to predate on mice as a food source during certain times of the year. There is a possibility that trout will ingest 1080 indirectly by consuming mice that have eaten 1080 bait. This in turn presents a potential human health risk if people catch and consume trout. After discussion between DOC, Fish & Game New Zealand and the New Zealand Federation of Freshwater Anglers. The Department of Conservation commissioned Cawthron Institute (Cawthron) to undertake a study to help determine the likely risks associated with trout that have consumed 1080-poisoned mice.

A model of the uptake and fate of 1080 in trout flesh was developed, as there was very little information available on the likely uptake and elimination rates of 1080 in trout. A laboratory experiment was also conducted to provide information on the key model variables, with the intention of revisiting the model so that it could be improved for other scenarios and risk-assessment purposes.

A very high 1080 dose was used in the experiment, to enable the measurement of the uptake and decay of 1080 in the flesh of rainbow trout (*Oncorhynchus mykiss*). Live rainbow trout, with an average weight of 0.78 ± 0.13 kg, were held in large tanks and given an oral 5 mg dose of 1080 in capsules. This dosage is higher than what could occur in a field scenario. This high dose represents a quantity of 1080 under which no direct effect was expected for the trout, which are less susceptible to the effects of 1080 than mammals. The concentration of 1080 was measured in fillets removed from trout at post-mortem. Trout fillets were sampled at set intervals over 120 h. Sampling was a balance between number of replicates, achieving a high enough frequency at the beginning to capture uptake, and having enough samples at designated time points after dosing to capture the rate of elimination.

There have been no previous studies on 1080 uptake and elimination in rainbow trout, so assumptions for this study were based on previous research done on warm-blooded animals, eels and crayfish.

The maximum concentrations measured in trout tissue (up to 4.7 mg/kg) were observed at 24 h and 48 h after ingestion. The concentration decreased to close to 2 mg/kg after 84 h. An increase in 1080 concentration in the flesh was observed after 120 h, but the number of replicates at this interval was low (n = 2).

The maximum concentration in trout occurred later than expected, based on results from warm-blooded animals, where maximum concentrations are normally achieved within 6 h. However, the maximum concentrations measured in trout flesh (fillet) at 24 h and 48 h after dosing was the same as previously reported for the crayfish (*Paranephrops planifrons*).

Compound 1080 was detected in trout 5 d after ingestion in this study. This was similar to the results for the crayfish (*Paranephrops planifrons*) where it was still detected after 8 d, but different for warm-blooded animals where it was eliminated after 1 d–4 d.

With the relatively high variability in results from individual fish, and the considerable amount of 1080 remaining in the flesh at the end of the experiment, it was also not possible to accurately determine the half-life (time for the concentration in the body to be reduced by half) or the elimination rate of 1080 in trout. Consequently, it was not possible to calibrate the model used in this study. However, this study generated preliminary data on 1080 uptake in trout and timeframes involved, which can be used to design more accurate studies to assess the potential risk, if required.

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ABBREVIATIONS

Acronym	Term
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
C_{max}	Maximum concentration
NOEC	No observed effect concentration
K_{OW}	Octanol-Water coefficient (partition coefficient)

GLOSSARY

Term	Definition
Half-life	Time it takes for the concentration in the body to be reduced by 50%
K_{ow}	Measures of how a chemical will distribute between two immiscible solvents: water (a polar solvent) and octanol (a relatively non-polar solvent). K_{ow} is commonly expressed as the log of the coefficient (log K_{ow}). It is used in environmental fate studies and large values (log $K_{ow} \geq 4$) are regarded as an indicator that a substance may bioaccumulate in aquatic organisms (accumulation of chemicals by direct uptake from the water (bioconcentration), uptake of suspended particles (ingestion) or consumption of contaminated food (biomagnification)). Conversely, a low value (≤ 4) indicates that a substance will more likely be mobile through the environment.
LC/LD_{x-t}	Concentration / dose of substance or material that is estimated to be lethal to a proportion (x%) of the test organisms after a defined period of exposure (t). This is an acute toxicity indicator.
NOEC	No observed effect concentration is the highest concentration of a test substance or material which is observed not to have a statistically significant adverse effect on the test organisms for a defined time of exposure and under the test conditions, relative to the control.
Terminal elimination phase	Following the initial distribution, equilibrium between the central compartment and peripheral compartment is attained, which means that drug movement between the peripheral and central compartments is bidirectional. This is represented by a reduction in the decline of the plasma concentration–time curve (Gustafson & Bradshaw-Pierce 2011).

1. INTRODUCTION

The Department of Conservation (DOC) conducts aerial sodium fluoroacetate (compound 1080) operations to control possum, rat and stoat numbers. Trout are known to predate on mice as a food source during certain times of the year. There is a possibility that trout will ingest 1080 indirectly by consuming mice that have eaten 1080 bait. This presents a potential health risk to humans, which may in turn catch and consume trout.

There are two other New Zealand studies that have examined the uptake *via* food and persistence of 1080 in freshwater animals. One was in eels where there was low level exposure to 1080 (Lyver *et al*. 2005). Residues of 1080 were not found in the majority of eels in this study, although levels up to 0.06 mg/kg were still found in eels 24 h after being presented with contaminated minced possum gut and muscle tissue. In the second study, crayfish ingested bait containing 1080, which ensured a more substantial dose was administered. A maximum concentration of between 1.0 to 1.5 mg/kg of 1080 was recorded after 24 h–48 h, with residues still detected after 8 d (192 h; Suren & Bonnett 2006).

After discussion between DOC, Fish & Game New Zealand and the Federation for Freshwater Anglers, DOC commissioned Cawthron Institute (Cawthron) to undertake the present study to help assess the risk involved with trout eating mice that have consumed 1080 bait. The aims of this study were: 1) to develop a model to predict the uptake and fate of 1080 in trout flesh and 2) to investigate the uptake and elimination of a known dose of 1080 in trout following ingestion of 1080 to validate the model.

2. MATERIAL AND METHODS

2.1. Modelling

The model simulating a single dose of 1080 to a trout was built in the freely available Cloud-based system modelling package, 'Insight Maker' (http://insightmaker.com). The model was developed to be mass conservative, so that the fate of the initial mass of 1080 parameterised in the model could be tracked. The model was built to simulate 1080 mass and flow in the gut and flesh of the fish.

The trout model was formulated based on two simple equations to model the uptake (U) of 1080 mass from the gut (M_g) to the flesh (M_f) and degradation / excretion loss processes in the flesh and gut as:

$$\frac{dM_g}{dt} = -U - k_g.M_g$$

Equation 1: Uptake in the gut

$$\frac{dM_f}{dt} = U - k_f.M_f$$

Equation 2: Uptake in the flesh

Where k_g and k_f are decay rate constants defined by half-life values ($T_{50\%}$ in days) obtained from degradation of 1080 in mammalian tissues of < 11 h (< 0.458 d) (Eason *et al.* 2011). Model values for the half-life were defined separately to allow for decreased gut half-life values if gut evacuation or other degradation processes need to be considered. After appropriate half-lives for the gut and flesh are determined, the flesh and gut decay rate constants (k_f and k_g) are calculated as:

$$k = \frac{-ln(0.5)}{T_{50\%}}$$

Equation 3: Elimination rate

This modelling assumed a maximum calorie uptake for brown trout (Elliott & Hurley 1998, 1999, 2000a, b; Hayes 2013) to estimate a constant uptake rate for 1080 (U) in the absence of available information on uptake rates for 1080 in fish.

The model was implemented in the 'Insight Maker' website[1]. Several input parameters can be easily modified, including:

- Fish weight (default = 1 kg)
- Water temperature (default = 14°C)
- Maximum gut size
- Food portion size (default = 20 gms)
- Mouse LD_{50} for 1080 (8 mg/kg)

Unfortunately no information on uptake or elimination rates of 1080 in trout was available when the model was developed. Therefore, there was considerable uncertainty about the accuracy of the model predictions. Key limitations of the model can be summarised in order of importance:

1. The model assumes no bioaccumulation (*i.e.* that flesh concentration cannot exceed gut concentration of 1080). The low K_{ow} of 1080 suggests this is a valid

[1] http://insightmaker.com/insight/15117# - Note that model is currently set to be private with insight user account and appropriate permissions required to access the model.

assumption but could influence the model accuracy when estimating the safety for human consumption.

2. The model only calculates a single 1080-dose scenario.
3. In the absence of a 1080 half-life value for trout, it was assumed to be < 11 h based on data from mammals (Eason *et al.* 2011).

Given the uncertainties in the model, we considered that laboratory experiments were required to assess the uptake of 1080 into trout flesh and to estimate the time to achieve total 1080 clearance.

2.2. Laboratory experiment

2.2.1. Equipment

The experimental system consisted of four 500 L polyethylene tanks (Promax Engineered Plastics Ltd, Auckland) with water recirculated through two 200 L header tanks (Figure 1). The average flow rate in the tanks was 16.2 ± 0.6 L/min. The system was filled with de-chlorinated tap water using a bubbler during the acclimation period.

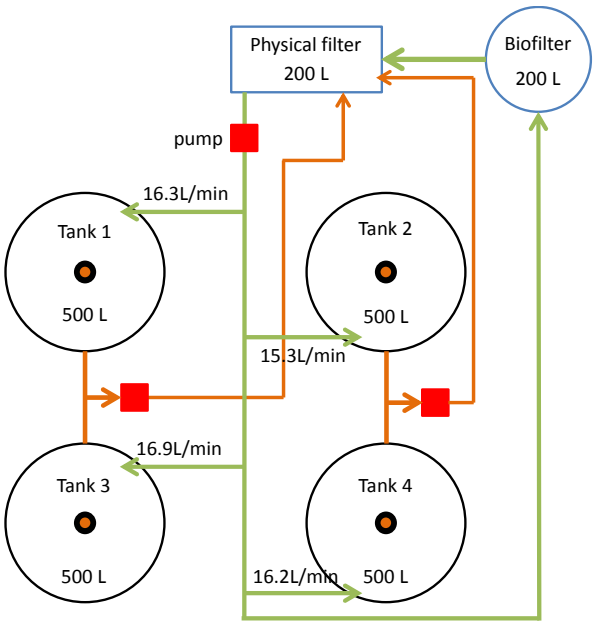

Figure 1. Fish holding tanks and recirculation system.

2.2.1. Animals

Thirty rainbow trout (*Oncorhynchus mykiss*) were obtained from the Fish & Game North Canterbury (Montrose) hatchery. Trout were transported in an aerated Fish &

Game tanker. They were treated with 50 mg/L H_2O_2 for 1 h to reduce fungal infections (AADAP 2011) prior to transfer into the holding tanks at Cawthron. There were eight trout per tank in three tanks, and six trout in the fourth tank. The fish were acclimated for 7 d prior to the trial and were monitored daily for signs of distress / disease. The few trout that exhibited these symptoms were removed, anesthetised with Aqui-S® and killed by spinal severance. Fish were fed daily during the acclimation period with a handful of trout pellets (provided by Fish & Game) until 2 d prior to the 1080 dosing. Eight fish were used as control and 22 for the 1080 dietary exposure. The experiment was conducted under animal ethics approval AEC2014-CAW-01 (Appendix 2).

Trout condition was assessed using the condition factor K (Equation 1) for salmonid fish (Fulton 1902; Ricker 1975).

$$K = \frac{W \times 10^5}{L^3}$$

Equation 4. Condition factor K

Where:

K is the condition factor ('K factor')
W is the weight of the fish in grams (g)
L is the length of the fish in millimetres (mm) from the tip of the snout to the rear edge of the fork at the centre of the tail fin.

2.2.2. Exposure

Anglers have reported catching large trout with up to 23[2] mice in their guts. Therefore, to verify the model predictions, a high dose was chosen to measure uptake and persistence of 1080 in trout flesh. The dose used for the experiment is beyond a worst-case scenario. This dose would be the equivalent of 30 mice with a dose of 8.33 mg/kg equivalent to the LD_{50}. Assuming each mouse weighs 20 g, the total dose for this scenario is 5 mg (8.33 mg/kg × 0.02 kg/mouse × 30 mice). The number of mice to be ingested is far above what a trout of the size used in this experiment (0.78 kg) could possibly eat.

The 1080 used in this experiment was sourced as powder (1.5 g) from the Tull Chemical Company, with a claimed purity of 99.49 ± 2.61%. A stock solution of 25 g 1080 /L was prepared in distilled water containing 2% agar under sterile conditions. There was 200 µL of this solution (*i.e.* 5 mg of 1080) transferred into a No 4 gelatine capsule (14.3 mm height) and dried overnight at room temperature. The average weight of the dried capsules, containing 1080 in agar, was 74.7±1.8 mg, which corresponds to a concentration of ~ 67 g 1080/kg.

[2] *www.anglerspassport.com/media/documents/pdf/fg_issue32_18-26.pdf*[3]
http://www.landcareresearch.co.nz/resources/laboratories/toxicology-laboratory/services/advice-and-protocols/protocol-for-sampling-and-testing-water-for-1080 (accessed on the 19/09/2014)

Trout were orally dosed on 28 August 2014 with the capsules containing 1080 and control fish were given capsules with agar only using a method slightly modified from that described by Hung (1991). To administer the oral dosage, the trout were turned ventral side up and restrained with a wet towel covering their eyes to reduce stress. A flexible tube (25 cm in length) was used, holding the capsule at the distal end, and pushed gently from the mouth into the oesophagus. A slight resistance was felt when the tube reached the end of the distal oesophagus where the capsule was pushed in. Fish were returned to the tank in less than 30 sec and monitored for signs of distress and regurgitation. Fish were not fed after dosing until sampling.

2.2.3. Sampling

The experiment was designed to balance between achieving a sufficient sampling frequency to capture initial 1080 uptake and the expected rate of elimination, assumed from other vertebrates (Eason *et al*. 1994).

Water samples were collected at all sampling times. Fish sampled were anaesthetised with Aqui-S® and killed by spinal severance, measured and weighed at 6 h, 12 h, 24 h, 48 h, 84 h and 120 h after dosing as shown in Figure 2.

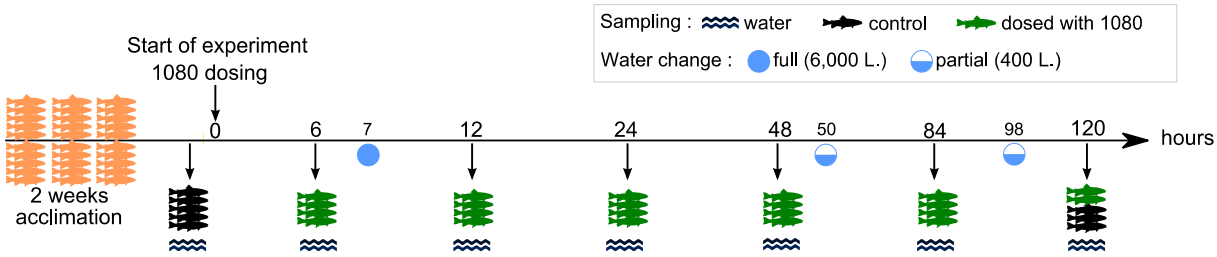

Figure 2. Timeline for fish and water sampling.

Liver, gut, and fillets from both sides were removed from the trout. Some guts were opened to verify ingestion of the capsules in the 6 h fish samples. Dissection equipment and gloves were rinsed or changed between samples to minimise 1080 cross-contamination. Tissues and water samples were stored at -70°C to prevent 1080 degradation (ERMA 2007a). Muscle tissue (right-side fillet) and water samples were frozen on dry ice (-50°C) and sent to the Landcare Research Lincoln laboratory for 1080 residue analysis. Other tissues were stored at -70°C for further analysis if required.

2.2.4. 1080 residue analysis

The 1080 chemical analyses were carried out using the gas chromatography method TLM 005, 'Assay of 1080 in water, soil, and biological materials by GLC'. This method

is accredited with IANZ (International Accreditation New Zealand) under Environmental Monitoring[3]. The method detection limits (MDL) are 0.1µg/L ± 12% (95% confidence interval) and 1µg/kg ± 9% (95% confidence interval) for the water and the biological material, respectively.

2.2.5. Water monitoring and renewal

Water quality within the trout tanks was regularly monitored for pH, temperature and ammonia levels with a SenEye® (Norfolk, UK) monitoring probe. Dissolved oxygen was checked daily with an HQ40d Hach® multi-parameter probe. Water was tested every second day for ammonia, nitrites and nitrates with aquarium test kits (API® test kits).

During the acclimation period, 10% of the water was renewed daily with de-chlorinated tap water that had been de-chlorinated by bubbling for at least 24 h before being changed. This ensured that ammonia, nitrate and nitrite levels were low.

Water was totally (6,000 L) renewed 8 h after dosing with certified standard drinking water from a Tasman District Council Motueka artesian well[4] (bore water) (Tasman Watercare Distribution Ltd, Motueka). A 15 % water change with de-chlorinated tap water (400 L treated with 10 mL of Seachem Prime®) was conducted 50 h and 98 h following dosing.

2.2.6. Statistics

A Dixon test was applied to detect outliers in the dataset. If any outliers were identified, they were excluded from the dataset for subsequent analysis. A non-parametric Kruskall-Wallis test was applied to test the null hypothesis that concentration of 1080 in the flesh did not change over time. If H_0 was rejected, the Wilcox-test was used to detect the significant difference between sampling times. Statistical analyses were conducted using the R statistical software (R Core Team 2014). The level of statistical significance selected was $P < 0.05$. Data are reported as the average ± the standard deviation.

[3] http://www.landcareresearch.co.nz/resources/laboratories/toxicology-laboratory/services/advice-and-protocols/protocol-for-sampling-and-testing-water-for-1080 (accessed on the 19/09/2014)
[4] http://www.drinkingwater.esr.cri.nz/

3. RESULTS

3.1.1. Water quality

Water parameters and variations of pH, ammonia and temperature during the acclimation and the experiment are presented in Table 1 and Figure 3, respectively.

Table 1. Physico-chemical parameters (median with 25% and 75% percentiles) during acclimation and experiment period.

Parameters	Acclimation period	Experiment period
Temperature (°C)	13 (11.3–14)	14.3 (13.4–14.5)
Dissolved oxygen (mg/L)	9.4 (9.3–9.8)	9.2–10.87 (range)
pH	7.3 (7.2–7.5)	8.11 (8.05–8.16)
Ammonia (NH_3) (mg/L)	0.115 (0.099–0.186)	0.042 (0.036–0.048)
Nitrates (mg/L)	< 80	< 40
Nitrites (mg/L)	< 5	< 3
General hardness	n/m	60–120 (range)
Potassium hardness	n/m	60–180 (range)

n/m not measured

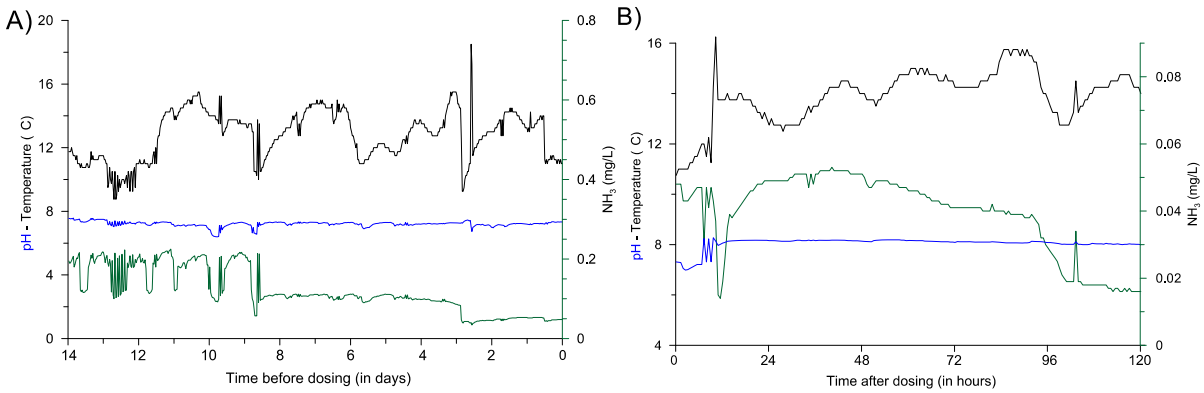

Figure 3. Variations of the physico-chemical parameters continuously monitored during A) acclimation and B) experiment periods.

3.1.2. Animal health condition

Mean trout weight and length were 777 ± 132 g and 400.7 ± 23.5 mm, respectively. Almost all trout (except for four) had condition factors indicative of fair to excellent condition at the end of their exposure time (Figure 4). Two of the fish were mature females with eggs (Figure 5). The weight of the eggs was about 12% of the animal total weight. The K indices from the two females were 1.19 and 1.13. Without eggs these indices were 1.05 and 1. (Raw data are presented in Appendix 3, Table A3.1).

Figure 4. The distribution of fish condition (K index) at the end of the exposure period (blue and pink colours represent the males and females, respectively)

Figure 5. Gravid females.

3.1.3. 1080 chemical analysis

Certificates of analyses carried out by Landcare Research for the concentration of 1080 residues in water and fish flesh are provided in Appendix 4.

Water

The concentration of 1080 in water during the experimental period was high following dosing and declined to the method detection limit after 84 h (Figure 6). Declines occurred over periods when the water was changed (6 h–12 h) and also during periods when no water change occurred (12 h–48 h) (Figure 5). Raw data are presented in Appendix 3, Table A3.2.

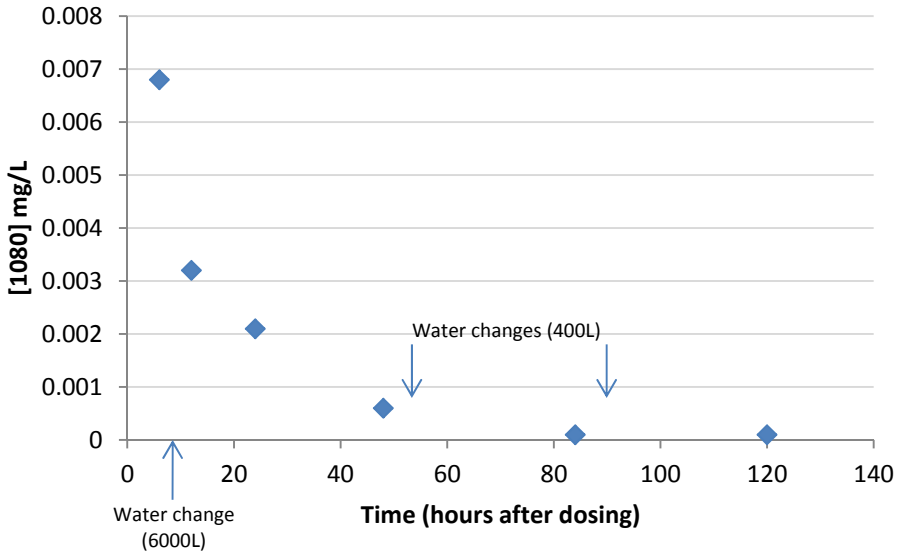

Figure 6. Concentration of 1080 (sodium fluoroacetate) in water during the experiment (in mg/L) (n = 1) with water renewal indicated by the arrows.

Fish

No mortality or changes in fish behaviour were observed following the dosing. Visual observations during the dissections did not indicate damage to gills or the liver.

The maximum concentration (C_{max}) of 1080 measured in trout flesh (3.8 ± 0.2 mg/kg) was observed 24 h after ingestion (Figure 7) (raw data are presented in Appendix 3, Table A3.1). The concentration decreased to about 21% (ranging from 16% to 39% of the dose delivered) after 84 h (3.5 d). An increase in 1080 concentration in the flesh was observed after 120 h (5 d) but the sample size was small for this final measurement (n = 2).

An effect of time on 1080 concentration in flesh was detected (χ^2 = 10.4, df = 4, P = 0.03). The concentration of 1080 in flesh at 84 h was significantly different from 48 h (P = 0.028). Although not significant at the P < 0.05 level, there was some indication that concentrations were higher at 24 h than at 84 h (P = 0.057) (Figure 6). Details of the statistics are presented in Appendix 4.

Five controls were killed before the feeding trial and two control fish were killed at the end of the experiment. 1080 concentrations in all control fish were below the method detection limit (MDL) of the assay.

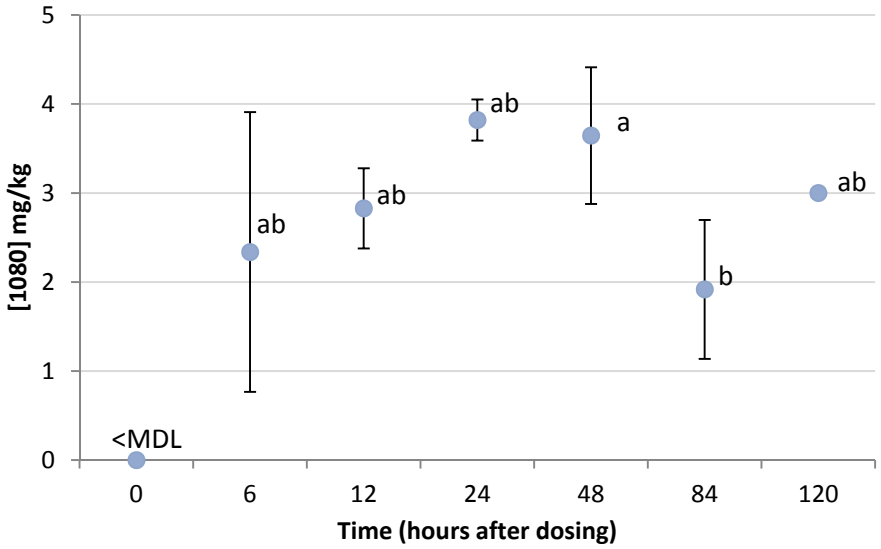

Figure 7. Trout flesh 1080 (sodium fluoroacetate) concentration (mg/kg) ± the standard deviation following ingestion of a 5 mg dose. Data points with the same letter are not statistically different ($P < 0.05$). MDL: method detection limit (1 µg/kg).

4. DISCUSSION

The results provide valuable insight into the uptake of 1080 by trout following ingestion of a very high dose. This dosage used in the experiment is higher than what could occur in a field scenario. The trout used for this experiment (average weight 777 g) would be physically unable to contain 30 mice in their gut. Reports of large numbers of mice in guts were in much bigger trout than those used in this investigation. However, no direct effect of this level of 1080 was expected (or observed) for the trout, as they are known to be much less susceptible to the effects of 1080 than mammals. For example, the LD_{50} for 1080 for a number of mammalian species is < 1 mg/kg, whereas the LD_{50} for 1080 in trout (by injection) is 50 mg/kg (Bauermeister *et al.* 1977). A study on trout found no visible effect at 4 mg 1080 per fingerling (approximately 80 mg/kg) and at 8 mg per adult (Rammell & Fleming 1978). The 1080 dose used in this study was about 600 times higher than the dose given in previous mammalian studies (Eason *et al.* 1993; Gooneratne *et al.* 2008). It is also worth noting that the trout were fasted for 2 d before being given a bolus of 1080.

The maximum concentration of 1080 in the trout was reached at later time points than we had expected. This was based on results from studies done with warm-blooded animals, where maximum concentrations are normally achieved within 6 h (Eason *et al.* 1994; Feldwick *et al.* 1994; Gooneratne *et al.* 1994; Gooneratne *et al.* 2008). The apparent elimination rates observed were also slower than anticipated. Warm-blooded animals receiving small sub-lethal doses, metabolised and excreted 1080 within 1 d–4 d (Eason *et al.* 1994; Eason *et al.* 1996).

The bioavailability (*i.e.* the amount of 1080 absorbed from a given dosing vehicle) is likely to be greater from an oral dosage of 1080 in a 0.075 g capsule, than 1080 derived from a contaminated mouse or bait. This would therefore lead to a higher concentration of 1080 in the fish. Compared to other studies, the persistence of 1080 in trout appears to be similar to that in crayfish, where 1080 was still detected for over 8 d after feeding (Suren & Bonnett 2006). Consequently, the half-life of 1080 in crayfish and trout is longer than in warm-blooded animals. The persistence of 1080 in terrestrial invertebrates appears to be even longer. Residues in tree weta dosed with 15 mg/kg of 1080 were still detected 14 d after dosing (Eason *et al.* 1993) while in the land crab (*Gecarcinus lagostoma*) residues of 1080 were still detected 9 d–11 d after ingestion of 2 mg of 1080 in 10 g–15 g of fish bait (Pain *et al.* 2008).

The persistence of 1080 in trout appears longer than the estimated 1 d–4 d, which is typical of mammals (Eason *et al*. 1994). This could be due to the high 1080 dose used, taking longer to be eliminated. The elimination rate and the half-life for 1080 in trout flesh could not be accurately determined because of the considerable amount remaining at the last sampling time. The data from this study did not allow the calibration of the model.

The degradation rate of 1080 in water found in this experiment is equivalent to previous studies, where it is quickly degraded by microbial metabolic processes. Temperature plays an important role in the degradation of 1080 (in either water or animals). In controlled conditions, biologically active aquaria spiked with 0.1 mg/L of 1080 (equivalent to two to three possum baits) the concentration declined by 70% in 24 h and after 100 h 1080 levels were below detection limit at a water temperature of 21°C (Parfitt *et al.* 1993; Ogilvie *et al.* 1996). At colder temperature (11°C) the rate of breakdown is slower and 1080 was still detectable after 192 h (Ogilvie *et al.* 1996). After a large-scale possum operation, 1080 concentration in surface water decreased rapidly and is likely to be very low 24 h after such an operation (Suren 2006; Suren & Lambert 2006; Eason *et al.* 2011).

Two other studies examined the uptake and persistence of 1080 in freshwater animals. One study was in the longfin eel (*Anguilla dieffenbachii*), where low concentrations of 1080 were detected in muscle tissue of eels that had consumed contaminated possum muscle and gut tissue boluses (Lyver *et al.* 2005). he amount of contaminated possum that was eaten by each eel could not be determined. In the second study, crayfish were exposed to 1080 directly through ingestion of bait, ensuring a more substantial dose was administered. A maximum concentration of 1080 was recorded at 24-48 h with residues detected over 8 d (Suren & Bonnett 2006). The maximum concentration in crayfish and trout was reached at later time points in these aquatic species when compared to warm-blooded animals where maximum concentration was achieved within 1 h (Eason *et al.* 1994).

In both crayfish and trout the maximum concentration (C_{max}) of 1080 in the test organism was reached later than would be expected for warm-blooded animals, which have been studied more intensively. C_{max} is normally achieved within 6 h in warm-blooded animals (Eason *et al.* 1994; Feldwick *et al.* 1994; Gooneratne *et al.* 1994; Gooneratne *et al.* 2008).

It is well-documented that the bioavailability of chemicals is modulated by the presence of food (Toothaker & Welling 1980; Welling 1984; Welling 1989). The fish were fasted for 2 d before being dosed with a small capsule containing 5 mg of 1080. The fact that the trout would have had empty guts prior to dosing and that the 1080 was delivered in a very concentrated form meant that the bioavailability of 1080 and its adsorption by the fish was very high and much higher than would be expected if the 1080 was delivered at a lower concentration in flesh (as would be the case for a trout consuming a 1080-poisoned mouse).

5. CONCLUSION

No trout died from the 1080 dosing. The results showed that following dosing of a capsule containing 5 mg of 1080 without food, a large residue was measured in the trout flesh between 24 h and 48 h, and decreased after 84 h. This profile is similar to that previously observed in crayfish. In our study we did not determine the time for the concentrations of 1080 to decrease below the method detection limit. Therefore, it was not possible to accurately determine the elimination rate. Our attempt to provide data to improve a model that could be used to extrapolate to other scenarios and for risk assessment purposes was unsuccessful.

It is unlikely that the extremely high 1080 dose provided in capsules to fasted trout in this experiment, will occur in the field. The data generated in this study provides insight into 1080 residue in trout flesh for risk assessment purposes. This study generated preliminary data on the 1080 uptake in trout and timeframes involved, which can be used to design more accurate studies to assess the potential risk, if required.

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7. APPENDICES

Appendix 1. Details of the model parameters.

Simulation Settings	• **Time Start:** 0 • **Time Length:** 10 • **Time Step:** 0.01 • **Time Units:** Days • **Algorithm:** RK4
Model Variables	
% of fish eaten	• **Value:** 70 • **Units:** Unitless
% of Ld50 human	• **Value:** 1000*[Flesh microg1080]*[% of fish eaten]/[Human Weight]/[Ld50 Human] • **Units:** Unitless
BCFfish	• **Value:** Exp(0.76*Ln([K_{ow}])+0.23) • **Units:** Unitless • **Note:**
Bh	• **Value:** IfThenElse([Water Temperature]<=18, 3.002, -20.376) • **Units:** Unitless
Calories per WWg of mouse	• **Value:** 1717 • **Units:** Unitless • **Note:** calculated from dry weight values given here.. http://www.rodentpro.com/qpage_articles_03.asp
CalTo1080	• **Value:** [LC50 - 1080 mouse]/[Calories per WWg of mouse] • **Units:** Unitless • **Note:** mg1080 per calorie
Cmax Piscivory	• **Value:** [Max Calories per day]*1.58 • **Units:** Unitless • **Note:** Cmax assuming whole fish diet
Fish Conc mug1080/kg fish flesh	• **Value:** [Flesh microg1080]/[Fish Weight] • **Units:** Unitless
Fish Weight	• **Value:** 0.5 • **Units:** Unitless • **Note:** kg
Flesh Conc	• **Value:** [Flesh microg1080]/[Flesh Volume] • **Units:** Unitless
Flesh Decay Rate	• **Value:** IfThenElse([Flesh microg1080]>0,-Ln(0.5)/[Flesh Half Life], 0)

	• **Units:** Unitless • **Note:** mg1080/day
Flesh Half Life	• **Value:** 0.458 • **Units:** Unitless • **Note:** time for 50% decay (in days) from (Eason *et al.* 2011)
Flesh Volume	• **Value:** [Fish Weight]*1000 • **Units:** Unitless • **Note:** cm3
Gut Conc	• **Value:** [Gut microg1080]/[Gut Volume] • **Units:** Unitless
Gut Decay Rate	• **Value:** IfThenElse([Gut microg1080]>0,-Ln(0.5)/[Gut Halflife], 0) • **Units:** Unitless
Gut Diffusion Rate	• **Value:** [Cmax Piscivory]*[CalTo1080] • **Units:** 1/Days • **Note:** mg1080 per day
Gut Halflife	• **Value:** 0.458 • **Units:** Unitless • **Note:** time for 50% decay (in days) - (Eason *et al.* 2011)
Gut Volume	• **Value:** [Mouse size]*[Max Gut Size] • **Units:** Unitless • **Note:** cm3 (assuming mouse size 1g =1 cm3)
Human Weight	• **Value:** 70 • **Units:** Unitless
K_{ow}	• **Value:** 0.000165959 • **Units:** Unitless
LC50 - 1080 mouse	• **Value:** 13 • **Units:** Unitless • **Note:** microg/g
Ld50 Human	• **Value:** 500 • **Units:** Unitless • **Note:** microg1080/kg - from http://www.toxipedia.org/pages/viewpage.action?pageId=6015922
Max Calories per day	• **Value:** 255.49 * ((((([TR] +7.48) / (6.8 +7.48)) ^ 4.899) * (([TS] +7.48) / (18 +7.48)) ^ [Bh]) * [Fish Weight] ^ 0.7658 • **Units:** Unitless • **Note:** sourced from John Hayes model

Max Gut Size	• **Value:** 19 • **Units:** Unitless • **Note:** max number of mice (assume 20 mice per kg?)
Mouse 1080 Mass	• **Value:** [LC50 - 1080 mouse]*[Mouse size] • **Units:** Unitless • **Note:** mg1080 per mouse
Mouse size	• **Value:** 13 • **Units:** Unitless • **Note:** weight of mouse (g, assume 1g=1cm3)
TR	• **Value:** IfThenElse([Water Temperature]<=6.8, [Water Temperature], 6.8) • **Units:** Unitless
TS	• **Value:** IfThenElse([Water Temperature]>6.8, [Water Temperature], 6.8) • **Units:** Unitless
Uptake Rate	• **Value:** IfThenElse([Flesh Conc]<((1+[BCFfish])*[Gut Conc]),[Gut Diffusion Rate],0) • **Units:** 1/Days • **Note:** mg1080/day
Water Temperature	• **Value:** 16 • **Units:** Unitless
Model Stocks	
Flesh microg1080	• **Initial Value:** 0 • **Non-Negative:** Yes • **Units:** Unitless
Gut microg1080	• **Initial Value:** [Mouse 1080 Mass]*[Max Gut Size] • **Non-Negative:** Yes • **Units:** Unitless • **Note:** microg1080
Model Flows	
Flesh 1080 Decay	• **Rate:** [Flesh microg1080]*[Flesh Decay Rate] • **Alpha:** Flesh microg1080 • **Omega:** *None* • **Positive Only:** Yes • **Units:** Unitless • **Note:** mg 1080
Gut Decay	• **Rate:** [Gut microg1080]*[Gut Decay Rate] • **Alpha:** Gut microg1080 • **Omega:** *None* • **Positive Only:** Yes

	• **Units:** Unitless
Uptake	• **Rate:** [Uptake Rate] • **Alpha:** Gut microg1080 • **Omega:** Flesh microg1080 • **Positive Only:** Yes • **Units:** Unitless

Appendix 2. Animal ethics committee approval.

Animal Ethics Committee

Tuesday 17 June 2014

EMAIL VOTE RESULTS

The Application for Approval for Proposed Experimental Procedures using Live Animals: Protocol # AEC2014-CAW-01, was circulated.

Members were asked to vote on their approval/rejection of this application.

The results are as follows:

Approve:	5
Reject:	0
No Response:	1

It was resolved that the Animal Ethics committee approves the Application for Approval for Proposed Experimental Procedures using Live Animals: Protocol # AEC2014-CAW-01

Dr Mark Burdass
Aquaculture Programme Coordinator, NMIT Primary Industries

17 June 2014
Date

Appendix 3. Raw data.

Table A3.1 Fish parameters and, dose fed and concentration of 1080 (sodium fluoroacetate) in fillets.

Fish	Weight (kg)	Length (mm)	Sex	Sampling time (hr)	K index	Dose fed (mg)	[1080]fed (mg/kg)	[1080] fillet (mg/kg)
1	1.1329	465	M	0	1.13	5	0	0
2	0.9601	440	M	0	1.13	5	0	0
3	0.8805	430	M	0	1.11	5	0	0
4	0.853	420	M	0	1.15	5	0	0
5	0.812	410	M	0	1.18	5	0	0
6	0.726	360	M	6	1.56	5	6.89	3.94
7	0.8998	420	M	6	1.21	5	5.56	1.56
8	0.7996	390	M	6	1.35	5	6.25	0.53
9	0.6029	380	M	6	1.10	5	8.29	3.32
10	0.8472	410	M	12	1.23	5	5.90	2.67
11	0.5796	370	M	12	1.14	5	8.63	2.83
12	0.6967	380	M	12	1.27	5	7.18	3.44
13	0.8238	410	M	12	1.20	5	6.07	2.37
14	0.8808	405	M	24	1.33	5	5.68	4
15	0.8662	408	M	24	1.28	5	5.77	0.56
16	0.6782	400	M	24	1.06	5	7.37	3.9
17	0.6551	380	M	24	1.19	5	7.63	3.56
18	0.712	390	M	48	1.20	5	7.02	3.01
19	0.7056	390	F	48	1.19	5	7.09	4.7
20	0.825	410	M	48	1.20	5	6.06	3.72
21	0.8907	430	M	48	1.12	5	5.61	3.15
22	0.7106	392	M	84	1.18	5	7.04	2.77
23	0.4708	366	M	84	0.96	5	10.62	2.33
24	0.6409	360	M	84	1.37	5	7.80	1.54
25	0.7767	396	M	84	1.25	5	6.44	1.03
26	0.7317	400	M	120	1.14	5	6.83	2.93
27	0.7237	400	F	120	1.13	5	6.91	3.07
28	0.95504	410	M	0	1.39	5	0	0
29	0.7	390	M	0	1.18	5	0	0
30	0.7836	410	M	0	1.14	5	0	0

Table A3.2. Concentration of 1080 (sodium fluoroacetate) in mg/L in water during the experiment.

Time (hours)	$[1080]_{water}$ (mg/L)
0	< MDL
6	0.0068
12	0.0032
24	0.0021
48	0.0006
84	0.0001
120	0.0001

Appendix 4. Landcare Research reports on 1080 (sodium fluoroacetate) chemical analysis.

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Landcare Research

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Toxicology Laboratory Analysis Report

Report No: T5594

CLIENT: Olivier Champeau, Cawthron Institute, Ecotoxicology, 98 Halifax Street East Nelson 7010

CLIENT REFERENCE No.: **Telephone No:** 03 548 3290

SAMPLES: One gelatine capsule

REQUIREMENT: Measure 1080 content

RECEIVED: 09 September 2014

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 1

LabNo.	Description	1080, µg
18412	Active ingredient, 1080 capsule	4996

All results are reported to two significant figures.

The determination was carried out using TLM023, the assay of 1080 in baits and formulations by gas chromatography. The method detection limit (MDL) is 2mg/kg and the uncertainty (95% c.i.) is ± 9%.

TESTED BY: mrc **WORKBOOK REF:** 44/2

TEST PERIOD: 11-13/9/14

AUTHORISED BY:

L.H.Booth, L.E. Brown

Date: 15/09/2014

547 ilac-MRA laboratory

All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results are confidential to the client and relate only to the samples as received and tested. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in anonymised form in the National Vertebrate Pesticide Residue Database.

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Toxicology Laboratory Analysis Report

Report No: T5608

CLIENT: Olivier Champeau, Cawthron Institute, Ecotoxicology, 98 Halifax Street East Nelson 7010

CLIENT REFERENCE No.: **Telephone No:** 03 548 3290

SAMPLES: One gel capsule

REQUIREMENT: Examine for 1080

RECEIVED: 10 September 2014

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 1

LabNo.	Description	1080, % wt
18471	Active ingredient, 1080 capsule, ctrl, 17/8/14	<MDL

All results are reported to two significant figures.

The determination was carried out using TLM023, the assay of 1080 in baits and formulations by gas chromatography. The method detection limit (MDL) is 2mg/kg and the uncertainty (95% c.i.) is ± 9%.

TESTED BY: mrc **WORKBOOK REF:** 44/5

TEST PERIOD: 22-24/9/14

AUTHORISED BY:

L.H.Booth, L.E. Brown

Date: 25/09/2014

547 All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results are confidential to the client and relate only to the samples as received and tested. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in anonymised form in the National Vertebrate Pesticide Residue Database.

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Toxicology Laboratory Analysis Report

Report No: T5601

CLIENT: Olivier Champeau, Cawthron Institute, Ecotoxicology, 98 Halifax Street East Nelson 7010

CLIENT REFERENCE No.: **Telephone No:** 03 548 3290

SAMPLES: Three water samples

REQUIREMENT: Examine for 1080

RECEIVED: 10 September 2014

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 3

LabNo.	Description	1080, µg/mL
18468	Water sample, +48 hr, 9.00 am, 28/8/14	0.0006
18469	Water sample, +96 hr, 9.00 am, 1/9/14	0.0001
18470	Water sample, +120 hr, 9.15 am, 2/9/14	0.0001

All results are reported to two significant figures.

The determination was carried out using TLM005, the assay of 1080 in water, soil and biological materials by GLC. The method detection limit (MDL) is 0.0001µg/mL and the uncertainty (95% c.i.) is ± 12%.

TESTED BY: leb **WORKBOOK REF:** 90/6

TEST PERIOD: 19-23/9/14

AUTHORISED BY:

L.H.Booth, L.E. Brown

Date: 24/09/2014

547 ilac-MRA laboratory

All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results are confidential to the client and relate only to the samples as received and tested. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in anonymised form in the National Vertebrate Pesticide Residue Database.

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Toxicology Laboratory Analysis Report

Report No: T5591

CLIENT: Olivier Champeau, Cawthron Institute, Ecotoxicology, 98 Halifax Street East Nelson 7010

CLIENT REFERENCE No.: **Telephone No:** 03 548 3290

SAMPLES: Four water samples

REQUIREMENT: Examine for 1080

RECEIVED: 09 September 2014

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 4

LabNo.	Description	1080, µg/mL
18430	Water sample, T0 - 9.45 AM, 28/8/14	<MDL
18431	Water sample, + 6 hr - 3 pm, 28/8/14	0.0068
18432	Water sample, + 12 hr - 9.15 pm, 28/8/14	0.0032
18433	Water sample, + 24 hr - 9.15 am, 29/8/14	0.0021

All results are reported to two significant figures.

The determination was carried out using TLM005, the assay of 1080 in water, soil and biological materials by GLC. The method detection limit (MDL) is 0.0001µg/mL and the uncertainty (95% c.i.) is ± 12%.

TESTED BY: leb **WORKBOOK REF:** 90/1

TEST PERIOD: 10-11/9/14

AUTHORISED BY:

L.H.Booth, L.E. Brown

Date: 12/09/2014

547 ilac-MRA laboratory

All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results are confidential to the client and relate only to the samples as received and tested. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in anonymised form in the National Vertebrate Pesticide Residue Database.

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Toxicology Laboratory Analysis Report

Report No: T5609

CLIENT: Olivier Champeau, Cawthron Institute, Ecotoxicology, 98 Halifax Street East Nelson 7010

CLIENT REFERENCE No.: 15495/01/01 **Telephone No:** 03 548 3290

SAMPLES: Thirteen fish muscle samples

REQUIREMENT: Examine for 1080

RECEIVED: 10 September 2014

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 13

LabNo.	Description	1080, µg/g
18455	Muscle tissue, +48 hr, #18, 30/8/14	3.01
18456	Muscle tissue, +48 hr, #19, 30/8/14	4.70
18457	Muscle tissue, +48 hr, #20, 30/8/14	3.72
18458	Muscle tissue, +48 hr, #21, 30/8/14	3.15
18459	Muscle tissue, +84 hr, #22, 31/8/14	2.77
18460	Muscle tissue, +84 hr, #23, 31/8/14	2.33
18461	Muscle tissue, +84 hr, #24, 31/8/14	1.54
18462	Muscle tissue, +84 hr, #25, 31/8/14	1.03
18463	Muscle tissue, +120 hr, #26, 2/9/14	2.93
18464	Muscle tissue, +120 hr, #27, 2/9/14	3.07
18465	Muscle tissue, Ctrl End, #29, 2/9/14	0.03
18466	Muscle tissue, Ctrl End, #30, 2/9/14	<MDL
18467	Muscle tissue, Ctrl End, #31, 2/9/14	<MDL

All results are reported to two significant figures.

The determination was carried out using TLM005, the assay of 1080 in water, soil and biological materials by GLC. The method detection limit (MDL) is 0.001µg/g and the uncertainty (95% c.i.) is ± 9%.

TESTED BY: leb **WORKBOOK REF:** 90/9

TEST PERIOD: 19-25/9/14

AUTHORISED BY:

L.H.Booth, L.E. Brown

Date: 25/09/2014

547 All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

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Toxicology Laboratory Analysis Report

Report No: T5595

CLIENT: Olivier Champeau, Cawthron Institute, Ecotoxicology, 98 Halifax Street East Nelson 7010

CLIENT REFERENCE No.: **Telephone No:** 03 548 3290

SAMPLES: 17 Water samples

REQUIREMENT: Examine for 1080

RECEIVED: 09 September 2014

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 17

LabNo.	Description	1080, µg/g
18413	Muscle tissue, Control #1, 18/8/14	<MDL
18414	Muscle tissue, Control #2, 18/8/14	<MDL
18415	Muscle tissue, Control #3, 18/8/14	<MDL
18416	Muscle tissue, Control #4, 18/8/14	<MDL
18417	Muscle tissue, Control #5, 18/8/14	<MDL
18418	Muscle tissue, +6 hr #6, 28/8/14	3.94
18419	Muscle tissue, +6 hr #7, 28/8/14	1.56
18420	Muscle tissue, +6 hr #8, 28/8/14	0.53
18421	Muscle tissue, +6 hr #9, 28/8/14	3.32
18422	Muscle tissue, +12 hr #10, 28/8/14	2.67
18423	Muscle tissue, +12 hr #11, 28/8/14	2.83
18424	Muscle tissue, +12 hr #12, 28/8/14	3.44
18425	Muscle tissue, +12 hr #13, 28/8/14	2.37
18426	Muscle tissue, +24 hr #14, 29/8/14	4.00
18427	Muscle tissue, +24 hr #15, 29/8/14	0.56
18428	Muscle tissue, +24 hr #16, 29/8/14	3.90
18429	Muscle tissue, +24 hr #17, 29/8/14	3.56

All results are reported to two significant figures.

The determination was carried out using TLM005, the assay of 1080 in water, soil and biological materials by GLC. The method detection limit (MDL) is 0.001µg/g and the uncertainty (95% c.i.) is ± 9%.

TESTED BY: leb **WORKBOOK REF:** 90/3

TEST PERIOD: 15-18/9/14

AUTHORISED BY:

L.H.Booth, L.E. Brown

Date: 18/09/2014

547 All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results are confidential to the client and relate only to the samples as received and tested. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in anonymised form in the National Vertebrate Pesticide Residue Database.

Report No: T5595

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Toxicology Laboratory Analysis Report

Report No: T5614

CLIENT: Olivier Champeau, Cawthron Institute, Ecotoxicology, 98 Halifax Street East Nelson 7010

CLIENT REFERENCE No.: 15495/01/01 **Telephone No:** 03 548 3290

SAMPLES: One fish muscle sample

REQUIREMENT: Examine for 1080

RECEIVED: 10 September 2014

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 1

LabNo.	Description	1080, µg/g
18465	Muscle tissue, Ctrl End #29, 2/9/14	<MDL

All results are reported to two significant figures.

The determination was carried out using TLM005, the assay of 1080 in water, soil and biological materials by GLC. The method detection limit (MDL) is 0.001µg/g and the uncertainty (95% c.i.) is ± 9%.

TESTED BY: leb **WORKBOOK REF:** 90/14

TEST PERIOD: 2-6/10/14

AUTHORISED BY:

L.H.Booth, L.E. Brown

Date: 7/10/2014

547 All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results are confidential to the client and relate only to the samples as received and tested. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in anonymised form in the National Vertebrate Pesticide Residue Database.

Appendix 5. Statistical comparisons of 1080 (sodium fluoroacetate) concentration in trout flesh between sampling times.

Comparing	with	K.-W Statistics	*P* value
6 h	12 h	6	0.685
6 h	24 h	2	0.228
6 h	48 h	2	0.114
6 h	84 h	11	0.485
6 h	120 h	6	0.533
12 h	24 h	1	0.114
12 h	48 h	2	0.114
12 h	84 h	14	0.114
12 h	120 h	8	0.133
24 h	48 h	6	1
24 h	84 h	12	0.057
24 h	120 h	6	0.2
48 h	84 h	16	0.029
48 h	120 h	8	0.133
84 h	120 h	6	0.533