Efficacy and welfare assessment of an encapsulated para-aminopropiophenone (PAPP) formulation as a bait-delivered toxicant for feral cats (*Felis catus*)

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Abstract

**Context.** Feral cats are invasive predators of small and medium-sized fauna throughout Australia. The only broad-scale population-management technique for feral cats currently available in Australia is poison baiting. As poison baits for feral cats must be surface-laid, this can lead to the unintended exposure of non-target species consuming the baits. Encapsulation of a toxin within a robust, controlled-release pellet implanted within the meat lure (the combination of which is termed the Curiosity® bait) substantially reduces the potential risk to non-target species. Para-aminopropiophenone (PAPP) has been shown to be an effective toxin to which cats are highly susceptible.

**Aims.** The present study aimed to measure the efficacy of encapsulating PAPP toxin in a controlled-release pellet on feral cats in a pen situation and to document the observed behaviours through the toxication process.

**Methods.** Pen trials with captive cats were undertaken to document efficacy of encapsulating PAPP toxin in a controlled-release pellet and to assess the behaviours during toxicosis. These behaviours inform an assessment of the humaneness associated with the Curiosity bait using a published relative humaneness model.

**Key results.** The trials demonstrated a 95% consumption of the toxic pellet and observed the pattern of behaviours exhibited during the intoxication process. There was a definitive delay in the onset of clinical signs and death followed at ∼185 min after the first definitive sign. The humaneness using the relative humaneness model was scored at ‘mild suffering’.

**Conclusions.** The encapsulating PAPP toxin in a controlled-release pellet for feral cats is effective. The feral cats display a range of behaviours through the toxication process, and these have been interpreted as mild suffering under the relative humaneness model.

**Implications.** The documented efficacy and behaviours of encapsulating PAPP toxin in a controlled-release pellet provides knowledge of how the PAPP toxin works on feral cats, which may assist in decision-making processes for conservation land managers controlling feral cats and whether to incorporate the use of the Curiosity® bait into existing management techniques.

**Additional keywords:** behaviour, conservation management, invasive species, pest management, threatened species, toxicology.

Received 20 September 2019, accepted 25 May 2020, published online 1 July 2020

Introduction

Invasive species, particularly the feral cat (*Felis catus*), are a primary cause of population declines and the extinction of endemic animals (Burbidge and Manly 2002; Veitch and Clout 2002; Gurevitch and Padilla 2004; Clavero and Garcia-Berthou 2005), and removing invasive predators has been demonstrated as providing substantial positive benefits to the conservation of such species (Jones et al. 2016). In Australia, feral cats (defined in Department of the Environment (2015)) have been implicated and directly linked to the extinction of endemic-species populations (McKenzie et al. 2007; Frank et al. 2014; Woinarski et al. 2015), while also posing a threat to a further 142 species and subspecies of endemic animals (Coutts-Smith et al. 2007; Woinarski et al. 2014). This provides a clear stimulus for wildlife managers to intervene using techniques that minimise the potential for...
impact on endemic fauna. There have been few techniques that enable targeted management of feral cat populations. Generally, trapping and shooting, which are labour intensive and costly, are only suitable for small areas (Reddiex et al. 2006; Fisher et al. 2015). In respect of this, the Australian Government, under the Environment Protection and Biodiversity Conservation Act 1999, has developed a broad-scale baiting technique that meets the six major principles for design and execution of vertebrate pest-control programs (Littin et al. 2004).

In developing the Curiosity bait, the toxin para-aminopropiophenone (PAPP) was selected to minimise unnecessary and unintentional negative impacts on animal welfare (Littin 2010). Poison baits intended for feral cats must be surface-laid because cats are likely to consume only surface-laid bait items (Denny and Dickman 2010) and this practice may present a hazard to non-target species (Seebeck and Clunie 1997). PAPP provides the benefit of being highly toxic to felids and canids, but is less toxic to many native species (Australian Pesticides and Veterinary Medicines Authority 2015). Baits containing PAPP still present a hazard to some Australian species, such as varanid lizards, which means that Curiosity is unlikely to completely replace existing products such as the Eradicat® feral cat bait (containing directly injected 1080 used in the south-west of Western Australia), but rather become an additional tool for land managers to be used at sites where the risk to varanids can be mitigated.

The Curiosity bait for feral cats combines a moist meat food lure and a hard, controlled-release pellet (hard-shelled delivery vehicle, or HSDV) containing a rapidly dispersing and acting toxicant formulation. The intent is to exploit differences in the biochemistry and feeding behaviours between felids and many Australian native species (Marks et al. 2006; Hetherington et al. 2007; Johnston et al. 2007; Forster 2010; Gigliotti 2011; Buckmaster et al. 2014).

The second aspect of mitigating intoxication of non-target species is achieved through the use of the PAPP, which reacts with haemoglobin (Hb) to produce the non-oxygen carrying methaemoglobin (MetHb; Vandenbelt et al. 1944). MetHb is normally present at low concentrations in mammalian blood; however, felids lack the enzyme present in most mammal species to convert MetHb back to Hb. PAPP intoxication causes hypoxia, depriving the brain and heart of oxygen (Savarie et al. 1983; Marks et al. 2004; Eason et al. 2010, 2014; Shapiro et al. 2018). Assessments of the behaviours and clinical signs associated with PAPP intoxication have been reported in a range of species (Marks et al. 2004; Gibson et al. 2011), with Eason et al. (2014) proposing that it is relatively humane for cats.

Para-aminopropiophenone (PAPP) has been evaluated as a vertebrate pesticide in the United States, Australia and New Zealand (Savarie et al. 1983; Marks et al. 2004; Fisher et al. 2005; Johnston et al. 2011; Murphy et al. 2011), with the LD₅₀ rate for cats determined to be ~6 mg kg⁻¹. However, the nominal LD₅₀ varies according to the formulation and delivery methodology used (Savarie et al. 1983). Thus, for directly injected PAPP-based bait products, greater amounts of PAPP are required to compensate for in situ toxicant loss through ‘degradation’, and the irreversible adsorption of the toxicant by the meat lure. Although the rate used is lower than will necessarily affect many Australian native mammals (e.g. for the spotted-tail quoll (Dasyurus maculatus) the LD₅₀ is 28.8 mg/kg, for the Tasmanian devil (Sarcophilus harrisii) it is 120 mg/kg, for the brown antechinus (Antechinus stuartii) it is 571 mg/kg (Nocturnal Wildlife Research (2006), as reported in Eason et al. (2014)), smaller-bodied species may receive a lethal dose if they consume only part of the toxic dose in a single direct-injected bait. The HSDV encapsulation of the PAPP seeks to mitigate the exposure of these species by using only the minimum amount of toxicant that is necessary for the target species. Encapsulation also protects the PAPP from environmental and biological degradation.

Australian native carnivores chew food items thoroughly in the mouth before swallowing and tend to reject large hard objects (Marks et al. 2006; Hetherington et al. 2007). Conversely, felids often consume large items intact (Fitzgerald 1988; Hilmer et al. 2010). Risk mitigation for smaller carnivores is achieved by implanting the HSDV inside the meat lure where the size of the HSDV is too large to be ingested whole and the structure is too robust to break when chewing, encouraging rejection (Marks et al. 2006; Buckmaster et al. 2014). The HSDV is formed from an impermeable material which is stable in the meat lure, but dissolves rapidly in the gastrointestinal tract following ingestion.

The HSDV is ~10 × 8 mm long (Buckmaster et al. 2014) and contains 78 mg of PAPP and other excipients. This was determined to be the preferred dose, calculated from the LD₅₀ of PAPP in cats being ~5.6 mg/kg (Savarie et al. 1983) and undertaking pen studies that compared the efficacy of various PAPP doses and formulations (Johnston et al. 2012; M. Johnston, unpubl. data). The current paper presents the outcomes of pen trials with 30 captive feral cats that documented the consumption of a meat lure, either Curiosity, chicken or beef, and HSDV, measured the efficacy of the PAPP toxin, and observed the behaviours following consumption of a HSDV.

The welfare of animals can be described in the following five domains: the four physical components, namely, nutrition, environment, health and behaviour; and the mental components (Mellor and Reid 1994; Mellor et al. 2009). Compromise in one or all of the physical domains can be used to infer potential negative effects in the fifth domain (Beausoleil and Mellor 2015). The negative experiences of the intoxication by PAPP can be described against the states defined in the mental domain. That is, thirst, hunger, discomfort and pain, breathlessness, nausea, dizziness, debility, weakness and sickness (Beausoleil and Mellor 2015). Although these behaviours may be mapped to the states defined in the mental domain, the degree, if any, of the negative experience the cat is having is unclear and whether one particular state dominates the experience. Within the constraints of the study design, the observed behaviours during toxicoses as a result of consumption of a HSDV were documented to potentially provide insight to the experiences of discomfort and pain, breathlessness, nausea, dizziness, debility, weakness and sickness. The cats were provided with water and food (bait) so did not experience thirst and hunger.

The present paper does not attempt to compare the observed behaviours of PAPP toxicoses with those of other toxins, such as 1080, but, rather, provide a description of clinical signs and behaviours observed in feral cats following consumption of the HSDV. Literature and reviews of toxicosis by 1080 (e.g. Eason and...
Materials and methods

Formulating of the HSDV was according to the methods described in Australian Patent Application No. 2009202778 (http://pericles.ipaustralia.gov.au/ols/auspat/applicationDetails.do?applicationNo=2009202778, accessed 12 June 2020) and were made for the trial by Scientec Research Pty Ltd in Melbourne, Australia. The Curiosity meat lures were prepared from kangaroo meat and chicken fat (Johnston et al. 2014). A single HSDV was inserted into a meat lure towards one end. Alternative lures of chicken and beef were available, with the HSDV inserted so that it could not fall out.

Pen trials were conducted in March 2015. In general terms, the study protocol entailed (1) health check, (2) housing in holding pens, (3) relocation to the test pen, (4) pre-test monitoring, (5) bait presentation, (6) post-test data collection (weight, sex and pregnancy status, vomit inspection), (7) video data review and (8) data collation and analysis.

With the exception of one instance, disturbance to and handling of cats was avoided before and during trials to minimise a stress response, because observations during earlier pen trials suggested an improved survival rate from PAPP toxicosis with cats experiencing frequent handling (M. Lindeman, and M. Johnston, unpubl. data).

Local site managers and the Western Australian Government Department of Biodiversity, Conservation and Attractions (formerly the Department of Parks and Wildlife) Animal Ethics Committee (DPaW AEC 2015-03) endorsed the capture of 30 un-owned cats from rural rubbish tips in Western Australia. Thirty was determined to be the minimum number of cats to provide a statistically robust result, taking into account the variables of sex, size and age of the cats. Captured cats were given a health check by a researcher using a protocol approved by the ethics committee while the cats were under light sedation by Zoletil 100 (Virbac, Sydney, NSW, Australia) before admission, with all cats found to be healthy and to have no identifying tattoos or PIT tags. None of the female cats was pregnant or lactating. The cats were housed in enclosures measuring 3 × 5 × 2 m at the Wildlife Research Centre, Woodvale, for a period of at least 7 days. Commercially prepared tinned pet food was supplied daily and water was available ad libitum. Cats were fasted for 8–24 h before being caught in cage traps and relocated ~100 m to the test pens (metal pens with a mesh front and back, 1500 × 500 × 500 mm) where water was provided ad libitum. The fasting period should be long enough so that the cat stomachs were empty or nearly empty because food starts to leave a cat stomach within 30 min of being taken in by mouth (Briggs 1994). Cats were remotely observed via a video-camera system during a 30–360-min familiarisation period in the test pen, until they appeared settled.

Remote monitoring was undertaken using infrared illuminated cameras (QC8653, Swann Communications, Melbourne, Vic., Australia) at both ends of the test pens. The camera system was connected to a 16-channel digital video recorder (Omnivision, Melbourne, Vic., Australia) located in an adjacent building ~10 m from the test pens. This permitted constant viewing and image recording, without disturbing the cats.

A single Curiosity bait, chicken or beef attractant containing a HSDV was placed in the test pen. If the HSDV was rejected while the bait was consumed, or the bait was not consumed, it was replaced with another lure containing a HSDV. The events recorded for each cat during the trials to provide a measure of efficacy included the time of (1) bait ingestion, (2) first signs, i.e. generally nodding or unsteadiness, (3) collapse, and (4) time of death or recovery. The cat behaviours were also recorded to provide an observation of the relative humaneness. A nominal time of death was recorded by the cessation of chest movement as viewed on video and was subsequently confirmed via absence of corneal blink reflex. It is possible that this method might overestimate the time of death (M. Lindeman, and M. Johnston, unpubl. data; Marks et al. 2009) but was considered to be more suitable than repeatedly disturbing the cat while alive.

The data were analysed using the Statistica 7 (StatSoft, Inc., Tulsa, OK, USA), with single factor ANOVA and regression analyses being performed after log-transformation of the data (Table 1).

Behaviours of the cats were also observed and recorded for each cat during the trials. The behaviours were divided into states

| Parameter | Overall mean ± s.d. | Range | Mean ± s.d. (♂) | Mean ± s.d. (♀) | Significance between ♂ and ♀ | F | P |
|-----------|---------------------|-------|-----------------|-----------------|-----------------------------|---|---|
| Time from HSDV ingestion to: | | | | | | | |
| onset of 1st signs | 242 ± 190 | 43–904 | 305 ± 236 | 179 ± 102 | 3.95 | 0.06 |
| collapse | 316 ± 228 | 70–1045 | 401 ± 284 | 230 ± 103 | 4.65 | 0.04 |
| death | 427 ± 270 | 122–1348 | 487 ± 350 | 371 ± 160 | 0.73 | 0.41 |
| Time from onset of 1st signs to: | | | | | | | |
| collapse | 74 ± 88 | 0–323 | 96 ± 100 | 51 ± 70 | 1.19 | 0.17 |
| death | 185 ± 153 | 43–643 | 177 ± 162 | 192 ± 150 | 0.21 | 0.65 |
| Time from collapse to death | 113 ± 122 | 28–513 | 84 ± 86 | 140 ± 146 | 1.65 | 0.21 |
and point-in-time activities, being the four states of (1) unaffected, (2) lethargy and inactivity, (3) unsteady walk or movement, and (4) respiration rate, and 14 points of (5) respiration, (6) clawing, (7) collapse, (8) convulsion, (9) head dipping, (10) head nodding, (11) involuntary urination, (12) paddling, (13) rolling, (14) salivation, (15) stiff legged arching of the back, (16) uncoordinated movement, (17) vocalisation and (18) vomiting. Three people observed the video records separately, with inter-observer reliability being obtained by consensus on observations and timing. A consensus approach was adopted where the first observer provided the gross timings on events to save the other observers needing to watch hours of inactivity. These, together with the timing on events recorded, were used as input to an assessment of the relative humaneness of the toxicant, as per the guidelines in Sharp and Saunders (2011a).

The calculation of relative humaneness is a two-stage assessment. Part A of the assessment measures the impacts on the target animal before the application of the control method. In the case of a lethal toxin, this occurs at the time of consumption and, as a result, there is no discernible impact for Part A of the assessment, giving a minimum score of 1. Part B of the assessment of relative humaneness (Sharp and Saunders 2011a) considers the mode of death, on the basis of the knowledge of the mode of action and observations of the physiological, behavioural and pathological responses. The assessment describes the levels of suffering as no impact, mild, moderate, severe and extreme, and presents these in a matrix against time levels of immediate to seconds, minutes, hours, days and weeks. Sharp and Saunders (2011a) provided an impact scale with examples as a guide to determine the level of suffering experienced by the animal after application of the method that causes death, but before the onset of insensibility.

An independent assessment of the relative humaneness was conducted by New Methods Humaneness Assessment Panel. This panel comprised the relative humaneness-model authors, a veterinarian, RSPCA-Australia representative, and species experts. The panel used the process outlined in Sharp and Saunders (2011a), viewing the video data provided from the present study.

### Results

Thirty cats (15 male and 15 female) ingested a HSDV implanted within a meat lure. The mean (±s.d.) dose rate of PAPP ingested was 35.9 (±13) mg kg⁻¹. On eight occasions, the cat took two to four baits before consuming the bait and HSDV, with either the bait not being consumed, or the HSDV falling out while the bait was being consumed, or the HSDV being rejected. Although this may have implications for the efficacy of the Curiosity bait, the number of baits consumed should not alter the intoxication observations or humaneness assessment. The time from consumption to the first clinical signs does not vary with consumption of multiple baits (time (mean ± s.d.) from HSDV ingestion to the onset of the first signs for one bait was 248 ± 206 min and for multiple baits it was 231 ± 140 min). All cats collapsed following ingestion of the HSDV, with 97% (n = 29) progressing to death and 3% (n = 1) recovering from the effects of toxicosis. The time (mean ± s.d.) from HSDV ingestion to the onset of the first signs was 242 ± 190 min; the time from HSDV ingestion to collapse was 316 ± 228 min; and the time from HSDV ingestion to death was 427 ± 270 min. Importantly, for an assessment of the relative humaneness, the mean time from the onset of first signs to collapse was 74 ± 88 min, and from the onset of first signs to death it was 185 ± 153 min. From collapse to death, the mean time was 113 ± 122 min (Table 1).

Sexual dimorphism was apparent in the study, with male cats being significantly heavier than females (F₁,2₈ = 5.41, P = 0.03). This resulted in the mean dose rate for males being lower than that for females (31.7 vs 40.0 mg kg⁻¹), even though the HSDV delivered a consistent 78-mg dose. The time from ingestion of the HSDV to collapse was significantly longer for males than for females (F₁,₂₈ = 4.46, P = 0.04); however, there were no significant differences in the time from ingestion to death, the time from onset of first signs to collapse or death, or the time from collapse to death (Table 1).

Figure 1 demonstrates that there was sufficient PAPP in each HSDV to cause toxicosis and that a higher dose rate does not hasten the onset, time to collapse or time to death.

#### Vomiting

Vomiting in animals receiving an orally delivered toxicant may reduce the amount of toxicant available for absorption, thus affecting the outcome. Six cats (2 females, 4 males) vomited during the study (Table 2). No other cats were observed to show any signs of gastro-intestinal discomfort (e.g. retching). There was no significant difference in the mean dose rate for the cats that vomited (F₁,₂₈ = 1.73, P = 0.20). The mean time (± s.d.) from consumption to vomiting was 548 (±387) min. Vomiting did not increase the time from consumption to display of the first sign of intoxication, but it increased the time from the onset of the first sign to collapse (F₁,₂₇ = 16.13, P = <0.001) and death (F₁,₂₇ = 15.16, P < 0.001). One male cat survived after vomiting, while the remaining six cats died. With the exception of the surviving cat, vomiting was closely associated with the

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**Fig. 1.** Time from hard-shelled delivery vehicle (HSDV) ingestion to collapse and death against dose rate. Observed times from cats consuming a HSDV with para-aminopropiophenone (PAPP) to collapse and death. The lack of a correlation between dose rate and the observed responses indicates sufficient toxin is contained in each HSDV.
time of collapse (−10 min to +7 min). The vomiting in 20% of the cats was a consideration in the assessment of welfare.

General behaviours
Prior to becoming familiarised with the test pen, the cats typically paced the pens, looking into corners and attempting to force openings. Once familiarised, all cats behaved normally, exhibiting behaviours comparable to those displayed by the cats after they had become familiarised with their pens, before presentation of the bait. These behaviours included sitting quietly, walking or passive exploring, grooming, social vocalisations and resting or sleeping.

Clinical signs of intoxication (events)
Table 3 provides a description of behaviours observed. In general, cats were alert, sitting or squatting, before the first observed indications of intoxication, with this generally being slight dipping of the head (as if falling asleep). In response, cats often did a short walk or rearranged themselves, then settled again. With progression of toxicosis, cats became increasingly lethargic, remaining inactive in a sitting or sternal recumbent position. The first definitive sign of intoxication was collapse, the action of which generally entailed the cat rolling into a laterally recumbent position with the legs and tail extended.

Cats were left undisturbed during the entire test, including the time post-collapse. Preliminary trials of intoxication of feral cats from PAPP observed that cats that had their levels of consciousness checked during the testing had a response to the disturbance, whereby the process was prolonged or disturbance promoted recovery from the intoxication (M. Lindeman, and M. Johnston, unpubl. data). Hence, the level of consciousness from collapse to death was not determined by physical inspection, but the visual clues of deep single breaths and lack of movement were employed.

Following collapse, individual cats displayed some, none or all of the intoxication behaviours listed in Table 3.

Intoxication behaviours
Following collapse, individual cats exhibited a range of behaviours including unsteadiness or uncoordinated movements (57% cats), paddling (83%), back arching (67%), involuntary urination, defecation or both (60%), rolling (53%), vomiting (23%) and salivation (10%) (Table 3). These behaviours were not exhibited by all cats, nor did they necessarily follow sequentially. The range, repetition and duration of occurrence of

| ID  | Cat weight (kg) | Number of baits | Dose rate (mg/kg) | Vomit | Time to first clinical sign (min) | Time to collapse (min) | Time to collapse from first clinical signs (min) | Time to death (min) | Time to death from collapse (min) | Time to death from first clinical sign (min) |
|-----|----------------|----------------|------------------|-------|----------------------------------|-----------------------|-----------------------------------------------|-------------------|----------------------------------|--------------------------------------------|
| 01  | 2.9            | 1              | 27.7             | Yes   | 240                              | 396                   | 87                                            | Survived          | Survived                         | Survived                                   |
| 02  | 2.5            | 1              | 32.2             | No     | 83                               | 148                   | 65                                            | 309               | 161                              | 226                                        |
| 03  | 3.2            | 1              | 25.1             | Yes   | 705                              | 1028                  | 323                                           | 1348              | 320                              | 643                                        |
| 04  | 3.1            | 2              | 25.4             | Yes   | 63                               | 294                   | 294                                           | 408               | 51                               | 345                                        |
| 05  | 2.0            | 3              | 40.2             | No     | 167                              | 432                   | 265                                           | 468               | 36                               | 301                                        |
| 06  | 2.9            | 1              | 27.4             | No     | 246                              | 262                   | 16                                            | 519               | 257                              | 273                                        |
| 07  | 2.9            | 3              | 27.4             | No     | 378                              | 388                   | 10                                            | 421               | 33                               | 43                                         |
| 08  | 2.9            | 1              | 32.5             | Yes   | 197                              | 405                   | 208                                           | 450               | 45                               | 253                                        |
| 09  | 1.9            | 1              | 41.8             | No     | 189                              | 264                   | 75                                            | 484               | 220                              | 295                                        |
| 10  | 2.6            | 3              | 30.6             | No     | 177                              | 219                   | 42                                            | 247               | 28                               | 70                                         |
| 11  | 2.7            | 3              | 29.4             | No     | 171                              | 187                   | 16                                            | 231               | 44                               | 60                                         |
| 12  | 2.3            | 1              | 34.2             | No     | 61                               | 70                    | 9                                             | 122               | 52                               | 61                                         |
| 13  | 2.9            | 4              | 27.7             | No     | 369                              | 375                   | 6                                             | 527               | 152                              | 158                                        |
| 14  | 2.9            | 2              | 27.1             | No     | 86                               | 95                    | 9                                             | 135               | 40                               | 49                                         |
| 15  | 3.0            | 3              | 26.0             | No     | 248                              | 283                   | 35                                            | 325               | 42                               | 77                                         |
| 16  | 2.9            | 2              | 26.9             | No     | 523                              | 554                   | 31                                            | 600               | 46                               | 77                                         |
| 17  | 1.9            | 1              | 41.1             | No     | 341                              | 392                   | 51                                            | 464               | 72                               | 123                                        |
| 18  | 1.9            | 1              | 41.4             | No     | 150                              | 180                   | 30                                            | 213               | 33                               | 63                                         |
| 19  | 1.8            | 1              | 43.1             | No     | 230                              | 299                   | 69                                            | 368               | 69                               | 138                                        |
| 20  | 1.4            | 1              | 55.8             | No     | 43                               | 91                    | 48                                            | 137               | 46                               | 94                                         |
| 21  | 4.4            | 1              | 19.0             | No     | 129                              | 175                   | 157                                           | 222               | 47                               | 93                                         |
| 22  | 2.9            | 1              | 28.8             | No     | 319                              | 346                   | 27                                            | 397               | 51                               | 78                                         |
| 23  | 2.5            | 1              | 31.2             | No     | 142                              | 153                   | 11                                            | 205               | 52                               | 63                                         |
| 24  | 1.8            | 2              | 43.4             | No     | 130                              | 159                   | 29                                            | 253               | 94                               | 123                                        |
| 25  | 1.4            | 1              | 84.3             | No     | 159                              | 200                   | 41                                            | 579               | 379                              | 420                                        |
| 26  | 2.9            | 1              | 40.7             | No     | 282                              | 282                   | 0                                             | 551               | 251                              | 251                                        |
| 27  | 2.4            | 1              | 49.2             | No     | 313                              | 345                   | 32                                            | 376               | 31                               | 63                                         |
| 28  | 1.7            | 1              | 45.4             | No     | 122                              | 229                   | 107                                           | 265               | 36                               | 143                                        |
Table 3. Description of behaviours observed and recorded

| Behaviour                 | Type   | Description                                                                                           | Observations recorded                                      |
|---------------------------|--------|-------------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| Unaffected                | State  | Animal undertakes normal actions including feeding, grooming, standing, walking, crouching, stretching, sitting, urinating, voiding bowel, and vocalising. | All 30 cats displayed this state (see Table 2). Cats were in the unaffected state for 242 ± 190 min from consumption of a bait. |
| Lethargy and inactivity   | State  | Spending extended time without movement, generally while in sternal recumbency. May involve drooping of the head. Includes time laying still on the side without other actions. The cat may be sitting, crouching, in a sternal recumbency 'sit or crouch', lying on its side. | All 30 cats displayed this state. |
| Unsteady walk or movement | State  | Taking steps or moving with an unstable gait, occasionally resulting in a fall.                        | 37% (11/30) of cats were observed with an unsteady walk. The mean number of observations of this state was 5.4, with a range from 2 to 11 observations. |
| Respiration               | State  | Description of breathing, typically measured as a variance from an unaffected animal in the same position. The breathing may be shallow, deep, rapid or irregular. | Breathing rates for all cats changed throughout toxicosis from normal to 'rapid and shallow' before progressing to single deep breaths in the minutes before death. |
| Respiration               | Point  | Respiration may be considered a point type when it is a single deep breath.                           | In several instances (unrecorded), once an animal had collapsed, there was observed to be a deep breath preceding possible rapid breathing. This type of respiration often proceeded, but has not been determined to be in direct association, with a whole body or larger movement such as arching of the back or paddling. |
| Clawing                   | Point  | The animal stretches out legs with claws extended as if trying to get purchase on an object. Repeated more than once. The cat may be clawing at the cage or in the air. | 13% of cats were observed clawing to some degree. All 11 observed records (some cats clawed more than once) were ≤30 s. |
| Collapse                  | Point  | Previous trials using the PAPP toxin (M. Lindeman and M. Johnston, pers. obs.) showed that human intervention produced a stress response that affected the survival rate from PAPP toxicosis. Collapse for this trial was determined from video footage of the animal laying on one side with the head recumbent, with no visible muscle tension. Some animals displayed twitching of the tip of the tail for a few minutes after the recorded time of collapse. | The time of collapse of each cat was recorded (see Table 2). |
| Convulsion                | Point  | Involuntary contraction of muscles causing sudden irregular movements of the body and limbs. All four limbs may be stiff. | Convolutions were observed on seven occasions with five cats (17%). They lasted for 5 s, 5 s, 9 s, 1 s each × 3 within 12 s, and 16 s. |
| Head dipping               | Point  | Small, vertical head movements. The movements may be slow, rapid or intermittent.                      | Head dipping was observed but not recorded other than to note the time as a first observation. |
| Head nodding              | Point  | Large, vertical head movements. The movements may be slow, rapid or intermittent.                      | Head nodding was observed to occur but not recorded.      |
| Involuntary urination     | Point  | Unintended emptying of the bladder contents                                                            | 60% (18/30) of cats were observed to urinate. The 18 cats were observed to urinate once. |
| Paddling                  | Point  | Movement of the legs in repetitive a front to rear motion while lying on the side. Can involve one, two or all four legs, may involve all the leg or just the lower portion of the leg. The rate of motion may be slow or rapid. May be linked to rolling or an attempt at regaining sternal recumbence. The animal is usually breathing rapidly at the same time. | 83% (25/30) cats were observed to paddle to some degree. The mean number of observations of this behaviour was 6.1. Observations of the behaviour: 79% were ≤30 s; 95% were ≤60 s; 22% were associated with back arching; 9% were associated with definitive attempts to sit, stand or walk; 3% were associated with major paddling or a convulsion and lasted ≥30 s. Some of the uncoordinated movements and paddling are suggestive of attempts to regain sternal recumbency or to stand. |
| Rolling                   | Point  | Moving from laying on one side to the other or from sternal recumbency to one side. The rolling may occur once or multiple times during one movement. | 53% (16/30) of cats rolled. There were 64 observations of rolling, with a mean number of four times per cat. Observations of the behaviour: 37% were not associated with any other point behaviour; 18% were associated with padding; 35% were associated with attempting to sit or stand; 10% of cats rolled after collapsing. |
| Salivation                | Point  | Unintended drooling of saliva from the mouth with no attempt to remove via grooming. Separate from vomiting. | 10% (3/30) of cats were observed with salivation. |

(Continued)
behaviours exhibited was also not consistent. In all, 40% of cats displayed ≤5 discrete behaviours; 33% of cats displayed from 6 to ≤10 discrete behaviours; 16% of cats displayed from 11 to ≤20 discrete behaviours and 10% displayed >20 discrete behaviours. Three of the cats displayed all the noted behaviours with a high rate of occurrence. These three cats were female, between 2.4 and 2.9 kg and consumed the HSDV on the first presentation. The behaviours were generally of short duration (<30 s – the longest was 3 min, comprising of one cat paddling) and interspersed with lengthy periods of inactivity.

**Assessment of relative humaneness**

After a cat has ingested the HSDV, there was a latent period of about 4 h before the first observed indications of intoxication, because of the time required to dissolve the HSDV and absorb the toxin. It is proposed that there was no abnormal pain or distress experienced during this time.

The mean time from the first indication of intoxication to collapse was 74 (±87) minutes. During this time, the cats exhibited occasional changes of position, grooming or stretching. Uncoordinated or unsteady movements were observed in 20 cats (67%) during this time, generally only once, but up to three times. Although not apparent to the human observers, this unsteadiness period could be interpreted as resulting in minor mental distress.

The mean time from collapse to death was 114 (±122) minutes. During this time, most cats exhibited a short period of paddling (potentially as an attempt to recover to sternal recumbency), occasional rolling over, and back-arching with stiff legs. These occurrences were generally short (<30 s) and were interspersed with lengthy periods of inactivity. The state of consciousness was unknown during these periods of movement. However, if the cats were lucid, they may have experienced confusion, distress or anxiety.

The mean time from the first observed indications of intoxication to death was approximately 3 h (185 ± 153 min; range 43–643 min).

For the purposes of the assessment of the relative humaneness of Curiosity, Sharp and Saunders (2011a) noted for the assessment of lethal toxins that, with methods involving toxic baits, it is likely that there will be no welfare impact prior the animal ingesting the bait; therefore, it is not necessary to assess both Part A and Part B. Only Part B is required. The encapsulation of the toxin in a HSDV delays the effects of the toxin absorption

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**Table 3. (Continued)**

| Behaviour                     | Type  | Description                                                                 | Observations recorded                                                                 |
|-------------------------------|-------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Stiff-legged arching of the back | Point | Backwards arching of the back generally while lying on the side with legs held stiffly in front; no bending of joints in the legs. Tail is extended straight and up. There may be single or multiple moves. Associated with toxicosis rather than limb-stretching behaviour. | 67% (20/30) of cats were observed with stiff-legged arching of the back. There were 46 observations of the point behaviour, with a mean number of observations per cat of 1.9 for the 20 cats. 37% lasted less than 10 s. 74% lasted less than 20 s. 100% lasted less than 60 s. 57% (17/30) of cats were observed displaying uncoordinated movements. All moves were ≤40 s. There were 26 observations (from the group of 17 cats) attempting to sit from a sternal recumbent or lying on side position. There were 15 observations (from the group of 17 cats) of the cats attempting to stand. Some of the uncoordinated movements and paddling are suggestive of attempts to regain sternal recumbency or to stand. |
| Uncoordinated movement        | Point | Movement generally occurs when animal is recumbent. The movement is similar to the regular action of the animal but the degree of movement may be reduced, timing of the movement between limbs may be different, and the animal’s sense of balance may be affected causing the animal to roll or fail in the action. An example is the pushing out of the legs in an attempt to gain recumbence or a sitting position. Generally resulting in moving across the floor or rotating on the spot. The leg action is uncoordinated between the legs. The movement may be associated with other actions such as walk, fall, roll, attempting to stand, attempting to sit. | 57% (17/30) of cats were observed displaying uncoordinated movements. All moves were ≤40 s. |
Table 4. Impact scale for Sharp and Saunders (2011a) model for assessing the relative humaneness of pest animal control methods, part B: assessment of mode of death

| Impact category | Description of impact                                                                 | Examples                                                                 |
|----------------|---------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| No suffering   | No suffering before death. There is immediate death or immediate loss of consciousness lasting until death. Note that components of suffering include (but are not limited to) fear, anxiety, pain, distress, apprehension, sickness, fatigue, thirst, hunger. Aversion refers to the avoidance or attempted avoidance of unpleasant, noxious stimuli and distressing stimuli. | Direct destruction or concussion of brain tissue resulting in rapid unconsciousness (e.g. accurate shooting in the head). |
| Mild suffering | Loss of consciousness is not immediate and there is no or only minimal aversion and no or only mild suffering before death. | Mild dyspnoea (breathlessness). Moderate degree of sickness, for example, vomiting or retching, diarrhoea, lethargy or weakness. Moderate dyspnoea. |
| Moderate suffering | Loss of consciousness is not immediate and there is moderate aversion and suffering before death. | Convolusions occurring during unconsciousness when animal recovers consciousness before death (i.e. muscle spasms with periods of relaxation as in clonic convulsions). Severance of major arteries resulting in rapid blood loss, hypovolaemia and shock. Severe degree of sickness, for example, vomiting or retching, diarrhoea, lethargy or weakness. Severe dyspnoea. Partial or full paralysis while conscious. Convolusions, while conscious (i.e. prolonged muscle spasm without periods of relaxation as in tonic convulsions). Extreme degree of sickness, for example, vomiting or retching, diarrhoea, lethargy or weakness. Extreme dyspnoea. |
| Severe suffering | Loss of consciousness is not immediate and there is severe suffering before death. | |
| Extreme suffering | Loss of consciousness is not immediate and there is extreme suffering before death. | |

until the HSDV dissolves so the assessment has been taken from the time of the first sign rather than of ingestion of the bait.

Should Part A be considered for the time between ingestion and first signs after a Curiosity bait is consumed, (1) there is no effect on food and water intake, (2) exposure to environmental challenge is not a feature of, or consequence of, the mode of action, (3) disease, injury or functional impairment is not a feature of, or consequence of, the mode of action, and (4) no interference with the behavioural needs of an animal; anxiety, fear, pain, sickness, breathlessness, nausea, lethargy or weakness, dizziness, greater than normal thirst and hunger or other negative affective experiences causing distress are not a feature, or consequence of, the method. Hence, the welfare impact at this time has been defined as no impact, with the minimum score of one.

Part B has been assessed as mild suffering, which is defined as the loss of consciousness is not immediate and there is no or only minimal aversion and no or only mild suffering before death. Table 4 provides the impact scale of Sharp and Saunders (2011a) reproduced with the applicable examples. The relevant example provided by Sharp and Saunders (2011a) is a mild degree of sickness, for example, vomiting or retching, diarrhoea and lethargy or weakness. Together with the time being from minutes to hours, this corresponds to a Part B assessment score of C–D (Fig. 2).

The independent assessment, conducted by the New Methods Humaneness Assessment Panel, using the same video footage, also obtained an assessment score of 1C–D (Sharp and Saunders 2011b: additional assessment for feral cat baiting with PAPP).

Fig. 2. Relative humaneness of feral cat control methods, adapted from Sharp and Saunders (2011a). Assessed animal-welfare impact of control methods. The vertical axis describes impact before death and the horizontal axis describes duration of suffering associated with the mode of death. Methods 1–7 are taken from Sharp and Saunders (2011a). 1, ground shooting – head (1A); 2, ground shooting – chest (1C); 3, padded foothold trap (5B); 4, cage trap – shooting (4B); 5, cage trap – lethal injection (4D); 6, cage trap, transport – shooting (5B); 7, cage trap, transport – lethal injection (5D); and 8, encapsulated para-aminopropiophenone (PAPP) toxicosis [1C–1D].
Discussion

The present paper has provided intoxication data and behavioural observations from pen trials with captive feral cats presented with toxic HSDVs. The trials demonstrated consumption of the HSDVs and >95% efficacy from the PAPP toxicant.

Efficacy

There was a delay in the onset of the first observed indications of intoxication following Curiosity, chicken or beef bait consumption, attributable to the time required to dissolve the HSDV and absorb the PAPP. However, the mean time from the first observed indications of intoxication to death was 185 min (range of 43–643 min), and once collapse was observed, the mean time to death was 114 min. Aside from the delay to the first observed indication of intoxication, the pharmacokinetics of the intoxication process were comparable to those reported previously (Murphy et al. 2007; Eason et al. 2010; Read et al. 2014). The assumption was made that the cats started the trial with an empty or near empty stomach so that there were no confounding delays due to digestion of additional food. However, where multiple-bait consumption occurred as a result of the cat rejecting the HSDV, there was no significant delay to the onset of the first observed indications of intoxication, suggesting that the presence of food in the stomach does not significantly alter the rate of adsorption of the toxin.

Cats of varying weights consuming uniformly dosed HSDVs will receive varying dose rates (mg/kg). The lack of a relationship between dose rate and overall time from consumption to death indicated that, within those rates evaluated (19–84 mg/kg), the HSDV contained sufficient PAPP for cats up to 4.4 kg. The approximate LD₅₀ of 5.6 mg/kg for cats suggests that the 78 mg of PAPP contained in a HSDV would also be effective on larger cats than those tested (1.4–4.4 kg); however, the relationship between the dose and effect may not be linear for cats over 4.4 kg.

Cats consumed the HSDVs on their first bait presentation in 63% of trials. Rejection of the HSDV is likely to be the result of both voluntary (i.e. active rejection) and involuntary factors (i.e. the HSDV inadvertently falling out of the bait during eating). Regardless of the reason, this rate is not expected to reduce the field efficacy of the Curiosity bait, given that some cats have been observed as having consumed multiple toxic baits in field studies before any intoxication occurred as a result of the delay of onset (Johnston et al. 2011, 2014).

Six cats (23%) vomited in the present study. Vomiting appears to slow the pharmacokinetics, possibly by slowing the dispersion and dissolution of the PAPP. One cat survived the study, and this was one that had vomited. However, no conclusions are drawn regarding the impact of vomiting on survival because of the small number of cats.

Welfare impacts

Familiarisation periods for cats in the holding and test pens were shorter than the 14 days recommended for full acclimatisation (Kessler and Turner 1997); however, this was undertaken to minimise the time that animals were held in captivity and the likely welfare impacts that this presents.

The behaviours exhibited during PAPP toxicosis observed in these trials are consistent with those reported previously (Murphy et al. 2011), with cats remaining largely inactive, becoming increasingly lethargic for extended periods before collapsing into some form of unconsciousness, irregularly interspersed with periods of the behaviours described in Table 4.

Death in a vertebrate species occurs in stages as organs and tissues progressively cease to function. As such, it is difficult to define an exact point in time when death occurs. When assessing the welfare impacts of death for a terrestrial vertebrate species, it has been argued (e.g. Newhook and Blackmore 1982; Mellor and Littin 2004; Warburton et al. 2008) that the time of onset of permanent insensibility or unconsciousness provides a suitable alternative point in time. Prior to this time, the animal may experience welfare issues, but it is proposed that once an animal becomes unconscious, brain responses become dulled enough to reduce any suffering to acceptably low levels.

There are different ways to measure this point in time. Some studies have used invasive techniques of measuring the electroencephalograms traces of sheep (Ovis aries) brains (Newhook and Blackmore 1982) or designs where physical intervention took place to measure corneal reflexes by touch or heartbeats with a stethoscope (Warburton et al. 2008). However, other studies have been conducted on wild animals where these methods were impossible. Hampton and Forsyth (2016) undertook an assessment of welfare of shooting of kangaroos from a distance by using video footage to calculate the time to insensibility. Marks et al. (2009), in measuring the behaviours of foxes (Vulpes vulpes) undergoing toxicoses, used video observation and took the time of death to be no activity observed for 10 min, with subsequent confirmation by corneal reflex. Because there was concern, on the basis of previous pen trials with feral cats and PAPP being administered (M. Lindeman, and M. Johnston, unpubl. data) that human presence and disturbance of the feral cats may affect the progress of intoxication and, potentially, affect the outcome, the study was designed to use video evidence to observe the behaviours and estimate timing of key transitions through the toxicosis. This included the recorded time to loss of consciousness and death, and the loss of consciousness was determined by collapse.

Associating the behaviours observed to the mental states, as described by Mellor et al. (2009), is difficult for all of the behaviours. Whereas others (e.g. Newhook and Blackmore 1982; Mellor and Littin 2004; Warburton et al. 2008) have argued that the cats do not have any negative welfare experiences once unconscious, it is possible that, should the cats retain a state of consciousness once they collapse, back arching, convulsions and possibly some of the paddling may cause discomfort or pain. The time periods were predominantly less than 30 s and there was no visual evidence that the cats experienced any discomfort or pain in-between when there were multiple bouts. Some of the paddling, where it might be attributed to an attempt to gain sternal recumbency, may be associated with a mental state of debility, discomfort or pain. This same paddling action has been observed in cats as they attempt to return to sternal recumbency when recovering from anaesthesia (Cleale et al. 2009; Muir et al. 2009).

Although sound was not recorded, observations of possible vocalisations from head and body movements of the cats were rare (13% of cats). In one instance, where the cat had been observed grooming 2 min prior, this was attributed as social
rather than communicating discomfort or pain. The other seven instances with four cats may have been communicating confusion, discomfort or pain, because they were associated with paddling observations or between observations of paddling. These observations and possible interpretations were considered in the welfare assessment.

Breathlessness may be associated with PAPP toxification, owing to its hypoxic action (Savarie et al. 1983; Marks et al. 2004; Eason et al. 2010, 2014) and this state may increase respiratory effort, air hunger or chest tightness (Beausoleil and Mellor 2015). With the exception of observations of individual deep breaths being taken once the cat had collapsed (see Table 3, respiration), the video quality and movement of the cats around the pens was insufficient to monitor respiration rates as an indicator of the development of hypoxia and any subsequent mental state of breathlessness.

Nausea is likely to have been experienced by all the cats that vomited. Vomiting was assessed under the impact scale of Sharp and Saunders (2011a) model as a mild, rather than moderate, because the cats vomited only once, with no observation of further retching, and the duration was short. Dizziness may have been experienced by cats that displayed uncoordinated behaviours, such as an unsteady walk or movement. Debility, weakness and sickness of some degree is likely to be a mental state experienced by the cats, depending on their level of consciousness, and may be associated with the behaviours of head dipping or nodding, involuntary urination, salivation, uncoordinated behaviours, paddling, rolling and clawing.

In undertaking the relative welfare assessment using the Sharp and Saunders (2011a) model, benchmarks were not ascribed, for example, for the number of cats displaying behaviours or the length of time on the behaviours, to differentiate among the degrees of impact in the model. The model has been designed to be subjective to help generate consensus among diverse stakeholders and to be able to compare the relative humaneness of different techniques. Further pen trials could provide additional information that may lend itself to statistical analysis on the behaviours across the population of feral cats. Whether this would alter the relative welfare assessment is unknown. Eason et al. (2010) and Read et al. (2014) provided some basic timing for PAPP toxicoses, such as the time to first symptoms and time to death for 20 and 10 cats respectively. However, the observed behaviours are provided generically and are unable to be used in this relative welfare assessment, other than to note that they are largely consistent.

The assessment of a mild impact under the Sharp and Saunders (2011a) model follows from the description that the of loss of consciousness is not immediate and there is no or only minimal aversion and no or only mild suffering before death, and includes examples of mild breathlessness, a degree of sickness and does not involve inhaled vapours or physical handling or restraint.

The behaviours exhibited by the cats and described in Table 3 indicated that there was some suffering, but without the degree of suffering to suggest a higher category (i.e. movements were not large or could not be described as violent in their action).

The assessment performed by the New Methods Humaneness Assessment Panel (Sharp and Saunders 2011b: additional assessment for feral cat baiting with PAPP) also noted the time lag to loss of consciousness, and noted that the animals are likely to experience some distress, confusion and anxiety because they cannot perform normal behaviours.

Conclusions

Invasive species management requires continual methodological improvement to increase both the range of techniques available and the efficacy of those techniques. The data and observations in the present paper have demonstrated that the HSDV-encapsulated PAPP is an effective toxin for the management of feral cats, exhibiting both efficacy when the toxin is encapsulated and likely humaneness of action. Although there is a delay to onset of intoxication when compared with PAPP directly injected within the meat lure, encapsulation does not reduce the efficacy of the toxin. At the same time, the combination of encapsulation of PAPP in a HSDV, and the use of a toxicant to which the target species is specifically sensitive, minimises impacts on non-target wildlife species (Buckmaster et al. 2014). Thus, the Curiosity bait containing a HSDV demonstrated potential to be an effective tool for the landscape-scale management of feral cats within Australia.

Conflicts of interest

The Australian Government and Western Australian Government Department of Biodiversity, Conservation and Attractions have developed the Curiosity® bait for feral cats and the Commonwealth of Australia owns patent AU2009202778. Scientec Research Pty Ltd has no conflicts of interest per se, noting that it is anticipated that Scientec will continue to provide, on an as-needed and fee-for-service basis, technical and technology transfer support for application of the Curiosity® technology for feral animal control programs. The other authors declare no conflicts of interest.

Acknowledgements

Pen trials were conducted in accordance with Animal Ethics approval number DPaW AEC 2015-03. Animal husbandry was undertaken by several Western Australian Government Department of Biodiversity, Conservation and Attractions staff at Woodvale. The Curiosity® bait for feral cats is protected by patent AU2009202778 Improved baiting method and composition.

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Handling Editor: Andrea Taylor