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Prepared by: Frank Gigliotti
General Dogsbody, Technical Services

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A Multi-Dose Ejector (MDE) for Control of Predator Pests

Stage1: Development of prototype

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Abstract

Predator pest species pose a serious threat to Australian agriculture and its biodiversity. Control of these species in remote and regional Australia is difficult and problematic due to inaccessibility, a small labour force and the immense size of the area infested. Stage 1 of the MLA project **B.AHE.0067** furthered previous research into the development of a chemical dosage dispenser or multi-dose ejector (MDE). The MDE provides a target specific, multiple-dose toxin delivery system, capable of remaining field active over an extended period of time with minimal operational maintenance, through the use of new innovative technology.

Pen trials conducted during Stage 1 evaluated the components of the MDE successfully demonstrating its capabilities, highlighting also shortcomings that need additional investigation to enable a commercially viable and effective product. This phase has confirmed the formulation and delivery of an 'aerosol toxin formulation', and the fabrication of a 'polymer bait' with the durability to withstand multiple visitations by foxes, and the chemical stability to contain an attractant lure. The exclusion collar and pull force technologies provide a target specific delivery technique which significantly reduces the risk of exposure to non-target species such as native fauna and working dogs. The aerosol toxin formulation has not been assessed on dogs and non-target species to date. Para-aminopropiophenone (PAPP), was selected as the toxin of choice for these trials due to initial restrictions placed on identified alternative toxins. With the current MDE configuration some sub-lethal results occurred using a standard on/off valve, however a metered dose valve would overcome this issue.

Based on the toxin PAPP the required lethal dose for dogs and foxes cannot be delivered with currently available metered dose valves. Therefore, an alternative toxin that is effective at a lower dose rate could be used.

Stage 2 of the project will consolidate the MDE components, provide a clear direction forward in terms of the desired toxin for aerosol formulation using a metered dose valve, to target both foxes and dogs. A potential range of polymer lures to target seasonal and individual attraction will also be developed. Field trials will be conducted to confirm the target specificity of the MDE for non-target species, demonstrate the efficacy of the MDE in a fox control program and examine its effectiveness for the control of wild dogs.

The MDE will provide land managers with a cost effective control technique, which enables targeted control programs without restraints of seasonal accessibility or the continual replacement of baits. The MDE allows for the establishment of long term sentinel sites to protect agricultural values and create buffer zones between private and public lands.

Executive Summary

The focus of this research is to provide land managers with a cost effective control technique in remote and regional Australia that enables the timely and effective management of predator pest species. These species, the European red fox and wild dogs (dingo hybrids and domestic dogs gone wild), pose a serious threat to farmer's livelihood and wellbeing.

The Multi-Dose Ejector (MDE) enables targeted control without restraints of seasonal accessibility, or available manpower, by providing a field baiting technique with multi dosing capability, without the continual need to replenish baits. The MDE can be deployed to provide long-term sentinel sites to protect agricultural values and create buffer or exclusion zones between private and public lands.

Stage 1 of the MLA project **B.AHE.0067** furthered the research commenced in 2009 under a Caring for our Country (CfoC) Federal Open Grant, which demonstrated proof of concept of a chemical dosage dispenser or multi-dose ejector. The research focused on developing an innovative new technique for the control of invasive predator species in remote and regional Australia for the protection of both agricultural and conservation values.

This research allowed an evaluation of previous data and furthered the development of the various components of the MDE prototype via controlled pen trials. Five main objectives were set as part of this 'development stage' of the project. These objectives are listed below along with a description of the successful output or achievement.

- **The evaluation and preparation of a toxicant-aerosol formulation:** - resulted in the preparation of three Para-aminopropiophenone (PAPP) formulations, each resulting in symptoms of toxicosis in the target species (fox) during pen trials.

- **Refinement and efficacy of the delivery system:** - achieving an aerosol delivery system incorporating target specific pull force., With the current MDE configuration and using Para-aminopropiophenone (PAPP) some sub-lethal results occurred due to the use of a standard on/off valve, however a metered dose valve (mdv) would overcome this issue.

- **The fabrication of an efficacious deployment anchoring system:** – the resulting 'ground screw' providing a secure deployment technique and protective housing for the MDE delivery system.

- **The refinement and efficacy of the exclusion and open collar:** - provides the means of delivering the aerosol formulation directly into the target animals' mouth. Additionally, the exclusion collar provides a target specific means of controlling the European red fox while greatly reducing the risk of toxin exposure to non-target species, including working dogs. Trials have demonstrated that foxes readily activated the MDE using both the open and exclusion collar once initial neophobia was overcome.

- **The development of a prototype polymer-lure bait matrix:** - resulted in the fabrication of a polymer matrix, which provides both durability and the chemical stability to allow the addition of scents and lures without the risk of denaturing the additive.

In brief, the project has progressed from proof of concept to preparedness for field trials. The research conducted during this first stage of the project has successfully demonstrated that an aerosol toxin formulation can be effectively delivered in a target specific manner using the MDE for control of foxes. The anchoring system was demonstrated to secure the device, preventing removal of the bait/toxin from the site of deployment and provided a secure housing for the MDE delivery system and aerosol canister. Work to date on the manufacture of a long-life bait has shown that polymer technology can be used to provide a durable and chemically stable matrix to allow the incorporation of selected lures and scents to achieve an attractive long-life field bait.

Assessment of the MDE components and subsequent pen trials were initially proposed using a 1080 aerosol formulation due to its current registration status and high water solubility. However the Animal Ethics Committee (AEC) over-seeing the research did not permit the proposal on the grounds of the humaneness of 1080 toxicosis. An alternative formulation was proposed containing Copper indomethacin (CuI) . The co-administration of a 1080 / CuI formulation was shown to significantly reduce the incidence of retching in foxes and also reduced the duration of the toxicosis compared to 1080 alone. However, this alternative proposal was also rejected and an alternative toxin was sought. Sodium cyanide (NaCN) was initially chosen as the replacement with consent from the AEC. However its indiscriminant nature and potential operator safety issues excluded it for aerosol formulation. Aerosol companies were reluctant to use the active therefore further development of a toxin formulation to date has not been possible. Pen trial work was all based on PAPP, itself, not yet approved by the Australian Pesticides & Veterinary Medicines Authority (APVMA).

Application to dogs will require additional testing to overcome constraints by animal ethics approval, commercially available delivery mechanisms, and an "acceptable" toxin to enable commercial production and delivery.

While project objectives were successfully achieved, within the constraints of animal ethics approval, and OH&S of a production system, the research identified a number of critical aspects, which require further investigation in order to achieve a final commercially viable product. These include:

1. **Limitations with the delivery of a lethal dose mass using PAPP.**

   The lethal dose mass required with PAPP is comparatively large i.e. 1.0ml for fox and 2.5ml for wild dog at the current formulation concentration of 20%. The ‘standard’ on/off aerosol valve currently used is reliant on the animal activating the triggering mechanism for sufficient time to expel the required lethal mass. As identified in the pen trial results, the dose delivered can therefore vary considerably. The large dose mass required, particularly for the control of wild dogs, most likely means that PAPP may not be able to be delivered from an aerosol unless the dose mass can be significantly reduced. Commercially available mdv’s with the capacity to deliver a lethal dose of PAPP to either species are not available.

2. **The availability and formulation of an alternative toxin to overcome identified ‘dose mass’ delivery issues.**

   PAPP was the chosen toxin for Phase 1 of the study. However, delivering a required lethal dose mass with PAPP is problematic. . As such, alternative toxin options, which require lower dose rates, should be investigated for use with a commercially available metered dose valve. Potential options include

cyanide ions and sodium monofluoroacetate (1080) formulations. Each alternative toxin requires further investigation to determine availability, potential registration and delivery options.

3. **The acquisition of an appropriate capacity 'metered dose valve' that cost effectively ensures the accurate and reliable delivery of a lethal dose mass.**

   The use of a mdv would ensure that a lethal dose is reliably and consistently delivered on each activation. The largest commercially available mdv at present is 0.2ml. At this capacity both of the suggested alternative toxins can be potentially delivered via an aerosol formulation. However, a lethal dose of PAPP for either dog or fox control cannot be delivered using a 0.2 ml mdv.

4. **Evaluation of specific lures for impregnation within the polymer matrix with a focus on (i) attractiveness, (ii) field durability and (iii) scent retention.**

   Trials to date have confirmed the choice of polymer and the ability to impregnate the matrix with a lure without denaturing that lure. Further work is required in i) the selection of appropriate attractive lures and ii) the impregnation and scent retention of the polymer matrix.

Stage 2 of the project will consolidate the MDE components for both fox and dog control, provide a clear direction forward in terms of the desired toxin for aerosol formulation and deliver a potential range of polymer lures to target seasonal and individual attraction. Field trials will be conducted to confirm the target specificity of the MDE for specific non-target species, demonstrate the efficacy of the MDE for fox control, and examine its effectiveness for the control of wild dogs. Field days and community workshops are also planned for this stage to increase awareness, enlist support and potential participation in field trials.

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Background

Predator pest species such as the European red fox (*Vulpes vulpes*) and wild domestic dog species (*Canis lupus familiaris*), and their Dingo hybrids (*Canis lupus dingo*) pose a serious threat to Australian agriculture and its biodiversity. Control of these species in remote and regional Australia is difficult and problematic due to limited accessibility, a small labour force and the immense size of the area infested. Currently the most effective means of managing the impact of these predator pests is through the use of broad scale poison baiting using sodium monofluoroacetate (1080) applied to meat baits. Although some level of success has been achieved through the use of poison baiting, these baits often create a potential risk to other species, and because their longevity and attractiveness under field conditions is limited, they require continual replacement. Another critical issue with current baiting techniques is caching, which is a common behaviour of foxes, where baits are removed and relocated some distance away in a shallow dug-out as a food store. This behaviour creates a potential risk, to both native species and working dogs, of exposure to the poison baits.

Stage 1 of the MLA project **B.AHE.0067** furthered research initiated in 2009 under a Caring for our Country (CfoC) Federal Open Grant (OG08960). This initial research focused on developing an innovative new technique for the control of invasive predator species in remote and regional Australia for the protection of both agricultural and conservation values. The resulting outcome demonstrated 'proof of concept' of a chemical dosage dispenser or multi-dose ejector (MDE) capable of delivering multiple doses, with minimal operational maintenance, over an extended period of time.

The MDE builds on previous research and technology from both here and abroad, incorporating innovative new developments such as polymer engineering technologies, aerosol toxin delivery, and target specific pull force and exclusion collar designs to improve target specificity.

The MDE aerosol toxin formulation and delivery system can provide 20+ doses of a chosen toxin, and safely protects the chemical from environmental degradation. The target specificity of the MDE is achieved through the combination of the 'exclusion collar', derived from the work by Nicholson and Gigliotti (2005) [1] and pull force technology. Marks and Wilson (2005) [2] found that a statistically significant relationship exists between an animal's body mass and the pull force it can exert. By exploiting the size difference between the target species and 31 indentified potential bait consuming native mammals [2], it was determined that 26 of the 31 mammals could be excluded from taking a bait if doing so required a specified minimum pull force. The remaining 5 larger mammal species which includes spotted-tailed quolls (*Dasyurus maculatus*) can potentially be excluded from exposure to the toxin by the use of an exclusion collar, which has been morphologically designed to only allow the long slender snout of a fox to penetrate deep enough to access the bait material [1]. Polymer technology has been used to provide a durable bait matrix able to accommodate multiple visitations by target animals and retain an attractive scent for an extended period of time (potentially up to 2 months), greatly reducing the need for bait replacement.

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1. Nicholson, E. and Gigliotti, F. (2005) Increasing the target specificity of the M-44 ejector by exploiting differences in head morphology between foxes and large dasyurids. Wildlife Research 32: 7333-736.

2. Marks C.A. and Wilson R.L. (2005). Predicting target specificity of the M-44 ejector in south-eastern Australia. Wildlife Research 32:1-6

The design of the MDE is such that, once the target animal is attracted to the bait and exerts the required upward pulling force, triggering the aerosol canister, a dose of toxin is released directly into the mouth of the animal. This mode of action potentially ensures a lethal dose of toxin is delivered, eliminating sub-lethal dosing due to partial consumption of a bait and thereby avoiding learnt aversion behaviour that can occur with current baiting practices. The polymer bait eliminates bait degradation while the deployment technique precludes bait caching or removal. By establishing permanent baiting sites using the MDE, baiting activities can occur in otherwise seasonally inaccessible and/or remote areas. A series of MDE sentinel sites, for example, can create buffer zones between public and private land providing long-term protection to livestock by reducing the dispersal of predators.

Toxin selection

A comprehensive literature review of potential toxins identified 3 potential actives as having the desired characteristics; humaneness, mode of action, formulation potential for aerosol delivery, dose mass requirements, selectivity (native tolerance and native vs. felid/canid sensitivity), potential environmental impacts, and cost. The 3 actives chosen for further consideration were i) sodium monofluoroacetate (1080), ii) sodium cyanide (NaCN) and iii) Para-aminopropiophenone (PAPP).

Assessment of the MDE components and subsequent pen trials were initially proposed using a 1080 aerosol formulation due to its current registration status and high water solubility. However the Animal Ethics Committee (AEC) over-seeing the research did not permit the proposal on the grounds of the humaneness of 1080 toxicosis. This issue is difficult to substantiate and has become an emotive argument. The retching and manic running activities commonly associated with 1080 poisoning occur early in the toxicosis when the animal is clearly conscious and responsive to external stimuli [3,4]. Some pain and distress thereby can be assumed prior to the collapse of the animal. Once collapsed, however, it is thought that the animal is unconscious and therefore unable to perceive pain during the convulsions and spasms that occur prior to death.

An alternative formulation was proposed containing Copper indomethacin (CuI) (Nature Vet, Glenorie, Australia) a potent non-sedating analgesic, reported as having central nervous system activity as well as peripheral analgesia [5]. Previous research [4] has shown that symptoms associated with 1080 toxicosis are reduced by the combination of the toxin with CuI. The co-administration of a 1080 / CuI formulation was shown to significantly reduce the incidence of retching in foxes compared with those receiving 1080 alone. The combination also reduced the duration of the toxicosis from the onset of first symptoms until death compared with the toxicosis produced by 1080 alone [4]. However, this alternative proposal was also rejected by the AEC committee and an alternative toxin was sought.

Sodium cyanide (NaCN) was initially chosen as the replacement with consent from the AEC. Though not currently registered for use as a predator pest control agent in

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3. Marks, C. A., Hackman, C., Busana, F. and Gigliotti, F. (2000). Assuring that 1080 toxicosis in the red fox (Vulpes vulpes) is humane: Fluoroacetic acid (1080) and drug combinations. Wildlife Research 27, 483-494.

4. Marks, C. A., Gigliotti, F. and Busana, F. (2009). Assuring that 1080 toxicosis in the red fox (Vulpes vulpes) is humane. II. Analgesic drugs produce better welfare outcomes. Wildlife Research 36, 98-105.

5. Barnett, J. and Jongman, E. (1996). Techniques for the assessment of humaneness and measuring pain and stress in animals. In 'Humaneness and Vertebrate Pest Control'. (Eds P. M. Fisher and C. A. Marks.) pp. 22–26.

Australia, the Australian Pesticides & Veterinary Medicines Authority (APVMA) is considering the active for registration as a vertebrate pesticide for use with the M-44 ejector (US manufactured single shot control device). Delivered orally, NaCN produces hydrogen cyanide on contact with the mucus membrane and moisture in the mouth [6]. The inhalation and absorption of this gas results in the rapid onset of toxicosis and death. Cyanide is a very toxic chemical asphyxiant and inhibits cytochrome oxidase preventing oxygen utilization leading to cytotoxic anoxia. Death results from central nervous system failure and anoxia although venous blood remains oxygenated [6]. Death by NaCN poisoning is regarded as a humane mode of action due to the rapid onset of toxicosis and suspected onset of insensibility before convulsions and death. Sodium cyanide is also high water soluble, however its indiscriminant nature and potential operator safety issues excluded it for aerosol formulation. Aerosol companies were reluctant to use the active therefore further development of a toxin formulation to date has not been possible.

Para-aminopropiophenone was thereby selected as the toxin with which to progress the project. The active is currently being assessed by a number of agencies as a potential alternative to 1080 because of its humane mode of action and high level of susceptibility of canids [7]. PAPP converts oxygen-carrying haemoglobin to methaemoglobin thereby inhibiting the supply of oxygen to the brain and tissues. Animals that consume a lethal dose become progressively lethargic, slipping into unconsciousness and then death due to brain hypoxia with few symptoms of toxicosis[8]. Previous trials with foxes have demonstrated that animals progressively became more lethargic and collapsed 14-25 minutes after dosing. A mean time to death of 43 minutes has been achieved with no observed distress behaviour [8].

Polymer technology

Shortcomings of current predator baits are their susceptibility to environmental degradation, which greatly reduces their viability in the field, and their palatability and attractiveness to the target species. The attractiveness of the bait determines whether the target animal is lured to the bait. Its palatability will then determine whether it is immediately eaten, cached or ignored. Current poison baits need to be fully consumed as partial consumption leads to sub-lethal dosing and potentially learnt aversion behaviour due to the unpleasant symptoms associated with toxicosis.

The use of polymer technology to create ‘the bait’ for the MDE potentially overcomes the issue of bait degradation. The involuntary aerosol delivery of the toxin, directly into the animal’s mouth and subsequent absorption through the mucus membrane, also removes the palatability need as there is no ingestion of a bait required. The attractiveness of the lure is therefore the crucial element of a polymer bait.

During previous research a comprehensive assessment of 22 potential polymer materials ranging from polyethylene to silicon and rubber, was conducted to identify a suitable matrix for the proposed application.

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6. Marks, C. A. and Gigliotti, F. (1996). 'Cyanide Baiting Manual: Practices and guidelines for the destruction of Red Foxes (Vulpes vulpes) ' Department of Natural Resources and Environment.

7. Fleming, P.J.S., Allen, L.R., Lapidge, S.J., Robley, A., Saunders, G.R. and Thomson, P.C. (2006) A strategic approach to mitigating the impacts of wild canids: proposed activities of the Invasive Animals Cooperative Research Centre. Australian Journal of Experimental Agriculture, 46: 753-762

8. Marks, C. A., Gigliotti, F., Busana, F., Johnston, M. and Lindeman, M. (2004b). Fox control using a para-aminopropiophenone formulation with the M-44 ejector. Animal Welfare 13, 401-407.

A number of considerations were applied including;

- the durability of the polymer to withstand multiple visitations by target animals,
- lure compatibility with the polymer to retain integrity,
- lure permeability through the polymer and
- cost and availability.

The results from these trials indicated that polyethylene type polymers provided the necessary features.

A further review of current lures and attractants commonly used in canid control programs identified that attractant compounds elicit different behaviours, from simply luring the animal to the site, to eliciting various responses such as defecation, scratching/digging, rubbing/rolling etc. For this application the 'attractant' is required to provide olfactory stimulus to draw the target animal to the site and also elicit a biting / pulling behaviour. A number of compounds identified from the review as eliciting the required behaviour are to be assessed for potential use within the polymer matrix.

Three options for the fabrication of a prototype bait head were initially considered for evaluation. These were impregnated polymer heads, polymer heads with lure reservoirs or wells and the use of natural materials such as rawhide to hold the lure. The choice of natural materials to hold the lure was not pursued, as the applied lures were prone to climatic degradations as well as bacterial and insect attack, thereby reducing their longevity in the field.

Project Objectives

Achievement Criteria		Due Date
1	Execution of agreement **[Achieved]**	15-Sep-2011
2	Evaluation of research data and selection of toxin completed **[Achieved]**	15-Jan-2012
3	Preparation of toxicant-aerosol formulation completed. Refinement of MDE delivery system completed **[Achieved based on PAPP]**	11-Nov-2012
4	Efficacy trials of the various components completed (MDE delivery system, toxicant-aerosol formulation, exclusion collar(s), deployment technique) Fabrication of prototype polymer- lure formulation completed **[Achieved]**	1-May-2013
5	Efficacy trials of the prototype polymer bait completed with foxes **[Achieved]**	15-Jul-2013
6	Final report to MLA on AHE.0067 received and accepted including: • 6.1 Progress report submitted to MLA 6.2 indicating completion of each project objective and contracted outputs • Final budget report submitted to MLA • Recommendations on a Phase 2 and subsequent stages of the project to deliver a commercially available MDE	15-Sep-2013

Objectives (Stage 1): Activities completed during the development stage:

Toxicant-aerosol formulation

· Toxicant Selection
· Aerosol formulation of selection
· Evaluate data in respect to toxicant-aerosol options
· Prepare toxicant-aerosol formulations for pen trials
· Pen trials of toxicant-aerosol formulation efficacy

MDE delivery system

· Refinement of the metered dose valve - lethal load and delivery
· Refinement of the MDE pull force mechanism
· Reliability pen trials of the pull force mechanism
· Efficacy pen trials to assess MDE delivery (dose delivery and lethality)

Deployment technique

· Field deployment efficacy pen trails in different soil types

Exclusion collar(s)

· Efficacy pen trials using target species with various collar types
· Design Refinement (as required)

Long-life bait material

· Evaluate data - polymer/lure affinity and options
· Fabricate polymer / lure formulations for testing
· Efficacy (pen) trials to assess 'bait' (i) attractiveness, and (ii) durability (with a particular focus on scent retention and field life 'toughness'

Methodology

The body of work during Stage 1 of the project culminated in pen trials where each of the components which make up the MDE were tested to determine their efficacy against one of the target species, the European red fox. It is anticipated that the MDE will provide a control technique for both foxes and wild dogs. This will entail the repeat a number of trials with dogs once animals and facilities become available.

The Methodology and Results provide a brief summary of the trials and achievements. A more descriptive explanation can be found in the relevant Appendices.

All animal experimentation was conducted at the Department of Environment and Primary Industries (DEPI) facilities in Frankston Victoria under the relevant permits.

- DEPI Pest Animal Research Permit RE73-13/14
- DEPI Scientific Procedures Fieldwork Licence SPFL348
- DEPI Wildlife and Small Institutes Animal Ethics Committee Permit 05.12
- DEPI Ministerial Approval for 'death as an end-point' Ref IR/04/0187

Two separate series of trials were conducted, one to assess the efficacy of the MDE components, the other to further investigate the potential of polymer technology to create a durable, stable matrix to contain selected lures.

1. The evaluation of the MDE components was conducted in combination during lethal pen trials. This approach reduced the number of animals required while still allowing the examination of the efficacy of each of the MDE components (Appendix 1). The components assessed during the trials were:

> The toxicant-aerosol formulation – evaluating the efficacy of the aerosol formulation (based on PAPP) and symptoms of toxicosis in foxes.
>
> The MDE delivery system - investigating the effective delivery of a lethal active and reliability of the mechanism, together with the animals' ability and willingness to trigger the unit.
>
> The deployment technique – demonstrating the ability of the 'soil anchor' to secure the MDE.
>
> The exclusion collar - determining the level of neophobic behaviour towards the collar, and the animals ability to place their snout into the collar to access a bait.

Units were deployed using the soil anchor in a number of pens and animals introduced and allowed to voluntarily interact with the MDE over a 16 to 18 hour period (overnight). Each trial was remotely monitored and recorded via infrared video surveillance cameras located within each pen (Appendix 1). Review of the recorded footage enabled examination of behavioural involvement and process of toxicosis in animals during the trial. The lethal trials were conducted using a PAPP aerosol formulation. Spraypack Pty Ltd formulated and supplied the formulation in aerosol canisters with collaboration from Scientec Research Pty Ltd. The primary PAPP formulation used was composed of:

Propellant, 40g Butane / Propane; Poison, PAPP HCl 20% concentrate solution and Solvent, 90%.DMSO 10% H_2O.

Three formulation variations were used during the trials,

- Primary formulation,
- glycerine form, and
- beef flavoured (primary formulation).

2. Through collaborative investigations with P.D Plastics Aust. Pty Ltd, cast polyurethanes (CPU) were chosen as the polymer of choice due to their abrasion resistance, chemical resistivity, stability in water, relative low cost and of particular importance their ease of processing. Cast polyurethanes are thermoset elastomers, created by the reaction between the two primary chemical components when mixed together. Temperatures during the chemical reaction remain low, unlike injection moulded materials, which are thermoplastics, created under conditions of high temperatures (200^{o}C+). The mixture is poured into a heated mould where the components react to form a solid elastomeric piece. The lower temperature chemical manufacturing process of CPU reduces the risk of denaturing additives thereby allowing those additives to impart different characteristics to the finished product (Appendix 3).

Having chosen the polymer matrix, bait heads were fabricated in 85% Shore A strength as an over-mould onto existing M-44 ejector capsule holders. For the purposes of these preliminary pen trials a number (7) of lures or scents were selected for trial impregnation within the CPU (Appendix 3). The scents were selected after discussion with other researchers and Pest Control Operators, as having an attraction to foxes.

Two prototype bait head designs were tested; i) scent impregnated polymer and ii) polymer bait heads with scent reservoirs or wells. The trials were conducted as a free choice cafeteria-style trial. Due to the number of lure/bait head varieties (8), two sets of 4 combinations were trialled over 2 nights. The location of each bait head in the 'cafeteria' was randomly selected. The sets of 4 bait heads were secured to the ground along the pens' length approximately 2 metres apart, with position 1 located closest to the pen entrance and position 4 located to the rear of the pen. Animals were introduced to the pen and allowed to voluntarily interact over an 18 hour period. The next day the alternative set of 4 bait heads were deployed and the experiment repeated. Fox activity was recorded to ascertain any preference to particular bait heads and potential behaviour elicited by the lure (Appendix 3).

Results

Toxicant-aerosol formulation

Symptoms of toxicosis were seen using each of the 3 PAPP formulations. Time to first symptoms and death were within expectations and supported previous research. The results indicate that the PAPP HCl aerosol formulation and delivery system combined, could deliver a lethal dose mass. The glycerine aerosol formulation was formulated to examine whether a gel / foam formulation increased toxin availability by reducing potential 'splash back' from the aerosol delivery, and improved toxin absorption by adhering to the mucus membrane and mouth cavity of the animal. Results however were inconclusive due to the small sample size and difficulties experienced in delivering a reliably consistent dose using the standard on/off valve of the delivery system (refer to Appendix 2).

Project objectives were achieved, a toxin aerosol formulation was successfully prepared and a lethal outcome was achieved. However, the dose mass delivered using the current standard on/off aerosol valve was totally dependent on the length of

time the animal activated the MDE. This explained the variation in dose rates delivered and the subsequent sub-lethal results (refer to Appendix 2). The anticipated large dose mass required for the control of wild dogs (2.5ml), most likely means that PAPP may not be able to be delivered from an aerosol unless the dose mass can be significantly reduced.

MDE delivery system

The aerosol delivery system provided an ideal multi-dosing capability. The sealed canister prevented contamination and deterioration of the toxin, and allowed the active to be delivered in a purer form improving bio-availability and absorption rates. As toxin transfer is via absorption through the mucus membrane, rather than requiring the animal to ingest a 'bait' (which can be rejected) the time to onset of toxicosis is reduced, as well as the doss mass due to the faster absorption into the blood stream. The results from the trials demonstrate that the design of the delivery system can successfully activate the aerosol canister via the upward motion. The use of rare earth magnets to provide the 3kg 'pull force' performed reliably and consistently, and has the flexibility to increase the pull force to 6kg if required for dog control (Appendix 2).

Sub-lethal dosing of animals during the pen trials was attributed to a combination of i) the open collar, allowing the animal to quickly release the bait, retracting its snout from the collar, and ii) the standard on/off valve currently used. The dose delivered using a standard valve was totally dependent on the length of time the animal activated the delivery system (i.e. pulls on the bait). Video footage of the trials confirmed the startled reaction and quick retraction of the foxes' head in response to the spray of aerosol into their mouth. Animals were unlikely to re-activate the unit though interest in the unit remained high (Appendix 2). The efficacy of the delivery system, in terms of delivering a specific dose mass, lies in the acquisition of a metered dose valve (mdv).

Deployment technique

Trials conducted with foxes have demonstrated that the screw anchor provides adequate resistance against being pulled out of the ground. The sand/sandy loam soils within the pens are regarded as difficult to anchor in due to the lack of structural cohesion. The large flange (10mm) of the screw however, cuts a thread into the soil greatly increasing surface area and maximising resistance to being removed. It is recommended that an undersized auger hole is pre-cut into the soil prior to deploying/screwing in the anchor, to avoid damage. The hollow tubular design of the anchor also provided an ideal protective housing for the aerosol canister.

Exclusion collar(s):

Both the exclusion and open collar types provided adequate access to the bait allowing activation of the MDE. The results indicated 76% of animals tested with the exclusion collar were prepared to place their snout into the collar, 82% animals did so with the open collar. The remaining animals did not interact at all with the units. Both collar types provided the necessary restriction forcing the foxes to take the bait using their front teeth, thereby ensuring the line of 'fire' was directly into their mouth. Recorded video footage during the trials demonstrated an initial level of neophobic behaviour by animals to the collars. However, once overcome, foxes did not appear to have any difficulty in placing their snout into either the exclusion or open collar (Appendix 2).

Long-life bait material

The results from the trial impregnation of CPU with the 8 selected lures showed that all but one, OUTFOXED Pty Ltd – Cat lure, could be successfully incorporated and retain a sufficient level of integrity in terms of smell and flavour to attract an animal. Chemical incompatibility with the ingredients of the cat lure (or part of) was thought to be the cause of the failed impregnation. The resulting material was not cohesive, with multiple lesions throughout the structure.

The pen trials compared the 7 impregnated CPU bait heads with the equivalent 'well type' heads. The results showed a significant preference by animals to the well type bait heads (in relation to time spent at a bait station and activity). This was attributed to the 'reward' (lure taste) animals received from the well type heads when chewing the bait. In contrast the impregnated heads had no reward and so interest declined more quickly. It is, however, important to remember that the role of the polymer bait head is to simply attract the animals and elicit the desire to bite and pull on the bait. Prolonged time at the bait is unnecessary, as long as the animal exerts the required pull force to activate the aerosol. The results demonstrated that impregnation of CPU polymer can be achieved and that their homogeneous and reactive properties allow impregnation with additives, which can impart different characteristics to the bait head matrix (refer to Appendix 4).

Discussion

The distress and neophobic behaviour demonstrated by foxes during the pen trials was attributed to being in captivity. This modified behaviour reduced animal participation in the trials, affecting the results. Conducting the trials during mating season meant that females in particular, were more preoccupied with escaping, due to establishment of territories and den sites. The manner of capture, i.e. the use of lures to attract animals to trap sets, also meant that animals were very wary of unfamiliar smells and objects due to the stressful experience of capture. The bias towards bait station location during the polymer bait head trials and the reaction to the infrared pen lighting, also contributed to the 'uncharacteristic' behaviour of the animals. Progress to Stage 2 of the project where proposed trials are to be conducted in a 'natural' field environment is expected to produce more characteristic behaviour.

Not all the issues, which came to light during the pen trials were detrimental, at least not in terms of the field efficacy of the MDE. For example, the deterioration (rusting) of the valve caps, which resulted in the aerosol canisters depressurising, affected the pen trial results but its identification and subsequent resolving will prevent such issues in the field. Discussion with the supplier will determine whether alternatives to tinplate are available and their cost. Further examples included the animals caching the MDE, thereby filling the collar with dirt and rendering it inoperable, also the faulting of the magnetic pull force mechanism by iron stones etc. A simple change in deployment technique and the use of a plastic shroud between the collar and the mechanism easily resolved these issues.

Toxicant-aerosol formulation

Para-aminopropiophenone (PAPP): The trials demonstrated that PAPP can be formulated for aerosol delivery and if delivered at a lethal dose will result in a humane death in foxes. All three of the formulations tested resulted in symptoms of toxicosis. The trials resulted in 2 deaths of foxes at doses of 0.8ml and 1.8ml and 6 sub-lethal doses, ranging from 'minimal' to 0.6ml. This variability in the delivered dose

highlights the issue with the current aerosol valve. Animal survival was due to sub-lethal dosing due to the aerosol valve, rather than an issue with the formulation . No vomiting and/or convulsions were recorded during the trials. This is noteworthy, as vomiting of meat based PAPP baits has resulted in significant sub-lethal dosing events both in foxes and feral cats.

The lethal dose mass required of PAPP for both foxes and wild dogs, however, appears to be unachievable using the current delivery mechanism of the MDE. A reliable and consistent delivery can only be achieved using of a mdv, which expels a measured dose upon activation and is not reliant on the animal holding open the valve for a required period of time. However, the largest commercially available mdv has a capacity of only 0.2ml. Mitani Valves Co. Ltd. in Japan has developed a prototype 1.0ml mdv, but this is not commercially available. At this capacity it would potentially deliver a lethal dose of PAPP to foxes. The larger dose mass required for the control of wild dogs (2.5ml or 500mg) however, most likely means that PAPP may not be able to be delivered from an aerosol unless the dose mass can be significantly reduced. Due to the recognised humaneness of PAPP and its likely registration as a predator control pesticide in Australia, means of reducing the dose mass maybe worth of further consideration. PAPP is currently under review by the Australian Pesticides & Veterinary Medicines Authority (APVMA).

Potential options for reducing the required lethal doss mass of PAPP include:

i) Increasing the current formulation concentration of 20% PAPP. While this appears a relatively straightforward approach, Spraypack Pty Ltd, manufacturers and suppliers of the toxin aerosol formulation, suggests that in doing so the formulation may become less stable and the PAPP is likely to fall out of solution at lower temperatures. The sticky nature of the glycerine formulation potentially increases the quantity of toxin available for absorption through the mucus membrane due to it adhering to the mouth cavity. Additionally, using a high-pressure butane/propane B75 mix as the formulation propellant may also potentially increase the overall percentage of concentrate being expelled.

ii) Chemical excipients have the potential to improve solubility, permeability and absorption, thus potentially decrease the required dose mass. Excipients designed to improve absorption through the mucus membrane and also to reduce the risk of rejection i.e. improve palatability, have been determined for potential inclusion into the toxin formulation(s). Membrane permeability enhancers have the potential to increase the rate of absorption and reduce the time to first symptoms and death, providing improved humaneness and efficacy. Two materials selected as likely aiding membrane permeability are, Vitamin E d-α-ocopheryl polyethyleneglycol succinate (Vitamin E TPGS), and polyethylene glycol 600 (PEG600).

The other means of overcoming the toxin dose mass issue is to select an alternative toxin with lower dose mass requirement. Two candidates are available, Sodium cyanide (NaCN) and a Sodium monofluoroacetate (1080) / Copper indomethacin (CuI) combination. Please refer to Appendix 5 for a full evaluation of each of the toxins.

Cyanide compounds: Sodium cyanide was considered for a number of reasons including its high water solubility, humane rapid mode of action, low lethal dose rates and the toxins' use with the USA M-44 ejector, currently under review for registration in Australia by the APVMA. The lower lethal dose mass required using NaCN (Coyote (*Canis latrans*) 4.1mg/kg) means that the commercially available 0.2ml mdv could potentially be used to deliver a lethal dose to both foxes and wild dogs. At a 40% concentration formulation (40mg active/100ml of water) a dose of 90mg or

0.225ml can be achieved. At this dose level an animal of 20kg could be destroyed. Add to this the direct delivery technique of the MDE and the mode of action of NaCN, and the amount of toxin required for a lethal dose may potentially be further reduced.

The main issues associated with this toxin is its indiscriminate nature, and the human safety issues that it presents both during manufacture and field deployment, which could impact on the likelihood of commercial production. A number of strategies are available to potentially overcome the indiscriminate nature of CN. namely the exclusion collar and target specific pull force activation. This greatly reduces the risk of exposure to non-target species. In terms of human safety, options such as;

- the use of a venturi system, which contains the toxin formulation in a sealed, tamper proof, un-pressurise cylinder;
- the formulation of a 'foam' to contain the 'splash effect' of the toxin upon activation and decreasing potential contamination; and
- the inclusion of a highly visible inert dye to indicate where possible contamination may have occurred; all contribute towards better operator safety.

Due to NaCN's highly toxic nature and 'reputation' it has been difficult to obtain industry support, although use of a venturi system would greatly reduce handling and manufacturing risks.

Sodium monofluoroacetate (1080) The high susceptibility of canids to 1080 means a low lethal dose mass is required to induce toxicosis and death. Toxicity, in terms of dose is similar to cyanide, current dose rates in meat baits are 3.0mg for foxes and 4.5mg for dogs. Although the humaneness of 1080 has been questioned previously, a 1080 /CuI combination has been shown to reduce the symptoms of toxicosis (Appendix 5) and could therefore provide a cost effective, metered dose, aerosol formulation.

While requests to use 1080 'experimentally' within the project were initially denied by the AEC, further discussions with DEPI have renewed possibilities and therefore this remains a viable option for use with the MDE.

MDE delivery system

Although aerosol technology has been widely used for the control of insect pests, the innovative step has been to develop a 'self-dosing' system for large vertebrates and overcome the difficulty of the potentially large dose. The other innovative step in the multi-dose delivery system is the use of the specific pull force technology. This is achieved using rare earth magnets, allowing the unit to be set at a specific 'pull force' to activate the unit. This together with the exclusion collar gives the MDE its unique target specificity.

As identified from the pen trials, the delivery of a reliable and consistent lethal dose of active is reliant on the use of a mdv of appropriate capacity. Investigations to date have revealed that the largest mdv currently available 'off the shelf' has a capacity of 0.2ml. Using the current formulation concentration of 20% PAPP HCl, a mdv of 1.0ml (200mg) capacity is required to deliver a lethal dose to foxes and 2.5ml (500mg) capacity for wild dogs. Discussions with the Mitani Valve Co. Ltd in Japan, which have developed a prototype 1.0ml mdv, has revealed that they are not continuing that line making the current PAPP formulations unviable for wild dogs and foxes.

However, using either of the suggested alternative toxins in combination with the commercially available 0.2ml mdv, a lethal dose of aerosol formulation can be delivered to both species.
The issue with the alternative actives is their toxicity and the human safety issues that it raises both in manufacture and field operations. The MDE was designed as a 'chemical dosage dispenser', i.e. the ability to dispense a variety of chemicals including, but not restricted to toxins. As such the acquisition or fabrication of a 'venturi system', which allows an active to be housed separately from the pressurised aerosol canister, is a viable option and worthy of further consideration, particularly in light of the difficulties experienced in obtaining industry assistance/collaboration in the formulation and manufacture of toxin related aerosol products. A venturi system potentially overcomes the human health risks associated with the aerosolisation of toxins and operator safety in the field, as the active solution is contained in a sealed non-pressurised canister until attached to the venturi.

The adoption of the 0.2ml mdv and acquisition/fabrication of a venturi system greatly improves the efficacy and safety of the MDE delivery system.

Deployment technique

The screw anchor is an off-the-shelf product, which requires only minor modification for adoption to the MDE. As discussed, the large flange and cylinder type construction aid the stability of the unit in the ground and provides a protective housing for the aerosol canister and delivery mechanism. Although extensive trials in different soil types have yet to be completed, the pen trial conducted in sandy soils are considered a worse case scenario. As seen in the recorded footage of the pen trials animals are not inclined to reactivate the MDE after the experience of the spray delivery. This experience therefore potentially reducing the time the animal interacts with the unit and thus decreasing the likelihood of animals persisting to pull on the unit. The size of the anchor is believed to have the capacity to cater for the potential use of a venturi system.

Exclusion collar(s)

The pen trials demonstrated that both collars prevent foxes from activating the MDE using their rear carnassial teeth, thereby ensuring that the dose delivered is directly into the animal's mouth. The use of the exclusion collar, in combination with the MDE pull force technology, potentially allows target specific baiting of foxes without risk of exposure to non-target species. The morphological design of the exclusion collar restricts access to the bait forcing the fox to insert it whole snout into the collar. With the exclusion collar it is important that the diameter of the bait head does not exceed 30mm as the collar only allows a certain gape to grip the bait.

If targeting wild dogs the open collar is required. This in itself does pose a greater risk to non-target species, however, due to the inbuilt flexibility of the pull force mechanism, the force required to activate the unit can be potentially increased to 6 kg significantly reducing the number of species capable of exerting such a force.

Long-life bait material

The chosen inert polymer matrix is not susceptible to climatic or biological degradation and has the durability to withstand numerous visitations. The pen trials were conducted with first generation CPU heads with no specific dimensions or formulation. Oil based attractants were identified as preferable, creating less binding issues and allowing greater retention of the lures integrity. The oil based lures are

also suspected of permeating from the polymer matrix better as they are less bound by the polymer structure. The smell of some of the impregnated heads was not necessarily recognisable as the original lure preparation used, therefore further refinement of the formulations is required. Formulations of lure compounds previously identified as eliciting desirable pulling behaviour should be investigated.

The option of using 'well type' polymer bait heads is regarded as a viable alternative where land managers are able to regularly inspect and re-service the MDE. This would allow greater flexibility to farmers allowing a variety of lures to be used during a baiting program.

Conclusion

The evaluation of the MDE components as derived from the original 'proof of concept' were successfully achieved during the Stage 1 pen trials. This study has outlined an effective MDE based on the toxin PAPP and targeting foxes. This prototype could be tested in field trails in a stage 2 project. However, Stage 1 also identified a crucial issue in terms of delivering a reliable dose of aerosol toxin formulation, without the use of a metered dose valve. The required capacity of the mdv is dependent on the lethal dose mass of the chosen toxin.

Toxicant-aerosol formulation

Summarising the option put forward in the discussion;

i) Re-formulation of Para-aminopropiophenone - while increasing the concentration of PAPP and adding excipients to the aerosol formulation may potentially reduce the dose mass required, it is unlikely that the dose mass can be reduced sufficiently to allow the use of currently available mdv.

If PAPP is the toxin of choice then the only foreseeable alternative is to further investigate the option of the 1.0ml mdv. However, Mitani has discontinued that line of development and in discussions appear unlikely or willing to revisit its production. Prototype examples of the mdv are available so potentially local production could be sought. This, however, would only accommodate fox baiting.

ii) The use of sodium cyanide – from a project point of view, NaCN remains the most desirable toxin. It is widely accepted as having a humane mode of action and the fast onset of toxicosis allows the retrieval of poisoned animals. This attribute provides both a precise measure of the success of the baiting program and provides land managers a 'result' (fox or dog body) for their investment and labour.

While unarguably toxic, its 'reputation' out weighs its dangers. Cyanide is extensively used in the mining industry and has been used in the United States for well over 50 years as the toxin of choice with the M-44 ejector for the control of Coyotes (*Canis latrans*), and in New Zealand for the control of possums. The acquisition of a venturi system for the MDE would significantly reduce the risk of exposure to operators both during manufacture and field operations.

iii) Sodium monofluoroacetate – the use of 1080 has a number of advantages, it is currently the registered pesticide for predator pests, target species are highly susceptible and it is highly suitable as an aerosol delivered toxin due to its stable, water-soluble nature. To overcome the perceived humaneness issues associated with 1080 toxicosis, copper indomethacin, a potent non-sedating analgesic, has been identified as a suitable additive for minimising any associated pain and distress which may occur during late symptoms of toxicosis. However,

further investigation of the suitability of CuI for aerosol delivery is required. Unlike NaCN, the time to death using 1080 is much greater, therefore the recovery of animal carcasses is unlikely, hence land managers' evidence of success.

MDE delivery system

The efficacy of the MDE delivery system was successfully demonstrated in delivering an aerosol formulation and providing a level of target specificity through the pull force activation technique. The acquisition of an appropriate mdv would complete the package by providing a reliable means of delivering a measured lethal dose of active. Sub-lethal dosing was observed in the majority of test foxes. The delivery mechanism is functional, however the required dose and toxin combination requires refinement.

Deployment technique

While not tested with wild dogs the anchor system of the deployment technique appears to satisfactorily secure the MDE with foxes. Considering the timid and wary nature of wild dogs it is unlikely that, once they have activated the MDE, they will approach the unit again.

Exclusion collar(s)

The efficacy of the MDE collars was successfully demonstrated showing that the animals did not display any long-term aversion to the units and voluntarily inserted their snout into the collars to access a bait. Furthermore the collars restricted access to the bait preventing animals from gripping the bait with their rear carnassials and therefore ensuring direct delivery of the active into the animals' mouth.

Long-life bait material

The 'bait' is crucial to the success of the unit in terms of attracting the animal to the device and eliciting a pulling response, field longevity of scent release and durability, are essential characteristics. It is important, therefore, to evaluate a broader range of lures for impregnation into the polymer matrix, including lure compounds previously identified as eliciting desirable pulling behaviour. This will provide a better understanding of animal preference and potential seasonal variability. While impregnated polymer heads are the preferred direction forward for long-term field deployment of the MDE, further evaluation of specific lures is required with a focus on (i) attractiveness, (ii) scent retention and (iii) field durability. The objective of Phase 1 was to conduct efficacy trials to assess bait materials' (i) design, (ii) durability and (iii) manufacturing processes.

In summary, each of the toxins under consideration have particular issues which require further investigation.

- The use of PAPP will only be possible for the control of foxes and only if a 1.0ml mdv can be locally manufactured.

- Due to operator safety issues the use of NaCN will only be possible if a venturi system to suit the application can be obtained.

- Using 1080 appears the simplest solution. The option of incorporating CuI to alleviate the concerns regarding the humaneness of the toxin is valid although the availability and solubility of the agent needs to be further explored. Discussions with DEPI has indicated that AEC approval would be considered from their internal committee.

The acquisition of an appropriate mdv for the MDE delivery system would complete the package by providing a reliable means of delivering a measured lethal dose of active. Lethal doses of both NaCN and 1080 formulations can be delivered using the commercially available 0.2ml mdv, to both foxes and wild dogs.

The deployment technique and exclusion collars performed to expectations and are considered ready for field assessment.

In terms of the long-life bait heads, the CPU polymer provides the ideal matrix for impregnation. Further evaluation of specific lures for impregnation within the matrix is required with a focus on (i) attractiveness, (ii) scent retention and (iii) field durability.

Recommendations

Stage 2 of the MLA project is designed to consolidate the results from the pen trials and take forward into field trials the 'best option' multi-dose ejector (MDE) prototype for foxes.

As discussed in the conclusion both the deployment technique and exclusion collars performed to expectation and are considered ready for field assessment. Similarly, the delivery system is capable of reliably delivering a dose of active for foxes. Combined with a 0.2ml metered dose valve (mdv), the MDE has the capacity to deliver a lethal dose of both sodium cyanide (NaCN) and/or Sodium monofluoroacetate (1080) aerosol formulations to both foxes and wild dogs.

The preferred active for use with the MDE is NaCN. However, due to operator safety concerns a number of issues need to be clarified prior to any field operations.

1. Seek clarification from the Australian Pesticides & Veterinary Medicines Authority (APVMA) on the likelihood of registration of NaCN as a predator control pesticide for use with the MDE.

2. Acquisition of a venturi system; two options are available –
    - Collaboration with Precision Valves in the USA, manufacturers of Preval® spray unit.
    - Local fabrication of venturi system.

3. Collaboration with Industry partner with mdv capabilities.

4. Collaboration with Industry partner for NaCN formulation.

5. Toxin aerosol formulation 'shelf life' (stability and climatic variations)

Alternatively, investigate the option of incorporating copper indomethacin (CuI) into a 1080 formulation in order to alleviate some of the community concerns about the humaneness of 1080. Taking this option, though not perceived as humane as NaCN, would demonstrate to community groups that MLA is aware of their concerns and trying to improve animal welfare while still protecting agricultural values.

If the availability and/or solubility of CuI can't be resolved, then, the final alternative is to move forward with a 1080 (alone) aerosol formulation. Although not ideal, it would allow field assessment of the MDE to progress as recommended. Points 2, 3, 4 and 5 above, are relevant regardless of the toxin.

The venturi system provides the MDE greater flexibility and scope as a 'chemical dose dispenser'. The potential application of such technology, extends beyond the current focus of predator pest control in Australia. A 'Chemical Dosage Dispenser' (patent applied) has a number of potential applications around the world such as: rabies vaccines delivery, species population management through the delivery of anti-fertility drugs, antibiotics/medication delivery and sedative delivery to enable safe relocation programs.

The use of polymer technology has the potential to provided a durable, long-life bait capable of withstanding multiple visitations and retention of an attractive lure. Trials to date have confirmed the polymer matrix of choice, however, further evaluation of specific lures for impregnation within the CPU polymer matrix are required with a focus on (i) attractiveness, (ii) scent retention and (iii) field durability. Bait attraction and durability are essential to the success of the MDE. If facilities at DEPI Frankston remain available pen trials to assess the efficacy of these lure/baits will be conducted. Alternatively, these trials could be conducted in the field, which may provide a better behavioural assessment.

Field trials would commence once the most viable option(s) is confirmed and a toxin aerosol formulation is available.

- Not recommended continuing work with PAPP because the best case scenario is the production of a fox control technique only, and that is only possible if/with the local manufacture of a 1.0ml mdv.

- Recommend the option of acquiring or producing a venturi system to allow greater versatility of the MDE as a chemical dose dispenser, and potentially the safe handling of NaCN. Although not currently registered as a predator pest pesticide NaCN provides a number of advantages not possible with other toxins investigated.

- Recommend formulating a NaCN aerosol formulation.
  (If a venturi system can be acquired.)

- Recommend pursuing the availability and solubility of CuI for formulating a 1080/CuI aerosol formulation. This option potentially provides the fastest path to a final product. However, it does not overcome community perception of the humaneness of 1080.

- Recommend formulating a 1080/CuI aerosol formulation.
  (If CuI becomes available.)

- Recommend formulating a 1080 aerosol formulation to allow field efficacy of the MDE.
  (If 1080/CuI formulation not possible.)

- Recommend further assessment of specific lures for impregnation within the polymer matrix. The attraction of the 'bait' is crucial to the success of the MDE.

- Recommend that trials to confirm the target specificity of the MDE delivery system and exclusion collar with specific non-target species be commenced.

- Recommend that field trials to confirm the efficacy of the MDE be undertaken with the European red fox. The proposed Werribee 'before and after' style control program will demonstrate the efficacy of the toxin formulation, multi-dose capability and effectiveness and durability of the polymer bait.

- Recommend that trials with wild dogs be commenced. These will include lethal dose trials with the chosen aerosol formulation and efficacy of the MDE. Trials will be conducted opportunistically i.e. pen or field trials depending on availability of animals and facilities.

Appendix 6 provides the proposed Stage 2 activities and timelines to achieve these recommendations.

Stage 2 Consolidation of MDE and efficacy field trials.

Objectives

Chronological outputs in Stage 2 of the MDE project are outlined below:

Consolidation phase:

- Confirmation of toxin:

- Clarification from APVMA on the likelihood of NaCN / MDE registration.
  (An appointment to discuss potential requirements and legislative status, provide demonstration of MDE and venturi system to allow informed decision.)
- Investigate the availability and solubility of copper indomethacin (CuI).
- Acquisition of venturi system, investigate two available options.
- Seek collaboration with Industry partner with mdv capabilities.
- Seek collaboration with Industry partner re toxin (aerosol) formulation, supply and manufacture.
- Toxin aerosol formulation 'shelf life' (stability and climatic variations)
- APVMA Cat.23 field trial permit application.

- Selection of specific lures for impregnation within the CPU polymer matrix:

- In collaboration with Scientec Research Pty Ltd and OUTFOXED Pest Control Company formulate lure compounds for trial impregnation.
- In collaboration with P.D Plastics fabricate polymer bait heads with selected impregnated lure.
- Conduct non-lethal assessment trials with fabricated bait heads.
  (Trials would be done opportunistically either as pen trials at DEPI or as a field evaluation.)

- Fabrication of MDE units:

- Local fabrication of venturi system.
- Fabrication of MDE units (20) for field deployment.

Field trial phase:

– Target specificity trials:

- The target specificity of the MDE is demonstrated on 8 non-target species.

  The species selected for non-lethal trials are:
  Spotted tailed quoll
  Both Eastern and Northern quoll species
  Tasmanian devil
  Southern and Northern brown bandicoots
  Large reptiles i.e. Goanna
  Dingoes and working dogs

Discussions with various wildlife agencies are in progress and access to animals has already been granted in certain cases (Appendix 7). Trials will require an animal ethics approval, though being non-lethal trials this should not be an issue. Where animal species are held in captivity, MDE(s) will be deployed within their enclosure and animals allowed to interact voluntarily. The MDE will be baited to encourage interaction, and adapted with a mechanical counter to record any activation of the unit. A water aerosol canister will be used in place of the toxin to mimic animal behaviour to the resulting spray. It is anticipated that with the combination of the pull force and exclusion collar, animals will not be able to activate the MDE. Small animals, which can penetrate into the collar, should not have the physical strength to activate the unit and those larger individuals with the potential strength will not be able to access the bait due to the restriction of the collar. All trials will be recorded via remote video cameras to avoid any adverse behaviour or stress to the animals due to human presence. In 'wild' field situations MDE bait sets will be monitored using motion detection cameras.

- Fox control field trials:

- Demonstrated efficacy of the MDE as a fox control technique at selected field sites (2).

An initial trial to confirm the efficacy of the MDE as a fox control technique using the selected toxin from the 'consolidation phase' is proposed at Melbourne Water, Werribee Treatment Plant. The site, regarded as atypical, has a high resident fox population with limited control and restricted access to the public. Melbourne Water has been approached in relation to the trial and appear supportive. The proposed 'before and after' style control program will demonstrate the efficacy of the toxin formulation, multi-dose capability of the MDE and the attraction and durability of the polymer bait. The trial will require an animal ethics and Ministerial 'death as an endpoint' approval. Trial will be conducted under an APVMA 'Small scale trial (7250)' permit thereby not requiring lengthy approval procedures. Monitoring the success of the 'before and after' style trial will include DNA technology. Analysis of DNA extraction from scats can be used to identify individual animals, therefore their presence before and after baiting can be monitored to provide an accurate assessment of the number of animals likely to have been removed from the population. As with the non-target trials above, MDEs' will be adapted with counters to record the number of activation at each unit.

A further trial conducted in a regular fox control area i.e. conservation and/or agricultural environment will also be required to provide the necessary supportive data to the APVMA application for registration. A location for this trial is yet to be

confirmed. Such a trial would require a Cat.23 'Restricted Use Permit' from the APVMA to allow 'off label' use of 1080 or an experimental permit in the case of NaCN.

- MDE for wild dog control:

- Demonstrated efficacy of the MDE for wild dog control.

With the closure of the Queensland Government facilities at Inglewood pens capable of housing wild dogs are now difficult to access. In order to assess the efficacy of the MDE for the control of wild dogs it is necessary to duplicate some of the previous work. Trials will therefore be conducted opportunistically i.e. pen or field trials depending on availability of animals and/or facilities. Evaluation of;

i) the efficacy of the aerosol formulation to confirm lethality and dose mass;
ii) the efficacy of the delivery system's 'pull force' and open collar;
iii) the efficacy of the deployment system to maintain secure anchor; and
iv) the efficacy of the polymer bait head to withstand multiple visitations.

All of the above could be potentially achieved during a single trial, as was the case with foxes (i.e. Milestone 4). Communication with the South Australian, Department of Primary Industries has indicated that a field site in the northwest of the state could potentially be available for such a trial. This would also require a Cat.23 'Restricted Use Permit' for 'off label' of an active from the APVMA. Field efficacy data gathered would contribute towards a final submission to the APVMA for registration and product manufacture.

- Field reliability/longevity assessment:

- The reliability and longevity of the MDE under field conditions is demonstrated.

The proposed fox and dog field trials will also allow assessment of MDEs' reliability and longevity under different climatic and environmental conditions. Field sites will be chosen to provide the necessary variability such that supportive field efficacy data can be obtained for National registration.

Communications Plan:

As part of the activities in Stage 2, a communication plan can be developed in collaboration with MLA and include demonstrations of the MDE at agricultural field days and community workshops. The objective of the communication plan is to increase awareness, enlist support and potential participation in the (APVMA) field trials. The potential to raise community awareness and enhance skills is significant, particularly in areas where producers abut public land and areas of high conservation value. Community / stakeholder involvement in the field trials would be encouraged and potentially go some way to increasing confidence and adoption rate of the technique. Information would be made available through existing MLA circulations.

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Appendices

1. Experimental protocol - MDE components pen trials
2. Tabulated results of MDE component pen trials
3. Experimental protocol – polymer baits pen trials
4. Results of fabrication and trials with polymer bait heads
5. Toxin specifications
6. Stage 2 activities and timelines
7. List of wildlife agencies.

Appendix 1:

Experimental protocol - MDE components pen trials
(Extract from Milestone 4 Report)

In order to reduce animal usage, pen trials were conducted in combination examining the efficacy of the MDE components, rather than individually, during lethal trials. The four components of the MDE assessed during the pen trials were:

a) MDE delivery system - investigate the animals' ability and willingness to trigger the mechanism.

b) Toxicant-aerosol formulation – evaluate the aerosol formulation and demonstrate the effective delivery of a lethal active by the MDE.

c) Exclusion collar - determine whether animals show any aversion or neophobia to the collar, and willingness to place their snout into the collar.

d) deployment technique – demonstrate the ability of the soil anchor to secure the MDE.

This method allowed researchers to assess each of the components [i.e. a) the MDE delivery system, b) toxicant-aerosol formulation, c) exclusion collar and d) anchor] while conducting a single lethal trial. MDE units were deployed using the soil anchor in a number of pens and animals introduced and allowed to voluntarily interact with the units over the following 16 to 18 hour period (overnight). Each trial was remotely monitored and recorded via the infrared video surveillance cameras (2) within each pen. If for whatever reason the animal did not interact and was not exposed to the toxin, the trial was run again the following night.

It was anticipated that animals would voluntarily interact with the MDE, trigger the device and receive a dose of toxin and in doing so, also allow an assessment of each of the components. Review of the recorded footage enabled observations to be made of behavioural involvement during the trial and if toxicosis occurred. Key activities and time points (incl. behaviour to collar, toxin delivery, exposure, first symptoms, collapse and death) were recorded.

The trials were conducted using para-aminopropiophenone (PAPP) formulated by Scientec Research Pty Ltd and aerosol canisters were manufactured and supplied by Spraypack Pty Ltd.

The primary PAPP formulation used was composed of:

100g Concentrate, 40g Butane / Propane, Poison PAPP HCl 20% solution, DMSO 90%.H_2O 10% solvent.

Three (3) variations of the formulation were used during the trials:

a) primary formulation;
b) glycerine form; and
c) beef flavoured (primary formulation).

Appendix 2:

Tabulated results of MDE component pen trials
(Extract from Milestone 4 Report)

Efficacy trials of the various components

a) MDE delivery system;
b) toxicant-aerosol formulation;
c) exclusion collar; and
d) deployment technique

a) MDE delivery system

In accordance with Marks and Wilson (2005) all animals used during the pen trials had the body weight / strength to pull the required 3kg pull force to activate the MDE (Table 1). The magnetic based delivery system provided a reliable and consistent force (Table 2) throughout the trials.

The results presented in Table 3 show that the MDE was activated 8 times, yielding 2 successful kills and delivering another 6 sub-lethal doses. Of the 21 animals used during the trials, seven individuals cached the MDE. Three animals (F32, F43 and F45) cached the MDE without attempting to remove the bait. The remainder (n=4) cached the unit after receiving a quantity of aerosol spray in their mouths. The Table 8 data (Appendix 1), which includes all the trials conducted, indicated that of the 52 trials;

- 12 occasions the MDE was activated;
- 22 occasions the delivery system was not activated (No pull);
- 18 occasions there was no interaction.

Of the 22 'No pulls' - 10 related to either caching (n=7) of the MDE so no attempt at pulling the bait was made, or the natural meat bait used was pulled from the MDE without activating the unit. On the occasions when the MDE was activated (n=12) the delivery system successfully activated to deliver a dose of active.

Table 2: MDE delivery mechanism, pull force accuracy test.

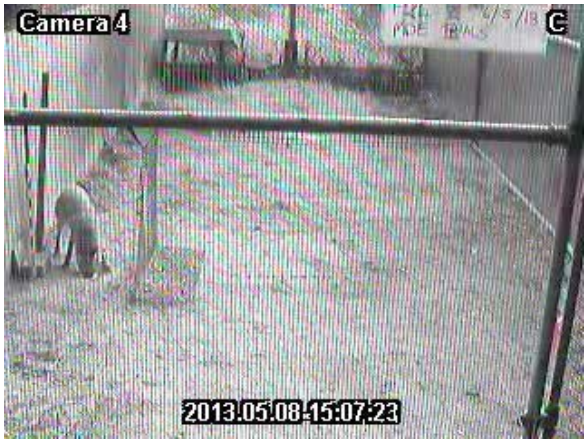
Unit 1 (kg) Set for max. pull force	Unit 2 (kg) Set at 3.2kg pull force	Unit 3 (kg) Set at 2.8 kg pull force
6.40	3.20	2.80
6.58	3.10	2.80
6.50	3.10	2.90
6.50	3.00	2.86
6.40	3.10	2.86
6.40	3.05	2.87
6.45	3.10	2.80
6.47	3.10	2.87
6.40	3.05	2.88
6.40	3.10	2.84
Average 6.45kg	**Average 3.09kg**	**Average 2.85kg**

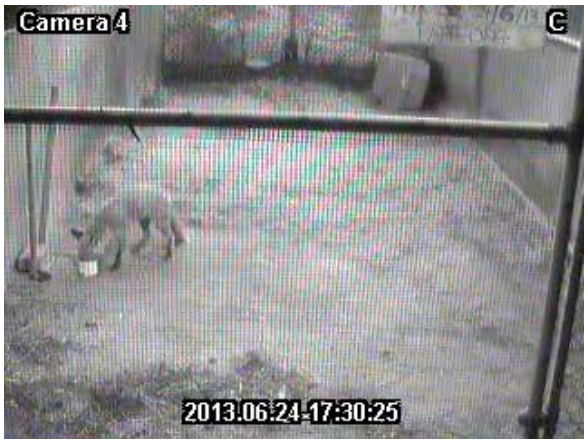
Table 3: Summary of trial results.

Animal	No of trials	Snout in collar	Collar type	Interaction			Bait types	MDE Cached	MDE fired	Time to death (min)	Canister formulation	Result
				Pull	YES	NO						
F4	1	n/a	Exclusion	n/a	n/a	n/a	Raw hide washers - liver oil	No	No		PAPP HCL	No footage
F6	2	Yes (2)	Exclusion (2)	**1**	1		Chorizo sausage, **Deep fried liver**	No	**Yes**	39	PAPP HCL	Death (160mg)
F10	1	No	Exclusion			1	Deep fried liver	No	No		PAPP HCL	
F24	6	Yes (4)	Exclusion (6)	**2**	2	2	Deep fried meat, **Deep fried liver** PU - chicken liver puree Deep fried liver - sugar sol^{n}	Yes	**Yes**		PAPP HCL (glycerine)	Bait removed Sub-lethal
F25	5	Yes (2)	Exclusion (5)		2	3	Deep fried meat, Deep fried liver PU - chicken liver puree Deep fried liver - sugar sol^{n}	No	No		PAPP HCL	
F26	1	Yes	Exclusion	**1**			**Deep fried liver**	No	**Yes**	>49	PAPP HCL (glycerine)	Death (360mg)
F29	2	Yes (1)	Exclusion (2)		2		Deep fried liver	No	No		PAPP HCL (glycerine)	
F30	3	Yes (1)	Exclusion (3)		3		Deep fried liver	No	No		PAPP HCL PAPP HCL (glycerine)	
F31	4	Yes (4)	Exclusion (2), Open (2)	**2** (O)	2 (E)		**Deep fried liver**	Yes (3)	**Yes**		**PAPP HCL** PAPP HCL (glycerine)	Bait removed Sub-lethal
F32	2	Yes (2)	Open (2)	**1**	1		Deep fried liver, **Deep fried meat**	Yes	No		**PAPP HCL** PAPP HCL (glycerine)	Bait removed
F33	3	No	Exclusion (2), Open (1)			3	Deep fried meat	No	No		PAPP HCL (beef flavour) PAPP HCL (glycerine)	
F34	3	Yes (1)	Exclusion (1), Open (2)	**1** (O)		2	**Deep fried liver**	No	**Yes**	n/a	PAPP HCL (glycerine)	Sub-lethal Depressurised canister
F35	1	Yes	Exclusion	**1**			**Deep fried meat**	No	No		PAPP HCL (beef flavour)	Not fire

F36	1	Yes	Open		1		Deep fried meat	No	No		PAPP HCL (glycerine)	Depressurised canister
F37	1	No	Exclusion			1	Deep fried meat	No	No		PAPP HCL (beef flavour)	
F38	4	Yes (1)	Exclusion (3), Open (1)		1 (E)	3	Deep fried meat	No	No		PAPP HCL (beef flavour)	
F39	2	Yes (2)	Exclusion (1), Open (1)	**1** (O)	1 (E)		**Deep fried meat – Fox lure**	Yes	**Yes**	n/a	PAPP HCL (beef flavour)	Sub-lethal (120mg)
F40	1	Yes	Open	**1**			**PU - Fish oil**	Yes	**Yes**	n/a	PAPP HCL (beef flavour)	Sub-lethal (100mg)
F41	3	Yes (2)	Open (3)	**1**	2		Fox lure PU - Fox lure, **Deep fried liver**	No	**Yes**	n/a	PAPP HCL (beef flavour)	Sub-lethal (20mg)
F43	2	Yes (1)	Open (2)		1	1	PU – SFE - liver & oil lure	Yes	No		PAPP HCL (beef flavour)	
F45	2	Yes	Open (2)		1	1	Deep fried liver	Yes	No		PAPP HCL (beef flavour)	

- ‘Bait type’ in bold equals that which was pulled.
- ‘PU’ bait type equals polyurethane head with vertical wells and specified lure.
- ‘Fox lure PU’ equals polyurethane head impregnated with fox lure and specified lure.





Photos show willingness of foxes to place their snout into the device in order to access the bait head.

b) Toxicant-aerosol formulation

Results from the Trial (Table 4) demonstrated that each of the 3 formulations tested produced toxicosis in animals. Time to first symptoms and death were within expectation and support previous research. The results indicate that the formulation and delivery system combined reduce the dose mass required in comparison to proposed conventional PAPP meat baits (400mg dose for foxes, pers. com. IA CRC).

Table 4: Comparison table between three formulation types.

Toxin canister formulation	Animal	Sex	Bait head pulled	Result	Time to 1st symptoms (min)	Time to death (min)	Bait Type	Collar type	Quantity of PAPP released	Notes
PAPP HCL	F6	Male	Yes	**Death**	23	39	Deep fried beef liver	Exclusion	160mg	Head shake
PAPP HCL	F31	Male	Yes	Sub-lethal		Survived	Deep fried beef liver	Open	Not detected	Pulled bait off ejector easily / head shake
PAPP HCL	F31	Male	Yes	Bait removed			Deep fried beef liver	Open		Pulled bait off ejector easily
PAPP HCL	F32	Male	Yes	Bait removed			Fried meat	Open		Pulled bait off ejector canisters corroded /depressurised
PAPP HCL (glycerine)	F24	Male	Yes	Bait removed			Deep fried beef liver	Exclusion		Bait was pulled and whole unit came out, mechanism was sticking & unit didn't fire
PAPP HCL (glycerine)	F24	Male	Yes	Sub-lethal		Survived	Deep fried beef liver	Exclusion	Not detected	Head shake but no toxicosis exhibited
PAPP HCL (glycerine)	F26	Male	Yes	**Death**	16	>49	Deep fried beef liver	Exclusion	360mg	Head shake
PAPP HCL (glycerine)	F34	Male	Yes	Sub-lethal		Survived	Deep fried beef liver	Open	Not detected	Head shake but no toxicosis exhibited
PAPP HCL (beef flavour)	F35	Male	Yes	Miss fire			Fried meat	Exclusion		MDE did not fire
PAPP HCL (beef flavour)	F39	Male	Yes	Sub-lethal		Survived	Fox lure PU - Fox lure	Open	120mg	Head shake
PAPP HCL (beef flavour)	F40	Male	Yes	Sub-lethal	21	Survived	PU - Fish oil	Open	100mg	Head shake
PAPP HCL (beef flavour)	F41	Female	Yes	Sub-lethal		Survived	Deep fried beef liver	Open	20mg	Head shake

c) Exclusion collar

Table 5 results show that once animals overcame their initial neophobia to the unit, there was no further difficulty or hesitation in placing their snout into either the (a) exclusion (table 5a) or open collar variety (Table 5b).

Table 5a: Collar comparison trial summary – exclusion collar
(*Highlight indicates animals first encounter)*

Animal	Sex	Collar type	Snout in collar	Time to snout in collar (mins)	Activity	Bait Type
F6	Male	Exclusion	Yes	35	no pull	Chorizo sausage
F6	Male	Exclusion	Yes	72	pull	Deep fried beef liver
F7	Male	Exclusion	Yes	90	no pull	Raw hide washers - tuna oil
F10	Male	Exclusion	No	n/a	no interaction	Deep fried beef liver
F12	Male	Exclusion	Yes	220	no pull	Deep fried beef liver
F14	Female	Exclusion	No	n/a	no interaction	Deep fried beef liver
F24	Male	Exclusion	No	n/a	no interaction	PU -chicken liver puree
F24	Male	Exclusion	Yes	5 hours	no pull	Fried meat
F24	Male	Exclusion	Yes	3	Pull	Deep fried beef liver
F24	Male	Exclusion	Yes	<1	Pull	Deep fried beef liver
F24	Male	Exclusion	Yes	<1	no pull	Deep fried beef liver
F24	Male	Exclusion	No	n/a	no interaction	deep fried beef liver - sugar soln
F25	Male	Exclusion	No	n/a	no interaction	PU -chicken liver puree
F25	Male	Exclusion	No	n/a	no interaction	Fried meat
F25	Male	Exclusion	No	n/a	no interaction	Deep fried beef liver
F25	Male	Exclusion	Yes	100	no pull	Deep fried beef liver
F25	Male	Exclusion	Yes	immediately	no pull	Deep fried beef liver - sugar soln
F26	Male	Exclusion	Yes	immediately	pull	Deep fried beef liver
F29	Male	Exclusion	No	n/a	no pull	Deep fried beef liver
F29	Male	Exclusion	Yes	< 60	no pull	Deep fried beef liver
F30	Female	Exclusion	Yes	>14hours	no pull	Deep fried beef liver
F30	Female	Exclusion	Yes	14	no pull	Deep fried beef liver
F30	Female	Exclusion	No	n/a	no pull	Deep fried beef liver
F31	Male	Exclusion	Yes	<6hours	no pull	Deep fried beef liver
F31	Male	Exclusion	Yes	15hours	no pull	Deep fried beef liver
F33	Male	Exclusion	No	n/a	no interaction	Fried meat
F33	Male	Exclusion	No	n/a	no interaction	Fried meat
F34	Male	Exclusion	No	n/a	no interaction	Deep fried beef liver
F35	Male	Exclusion	Yes	17hours	Pull	Fried meat
F37	Female	Exclusion	No	n/a	no pull	Fried meat
F38	Female	Exclusion	Yes	100	no pull	Fried meat
F38	Female	Exclusion	No	n/a	no interaction	Fried meat
F38	Female	Exclusion	No	n/a	no interaction	Fried meat
F39	Male	Exclusion	Yes	25	no pull	Fried meat - Fox lure

Table 5b: Collar comparison trial summary – open collar

Animal	Sex	Collar type	Snout in collar	Time to snout in collar (mins)	Activity	Bait Type
F31	Male	Open	Yes	35	Pull	Deep fried beef liver
F31	Male	Open	Yes	40	no pull	Deep fried beef liver
F32	Male	Open	Yes	140	no pull	Deep fried beef liver
F32	Male	Open	Yes	68	Pull	Fried meat
F33	Female	Open	No	n/a	no interaction	Deep fried beef liver
F34	Male	Open	No	n/a	no interaction	Deep fried beef liver
F34	Male	Open	Yes	32	Pull	Deep fried beef liver
F36	Female	Open	Yes	9hours	no pull	Fried meat
F38	Female	Open	No	n/a	no interaction	Fox lure PU - Fox lure
F39	Male	Open	Yes	160	pull	Fox lure PU - Fox lure
F40	Male	Open	Yes	>7hours	pull	PU - Fish oil
F41	Female	Open	Yes	18hours	no pull	Fox lure PU - Fox lure
F41	Female	Open	Yes	100	pull	Deep fried beef liver
F41	Female	Open	Yes	9hours	no pull	PU - liver and oil
F43	Male	Open	Yes	60	no pull	PU - SFE
F43	Male	Open	No	n/a	no interaction	PU - liver and oil
F45	Female	Open	Yes	150	no pull	Deep fried beef liver
F45	Female	Open	No	n/a	no interaction	Deep fried beef liver

Table 6: Summary of first encounter collar comparison trials

Collar type	Sex	Snout in collar	Snout in collar %	Average time (min)	Activity
Exclusion	Male 13	7	54%	250	P=(2) NP=(5) NI=(5)
Exclusion	Female 4	2	50%	470	P=(0) NP=(2) NI=(1)
Open	Male 6	5	83%	163	P=(3) NP=(2) NI=(1)
Open	Female 5	3	60%	590	P=(0) NP=(3) NI=(2)

Results from animals first encounter; P= pull; NP= no pull; NI= no interaction.

The results tabulated in Table 6 indicate that during the trials with the exclusion collar 50% of the animals (both males and females) voluntarily put their snouts into the collar (12/17). However, only 29% (2/7) of those males then pulled on the bait. Females appeared much more wary, with none pulling on the bait with the exclusion collar. In contrast, using the open collar 83% (5/6) of male foxes put their snouts into the collar and 50% (3/6) of those then pulled on the bait material. Females again appeared much more wary, with none pulling on the bait although 60% (3/5) were willing to put their snout into the open collar. On average animals took over 6 hours (368min) before being confident to enter the collars. Females again were more wary taking 470 and 590 minutes to enter the exclusion and open collars respectively. For females, exclusion collar times ranged from 14min to 14 hours and for the open collar 100min to 18hours. Males in contrast ranged from <1min to 17 hours for the exclusion collar and 32min to 7 hours for the open. The varying times are to a degree due to animal behaviour in captivity. Those preoccupied with escaping took longer to investigate the

unit. Nine of the 17 animals tested with the exclusion collar place their snout into the collar, while 8 of the 11 animals do so with the open collar.

d) **Deployment technique**

The soil type in the pens where the trials took place is sand/sandy loam. The screw anchor used during the trials successfully secured the MDE at the bait station. Footage from the trials demonstrates that the anchor allowed animals to exert significant force (>3kg) to the bait head and collar without dislodging the unit. On the occasion reported when the MDE was pulled from the set (F24) the unit was deployed with an earlier designed anchor.

Summary of efficacy trial results

As a summation of the pen trial results the following concluding remarks are provided;

a) MDE delivery system;

The magnetic based delivery system provided a reliable, consistent pull force, and controlled activation allowed a dose mass to be sprayed directly into the target species mouth.

b) toxicant-aerosol formulation;

Current toxin formulation(s) successfully produced toxicosis in the target species. The oral aerosol formulation exhibited good solubility and membrane permeability, and provided minimal lethal dose mass (160mg).

c) exclusion collar;

Both collar types provided access to the bait allowing activation of the MDE. After initial neophobia, 75.6% of animals tested with the exclusion collar placed their snout into it, 81.8% animals did so with the open collar. Both collar types successfully restricted access to the bait, forcing foxes to take the bait using their front teeth thereby ensuring the line of 'fire' was directly into their mouth.

d) deployment technique;

The deployment anchor successfully held the MDE in the sand/sandy loam soil within the pens. This soil type is often regarded as the most difficult due to the lack of structural cohesion.

Appendix 3:

Experimental protocol – polymer baits pen trials
(Extract from Milestone 4 & 5 Report)

Initial assessment of several soft feel materials, were conducted by P.D Plastics including thermoplastic elastomer (TPE) such as (polyethylene #4997, 4998 and Flexene), thermoplastic vulcanizates (TPV) and thermoplastic polyurethane (TPU). Their results indicated that TPU, as it is the strongest and most resilient of the soft feel materials, was potentially the material to use for the fabrication of the prototype polymer bait head.

A sample of injected moulding TPU containing vanilla essence was acquired to allowed a preliminary assessment of the material. The pen trials investigated an animals' behaviour to the bait head and the durability of the material. The polymer samples were modified so that a M-44 ejector 'capsule holder' could be inserted. This allowed the bait head to be secured to the ground. A set ejector was also incorporated into the trial to indicate whether sufficient force had been applied to the bait head to trigger the ejector. Individual foxes were allowed to voluntarily interact and their behavioural response recorded. Each bait head was visually assessed to determine the damage sustained.

Having selected a potential polymer matrix, we then looked at the option of impregnating the polymer with selected scents. Initial attempts were done with injection moulding. The main issue with this process was to find additives that were stable enough to withstand the high processing temperatures of around 200°C. To overcome the issue of denaturing the selected scents, cast polyurethane was considered. Removing the high temperature processing allowed more scents to be tested as the casting process is regarded as a more stable process for scented oils and lures.

Cast polyurethanes are a diverse and versatile group of materials that are known for abrasion resistance, chemical resistivity, stability in water, ease of processing and relative low cost. Cast polyurethanes are elastomers and are created by the reaction of a prepolymer, which contains reactive isocyanate groups and a curative, which contains hydroxyl or amine groups. In their simplest form, these two components, the prepolymer and curative are the only chemicals in the mix. The mixture is then poured into a heated mould where the components react to form a solid elastomeric piece. Unlike injection moulding materials, which are thermoplastic, cast polyurethanes are thermosets. Thermoset materials are chemically different to the chemicals that were initially mixed together, once reacted they are then unable to be remoulded. Because of the homogeneous and reactive properties of cast polyurethane, they form a great matrix for specialty additives, which can impart different characteristics to the finished product.

A number of lures or scents were selected for trial impregnation within the cast polyurethane. These scents were selected after discussion with other researchers and Pest Control Operators as having an attraction to foxes and included:

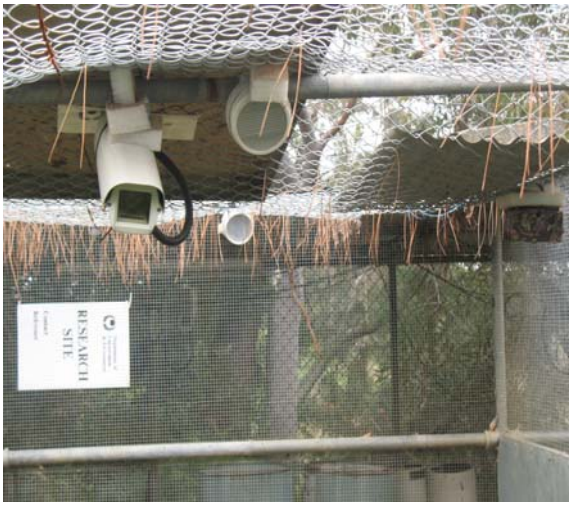
- Synthetic fermented egg (SFE)
- Honey
- Outfoxed fox lure
- Red cordial
- Molasses
- Fish oil
- Vanilla essence
- Outfoxed cat lure

The prototype polymer bait heads will form the basis of comparative efficacy trials to be conducted as part of Milestone 5.

Verbal approval to use the animal facilities at the Department of Environment and Primary Industries (DEPI) Frankston Victoria was received in July 2013 and Animal ethics approval for the (pen) trials was granted through the DEPI – Wildlife and Small Institutes Animal Ethics Committee (January 2013). Animals were kept at the facilities under a Pest Animal Research/Education Collections permit RE73-2012/13.

Animals were sourced from commercial pest control operators licensed in the control of feral animals and experienced in the capture and supply of animals for research purposes. Animals were opportunistically taken from the greater Melbourne Area, including private property, National Parks and other land tenure, using Victor® soft-catch foothold traps. Animals that showed signs of disease, physical impairment or old age that might potentially confound the trial results were humanely euthanased on sites by the pest control operator. Juvenile foxes weighing less than 3kg were also humanely euthanased at the point of capture in accordance with the relevant Code of Practice and contractual obligations.

The facilities at DEPI Frankston were purpose built for the housing and monitoring of animals. The pens measure 9 x 3 x2m and are equipped with automatic water drinkers, shelter and remote video cameras. Upon arrival animals are lightly sedated, inspected for any injuries and treated as deemed necessary. Sex and body morphology is recorded. Animal behaviour is monitored and continually recorded, via remote video camera system in order to reduce the potential distress caused to animals by human presence and allow review of the nightly activity.



Two video cameras were used to monitor activity, one giving a complete view of the pen with the second positioned to give a close-up view of the rear of the pen.

Two prototype bait head designs were tested; i) scent impregnated PU and ii) PU bait heads with scent reservoirs (wells). The free choice cafeteria-style trial was designed in consultation with Andrew Gormley, former DEPI Biometrician. The design of the trial was such that each fox was given four bait heads per day, and all eight bait heads were offered to each fox once during the trial. The location of each bait head in the 'cafeteria' was randomised (Table 1). The sets of 4 bait heads were secured to the ground along the pens' length approximately 2 metres apart, with position 1 located closest to the pen entrance and position 4 located to the rear of the pen. Animals were introduced and allowed to voluntarily interact over the following 18 hours. The next day the alternative set of 4 bait heads were deployed and the experiment repeated. Fox

activity was recorded to ascertain any preference to particular bait heads and potential behaviour elicited by the lure.

Cat lure provided by OUTFOXED Pty Ltd was initially to be the eighth lure to be impregnated into the PU matrix, however, it could not be successfully impregnated due to chemical incompatibility. This created a 'gap' in the deployment of the impregnated PU bait heads but was regarded necessary as the lure was used in the PU 'wells' bait head trials (Table 1).

Table 1. Random Order Grid for assessing the attractiveness and durability of each bait head and the elicited behaviour in the foxes.

PU BAIT HEADS IMPREGNATED WITH LURE DURING MANUFACTURE						
BAIT HEAD LURES	FOX POSITION	F35	F33	F38	F32	F39
1. SFE	Day 1 1	4	7	4	1	6
2. HONEY	2	2	4	5	4	*8
3. FOX LURE	3	6	6	2	6	3
4. RED CORDIAL	4	5	1	7	3	2
5. MOLASSES	Day 2 1	3	3	6	5	7
6. FISH OIL	2	*8	5	*8	2	4
7. VANILLA	3	7	*8	1	*8	5
*8. *CAT LURE*	4	1	2	3	7	1
PU BAIT HEADS MANUFACTURED WITH WELLS TO CONTAIN LURES						
BAIT HEAD LURES	FOX POSITION	F40	F42	F45	F43	F47
1. SFE	Day 1 1	4	4	7	8	6
2. HONEY	2	5	5	3	4	7
3. FOX LURE	3	2	2	1	1	2
4. RED CORDIAL	4	6	1	8	6	3
5. MOLASSES	Day 2 1	1	6	6	2	*4*
6. FISH OIL	2	8	8	5	5	*1*
7. VANILLA	3	3	3	2	7	*5*
8. CAT LURE	4	7	7	4	3	*8*

*(*8)* In the impregnated table indicates the position of the cat lure in the random distribution, however it was not available in this form.

Typical pen set-up during trials shows shelter at the rear and bait heads secured along the length of the pen.

Appendix 4:

Results of fabrication and trials with polymer bait heads

(Extract from Milestone 5 Report)

Table 2 presents two data sets, on the left; the bait head first investigated by each animal, its position and time spent at the station. The right; indicates the bait head that elicited the greatest activity, it position and duration of time animal spent at that station. During the impregnated bait trials, SFE received the most first visits, but did not elicit any desirable activity. Vanilla was most likely to elicit pulling. With well type bait, fox lure and fish oil received the most first visits. Though fish oil elicited greater pulling activity in comparison to the other lures.

Table 2: Summary of bait head trial results in terms of fox activity.

Sex	Day Used	Fox ID	Bait Head Type	First Lure Visited	Time To Visit	Time At Bait Stn.	Stn.		B/H eliciting Greatest Activity	Stn.	Greatest Activity	Time To This Event	No. Visits To Stn.	Ratio of Greatest Activity	Total Time At Station
Male	2	F32 Day1	PU Imp.	Fox lure	73sec	85sec	4		Fox lure	4	Pulling	13hours	7	3/7	85s
	3	Day2	PU Imp.	Vanilla	12sec	14sec	4		Vanilla	4	Pulling	12secs	1	1/1	14s
Female	5	F33 Day1	PU Imp.	SFE	15sec	6sec	4		Vanilla	1	Pulling	38mins	1	1/1	6s
	6	Day2	PU Imp.	None	-	-	-		None	-	None	-	-	-	-
Female	3	F38 Day1	PU Imp.	Vanilla	39sec	5sec	4		Vanilla /Molasses	4	Sniffing	39secs /18mins	3/3	3/3	5s/5s
	4	Day2	PU Imp.	Fox lure	100sec	2sec	4		Fox lure	4	Licking	100secs	1	1/1	4s
Male	2	F35 Day1	PU Imp.	Red cordial	80sec	17sec	1		Red cordial	1	Biting	80s	9	2/9	17s
	3	Day2	PU Imp.	SFE	29sec	5sec	4		Fox lure	1	Biting	16hours	6	2/6	30s
Male	4	F39 Day1	PU Imp.	Honey	32sec	65sec	4		Honey	4	Pulling	20mins	6	5/6	65s
	5	Day2	PU Imp.	SFE	48sec	5sec	4		Molasses	3	Pulling	94s	3	3/3	48s
Male	1	F40 Day1	PU wells	Red cordial	33sec	94sec	1		Fish oil	4	Pulling	4hours	13	6/13	312s
	2	Day2	PU wells	Fox lure	4mins	6sec	3		Cat lure	2	Pulling	15mins	4	2/4	45s
Female	1	F42 Day1	PU wells	None	-	-	-		None	-	None	-	-	-	-
	2	Day2	PU wells	Vanilla	8hours	2sec	4		Vanilla	4	Sniffing	8hours	1	1/1	2secs
Female	1	F45 Day1	PU wells	Fox lure	30mins	3sec	2		Cat lure	4	Sniffing	30mins	13	13/13	13s
	5	Day2	PU wells	Fish oil	10mins	48sec	1		Honey	3	Licking	4mins	5	2/5	70s
Male	2	F43 Day1	PU wells	Fish oil	10sec	89sec	4		Fish oil	4	Pulling	12hours	6	2/6	89s
	6	Day2	PU wells	Fox lure	34sec	39sec	4		Fox lure	4	Pulling	15mins	2	1/2	39s
Male	4	F47 Day1	PU wells	Honey	20mins	78sec	3		Fish oil	1	Pulling	2hours	12	3/12	83s
		Day2	euthanased												

On day 1 for each animal, five of the ten animals tested visited station 4 first, a further two chose station 1 while one animal (F42) did not interact with any bait head (i.e. 7/10 animals visited stn4 or stn1 first). In five of the seven cases this first encounter also was the site of greatest activity. On day 2 six of the nine animals chose station 4. Female F42 did not interact with the bait heads on either day, while female F33 had no interaction on the first day.

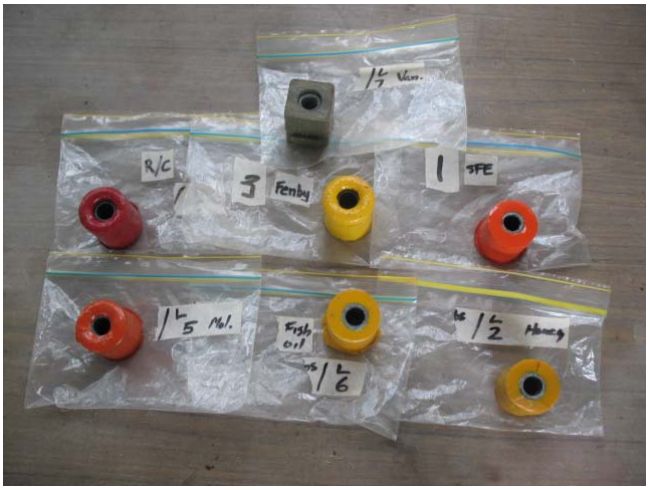
The data in Table 3 illustrates that, although vanilla impregnated heads did not receive the longest attention by each of the foxes, they did obtain a more consistent time by each fox and elicited the greater desired activity. The same can be said of fish oil in the well type bait heads. Interestingly when you look at the least visited lures from each of the bait head type the complete reverse occurs, fish oil is the least visited for impregnated heads and vanilla for well type heads.

Table 3: Illustrates the time each lure station was visited and the behaviour that lure elicited.

Bait Head Type		**Fox Time At Station (Secs)**						**Comment**	**Activity Type**				**Desirable Activity score**
Impregnated	**Lures**	**F32**	**F35**	**F33**	**F38**	**F39**	**Total time**		**Sniff**	**Lick**	**Bit**	**Pull**	
	SFE	2	5	6	1	5	19		4			1	1
	Red cordial	2	17	21	1	1	42		5	1	1		1
	Molasses	0	8	0	5	48	61	2 no activity	2		1	1	2
	Honey	0	7	0	3	65	75	2 no activity	3		1	1	2
	Fish oil	2	5	3	0	3	13	Least time	4				0
	Fox lure	85	30	0	2	2	119	Longest time	3		2	1	3
	Vanilla	14	7	6	5	1	33	Consistent times	4		2	2	4
	Cat lure	n/a	n/a	n/a	n/a	n/a							
Wells	**Lures**	**F40**	**F42**	**F45**	**F43**	**F47**	**Total time**						
	SFE	67	0	8	447		522	Longest time	2	1	2	2	4
	Red cordial	94	0	32	34		160		3	2	2	2	4
	Molasses	56	0	26	3		85		3	1	1	1	2
	Honey	56	0	75	3	*78*	134		3	1	2	2	4
	Fish oil	312	0	48	89	*83*	449	Consistent times	4	2	3	3	6
	Fox lure	6	0	3	39	*52*	48		4		3	2	5
	Vanilla	13	2	4	0	*28*	19	Least time	3			2	2
	Cat lure	45	0	13	420		478		3			2	2

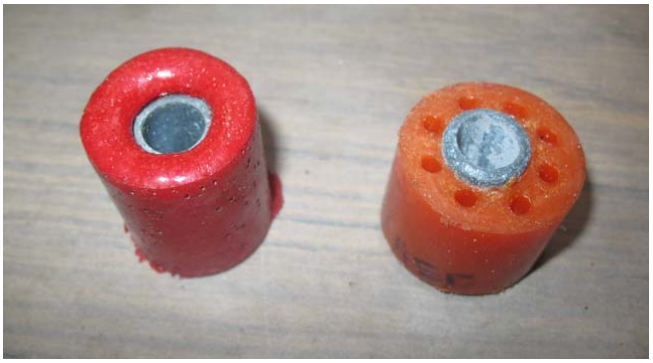
**not included in 'wells' Total time*

Shore hardness scales (A00, A and D) are a measure of the hardness of different materials. The **Shore A** Hardness Scale measures the hardness of flexible mold rubbers that range in hardness from very soft (gels) to semi-rigid plastics with little flexibility (e.g. shopping cart wheels). Shore 85A is at the high end of the Shore A Scale. The cast, impregnated samples and rod for the well type bait heads were all manufactured to 85% Shore A strength.



Seven lure impregnated bait heads used in the trials.
(top, left to right)
Vanilla
Red cordial
Fox lure
SFE
Molasses
Fish oil and
Honey

The resulting smell of each of the impregnated samples was not necessarily recognisable particularly red cordial, vanilla and honey samples, although these have a stronger flavour attraction rather than a strong scent. The comparison with the well type heads was to investigate these differences. From the results in Table 2 one would say that the well type heads performed better; longer time spent and a greater degree of activity. However, the practicality of using these heads in the field and the longevity of the attraction created were questioned. It is likely that the process and/or chemical reaction in producing the cast impregnated heads, however more flexible to the addition of additives, may still in some way have altered the chemical structure and volatile composition of these materials potentially altering their smell. Discussions with P.D Plastics have highlighted that oil based materials are preferred. As experienced with the fox lure, suspected binding issue during the casting, created lesions in the final solidified material altering structure. The red cordial and molasses samples also had what appear to be trapped air bubble imperfections possibly due to the rate of the chemical reaction trapping the air bubbles within the solidifying material. It is for these reasons that further development work with the cast polyurethane is recommended.



Impregnated Red Cordial bait head and bait head with wells

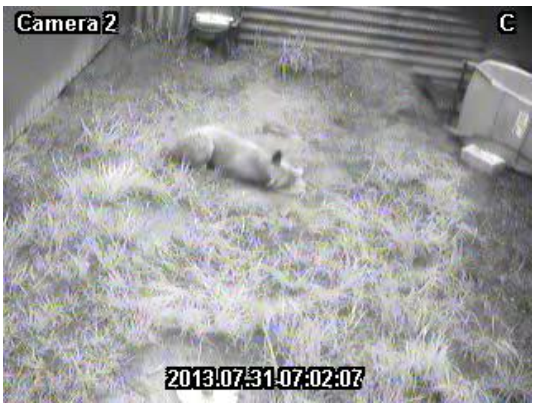
As described in the Experiment Protocol, the sets of four bait heads were positioned along the pens length approximately 2 metres apart. Station 1 being the closest to the

pens entrance, while station 4 was at the rear of the pen and closest to the animals' shelter. Data from Table 2 suggests that there may have been a bias created towards the bait head located at station 4 and to a lesser extent station 1 due to their location. Animals were generally preoccupied with escaping therefore spending the majority of their time either at the rear of the pen or at the front. Interaction may therefore have been more of a reactive behaviour of biting /chewing on the closest accessible object. This appeared to be the case regardless of the lure and occurred with both bait head types. Of the 19 trial days, station 4 was the first visited on 11occasions, station 1 on 3 occasions, two foxes (both female) did not interact at all and on the remaining 3 days stations 2 and 3 twice were chosen. All three occasions occurred during the well type trials and on two of those vanilla, which was the least visited bait head, was located on station 4 and 1 (Day 2-F40 and Day 1-F45) (Appendix 1).

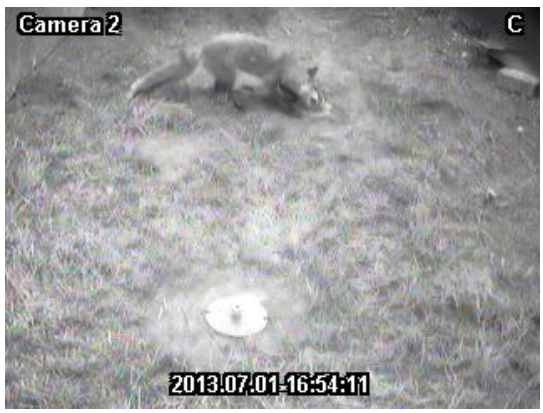
Table 3 indicates that time spent at each station for the 'well type' bait heads was generally longer than that at the equivalent impregnated heads, except for vanilla and fox lure. Fox lure, in the impregnated trials, scored the highest in the 'total time spent' (119sec), however the majority of this was attributed to only one animal (F32-85sec). High scores to molasses and honey were also due to one animal (F39-48sec and 65sec respectively). All impregnated bait heads elicited a desirable outcome other than fish oil, which only received minimal interaction time from all five animals and their activity was limited to occasional passing sniffs only. Red cordial recorded high activity but did not result in any pulling behaviour. The most consistent, in time spent by each of the five animals was vanilla, and recorded the highest desirable outcome score (4).

Similar results were seen in the well bait head group, SFE scored high due to F43 (447sec), and fish oil and cat lure similarly due to the interest from F40 (312sec) and F43 (420sec) respectively. All well type bait heads elicited a pulling behaviour, though fish oil had the most 'consistent in time spent' across the five animals and the highest desirable outcome score (6). In contrast vanilla essence used in the well trial had the least time spent at the bait station (19sec) and only elicited a pulling response twice as did molasses and cat lure.

There was no apparent difference in the animals behaviour or station choice based on the days in captivity prior to the bait head trials.



Fox 40 pulling on PU bait head with wells

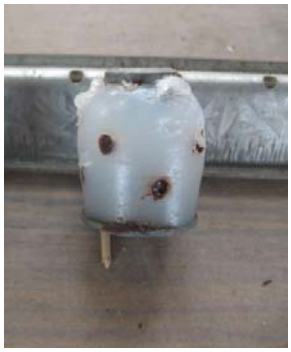


Fox 39 chewing impregnated PU

It is noteworthy to highlight that the bait head trials were conducted without any restricting collars thereby allowing foxes full access to the bait heads. Video footage clearly shows animals biting and pulling on the heads with their carnassials teeth. Damage to the bait heads, in both trial types, was minimal other than with the

impregnated fox lure type. This bait heads sustained significant damage due to the lesions created during the casting process. A moulded EVA rubber (Ethylene vinyl acccetate) bait head was also initially trialed (Appendix 1). This proved to be to soft a material and further trials were abandoned.

Bait head impregnated with fox lure



EVA bait head

Maximum damage on PU imp. head

Appendix 5:
Potential MDE toxin specifications

1. Cyanide compounds

Sodium cyanide (NaCN) is not registered for use as a predator pest control agent in Australia, although it has been assessed in a number of States under an experimental research permit from the Australian Pesticides & Veterinary Medicines Authority (APVMA) and is currently being considered for registration as a vertebrate pesticide for use with the M-44 ejector. Cyanide is commonly used in the United States for the control of canids and in New Zealand for the control of brushtail possums (*Trichosurus vulpecula*). A number of registered cyanide products are used for the control of possums ranging from cyanide paste to encapsulated potassium cyanide pellets (pers. comm. Penny Fisher). The Connovation Limited (New Zealand) registered cyanide paste is also being considered for use with the Lethal Trap Device (LTD) as a means of improving the humaneness of trapping. Sodium cyanide's high water solubility makes it an attractive toxin for aerosol formulation. However, its indiscriminant nature and potential operator safety issues need to be addressed if it is to have any potential of being registered.

Toxin type: Cytochrome C oxidase inhibitors - inhibits oxidative enzymes causing death through anoxia

LD_{50} values: Coyote (*Canis latrans*) 4.1mg/kg (6.44 mg/kg Rat), (4.6 mg/kg brushtail possums)

Hooke *et al* (2006) report successfully using the Pocatello Supply Depot (US Department of Agriculture: Pocatello, ID) cyanide capsules with the M-44 ejector to control wild dogs. The capsules contain 0.88 g of sodium cyanide delivered as a powder into the animals' mouth. The quantity of cyanide delivered in this manner appeared sufficient to kill animals weighing up to 17.5 kg (Hooke *et al* 2006).

Mode of action: Delivered orally, NaCN produces hydrogen cyanide on contact with oral mucosa and the acids in the stomach (Marks and Gigliotti, 1996). Cyanide is a very toxic chemical asphyxiant and inhibits cytochrome oxidase preventing oxygen utilization leading to cytotoxic anoxia. Death results from central nervous system (CNS) failure and anoxia although venous blood remains oxygenated.

Toxicosis: The onset of clinical signs is in seconds and can include irritation/burning of eyes, nose, throat, lungs, shortness of breath, breathing difficulties, hot flushes throughout the body, headache, drowsiness, dizziness, heaviness of arms and legs, nausea, vomiting, CNS suppression, respiratory suppression, cardiac arrest, coma and death (DEFRA, 2005; Marks and Gigliotti, 1996; Sigma-Aldrich, 2009).

Hooke *et al* (2006) reported: Sodium cyanide poison is potentially a more humane method to control wild dogs than sodium fluoroacetate (1080) poison. This study quantified the clinical signs and duration of cyanide toxicosis delivered by the M-44 ejector. The device delivered a nominal 0.88 g of sodium cyanide, which caused the animal to lose the menace reflex in a mean of 43 s, and the animal was assumed to have undergone cerebral hypoxia after the last visible breath. The mean time to cerebral hypoxia was 156 s for a vertical pull and 434 s for a side pull. The difference was possibly because some cyanide may be lost in a side pull. There were three distinct phases of cyanide toxicosis: the initial phase was characterised by head shaking, panting and salivation; the immobilisation phase by incontinence, ataxia and loss of the righting reflex; and the cerebral hypoxia phase by a tetanic seizure. Clinical

signs that were exhibited in more than one phase of cyanide toxicosis included retching, agonal breathing, vocalisation, vomiting, altered levels of ocular reflex, leg paddling, tonic muscular spasms, respiratory distress and muscle fasciculation of the muzzle.

Humaneness: Death by NaCN poisoning is regarded as a humane mode of action due to the rapid onset of toxicosis and suspected onset of insensibility before convulsions and death. Foxes that ingest a lethal dose of NaCN succumb quickly and show only moderate distress. Mild irritation upon contact with cyanide is expected and progression of toxicosis may elicit a level of anxiety (Marks and Gigliotti 1996).

Environment and Secondary poisoning: As a result of (i) the relatively small quantities required, (ii) its high water solubility, and (iii) its rapid dissipation as hydrogen cyanide, NaCN is regarded as having low environmental persistence. The potential of secondary poisoning is also low due to it being rapidly metabolised and/or oxidised once ingested or inhaled (Marks and Gigliotti 1996). Combined with the target specificity of the MDE, it is suggested that the risk of primary exposure to the toxin by non-target species is minimal.

Human safety: Cyanide is highly toxic and can be absorbed through the skin and eyes, inhaled and ingested. An LD_{50} range of 0.5 - 3.5 mg/kg has been suggested by Eason and Wickstrom 1997.

2. Sodium monofluoroacetate (1080)

This compound is registered in Australia for the control of mammalian pest species, both herbivore and predator. Favourable attributes associated with 1080 for the intended application include high water solubility and target specificity. Many Australian natives (particularly in Western Australia) have a tolerance to 1080 due to fluoroacetate derivatives occurring in certain plants. Contrary to this is the concern about the humaneness of the toxin by animal welfare groups.

Toxin type: Citrate accumulation causing energy deprivation.

LD_{50} values: Foxes 0.13 mg/kg; Wild dogs 0.11 mg/kg (McIlroy 1981 and 1986)

In Victoria, 1080 is currently registered for use in predator baits at concentrations of 3.0 mg and 4.5 mg for foxes and wild dogs respectively.

Mode of action: 1080 is converted to fluorocitrate, which is subsequently converted to hydroxy-trans-aconitate (HTn) which binds and inactivates the enzyme aconitase resulting in inhibition of citrate oxidation (Goh *et al.* 2005). This binding causes the tricarboxylic acid (Krebs) cycle to stall, and inhibiting energy production (Eason 2002). The result is an accumulation of citrate in the tissues and blood, resulting in energy deprivation, eventually leading to death. In carnivores, death is a result of central nervous system failure (Goh *et al.* 2005; Marks *et al.* 2000).

Toxicosis: The clinical symptoms of 1080 toxicosis (Goh *et al.* 2005; Marks *et al.* 2000) entail:

1. Initial anxiety, frenzied activity, running, howling and either hypersensitivity or non-responsiveness to external stimuli;
2. Excessive salivation, vomiting, inappropriate urination/defecation, difficulty during defecation and hyperthermia; and
3. Collapse, unconsciousness, convulsions, shortness of breath, and cardio-respiratory arrest.

Humaneness: Death from 1080 toxicoses can occur anywhere from 2 to 24 hours after dosing. The occurrence of repeated convulsions and seizures (DEFRA 2005; Goh *et al*. 2005; Marks *et al.* 2000; Sherley 2007) raise issues in relation to its humaneness. Presently there is no specific antidote for 1080 and treatment provided by veterinarians is often unsuccessful because once symptoms become apparent, the toxicosis is difficult to reverse.

Environment and Secondary poisoning: Under favourable conditions (11–20^{o}C and 8-15% moisture) 1080 is successfully defluorinated by micro-organisms in 1 - 2 weeks in soil and/or water. However, this time can increase to several months in unfavourable conditions (DEFRA 2005; Eason 2002). Secondary poisoning can be an issue as 1080 persists in the carcasses of dead animals resulting in significant risk to raptors and other scavenging carnivores (Meenken and Booth 1997). Strict government legislation is in place to govern the use of 1080 and reduce the risk to non-target animals and the environment.

Human safety: 1080 can be absorbed through the skin and nose thereby requiring an appropriate level of care when preparing baits. LD_{50} in humans' 0.71 mg/kg (McIlroy 1986).

Summary of research results from 1080 / CuI combination trials:

For a poison to be considered humane it should produce a minimum number of symptoms and a rapid loss of consciousness before death (Mason and Littin 2003). The humaneness of 1080 toxicosis is difficult to substantiate. Retching and manic running activities commonly associated with 1080 poisoning occur early in the toxicosis when the animal is clearly conscious and responsive to external stimuli (Marks *et al.* 2000; Marks *et al*. 2009). Some pain and distress thereby can be assumed prior to the collapse of the animal. Once collapsed, however, it is thought that the animal is unconscious and therefore unable to perceive pain during the convulsions and spasms that occur prior to death.

Copper indomethacin (CuI) (Nature Vet, Glenorie, Australia) is a potent non-sedating analgesic. Okuyama *et al.* (1987) reported evidence of CuI having central nervous system activity as well as peripheral analgesia (Barnett and Jongman 1996). As such it is a potentially suitable additive for minimising any associated pain and distress during the1080 toxicosis.

Previous research (Marks *et al*. 2009) has shown that symptoms associated with 1080 toxicosis are reduced by the combination of the toxin with CuI. The co-administration of a 1080 / CuI formulation was shown to significantly reduce the incidence of retching in foxes compared with those receiving 1080 alone (Marks *et al.* 2009). The combination also reduced the duration of the toxicosis from the onset of first symptoms until death compared with the toxicosis produced by 1080 alone. The 1080 / CuI combination was delivered to penned foxes using an M-44 ejector. The ejector capsule contained 2.7 mg of 1080 with 2.8 mg CuI. At the given dose rate CuI did not appear to affect the lethality of 1080. The first signs of abnormal behaviour observed in foxes dosed with 1080 alone were retching and manic running, followed by periods of intense spasms, convulsions and uncoordinated paddling subsequent to collapse. While there was no significant difference in the incidence of paddling and convulsions after collapse a significant reduction in the incidence of retching and manic running prior to collapse was found for foxes co-administered with 1080 and CuI compared with those foxes given 1080 alone (Marks *et al*. 2009). In foxes dosed with 1080 alone the mean time to death was 310 minutes compared to 280 minutes for the 1080 / CuI co-formulation (Marks *et al*. 2009).

Thus, the humaneness of 1080 maybe improved when formulated with an analgesic such as CuI. Research has shown an improved efficacy (increased toxicity, reduced duration of toxicosis, and diminution of clinical symptoms) using the combination (Marks *et al*. 2009).

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Appendix 6:

Stage 2 activities and timelines

The table below provides potential Stage 2 Milestones and timelines.
The timelines against each criterion is based on an estimate of time required to achieve the output. Where possible outputs will run concurrently.

Milestones

Achievement Criteria		Time duration
1	Execution of agreement	
2	**Consolidation phase** *- Confirmation of toxin:* • Industry negotiations • APVMA Application • Toxin formulation 'shelf life'	 3 months 2 months 12 months
3	*- Selection of specific lures for impregnation within the CPU polymer matrix:* • Lure formulation and fabrication • Efficacy pen trials	 2 months 3 months
4	*- Fabrication of MDE units:* • Local fabrication of venturi system • Fabrication of MDE units (20) for field deployment	 4 months 2 months
5	**Field trial phase** *– Target specificity trials:* • Spotted tailed quoll • Eastern quoll • Northern quoll • Tasmanian devil • Southern brown bandicoot • Northern brown bandicoot • Large reptile species • Goanna • Dingo and working dog	 12-18 months
6	*- Fox control field trials:* • Melbourne Water, Werribee trial • Fox control program	 3 months 4 months
7	*- MDE for wild dog control:* • MDE assessment trial • Wild dog control program	 3 months 4 months
8	*- Field reliability/longevity assessment* • Demonstrated reliability and longevity of the MDE under field conditions	 12 months
9	**Communication Plan**	12 months

The successful completion of the Stage 2 milestones would then allow progression to the final Stage 3 phase, which in broad terms would involve the;

- evaluation of the research data,
- preparation of the APVMA registration application, and
- negotiations to determine an commercial industry partner.

Appendix 7:
Correspondence with wildlife agencies

Species *Include both scientific and common name*	Source Include location details	Number of animals available	Contact details
Spot tailed quoll *(Dasyurus maculates)*	**Captive:** Featherdale W/P Ballarat W/park **Wild population:** Tasmania NSW	≤20	Chad Staples, (02) 9622 1644 (Gen.) Ballarat, (03) 5333 5933 (Gen.) Stephen Harris, 0427 330 945 Andrew Claridge, 0427 896 827
Tasmanian devil *(Sarcophilus harrisii)*	**Captive:** Ballarat W/park **Wild population:** Tasmania	≤20	Ballarat, (03) 5333 5933 (Gen.) Stephen Harris, 0427 330 945
Eastern quoll *(Dasyurus viverrinus)*	**Captive:** Woodley school Mt Rothwell **Wild population:** Tasmania	≤20	Gary Simpson, (03) 5971 6100 (Gen.) Annette Rypalski, 0434 295 355 (Gen.) Stephen Harris, 0427 330 945
Northern quoll *(Dasyurus hallucatus)*	**Captive:** Territory W/park **Wild population:**	≤20	Dion Wedd, 0439 991 240
Sthn brown bandicoot *(Isoodon obesulus)*	**Captive:** Mt Rothwell **Wild population:** RBG Cranbourne	≤20	Annette Rypalski, 0434 295 355 (Gen.) Terry Coates, (03) 5990 2200 (Gen.) (Approved: Research permit RBGC-1301)
Nthn brown bandicoot *(Isoodon macrourus)*	**Captive:** **Wild population:** Territory W/park	≤20	Dion Wedd, 0439 991 240
Lace monitor *(Varanus varius)*	**Captive:** **Wild population:** East Gippsland	≤20	Andrew Murray, 0419 396 948
Monitors *(Varanus spp)*	**Captive:** Territory W/park **Wild population:** Nth Territory		Dion Wedd, 0439 991 240
Dingoes *(Canis lupus)*	**Captive:** Featherdale Austn dingo Con. Assoc. **Wild population:**		Chad Staples, (02) 9622 1644 (Gen.) Amanda McDowell, (02) 4888 9289

Source location details:

- Austn Dingo Conservation Assoc. Inc. Colong Station POB 146 Oberon, NSW 2787
- Ballarat Wildlife Park, 250 Fussell St, Ballarat East, Victoria.
- Featherdale Wildlife Park, 217 Kildare Rd Doonside, NSW.
- Mt Rothwell Conservation and Research Centre, 5 Mt Rothwell Road, Little River, Vic.
- Royal Botanic Gardens Cranbourne, 1000 Ballarto Rd, Cranbourne, Victoria.
- Territory Wildlife Park, Cox Peninsula Rd Berry Springs, Northern Territory.
- Woodley School, 485 Golf Links Road, Langwarrin South, Victoria.

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