Testing the efficacy of Pestoff® 20R to kill House Mice *Mus musculus* on Lord Howe Island.

Prepared for

The Lord Howe Island Board

By

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Executive Summary

An invasive rodent eradication programme targeting Ship Rats (*Rattus rattus*) and House Mice (*Mus musculus*) is proposed for Lord Howe Island in the winter of 2017. The proposed bait to be used in the trial is Pestoff® Rodent Bait 20R containing brodifacoum at 20 ppm. The bait will be distributed by hand broadcasting or in bait stations within the settlement area, and by helicopter outside the settlement area. The aims of this study were to demonstrate that Pestoff 20R will be effective against mice when the bait is provided in a manner that is consistent with its application in the field.

Between 4 and 8 April 2016, 90 mice were captured at various locations across the settled parts of the island. After an up to seven day acclimatisation period the mice were placed in three treatment groups: Control (C) where 29* mice were fed *ad libitum* with commercial pet feed; Aerial Simulation (AS) where 30 mice were given Pestoff 20R for three days followed by seven days of pet food, followed by another three days of Pestoff 20R; Bait Station Simulation (BS) where 30 mice were provided with Pestoff 20R *ad libitum*.

The first death in the AS group occurred after 2 days and the last mouse died (euthanized as per animal ethics requirements^) after 22 days. In the BS group, the first death occurred after 4 days and the last mouse died after 22 days (euthanized as per animal ethics requirements^). After 16 days more than 90% of mice had died or had been euthanized in both the AS group and the BS group. All 29 mice in the Control Group were alive at the end of the trial.

This study shows that, while there is a wide range in the time until death following ingestion of Pestoff 20R, the bait will kill Lord Howe Island mice when the bait is provided in a manner that is consistent with field conditions. Initially, the mice in the AS trial died faster than those in the BS trial. Only six mice of the original 30 in the AS group survived to receive the second dose of poison. This indicates that a single ingestion of the bait (from a limited exposure) will be sufficient to kill the majority of mice relatively quickly. During the actual eradication, the period between poison exposure and death is likely to be faster than in this simulation. The mice in this trial were not challenged physically due to the confinement of their holding cages. In a natural setting with normal physical activity and exertion, there should be more likelihood of bleeding leading to death.

**One mouse died during the acclimation period, presumably from poison consumed prior to being captured.*

^In a moribund state as measured by immobility and a lack of response to stimuli

Introduction

An invasive rodent eradication programme targeting Ship Rats (*Rattus rattus*) and House Mice (*Mus musculus*) is proposed for Lord Howe Island in the winter of 2017. The proposed bait to be used in the trial is Pestoff® Rodent Bait 20R (Pestoff 20R) in the form of pellets containing the anticoagulant brodifacoum at 20 ppm. In 2013, a trial was performed to test the efficacy of brodifacoum on Lord Howe Island rodents (Wheeler & Carlile 2013). In those trials, rats and mice were fed a measured and restricted amount of brodifacoum in line with their respective accepted LD50. The results showed that, while rats died as expected, the LD50 of mice caught from within the settlement area of the island was five times the accepted value of 0.4 mg/kg. Moreover, some mice could survive a dose of 15 times the accepted LD50. As brodifacoum has long been used by island inhabitants in an effort to control rodent numbers (particularly in and around the settlement area), the results of the 2013 trial suggested that the mice had developed some resistance to brodifacoum.

In the proposed rodent eradication, Pestoff 20R will be applied across the entire island. Within the settlement area, pellets will be either broadcast by hand or made available to rodents in bait stations. Outside of the settlement area, pellets will be broadcast by helicopter in two drops. The first drop will spread pellets at a density of 12 kg/ha (one bait every two square metres). The second drop will occur 14 to 21 days later (depending on conditions) and will spread bait at a density of 8kg/ha. A single 2 g pellet of the bait will provide mice with the LD50 of brodifacoum as determined in the 2013 study.

This toxicity trial was designed to simulate potential exposure to bait that mice will experience under field situations. The main aim of the trial was to examine the efficacy of Pestoff 20R to kill Lord Howe Island mice, when the bait is provided in a manner that is consistent with its application in the proposed eradication.

Methods

With the aim of catching 90 mice, 250 Elliot traps were set between 4 and 6 April 2016, at various locations across the island: Southern Settlement (200 trap nights); Waste Management Facility (WMF)/Airport (100 trap nights); and Nursery (200 trap nights). The locations were chosen to include mice from within and on the edge of the settlement area to reflect potential differences in previous exposure to brodifacoum in mice from different parts of the inhabited sections of the island.

The majority of the 90 mice were caught in the Nursery area (63%), followed by the WMF/Airport (37%). No mice were caught at the Southern Settlement. The mice were weighed, and then placed in individual purpose-built mouse cages. Every seven days the cages were cleaned and the bedding was replaced. The mice were allowed to acclimatise for up to seven days in a mouse housing facility which provided 12 hours of natural/artificial light/12 hours of darkness each day throughout the trial. On 12 April 2016 the mice were placed in three treatment groups:

Control (C; N =29*. Mice fed *ad libitum* with pet food pellets and mixed seeds)

Aerial Simulation (AS; N = 30. Mice given Pestoff 20R for three days followed by seven days of pet food, followed by another three days of exposure to Pestoff 20R)

Bait Station Simulation (BS; N = 30. Mice provided with Pestoff 20R *ad libitum*)

The distribution of mice by treatment group and capture location is shown in Fig 1. At the beginning of the trial, there was no significant difference in the mean body mass of mice in different treatment groups ($F_{2,86}$= 3.10, P = 0.12; Fig. 3).

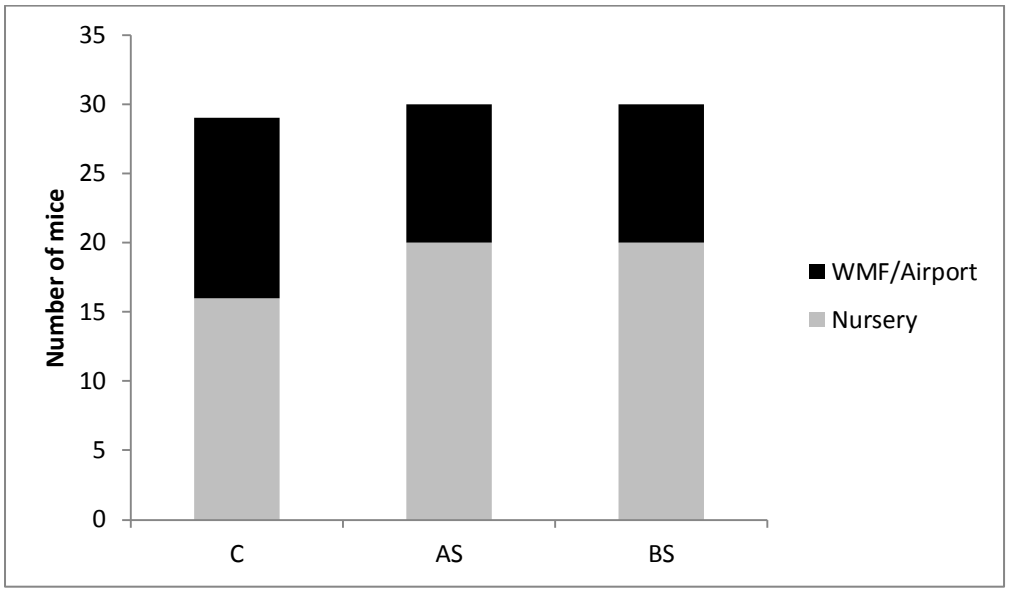

Figure 1. The proportion of mice used in the trial by treatment group and capture location.

The condition of mice was checked every six hours. The characteristics examined included, activity level, gait, posture, respiration, condition of fur, and condition of eyes. If a mouse was found to be prostrate, it was checked every hour for the next three hours. As per Office of Environment and Heritage Animal Ethics Committee requirements (AEC 160202 02), the mouse was euthanized if its condition had not changed after those three hours. Mice were also euthanized if they became moribund to the extent that they were found to be immobile and unresponsive to stimuli in two consecutive 6-hourly checks.

Results

The first death in the AS group occurred after two days of exposure to toxic bait and the last death occurred after 22 days after commencement of exposure (the mouse was in a severe moribund state and was therefore euthanized). After 15 days, more than 90% of the mice in the AS group had died or been euthanized. The average time until death in the AS group was 8.7 ± 4.4 days. In the BS group, the first death occurred four days after exposure and the last mouse died after 22 days after commencement of exposure (the mouse was in a severe moribund state and was therefore euthanized). More than 90% of mice were dead 16 days after commencement of exposure. The average time until death in the BS group was 9.9 ± 4.8 days. There was no significant difference in the mean number of days until death between the AS and BS groups ($t_{(1),\,58}$ = 2.00, P = 0.33). All 29 mice in the Control group were alive at the end of the trial, at which time these mice were euthanized as per ethics licence requirements. The attrition of mice in each treatment group is shown in Figure 2.

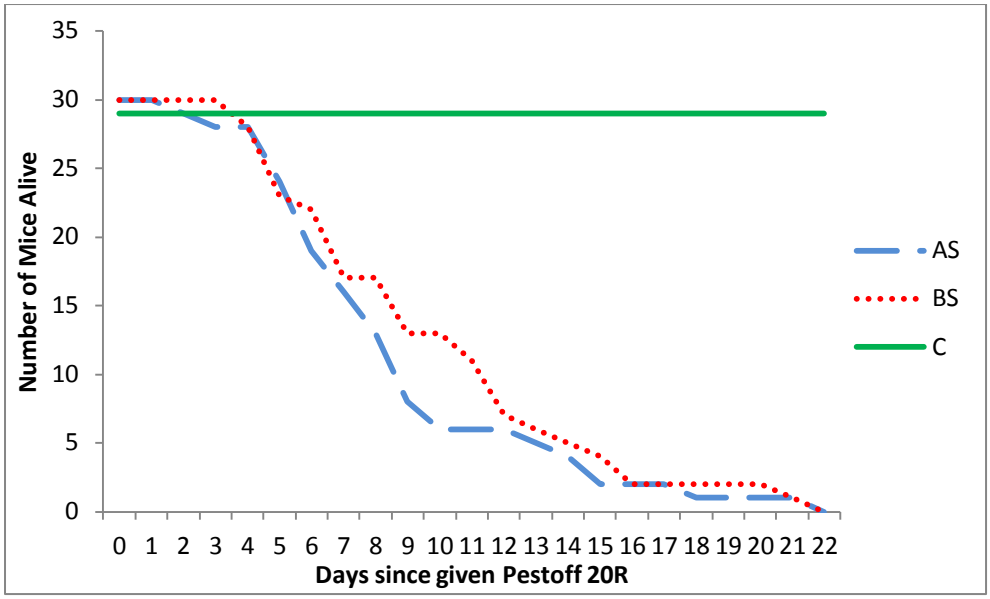

Figure 2. Survival of mice *Mus musculus* in the baiting trial.

Mice in all three groups had a lower body mass at the end point (i.e. death in the baited groups or at 29 days in the control group); however the average decrease in the baited mice was more than twice that of the control mice (Fig 3).

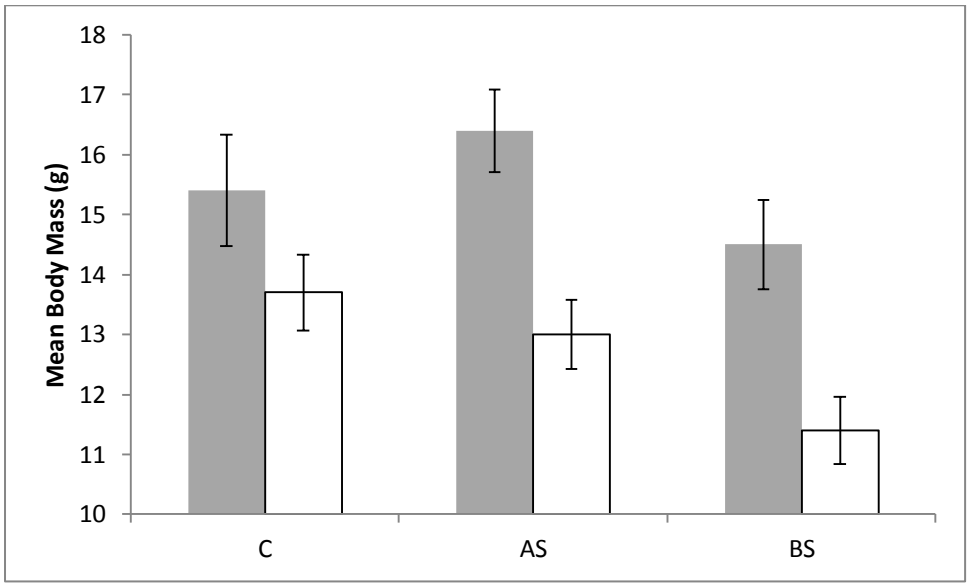

Figure 3. Body mass of mice *Mus musculus* at the beginning and end of the trial. Filled columns represent initial body mass, open columns represent body mass at the end point.

**One mouse died during the acclimatisation period. The mouse had blood around its nose and mouth and thus had presumably consumed poison prior to being captured.*

Discussion

The finding that no mice died in the Control group while all mice died in the groups given Pestoff 20R, indicates that the poison was effective against Lord Howe Island mice when provided in a manner that is consistent with field conditions. Initially, the mice in the AS group (mice that had access to bait for three days) died faster than those in the BS group (mice with *ad libitum* access to bait). Only six of the original 30 mice in the AS group survived to receive the second dose of poison on day 10. This indicates that a single ingestion of the bait will be sufficient to kill the majority of mice relatively quickly.

The results also confirmed that there is a broad range in tolerance to brodifacoum, with the time until death following ingestion of Pestoff 20R varying from just two days to 22 days. The fact that 90% of mice were dead after 16 days in both of the baited groups and that only three mice made it past 18 days, suggests that an extensive range of tolerance levels in the mouse population have been captured in the trial. The results of this trial, therefore, provide some level of confidence that all wild mice will receive a lethal dose of brodifacoum in the proposed eradication. Indeed, death from ingestion of brodifacoum is expected to be faster during the eradication. The mice in this trial were not challenged physically due to the confinement of their holding cages. In a natural setting percussive damage from normal activities, and therefore the likelihood of more excessive bleeding leading to death, would be expected. In addition, once mice in the trial became strongly affected by the poison they became unresponsive to gentle stimuli and did not readily seek refuge within their cardboard tubes or beneath shredded paper but rather sat out in the open. Wild mice displaying these behaviours would be vulnerable to predation and, because the eradication is planned for winter, they would be exposed to cold temperatures, both of which are likely to reduce the survivability of wild mice following consumption of bait.

One mouse died during the acclimatisation period. Bait is used by residents in and around the settlement area, and there was evidence that this mouse had been poisoned prior to capture. It is possible that the mice that were the quickest to die during the baiting trial had also eaten bait prior to being captured. However, no mice in the control group died once the baiting component of the trial had begun, suggesting that few if any of the mice used in this trial had previously ingested a lethal dose of bait and that the deaths in the baited groups were a result of ingestion of Pestoff 20R during the trial.

While there is little doubt that the death of mice was due to ingestion of Pestoff 20R (i.e. no mice in the control group died), necropsies of dead mice were performed to provide confirmation that the cause of death was brodifacoum poisoning. A number of mice examined showed external signs of haemorrhaging as evidenced by bleeding around the nose and mouth and darkening in the rear leg joints. Mice that showed no external signs of haemorrhaging were dissected. These mice exhibited various signs of being affected by brodifacoum including bleeding in the pericardium, subcutaneous bleeding along the flanks, discoloured kidneys, and blotchy lungs.

Mice in the baited groups lost more body mass throughout the trial than did mice in the Control group. It is likely that this loss of body mass is due to illness and a loss of appetite once the poison had begun taking an effect rather than an aversion to the bait and therefore a lack of overall food intake. Pestoff 20R does not contain the bitter compound (Bitrex®) found in commercially available rodenticides containing brodifacoum. Previous trials on LHI

have shown that Pestoff 20R is palatable to LHI rodents (Wheeler & Carlile 2013) and in this trial a number of mice were seen to almost immediately begin to chew the pellets following the provision of them. Conversely, mice that were fed commercial pet food during the acclimatisation period and those in the control group, were rarely seen consuming food.

In conclusion, this trial demonstrated that, despite there being a broad range of tolerance levels to brodifacoum in the Lord Howe Island mouse population, Pestoff 20R, when provided in a manner consistent with the methods proposed for the rodent eradication, will be effective against Lord Howe Island mice.

REFERENCES

Wheeler, R. & Carlile, N. Testing for brodifacoum resistance in invasive rodents on Lord Howe Island: Summary of Work Undertaken by the Office of Environment and Heritage in 2013. Unpublished report prepared for the Lord Howe Island Board.