Ministry for Primary Industries
Manatū Ahu Matua

Fumigation of Giant African Land Snails (*Achatina fulica*) Using Methyl Bromide and Methyl Iodide

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Prepared for the Ministry for Primary Industries
by Dr Gene Browne[1*], Mike Goss[2], Shane Jefferies[2], Kuatemani Tuapola[3]

[1]FBA Consulting, PO Box 100-287, NSMC 0745, Auckland, New Zealand
[2]Genera Ltd, Tauranga, New Zealand
[3]MAF Quarantine, Nu'u Research Station, Nu'u, Samoa

gene@FBAconsulting.co.nz

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Ministry for Primary Industries
PO Box 2526
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Executive Summary

The Ministry for Primary Industries New Zealand (MPI) sought to evaluate methyl iodide as a replacement fumigant for methyl bromide, as eventually it may be needed for quarantine and pre-shipment use in New Zealand and elsewhere. Due to an absence of giant African snails (*Achatina fulica*) in New Zealand, a trial was run in Samoa where this introduced species is abundant. Permission was obtained from the Samoan Ministry of Agriculture and Fisheries and the Samoan Port Authority to conduct a trial on the Apia wharf.

The trial evaluated aestivating *A. fulica* snails housed in secure cages inside replicate refrigerated shipping containers. The containers were set at either 12 ^{o}C, 17 ^{o}C, or a maximum of 30 ^{o}C to mimic mean New Zealand temperatures in winter and summer, and ambient Samoan/Pacific Island conditions. Various concentrations of both methyl iodide and methyl bromide were independently fumigated into each container. Acute snail mortality within each replicate cage was determined after 24 hours. The trial was analysed as a two-factor logistic regression. Probability of mortality was fit to a logistic cumulative distribution function applied to a linear model that used temperature, fumigant concentration, and the interaction between temperature and fumigant concentration as explanatory variables.

Based on the results obtained within this report, it can be concluded that mortality results for *A. fulica* are strongly influenced by temperature. The impact of temperature also showed significant interactions with each fumigant concentration, which resulted in varying mortality responses for *A. fulica*. For incursion response at the shipping container level, it would appear that 260 g/m^3 of methyl iodide would be needed to obtain complete control (i.e., 100% mortality after 24 hours) at 12^{o}C; 81 g/m^3 at 17 ^{o}C and 37 g/m^3 at 30 ^{o}C. Similarly, for methyl bromide it would appear that 465 g/m^3 would be needed to obtain complete control at 12^{o}C; 132 g/m^3 at 17 ^{o}C; and as low as 27 g/m^3 at 30 ^{o}C. Only at 30 ^{o}C is the fatal concentration needed for complete control lower than the 128 g/m^3 rate that is currently used in such scenarios.

As the trial only assessed acute mortality after 24 hours, it is the consideration of the authors that mortality rates would have been greater if chronic impacts had been assessed. However, these considerations were outside the scope of the trial. The results reported within this report are therefore likely a conservative estimate of the fumigant concentration needed for complete mortality of *A. fulica*. In practical terms, this trial provides very positive results for tropical countries where fumigation occurs against *A. fulica* at temperatures approaching or exceeding 30 ^{o}C, where a much lower rate of methyl bromide can be applied. The results were more variable for lower temperatures, and although high application concentrations of methyl iodide and methyl bromide were required to achieve 100% acute mortality after 24 hours, the chronic impacts on mortality should also be considered.

Similar to methyl bromide, methyl iodide is a broad spectrum fumigant with similar effectiveness. Operators who are familiar with methyl bromide fumigation should therefore be able to easily manage methyl iodide fumigation in the field or industrial settings. The only challenge observed during this trial was that methyl iodide needs a higher initial temperature for vaporization. As methyl iodide has been found to pose no threat to ozone depletion, it should be further considered as a suitable replacement for methyl bromide.

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Introduction

"The importance of terrestrial gastropods as crop pests has greatly increased over the past 30 years, and in the process demands for effective control have outstripped the development of chemical control measures".

Henderson and Triebskorn 2002

The Ministry for Primary Industries New Zealand (MPI) seeks to evaluate a replacement fumigant for methyl bromide, as eventually it may be needed for quarantine and pre-shipment use in New Zealand. This follows a world-wide phase-out of methyl bromide, which is a severe ozone depletor. New Zealand's use of methyl bromide is increasing due to log exports and scrap metal imports, where mandatory quarantine and pre-shipment fumigation is required to kill insects and other unwanted pests, such as the giant African snail (*Achatina fulica*).

One compound with potential to replace methyl bromide is methyl iodide, a non-ozone depleting fumigant that is already effective against other biosecurity-targeted pests, such as arthropods and nematodes.

FBA Consulting and Genera were commissioned by MPI to undertake trails in Samoa to determine the effectiveness of methyl iodide. On-going logistical issues not only delayed the start of the trial, but almost prevented it from occurring (e.g., methyl iodide being pulled out of Australasia for all uses, including possible quarantine use). Mitigation measures were enacted (e.g., substituting methyl bromide) to ensure that the trial would proceed. This report provides a limited review of the effectiveness of methyl iodide and methyl bromide as fumigants, and provides empirical results from field trials against the giant African snail using real world fumigation methods and techniques.

CONTROL AGAINST SNAILS

Chemicals have been used for centuries to control various gastropods, with discovery of effective methods occurring through trial and error using whatever materials were to hand. These included lime, salt, soot (Theobald 1895), carbolic acid, sawdust (French 1906), and tobacco dust (Gahan 1907). Simple chemicals have included aluminium sulphate, copper sulphate and iron sulphate (Durham 1920, Anderson and Taylor 1926). As opposed to directly killing the target pest, repellent barriers have also been tried; however, these were often phytotoxic and therefore typically lost favour amongst snail control practitioners (Henderson and Triebskorn 2002).

A major advance in chemical control against molluscs was found around 1934 with the use of metaldehyde (Gimingham 1940). This remained a key control method against many gastropods until the reversible carbamate-based baits were invented in the 1950s. Fortunately, researchers now have a better understanding of the interactions different compounds have to biological organisms, and have developed better approaches to understand what may or may not be effective at controlling pests.

Currently, most terrestrial gastropod control is achieved by toxic baits that are either effective by dermal contact or food uptake (Henderson and Triebskorn 2002). However, *A. fulica* still causes a significant pest issue, despite use of various methods of physical, chemical and biological control (Raut and Barker 2002).

Small scale eradication can be successful from physical methods, such as collecting and destroying the snails and eggs, and a considerable number of repellents and toxicants have been tested against *A. fulica* (best summarised by Raut and Ghose 1984; and Srivastava 1992). Although Venette and Larson (2004) report on a number of small scale eradications of *A. fulica* incursions in the US, only a few large scale eradications have been undertaken there (Poucher 1975). Additionally, Venette and Larson (2004) report that this species is intolerant of sunlight and individuals die quickly of exposure to sun. Based on the continuing threat that this species presents and the various methods that are on-going in evaluation, it can be concluded that there is currently no single silver bullet or combined strategy that completely controls this species.

BIOLOGY OF *ACHATINA FULICA*

The giant East African land snail, *Achatina fulica* (Bowdich 1822), is a member of the Achatinidae family, which includes some 200 species of large tropical land snails and pulmonate gastropods from Africa. The Pulmonates are an informal group of snails and slugs characterized by the ability to breathe air by virtue of a pallial lung. The group includes many land and freshwater families and several marine families. Pulmonates have a single auricle and kidney and a concentrated symmetrical nervous system. The mantle cavity is located on the right side of the body, lacks gills, and functions as a vascularised lung. Most species have a shell, but no operculum (i.e., lid used to seal the shell). Sexually, pulmonates are hermaphroditic and the snails can lay up to ~1,200 eggs per annum, giving rise to expansive populations.

A. fulica is similar to its relative the giant African snail *Achatina achtina* (known as either the giant Ghana snail or giant tiger land snail), which can reach shell lengths of up to 27 cm (Venette and Larsen 2004). Often, *A. fulica* is simply called the giant African snail (or GAS as a common quarantine abbreviation), which may give grounds for confusion.

Looking at the animal itself, *A. fulica* has a narrow conical shell twice as long as it is wide. The shell is generally reddish-brown in colour with weak yellowish vertical markings. Adults of the species may exceed 20cm in shell length but generally average about 5-10cm. The average and maximum weight of the snail is approximately 32g and 750g respectively (Venette and Larsen 2004).

A. fulica is a macrophytophagous herbivore eating a wide range of plant material, including fruit and vegetables. It will sometimes eat sand, very small stones, bones from carcasses and even concrete as calcium sources for its shell. *A. fulica* specimens of sizes up to 60 mm shell length have been reported to consume up to 10% of their body weight daily (Schreurs 1963).

The natural home range for *A. fulica* is coastal East Africa, and includes many associated islands (Pilsbry 1904, Bequaert 1950). However, *A. fulica* has become well established in its expanded home range from Africa through Indo-Asia into the Pacific. Its wide distribution is due to human translocation, which was mostly deliberate, but in rare instances by accident (Berquaert 1950; Raut and Barker 2002 and references within). Raut and Barker (2002) further reported that around the time of the Second World War *A. fulica* was deliberately

introduced into Asia and the Pacific to provide a source of food. The presence of *A. fulica* was recorded in mainland Papua New Guinea by 1977; Tahiti by 1967; New Caledonia and Vanuatu by 1972; and both western and American Samoa by 1974.

Throughout this time period, small incursions of this species were successfully eradicated in California, Florida, Fiji, and Queensland (Raut and Barker 2002). Occasional but regular finds are recorded in New Zealand in consignments of scrap metal, shipping containers and on visiting vessels. All these have been successfully managed or eradicated to date. A recent (February 2012) larger incursion/find of *A. fulica* occurred in New Zealand in a ship from Samoa, demonstrating that this pest is an enduring threat. In this particular case, a number of containers and the ship's hold recorded several viable snails.

Raut (1983) reported that, based on its home range and finds of *A. fulica* in high altitude areas, this species has the potential to inhabit areas through to 40^{o} latitude. This suggests *A. fulica* is potentially capable of establishing in the majority of the North Island. Venette and Larson (2004) provide a risk assessment of *A. fulica* for the US and reported that while the ecological suitability of this species is low their appetite for a wide range of plants this makes their host availability relatively high.

Raut and Ghose (1984) reported that *A. fulica* can survive within a temperature range of 0 – 45^{o}C and needs temperatures in the range of 22 - 32^{o}C to increase in population size. Smith and Fowler (2003) also reported that *A. fulica* remains active in a range between 9 - 29^{o}C (but concede that continuous activity is restricted to areas with 80% relative humidity), hibernates at ~2^{o}C and aestivates around 30^{o}C. *A. fulica*, like other Achatinidae, are typically nocturnal and dependant of the availability of moisture, preferring high humidity conditions. Indeed, Takeda and Ozaki (1986) reported that *A. fulica* only becomes active once humidity increases. Extrapolating from this, the cooler winter conditions of New Zealand may slow any establishing population; however, summer conditions may prove more favourable for population growth and dispersal in selected areas.

Pest status of *A. fulica*

Raut and Barker (2002) record that *A. fulica* is a serious pest to agriculture in tropical regions and that there has been little advancement in the development of sustainable control measures against this species in the last 30 years. In New Zealand, the best control of *A. fulica* is considered to be vigilant quarantine with sharp incursion response measures. A review by Venette and Larsen (2004) reports that the potential economic and environmental impacts of this species are high.

Similar to many other significant pest species *A. fulica* has a predilection for a wide range of modified environments and can be a significant crop pest within eight months to 10 years of establishing (Smith and Fowler 2003; Raut and Barker 2002). Shortly after it establishes, the most severe infestations of *A. fulica* occur in disturbed areas, such as residential and crop areas, forest edges, shorelines, and road ways where it achieves dominance of other snail communities. It is the larger snails that are then capable of spreading into undisturbed areas (von Stanislaus et al., 1987; Numazawa et al., 1988).

Another potential threat to New Zealand is the increasing demand for *A. fulica* meat as a food source. To put this in context, South America presently has an established *A. fulica* trade (Teles et al., 1997; J. Coltro as reported in Raut and Barker 2002), and in 1982 Mead reported an increase in trade of *A. fulica* to Europe and America from Taiwan, China, and other Asian countries. Such commercial activities may increase the risk of people trying to set up similar

businesses in New Zealand, which could result in accidental escape or purposeful release of the snail into the environment. Whereas many other giant African land snails are regarded as edible species, *A. fulica* is not regarded as a food source throughout much its home range. Consequently, *A. fulica* populations often go unchecked, giving rise to fast-growing infestations. Based on the above, it is considered a similar scenario may occur if it were to establish in New Zealand, as edible snail meat as not normally regarded as a common source of nutrition.

Raut and Barker (2002) report that the cost of an incursion of this species can be threefold: loss of agricultural productivity caused by herbivory and potential disease transmission (i.e., the snail could be a vector); the cost of labour and materials for the control of the infestation; and opportunity losses due to enforced changes in agricultural practises. It is considered here that potential costs may also include additional funds associated with regional biosecurity measures that would be imposed to reduce/prevent further spreading around New Zealand.

Apart from significant economic and ecological impacts, giant African land snails can be intermediate hosts for several animal pathogens (Venette and Larson 2004). For example *A. fulica* is known to vector *Phytophthora palmivora* spores, the cause of black pod disease in cacao, in its faeces (Evans 1973). In this regard it is possible that it could also vector other plant pathogens.

Aestivation in *A. fulica*

Avoiding hot and dry periods in an inactive state is known as aestivation, which is different from the more commonly known hibernation (an inactive state used to avoid cold environmental conditions). Many achatinid species aestivate during the dry season, particularly at the onset of dry weather, to provide protection from light and desiccation effects. In the monsoonal tropics achatinids often estivate in winter with temperatures of 15 - 28^{o}C, and in some regions aestivation can occurs with temperatures as low as 10^{o}C (Raut and Barker 2002).

Previous research indicates that the cue for aestivation in achatinidae is air humidity (Raut and Ghose 1984; Raut and Barker 2002). The snails prefer aestivation in moist soils, but they will aestivate in sites above ground as well. During aestivation a snail's shell aperture orientation is downwards and the mantle is sealed with an epiphragm plug (Figure 1). Physiological changes also occur during aestivation, for example, there is a reduction in heart rate from 52 to 8 beats per minute (Raut and Ghose 1984; Raut and Rahman 1991).

Aestivated snails can still suffer from dehydration, hence the production of the epiphragm to reduce further water loss during this time. The protective epiphragm temporarily closes a snail's shell aperture and this acts as a replacement to the operculum that many other non-pulmonate snails have. It has been shown that the longer a snail remains in aestivation, the greater the water loss due to dehydration (Raut and Ghose 1981).

Figure 1: Comparison of aestivating *A. fulica* (apertural view) with epiphragm plug (left) with normal roaming *A. fulica* snail.

Although many snail species undergo aestivation, aestivation varies amongst and within species (Hodasi 1982).

The state of aestivation in *A. fulica* increases the risk of a barrier to successful fumigation treatments. With a sealed shell and reduced physiological state (e.g., heart rate and breathing), an aestivating snail may not be subject to the same concentrations as an active roaming snail. This aspect of the natural life cycle of *A. fulica* was therefore taken into account during the trial.

METHYL BROMIDE

Methyl bromide, or Bromomethane, is a colourless, odourless, non-flammable gas produced both industrially and biologically. Methyl bromide is used to kill a range of organisms, including microbes, insects and weeds. Worldwide, the primary use of methyl bromide is as a fumigant in soil to control fungi, nematodes and weeds. Methyl bromide is also used in commercial space for the fumigation of food commodities (e.g., grains) and in storage facilities (such as mills, warehouses, vaults, ships, and freight cars) to control insects and rodents.

As a quarantine treatment agent, methyl bromide fills air spaces in enclosed areas and penetrates cracks, crevices and pores in soil, commodities and structures. To be an effective treatment, an appropriate concentration of methyl bromide must be contained at the application site for a given period of time. Methyl bromide is vented out of the application site after the treatment is complete and is broken down rapidly by sunlight and chemical reactions in the air. It can also be broken down by water and microorganisms.

Methyl bromide is known to be extremely toxic to humans. Brief exposure to high concentrations and prolonged inhalation of lower concentrations are hazardous to health. Exposure levels leading to death vary from 1,600 to 60,000 ppm, depending on the duration of exposure. Respiratory, kidney, and neurological effects are of the greatest concern (Muir 1971).

Methyl bromide poisoning primarily occurs after inhalational exposure, but dermal exposure might also occur. Methyl bromide is irritating to the eyes, skin and mucous membranes of the upper respiratory tract. Studies in humans indicate that the lung may be most severely injured by acute inhalation exposure (ATSDR 1992, US EPA 1999). Breathing high concentrations of methyl bromide may cause pulmonary edema, impairing respiratory function. Acute exposure by inhalation of methyl bromide can lead to neurological effects in humans. Symptoms include headaches, dizziness, fainting, apathy, weakness, confusion, speech impairment, visual effects, numbness, twitching and tremors. In severe cases, paralysis and convulsions are possible. Inhalation of methyl bromide may cause kidney damage and the liver may become swollen and tender (ATSDR 1992, US EPA 1999).

In addition to toxicological effects, methyl bromide is recognised as a significant ozone depleting substance under the Montreal Protocol. As such, control measures were put in place in 1992 that require developed countries (including New Zealand) to phase out the production and consumption of methyl bromide by January 2005. However, three categories of methyl bromide use are exempted from phase-out under the control measures: use as a chemical feedstock; uses that the Parties to the Montreal Protocol deem ‘critical’ subsequent to complete phase-out; and use for quarantine and pre-shipment.

METHYL IODIDE

Methyl iodide, also called Iodomethane and commonly abbreviated to "MeI", is a dense, colourless, volatile, yet non-combustible, liquid. In terms of chemical structure, it is related to methane by replacement of one hydrogen atom with an atom of iodine. It is naturally produced and emitted by rice plantations in small amounts, and is also produced in vast quantities by algae and kelp in the world's temperate oceans. Lesser volumes are known to be produced on land by terrestrial fungi and bacteria.

Methyl iodide is a biocide used to control insects, plant parasitic nematodes, soil-borne pathogens and weed seeds. The compound is registered for use as a pre-plant soil treatment for field grown strawberries, peppers, tomatoes, stone fruits, tree nuts, grape vines, ornamentals and turf and nursery grown strawberries, stone fruits, tree nuts and conifer trees.

Methyl iodide has also been proposed as a replacement for methyl bromide as a fungicide, herbicide, insecticide, nematicide and soil disinfectant. Manufactured by Arysta LifeScience and sold under the brand name MIDAS, methyl iodide has been registered as a pesticide in the U.S., Mexico, Morocco, Japan, Turkey and New Zealand[1]. Registration in many other countries is pending. Various groups have looked at using methyl iodide as a fumigant. Fumigation methods often include mixing methyl iodide with CO_2 (e.g., Kawakami 2007). One of the key benefits of the use of methyl iodide as a fumigant is that it is not an ozone depletor.

[1] At the time of writing methyl iodide has been pulled from the US and Australasian market as a means for commercial and quarantine fumigation.

Methodology

TRIAL OUTLINE

As *A. fulica* does not naturally occur in New Zealand, it was decided that fumigation trials should be undertaken in Samoa, in conjunction with the Samoan Ministry of Agriculture and Fisheries (MAF) Quarantine Service and associated MAF Crops Division based at Nu'u. The trial was initially to be located at the Nu'u research station; however, to mitigate against potential power disruptions impacting on the trial, the trial site was relocated to the Samoan Port Authority's (SPA) Apia wharf.

The trial focused on determining the indicative concentrations needed for methyl iodide to effectively kill *A. fulica* over a range of temperatures. Methyl bromide was concurrently trialled for comparative purposes, as there is little definitive empirical data to prove effective concentrations of this fumigant. Historical use has resulted in the application of high concentrations to control regulated snail species. As temperature can have an impact of fumigation performance, the trial was conducted under three simulated temperature regimes:

- Average New Zealand winter conditions (10 – 14.9^{o}C);
- Average New Zealand summer conditions (15 – 20 ^{o}C); and
- Average Pacific Island ambient temperature (approximately 30 ^{o}C).

These temperatures were selected by MPI to represent temperatures that would likely occur during fumigation. The fumigation treatment itself was for 24 hours continuous treatment, evaluated across a series of concentrations. Initially, it was proposed that the following concentrations be evaluated:

- 0 g/m^3 (a control);
- 60 g/m^3;
- 80 g/m^3; and
- 120 g/m^3.

It was determined that at the start of the trial that fumigation would begin at the lower concentration levels (for example, 60 and 80g/m^3), as low concentrations could lead to mortality of the test snails. If this occurred, it would negate the need for testing at higher concentrations. To ensure consistent results, concentrations of fumigant were continually monitored over the fumigation period using a calibrated Riken fumigant analyser (F1-21 Gas monitor, Riken Keiki Co. Ltd).

To test the various fumigation concentrations and temperature combinations, a series of 20 ft refrigerated shipping containers (Reefers [RFRs]) were used. Initially, each combination of temperature and fumigant concentration tested in the containers was to be replicated four times; however, logistical issues prevented this from occurring. The cages that housed the test subjects (*A. fulica*) were therefore used as test units for statistical comparison. Prior to the trial starting, the RFRs were moved to the site inspected with all vent holes sealed and checked to ensure no fumigant could escape. To ensure a consistent trial, a SPA technician inspected each RFR three times per day to ensure there were no issues with power outages or alarms (indicating variations in temperature). RFRs were modified so that the standard defrost cycle was deactivated (which prevented a 12 hr defrost shut down cycle from occurring).

Snails were collected from wild populations around Nu'u and housed in suitable cages. The cages were plastic with secure lids and had dimensions of 250mm (l) x 250mm (w) x 300mm

(h). Each cage was drilled with a total of twelve 10mm holes in the lid, sides and base of the cage to allow airflow and fumigant access and venting. Snails were randomly allocated to cages to avoid any systematic bias and left in a secure shade hut without food or liquid to induce biological aestivation (Figure 2). Cages were routinely checked to determine the level of aestivation and remove any dead or infected individuals (Figure 3).

Where possible, each cage held 25 snails. Cages were inspected immediately before the trial began, and dead and maggot-infested snails were removed to prevent further contamination. Cages were randomly collected each day for testing from the Nu'u station. Between 3 and 5 cages were randomly allocated to each test RFR (i.e., between 75 – 125 snails in total per RFR).

Figure 2: Snail aestivation post quarantine room, Samoa MAF Nu'u Station.

Placement of the cages in each RFR was recorded as either near the door, near the middle of the RFR or near the far end of the RFR (Figure 4). These positions were recorded to check for any biases in mortality due to distance from the RFR air circulatory system located at the rear of the RFR.

Although the trial focused on *A. fulica* in a state of aestivation, occasional live roaming snails were present in the cages. These were left in for observational purposes.

Both methyl bromide and methyl iodide were applied through a standard Genera vaporiser unit. Such units are frequently used throughout New Zealand for routine fumigation treatments. Based on data from the MSDS and pre-trip trial work by Genera, it was known that methyl bromide vaporises at temperatures >4.6^{o}C, whereas methyl iodide requires a minimum temperature of 42.5^{o}C to vaporise. Methyl bromide application was measured via a calibrated sight glass (as used for routine fumigation). By contrast, methyl iodide application was measured using digital scales to calculate how much product had been released from the cylinder into the vaporiser. Figure 5 shows the setup used during the methyl iodide fumigation.

Target dose concentrations of both fumigants were calculated in addition to actual dose concentrations introduced into the test RFRs. For accuracy, analyses were carried on the actual dose concentrations of each fumigant.

Figure 3: Kuatemani checking aestivation in the snail cages, Nu'u.

Figure 4: Snail cages set at rear, middle and front of RFR.

Figure 5: Fumigation equipment during Methyl iodide application.

Figure 6: SPA restricted area surrounding trial site.

As methyl bromide and methyl iodide are dangerous goods, a secure perimeter was established around the trial site by SPA (Figure 6). During the trial, only experimental personnel were allowed into the area. When venting, only Genera staff using breathing apparatus equipment were allowed into the identified danger zone. A calibrated IBRID M22 (Industrial Corp.) meter was used to measure fumigant levels in and around the danger zone and to provide a warning of any remnant gases in the area. During each trail, the door seals were rechecked using the IBRID meter and resealed.

Once the 24 hour test period was completed, each individual RFR was opened for venting and the cages were individually removed. The RFRs were left for between 0.5 – 1 hours to vent fully (as measured by the IBRID meter), and were reset for the next test conditions. The cages were moved to a clear area within the secure zone and opened to aid venting. Fumigant levels within the cages were monitored using the IBRID meter. Snails were processed after a minimum of five minutes after a 'no gas level detected' reading was recorded.

Mortality was determined by two methods. Post treatment snails were placed so that their shell opening was upward facing (apertural view) and clean ambient temperature water was added to fill the void in the shell. Snails were then set aside for at least 0.5 hour to check for movement. If no movement was detected, then a foot retraction test was performed. As the aestivated snails had often retracted some distance into their shells, the shells were broken open so that the foot retraction test could be applied. Mortality was recorded if no movement to either the water or foot retraction methods was observed.

STATISTICAL METHODOLOGY

The trial was designed to be a 3 x 4 factorial design (with 3 temperature ranges and 4 fumigant concentrations). However, this strategy changed as a consequence of developments in the results as they occurred.

As the response of the trials was binary (i.e., mortality or survival), a linear model was fitted to a multi-level logistic response function using maximum likelihood. Likelihood-ratio test statistics were computed for the whole model and compared to 'lack of fit' tests to check for suitability. Wald Chi-square test statistics were computed for each effect in the model to check for its overall appropriateness in the model.

Logistic regression fits probabilities for the response level (i.e., mortality) using the following logistic function:

$$prob(\gamma = mortality) = (1 + e^{\overline{Xb}})^{-1}$$

Or equivalently

$$\log\left(\frac{prob(Y = mortality)}{prob(Y = survival)}\right) = Xb$$

For this trial, the analysis can be considered a full two-factor logistic regression model fitting the probability of mortality to a logistic cumulative distribution function applied to the linear model with temperature, fumigation concentration, and the interaction between temperature and fumigation concentration as the regressors. The parameters were estimated by minimizing

the sum of the negative logs of the probabilities attributed to the observations by the model (i.e., maximum likelihood).

$$prob(mortality) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 temp + \beta_2 conc + \beta_3 temp \times conc)}}$$

Analyses were undertaken using JMP Statistical software (SAS Institute 2003). Statistical significance was determined at the 95% confidence level.

Results and Discussion

SIMPLE STATISTICS

A total of 2,690 snails were tested and evaluated for response to varying concentrations of both methyl iodide and methyl bromide. A total of 100 snail cages, each containing a mean of 36.9 *A. fulica* snails (+/- S.E. = 0.34), were treated. The number of snails per cage ranged from 20-35 individuals. A total of 26 cages held less than 25 aestivated snails, as per the specifications (mean = 22.6, range 20 – 24 snails per cage).

Approximately 100 snails were removed either prior to the trial or post treatment due to natural mortality. Pre-trial mortality was identified by fly maggot infestation.

GAS MONITORING

The Riken unit started to give spurious results early on the trial, and it was later determined that the optic inferometer sensor may have become contaminated with additional moisture, which caused consistent errors. Although the meter was operated using both CO_2 and moisture pre-filters, the manufacturers of the unit indicated that excess moisture must still have entered the unit. Despite the aforementioned technical issues, results are presented here to inform future research and related programmes.

Figure 7 compares the readings from within RFR 2. This RFR was configured with top (roof level), middle (midpoint located), and bottom (floor level) air sampling hoses, from which representative air samples were collected. Air samples were collected hourly over the course of 4 hours and processed through the Riken meter. Although the initial readings were all consistent with low variability (S.E. = 0.6), the final reading was much higher (S.E. = 3.9). The concentration of fumigant added at the beginning of the trial was 80 g/m^3. The cause of the markedly lower initial two readings is unknown (means 54.1 and 27, respectively).

Figure 8 displays fumigant level readings collected hourly from each of the test RFR units from 1 – 4 hours and processed through the Riken meter. Each RFR had an air sample tube located in the middle of the container (i.e., hanging centrally from the roof), and this was the point used for comparisons.

Table 1 gives summary statistics based on results displayed in Figure 8. For the 60 g/m^3 concentration, mean readings ranged from 31.5 to 99.7 g/m^3. The variability of the means (as determined by the standard error) increased across time from 6.63 to 11.39 g/m^3. Mean values for the 80 g/m^3 concentration RFRs ranged from 38.0 to 84.2 g/m^3. The variability of these means decreased across time from 15.02 to 1.79 g/m^3.

It was the higher than possible readings in the lower 60 g/m^3 RFRs that alerted us to problems with the meter. All figures were independently checked by Genera and FBA Consulting staff, in case errors were made in terms of unit volumes of fumigant; however, this was not the case. The Riken meter also began producing default ‘contrast’ errors which signalled that the optic inferometer was damaged or incapacitated.

Table 1: Summary statistics for fumigant readings of RFRs for first 4 hours following fumigation.

Concentration	60 g/m^3		80 g/m^3	
Time	Mean	S.E.	Mean	S.E.
1 hr	31.5	6.63	38.0	15.02
2 hr	62.0	10.54	43.7	14.71
3 hr	99.7	11.39	84.2	12.91
4 hr	90.3	11.33	74.4	1.79

It is known that with partially filled containers undergoing fumigation, the fumigant levels start high and quickly drop within the first few hours as the objects within the containers absorb the fumigant. Figure 9 provides a theoretical example of this decline over time based on data from Kawakami (2007) and discussions with Genera staff.

As the test containers in the Apia trial were relatively empty (except for the test cages with snails), it was expected that the fumigant levels would followed a more linear trajectory (as provided in Figure 10). Although some of the fumigant would likely break down or be lost through leakage, the majority should still remain during the course of the trial run.

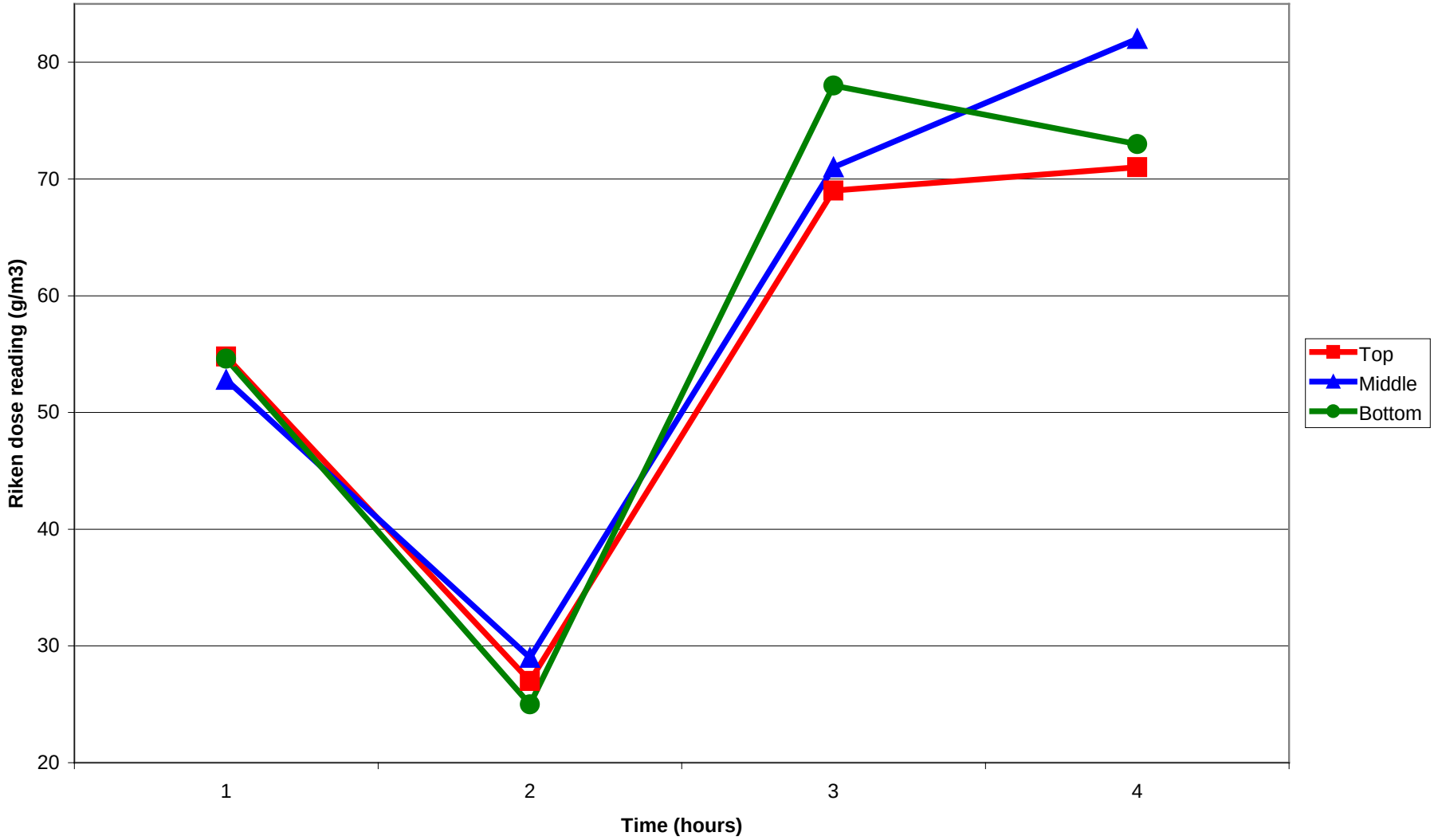

Figure 7: Comparison of fumigant levels at top, middle and bottom of RFR for first four hours following fumigation.

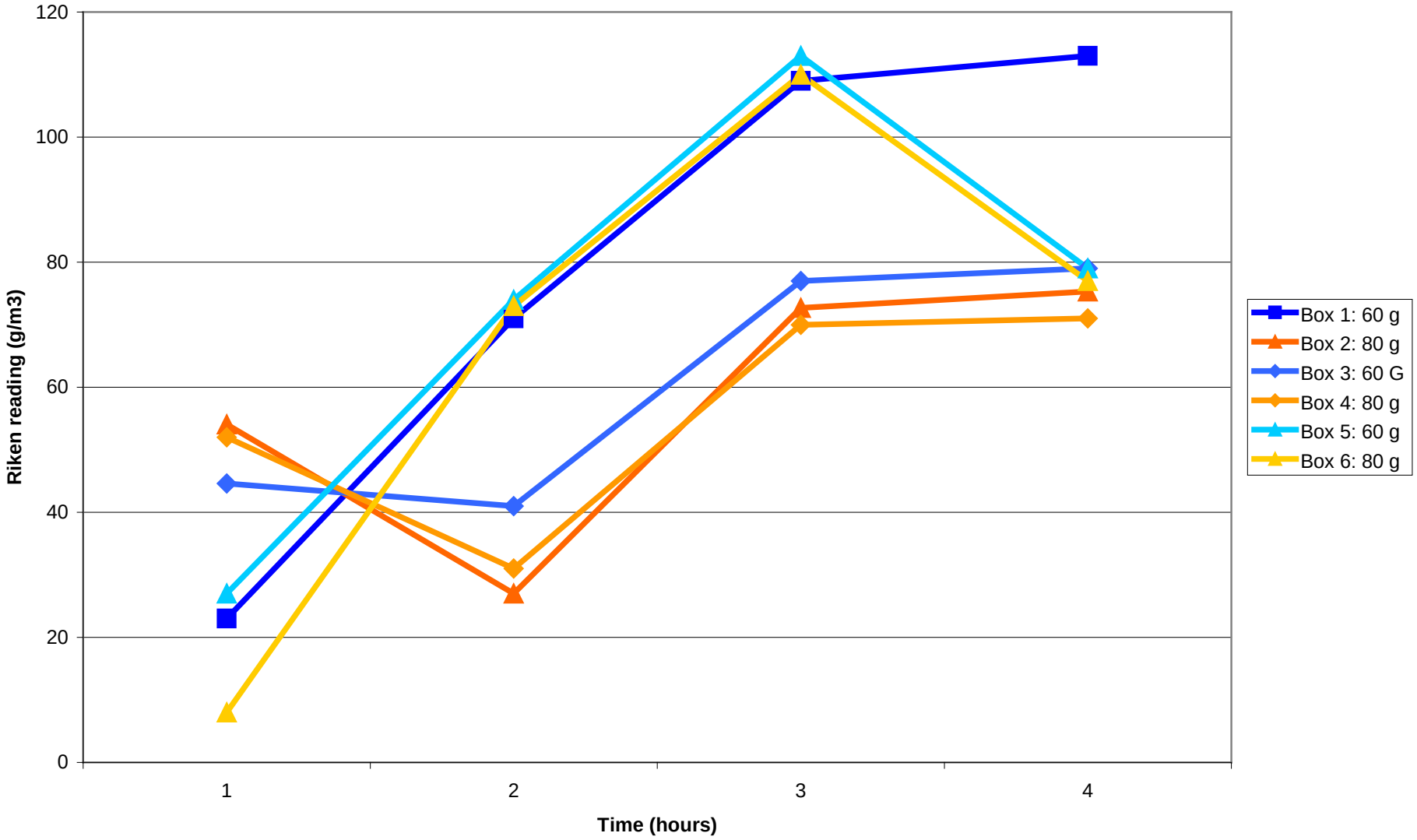

Figure 8: Comparison of fumigant levels between test RFRs for first four hours following fumigation.

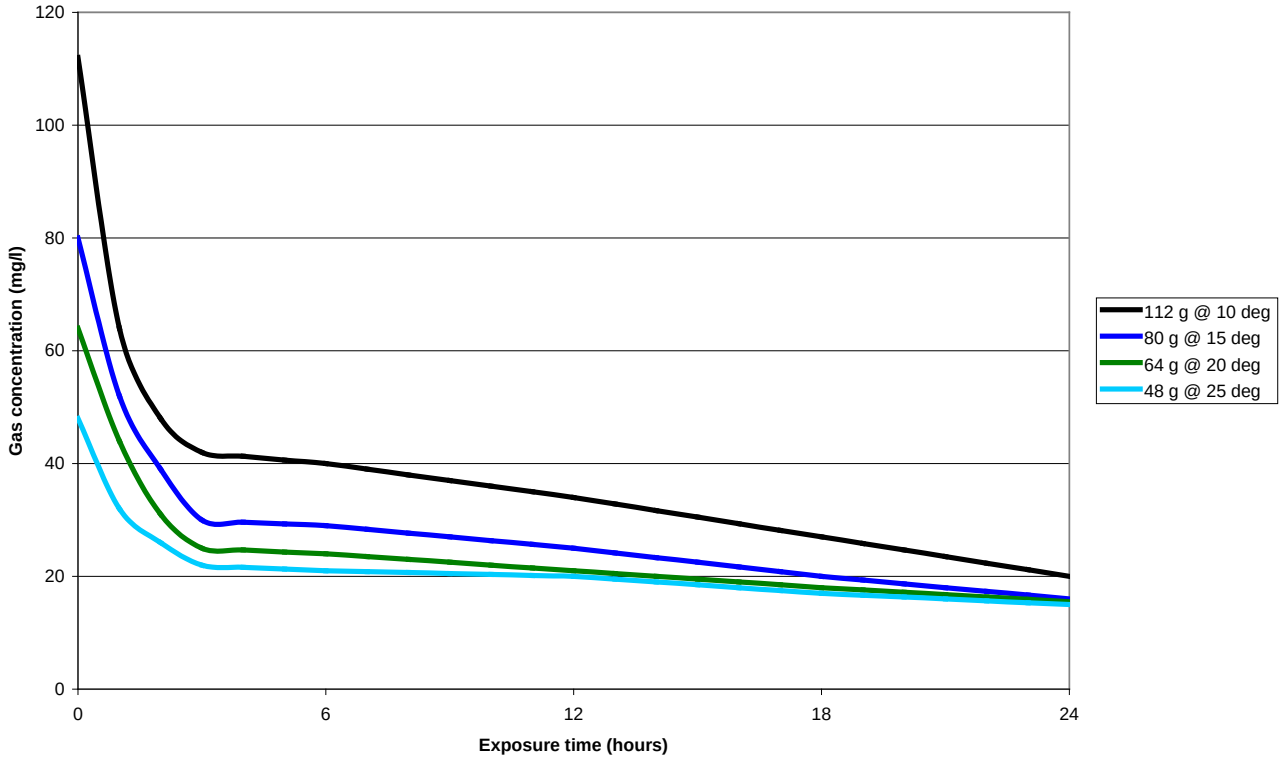

Figure 9: Theoretical progressive gas concentrations with 50% loading on container (based on Kawakami 2007).

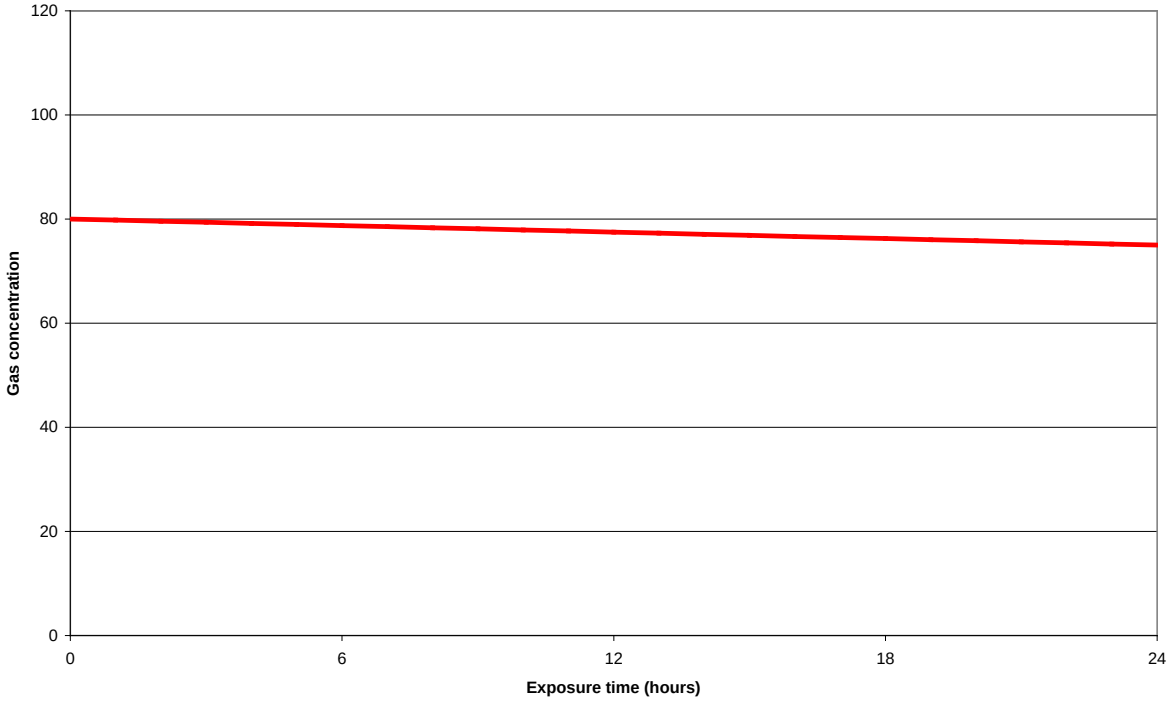

Figure 10: Expected progressive gas concentrations with no loading on container.

Genera has suggested that a trial could be rapidly undertaken in New Zealand to confirm the fate of methyl bromide during fumigation. This would provide MPI with data on fumigant levels inside empty containers infected with an exotic organism.

TEMPERATURE READINGS

The RFRs were considered excellent test chambers, and across the experiment they maintained their respective temperatures with little variation (Table 2).

Four of the RFRs were 32.2 m^3 in internal volume and another two were 32.1 m^3. Temperatures were set at 12 ^oC to simulate the NZ winter 10 – 14.9^oC temperature range; 17 ^oC for the NZ summer 15 – 20 ^oC temperature range; and a maximum of 30 ^oC for the Pacific Island ambient range.

Table 2: Summary statistics for temperature readings (degrees Celsius) of RFRs during the fumigation trial in Apia.

Conditions	Mean	S.E.
NZ Winter	12.13	0.062
NZ Summer	17.06	0.048
Pacific Ambient	30.04	0.034

SNAIL MORTALITY

This trial focussed on acute mortality of the test subjects and even with two methods by which to evaluate this, it became clear that many of the snails were morbid (i.e., technically alive but well on their way to mortality). For the purposes of the trial, a strict evaluation of live/dead was used (i.e., a binary response). It was highly probable that many of the snails that exhibited no response to shell flooding and only a very weak foot retraction response would most likely have not survived for much longer. Unfortunately, assessing this was beyond the scope or considerations of this trial. It is recommended that chronic assessments of mortality are undertaken in any future assessments.

Based on the above findings, the authors of this report consider that higher levels of mortality should be expressed for some of the lower dose concentrations. It is likely that individual snail morbidity would eventually result in mortality, and that mortality of all snails would reach 100%.

Some snails died prior to entering their trials, as indicated by maggot infestation. It was observed that all maggots died at all concentrations (except the zero dose controls) (Figure 11). It was also observed that any *A. fulica* that were not in an aestivated state prior to the trial beginning (i.e., either not fully aestivated or had been awoken during the trip to the test site) succumbed to the fumigant at most concentrations. This was determined by observing no epiphragm inside the shell and a dehydrated appearance on the exposed portions of the foot (Figure 12).

Figure 11: An example of a rotten snail with maggots.

Figure 12: An example of a desiccated free roaming snail post treatment.

It is possible that some snails were stimulated into emerging from aestivation, although they had not completely emerged. These snails were identified by the presence of a partially intact epiphragm within the confines of the shell and partial dehydration of the snail foot. Whether

the trip to the test site or the fumigant stimulated them to come out of aestivation is unknown, and outside the scope of these trials.

Snails that were clearly not impacted by the fumigation readily emerged, following a brief rest, after their shells had been flooded (Figure 13). The snails designated to the control fumigation (i.e., 0 concentration) emerged rapidly and started to roam about (Figure 14).

Snails that were still in an aestivated state, as determined by remaining secure behind their epiphragm plug, had a better chance of surviving the fumigation applications. The evaluation criteria were subjective, however. Snails had to be broken out of their shells to test for foot retraction. Foot retraction responses ranged from no apparent response to a faint retraction response and a strong retraction response.

Determining survival vs. mortality of the snails was difficult in many of the morbid cases. A comparison of morbid snails to healthy roaming or emerging snails indicated that many of the morbid snails were clearly not in a normal state. It was often queried whether the faint response to foot stimulation was real or some factor of remnant muscle tension remaining in the animal following the shell being broken open. Often, torsion of the foot into or across the mantle could be observed and this was carefully examined and discounted from a true foot retraction response.

No evidence could be found for differences in placement of the cages within the test RFRs. This meant that each replicate snail cage could be considered an independent replicate within each treatment regime.

RESULTS FOR CONTROL INDIVIDUALS

Figure 15 shows an overview comparison between treatments for methyl iodide and the control test/individuals (i.e., 0 dose concentrations). Mean mortality for the controls were 4.2 % at 12^{o}C (S.E. = 2.74), 1.0 % at 17^{o}C (S.E. = 1.04) and 2.2 % at 30^{o}C (S.E. = 2.15).

The surviving snails mostly woke from aestivation within 5-10 mins following water flooding of their shells. Only a small number of aestivating snails needed to be broken out from within their shells and checked for survival via foot retraction methods.

As a side observation, any cages that had remnant Dipteran maggots inside still had live maggots after the 24hr treatment period. This would indicate that such life was not impacted by any of the experimental conditions. This contrasts with results of maggots observed from the fumigated RFRs.

These results suggest that the RFR units were well suited for this trial and that the three temperature settings had no significant impact upon survival.

Figure 13: An example of live snails emerging following being flooded post treatment.

Figure 14: An example of control snails being roaming following being flooded post treatment.

RESULTS FOR METHYL IODIDE

Based on the raw data, Figure 16 displays the mean mortality results (+/- S.E.) of methyl iodide for all concentrations >20 g/m^3. It was never an aim of the trial to evaluate fumigation concentrations this low; however, positive results in the higher temperature allowed trials to progress further down the concentration gradient.

To obtain mortality above 90%, concentrations of methyl iodide typically needed to be above 75 g/m^3 across all temperatures, although there were some conflicting results (e.g., poor results demonstrated for 80g/m^3). It was evident that mortality increased with temperature and that less methyl iodide was needed to achieve snail mortality at higher temperatures. Based on raw data at the maximum temperature of 30^{o}C, it was concluded that any dose >50 g/m^3 would likely result in 100% mortality after the 24 hour fumigation period. Note: Considerations of chronic mortality of the snails are excluded.

The logistic model that included temperature and methyl iodide concentration regressors was compared to a reduced model (i.e., using just the regression intercept parameter) to ensure that the linear responses were valid for these analyses. The ratio of the difference between the whole model and reduced model and the reduced model negative log-likelihood values determined that the proportion of uncertainty attributed to the fit of the full model was 42%. Based on the 1,745 snails evaluated, analyses indicated that significant differences in mortality could be explained by temperature and concentrations of methyl iodide, as well as their interaction effect ($p<0.0001$; Appendix 1).

Wald Chi-square tests indicated that the linear temperature, methyl iodide concentration, and interaction responses were significant to the negative log-likelihood values ($p<0.0001$; Appendix 1).

As the interaction between temperature and concentration was significant, the response (mortality) to the methyl iodide concentration was analysed separately for each of the three set temperature ranges. This approach does not use interaction term, which normally would impede further analysis. Temperatures are best analysed individually, as they are fixed parameters that can significantly influence mortality results.

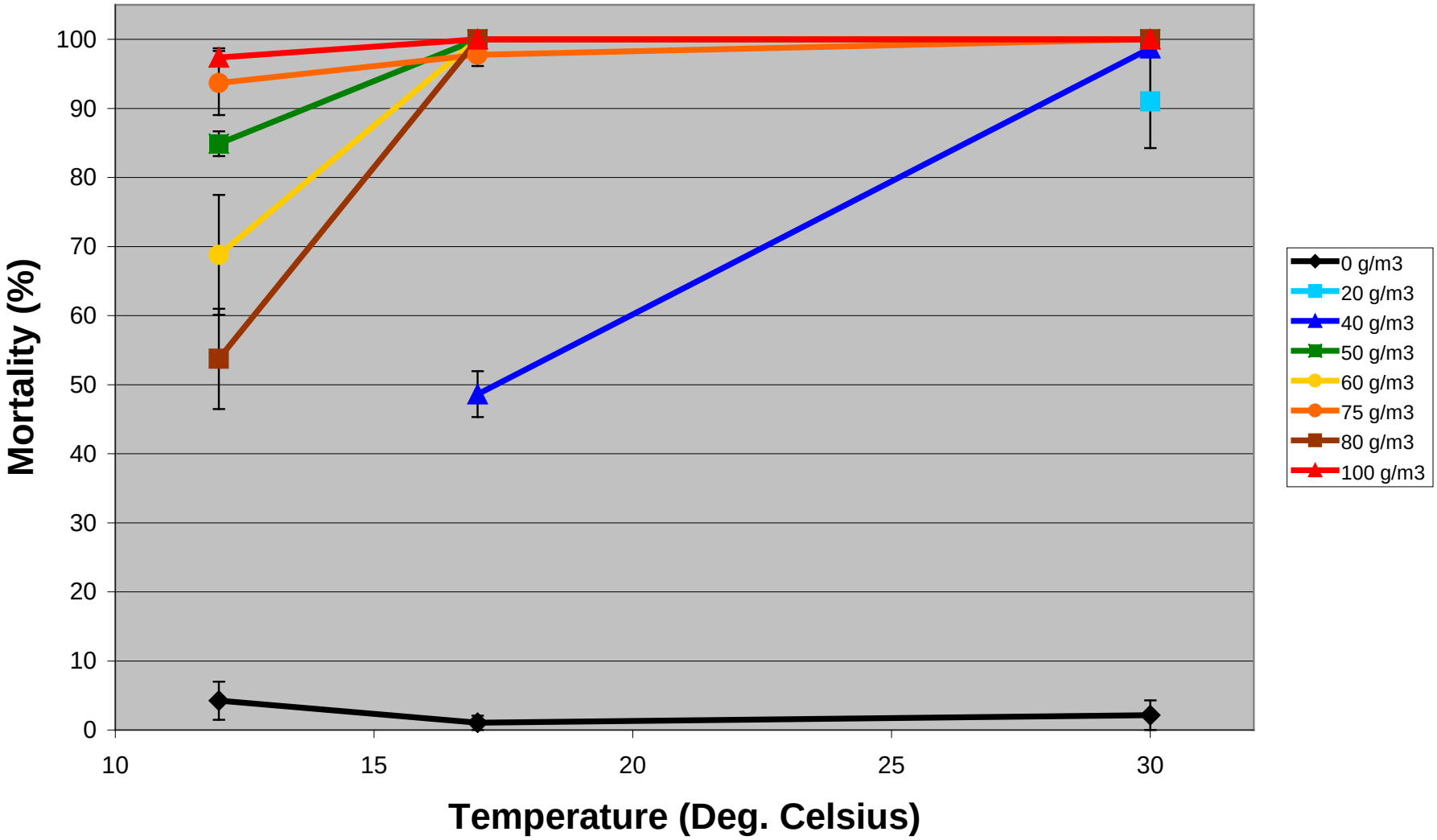

Figure 15: Mean mortality of *A. fulica* (+/- S.E.) across temperature and all methyl iodide concentrations.

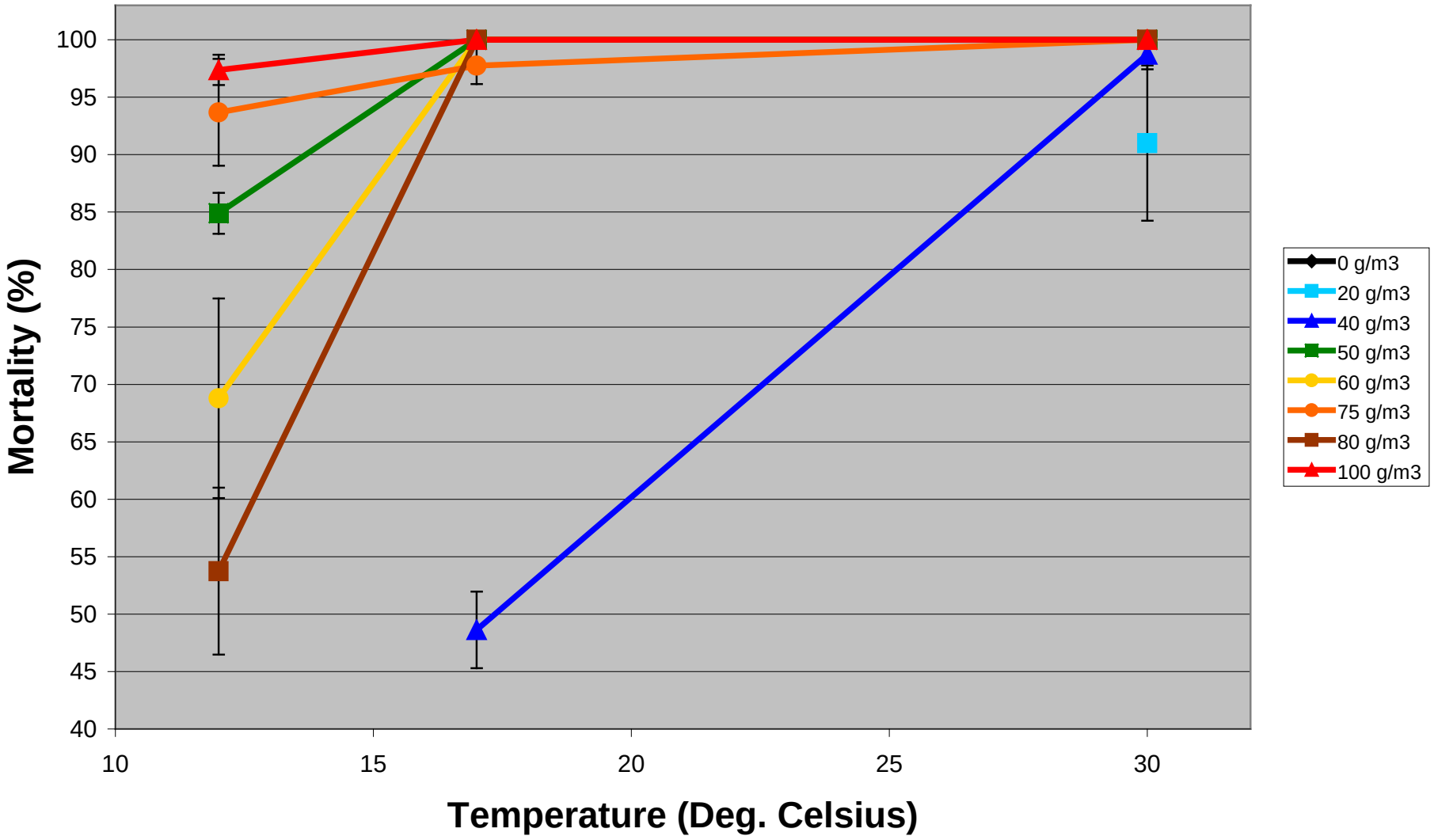

Figure 16: Mean mortality of *A. fulica* (+/- S.E.) across temperature and methyl iodide concentrations >20 g/m^3.

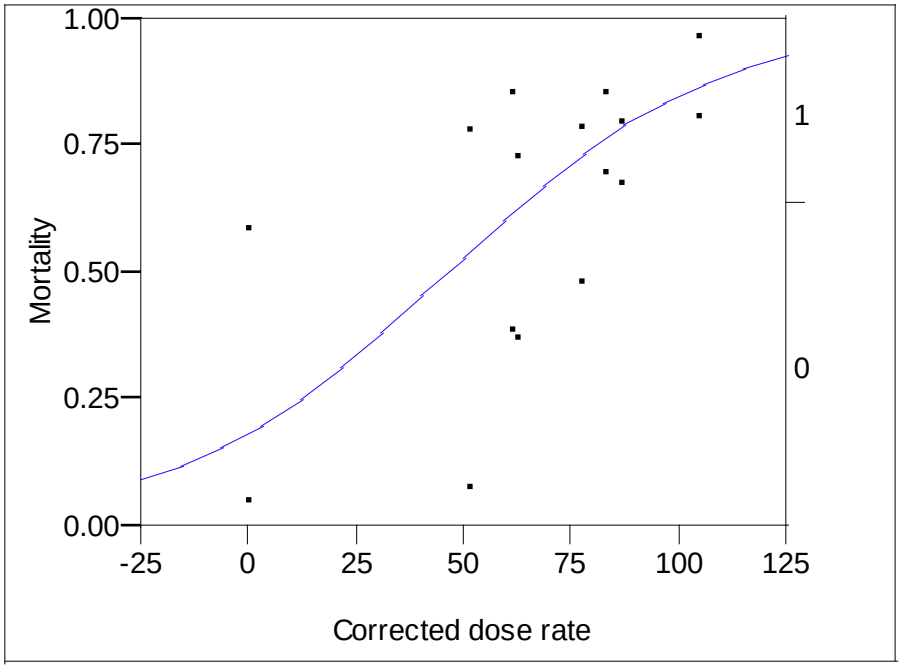

Figure 17: Logistic fit of *A. fulica* mortality vs. corrected methyl iodide fumigation dose rate at 12°Cel.

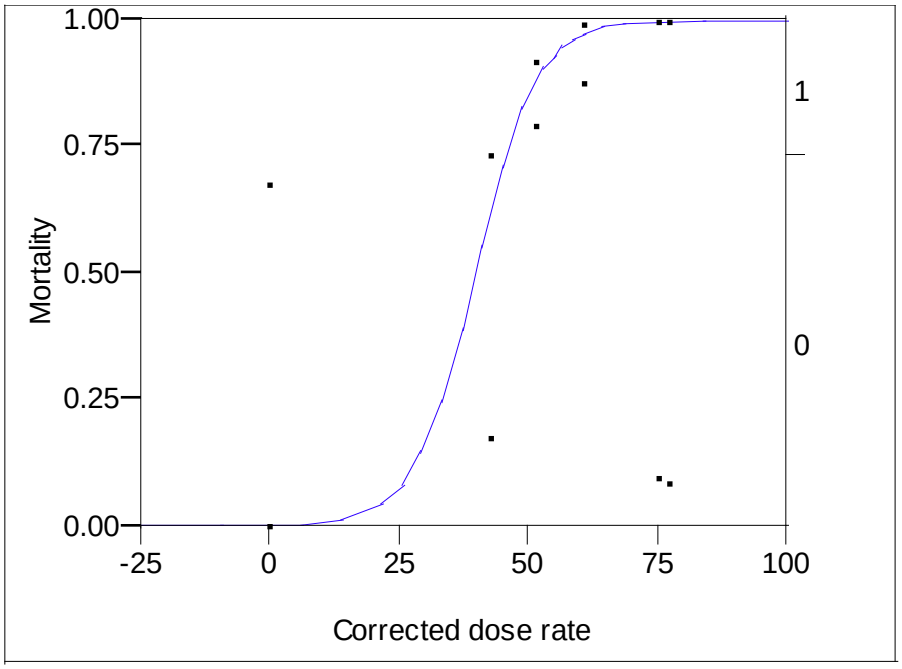

Figure 18: Logistic fit of *A. fulica* mortality vs. corrected methyl iodide fumigation dose rate at 17°Cel.

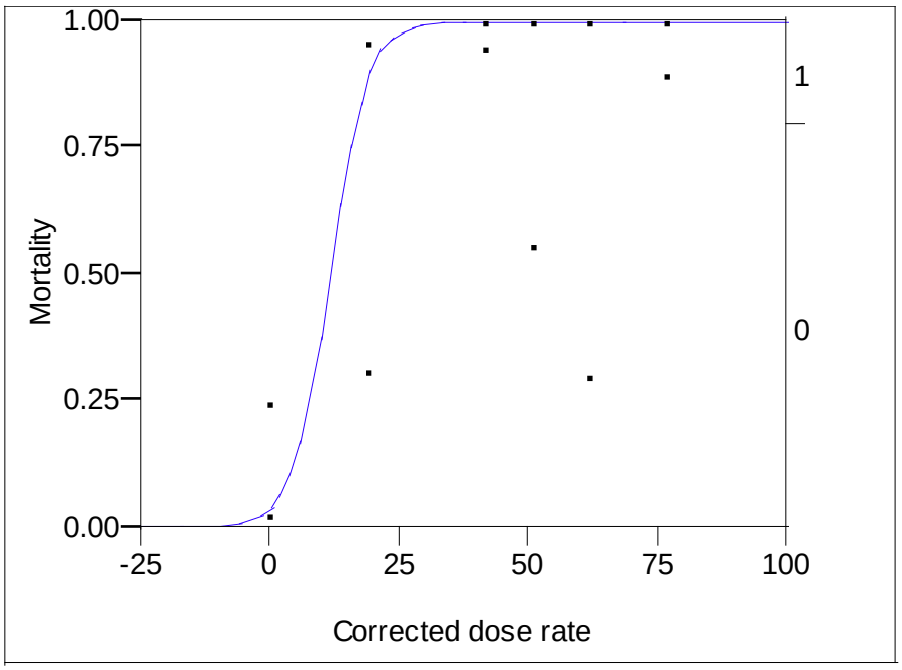

Figure 19: Logistic fit of *A. fulica* mortality vs. corrected methyl iodide fumigation dose rate at 30°Cel.

Removing temperature as a regressor from the model (and its associated interaction term) improved the fit for each of the three new models (as evaluated as a reduction in the 'whole model' degrees of freedom Chi-square value [~53-85%]) (Appendix 1).

Figures 17 - 19 display the fit of the logistic regression for mortality data to the corrected methyl iodide fumigation dose rates across the three temperature ranges. The mortality response is not as dramatic in the lower temperatures, compared to the higher temperatures (as determined by the relative changes to slope in the logistic curve).

Across all three temperatures, methyl iodide concentration independently had a significant impact on mortality ($p<0.0001$; Appendix 1). For practical and operational purposes, a reverse prediction method was undertaken based on the logistic regression model fitted above.

Figures 20 – 22 plot the predicted methyl iodide concentrations (including a 95% confidence interval) needed to obtain a set mortality (note the highest prediction is for 99.9% mortality). Tabulations of the data for Figures 20 – 22 are provided in Appendix 1.

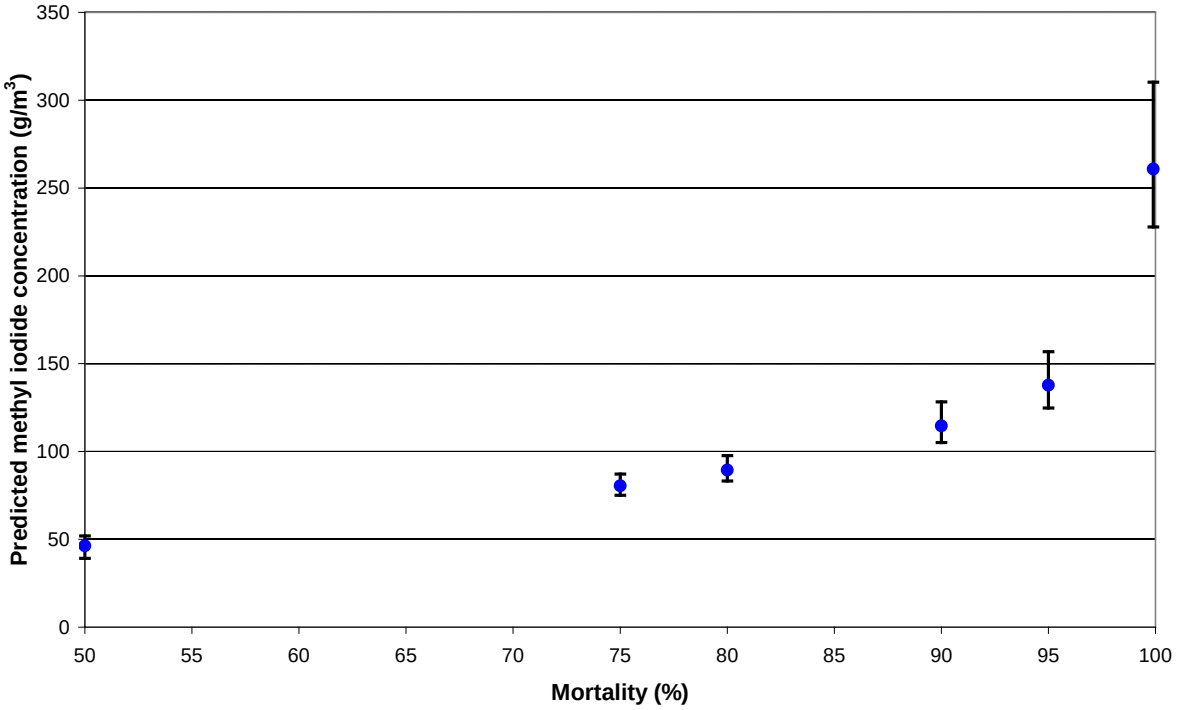

Figure 20: Inverse prediction from percentage mortality of *A. fulica* to provide methyl iodide fumigation dose rates at 12°Cel.

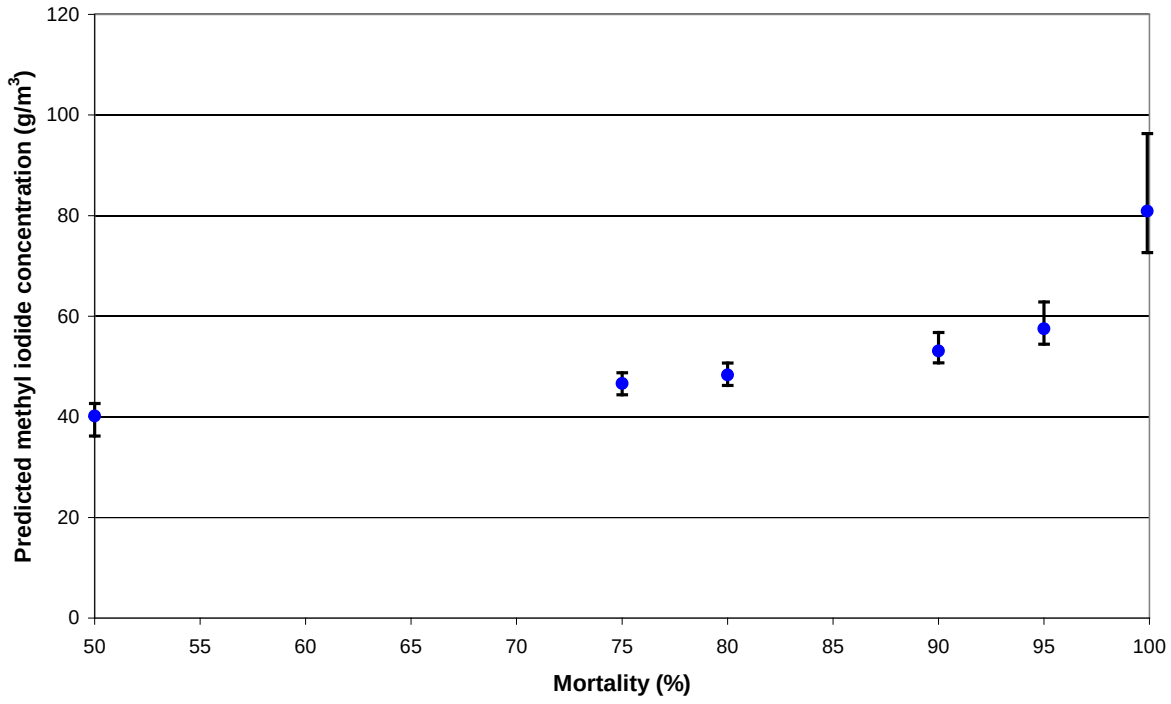

Figure 21: Inverse prediction from percentage mortality of *A. fulica* to provide methyl iodide fumigation dose rates at 17°Cel.

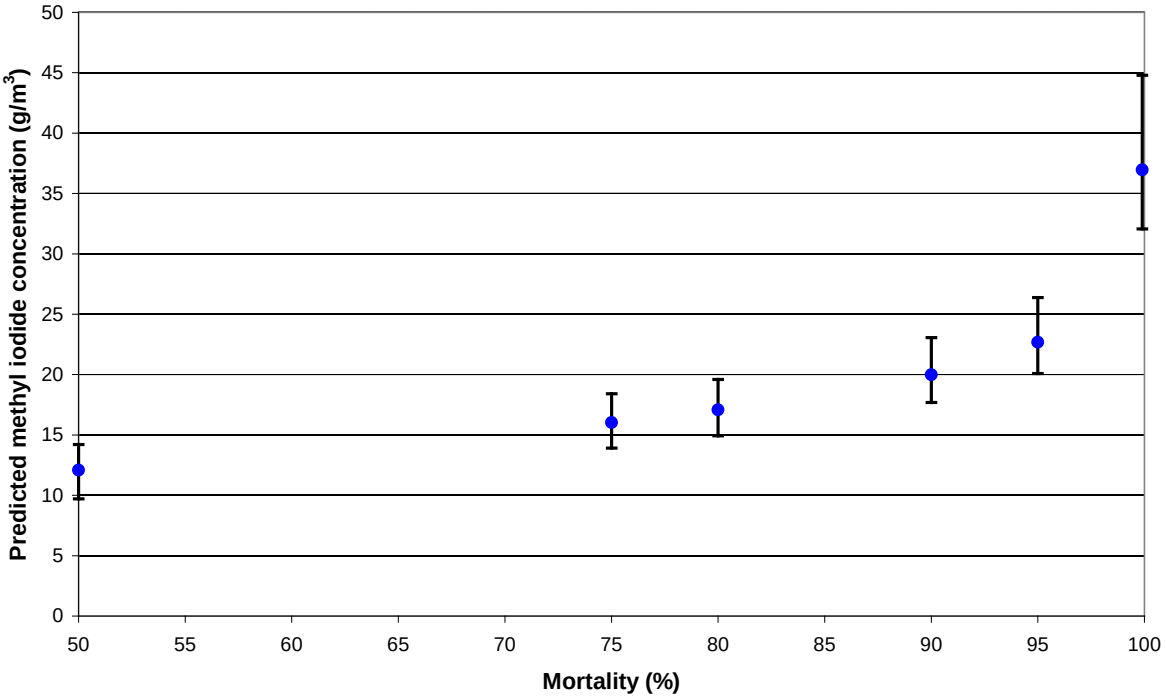

Figure 22: Inverse prediction from percentage mortality of *A. fulica* to provide methyl iodide fumigation dose rates at 30°Cel.

Based on the figures above and tabulations in Appendix 1, it can be seen that increasing temperatures in which the fumigation is conducted results in decreasing methyl iodide concentrations required to obtain complete mortality (i.e., 100% control).

All remnant Dipteran maggots within cages died during fumigation, irrespective of treatment temperature. This indicates that a methyl iodide concentration above 20 g/m^3 is sufficient to achieve Dipteran mortality at all treatment temperatures.

RESULTS FOR METHYL BROMIDE

Figure 23 displays the raw mean mortality results after 24 hours of the methyl bromide trial for all concentrations >20 g/m^3. Initially, methyl bromide was not part of the trial programme; however, due to unexpected logistical issues with obtaining methyl iodide, a canister of methyl bromide was taken to Samoa in case insufficient methyl iodide was available. The logistical nature of combining an experimental mortality trial with fumigation meant that some time was needed to process mortality from the previous day's mortality trial and then process the results in view of the overall programme. This allowed for occasional methyl bromide trials between methyl iodide runs.

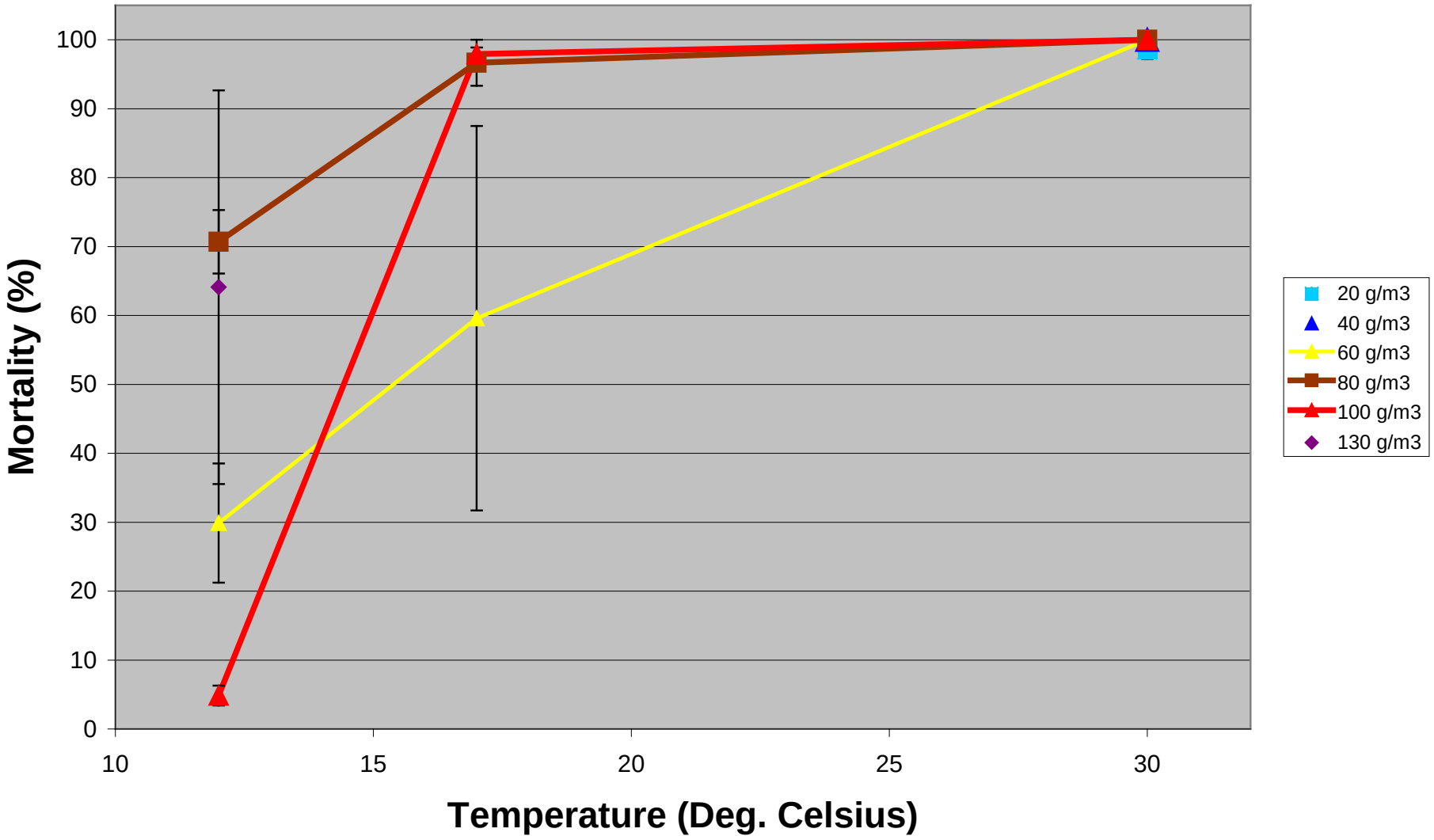

Figure 23: Mean mortality of *A. fulica* (+/- S.E.) across temperature and methyl bromide concentrations >20 g/m^3.

In New Zealand, the concentration of methyl bromide applied to *A. fulica* is 128 g/m^3. Based on the results from this trial, to obtain >90% mortality, at least 80 g/m^3 of methyl bromide would need to be applied at temperatures no less than 17^{o}C. As with results from methyl iodide, higher temperatures resulted in higher mortality. Consequently, lower concentrations of methyl bromide were required to achieve high rates of mortality at high temperatures. At the maximum temperature of 30^{o}C, any dose >40 g/m^3 resulted in 100% mortality after 24 hours.

Similar to the analyses undertaken on methyl iodide, the whole logistic model was compared to the reduced model to ensure that the linear responses of temperature and methyl bromide concentrations were valid. The ratio of negative log-likelihood values of the difference between the whole model and reduced model and the reduced model determined that the proportion of uncertainty attributed to the fit of the full model was 54% (Appendix 2). Based data from 1,213 snails, analyses indicated that significant differences in mortality could be explained by temperature and concentrations of methyl bromide, as well as their interaction effect ($p<0.0001$; Appendix 2). Wald Chi-square tests indicated that each of the linear responses (i.e., temperature, concentration, and the interaction between temperature and concentration) were significant for the negative log-likelihood values.

Similar to the methyl iodide analyses, further analyses were conducted on the response of mortality to methyl bromide concentration for each of the three temperatures tested. Figures 24 – 26 display the fit of the logistic regression for mortality to the corrected methyl bromide fumigation dose rates across the three treatment temperatures. The mortality response was not as dramatic in the lower temperatures, as compared to the higher temperatures (as determined by the relative changes to slope in the logistic curve).

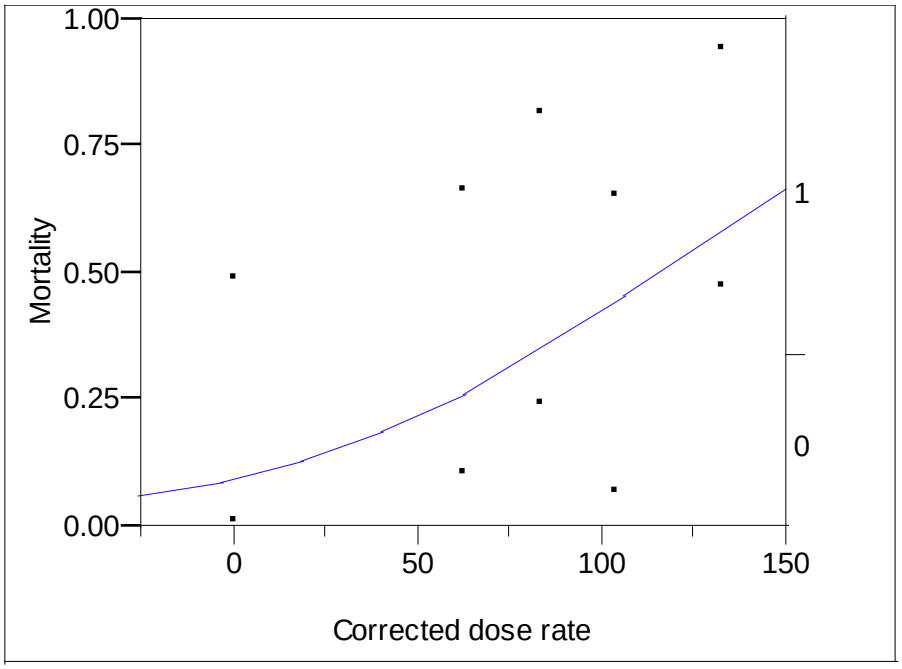

Figure 24: Logistic fit of *A. fulica* mortality vs. corrected methyl bromide fumigation dose rate at 12^{o}Cel.

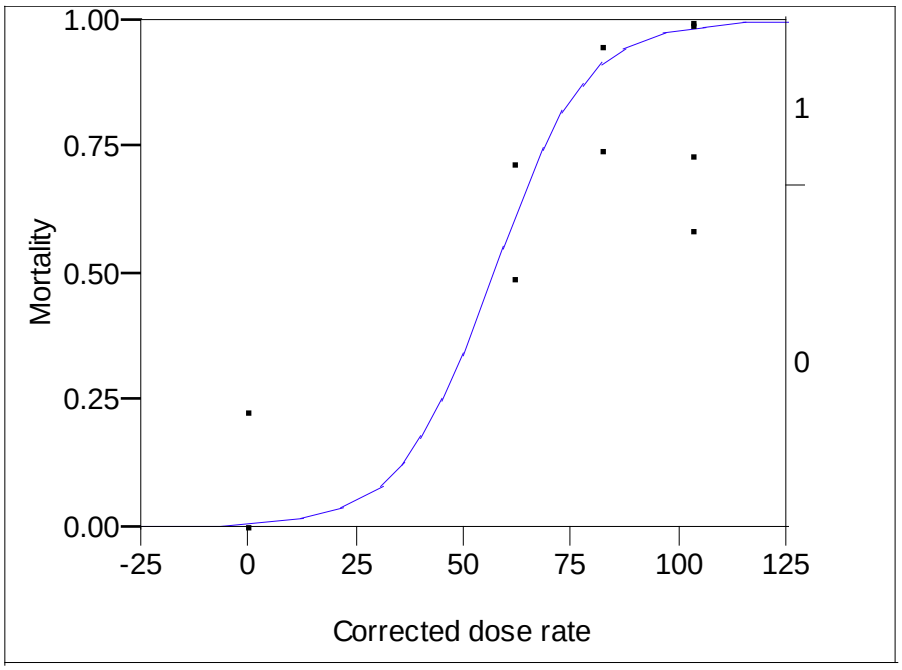

Figure 25: Logistic fit of *A. fulica* mortality vs. corrected methyl bromide fumigation dose rate at 17°Cel.

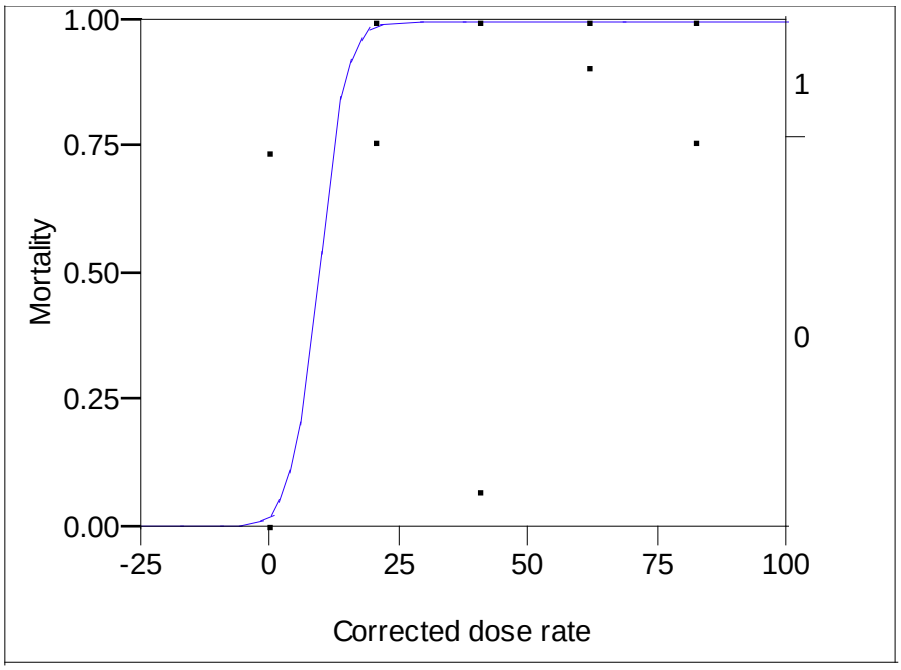

Figure 26: Logistic fit of *A. fulica* mortality vs. corrected methyl bromide fumigation dose rate at 30°Cel.

Across all three temperatures, methyl bromide concentrations independently had a significant impact on mortality ($p<0.0001$; Appendix 2). For practical and operational purposes, a reverse prediction method was undertaken, based on the logistic regression model fitted above. Figures 27 – 29 plot the predicted methyl iodide concentrations (including a 95% confidence interval) needed to obtain a set mortality (note: the highest prediction is for 99.9% mortality). Tabulations of the data for Figures 27 – 29 are provided in Appendix 2.

Based on the figures below and tabulations in Appendix 1, it is evident that, similar to methyl iodide, increasing temperatures in which the fumigation is conducted results in decreasing methyl bromide concentrations required to obtain complete mortality (i.e., 100% control).

Similar to methyl iodide, all remnant Dipteran maggots perished in all temperatures at concentrations above 20 g/m^3.

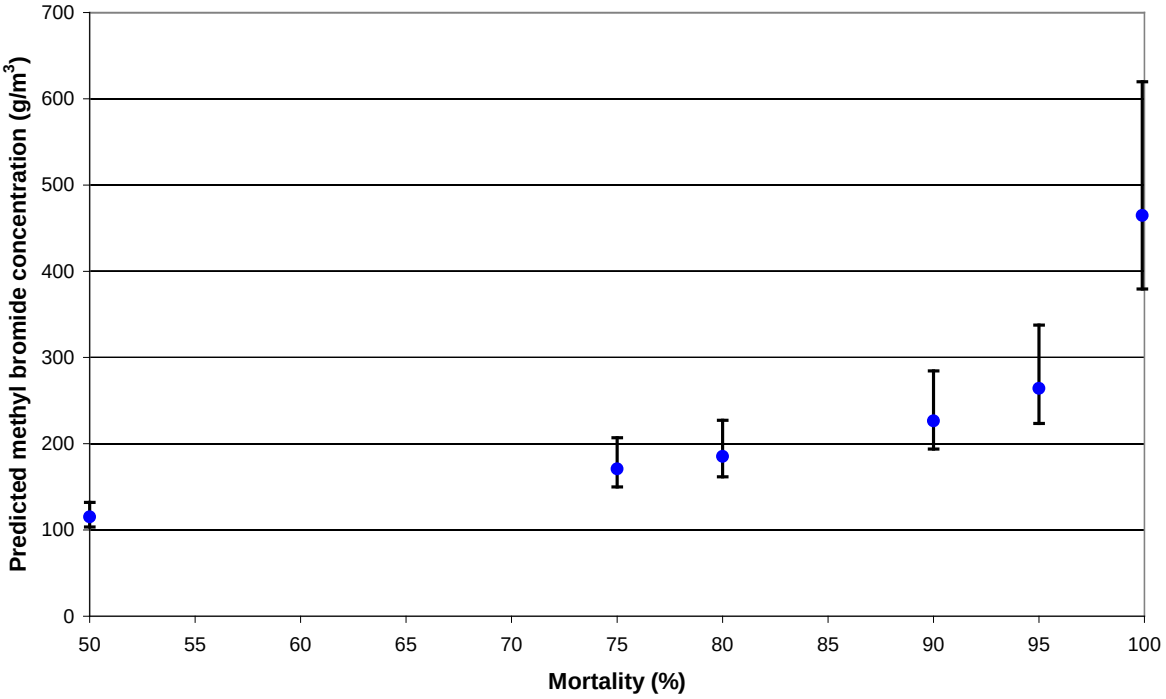

Figure 27: Inverse prediction from percentage mortality of *A. fulica* to provide methyl bromide fumigation dose rates at 12°Cel.

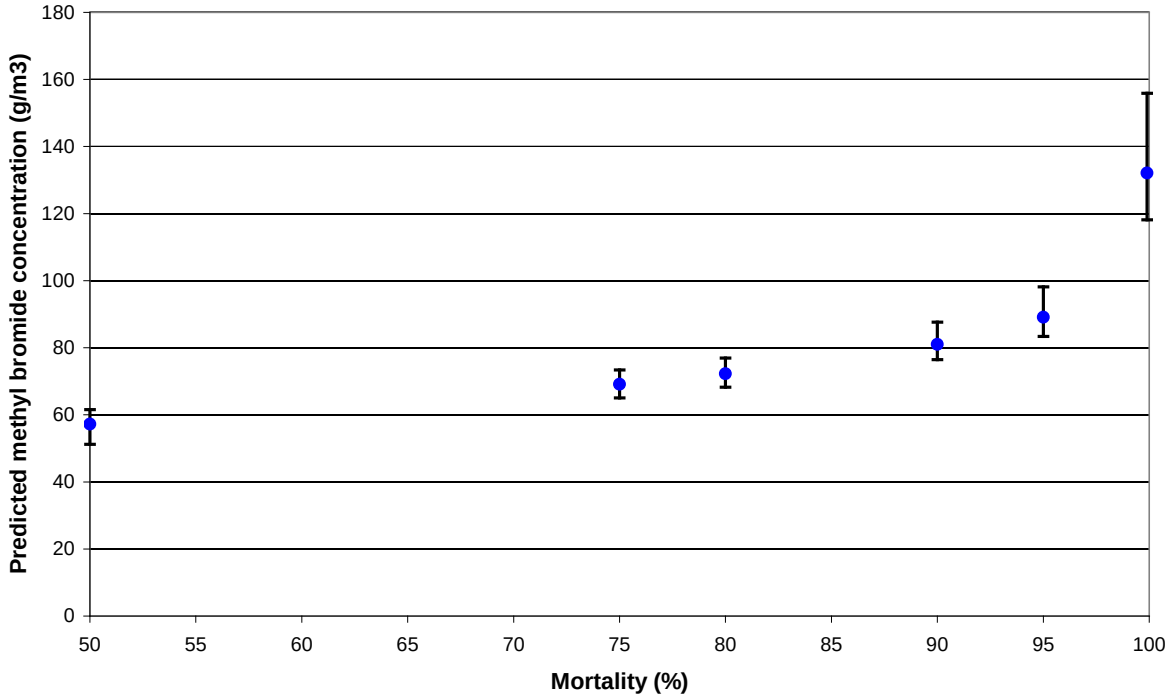

Figure 28: Inverse prediction from percentage mortality of *A. fulica* to provide methyl bromide fumigation dose rates at 17°Cel.

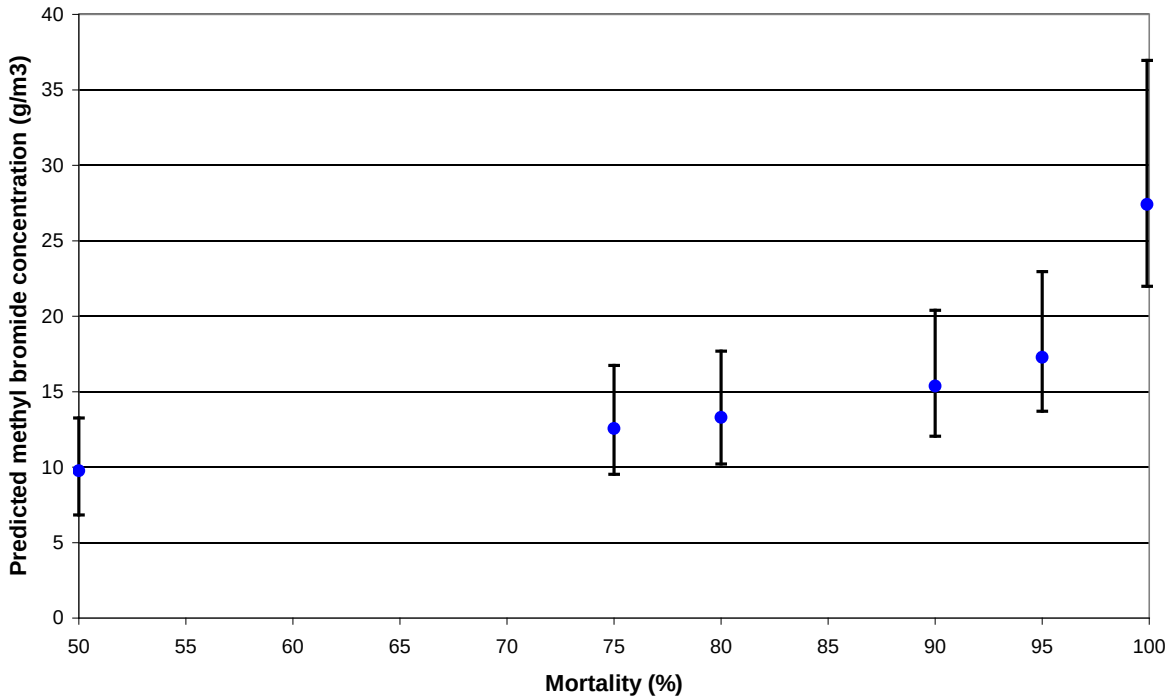

Figure 29: Inverse prediction from percentage mortality of *A. fulica* to provide methyl bromide fumigation dose rates at 30°Cel.

Conclusions

Both methyl iodide and methyl bromide analyses suggest that mortality results for *A. fulica* are strongly influenced by temperature. Temperature was a complex effect (as opposed to a simple independent effect), with significant interactions recorded between temperature and fumigant concentrations. This resulted in varying mortality of *A. fulica*.

The temperature – concentration interaction for methyl iodide resulted in much higher predictions of required concentrations to achieve high levels of mortality at 12^{o}C compared to 17 ^{o}C and 30 ^{o}C temperatures.

For incursion response at the shipping container level, it would appear that 260 g/m^3 of methyl iodide would be needed to obtain 100% mortality at the immediate conclusion of the 24 hour fumigation period (complete control) at 12^{o}C; 81 g/m^3 at 17 ^{o}C; and 37 g/m^3 at 30 ^{o}C. At temperatures above 17 ^{o}C, all of these concentrations are much lower than the current 128 g/m^3 rate for methyl bromide that is currently used in such scenarios.

The fumigation rates at which complete mortality was estimated for *A. fulica* are higher than those published for other fauna (e.g., nematodes and forest wood insect pests, Soma et al., 2005; Soma et al., 2007; Kawakami 2007). This may be in part due to the size of *A fulica* which is much larger than the abovementioned species, their ability to seal themselves inside their shells during aestivation, physiological changes during aestivation, or simply due to being a different organism (all of which were outside of the scope of this project to comparatively evaluate).

As with methyl iodide, the temperature-concentration interaction for methyl bromide resulted higher predictions of required concentrations to achieve high levels of mortality at 12^{o}C compared to 17 ^{o}C and 30 ^{o}C. For incursion responses at the shipping container level, it would appear that 465 g/m^3 of methyl bromide would be needed to obtain 100% mortality at the immediate conclusion of the 24 hour fumigation period (complete control) at 12^{o}C; 132 g/m^3 at 17 ^{o}C; and as low as 27 g/m^3 at 30 ^{o}C. Only at 30 ^{o}C is the fatal concentration much lower than the current 128 g/m^3 rate that is currently used in such scenarios.

This trial provides positive results for tropical countries or countries where fumigation occurs against *A. fulica* at temperatures approaching 30 ^{o}C. At higher temperatures, lower rates of methyl bromide can be applied during fumigation. This has associated reductions in terms of product costs, reduced impacts to the environment etc.

Across a range of temperatures, methyl bromide also appeared to be more heteroscedastic than methyl iodide in terms of the mortality response of snails. At temperatures greater than 17^{o}C, the methyl iodide application rate required to achieve 100% mortality of *A. fulica* was lower than the methyl bromide application rate (128 g/m^3) that is currently used for fumigation by New Zealand. At each of the lower treatment temperatures, the application rate required to achieve 100% mortality of *A. fulica* at the immediate conclusion of the 24 hour fumigation was lower for methyl iodide than methyl bromide.

Similar to methyl bromide, methyl iodide is a broad spectrum fumigant with comparable effectiveness. Operators who are familiar with methyl bromide fumigation should be able to easily manage methyl iodide fumigation in the field. The only challenge observed during this trial was that methyl iodide needs a higher temperature for vaporization. As methyl iodide has been found to pose no threat to ozone depletion, it should be further considered as a suitable replacement for methyl bromide in many, if not all, use areas.

It must also be considered that the results of this trial were based on absolute acute mortality, and it is firmly considered by the authors that chronic impacts against the test subjects would have resulted in greater mortality post treatment. Therefore, the results provided in this report may be conservative in terms of estimates of *A. fulica* mortality.

The refrigerated shipping containers proved to be reliable experimental test units. There were no issues relating to operation of the units and temperature regulation results were consistent. Unfortunately, failure of the fumigant monitoring equipment meant that no data relating to real world fumigation conditions inside refrigerated shipping containers were obtained. It is recommended that a further evaluation is considered for this aspect of fumigation. Currently, there is the expectation that fumigant concentrations decline to a threshold asymptote within about 4 hours due to inherent cargo absorption. Further gains in the reduction of fumigant use by the government could be made by evaluating if this threshold asymptote exists for empty, or near empty shipping containers.

Acknowledgements

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References

Anderson, A.W.; Taylor, T.H. 1926: *The slug pest*. Bulletin of University of Leeds Department of Agriculture. No. 143, University of Leeds 14 pp.

[ATSDR] Agency for Toxic Substances and Disease Registry. 1992: *Toxicological Profile for Bromomethane.* U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta.

Bequaert, J.C. 1950: Studies on the Achatinidae, a group of African land snails. *Bulletin of the Museum of Comparative Zoology*, Havard. 105:1-216

Durham, H.E. 1920: Of slugs. *Gardeners Chronicle*. 68, 46, 85-86

Evans, H.C. 1973: Invertebrate vectors of *Phytopthora palmivora*, causing black pod disease of cocoa in Ghana. *Annals of Applied Biology*. 75: 331-345

French, C. 1906: Slugs. *Journal of the Department of Agriculture of Victoria*. 8: 445-447

Gahan, A.B. 1907: *Greenhouse pests of Maryland*. Bulletin of the Maryland Agricultural Experimental Station. No. 119. 36 pp

Gimingham, C.T. 1940: Some recent contributions by English workers to the methods of insect control. *Annals of Applied Biology*. 27:161-175

Godan, D. 1983: *Pest slugs and snails: biology and control*. Springer Verlag, Berlin. 445pp.

Henderson, I; Triebskorn, R. 2002: Chemical control of terrestrial gastropods. IN Barker, G.M (ed.) *Molluscs as Crop Pests*. CABI Publishing. Oxon, Uk. Pp: 1-31

Hodasi, J.K.M. 1982: Some aspects of the biology of *Achatina (Achatina) achatina* (Linne). *Bulletin de l'Institut Fondamental d'Afrique Noire*. 44:100-114

Kawakami, F. 2007: *Additional treatment options available for inclusion in ISPM No.15(I) – Methyl iodide fumigation-*. Japan Fumigation Technology Association, Tokyo. Pp. 9

Mead, A.R. 1982: Anatomical studies in the African Achatinidae – a preliminary report. *Malacologia*. 18:133-138

Muir, G.D. 1971: *Hazards in the Chemical Laboratory*. The Royal Institute of Chemistry, London.

Numazawa, K.; Koyano, S.; Takeda, N.; Takayanagi, H. 1988: Distribution and abundance of the giant African snail, *Achatina fulica* Ferussac (Pulmonata: Achatinidae), in two islands, Chichijima and Hahajima, of the Ogasawa (Bonin) Islands. *Japanese Journal of Applied Entomology & Zoology*. 32: 176-181

Pilsbry, H.A. 1904: Family Achatinidae. IN Tryon, G.W.; Pilsbry, H.A. (eds). *Manual of Conchology*, Vol. 16. Academy of Natural Sciences, Philadelphia. Pp: 205-329

Poucher, C. 1975: Eradication of the giant African snail in Florida. Florida State Horticultural Society. Pp:523 - 524

Raut, S.K. 1983: Altitudinal immobilization of the giant land snail *Achatina fulica* Bowdich. IN: *Proceedings of a Workshop on High Altitude Entomology and Wildlife Ecology*. Zoological Survey of India. Pp: 359-366

Raut, S.K.; Barker, G.M. 2002: *Achatina fulica* Bowdich and other Achatinidae as pests in tropical agriculture. IN Barker, G.M (ed.) *Molluscs as Crop Pests*. CABI Publishing. Oxon, Uk. Pp: 55-114

Raut, S.K.; Ghose, K.C. 1981: Factors influencing mortality in land snails, *Achatina fulica* Bowdich and *Macrochlamys indica* Godwin-Austen during aestivation. *Proceedings of the Zoological Society of Calcutta*. 32: 107-120

Raut, S.K.; Ghose, K.C. 1984: *Pestiferous land snails of India*. Zoological Survey of India. No. 11, Bani Press, Calcutta. 151 pp.

Raut, S.K.; Rahman, M.S. 1991: Influence of temperature on the heart beat rate in two land snails *Achatina fulica* Bowdich and *Macrochlamys indica* Godwin-Austen (Gastropoda, Stylommatophora). *Malakologische Abhandlungen*. 15: 165-172

Schreurs, J. 1963: *Investigations on the biology, ecology and control of giant African snail in West New Guinea.* 18 pp.

Smith, J.W.; Fowler, G. 2003: *Pathway risk assessment for Achatinidae with emphasis on the giant African land snail Achatina fulica (Bowdich) and Limicolaria aurora (Jay) from the Caribbean and Brazil, with comments on related taxa Achatina achatina (Linne), and Archachatina marginata (Swainson) intercepted by PPQ*. USDA-APHIS, Centre for Plant Health Science and Technology (internal report), Raleigh,NC.

Soma, Y. Goto, M., Ogawa, N., Naito, H., Hirata, K. 2005: Effects of some fumigants on mortality of pine wood nematode, *Bursaphelenchus xylophilus* infecting wooden packages: 5. Mortality of pine wood nematode and fumigation standards by methyl iodide. *Res. Bull. Plant Prot. Japan*. 41:1-7

Soma, Y. Komatsu, H., Oogita, T., Nakamura, Z., Nomura, N., Abe, Y., Itabashi, T., Mizobuchi,M. 2007: Mortality of forest insect pests by methyl iodide tarpaulin fumigation. *Res. Bull. Plant. Prot. Japan*. 43:9-15

Srivastava, P.D. 1992: *Problem of land snail pests in agriculture: a study of the giant African snail*. Concept Publishing Company, New Delhi. Pp 234.

Takeda, N.; Ozaki, T. 1986: Induction of locomotor behaviour ion the giant African snail, *Achatina fulica. Comparative Biochemistry and Physiology*. 83A: 77-82

Teles, H.M.S.; Vaz, J.F.; Fontes, L.R.; de Fatima Domingos, M. 1997: Registro de *Achatina fulica* Bowdich, 1822 (Mollusca, Gastropoda) no Brazil: *caramujo hospedeiro intermediaro da angiostroingiliase.* Revista de Saude Publica. 31: 310-312

Theobald, F.V. 1895: Mollusca injurious to farmers and gardeners. *Zoologist*. 19:2301-211

Triebskorn, R.; Ebert, D. 1989: The importance of mucus production in slugs' reaction to mulluscicides and the impact of molluscicides on the mucus producing system. IN:

Henderson, I.F. (ed.) *Slugs and Snails in World Agriculture*. Monograph No. 41, British Crop Protection Council, Thornton Heath. 373-378

Triebskorn, R.; Henderson, I.F.; Martin, A.P.; Kohler, H.R. 1996: Slugs as target and non-target organisms for environmental chemicals. IN: Henderson, I.F. (ed.) *Slug and Snail Pests in Agriculture*. Symposium Proceedings No. 66, British Crop Protection Council, Farnham. Pp: 65-72

[US EPA] U.S. Environmental Protection Agency. 1999: *Integrated Risk Information System (IRIS) on Bromomethane*. National Center for Environmental Assessment, Office of Research and Development, Washington.

Venette, R.C.; Larson, M. 2004: *Mini risk assessment. Giant African snail, Achatina fulica Bowdich [Gastropoda: Achatinidae]*. Dept of Entomology, University of Minnesota. Pp: 30

von Stanislaus, K.; Mrkramer, G.; Peters, K.J.;Waitkuwait, E. 1987: Opportunities for utilizing the African giant snail. IN Siegmund, R. (ed). *Untersuchungen fur Wachstumsund Reproducktionsleitung Beider Achaschnecke*. Diplomabiet am Instutute fur Tierzucht und Hanstiergenetik der Universtat Gottingen, Gottingen. Pp: 60-71

Appendix 1: Statistical Analysis Tables: Methyl iodide

Nominal Logistic Fit for Mortality

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	443.0897	3	886.1795	<.0001
Full	605.8236			
Reduced	1 048.9133			
RSquare (U)		0.4224		
Observations (or Sum Wgts)		1 745		

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	-11.275909	0.7823042	207.76	<.0001
Corrected dose rate	0.10149801	0.006069	279.69	<.0001
Temp	0.48271606	0.0364765	175.13	<.0001
Temp * Corrected dose rate	0.00955183	0.0008943	114.08	<.0001

For log odds of 0/1

Effect Wald Tests

Source	Nparm	DF	Wald ChiSquare	Prob>ChiSq
Temp	1	1	175.129012	0.0000
Corrected dose rate	1	1	279.691379	0.0000
Temp*Corrected dose rate	1	1	114.079472	0.0000

Temp=12 deg Cel.

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	62.37627	1	124.7525	<.0001
Full	440.50473			
Reduced	502.88100			
RSquare (U)		0.1240		
Observations (or Sum Wgts)		771		

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq	Lower 95%	Upper 95%
Intercept	-1.4920972	0.2289721	42.46	<.0001	-1.9579815	-1.058099
Corrected dose rate	0.03219758	0.0032887	95.85	<.0001	0.02595835	0.0388816

For log odds of 0/1

Effect Wald Tests

Source	Nparm	DF	Wald ChiSquare	Prob>ChiSq
Corrected dose rate	1	1	95.8531284	0.0000

Inverse Prediction

Probability	Predicted Corrected dose rate	Lower Limit	Upper Limit	1-Alpha
0.99900000	260.853544	227.827813	310.285647	0.9500
0.95000000	137.790997	124.803044	156.875893	
0.90000000	114.583834	105.116766	128.203692	
0.80000000	89.397771	83.210768	97.627629	
0.75000000	80.462872	75.074465	87.145624	
0.50000000	46.341910	39.139333	51.980387	

Temp=17 Deg Cel

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	182.02488	1	364.0498	<.0001
Full	99.51314			
Reduced	281.53802			
RSquare (U)		0.6465		
Observations (or Sum Wgts)		487		

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq	Lower 95%	Upper 95%
Intercept	-6.8127289	1.2764309	28.49	<.0001	-9.6377606	-4.6581131
Corrected dose rate	0.16963288	0.0268293	39.98	<.0001	0.12497849	0.229925

For log odds of 0/1

Effect Wald Tests

Source	Nparm	DF	Wald ChiSquare	Prob>ChiSq
Corrected dose rate	1	1	39.976085	0.0000

Inverse Prediction

Probability	Predicted Corrected dose rate	Lower Limit	Upper Limit	1-Alpha
0.99900000	80.8774999	72.6671583	96.3083271	0.9500
0.95000000	57.5193179	54.4374749	62.8277071	
0.90000000	53.1144267	50.7503659	56.7632754	
0.80000000	48.3339255	46.2416843	50.6888916	
0.75000000	46.6380157	44.3991268	48.7770468	
0.50000000	40.1616047	36.1835412	42.6551647	

Temp=30 deg Cel

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	204.08045	1	408.1609	<.0001
Full	41.81724			
Reduced	245.89769			
RSquare (U)		0.8299		
Observations (or Sum Wgts)		487		

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq	Lower 95%	Upper 95%
Intercept	-3.3541937	0.5710794	34.50	<.0001	-4.7123981	-2.3975094
Corrected dose rate	0.2776229	0.0346068	64.36	<.0001	0.21706926	0.3557686

For log odds of 0/1

Effect Wald Tests

Source	Nparm	DF	Wald ChiSquare	Prob>ChiSq
Corrected dose rate	1	1	64.3559294	0.0000

Inverse Prediction

Probability	Predicted Corrected dose rate	Lower Limit	Upper Limit	1-Alpha
0.99900000	36.9600225	32.0596679	44.7865892	0.9500
0.95000000	22.6877277	20.0945459	26.3854541	
0.90000000	19.9962551	17.6891773	23.0643470	
0.80000000	17.0752777	14.9375068	19.6012389	
0.75000000	16.0390444	13.9125529	18.4214651	
0.50000000	12.0818337	9.7059637	14.2085478	

Appendix 2: Statistical Analysis Tables: Methyl bromide

Nominal Logistic Fit for Mortality
Freq: Count

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	439.87262	3	879.7452	<.0001
Full	381.39989			
Reduced	821.27251			
RSquare (U)		0.5356		
Observations (or Sum Wgts)		1 213		

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	-13.340924	0.7947848	281.76	<.0001
Temp	0.54571875	0.0345045	250.14	<.0001
Corrected dose rate	0.0870564	0.0052521	274.75	<.0001
Temp * Corrected dose rate	0.00807355	0.0006509	153.86	<.0001

For log odds of 0/1

Effect Wald Tests

Source	Nparm	DF	Wald ChiSquare	Prob>ChiSq
Temp	1	1	250.141339	0.0000
Corrected dose rate	1	1	274.752147	0.0000
Temp* Corrected dose rate	1	1	153.860273	0.0000

Temp=12 deg Cel

Nominal Logistic Fit for Mortality
Freq: Count

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	29.00848	1	58.01697	<.0001
Full	243.76193			
Reduced	272.77042			

RSquare (U)	0.1063
Observations (or Sum Wgts)	425

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq	Lower 95%	Upper 95%
Intercept	-2.2783714	0.2818422	65.35	<.0001	-2.8599773	-1.7522674
Corrected dose rate	0.01976616	0.0029288	45.55	<.0001	0.01423622	0.02574543

For log odds of 0/1

Inverse Prediction

Probability	Predicted Corrected dose rate	Lower Limit	Upper Limit	1-Alpha
0.99900000	464.689501	379.339570	619.740276	0.9500
0.95000000	264.229920	223.487134	337.514478	
0.90000000	226.427207	193.905282	284.483438	
0.80000000	185.401014	161.549633	227.181702	
0.75000000	170.846741	149.945580	206.979290	
0.50000000	115.266274	103.551743	131.909278	

Temp=17 deg Cel

Nominal Logistic Fit for Mortality
Freq: Count

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	153.85712	1	307.7142	<.0001
Full	90.73517			
Reduced	244.59229			

RSquare (U)	0.6290
Observations (or Sum Wgts)	388

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq	Lower 95%	Upper 95%
Intercept	-5.2796493	0.8698653	36.84	<.0001	-7.2203903	-3.7911135
Corrected dose rate	0.09226069	0.0125025	54.45	<.0001	0.07116429	0.12072747

For log odds of 0/1

Inverse Prediction

Probability	Predicted Corrected dose rate	Lower Limit	Upper Limit	1-Alpha
0.99900000	132.086634	118.123760	155.854754	0.9500
0.95000000	89.139678	83.389606	98.131620	
0.90000000	81.040732	76.433450	87.652160	
0.80000000	72.251179	68.239074	76.924165	
0.75000000	69.133035	65.049180	73.401241	
0.50000000	57.225339	51.236640	61.578593	

Temp=30 deg Cel

Nominal Logistic Fit for Mortality
Freq: Count

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	200.82607	1	401.6521	<.0001
Full	14.88446			
Reduced	215.71054			

RSquare (U)	0.9310
Observations (or Sum Wgts)	400

Converged by Gradient

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq	Lower 95%	Upper 95%
Intercept	-3.8179136	0.7148319	28.53	<.0001	-5.6210935	-2.6675473
Corrected dose rate	0.39126181	0.0600888	42.40	<.0001	0.29363197	0.5448889

For log odds of 0/1

Inverse Prediction

Probability	Predicted Corrected dose rate	Lower Limit	Upper Limit	1-Alpha
0.99900000	27.4104657	21.9896963	36.9490546	0.9500
0.95000000	17.2834466	13.7027588	22.9504526	
0.90000000	15.3736911	12.0541519	20.3964474	
0.80000000	13.3010885	10.2047078	17.6849181	
0.75000000	12.5658211	9.5293471	16.7422477	
0.50000000	9.7579511	6.8284229	13.2641672	