# final report

Project code: B.AHE0062

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Date published: 14 May 2018

PUBLISHED BY
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# Roll out of feral pig control solutions

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

# Abstract

Meat and Livestock Australia has previously supported the Invasive Animals Cooperative Research Centre and Animal Control Technologies Australia (ACTA) to develop the 1080 (sodium fluoroacetate) PIGOUT® bait, the more welfare and environmentally friendly sodium nitrite HOGGONE® bait, and indirectly the HogHopper™ feral pig-specific bait delivery device.

To complete the suite of feral pig control tools graziers/the market desires a cheaper bite-sized PIGOUT® bait (70g vs existing 250g bait) that can be deployed in hoppers and a sodium nitrite concentrate that can be added to grain. This project has successfully investigated these two needs. A commercialised smaller bait (1080 based) is not available with registration rejected by the APVMA. A new palatable bait matrix developed by the project (to carry sodium nitrite) is being evaluated as a carrier for 1080 in the smaller bait.

The sodium nitrite concentrate was developed and evaluated, but did not warrant further progress for a registration (delivery with grain). The laboratory and field work evaluating the sodium nitrite concentrate and delivery mechanisms, underpinned the associated development of the Hoggone bait

**With the completion of bait formulation, manufacturing processes and micro encapsulation processes of sodium nitrite for HOGGONE®, it was determined that composition of matter patents were both achievable and desirable. Patents have been applied for in both the USA (15/747,311) and Australia (2017301076). Both the US and Australian applications entered the national phase of the registration processes in late January 2018.** The national phase of the registration phase is, typically, 3 to 4 years in the USA and 2 to 4 years in Australia.

# Executive summary

In Australia, many land managers (mainly primary producers) use 1080 concentrate as a toxic additive to grain and meat baits for controlling feral pigs. This is because it is perceived that these substrates are preferred by pigs in some areas, it can be acquired relatively cheaply, and because 1080 is the only nationally registered toxicant for controlling feral pigs. Despite this, the use of 1080 as a feral pig management tool is not universally accepted as an ideal poison, due to the high doses required to control pigs, the prolonged and variable clinical phase in some animals and potential off-target impacts. A new approach is being explored.

Animal Control Technologies Australia (ACTA) in partnership with the US Department of Agriculture (USDA) and previously also with Invasive Animals Cooperative Research Centre (IA CRC), were developing HOGGONE® (HG) manufactured feral pig bait, which contains sodium nitrite (SN) as a new toxicant (**Sodium Nitrite Patent sub project**). A liquid concentrate SN formulation (LCSN) was evaluated to assess if this could provide a potential way to use sodium nitrite on grain under field conditions where a pre-prepared bait is not ideal (**Sodium Nitrite Concentrate sub project**).

In addition to the development of HG, Meat and Livestock Australia also supported the development of 1080 PIGOUT® (PO) manufactured feral pig bait. PO is commercially available and it is currently the only commercially manufactured feral pig bait in Australia. PO is made of an omnivorous matrix that is wrapped in a cellulose skin, and in the centre of the bait is a hydrophobic 1080 core that provides a non-toxic buffer zone in the remainder of the bait. PO is highly target specific and it can produce population knockdowns, equal to, or in excess of traditional baits.

Many pest managers currently use PO in HogHopper™s (a feral pig specific bait delivery device also developed by the IA CRC and marketed by ACTA) to control feral pigs. Whilst effective, the large 250g baits are sometimes removed from the hoppers by smaller pigs, resulting in crumbs being left outside the hoppers that have the potential to be lethal to small mammals. A smaller “bite-sized” bait is the potential solution, hence ACTA and the IA CRC tested a smaller (70 gram) version of the PIGOUT® registered product to determine efficacy under field conditions when applied in the HogHopper™ and also determined the stability of 1080 in the PIGOUT® Econobait (PO Econobait) when the 1080 is not contained within a central core (**PIGOUT® Econobait sub project see below)**) but spread throughout the bait matrix.

This project aimed to develop , field trial and register the 1080 based "PIGOUT Econobait" – a smaller and cheaper bait, and secondly to develop, field trial and register a delivery process for a new active, sodium nitrite as a concentrate or liquid form (that could be applied to grain), providing more options for pig management.

**Summary by sub projects:**

**PIGOUT® Econobait sub project**

Baits were prepared using the same matrix as the registered PIGOUT® bait, but with the 1080 mixed through the matrix instead of in an encapsulated core. The baits were manufactured as a smaller 70g bait, compared to the PIGOUT® bait which is 250g. A large scale field efficacy trial in Dirranbandi, Queensland achieved feral pig population decline of 78% at bait stations and independent monitoring stations. This level of efficacy exceeds the APVMA required 70% mark for registration.

However, stability data did not meet acceptable levels with the APVMA and the registration of product was rejected in May 2016 on this ground. In order to gain registration approval, the stability data had to demonstrate that the active constituent (1080) would still be within specifications within the proposed 1 year shelf life.

ACTA has since continued the project using a different matrix, with the aim of achieving the objective of having a smaller bait available that addressed the stability issues raised by the APVMA. This product is still in development.

**Sodium Nitrite Concentrate sub**

ACTA and the IA CRC assessed multiple methods of protection and masking of SN with bait stability and bait acceptance being evaluated. This involved extensive laboratory and field assessment.

The aim has been to minimise detection of saltiness by pigs and prevent degradation of SN in the bait, while simultaneously achieving rapid dumping (high bioavailability) of the full SN dose when the animals ingest the bait.

Subsequently a series of pen and free-range trials were undertaken using a candidate pour on microencapsulated sodium nitrite (meSN) slurry. A series of trials were undertaken at Mount Hope western NSW, on both free-range and penned feral pigs. During these trials, we assessed the palatability and lethality of several different meSN concentrations, to identify which concentration could produce maximum, and rapid, bait-uptake and consistent death.

Despite some feral pigs being killed, the efficacy was not sufficient to meet APVMA requirements for successful product registration. The relative failure of field trials of all variations of sodium nitrite concentrate indicates that no further testing is warranted at this stage. The work undertaken on this sub-project has increased knowledge of sodium nitrite and methods of micro-encapsulation to the benefit of the MLA-IA CRC project NBP.0502 (HOGGONE®).

With the completion of bait formulation, manufacturing processes and micro encapsulation processes of sodium nitrite for HOGGONE®, it was determined that composition of matter patents were both achievable and desirable. Patents have been applied for in both the USA (15/747,311) and Australia (2017301076). Both the US and Australian applications entered the national phase of the registration processes in late January 2018

**Sodium Nitrite Patent sub project**

The use of Sodium Nitrite as a toxin in omnivores is a patent already held by Invasive Animals Limited. This sub project was to cover any additional ‘composition of matter’ patents that could be registered to further enhance the patent protections around the use of sodium nitrite.

With the completion of bait formulation, manufacturing processes and micro encapsulation processes of sodium nitrite for HOGGONE®, it was determined that composition of matter patents were both achievable and desirable. Patents have been applied for in both the USA (15/747,311) and Australia (2017301076). Both the US and Australian applications entered the national phase of the registration processes in late January 2018. The national phase of the registration phase is, typically, 3 to 4 years in the USA and 2 to 4 years in Australia.

In summary:

APVMA did not support registration of a "PIGOUT Econobait" due to product instability.
Following conduct of extensive development, testing and field evaluation, the sodium nitrite concentrate product proposed at the commencement of the project was not progressed - evaluation did not warrant progress as a commercial product registration application to the APVMA.

# Table of contents

# 1 Background

Meat and Livestock Australia has previously supported the Invasive Animals Cooperative Research Centre (IA CRC) and Animal Control Technologies Australia (ACTA) to develop the 1080 (sodium fluoroacetate) PIGOUT® bait (where 1080 is enscapulated), the more welfare and environmentally friendly sodium nitrite HOGGONE® bait, and indirectly the HogHopper™ feral pig-specific bait delivery device.

MLA project NBP.0502 with the IA CRC was further developing the active ingredient (sodium nitrite) for HOGGONE®. NBP.0502 was developing a solid or paste bait based on sodium nitrite encompassed stably in an attractive and palatable matrix that can be marketed as a commercial product to humanely and reliably kill feral pigs.

To complete the suite of feral pig control tools graziers/the market desired a cheaper bite-sized PIGOUT® bait (70g vs existing 250g 1080 based bait) that could be deployed in hoppers but with 1080 spread throughout the bait (i.e. not encapsulated), and a sodium nitrite concentrate that can be added to grain.

This project aimed to develop the 1080 based "PIGOUT® Econobait" and secondly a delivery process for a new active, sodium nitrite as a concentrate or liquid form (that could be applied to grain), providing more options for pig management.

# 2 Project objectives

The objectives of this Project are:

1. To develop, field trial and register a PIGOUT® Econobait;
2. To develop, field trial and register a sodium nitrite concentrate; and
3. To obtain patent protection on product final embodiments containing micro-encapsulated sodium nitrite.

# 3 Methodology

## 3.1 PIGOUT® Econobait

Prototype 70g (bite-sized for feral pigs) baits were prepared by ACTA for field testing in the HogHopper™ in the Macquarie Marshes Nature Reserve, NSW, AND Dirrabandi in QLD.
The main issues assessed were:

a) that baits are not removed from the hoppers for consumption (a problem with the existing 250g PIGOUT® bait),

b) how many baits individual pigs of varying sizes consume; and

c) bait palatability and longevity in the hopper environment.

Such data was also directly relevant to the future registration of a solid form HOGGONE® bait, should one be developed.

Field trial data was compiled for a registration submission to the APVMA.

## 3.2 Sodium Nitrite Concentrate

This sub-project was conducted in parallel with the MLA-IA CRC project NBP.0502 (HOGGONE®), developing a bait matrix (solid/semi solid) for sodium nitrite

Various formulations of a micro-encapsulated/protected sodium nitrite paste were prepared by ACTA for taste and stability testing.

The preferred formulations were tested on grain in the Macquarie Marshes Nature Reserve, NSW, and other potential locations, including in Queensland with Biosecurity Queensland personnel.

Baiting for the all trials was undertaken according to the following four stage process:

**Stage 1**: Offer plain wheat in a series of trails to cluster the feral pigs at the bait station.

**Stage 2**: Offer placebo pour on slurry mixed with wheat in trails to confirm the slurry is palatable without meSN.

**Stage 3**: Offer toxic pour on slurry mixed with wheat in trails to confirm the slurry was palatable and lethal with meSN.

**Stage 4** (free-range trials only): Offer placebo pour on slurry mixed with wheat in trails to determine whether any animals remained post toxic baiting. This stage was not undertaken in the pens as it was possible to count any survivors.

Throughout all of the trials, the bait stations were monitored with Reconyx HC600 remote cameras. Bait stations were also visited daily to record bait-uptake and replace bait. Carcass and vomit searches were undertaken in the poisoning stage, during which, the number of dead animals were counted and their details were recorded (weight, gender and colour); a photograph and GPS waypoint was also taken.

Studies were undertaken near Roma in south-western Queensland. HogHopper™s were positioned at feral pig hotspots and were loaded with free-feed bait material (fermented grain). During this time, HogHopper™s were set to the free-feed position (doors fixed partially open) to encourage feral pigs to feed (Fig. below).

**Figure -** Feral pigs feeding from a HogHopper™ that is set to the free feeding position.

Free-feeding continued until the number of visiting individuals had begun to plateau and also once it was seen that pigs were feeding from both sides to remove placebo slurry treated grain.

Placebo grain was wheat grain mixed with other material, one being used to both mask flavour and act as a binder for the sodium nitrite in the toxic version, thus ensuring consistency of materials between the placebo and the toxic grain.

To the human observer, these formulations smelled highly attractive and the pigs readily ate the placebo material. Preliminary testing at ACTA showed no obvious reaction between the nitrite (even if unprotected) and the coating materials used to form the test slurries.

Prior to this time pigs had been encouraged to congregate at the stations by offering wheat, that had been moistened to allow some fermentation and also flavoured with molasses and a sugar cane derivative called Carasweet (which is used to encourage piglets onto creep feed during weaning in the pig industry). This is an industry standard grain mix used to bait pig traps or deliver poisons such as 1080 to feral pigs.

Once pigs were known to be eating the placebo treatments from both sides of the hoppers the placebo was scooped and swept out of one side of each hopper and replaced with the nitrite-treated test material. This grain contained the same coating materials plus the required quantity of protected sodium nitrite (SN) to achieve 20 grams of sodium nitrite a.i. per 200 grams of grain. Each test formulation was adjusted for stated % SN in the protected formulation.

Field trial data was compiled, and then assessed to determine if an application was warranted to the APVMA.

## 3.3 Embodiment Patent

Nearly $100,000 had been spent by the IA CRC, with some financial assistance from ACTA, on the international patent NITRITE SALTS AS POISONS IN BAITS FOR OMNIVORES (WO/2008/104028).

This initial patent included feral pigs, possums (NZ) and rodents and was very broad in its claims and may be challenged in the future. To ensure return-on-investment from HOGGONE® and a nitrite concentrate a patent on the final embodiment of products containing sodium nitrite is necessary.

ACTA/IA CRC used their existing patent attorneys, Davies Collison Cave, to file a provisional patent in Australia after seeking a validity opinion from US patent attorneys.

A complete patent is proposed to be prosecuted in Australia and the USA

# 4 Results

## 4.1 PIGOUT® Econobait

A trial was conducted determine the effectiveness of HogHopper™ delivered PO Econobait for controlling feral pigs in Dirranbandi, Queensland. The trial was undertaken on “Booligar Station”, which is a large agricultural property that is used for beef cattle production and cropping. HogHoppers were positioned at all feral pig hotspots at the site and remote cameras were installed at each HogHopper™ to record feral pig interactions.

Independent monitoring stations were also created, where remote cameras were placed near dams or on travel pads to gather an index of feral pig abundance away from the hoppers. Free-feeding was undertaken with dry wheat and non-toxic PO Econobait until the number of feral pigs visiting each HogHopper™ had reached a plateau (Fig. below).

**Figure –** feral pig free feeding from a HogHopper™ during the PO Econobait trail at Dirranbandi.

Thereafter, toxic (1080) PO Econobait was deployed over four consecutive days. On the first night of toxic baiting 887 toxic PO Econobait was consumed by a potential 136 feral pigs, an average of 6.5 baits per pig.

The results showed a significant ($t_{(2)}$=11.82, $p$=0.007) decline in the mean number of feral pigs visiting HogHopper™s per night before (134 ± 7 SE) and after (30±3 SE) toxic baiting; a 78% reduction.

A significant ($t_{(2)}$=4.37, $p$=0.049) and simultaneous 78% reduction was also achieved in the mean number of feral pig passes per camera, per night, at the independent monitoring stations before (68 ± 12 SE) and after (15±1 SE) baiting.

High bait uptake and simultaneous declines in the mean number of visits at bait stations and the mean number of passes at independent stations indicated considerable lethal poisoning, as opposed to toxic bait aversion.

Therefore, the trial showed that HogHopper™ delivered PO Econobait can provide population reductions that exceed the Australian Pesticide and Veterinary Medicine Authority required 70%.

ACTA also established a program to improve assays for 1080 in pest baits. We have worked with experts at ACS Laboratories in Melbourne to devise a direct detection method using a very high technology Liquid chromatograph column that can separate 1080 from other materials in aqueous extracts of baits. The 1080 is then detected using LC-MS with a $C_{14}$ labelled 1080 internal standard to account for detection losses.

This assay was then specifically validated for extraction and detection of 1080 from the PO Econobait matrix, as it was required that new stability data is provided on this product to support APVMA registration.

The assay was fully validated for Linearity, Precision, Specificity, and Accuracy. All recovery processes were confirmed using internal standards.

The 1080 assay and extraction procedures were used to determine the shelf stability of 1080 in PO Econobait under conditions of high temperature storage (>2 weeks at 52°c).

Unfortunately, on review of the registration application, the APVMA decided to refuse the registration on the grounds of product stability. In order to gain registration approval, the stability data had to demonstrate that the active constituent (1080) would still be within specifications within the proposed 1 year shelf life.

ACTA has since continued the project using a different matrix, with the aim of achieving the objective of having a smaller bait available that addressed the stability issues raised by the APVMA. This product is still in development.

## 4.2 Sodium Nitrite Concentrate

All formulated products, except unprotected SN, remained stable on manufacture and during transit to the study site. The moistened grain containing unprotected nitrite showed marked colour change to orange within days of preparation. Additional samples of slurry and normal grain were also taken to the site without premixing in the grain, so that absolutely fresh material could also be tested.

None of the formulations showed any signs of nitric oxide release, even when pails of each formulation were smell tested immediately after opening for the first time. All smelled (to human experience) to be highly palatable and none differed from the placebo slurries at the time of test, other than the colour change in formula mentioned above. It was anticipated that the pigs would find the protected nitrite treated grains very palatable.

For assessment of the toxic baits, one side of the hopper was cleaned of placebo grain residues and the side was filled with 5kg of treated grain with 5 kg of placebo grain added to the opposite side of the hopper.

This methodology provided the animals with a simple free choice test for uptake of nitrite treated vs. control slurry treated grain.

In some sites the pigs did not revisit the test hopper on the evening of the test. In this case the bait was left out and rechecked daily for four days. In some sites, despite initial set up over some weeks, the pigs did not return and no test could be concluded.

Due to low pig visitation rates to hopper placements it was not possible to get more than one replicate of each formulation tested.

For those hoppers where pigs did revisit the results were very clear, there was immediate and near total aversion to all of the nitrite treated grain slurries, regardless of the type of protection method used. Uptake of the placebo coated grain continued with 100% uptake from that side of the hopper in all tests.

Unprotected and protected nitrite formulations were all rejected.

These results, though only single tests of each formulation, indicated strong aversion to the grain that had been treated with all three of the protected SN formulations tested and for the

unprotected nitrite negative control (as expected). Treated slurries were all rejected due entirely to the presence of the nitrite (no other differences between sides and placebo slurry treated grain was taken).

ACTA and the IA CRC continued to assess several methods of protection and masking of SN with bait stability and bait acceptance being evaluated.

The aim was to minimise detection of saltiness by pigs and prevent degradation of SN in the bait, while simultaneously achieving rapid dumping (high bioavailability) of the full SN dose when the animals ingest the bait. This is a balancing process with conflicting aims that must be mutually satisfied. With a highly reactive chemical such as sodium nitrite, this has thus far proven difficult. Further testing of meSN options has resulted in a decision by ACTA and IA CRC to settle on the encapsulation process first proposed by Connovation Limited. However it is considered that this process can be further improved by utilising fluid bed coated product as opposed to pan coated product (original Connovation process). This improvement appears to have been successful in Hoggone trials in the USA but has not been replicated in the SN Concentrate sub project.
ACTA and the IA CRC have assessed several methods of protection and masking of SN with bait stability and bait acceptance being evaluated.

A series of pen and free-range trials have been undertaken using what was/is our greatest version of a pour on microencapsulated sodium nitrite (meSN) slurry. Several trials were undertaken at Mount Hope western NSW, on both free-range and penned feral pigs. During these trials, we assessed the palatability and lethality of several different meSN concentrations, to identify which concentration could produce maximum, and rapid, bait-uptake and consistent death.

Throughout all of the trials, the bait stations were monitored with Reconyx HC600 remote cameras. Bait stations were also visited daily to record bait-uptake and replace bait. Carcass and vomit searches were undertaken in the poisoning stage, during which, the number of dead animals were counted and their details were recorded (weight, gender and colour); a photograph and GPS waypoint was also taken.

Feral pigs (free range and penned) readily and rapidly consumed the grain mixed with placebo pour on slurry; indicating that the slurry itself was palatable. There was also very little change in feeding behaviour when the toxic slurry was mixed with grain at a low w/w rate of meSN.

However, all feral pigs returned to feed the night after poison baiting indicating this concentration was insufficient to cause fatal Methaemoglobinaemia. When the concentration was increased, there was a slight change in feeding behaviour with feral pigs generally consuming toxic bait more slowly, resulting in a knockdown rate of at least 17% of free range pigs and 30% of penned pigs. A final pen trial, with a further increased concentration, was undertaken 12 months later resulting in a knockdown of 31%. Again, the animals showed differences in feeding behaviour when they were feeding on the toxic bait material compared to the non-toxic bait material, suggesting they were less interested in the toxic bait.

Despite some feral pigs being killed, the efficacy was not sufficient to meet APVMA requirements for successful product registration.

ACTA has exhausted all potential options and tested the most promising formulation to failure, and decided to cease this project. The approach had merit and could be pursued again at some point in the future, as it is an excellent product in theory. It just means waiting until the technology required to manufacture a stable, palatable and efficacious pour on product becomes available.

## 4.3 Embodiment Patent

The use of Sodium Nitrite as a toxin in omnivores is a patent already held by Invasive Animals Limited. This sub project was there to cover if any additional 'composition of matter' patents could be registered to further enhance the patent protections around the use of sodium nitrite within a bait matrix and/or the micro-encapsulation process.

With the completion of bait formulation, manufacturing processes and micro encapsulation processes of sodium nitrite for HOGGONE®, it was determined that composition of matter patents were both achievable and desirable. Patents have been applied for in both the USA (15/747,311) and Australia (2017301076). Both the US and Australian applications entered the national phase of the registration processes in late January 2018.

# 5 Conclusions/recommendations

## 5.1 PIGOUT® Econobait

Whilst the submission for a registered product to the APVMA was refused, the success of the field work suggests that the option of cheaper bite-sized PIGOUT® bait (70g vs existing 250g 1080 based bait) that can be deployed in hoppers but with 1080 spread throughout the bait (i.e. not encapsulated) remains a valid proposition. The HOGGONE project (NBP.0502) has outlined a highly palatable bait matrix as a potential carrier for 1080.

ACTA will continue to work on this project using an alternative matrix to improve stability. Once this has been formulated, field tested and stability tested a registration will be submitted to the APVMA.

## 5.2 Sodium Nitrite Concentrate

The relative failure of field trials of all variations of sodium nitrite concentrate has led us to conclude that no further testing is warranted at this stage. The work undertaken on this sub-project has increased knowledge of sodium nitrite and methods of micro-encapsulation to the benefit of the MLA-IA CRC project NBP.0502 (HOGGONE®). However, it is anticipated that process of micro-encapsulating sodium nitrite will be improved in the future. As knowledge continues to be developed and enhanced, this may allow for a future project examining different encapsulation processes and carrying agents with the final result of achieving and in-field, shelf stable concentrate for adding to grain for feral pig control.

## 5.3 Embodiment Patent

No further work is required as this sub-project is now at the stage where composition of matter patents have been submitted in respect to the micro-encapsulation process of sodium nitrite in both Australia and the USA. Both patent applications entered the national phase of the registration processes in late January 2018. 22$^{nd}$ January 2018 for the Australian patent application and 24$^{th}$ January 2018 for the USA patent application.

The national phase of the registration phase is, typically, 3 to 4 years in the USA and 2 to 4 years in Australia.