

# final report

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# Development and delivery of a new feral pig toxin/HOGGONE®

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# **Executive summary**

1080 can be an effective toxin for feral pig control. However, is requires high doses of 1080 in feral pig control products, and feral pigs exhibit a high degree of variability to 1080, so there was an industry lead initiative to discover and register a new chemical-based product that could be added to the feral pig management tool kit. A suitable new chemical was identified. The chemical was sodium nitrite, and it was chosen because:

- 1. there was a considerable amount of data on its chemistry and toxicology as well as being approved for use in human food products,
- 2. sodium nitrite in a bait substrate is relatively safe for humans to use,
- 3. this active agent acts by an humane mode of action (methaemoglobinemia induction),
- 4. pigs are one of the most susceptible species to this mode of action as they exhibit uniquely low levels of methaemoglobin reductase, and
- 5. sodium nitrite is realtively environmental friendly.

Having identified a chemical that ticked many of the categories that would be required to register a safe to use, effective, humane, low environmental impact chemical, the technical challenge the project had to overcome was how to incorporate it into a bait substrate that would be highly attractive to feral pigs, less attractive to primary non-target species, stable, and bioactive once consumed.

The technical challenges turned out to be significant, delayed the final outputs of the project, required significantly more intellectual, time and monetary input, but did not prevent a new, effective and safe feral pig management product application being submitted to the Australian Pesticides and Veterinary Medicines Authority in the first quarter of the 2017/18 financial year. The new product containing a specialised formulation of sodium nitrite for feral pig management in Australia has been branded and trademarked as HOGGONE<sup>®</sup> in Australia and for future product sales also in the USA.

This will be the first time in over a decade that the APVMA has been asked to review and approve a new feral pig control product, and the first time ever that Australian R&D in this field has been used to support an application for a wild pig control product application to the US EPA.

Australian red meat producers will be the ultimate beneficiaries of this innovation as a proportion of all HOGGONE<sup>®</sup> sales in Australia and the USA flows back to Meat and Livestock Australia who will reinvest those dollars into R&D aimed at making red meat production in Australia even more globally competitive.

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# 1 Background

# 1.1 Overview of the project

### Industry Need and Overarching Aim

In 2003 a Pest Animal Control CRC-MLA co-sponsored workshop, "The Feral Pig Action Agenda", identified the need for greater feral pig control as well as improved tools. Between 2004 and 2008, PIGOUT<sup>®</sup>, a new feral pig-targeted bait was developed by Animal control Technologies and tested by the PAC-CRC with support from MLA to deliver a manufactured convenient and effective ready-to-use bait containing sodium fluoroacetate (1080). The product was registered in December 2007 and launched in March 2008. That bait matrix was also designed to deliver other actives, such as contraceptives or vaccines to feral pigs. The commencement of the PIGOUT<sup>®</sup> project, was one of three projects that MLA co-sponsored as a program of research aimed at improving the cost-effectiveness of feral pig management for Australia's red meat producers. Another of those projects was a PhD, that aimed to find an improved feral pig toxin, that, like the PIGOUT<sup>®</sup> bait matrix, reduced the risks to non-targets, while increasing target species efficacy. The third was to develop a concentrate of any new chemical identified in the PhD that could be used like the currently available 1080 concentrate so that end users could target feral pigs with food substrates that are locally preferred by feral pigs.

### Achiles Heel Approach to R&D

That PhD was considered an important plank in the bridge to enhanced feral pig management because 1080 is not an ideal toxin for feral pig management due to the high risk of sub-lethal dosing and learned aversion, and because very large doses of 1080 are used. The PhD project did uncover a physiological chink (Achiles Heel) in feral pigs that makes them highly susceptible to the effects of methaemoglobinemia (conversion of heamoglobin to methaemoglobin, which can't carry oxygen), because they have one of the lowest levels of methaemoglobin reductase in the animal kingdom (Cowled B.D., Elsworth, P. and Lapidge S.J. (2008) Additional toxins for feral pig (*Sus scrofa*) control: identifying and testing Achilles' heels. *Wildlife Research* 35: 651-662.

That enzyme is responsible for maintaining healthy levels of haemoglobin by converting oxidised haemoglobin (methaemoglobin) back to normal haemoglobin. The chemical selected to induce methaemoglobinaemia was sodium nitrite (SN). SN was selected because its used as a human food preservative, and there is a considerable amount of data on its chemistry, toxicology, environmental toxicology and fate. That makes it an attractive chemical to use in a feral pig management product because the regulatory pathway to approving it as a new agricultural chemical is less expensive as many of the required studies are already published. Sodium nitrite's mode of action (metabolic annoxia) is also accepted as humane. That acceptance stems from its clinical effect being similar to carbon monoxide poisoning and the unremarkable reports of pain or distress reported by people that have been exposed to high but sub-lethal doses of carbon monoxide.

# **Project Challenges**

Preliminary tests showed that SN could be lethally efficacious whether given by gavage or voluntarilly consumed in freshly made (in situ) PIGOUT baits. However, those results couldn't be replicated in baits that were more than a couple of days old due to SN instability in different bait substrates that made them unattractive and unpalabtable. SN's propensity to react with almost any substrate, and its highly salty taste neccessitated it being encapsulated so that it wasn't detectable by pigs in bait substrates and so that manufactured bait remained shelf-stable for a commercially relevant period. Macroencapsulation of SN

into encased pellets approximately 4-5mm in diameter was ineffective as pigs detected these in the bait substrate and spat out a majority of them during mastication of the bait. To overcome that obstacle the project turned to microencapsulation so that the encased SN was granule sized. This solved the initial issue, but more than 20 different microencapsulated prototypes were assessed in pen studies and pilot scale field studies before the project determined the best combination of coating material and the optimal coating thickness of formulated SN that would be stable and bioactive in a bait. This combination is proprietary to the project IP licensee and commercialiser of HOGGONE – Animal Control Technologies.

Formulating an optimal bait matrix was also a critical factor in the targetting of feral pigs and efficacy of HOGGONE. Initial studies used the PIGOUT matrix, which ultimately proved incompatible with microencapsulated SN (meSN). At this juncture of the project testing facilities in Queensland (Inglewood) were mothballed and were no longer available to the project. To circumvent this obstacle wild captured pigs were used as test subjects, but these freshly trapped animals often behaved aberantly and this approach was abandoned in favour of taking advantage of testing facilities and in-kind inputs from Texas Parks and Wildlife Department and the United States Department of Agriculture that the project gained access to via the related project (HOGGONE USA). Attractiveness and palatability testing of initial reformulated bait matrices also proved incompatible with the pre-formulated meSN. To overcome the technical issues the HOGGONE bait matrix prototype was modified from individual 70gram-250gram semihard baits to a paste format (penut butter consistency). This bait matrix is proprietary to the project IP licensee and commercialiser of HOGGONE – Animal Control Technologies.

# **Project Outputs**

The project proposal was to:

- 1. develop and register an effective and humane chemical that could be added to the PIGOUT bait matrix for enhanced feral pig management in Australia, and
- 2. if needed re-formulate the PIGOUT bait matrix to be compatible with the new chemical as well as selectively more attractive to feral pigs than the majority of non-targets, and could be trademarked and or patent protected as HOGGONE<sup>®</sup>

Both of these outputs have now been delivered by the project and a pictorial narrative of HOGGONE<sup>®</sup>'s development, final testing and registration application process is included in Appendix I.

# Project Outcome

By delivering the project outputs the outcome of the project will be a new tool for private and public land managers to incorporate into integrated feral pig management programs. This will mean that end users can tailor management programs so they use a combination of products that reduce the risk to non-target species, or a lack of participation due to personal preferences about chemical use. Ultimately this will enhance the Australian public's perception and the optics globally around Australia's stewardship and responsible use of chemicals on farming land used to grow healthy food. That can be used by Australian red meat production sectors to enhance our international reputation for growing environmentally friendly healthy food for global markets that will benefit the profitability of Australian red meat producers.

# 2 Project objectives

# 2.1 Development and Registration of HOGGONE® in Australia

# 2.1.1 Development of HOGGONE

HOGGONE<sup>®</sup> was originally intended to be a copy of the PIGOUT bait matrix containing the active ingredient identified during the achiles heel analysis - sodium nitrite. When the matrix and active ingredient proved incompatible the bait matrix formulation required a complete rework to produce a shelf-stable, attractive, palatable, and effective bait. That process, ultimately, required many (over 20) iterative improvements to the pre-formulation of sodium nitrite, and or the bait matrix, and each change needed to be tested for attractiveness and palatability using feral pigs, either in pens, or free ranging. That process resulted in the development of HOGGONE<sup>®</sup>.

# 2.1.2 Assessment of the final product prototype of HOGGONE®

Once HOGGONE<sup>®</sup> was developed and proof-of-concept was demonstrated in pilot scale studies the project objective changed from developing a product to assessing the final product prototype using regulatory compliant studies. These included studies on the sodium nitrite technical active as well as studies on the end use product (EUP). Those studies assessed their Physical Chemistry, Toxicology, and Ecological Effects for inclusion into both the APVMA and US EPA new product applications.

# 2.1.3 Submission of the APVMA registration application for HOGGONE®

The final project objective was to use the data from the regulatory compliant studies of sodium nitrite and HOGGONE<sup>®</sup> to compile a complete registration application for a new product and submit that to the APVMA in Australia.

Although not a milestone for this project, the submission of a new product application to the US EPA will also be made that will include data from this project.

# 3 Methodology

# 3.1 Overview

The project design and methods used were similar to the successful development and registration of PIGOUT<sup>®</sup>, but because sodium nitrite was not an approved agricultural chemical (as 1080 was for PIGOUT<sup>®</sup>, there was a significant investment made to assess the humaneness of sodium nitrite's mode of action, as well as to complete other regulatory studies required to register sodium nitrite as a new agricultural chemical and HOGGONE<sup>®</sup> as a new agricultural product.

The project included the following 9 Programs of work:

- development of a bait dose that best balances lethality, target-specificity, humaneness, operator safety, and manufacturing issues by exposing feral pigs to various doses and bait formulations (Assessing the attractiveness, palatability, and efficacy of product prototypes);
- an independent humanness assessment by the Institute of Medical and Veterinary Science (funded by DEWHA grant – Appendix II) <u>http://www.environment.gov.au/system/files/resources/091b0583-f35c-40b3-a530-f2e0c307a20c/files/pigs-imvs-report.pdf</u>);
- native non-target species desktop risk analysis, and empirical testing of sodium nitrite oral toxicity to possums and wallabies (Landcare Research and Connovation P/L respectively in New Zealand (funded by DEWHA Appendix III)
  <a href="http://www.environment.gov.au/system/files/resources/4ba0fe0d-af54-46b4-8289-42d6ed16f30a/files/sodium-nitrite-risk-assessment.pdf">http://www.environment.gov.au/system/files/resources/4ba0fe0d-af54-46b4-8289-42d6ed16f30a/files/sodium-nitrite-risk-assessment.pdf</a>);
- 4. population level efficacy ground baiting studies assessing the final HOGGONE<sup>®</sup> product;
- 5. an assessment of toxin residue levels in field poisoned feral pigs to determine secondary poisoning hazards (This report is commercial in confidence, but is provided on that basis and should not be publicly disclosed Appendix IV);
- 6. article-of-commerce regulatory studies;
- 7. the preparation and submission of an Australian Pesticide and Veterinary Medicine product registration package;
- 8. the preparation of results for popular articles, scientific publications (where possible) and international promotion; and
- 9. the preparation of product extension information, promotional material and advertising.

In this section the methods used for Program 1 through 6 will be detailed. Methods for Programs 7, 8, and 9 are detailed by the APVMA, relevant scientific publishers, and are proprietary to the commercialiser of HOGGONE<sup>®</sup>.

# **3.2** Program 1: Assessing the attractiveness, palatability and efficacy of product prototypes

# **3.2.1** Procedure for assessing the attractiveness, palatability and efficacy of product prototypes in pen studies

All pen studies assessing product prototypes during the development phase of the project were conducted at the Robert Wicks Research Station (RWRS - 5206 Millmerran Inglewood Rd Inglewood, Qld 4387). The RWRS was mothballed in 2012 and ultimately sold by the QLD state government in 2016. Pen studies at this facility were conducted until 2012, after which, the project had to change testing methods to account for the use of trapped feral pigs or pilot-scale field study sites using free-ranging feral pigs (see 3.2.2 and 3.2.3 sections below).

Pen studies used trapped wild pigs that were transported from the point of capture to the RWRS and acclimated for at least 7 days in a large 10acre pen containing native vegetation and man-made watering points. For each test the following procedure was used;

- 1. animals were drafted off into individual pens (see below), and acclimated onto placebo bait substrates over several days.
- 2. Once animals were consuming placebo bait substrate they were provided with test baits to assess each bait type attractiveness, palatability, and effectiveness if consumed.
- 3. Bait aversion, consumption or not and endpoint (death or survival) were all recorded.



# **3.2.2** Procedure for assessing the attractiveness, palatability and efficacy of product prototypes in large enclosure studies: Water point traps

These studies were all undertaken on two large wheat and sheep stations ('Yara' [Figure 2a] and 'Kilparney' [Figure 2b]) near Mount Hope in south-central New South Wales, Australia (Fig. 1). Both properties contained large areas of old growth Mallee scrub and a number of other woodland habitats that are common throughout south-central New South Wales including white cypress pine (*Callitris glaucophylla*), brimble box (*E. populnea*), black box (*E. largiflorens*) and belah (*Casuarina cristata*). Average March rainfall and daily maximum temperatures for the area are 36.5 mm and 30.2°C, respectively.



**Figure 1** – Approximate trial site location in western NSW.



Figure 2a – Yara station, with property boundary shown in orange. Other symbols represent the approximate locations of the water-point traps.



Figure 2b – Kilparney station, with property boundary shown in orange. Other symbols represent the approximate locations of the water-point traps.

# Product (For example)

**Placebo HOGGONE® Concentrate**: is a non-toxic peanut flavoured slurry. 1.25 kg of placebo HOGGONE® slurry was packaged in 1.5 litre polypropylene sealed containers. The contents of each container was mixed to homogeneity with 3.75kg of dry wheat and 1kg/pig was deployed at each bait station. Placebo peanut flavoured slurry was prepared and supplied by ACTA. Wheat grain was supplied by the landholders and mixing was undertaken in the field.

**Toxic HOGGONE® Concentrate (Batch# 16.03.01, DOM 01.03.16):** 1.25kg of slurry containing meSN 40% w/w was packaged in 1.5 litre polypropylene sealed containers. The contents of each container was mixed with 3.75 kg of dry wheat, resulting in a concentration of 10 % w/w a.i. Toxic bait (~1 kg per pig) was prepared and deployed at each bait station. Toxic HOGGONE® concentrate slurry was prepared and supplied by ACTA. Wheat grain was supplied by the landholders and mixing was undertaken in the field.

# Procedure

# <u>Sites</u>

Six water-point traps (bait sites) were used including three on Yara and three Kilparney (Figures 2a and 2b). The water-point traps were previously installed as part of another project to help manage feral goats and feral pigs on each property. The traps also provided an ideal opportunity to test bait products on closed feral pig populations, in the environment they are familiar with. The water-point traps are permanent, although they are left open throughout the year to allow animals to visit, drink and leave *at libitum*. The traps are approximately 1 hectare in size and they provide shade, water and a small amount of food (Fig. 3) and is as natural a state as possible under which to test feral pig behaviour toward test substances.



Figure 3 – Feral pigs and feral goats captured in a water-point trap.

Trapping was undertaken as the three stages, and they were:

1. "Open mode" - doors wired open to allow feral pigs to come and go from the trap.



2. "Training mode" - training doors (swing both ways) were lowered to enable animals to come and go from the trap, although they must push through a door to do so. Training doors are light to allow pigs to push in and out with little effort.



3. "Trapping mode" – training doors are removed and a one-way swing door put in position. This enables animals to enter but prevents them from leaving. The door is assisted by a gas strut to reduce its weight, and this door is reinforced to reduce damage caused by feral pigs.



Importantly, the trap doors were wired shut when sufficient animals were captured, or when animals were no-longer being captured, to prevent new animals from entering the trap during the study.

# <u>Baiting</u>

In the beginning, plain wheat was poured onto the ground (~ 1 kg per pig) in up to 3 parallel trails at each station (Fig. 4a). A Reconyx Hyperfire HC600 remote camera was also installed and they were placed 5 metres from the bait trails, and fixed to a tree at waist height. In addition, cameras were programmed to take one picture per trigger with no time delay. Bait stations were checked daily to record bait-take and to replace bait. Wheat mixed with placebo HOGGONE<sup>®</sup> concentrate slurry was deployed at each bait station once feral pigs consumed  $\geq$  80 % of the plain grain. During this stage, we provided approximately 1kg of placebo bait material per pig (Fig. 4b). Toxic HOGGONE<sup>®</sup> concentrate slurry treated grain (10% w/w a.i.) was deployed, as per the placebo slurry grain procedure (Fig. 4c), when  $\geq$  80% of the placebo slurry had been consumed on two consecutive nights.



Figure 4a – plain wheat.



Figure 4b – wheat mixed with placebo HOGGONE<sup>®</sup> slurry.



Figure 4c – wheat mixed with toxic HOGGONE<sup>®</sup> slurry.

# Monitoring

Bait uptake – all bait stations were checked daily to record bait-take, and to replace any bait that was consumed. The amount of wheat that was consumed was visually estimated, in kilograms. Remote camera images - where used to gather two data sets that were ultimately used to measure activity at bait stations, they included:

### Number of photos

The total number of images with feral pigs present during each 15 minute interval over 24 hours.

### Number of feral pigs

Maximum number of feral pigs present in a single image, within each 15 minute interval over the 24 hours.



Figure 5 - Feral pigs feeding on wheat mixed with placebo HOGGONE® slurry.

Carcass and vomit searches – were undertaken every morning during poison baiting, where researchers systematically searched each pen for carcasses and vomit. If any were found their details were recorded, and a photograph was taken (Fig. 6).



Figure 6 – Dead feral pig found during carcass search.

# **3.2.3** Procedure for assessing the attractiveness, palatability and efficacy of product prototypes in pilot scale field studies

Trials were undertaken within SE Queensland (Dirrinbandi/Tallwood/St George regions). The methods used to assess the attractiveness, palatability, and to a degree the efficacy of bait prototypes are described below. The sites used to assess these measures were within a 200km radius of one another and were selected because there was a known high density of feral pigs. The method below is specific to a private property, which is situated on the Culgoa River in south-central Queensland. The property is largely used for cropping and beef cattle production. It contains areas of dense remnant vegetation and cleared cropping areas (Fig. 1). In addition, the study site adjoins Cubbie Station, which is the largest irrigated cotton farm in the Murray-Darling basin. The region has experienced a prolonged dry season and thus alternate foods, and surface water, was scarce throughout the trial. The average maximum daily temperature was approximately 30°C.



Figure 1 – map of HOGGONE bait stations used on Oakey Park during the trial.

**Free-feed grain**: stale wheat was the primary free-feed grain used during the trial. Carasweet<sup>®</sup> and molasses were added to enhance site discovery and visitations by feral pigs.

**Procedure:** At the beginning of the trial, each potential bait station site was assessed for recent feral pig activity (tracks, scats and rooting). If fresh activity was found, a bait station was created and a motion sensing camera was installed (Reconyx PC85 or HC600) see Fig. 1 for bait station locations (n=11).

Trials always consisted of four stages:

Stage 1 – approximately 60kg of free-feed grain was deployed in one pile at each site for 6 – 7 days.

Stage 2 – two piles of 30 (n=60) non-toxic HOGGONE<sup>®</sup> baits were deployed at each site for two nights with a small amount of free-feed grain.

*Stage 3* - two piles of 30 (n=60) non-toxic HOGGONE<sup>®</sup> baits were deployed at each site for three nights without free-feed grain.

*Stage 4* - one pile of 30 non-toxic HOGGONE® baits and one pile of toxic HOGGONE baits containing DAFTA5 were deployed at each site for three consecutive nights without free-feed grain (Fig. 2).



**Figure 2** – image of stage 4 poison baiting site set up. A series of wooden stakes were positioned in the ground to differentiate between non-toxic HOGGONE bait (F) and toxic HOGGONE bait (T) piles.

Photographs taken by the remote cameras at the bait stations were analysed for the total number of feral pigs present per site per night; non-target species were also recorded. Bait-uptake was also recorded on standardised data sheets daily (see appendix 1). In addition, carcass searches spanning ~500m from each bait station were undertaken during poison baiting. If a carcass was discovered, a waypoint was recorded and a photograph was taken.

# 3.3 Program 2: Humaneness assessment

# **Study Design**

Adult pigs 30-50 Kg in mass were used, which represents the average weight of a feral pig population. Animals were fed nitrite-free baits for two to three days prior to the start of the study. All animals were anaesthetised and an arterial catheter was inserted for blood collection. The next day when the animals had recovered from surgery, baseline blood samples were taken (co-oximetry, clinical pathology). One or two HOGGONE<sup>®</sup> baits with 20 g of micro-encapsulated sodium nitrite spread throughout the matrix were placed in the pens of the treatment animals. The controls received the same amount of bait without the sodium nitrite. Animals were provided with water *ad libitum*. All animals were watched until they had eaten the bait.

The animals were closely monitored for clinical signs of distress, and physiological changes (respiration, haematology, biochemistry, cortisol and lactate and methaemoglobin levels) until death. Cortisol and lactate levels were assessed to monitor the internal stress response of the animals. Any animal still alive four hours post-consumption of bait would be humanely killed.

# 3.4 Program 3: Non-target risk assessment

# 3.4.1 Primary poisoning non-target risk assessment (see Appendix III for references)

### Pharmacokinetics and species variation.

The metabolism and pharmacokinetics of a vertebrate pesticide are often determinant factors in its ultimate manifestation of toxicity, non-target safety and residue profiles. The metabolic deactivation of toxins results from the actions of enzymes whose primary function is believed to be to protect the body against the accumulation and undesirable effects of foreign compounds naturally present in food and in the environment. Metabolism and excretion are protective processes attempting to limit persistence in the body.

Species differences in sensitivity to an individual toxicant may be linked to variation in the pharmacokinetic differences for the compound in different species. Savarie *et al.* (1983) clearly demonstrated this with PAPP, with a seventy-fold difference occurring between the LD<sub>50</sub> of a coyote and a Striped skunk or Golden eagle. Wood *et al.* (1991) later detailed that this was due to the metabolic activation of PAPP to a reactive, and more toxic, intermediate metabolite in susceptible species such as dogs. This is the case with 1080 and cholecalciferol. However, as is normal with toxic intermediates of other drugs or pesticides, metabolic processes in the body then go on to detoxify these intermediate compounds as well. In the case of 1080 and sodium nitrite the parent compound is itself highly water soluble; hence the normal metabolic processes that render a toxin more water soluble are less important with regard to their elimination from the body.

# Receptor site interactions and species susceptibility

Fortunately, the pharmacokinetics of the direct methaemoglobin (MtHb) former sodium nitrite is similar in different species as it does not require biotransformation to an active metabolite. As with the drug amrinone, there are rapid and similar patterns of absorption and excretion across a range of different species. Hence, extrapolation of chemical-receptor interactions can be used with greater confidence to predict the innate risk of sodium nitrite to non-target species. The receptor site for sodium nitrite poisoning is haemoglobin in the red blood cell. The mode of action of nitrite is the oxidization of the haem iron in red blood cells from the ferrous state (Fe2+) to the ferric state (Fe3+) to form MtHb. MtHb is incapable of carrying oxygen and cyanosis results, with death occurring if the dose is high enough (Egyed and Hanji 1987). The pattern of methaemoglobinaemic response induced when erythrocytes are exposed to sodium nitrite oxidant challenge will be a balance between MtHb formation and its subsequent reduction back to haemoglobin by the protective enzyme MtHb reductase.

The activity of the enzyme MtHb reductase varies in different animals, and is known to determine a species direct sensitivity to a direct methaemoglobin former (Smith and Beutler 1966; Stolk and Smith 1966; Agar and Harley 1972; Board *et al.* 1977; Chun-Lap Lo and Agar 1986; Whittington *et al.* 1995; Agar *et al.* 2000; Rockwood *et al.* 2003). Species differences in MtHb reductase are therefore critical when evaluating the risk to different species from MtHb formers such as sodium nitrite. Under normal conditions this enzyme is the only system within the erythrocyte that maintains haemoglobin in its oxygen-carrying reduced state. Toxicologically, MtHb reductase will therefore be the rate-limiting enzyme controlling the toxicodynamics of sodium nitrite's effect on the red blood cells. In theory, species with lower MtHb reductase activity convert MtHb back to haemoglobin more slowly than do species with higher activity, and will therefore be more susceptible to sodium nitrite. Conversely animals with greater MtHb reductase activity will be at less risk of sodium nitrite induced toxicity.

# Correlating methaemoglobin reductase activity, body size, diet and risk

To test this hypothesis we collated all published acute sodium nitrite toxicity data and MtHb reductase activity, as generally determined through nitrite challenge. Data for eight eutherian mammal species were obtained (Table 1). In those species for which published data exists on both acute toxicity and on red blood cell MtHb reductase activity there is a strong correlation between susceptibility and MtHb reductase activity (*r*=0.961). Simple linear regression analysis revealed a highly significant ( $F_{1,6}$ = 72.0;  $F_{0.001(1)1,6}$ = 35.5; P > 0.001) relationship, with a line of best fit accounting for 92% of the variance with the equation y=0.3777x-18.843 (Fig. 1).

| Species | Scientific name        | Lethal gavage dose<br>(mg/kg-1) | NADH metheamoglobin<br>reductase (IU/g Hb) | Reference               |
|---------|------------------------|---------------------------------|--|-------------------------|
| Pig     | Sus scrofa             | 90                              | 12   | Winks et al. 1950       |
| Human   | Homo sapien            | 94                              | 15   | Boink and Speijers 2001 |
| Rat     | Rattus norvegicus      | 104                             | 10   | Druckery et al. 1963    |
| Mouse   | Mus musculus           | 215                             | 53   | Rieman 1950             |
| Rabbit  | Oryctolagus cuniculus  | 250                             | 87   | Dollahite and Rowe 1974 |
| Dog     | Canis lupus familiaris | 60                              | 8.5  |                         |
| Sheep   | Ovis aries             | 50                              | 10   | Lewis 1950              |
| Cattle  | Bos taurus             | 67                              | 5  | Bartik and Pisac 1981   |

Table 1. Published sodium nitrite lethal gavage doses and NADH metheamoglobin reductase activities for eutherian mammals.

Figure 1. General linear regression between published sodium nitrite lethal gavage doses and NADH metheamoglobin reductase activities for eutherian mammals.



Underpinned by the comparatively straightforward nature of sodium nitrite pharmacokinetics extrapolation based on the regression analysis from individual species' MtHb reductase activity allows prediction of probable lethal doses in native Australian species. Further extrapolations are possible firstly to predict likely lethal doses of sodium nitrite in native Australian species. Secondly, based on animal size and eating habits, susceptible non-target species can be identified. The results of this modelling are summarised in Table 4 and identify susceptible non-target species that might eat sufficient toxic pig bait. Those are Common brushtail possum, Northern brown bandicoot, Tammar and Swamp wallabies, and dogs.

# Proofing model: direct non-target species testing

To examine the validity of the lethal dose predictions in Table 2, and to ascertain the direct risk to two key potential non-target species that have previously been observed to consume PIGOUT baits, direct toxicity testing on Common brushtail possums and Tammar wallabies was undertaken by Landcare Research (New Zealand) and Connovation (New Zealand) respectively. Trials occurred in New Zealand where both species are invasive and the use of sufficient animals received ethics approval.

Pen trials were undertaken on 12 individually caged brushtail possums at the Landcare Research Animal Facilities in Lincoln, New Zealand. Tammar wallaby trials were undertaken in outdoor pens in a new animal research facility at Rotorua, New Zealand. Additional non toxic trials were undertaken by the Tasmanian Department of Primary Industries and Water to assess the attractiveness of the HOG-GONE<sup>®</sup> baits to Bennett's wallaby (*Macropus rufogriseus*, n=5) and Tasmanian pademelon (*Thylogale billardierii*, n=17) held at the department's Launceston facilities. Twelve HOG-GONE<sup>®</sup> baits were placed in the wallabies 0.8ha enclosure and monitored using motion sensitive video camera continually for 5 nights. Although most wallabies investigated the baits at least once, they generally recoiled abruptly once smelling the bait material (Fish and Statham 2009). No wallabies showed any inclination to consume the baits.

Although low sample sizes for possums (n=5) and tammar wallabies (n=3), results from toxic trials supported the lethal dose predictions made in Table 2, and provide some validity to the model approach presented. Results from the overall trials do however clearly indicate that marsupials are generally repelled by the HOG-GONE<sup>®</sup> bait matrix itself or the smell of formulated sodium nitrite. Despite formulation, the nitrite smell is still present in the toxic matrix and the chemical is extremely salty to taste.

**3.4.2** Secondary poisoning canid predator/scavenger study: Snow, N. P., Foster, J. A., VanNatta, E. H., Horak, K. E., Humphrys, S. T., Staples, L. D., Hewitt, D. G. and VerCauteren, K. C. (2018), Potential secondary poisoning risks to non-targets from a sodium nitrite toxic bait for invasive wild pigs. Pest. Manag. Sci, 74: 181–188. doi:10.1002/ps.4692

http://onlinelibrary.wiley.com/doi/10.1002/ps.4692/abstract;jsessionid=1A6E1D6E85908017E8194F7 B3C45B1BB.f04t01

- 16 coyotes individually housed
- Fed 8 coyotes pig carcasses with HOGGONE®
- Fed 8 coyotes pig carcasses with placebo bait
- Monitored coyotes for symptoms of SN toxicity

# 3.4.3 Secondary poisoning avian scavenger study (2 Phases)

Phase 1 Gavage

- 30 Captive vultures in pens (dosed in groups of 5)
- Gavaged with micro-encapsulated SN in gelcap
- Started dosing with 75 mg/kg
- Ended dosing with 700 mg/kg

Phase 2 free feeding on poisoned carcass

- Groups of 5 vultures in pens
- Fed 3 groups pig carcasses with HOGGONE®
- Fed 1 group pig carcass dosed with placebo

# **3.5** Program 4: Population level ground baiting efficacy and non-target safety of HOGGONE<sup>®</sup>

Once proof of concept was satisfactorily demonstrated in water point traps and pilot scale field studies the project was ready to test the effectiveness of HOGGONE<sup>®</sup> in regulatory compliant studies in pens in the USA and if successful in those studies in large enclosure studies and a landscape scale population level ground baiting field trial in Australia.

### 3.5.1 Australian and USA collaborative studies (the value of the CRC model)

#### 3.5.1.1 USA pen studies

Testing occurred during October 2015 to June 2016. All captive wild pigs were group housed in a 0.02 km<sup>2</sup> (5 ac) outdoor holding pen at Kerr Wildlife Management Area (WMA) for ≥2 weeks prior to study initiation. The holding pen contained naturally-growing vegetation on the ground, trees, and shade structures. Wild pigs were maintained on Bluebonnet<sup>®</sup> 18% Sow Ration Pellet (AC Nutrition, LP, Ardmore, OK, USA) provided at 3–5% of group body mass daily. This maintenance diet had a recommended feeding rate of 3% of body weight for growing swine. Water was always provided *ad libitum* from self-maintaining water troughs. Water quality was tested (National Testing Laboratories, Ltd., Cleveland, OH) and no contaminants were detected above reference standards. The toxic HOGGONE<sup>®</sup> bait was manufactured on 02 October 2015 and stored indoors at ambient temperature and humidity after delivery to the Kerr WMA facilities until used.

Prior to each trial, wild pigs were moved into a sorting chute and 7 animals were randomly selected for each of 3 trial pens of 0.002 km<sup>2</sup> (0.5 ac) Random assignment to group was conducted under the following conditions: 1) sex ratio for each trial was 4:3 females to males, and 2) animal weights were between 20– 113 kg. Any animals weighing  $\geq$ 50 kg that were not deemed safe for handling (e.g., highly aggressive disposition) were excluded from study. After selection, wild pigs were moved into their respective pens for the trials. Daily temperatures during the study ranged from -3.8–32.2 °C and precipitation ranged from 0.0–4.0 cm.

All pens were outdoors and subject to natural climatic conditions. The pens contained naturally-growing vegetation on the ground, trees, and shade structures. Each pen was constructed with steel mesh fencing buried into the ground to hold wild pigs. Pens were immediately adjacent to each other so that all wild pigs experienced the same conditions. Each pen was identically equipped with 2 feeding troughs (approximately  $1.8 \times 0.3 \times 0.1$  m) that were fitted back to back, separated by a wire mesh panel, and covered with a structure to protect feed from direct precipitation. Food items were uniformly distributed along the length of the trough to allow feeding by multiple wild pigs at one time. The alternate food item,

rough rice (i.e., seed rice), was selected as the challenge diet for the 2-choice test portion of the study because wild pigs demonstrated a similar preference for rough rice as the placebo HOGGONE<sup>®</sup>.

# Study Design

Each replicate trial consisted of 3 pens containing 7 wild pigs per pen. A total of 4 replicate trials were conducted. The first 3 replicate trials were conducted while the HOGGONE<sup>®</sup> was 1–2 months post-manufacture. The fourth replicate trail was conducted approximately 8 months post-manufacture. For the first replicate trial, the pens were randomly assigned as the 2 toxicant pens and 1 control pen. The toxic bait and control treatments were then rotated in a randomized block design for the subsequent replicate trials of the study to control for any possible confounding effects from the individual pens.

Each replicate trial lasted 8 nights. During Nights 1 and 2, the wild pigs were allowed to acclimate to their new pens and fed their regular maintenance diet at a minimal maintenance ration of 1% of group body mass, equally split between the 2 troughs. During Nights 3–6, the wild pigs were pre-baited with the placebo bait at 1% of group body mass split equally between the 2 feeding troughs. During Night 7, the wild pigs were fed HOGGONE® toxic bait in the toxic-treated pens and placebo bait in the control pens at 1.74% of group body mass in a randomly assigned trough. In the opposite trough, the challenge diet was offered at 1.74% of group body mass (i.e., 2-choice trial period). During Night 8, surviving wild pigs were fed identical rations to Night 7 (i.e., 1.74% of group body mass) except the baits and challenge diets were switched to being offered in the opposite troughs to account for any possible confounding effects of individual troughs. Finally, any wild pigs that survived Nights 7 and 8 were humanely euthanized on Day 9. All pens were left vacant for ≥7 days between replicate trials to allow any residual scents and bait to dissipate naturally. For the fourth replicate, (8 month old bait) the prebaiting with placebo period was shortened 1 day to avoid testing during inclement weather.

# Observations

The wild pigs were fed each evening approximately 30 minutes before sunset, and checked the next morning approximately 30 minutes after sunrise. The amount of food consumed during the night was recorded by removing and weighing any remaining food in the troughs and dropped food in the immediate area during the morning check. We calculated the average amount of consumption per individual by dividing the total amount consumed by the number of wild pigs in each pen. During morning checks we recorded the number of wild pigs that were dead in each pen. Post-trial weights and age classification of wild pigs via tooth eruption were recorded for each animal, except during the fourth replicate. Sub-adults were considered as >2 months and <1 year, and adults were >1 year.

For the first 3 replicates, we used motion-activated cameras (Reconyx PC900, Holmen, WI, USA) to record feeding events and behaviour at each trough during Nights 6–8 for each trial. This allowed examination of feeding during the last night of pre-baiting and during the 2-choice nights. Cameras were mounted ~3 m away from troughs and ~1 m high on steel T-posts. We set the cameras to record 30 picture bursts at 2-second intervals per motion-activated trigger, without a delay between bursts if triggered. We examined each image using the Colorado Parks and Wildlife Photo Database (v3.0) for image processing. Individual animals were identified by ear tags or natural characteristics. We recorded which animals had their head directly above or in the trough to indicate feeding. We recorded the feeding times and durations for each

individual. We considered independent feeding bouts for each animal as feeding events separated by  $\geq$  30 minutes of non-feeding activity.

We subsampled the HOGGONE<sup>®</sup> toxic bait used in the first 3 replicate trials to ensure consistent concentrations of SN throughout the bait. For each replicate, we extracted 3, approximately 10 g samples stratified from within 1 bucket of HOGGONE<sup>®</sup> including the top, middle, and bottom portions (i.e., 9 samples total). We also extracted 1 sample from the control bait during each replicate to ensure the placebo bait did not contain SN. The samples were shipped to the USDA, National Wildlife Research Center Chemistry Unit for analysis of the concentration of SN using Method 180A, a validated enforcement analytical method for HOGGONE<sup>®</sup> using reverse-phase-ion-chromatography. This method was validated using samples containing 1% to 15% SN. The efficiency of recovery for SN averaged 92% (SD = 2.4%) and the method limit of detection was 0.00036%.

# **Statistical Analyses**

We compared the proportion of bait and challenge diets consumed between the treatment and control groups using a multivariate generalized linear mixed-effects model. The pre- and post-trial weights of groups were compared using a linear mixed-effects model. We used a similar model to examine whether the proportion of HOGGONE<sup>®</sup> bait consumed was influenced by group body mass during the first night offered. A similar analysis for the second night was not conducted because of a reduction in sample size in the HOGGONE<sup>®</sup> treatment group. For all mixed-effects models, we treated pens and treatment nights (first and second) as random effects.

A simple computation of the proportion of wild pigs (all individuals, males, females) that died across the two nights in the HOGGONE<sup>®</sup> treatment group was the metric of efficacy for HOGGONE<sup>®</sup>. For the HOGGONE<sup>®</sup> treatment group, we compared its efficacy for males compared to females using a two-tailed Fisher's Exact Test. We also compared mortality rates over the duration of the 2-choice trials between the toxic bait and control treatment groups as a whole and for females and males separately using two-tailed Fisher's Exact Tests.

From the camera data, we compared the number and length of feeding bouts between treatment- and control animals, and between the challenge diet and HOGGONE<sup>®</sup> or placebo bait, using multivariate generalized linear mixed-effects models. Within the HOGGONE<sup>®</sup> treatment group, we also compared the length and number of feeding bouts between wild pigs that died and did not die using multivariate generalized linear mixed-effects models. Again, we treated pens and treatment nights (first and second) as random effects for all mixed-effects models.

The mixed-effects models were performed using package lme4 in Program R (v3.1.1; R Development Core Team). Fisher's Exact Tests were also performed using Program R. For all statistical tests, we considered significant differences at the level of  $\alpha$  = 0.05 or where 95% confidence intervals for parameter (fixed effect) estimates did not overlap zero.

# 3.5.1.2 Australian large enclosure studies: Water point traps

# (see section 3.2.2 for site and pig trapping procedure)

Once the feral pigs were captured, baiting during the main trial was undertaken in three phases, they were:

 Phase 1 – we deployed three parallel trails of grain (20 kg total) at each bait station in each trap. The piles of grain were spaced 1 meter apart to prevent dominant animals from monopolising the bait material (Fig. 1). Phase 1 baiting was undertaken until >80% bait consumption was achieved.



**Figure 1** – Phase 1 bait configuration.

Phase 2 – we placed a minimum of 1kg of placebo HOGGONE<sup>®</sup> feral pig bait at each bait station. The paste trays were spaced > 1 meter apart to prevent dominant animals from monopolising the bait material. A small amount of grain (2.5 kg per tray) was also over the trays to help transition the feral pigs onto the HOGGONE<sup>®</sup> feral pig bait (Fig. 2). Phase 2 baiting continued until > 80% of the placebo HOGGONE<sup>®</sup> feral pig bait had been consumed on two consecutive nights. We did not deploy grain on the final night of phase 2 baiting to ensure the animals would consume HOGGONE<sup>®</sup> feral pig bait on its own before moving on to phase 3.



**Figure 2** – Phase 2 bait configuration.

Phase 3 (toxic) – we provided a minimum of 500g of toxic HOGGONE<sup>®</sup> feral pig bait per at bait stations during phase three baiting (Fig. 3). Grain was not deployed with the toxic HOGGONE<sup>®</sup> feral pig bait. Phase 3 baiting was undertaken for two nights irrespective of bait consumption. Feral pigs that were alive at the end of phase three were euthanased via rifle shot.



**Figure 3** – Phase 3 bait configuration.

*Bait station monitoring* – we installed remote cameras (Reconyx HC600) at all bait stations to determine the proportion of known animals in the pen that visited each station, per day (12 noon until 12 noon the following day). All cameras were positioned approximately 5 metres from the bait and they were fixed to a tree at waist height. Cameras were programmed to take 3 pictures per trigger with a 1-minute time delay. We identified individuals using coat patches, colour, sex and relative size, where possible (Fig. 4).



**Figure 4** – Feral pigs feeding on poison bait at a bait station in the pen.

We also checked the bait stations daily to record bait-uptake, and to replace any bait that had been consumed. Bait-uptake was measured by weighing the HOGGONE<sup>®</sup> past trays with a set of digital field scales each day, except during phase 1 baiting where bait-uptake was estimated proportion by volume as it was too difficult to accurately measure ground deployed grain. We expressed bait uptake as the proportion of bait consumed per bait station, per night.

*Carcass and vomit searches* – we implemented ground searches for feral pig and non-target species carcasses (and vomit) every morning post-toxic bait deployment. We took a photograph and a GPS waypoint whenever a feral pig or non-target species carcass (or vomit) was found, and sprayed the processed animals with marking paint (Fig. 5). We also recorded the weight (visual estimate), gender, reproductive status and condition of each animal. All dead pigs will be left to decompose.



Figure 5 – Example of a processed animal.

# Data

We investigated the number of feral pigs killed with toxic HOGGONE<sup>®</sup> feral pig bait compared to the number present, as a direct measure of HOGGONE<sup>®</sup> efficacy.

# 3.5.1.3 Australian landscape scale population level field study

# Study site.

The trial was undertaken on Burwah Station (treatment) and Maroota Station (control) near St. George in southern Queensland (28° 01' 48" S, 148° 34' 12" E). St. George is located on the Balonne River approximately 450 kilometres west of Brisbane, Queensland (Fig. 1). The mean maximum daily temperature at the time for the trial for the region is 32°C and the mean monthly rainfall is 50mm (Bureau of Meteorology 2016).



Figure 1 – Trial site location in south-west Queensland.

Product Placebo HOGGONE<sup>®</sup> paste: Batch Number: 16.07/01 Date of Manufacture: 08/07/2016

### Toxic HOGGONE® paste:

Batch Number: 16.07/01 Date of Manufacture: 11/07/2016

Toxic bait is stabilised peanut and grain paste containing 10% w/w sodium nitrite active ingredient added in the form of fluid bed coated microspheres prepared at 5% protein coating (pre-prepared at GEA in Switzerland batch "A" ACTA UIN 518, prepared March 2016). Packaged into plastic trays, nitrogen purged and sealed with barrier film; 2.5 kg per tray. As baits were manufactured in July 2016 and the trial tests will be conducted in November 2016, the bait will be approximately four months old.

#### Procedure

#### **Pre-trial**

GPS Collaring – we trapped, fitted and released feral pigs of mixed ages, sexes and sizes with Lotek Iriduim GPS Collars from the treatment area (Burwah Station) two weeks prior to the main trial to measure knockdown during poison baiting. We allowed a week "cool-off" period between collaring and baiting to enable animals to revert back to their normal daily activity patterns before baiting commenced. During the collaring process, we trapped feral pigs with swing door panel traps and wheat mixed with Carasweet<sup>®</sup>. The traps were positioned at known feral pig hotspots in the treatment area, and they were spaced relatively evenly across the site to ensure that different groups of feral pigs (sounders) were captured. Collars were fitted to at least one animal from each different sounder, rather than all animals from one sounder, to help determine feral pig population knockdown at a property scale. Once trapped, feral pigs that were deemed "fit" (good physical health and large enough to accommodate the collars [collar weight of 100-130 gm not exceeding 5% of animal's body weight]) were sedated with Zoletil. They were then fitted with an identifiable GPS/radio collar and released. They were monitored after their release to ensure they had fully recovered from the anaesthetic. Collars were programmed to take half hourly fix attempts, with an Iridium upload after 12 fixes; transferred 4 times per day. A mortality alert signal was also transmitted if an animal was inactive for an extended period, and thus enabling us to determine when an animal had either slipped a collar or been killed. The VHF beacon is on a sunrise to sunset schedule. The geographic coordinate system being used for the collars was WGS84.

| Date       | Collar<br>Freq. | Pig ID | Weight | Gender | Rep. Status                 | Condition |
|------------|-----------------|--------|--------|--------|-----------------------------|-----------|
| 31/08/2016 | 150.060         | 42129  | 30     | Male   | weaner                      | good      |
| 31/08/2016 | 150.160         | 42134  | 45     | Male   | Adult                       | good      |
| 31/08/2016 | 150.140         | 42133  | 42     | Female | Adult - preg                | good      |
| 31/08/2016 | 150.100         | 42131  | 38     | Female | Adult - preg                | good      |
| 31/08/2016 | 150.040         | 42128  | 35     | Female | Adult -<br>lactating        | skinny    |
| 16/09/2016 | 150.060         | 42129  | 60     | Female | Adult - preg                | good      |
| 16/09/2016 | 150.020         | 42127  | 40     | Male   | Adult                       | good      |
| 16/09/2016 | 150.000         | 42126  | 60     | Male   | Adult                       | good      |
| 16/09/2016 | 150.080         | 42130  | 65     | Male   | Adult                       | good      |
| 16/09/2016 | 150.180         | 42135  | 70     | Male   | Adult                       | good      |
| 21/09/2016 | 150.120         | 42132  | 60     | Male   | Adult                       | good      |
| 21/09/2016 | 150.100         | 42131  | 50     | Female | Adult - Just<br>had piglets | skinny    |
| 21/09/2016 | 150.160         | 42134  | 50     | Female | Adult - preg                | good      |

Table 1 – Details of collared feral pigs.

Slipped collars were collected and re-deployed in the study



**Figure 2** – A feral pig with the GPS collar attached. This animal was recaptured with another sounder, at another site, after its collar had already been fitted.

We calculated knockdown of collared animals as the proportion of collard animals killed in the treatment area during poison baiting, compared to the number of collared animals present at poisoned stations. Individuals were excluded from the analysis if they did not visit a bait station during poisoning (confirmed via remote camera images).

Landscape activity monitoring – we used remote cameras (Reconyx HC600) to gather an index of feral pig activity within the treatment and control area before and after poison baiting. All cameras were positioned on game trails one month prior to the main trial and they were left in the field for approximately one month after. We installed 15 landscape activity monitoring cameras in the treatment area (Fig. 3) and 7 activity monitoring cameras in the control area (Fig. 4). Fewer cameras were positioned in the control area, as the site was smaller and the number of cameras per site was relative to their size.



Figure 3 – Approximate landscape camera locations in the treatment area (Burwah).



Figure 4 – Approximate landscape camera locations in the control area (Maroota).

We spaced all landscape cameras ≥1km apart to reduce the likelihood of the same animal passing multiple cameras on a single night, and to gather a more accurate representation of activity at the property scale. Landscape cameras were programmed to take 1 picture per trigger with no time delay. They were also fixed to a tree (or post) 70cm above ground level and within 1 – 2 meters to the side of the game trail. The cameras faced across and down the trail at approximately 22 degrees, rather than at the trail directly (Fig. 5). This provided more opportunity to capture an image of the animal before they pass the camera. If the camera is pointed directly at the trail, at a right angle, the camera can be triggered and the animal can pass before an image is taken.



Figure 5 – Camera being installed on fresh game trail.

We obtained an index of activity by counting the total number of feral pigs per image per camera, per day (12 midnight to 12 midnight [24hrs]) and dividing the total by the total number of images containing feral pigs per camera, per day. This was expressed as the rate of pigs per image, per camera, per site. For example, in the below series of images we count a total of 21 feral pigs in three images of feral pigs, and thus giving us a rate of 7 pigs per image.



Figure 6a, b, c – A series of images taken of feral pigs passing a landscape activity monitoring camera. Main Trial

*Baiting* – We deployed ~20 kg of pre-feed grain at all feral pig hotspots (fresh and regular feral pig activity) in each property, one week prior to the main trial. Hotspots/bait stations were spaced > 1km apart to minimise the chances of the same animals feeding from multiple bait stations on any given night. These sites were checked every second day until the main trial commence; pre-feed grain was replaced if it had been consumed.

Baiting during the main trial was undertaken in four phases, they were:

Phase 1 – we deployed four piles of grain (~10 kg each) at each bait station and the piles of grain were spaced ~1.5 meters apart to prevent dominant animals from monopolising the bait material (Fig. 7). Phase 1 baiting was undertaken until >80% bait consumption was achieved on two consecutive nights. Bait stations that were inactive upon the completion of phase 1 were discarded thereafter.


**Figure 7** – Phase 1 bait configuration.

Phase 2 – we placed four trays (2.5 kg per tray) of placebo HOGGONE<sup>®</sup> feral pig bait at each bait station, as per phase 1 bait configuration. We also poured a small amount of grain (~5 kg per tray) over each paste tray to help transition the feral pigs onto the HOGGONE<sup>®</sup> feral pig bait (Fig. 8). Phase 2 baiting continued until > 80% of the placebo HOGGONE<sup>®</sup> feral pig bait had been consumed on two consecutive nights. We did not deploy grain on the final night of phase 2 baiting to ensure the animals would consume HOGGONE<sup>®</sup> feral pig bait on its own before moving on to phase 3.



Figure 8 – Phase 2 bait configuration.

*Phase 3 (toxic)* – we provided a minimum of 500g of toxic HOGGONE<sup>®</sup> feral pig bait per feral pig at bait stations in the treatment area (confirmed via remote cameras), and placebo HOGGONE<sup>®</sup> feral pig bait at bait stations in the nil-treatment area. Phase 3 baiting was undertaken for two nights irrespective of bait consumption.



Figure 9 – Phase 3 bait configuration.

**Phase 4** – we placed four trays of placebo HOGGONE<sup>®</sup> feral pig bait at all sites in both the treatment area and the control area (Fig. 10). Bait stations were not checked daily thereafter, but they were monitored continuously with remote cameras.



Figure 10 – Phase 3 bait configuration.

*Bait station monitoring* – we installed remote cameras (Reconyx HC600) at all bait stations to generate an index of feral pig activity before and after poison baiting. All cameras were positioned approximately 5 metres

from the bait and were fixed to a tree at waist height. Cameras were programed to take 3 pictures per trigger with a 1-minute time delay. To generate the activity index, we tallied the maximum number of identifiable individuals per station, per day (12 noon until 12 noon the following day). We identified individuals using coat patches, colour, sex, relative size and mob affinity, where possible.



Figure 11 – Feral pigs feeding on poison bait at a bait station in the treatment area.

We also checked the bait stations daily during the main trial to record bait-uptake, and to replace any bait that had been consumed. Bait-uptake was measured by weighing the HOGGONE<sup>®</sup> past trays with a set of digital scales each day, except during phase 1 baiting where bait-uptake was estimated proportion by volume as it was too difficult to accurately measure ground deployed grain. We expressed bait uptake as the proportion of bait consumed per bait station, per night.

*Carcass and vomit searches* – we implemented ground searches for feral pig and non-target species carcasses (and vomit) every morning during toxic baiting. We took a photograph and a GPS waypoint whenever feral pig or non-target species carcass (or vomit) was found. We also recorded the weight, gender, reproductive status and condition of each animal. Aerial searches were also undertaken, using an R22 helicopter, after the second night of baiting. During this time, we circled each bait station several times (to a radius of ~500m) to observe any carcasses that may have been missed with the ground searches (Fig. 12). We recorded a GPS waypoint whenever a carcass was sighted and later returned those carcasses on foot using the GPS coordinates to record their details (as above). All dead pigs will be left to decompose.



Figure 12 – Aircraft used for aerial surveys.

### **Data Analysis**

We used linear mixed models to examine the interaction of treatment (i.e., toxic or control) and period (preor post-treatment), including the main effects, on three indices of efficacy for HOGGONE<sup>®</sup> on the population of pigs using package lme4 (Bates et al. 2015) in Program R (v3.3.3, R Foundation for Statistical Computing, Vienna, Austria). For each model, we considered each baiting or camera site as a random grouping variable. For the first index, we examined the effect of the interaction on the number of individual pigs that were counted for two days pre- and post-baiting at the baiting sites. Data from two days before and after baiting were used as this is when feral pig numbers had plateaued prior to poison baiting, and thus allowing for a more accurate pre- and post-toxic baiting comparison. For the second index, we examined the proportion of bait consumed for one day pre- and post-baiting at each baiting site. One day before and after poison baiting was used because the placebo HOGGONE® paste that was placed at all bait stations on the final night of the trial was not replaced. Finally, for the third index, we examined the number of pigs viewed per image at independent camera sites for three days pre-free-feed being deployed and three days post-free-feed being deployed post-toxic baiting, ie activity when there is no bait (non-toxic or toxic) on either property. Three nights before and after were chosen to minimize the impacts of emigration and immigration from/to the area outside the study area (area/distance around our bait sites from which we attracted pigs, "radius of attraction". For each analysis, we examined the parameter estimates ( $\beta$ ) and 95% confidence intervals (CIs) of the interaction for a lack of overlap of 0 to indicate statistical and biological influences from the deployment of HOGGONE® on the population of pigs.

## 3.6 Program 5: Tissues residues assessment

A SymbioAlliance (Brisbane, QLD, AUS) in-house analytical method for nitrite determination was validated for the determination of sodium nitrite concentrations in tissues from feral pigs. Tissue samples were collected in situ within 18hrs of feral pig death and sent to SymbioAlliance (QLD) for analysis. Details of the sample analysis are contained in Appendix IV and are 'Commercial in Confidence' and should not be publicly disclosed.

## 3.7 Program 6: APVMA/EPA regulatory studies required for registration application

Studies required by the APVMA as well as the US EPA were paid for or significantly subsidised by the project (IAL). Studies that are not required by the APVMA, but were required by the US EPA were paid for by the NWRC, and or ACTA. The studies are listed in the below tables and methods for each can be found on the USA EPA or OECD websites under the listed Guideline numbers in columns 1/2.

#### Product Chemistry

| EPA OPPTS | OECD      |   |      |          |                  |
|-----------|-----------|---|------|----------|------------------|
| Guideline | Guideline | Study Title                                     | Cost | Estimate | Sponsor (funder) |
|           |           |   |      |          |                  |
| 830.6302  |           |   |      |          |                  |
| 830.6304  |           |   |      |          |                  |
| 830.6303  | None      | Color, Odor, Physical State (Hoggone)           | \$   | 400      | ACTA             |
|           |           |   |      |          |                  |
| 830.6314  | None      | Oxidation/Reduction (Hoggone)                   | \$   | 1,250    | ACTA             |
|           |           |   |      |          |                  |
| 830.6316  | None      | Explodability (Hoggone)                         | \$   | 7,050    | not submitted    |
|           |           |   |      |          |                  |
| 830.6317  | 101       | pH (Hoggone)                                    | \$   | 350      | ACTA             |
|           |           |   |      |          |                  |
| 830.632   | 109       | Density/relative density/bulk density (Hoggone) | \$   | 600      | ACTA             |
|           |           |   |      |          |                  |
|           |           | Stability to normal and elevated temperatures,  |      |          |                  |
| 830.6313  | None      | metals and metal ions (Hoggone)                 | \$   | 13,290   | ACTA 50%/IAL 50% |
|           |           |   |      |          |                  |

## Acute Toxicity

|   | EPA OPPTS | OECD      |   |      |          |               |
|---|-----------|-----------|---|------|----------|---------------|
|   | Guideline | Guideline | Study Title   | Cost | Estimate |               |
|   |           |           | Acute oral toxicity – rat (Hoggone)   |      |          |               |
|   |           |           | - study will cost less if only the limit test at  |      |          |               |
|   | 870.11    | 425       | 2,000 mg/kg is needed   | \$   | 5,520    | ACTA          |
|   |           |           | Acute dermal toxicity (BASF technical sodium  |      |          |               |
|   |           |           | nitrite - NOT the microencapsulated sodium<br>nitrite)  |      |          |               |
|   |           |           |   |      |          |               |
|   | 870.12    | 402       | <ul> <li>cost estimate assumes TGAI is non-toxic and<br/>only a limit test is needed</li> </ul>             | \$   | 1,650    | ACTA          |
|   |           |           |   |      |          |               |
|   |           |           | Acute dermal toxicity (Hoggone)   |      |          |               |
|   |           |           | - cost estimate assumes Hoggone is non-toxic  |      |          |               |
|   | 870.12    | 402       | and only a limit test is needed   | \$   | 1,650    | ACTA          |
|   |           |           | Acute inhalation toxicity – <u>rat</u> (BASF technical  |      |          |               |
|   |           |           | sodium nitrite - NOT the microencapsulated<br>sodium nitrite)   |      |          |               |
|   |           |           |   |      |          |               |
|   | 870.13    | 403       | <ul> <li>cost estimate assumes TGAI is relatively non-<br/>toxic and only a limit test is needed</li> </ul> | Ś    | 8.800    | ΑСΤΑ          |
|   |           |           |   | ,    | -,       |               |
|   |           |           | Acute inhalation toxicity – <u>rat</u> (Hoggone)  |      |          |               |
|   |           |           | - cost estimate assumes Hoggone is relatively   |      |          |               |
|   | 870.13    | 403       | non-toxic and only a limit test is needed   | \$   | 8,800    | ACTA          |
|   |           |           | Primary eye irritation – <u>rabbit</u> (Hoggone)  |      |          |               |
|   |           |           | <ul> <li>cost estimate assumes some irritation will</li> </ul>  |      |          |               |
|   | 870.24    | 405       | occur and persist   | \$   | 2,930    | ACTA          |
|   |           |           | Primary dermal irritation (Hoggone)   |      |          |               |
|   |           |           | ,   |      |          |               |
|   | 870.25    | 404       | <ul> <li>cost estimate assumes it is not irritating</li> </ul>  | \$   | 1,650    | АСТА          |
| ļ |           |           | Dermal sensitization (BASF technical sodium   |      |          |               |
|   | 870.26    | 406       | nitrite - NOT the microencapsulated sodium  | ć    | 9 440    | not submitted |
|   | 070.20    | 400       |   | ڔ    | J,++U    | not submitted |
|   | 870.26    | 406       | Dermal sensitization (Hoggone)  | \$   | 9,440    | ACTA          |
|   |           |           |   |      |          |               |

## Ecological Effects

| EPA OPPTS | OECD      |  |      |          |
|-----------|-----------|--|------|----------|
| Guideline | Guideline | Study Title                                      | Cost | Estimate |
|           |           |  |      |          |
|           |           | Honey bee acute contact toxicity (BASF technical |      |          |
| 850.302   | 214       | sodium nitrite)                                  | \$   | 5,000    |
|           |           |  |      |          |

IAL

| EPA OPPTS | OECD      | Study Title  |               |
|-----------|-----------|--|---------------|
|           | EUP Appli |  |               |
|           |           | Application materials preparation  | NWRC          |
|           |           | Study protocol   | NWRC          |
|           |           | Product label  | NWRC          |
|           |           | Data package assembly  | NWRC          |
|           |           |  |               |
| 830.155   | None      | Product identity and composition (Hoggone)                                 | NWRC          |
| 830.18    | None      | Description of materials used to produce<br>(Hoggone)                      | NWRC          |
| 830.162   | 104       | Description of production process (Hoggone)                                | NWRC          |
| 830.17    | 104       | Preliminary analysis (Hoggone) [start concurrently with storage stability] | NWRC          |
| 830.6317  | 113       | Storage stability (Hoggone)  | NWRC          |
| 830.632   | None      | Corrosion characteristics (Hoggone)  | Not submitted |

# 4 Results

## 4.1 Program 1: Assessing the attractiveness, palatability and efficacy of product prototypes

Summary of all developmental and final product prototype attractiveness, palatability and efficacy studies

| Study        | Bait type              | Methods        | Results                 | Bait (Go/No Go)     |
|--------------|------------------------|----------------|-------------------------|---------------------|
| Kangaroo     | Unprotected nitrite    | Penned         | Bait instability        | No Go               |
| Island       | in PIGOUT matrix       | animals        | Obvious bait            |                     |
| SA           |                        |                | aversion                |                     |
| Kangaroo     | Macroencapsulated      | Penned         | Macroencapsulated       | No Go               |
| Island       | SN in PIGOUT           | animals        | SN pellets (4-5mm       |                     |
| SA           | matrix                 |                | diameter) rejected      |                     |
|              |                        |                | and spat out.           |                     |
| Inglewood    | Unprotected SN in      | Penned         | Bait aversion           | No Go               |
| QLD          | a hydrophobic core     | animals        | Lack of efficacy        |                     |
|              | within PIGOUT          |                |                         |                     |
| Inglewood    | Unprotected SN in      | Penned         | Bait aversion           | No Go               |
| QLD          | differently            | animals        | Lack of efficacy        |                     |
|              | formulated             |                |                         |                     |
|              | hydrophobic cores      |                |                         |                     |
|              | within PIGOUT          |                |                         |                     |
|              |                        | to at CNI /m a |                         | ith hait and to     |
| Need for mic | roencapsulation to pro | otect SN (mes  | SN) from interacting w  | ith balt and to     |
|              | uour/laste             |                | miand the conseits of ( | N to interact with  |
| A new balt m | atrix was also develop | ed that mini   | mised the capacity of s | on to interact with |
| water in any | way                    |                |                         |                     |
| Kangaroo     | Balchem                | Penned         | 25% to 100%             | Go hut expensive    |
| Island       | microencansulated      | animals        | efficacy dependent      | and IP to           |
| SA           | SN in a fish-oil       | unnuis         | on dose                 | manufacture not     |
| 0, (         | based HOGGONE          |                |                         | provided at this    |
|              | matrix                 |                |                         | stage               |
|              | (HOGGONF1)             |                |                         | 50080               |
| Glenrock     | Balchem                | Free           | Highest dose (10%       | Tentative Go        |
| (NSW)        | microencapsulated      | ranging        | loading) in previous    | Repeat field trial  |
| (,           | SN in a fish-oil       |                | trial showed >80%       |                     |
|              | based HOGGONE          |                | efficacy by camera      |                     |
|              | matrix                 |                | activity analysis.      |                     |
|              | (HOGGONE1)             |                | but only 4 pigs         |                     |
|              |                        |                | found dead              |                     |
| Namadgi      | Balchem                | Free           | Highest dose (10%       | No Go               |
| (ACT)        | microencapsulated      | ranging        | loading) showed         | Expense of          |
| ( )          | SN in a fish-oil       | 00             | ~60% efficacy by        | Balchem             |
|              | based HOGGONE          |                | camera activity         | formulated SN       |
|              | matrix                 |                | analysis, but only 5    | Bait aversion and   |
|              | (HOGGONE1)             |                | pigs found dead         | instability remains |
|              |                        |                |                         | unacceptably high   |
| Inglewood    | Chinese enteric        | Penned         | Poor efficacy           | No Go               |
| QLD          | formulation meSN       | animals        |                         |                     |
|              | HOGGONE1               |                |                         |                     |

| Inglewood<br>QLD    | Chinese enteric<br>formulation meSN<br>at higher coating<br>HOGGONE1                         | Penned<br>animals | Poor efficacy  | No Go<br>Variable batch<br>quality   |
|---------------------|--|-------------------|--|--|
| Inglewood<br>QLD    | Chinese enteric<br>formulation meSN<br>at higher coating<br>HOGGONE1 +<br>saccharin or Talin | Penned<br>animals | Poor efficacy<br>Maximum efficacy<br>2/3 died  | No Go<br>Sweetening the<br>bait did not<br>overcome aversion<br>or efficacy<br>deficiencies  |
| Kimberly<br>WA      | Chinese enteric<br>formulation meSN<br>10% coating (10%<br>w/w loading)<br>HOGGONE1          | Free<br>ranging   | Bait instability<br>Bait aversion<br>High pig numbers<br>concentrated at<br>water holes due to<br>high temps. 13<br>found dead | No Go<br>New<br>microencapsulation<br>tech required  |
| Inglewood<br>QLD    | Reed Pacific meSN<br>(20:80) and<br>Connovation meSN<br>(10:90) in<br>HOGGONE1               | Penned<br>animals | 100% efficacy for<br>both baits<br>(n=3)/bait test.  | Satisfactory result<br>with freshly made<br>bait. IP to<br>manufacture not<br>provided for Reed<br>Pacific.<br>Connovation<br>formulation still<br>worth pursuing. |
| Goondiwindi<br>QLD  | Reed Pacific meSN<br>(20:80) in<br>HOGGONE1  | Free<br>ranging   | Efficacy 67%-83%<br>based on bait<br>station visitation<br>Still some bait<br>instability noted<br>13 dead pigs ID'd           | Satisfactory result<br>with freshly made<br>bait. IP to<br>manufacture not<br>provided by Reed<br>Pacific – No Go  |
| Lassie Creek<br>QLD | Reed Pacific meSN<br>(20:80) in<br>HOGGONE1  |                   | Efficacy ~65%<br>based on bait<br>station visitation<br>Still some bait<br>instability noted                                   | No Go<br>New SN<br>formulation<br>required   |
| Inglewood<br>QLD    | AromacoatV1<br>(40:60w/w) +<br>synthetic truffle oil<br>formulated in<br>HOGGONE1            | Penned<br>animals | 25% efficacy (n=8)   | No Go  |
| Inglewood<br>QLD    | AromacoatV2<br>(30:70w/w)<br>formulated in<br>HOGGONE1                                       | Penned<br>animals | ~80% efficacy (n=6)  | Go<br>AromacoatV2<br>HOGGONE1  |
| Byrne Valley<br>QLD | AromacoatV2<br>(30:70) and<br>Connovation meSN<br>(10:90) in<br>HOGGONE1                     | Free<br>ranging   | AromacoatV2 ~40%<br>efficacy<br>Connovation meSN<br>~60% efficacy  | No Go for either<br>bait combination   |
| Inglewood<br>QLD    | AromacoatV2 20% in bait  | Penned<br>animals | AromacoatV2 20% -<br>~70%  | No Go, poor<br>stability   |

|                           | DAFTA 1<br>DAFTA2<br>HOGGONE2<br>(refermulated bait)  |   | DAFTA 1 - ~55%<br>DAFTA2 - ~42%  | Aversion to even fresh baits  |  |  |  |
|---------------------------|---|---|--|---|--|--|--|
| Inglewood fac<br>required | Inglewood facility is mothballed so a new approach to testing baits in pilot studies is<br>required |   |  |   |  |  |  |
| Roma<br>QLD               | DAFTA 3 in new<br>HOGGONE2 baits  | Trapped/p<br>enned<br>animals                         | ~30% efficacy<br>No animal<br>acclimation to<br>being trapped  | Poor efficacy, but<br>animals did eat<br>baits older than 2<br>months. A first for<br>the project.                        |  |  |  |
| Roma<br>QLD               | DAFTA 3 in new<br>HOGGONE2 baits  | Free<br>ranging<br>palatabilit<br>y study             | Good Placebo<br>uptake<br>Rejection of toxic<br>baits  | No Go, poor<br>stability<br>Aversion to even<br>fresh baits when<br>given a choice<br>between placebo<br>and toxic baits. |  |  |  |
| Texas<br>USA              | DAFTA 3   | Penned<br>animals                                     | Poor placebo<br>uptake<br>No toxic bait<br>consumption   | No Go, poor<br>stability<br>Aversion to even<br>fresh baits   |  |  |  |
| Texas<br>USA              | DAFTA 3 (3<br>months)<br>DAFTA 5 (4 weeks)  | Penned<br>animals                                     | Satisfactory<br>placebo uptake<br><50% efficacy  | No<br>Still bait<br>discoloration<br>indicating stability<br>issues   |  |  |  |
| Dirranbandi               | DAFTA 5 ( 2 weeks)  | Free<br>ranging                                       | Excellent placebo<br>uptake<br>Some toxic bait<br>consumption but<br>aversion to baits<br>obvious  | No  |  |  |  |
| Roma                      | DAFTA 5 (2 weeks)<br>Concentrates<br>(DAFTA,<br>Connovation, RP)<br>on grain                        | Free<br>ranging<br>but caged<br>(trapped<br>on baits) | Excellent placebo<br>uptake<br>Highly variable<br>toxic bait<br>consumption<br>3/6 50% efficacy<br>100% rejection of<br>concentrate on<br>grain in sesame oil<br>based carrier | No  |  |  |  |
| Texas                     | ACTA Paste (4<br>weeks)<br>Connovation paste<br>(1 day)<br>Texas paste (1 day)                      | Penned<br>animals                                     | 2 nights before<br>100% consumption<br>of placebo<br>6/7, 1/1 100%<br>efficacy<br>5/7<br>7/7   | Yes<br>BUT need to<br>confirm result<br>using stored paste<br>bait  |  |  |  |

|             | 3 replicates (n=7)   |                 |   |   |
|-------------|--|-----------------|---|---|
| Dirranbandi | DAFTA 5 (2 weeks)<br>DAFTA 5 (13<br>months)<br>DAFTA 6 (2 weeks) | Free<br>ranging | Confirmed dead<br>pigs<br>Lower activity post<br>baiting<br>Obvious rejection<br>of toxic baits | No<br>Results too variable<br>Clear aversion to<br>toxic baits  |
| N/A         | DAFTA 7, 8, & 9  | Not tested      | Similar formulation<br>technology to<br>DAFTA 5 and 6   | No confidence that<br>using the same<br>formulating<br>technology, but<br>with increasing<br>concentration of<br>carrier compounds<br>would result in a<br>stable + disguising<br>encapsulation of<br>sodium nitrite. This<br>formulation<br>technology also<br>included water. |

# HOGGONE<sup>®</sup>-2 reformulated to HOGGONE<sup>®</sup>-3 Paste bait due to incompatabilitiy of meSN incorporation into harder single baits

| Texas       | ACTA Paste (14<br>weeks)<br>Connovation paste<br>(1 day)<br>Texas paste (1 day) | Penned<br>animals | 7/7<br>4/7<br>7/7   | Yes<br>No behavioural<br>difference between<br>placebo and toxic<br>paste                                 |
|-------------|---|-------------------|---|---|
| Dirranbandi | Paste (Connovation spheres)   | Free<br>ranging   | >90% reduction in<br>activity<br>Confirmed dead<br>pigs   | Yes<br>No behavioural<br>difference between<br>placebo and toxic<br>paste                                 |
| Mt Hope     | Paste (ACTA<br>spheres)   | Free<br>ranging   | Toxic bait uptake at<br>bait stations<br>showed it to be<br>attractive and<br>palatable to feral<br>pigs.   | Yes – Go<br>Pilot scale trial<br>demonstrated bait<br>is ready to test at<br>larger field trial<br>scales |
| St George   | Paste (ACTA<br>spheres)   | Free<br>ranging   | Toxic bait had to be<br>switched from<br>HOGGONE®-3<br>(paste) to 1080<br>laced grain due to<br>administrative<br>error by QLD DAFF<br>that impacted<br>Animal Ethics<br>Approval for trial | Abandoned   |

| Georgetown | Paste (ACTA<br>spheres) | Free<br>ranging   | Abandoned due to<br>lack of activity at<br>bait stations  | Abandoned   |
|------------|-------------------------|---|---|---|
| St George  | Paste (ACTA<br>spheres) | Free<br>ranging   | 64–80% reduction in<br>pigs on camera<br>66% reduction in<br>bait up-take<br>115 dead pigs<br>found, all ≤ <b>250</b> m<br>from bait<br>Only 3 collared pigs<br>visited on toxic<br>nights, 2 died<br>Non-targets killed –<br>3 corvids | Yes - GO<br>Use data in APVMA<br>application for a<br>HOGGONE®-3              |
| Mt Hope    | Paste (ACTA<br>spheres) | Free<br>ranging in<br>large<br>enclosure<br>surroundi<br>ng a dam | Deployment of toxic<br>HOGGONE® feral pig<br>bait provided a<br>knockdown of 87.5<br>% of feral pigs (91 of<br>104) feral pigs  | Yes - GO<br>Use data in APVMA<br>application for a<br>HOGGONE <sup>®</sup> -3 |

## 4.2 Program 2: Humaneness assessment

## Independent Humaneness Assessment (Appendix 1)

The study was undertaken in March, 2009, on 5 treated and 5 control animals. In summary, four of the five baited animals that voluntarily consumed near one final-formulation HOGGONE<sup>®</sup> bait or more died in 64±13 min. The symptoms of the toxicosis, and relative timeframes, are detailed in the below graph. A fifth pig that consumed less than 40% of one bait took near three hours to die, but showed no symptoms or increase in stress hormones until 2¼ hours after bait consumption, then progressed through the usual symptoms. Porter and Kuchel (2009) Appendix II, reported

"In the opinion of the authors, the symptoms leading to death and duration of display of these symptoms would suggest that sodium nitrite satisfies a general understanding of what a humane poison would be."



## 4.3 Program 3: Non-target risk assessment

## 4.3.1 Primary poisoning non target risk assessment

Primary risks, or susceptibility, have been estimated for 28 marsupial and nine eutherian mammal, four reptile and two bird species based on published doses and methaemoglobin reductase activity levels (see Appemdix III). A single compartment model of sodium nitrite pharmacokinetics facilitated a regression analysis from individual species' MtHb reductase activity and that analysis was used to estimate the probable lethal doses in native Australian species. The results of this modelling identified susceptible non-target species that might eat sufficient toxic HOGGONE<sup>®</sup>. Those are Common brushtail possum, Northern brown bandicoot, Tammar and Swamp wallabies, and dogs. To validate the model's predictions 2 species that were identified as likely to eat HOGGONE and be sensitive to the doses of sodium nitrite in HOGGONE, empirical testing was carried out in Bennet's Wallaby and brush tailed possums.

In the toxic trials nine of the 12 wallabies would not consume any toxic matrix after sniffing the mixture. Three wallabies consumed material receiving nitrite doses of 894, 335 and 625 mg/kg respectively. All doses were lethal, as predicted in Table 2 (minimum lethal dose 245 mg/kg), with the mean time to first symptoms being

63 min and 157 min to death. The three wallabies that died all displayed lethargy, shallow breathing, slight leg spasms, and unconsciousness before death (Shapiro and Eason 2009).

Maximum consumption by any individual possum was 229 mg/kg of sodium nitrite. Although five of the 12 possums showed visible signs of methaemoglobin formation (blue/cyanotic nose), none of the animals were affected behaviourally in terms of responses to stimuli, and as predicted no possums received a lethal dose and died. The model predicted a minimum lethal dose of 393 mg/kg. All possums were monitored for seven days following the trial, with the maximum weight changes being -3.3 % to +10.5 %, with all possums appearing healthy throughout the observation period (Fisher et al 2009).

**4.3.2** Secondary poisoning canid predator/scavenger study: Snow, N. P., Foster, J. A., VanNatta, E. H., Horak, K. E., Humphrys, S. T., Staples, L. D., Hewitt, D. G. and VerCauteren, K. C. (2018), Potential secondary poisoning risks to non-targets from a sodium nitrite toxic bait for invasive wild pigs. Pest. Manag. Sci, 74: 181–188. doi:10.1002/ps.4692

http://onlinelibrary.wiley.com/doi/10.1002/ps.4692/abstract;jsessionid=1A6E1D6E85908017E8194F7B3C45 B1BB.f04t01

- No symptoms/deaths in coyotes
- No difference in pig consumed

Implications: Low/no risk of secondary poisoning

### 4.3.3 Secondary poisoning avian scavenger study

Phase 1: Gavage study

First death recorded at 400 mg/kg - LD50 = 663 mg/kg (95%Cl = 540-813)

Implications: Low/no risk of vultures consuming this high of dose in natural setting

Phase 2: Free feeding study

- No symptoms/deaths in vultures
- Entire carcass consumed in all groups

Implications: Low/no risk of secondary poisoning

# 4.4 Program 4: Population level ground baiting efficacy and non-target safety of HOGGONE®

#### 4.4.1 Population pen efficacy

A total of n = 84 wild pigs (n = 56 HOGGONE<sup>®</sup> treatment animals and n = 28 control animals) were tested. Of the 63 animals in the first 3 replicates, 28 were adults and 35 were sub-adults. Overall, 53 of 56 (95%) of the HOGGONE<sup>®</sup> treatment animals succumbed from the toxic bait, including 24 of 24 (100%) males and 29 of 32 (88%) females (Table 1). The proportion of males killed was not statistically different from the proportion of females killed in the HOGGONE treatment group (p = 0.252) and none of the animals in the control groups died during the trials. Accordingly, the HOGGONE<sup>®</sup> treatment animals had significantly higher mortality than the control animals (p < 0.001). We found 52 of 53 (98%) of the HOGGONE<sup>®</sup> treatment animals that died succumbed during the first night the toxic bait was offered (Table 1).

Table 1. Sample sizes, proportions of food items consumed, and overall proportions of lethality for 2 treatment groups of invasive male (M) and female (F) wild pigs in 2-choice trials in 0.002-km<sup>2</sup> pens at Kerr Wildlife Management Area, Hunt, Texas, USA, during October 2015 to June 2016.

|                      |             | Days 1–2                     | Days 3–6                    | Days 7–8                                 |      |                            |      |                      |
|----------------------|-------------|------------------------------|-----------------------------|--|------|----------------------------|------|----------------------|
| Treatment<br>group   | n (M, F)    | Maintenance food<br>consumed | Placebo prebait<br>consumed | HOGGONE <sup>®</sup> or placebo consumed | SE   | Challenge diet<br>consumed | SE   | Lethality<br>(M, F)  |
| HOGGONE <sup>®</sup> | 56 (24, 32) | 1.00                         | 1.00                        | 0.62                                     | 0.11 | 0.26                       | 0.12 | 0.95<br>(1.00, 0.91) |
| Placebo<br>(control) | 28 (12, 16) | 1.00                         | 1.00                        | 1.00                                     | 0.00 | 1.00                       | 0.00 | 0.00<br>(0.00, 0.00) |

In all replicates, 100% of the maintenance food and placebo prebait were consumed during the acclimation and prebaiting periods, respectively (Table 1). During the 2-choice test period (2 consecutive nights), the HOGGONE<sup>®</sup> treatment groups consumed an average of 62% HOGGONE<sup>®</sup> and 22% challenge diet items, and the control groups consumed 100% of the placebo bait and challenge diet items (Table 1).



**Figure 2.** Average consumption (with SE) of baits (HOGGONE<sup>48</sup> and placebo) and challenge diets consumed per invasive wild pig housed in groups of 7 animals in 0.002-km<sup>2</sup> pens during 2 consecutive nights of 2-choice trial periods at the Kerr Wildlife Management Area, Hunt, Texas, USA, during October 2015 to June 2016.

The HOGGONE<sup>®</sup> treatment group consumed a significantly lower proportion of HOGGONE<sup>®</sup> than the control group consumed of placebo bait ( $\beta$  = -0.42, 95% CI = -0.612 -0.17) because the animals succumbed in the treatment group. Similarly, the HOGGONE<sup>®</sup> treatment group consumed lower proportions of the challenge diet than did the control group ( $\beta$  = -0.77, 95% CI = -1.07– - 0.50). The proportion of HOGGONE<sup>®</sup> bait consumed was not statistically influenced by group body mass ( $\beta$  = - 0.001, 95% CI = -0.003–0.001). On average, individual wild pigs consumed approximately 479 g/animal of HOGGONE<sup>®</sup> during the first night offered, but survivors

only consumed an average of 56 g/animal during the second night (Figure 2). The HOGGONE<sup>®</sup> treatment animals consumed an average of 16.0 g/kg body weight (SE = 1.2) of HOGGONE<sup>®</sup> per night.

Analyses of images from motion activated cameras indicated that HOGGONE<sup>®</sup> treatment animals underwent fewer feeding bouts on HOGGONE<sup>®</sup> than control animals on placebo HOGGONE<sup>®</sup> during the first night

HOGGONE<sup>®</sup> was offered ( $\beta$  = -1.74, 95% CI = -2.20– -1.28), but not during the second night when only 4 survivors remained in the HOGGONE<sup>®</sup> treatment group ( $\beta$  = -0.66, 95% CI = -3.19– 1.99; Figure 3). Similarly, the HOGGONE<sup>®</sup> treatment group also had shorter feeding bouts than the control group during the first night toxic bait was offered ( $\beta$  = -25.45, 95% CI = -40.54– -10.37), but not during the second night ( $\beta$  = -23.93, 95% CI = -50.93–10.89; Figure 4).



**Figure 3.** Mean number of feeding bouts (with SE) per invasive wild pig in HOGGONE<sup>®</sup> treatment (toxic bait) and control (placebo bait) groups in 0.002-km<sup>2</sup> pens during 2 consecutive nights of 2-choice trial periods at the Kerr Wildlife Management Area, Hunt, Texas, USA, during October 2015 to June 2016.



**Figure 4.** Mean duration of feeding bouts (with SE) per invasive wild pig in HOGGONE<sup>®</sup> treatment (toxic bait) and control (placebo bait) groups in 0.002-km<sup>2</sup> pens during 2 consecutive nights of 2-choice trial periods at the Kerr Wildlife Management Area, Hunt, Texas, USA, during October 2015 to June 2016.

Most of the feeding bouts for the HOGGONE<sup>®</sup> treatment groups occurred within 3 hours post-offering of baits (Figure 5). Only 4 wild pigs in the HOGGONE<sup>®</sup> treatment group survived the first night of exposure. Comparisons between those animals and non-survivors indicated that the survivors underwent more feeding bouts ( $\beta$  = 0.47, 95% CI = 0.70–0.25), but had similar durations of feeding ( $\beta$  = 0.58, 95% CI = -9.21– 10.38) on HOGGONE<sup>®</sup> during the first night offered. Feeding bouts in the control groups continued throughout the night but steadily decreased as the available food diminished.

Animals in the first 3 replicates lost a small proportion of weight during the trial ( $\bar{x} = -0.08$  proportion of body weight, SD = 0.06), but weight loss did not differ between the treatment and control groups ( $\theta = 0.03$ , 95% CI = -0.001–0.06) and none of the animals were emaciated post-trial. The average concentration of SN in HOGGONE<sup>®</sup> was 9.5% (SD = 0.02), suggesting an actual concentration of ~10.3%, including the 8% recovery loss reported for the analytical method. All placebo samples contained <0.01% SN.

(Snow et. al., 2016)

http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=2956&context=icwdm\_usdanwrc

## 4.4.2 Australian large enclosure studies: Water point traps

### Trapping

Six water-traps were set up at the beginning of the trial including three on Yara and three on Kilparney (Fig. 2 and 3). By the end of the trapping phase, we had captured feral pigs in Trap 1 at Yara, and at Trap 1 and Trap 2 on Kilparney; the remaining traps were discarded. A summary of results for each trap are presented below.

## Yara – Trap 1 (YT1)

87 feral pigs were captured during the trapping phase. Trap gates were locked prior to the final of placebo HOGGONE<sup>®</sup> baiting to ensure all animals had been exposed to HOGGONE<sup>®</sup> feral pig bait on at least one occasion before poison baiting.



**Figure 11** – Image of feral pigs, the morning after placebo baiting. Note the destroyed placebo HOGGONE<sup>®</sup> paste trays in the foreground.

Feral pigs readily transitioned onto the placebo HOGGONE<sup>®</sup> feral pig bait during baiting. It also appeared that all of the animals present in the trap visited the bait station on the final night of placebo HOGGONE<sup>®</sup> baiting, although it is hard to determine, due to the considerable numbers present. A total of 74 feral pig carcasses (28 male; 46 female; 19 kg ± 2 kg S.E.) were found in the pen after the first night of poison baiting. A majority of these animals were found in a group in one particular corner of the pen (Fig. 12a and 12b). No animals were killed on the second night of poison baiting, despite all animals visiting the station.



Figure 12a and 12b – Image of dead feral pigs, the morning after toxic baiting.

Feral pigs in YT1 consumed 45 kilograms of placebo HOGGONE® feral pig bait (100 %) the night before poison baiting, they consumed 13 kilograms of toxic HOGGONE® feral pig bait (28 %) on the first night of poison baiting and they consumed 1.95 kg kilograms of toxic HOGGONE® feral pig bait (19.5 %) on the second night of poison baiting. There was an obvious difference between placebo HOGGONE® baiting and toxic HOGGONE® baiting, and that was that feral pigs always consumed all of the placebo HOGGONE® bait and destroyed the bait trays when feeding on placebo bait, but they left a considerable amount of toxic HOGGONE® bait during toxic baiting, and the trays were intact (Fig. 13). We found one instance of vomit in YT1 and we did not find any dead non-target animals, despite feral goats being present in the pen at the time of poison baiting.



Figure 13 – bait station the after the first night of poison baiting.

## Kilparney – Trap 1 (KT1)

As there was only a small number of feral pigs (seven) present in KT1 leading up to the last night of placebo HOGGONE<sup>®</sup> baiting, we left trap gates in trapping mode hopeful that additional animals would enter the trap that afternoon and feed with the other animals. Four new animals did enter, but unfortunately, they arrived after the bait had been consumed and before we arrived to shut the gate prior to poisoning. Hence, there were ten feral pigs present in KT1 on the first night of poison baiting but four of these had not been exposed to HOGGONE<sup>®</sup> bait material. Seven feral pigs (5 male and 2 female; 32 kg ± 10kg) were killed on the first night of baiting including six of the original seven and one of the new animals (male; 47 kg). One of the original seven (2kg) was seen alive when we approached the trap in the morning, but it was small enough (~2kg) to escape through the trap mesh, one was shot (male; 70kg) and two could not be found; small enough (<5kg) to fit through the trap mesh.



Figure 14 – Original feral pigs feeding at the bait station.

Feral pigs in this pen took several days to start feeding, which is why we were only able to implement one night of placebo baiting and one night of toxic baiting. When they did start feeding, they consumed 7.5 kilograms of placebo HOGGONE® feral pig bait (100 %) the night before poison baiting and they consumed 2 kilograms of toxic HOGGONE® feral pig bait (20%) on the night of poison baiting. The obvious difference between placebo HOGGONE® baiting and toxic HOGGONE® baiting, was again that feral pigs consumed all of the placebo HOGGONE® bait and destroyed the bait trays, whereas there was a considerable amount of toxic HOGGONE left over and the trays were intact. We found 3 occurrences of vomit in this pen, all of which were within two meters from the largest animal (male; 85 kg). No non-target carcasses were found in the pen, despite numerous feral goats being present.





Figure 15a and 15b – Image of the largest animal and vomit nearby.

### Kilparney – Trap 2 (KT2)

13 feral pigs were captured during the trapping phase (Fig. 16). Although trap gates were locked prior to the final night of placebo HOGGONE<sup>®</sup> baiting, one trap gate failed and four new animals were able to push in on the first night of baiting. This meant that these four animals had not been exposed to placebo HOGGONE<sup>®</sup> bait prior to being exposed to the toxic bait. Notwithstanding, 10 feral pigs (7 male and 3 female; 13 kg ± 1kg S.E.) were killed on the first night of poisoning (likely to be 10 of the original 13) and an additional feral pig

was killed the following night (male 14kg). It was not possible to determine whether this was 1 of the original 13 that had been exposed to placebo or whether it was one of the new animals. The 6 remaining animals (3 male and 3 female;  $17\text{kg} \pm 4 \text{ kg}$  S.E.) were euthanased, including the large sow from the original group of 13.



**Figure 16** - The original group of 13 feral pigs feeding as a group on the first night of poison baiting, before an additional 4 animals managed to push into the trap.

15 kilograms of placebo HOGGONE<sup>®</sup> feral pig bait (100 %) was consumed the night before poison baiting. Unlike at the other traps, most was consumed by feral goats. Feral pigs consumed 2kg kilograms of toxic HOGGONE<sup>®</sup> feral pig bait (16 %) on the first night of poison baiting and they consumed 0.2 kg kilograms of toxic HOGGONE<sup>®</sup> feral pig bait (4 %) on the second night of poison baiting. As with the other two traps, feral pigs destroyed the placebo HOGGONE<sup>®</sup> bait trays, but left the toxic HOGGONE<sup>®</sup> trays intact after toxic baiting. We did not find vomit in this pen. One goat found dead that was also present at the bait station on the first night of poison baiting (Fig. 17a and 17b).



### **Overview of HOGGONE® efficacy.**

The deployment of toxic HOGGONE<sup>®</sup> feral pig bait provided a knockdown of 87.5 % of feral pigs (91 of 104) feral pigs that had been exposed to placebo HOGGONE<sup>®</sup> bait material. An additional 8 animals (n=112) were captured later in the trial and were not exposed to placebo HOGGONE<sup>®</sup> bait. Of these, only 1 was killed with toxic HOGGONE<sup>®</sup> feral pig bait and the remainder were shot. This highlights the importance of pre-feeding, particularly with respect to real life baiting programs. The likelihood of new animals arriving before toxic bait deployment in a field situation can be reduced with an extended free-feed period.

## 4.4.3 Australian landscape scale population level field study

#### Feral pig activity at bait stations

We created 19 bait stations in the treatment area and 7 bait stations in the control area. By the completion of phase 1 baiting there were 11 active bait stations in the treatment area and 3 active bait stations in the control area; inactive stations by this point were discarded and have not been included in the following analyses. Prior to poison baiting, there was a mean of  $23 \pm 2$  S.E. feral pigs per active station in the treatment area and a mean of  $19 \pm 7$  feral pigs per active bait station in the control area. After poison baiting there was a mean of  $8 \pm 1$  S.E. feral pigs per station in the treatment area, and a mean of  $25 \pm 5$  S.E. feral pigs per station in the control (means calculated using data from two nights pre poison baiting and two nights post poison baiting).



**Figure 13** – Treatment site bait station locations. Green dots represent active stations and yellow dots represent inactive stations that were abandoned.



**Figure 14** – Control site bait station locations. Green dots represent active stations and yellow dots represent inactive stations that were subsequently abandoned.

## Bait-uptake at active bait stations

Feral pigs readily consumed placebo HOGGONE<sup>®</sup> feral pig bait at sites where pre-feed grain was completely consumed. Bait-uptake was more variable at sites where pre-feed grain was being left over, although it improved on subsequent nights. There was an obvious difference between placebo HOGGONE<sup>®</sup> baiting and toxic HOGGONE<sup>®</sup> baiting, that was feral pigs typically consumed all of the placebo HOGGONE<sup>®</sup> bait and destroyed the bait trays during placebo baiting, whereas there was always some toxic HOGGONE left over (varying amounts) and the trays were intact after toxic baiting.



**Figure 15a** – Bait site after placebo HOGGONE baiting.

**Figure 15b** – Same bait site after toxic HOGGONE baiting.

Nevertheless, feral pigs consumed 143.5 kilograms of placebo HOGGONE<sup>®</sup> feral pig bait (97 % ± 3% S.E. of available bait) the night before poison baiting, they consumed 73.6 kilograms of toxic HOGGONE<sup>®</sup> feral pig bait (41% ± 9% S.E. of available bait) on the first night of poison baiting, they consumed 17.4 kilograms of

toxic HOGGONE<sup>®</sup> (17%  $\pm$  4% S.E. of available bait) on the second night of poison baiting and they consumed 35.5 kg (33%  $\pm$  11% of available bait) of placebo HOGGONE<sup>®</sup> feral pig bait on the night after poison baiting.

## Feral pig activity at independent camera sites

Feral pig activity across the landscape was monitored independently from bait station activity as a second activity index of abundance. 12 cameras across the treatment property and 7 cameras across the control property recorded feral pig activity 4 weeks pre-, during- and 4 weeks post- the toxic baiting program. Prior to poison baiting, there was a mean of  $7.5 \pm 3.3$  S.E. feral pigs per day in the treatment area and a mean of  $2.1 \pm 1.2$  feral pigs per day in the control area. After poison baiting, there was a mean of  $1 \pm 0.6$  S.E. feral pigs per day recorded over the treatment property, and a mean of  $25.4 \pm 23.8$  S.E. feral pigs per day/camera recorded over the control property.

## **Collared Feral Pigs**

10 collared animals on or surrounding the Treatment site were monitored pre-, during and post-baiting with Hoggone. In the week of free feeding that preceded the toxic phase of the trial only 6 collared animals remained on the treatment site (magenta, orange, red, cyan, dark grey, yellow traces, see below). 2 slipped collars were re-deployed on trapped feral pigs on the 21<sup>st</sup> Nov (lime and dark green traces). Movement of collared feral pigs was cross referenced to images of collared feral pigs at bait stations. In total, images of 7 collared animals were observed at bait stations during the non-toxic free feeding phase (2 nights). From individual characteristics and collar data these are 5 distinct animals. In total, images of 4 collared pigs were observed at bait stations during the toxic phase of the trial (2 nights). From individual characteristics and collar data these are 3, and of those 3, 2 were confirmed killed by toxic bait (60%). One was killed on the night of the 29<sup>th</sup> Nov (dark grey trace, see below) and one on the 30<sup>th</sup> Nov (yellow trace, see below). A total of 3 distinct collared animals were observed at bait stations during the toxic phase of the toxic phase of the study and included the most recently collared animals.



26 Nov (-2 day from toxic baiting)





28 Nov Toxic night 1



29 Nov Toxic night 2

#### Carcasses/vomit/non-targets

A total of 115 feral pigs were found during the carcass recovery phase of the trial. These animals were found at distances ranging from 1 meter to 178 meters from the station. Of the 115 carcasses that were found, 98 were found during ground-based after the first night of baiting and 17 were found during the aerial searches after the second night of baiting. It is difficult to determine whether some of the animals found during the aerial searches were killed after the first night of poison baiting and were missed during the ground-based searches, or whether they were killed on the second night of baiting. Also, during the ground searches, we found two non-target kills and three occurrences of feral pig vomit. The non-target species were Australian ravens and they were found at bait station 8 approximately 30 meters and 24 meters from the station. The three occasions of vomitus were located within 20 meters of bait station 13. Hereafter, we show a series of maps that depict all feral pig carcasses locations in proximity to the nearest bait station.









#### Overview of HOGGONE® efficacy

We found strong evidence that the deployment of HOGGONE<sup>®</sup> reduced the number of pigs that visited the toxic bait sites during the post-treatment period ( $\beta$  = -22.17, 95% CI = -30.01– -13.78; *P* < 0.001; Figure 12). Similarly, consumption of bait at the toxic sites was reduced during the post-treatment period ( $\beta$  = -0.64, 95% CI = -1.22– -0.05; *P* = 0.040). Finally, deployment of HOGGONE<sup>®</sup> also reduced the number of pigs seen per image at independent cameras sites during the post-treatment period ( $\beta$  = -1.67, 95% CI = -2.43– -0.91; *P* < 0.001).



## 4.5 Program 5: Tissue residues assessment



## Sodium nitrite residues in HOG-GONE® poisoned feral pigs

## Sodium nitrite residues in HOG-GONE<sup>®</sup> poisoned feral pigs and food products



## 4.6 Program 6 and 7: APVMA regulatory studies and submission of new product application

#### APVMA registration application submitted May 2017. The submission consisted of:

| Section 1  | Overview   |
|------------|--|
| Section 2  | Chemistry & Manufacture  |
| Section 3  | Toxicology   |
| Section 4  | MetabolismNo separate Section 4 was included as the product is not intended for use on food or fibre producing animals. Metabolism was covered within Section 1 and Section 3. |
| Section 5  | ResiduesNo separate Section 5 was included as the product is not intended for use on food or fibre producing animals. Residues was covered within Section 1.                   |
| Section 6  | Occupational Health and Safety   |
| Section 7  | Environmental toxicity and fate  |
| Section 8  | Efficacy and non-target safety   |
| Section 10 | Information/Finalisation   |

See Appendix V for the draft label.

## 5 Discussion

## 5.1 Learnings from a decade of R&D with feral pigs

After 10 years, we were able to develop a bait that is shelf-stable, palatable and efficacious under Australian and US conditions. It is possible in some situations in Australia a paste hopper will be required to prevent up-take by non-target species, but up-take be non-target species in our trials was minimal. In contrast, HOGGONE paste will always need to be delivered in a paste hopper in the US due to the greater presence of non-target species that find the bait attractive. In all situations it is going to be important to bait when the conditions are most suitable (only low quality natural food available) and to incorporate a methodical prefeeding phase that allows animals to become accustomed to the bait gradually. Even though we tested aged bait that had been exposed to hot ambient temperatures, whilst in storage, and it provided excellent knockdown results. It would also be advisable to store all bait in a locked dry and shaded facility to prevent the bait spoilage. Our results, in combination with what was found in the US studies, indicate it is fine to leave feral pig carcasses in the environment as secondary poisoning risk is minimal.

### 5.1.1 Insights from attractiveness, palatability and efficacy studies

Feral pigs are highly intelligent, but also creatures of habit.

Lethal doses of sodium nitrite are extremely challenging to formulate into a food substrate that feral pigs will readily consume in sufficient quantities.

The slightest (>1%) breakdown of lethal doses of sodium nitrite or its interaction with food substrates almost inevitably results in complete aversion by feral pigs.

#### 5.1.2 Practical implications for end users

Neophobia by feral pigs means that any change to food substrates requires a gradual (over 2-3 days) transition in order to optimise uptake.

Land managers must invest in pre-feed and transition feral pigs onto the placebo bait properly to maximise the cost effectiveness of baiting programs.

This involves:

• clustering animals with traditional substrate (grain)

• offering a combination of traditional substrate and placebo paste only when grain is being consumed regularly by itself.

- Offering paste by itself once pigs are eating a both the grain and the paste in the combination phase.
- Offering poison paste by itself once placebo paste is being consumed on its own.

#### 5.1.3 Draft extension messages

A new chemical (sodium nitrite) and product (HOGGONE<sup>®</sup>) containing that chemical is being assessed by the Australian Pesticides and Veterinary Medicines Authority so that red meat producers, as well as other feral pig affected land managers have additional tools in the future to help them reduce feral pig impacts and manage the profitability and sustainability of their enterprises.

#### 5.1.4 How the project met its objectives ?

#### 5.1.4.1 Development of HOGGONE®

Yes, but delayed due to technical challenges.

#### 5.1.4.2 Assessment of the final product prototype of HOGGONE®

Once the development technical challenges were overcome there were still regulatory technical challenges to address. These were successfully completed.

#### 5.1.4.3 Submission of the APVMA registration application for HOGGONE®

Completed, so now the planning starts for the market launch and end user/industry engagement/extension.

## 6 Conclusions/recommendations

## 6.1 How the practical application of the project outputs will benefit the red meat industry

The greatest practical application that this project delivers is choice for red meat producers in what they can use to manage the impacts of feral pigs. That choice comes with the promise that producers that currently don't use the most cost-effective option to control feral pigs, will participate in management programs in the future.

An analysis of the incremental benefits of introducing HOGGONE was conducted at the beginning of the project to ensure that the investment was warranted.

The industries directly affected by feral pigs are:

- wool;
- sheep meat;
- grains;
- sugar; and
- bananas.

For the wool, sheep meat and grain industries we have estimates based on pest density information reported in the recent assessment of the costs of invasive animals in Australia. The sugar industry information was obtained from the New South Wales Department of Primary Industry. Less information is available on the banana industry; however, like sugar, bananas tend to be grown in areas were feral pigs are relatively abundant. Feral pig density is therefore assumed to be the same as for the sugar industry.

#### Feral pig density by industry

|            | No impact | Low density | Medium density | High density |
|------------|-----------|-------------|----------------|--------------|
|            | %         | %           | %              | %            |
| Wool       | 96.2      | 1.5         | 1.9            | 0.4          |
| Sheep meat | 96.2      | 1.5         | 1.9            | 0.4          |
| Grains     | 94.4      | 3.6         | 1.2            | 0.9          |
| Sugar and  | 6.6       | 26.7        | 46.7           | 20.0         |

Source: Gong, Sinden and Jones (2008), Peter West per comm, ABS, TheCIE.

Total number of farming enterprises in Australia numbers 133,000, which is comprised of 13% (17,290) grains + mixed farming and 10% (13,300) sheep production (wool and meat), which I have split for convenience. The total area under sugar and banana production is 4400 and 107 km<sup>2</sup> respectively, which equates to approximately 2504 enterprises using the average enterprise size of 1.8km<sup>2</sup> (Australian Bureau of Statistics).

The effectiveness of HOGGONE in controlling feral pigs is estimated to be around the same as meat baiting. This can be relatively time consuming, and when labour costs are taken into account, would cost around \$3.00 per bait. This compares to around \$2.40 per bait for HOGGONE. This represents a saving of around 60 cents per bait. Therefore there is a direct economic benefit of using HOGGONE due to the reduction in input costs, mainly labour.

Pig baiting programs typically occur one to three times per year and recommendations are to use between 10 and 40 baits per square kilometer, depending on pig density. For the purposes of establishing

market size, it is assumed that land managers use 40 baits per square kilometer three times per year in high density areas, 25 baits per square kilometer (the mid-point of the estimated range) twice per year in medium density areas and 10 baits per square kilometer once per year in low density areas.

#### Market size for HOGGONE

|                                      |         | <b>•</b>   |         | Sugar<br>bananas | and |
|--------------------------------------|---------|------------|---------|------------------|-----|
|                                      | Wool    | Sheep-meat | Grains  |                  |     |
| Low density areas                    |         |            |         |                  |     |
| Cost per bait (\$)                   | 2.4     | 2.4        | 2.4     | 2.4              |     |
| Baits per Km <sup>2</sup>            | 10      | 10         | 10      | 10               |     |
| Average farm size (Km <sup>2</sup> ) | 55.6    | 55.6       | 24.9    | 1.8              |     |
| Baiting programs per year            | 1       | 1          | 1       | 1                |     |
| No of enterprises                    | 6,650   | 6,650      | 17,290  | 2504             |     |
| % affected by pigs                   | 1.5     | 1.5        | 3.6     | 26.7             |     |
| Market size (Number of baits)        | 55,549  | 55,549     | 154,988 | 12,035           |     |
| Medium density areas                 |         |            |         |                  |     |
| Cost per bait (\$)                   | 2.4     | 2.4        | 2.4     | 2.4              |     |
| Baits per Km <sup>2</sup>            | 25      | 25         | 25      | 25               |     |
| Average farm size (Km <sup>2</sup> ) | 55.6    | 55.6       | 24.9    | 1.8              |     |
| Baiting programs per year            | 2       | 2          | 2       | 2                |     |
| No of enterprises                    | 6,650   | 6,650      | 17,290  | 2504             |     |
| % affected by pigs                   | 1.9     | 1.9        | 1.2     | 46.7             |     |
| Market size (Number of baits)        | 351,253 | 351,253    | 258,312 | 105,243          |     |
| High density areas                   |         |            |         |                  |     |
| Cost per bait (\$)                   | 2.4     | 2.4        | 2.4     | 2.4              |     |
| Baits per Km <sup>2</sup>            | 40      | 40         | 40      | 40               |     |
| Average farm size (Km <sup>2</sup> ) | 55.6    | 55.6       | 24.9    | 1.8              |     |
| Baiting programs per year            | 3       | 3          | 3       | 3                |     |
| No of enterprises                    | 6,650   | 6,650      | 17,290  | 2504             |     |
| % affected by pigs                   | 0.4     | 0.4        | 0.9     | 20               |     |
| Market size (Number of baits)        | 177,475 | 177,475    | 464,962 | 108,173          |     |
| Total market potential (\$)          | 584,277 | 584,277    | 878,262 | 225,451          |     |

Total revenue from bait sales at market maturity (20%) = ~\$2.27M Royalty revenues @ 7.5% = ~\$170,000

Taking into account the different average farm size and use of chemicals across industries (as reported in ABARE's farm surveys), the estimated cost saving from using IA CRC developed pig baits are estimated in the following table.

#### Change to input costs using HOGGONE

|  | Wool   | Sheep-meat | Grains | Sugar and<br>bananas |  |  |
|--|--------|------------|--------|----------------------|--|--|
| Low density areas                      |        |            |        |                      |  |  |
| Change in cost per bait (\$)           | -0.60  | -0.60      | -0.60  | -0.60                |  |  |
| Baits per Km <sup>2</sup>              | 10     | 10         | 10     | 10                   |  |  |
| Average farm size (Km <sup>2</sup> )   | 55.6   | 55.6       | 24.9   | 1.8                  |  |  |
| Baiting programs per year              | 1      | 1          | 1      | 1                    |  |  |
| Change in annual cost per farm         | 334    | -334       | -149   | -11                  |  |  |
| Average labour costs <sup>a</sup>      | 57 200 | 57 200     | 62 510 | 63 677               |  |  |
| Change in labour costs (%)             | -0.58  | -0.58      | -0.24  | -0.02                |  |  |
| Medium density areas                   |        |            |        |                      |  |  |
| Cost per bait (\$)                     | -0.60  | -0.60      | -0.60  | -0.60                |  |  |
| Baits per Km <sup>2</sup>              | 25     | 25         | 25     | 25                   |  |  |
| Average farm size (Km <sup>2</sup> )   | 55.6   | 55.6       | 24.9   | 1.8                  |  |  |
| Baiting programs per year              | 2      | 2          | 2      | 2                    |  |  |
| Annual cost decrease per farm          | -1 668 | -1 668     | -747   | -54                  |  |  |
| Average labour costs <sup>a</sup>      | 57 200 | 57 200     | 62 510 | 63 677               |  |  |
| Change in labour costs (%)             | -2.92  | -2.92      | -1.20  | -0.09                |  |  |
| High density areas                     |        |            |        |                      |  |  |
| Cost per bait (\$)                     | -0.60  | -0.60      | -0.60  | -0.60                |  |  |
| Baits per Km <sup>2</sup>              | 40     | 40         | 40     | 40                   |  |  |
| Average farm size (Km <sup>2</sup> )   | 55.6   | 55.6       | 24.9   | 1.8                  |  |  |
| Baiting programs per year              | 3      | 3          | 3      | 3                    |  |  |
| Annual cost decrease per farm          | -4 003 | -4 003     | -1 793 | -130                 |  |  |
| Average labour costs <sup>a</sup>      | 57 200 | 57 200     | 62 510 | 63 677               |  |  |
| Change in labour costs (%)             | -7.00  | -7.00      | -2.87  | -0.20                |  |  |
| Change in industry labour costs<br>(%) | -0.090 | -0.090     | -0.047 | -0.090               |  |  |

<sup>a</sup> Including operator and family labour.

#### **Economy-wide impacts**

The direct impacts estimated above must be adjusted to reflect only partial adoption of the products. The adoption profile used for this study is shown below. The profile is based on typical patterns for the adoption of new technology, together with product-specific information provided by the IA CRC. The IA CRC estimates the maximum adoption rate for HOGGONE will be around 50 per cent. The shift away from 1080 to nitrite will also increase the potential for export markets. In this case, the benefits to Australia are from an increase in export demand for the product. However, this benefit is likely to be small in the context of economy-wide benefits and therefore has not been included in the quantitative analysis.
#### Share of sugar and bananas in 'other agriculture'

|  | Gross value of production \$m | Share of 'other agriculture' % |
|--|-------------------------------|--------------------------------|
| Gross value of agricultural production | 38 528                        |                                |
| Less: wool                             | 2 054                         |                                |
| Less: sheep meat                       | 2 112                         |                                |
| Less: grains                           | 8 238                         |                                |
| Less: beef                             | 7 685                         |                                |
| Less: dairy                            | 3 341                         |                                |
| Less: pigs                             | 890                           |                                |
| Less: chicken meat                     | 1 223                         |                                |
| Less: eggs                             | 376                           |                                |
| Other agriculture                      | 12 608                        |                                |
| Sugar                                  | 1 032                         | 8.2                            |
| Bananas                                | 431                           | 3.4                            |

Source: ABS, Value of Agricultural Commodities Produced, Australia, 2005/06, Catalogue No. 7503.0, Tables 1 and 3.

#### Economy-wide benefits of HOGGONE at maximum adoption

|         | Percent | 2007\$million |
|---------|---------|---------------|
| HOGGONE | 0.00024 | 1.88          |

#### Service Provider Analysis:

Identify what existing agents and programs provide extension or service the defined end user group/s that could increase the adoption rate for products, strategies and services. Highlight any relevant IA CRC Participant networks/programs.

#### • Participant Commercialiser(s) / Marketer(s)

| Animal Control Technologies (Core Participant) | Commercialisation SME. Company offices and manufacturing facility located in Victoria |
|--|---|
| Connovation (Supporting Participant)           | Research SME, manufacturing facility in Auckland NZ. Offices in Sydney and NZ         |

### Participant extension or service providers that will influence the adoption rate

| Industry or other<br>research users and<br>the basis of their<br>interaction | Type of activity and location of activity   | Nature and scale of benefits<br>to end-users   | Actual or expected benefit to user  |
|--|---|--|---|
| Invasive species control and research SMEs                                   |   |  |   |
| Animal Control<br>Technologies   | Commercialisation SME. Company offices<br>and manufacturing facility located in<br>Victoria | Licensed to manufacture,<br>distribute and sell CRC's wild<br>dog, fox and feral pig baits and<br>baits to control rodents in<br>industrial settings                           | New products once registered will<br>increase company economy of scale,<br>turnover and market share  |
| Connovation  | Research SME, manufacturing facility in<br>Auckland NZ. Offices in Sydney and NZ            | Access to proprietary<br>formulations. Export<br>opportunities<br>Licensed to manufacture,<br>distribute and sell PAPP-<br>based wild-cat and stoat<br>control products in NZ. | New products once registered will<br>increase company economy of scale,<br>turnover and market share<br>Enhances export potential, provides<br>expertise in research/development gaps |

| Commercial and NGO land managers                           |   |                                       |  |
|--|---|---------------------------------------|--|
| Australian Wildlife<br>Conservancy                         | Private natural resource conservation.<br>Sites in a number of States. Headquarters<br>in Perth   | Access to new tools and techniques    | Increased efficiencies and effectiveness<br>of feral animal control  |
| Regional and NRM mana                                      | agers and field officers  |                                       |  |
| Local Land Services  | Public sector natural resource<br>management. HQ in Orange, NSW   | Access to new tools and techniques    | Increased efficiencies, effectiveness and participation in feral animal control within agricultural production enterprises                                       |
| Government natural res                                     | ource managers and field officers   |                                       |  |
| Environment ACT  | Public sector environmental protection.<br>Offices in Canberra  | Access to new tools and techniques    | Increased efficiencies, effectiveness and<br>participation in feral animal control on ACT<br>state owned land.   |
| Murray-Darling Basin<br>Commission                         | Public sector natural resource<br>management. Provides strategic direction<br>for pest carp research, development and<br>extension through their <i>Native Fish</i><br><i>Strategy</i> . Offices in Canberra. | Access to new tools and techniques    | Increased efficiencies, effectiveness and<br>participation in pest fish control within the<br>Murray-Darling basin   |
| NSW Department of<br>Primary Industries                    | Public sector agricultural resource<br>management. HQ in Orange, NSW  | Access to new tools and techniques    | Increased efficiencies, effectiveness and<br>participation in feral animal control within<br>agricultural production enterprises in NSW                          |
| NSW Department of<br>Environment and<br>Conservation       | Public sector natural resource<br>management. HQ in Sydney  | Access to new tools and techniques    | Increased efficiencies, effectiveness and<br>participation in feral animal control on<br>public lands in NSW   |
| Tas Department of<br>Primary Industries                    | Public sector natural resource<br>management. HQ in Hobart  | Access to new tools and<br>techniques | Increased efficiencies, effectiveness and<br>participation in feral animal control on<br>public lands in Tasmania  |
| Vic Department of<br>Sustainability and the<br>Environment | Public sector natural resource<br>management. HQ in Melbourne   | Access to new tools and techniques    | Increased efficiencies, effectiveness and<br>participation in feral animal control within<br>agricultural production enterprises and<br>public lands in Victoria |

| Vic Department of<br>Primary Industry                             | Public sector natural resource<br>management. HQ in Melbourne  | Access to new tools and<br>techniques   | Increased efficiencies, effectiveness and<br>participation in feral animal control within<br>agricultural production enterprises in<br>Victoria                           |
|---|--|---|---|
| WA Department of<br>Environment and<br>Conservation               | Public sector natural resources management. HQ in Perth.   | Access to new tools and<br>techniques   | Increased efficiencies, effectiveness and<br>participation in feral animal control within<br>agricultural production enterprises and<br>public lands in Western Australia |
| Government, industry a  | nd NGO decision-makers, influencers and i  | networks  |   |
| Australian Veterinary<br>Association                              | Animal welfare policy development.<br>Headquarters in Canberra   | Advice to vets on new<br>products   | More informed professionals<br>Increased public awareness of the issues   |
|   |  |   | involved in managing invasive species   |
| Australian Wool<br>Innovation Ltd                                 | Funder. Industry R&D body representing<br>wool growers. Company offices in<br>Sydney and Melbourne                                     | Enhanced productivity<br>through a reduction in losses<br>from wild dog and fox attack,<br>an reduced rabbit impact | New tools and techniques, applied by<br>producers and land managers that<br>improve wool production enterprises'<br>efficiencies  |
| Cattle Council of   | Agricultural and natural resource policy   | Advice to governments and   | More informed decision-makers   |
|   | producer body representing Australia's<br>beef cattle producers. Headquarters in<br>Canberra   | natural resource policy<br>development.   | Increased public awareness of the issues<br>involved in managing invasive species in<br>the context of beef production enterprises  |
| Grains Research and<br>Development<br>Corporation                 | Funder. Industry R&D body representing 30,000 grain growers. Company offices in Canberra.  | Enhanced productivity<br>through reduction in damage<br>by rodents  | New tools and techniques, applied by<br>producers and land managers that<br>improve grain production enterprises'<br>efficiencies   |
| Meat and Livestock Ltd  | Funder. Industry R&D body representing<br>34,000 graziers. Company offices in<br>Sydney  | Enhanced productivity<br>through reduction in damage<br>by feral pigs   | New tools and techniques, applied by<br>producers and land managers that<br>improve meat production enterprises'<br>efficiencies  |
| WWF-Australia   | Environment and natural resource policy<br>development and advocacy. Peak<br>conservation body. Headquarters in<br>Sydney              | Advice to governments and<br>industry on environment and<br>natural resource policy                                 | More informed decision-makers   |
| Offshore collaborators  |  |   |   |
| New Zealand<br>Department of<br>Conservation                      | Collaborative research on PAPP in NZ   | Trialing of products in NZ  | Contract research – access to new actives and baits   |
| Landcare Research,<br>NZ  | Collaborative research   | Trialing of products in NZ  | Contract research – access to new actives and baits   |
| United States<br>Department of<br>Agriculture                     | Invasive and overabundant wildlife<br>management research. Potential research<br>collaborations. Import and/or export<br>opportunities | Trialing of products in USA   | Research outputs (new baits and actives)<br>with potential in the USA tested on relevant<br>target species.   |
| National Wildlife<br>Research Center                              | Headquartered in Colorado, USA.  |   |   |
| Animal Health and<br>Veterinary Laboratories<br>Agency (AHVLA) UK | Research and commercialisation<br>York, UK.  | Trialing of products in UK  | Research outputs (new baits and actives)<br>with potential in the UK tested on relevant<br>target species.  |
|   |  |   | 1   |

### 6.2 Communication and adoption strategies/planning

Commercialisation strategy will be similar to that used for PIGOUT (reference PIGOUT pathway to adoption business plan).

## 7 Key messages

# 7.1 The top six management practices that end users can implement to make feral pig management more cost-effective

- 1. Use bait stations to aggregate feral pigs to reduce time/labour costs.
- 2. Free-feed for between 7-10 days before deploying toxic bait.
- 3. Introduce any change in free-feed to what is going to be the toxic bait substrate over 1-3 days if possible.
- 4. Split up deployed free-feed and toxic bait so that dominant animals can't eat like pigs and leave less dominant animals without a feed.
- 5. Timing is essential. Work out when resources are scarce in your particular area and bait then.
- 6. Coordinate efforts with neighbours to maximise baiting program effectiveness.

# 7.2 The economic and biosecurity benefits of implementing those management practices into feral pig management programs

As of 2018 any land manager wanting to manage for the impacts of feral pigs can only use 1080 (or 1080 and CSSP in Queensland). This limited range of options impinges on some landholders from using any chemical control for managing feral pigs. This project will deliver an additional tool for land managers to integrate into their feral pig control programs, and as detailed in section 6.1 this will have incremental benefits for red meat producers that can help them address the following impacts.

Feral pigs inhabit nearly half of the Australian land mass with NSW and Queensland experiencing the largest populations and production losses

- Nationally feral pigs are calculated to have inflicted \$14.4 million in production loss costs per year in 2013-14, which is above the 2009 estimate of \$10 million
- Australia-wide losses are estimated to be \$5 million for wool producers, \$3 million for sheepmeat farmers and \$7 million for broad acre wheat and barley producers
- The production loss cost to NSW is estimated at \$13.5 million in 2013-14, which is more than 90 percent of the national total

https://www.pestsmart.org.au/wp-content/uploads/2017/10/Cost-of-Pest-Animals-in-NSW-and-Aus-2013-14-web-HR.pdf

## 8 Bibliography

All references cited within this final report are included within each Appendix.

## 9 Appendices

- 9.1 Appendix I HOGGONE® development and registration pictorial narrative
- 9.2 Appendix II Sodium nitrite humanness assessment
- 9.3 Appendix III Non target risk assessment report
- 9.4 Appendix IV Tissue residues assessment report
- 9.5 HOGGONE<sup>®</sup> draft label