

## Welfare assessment of fatal methaemoglobinaemia in adult rats (*Rattus norvegicus*)

Gibson, T.J.<sup>1</sup>, Gregory, N.G.<sup>1</sup>, Quy, R.J.<sup>2</sup>, Eason, C.T.<sup>3</sup>

<sup>1</sup>Department of Veterinary Clinical Sciences, Royal Veterinary College, University of London, UK, [tgibson@rvc.ac.uk](mailto:tgibson@rvc.ac.uk)

<sup>2</sup>The Food & Environment Research Agency, UK

<sup>3</sup>Lincoln University, New Zealand and Connovation Ltd, New Zealand

DOI: 10.5073/jka.2011.432.081

### Abstract

This study aimed to assess the welfare of rats poisoned with a lethal dose of a methaemoglobin (MetHb) inducing compound. Twenty rats were dosed orally with either the active compound (treated) or the vehicle only (control). Blood samples were collected post mortem for analysis of MetHb%. Female and male rats received a mean dose of 263 (SD 6) and 199 (SD 13) mg/kg respectively. Mean time to death was 67 (SD 36) and 354 (SD 158) minutes for female and male rats respectively. Control animals did not show any signs of intoxication. The time to death from methaemoglobinaemia in rats was significantly shorter than that previously reported for anticoagulants with no obvious signs of distress or pain.

Keywords: cerebral depression, death, methaemoglobinaemia, methaemoglobin (MetHb), welfare

### Introduction

Methaemoglobin-inducing compounds are being evaluated for use as vertebrate pesticides for the control of feral pigs (Anon, 2010), stoats (Fisher et al., 2005; Eason et al., 2010; Dilks et al., 2011), ferrets (Fisher and O'Connor, 2007), bushtail possums (Fisher et al., 2008) and feral cats (Murphy et al., 2007) with the aim of improving the humaneness of pest control. They target red blood cells and induce the formation of MetHb, which reduces the capacity of blood to carry oxygen to tissues and causes depressed consciousness, respiratory depression and leads to death over a shorter time period than anticoagulant agents. Rodents have previously been demonstrated to have a high MetHb reductase activity after treatment with sodium nitrite (Stolk and Smith, 1966) or *p*-aminopropiophenone (PAPP) (Scawin et al., 1984). Newly synthesised analogues of PAPP have shown promise during in-vitro and in-vivo testing as being more toxic in rodents. Currently there has been no comprehensive assessment of the welfare of rodents poisoned with MetHb-forming compounds. It is important to evaluate the welfare problems that could or do arise when killing by this means prior to further testing for efficacy. The aim of the study was to objectively examine the behaviour of rats during fatal methaemoglobinaemia. The observations will be used to develop a rat-specific ethogram for use during efficacy testing.

### Materials and methods

Twenty Wistar-strain, laboratory rats (*Rattus norvegicus*) (10♂, 10♀) were fasted overnight prior to experimentation. Rats were randomly selected and dosed with either the active compound (treated) or the vehicle only (control). Animals were matched for dosing time and sex. Treated male (5) and female (5) rats received the active compound in a PEG:TEA (9:1 ratio) vehicle at a mean dose of 231 (SD 35) mg/kg via oral gavage with matched controls receiving the vehicle only. The dosing volume was 1 ml.

Post dosing, rats were immediately placed in individual cages and observed under white lighting. After dosing the time to onset of sickness behaviours, the duration and frequency of abnormal behaviours, changes in posture, and the time to unconsciousness and death were recorded. As treated animals died, the matched controls were dispatched by cervical dislocation. Immediately post mortem, blood samples were collected to assess circulating MetHb% at death (CO-oximeter, Instrumentation Laboratories Ltd, Warrington, UK). Post-mortem examinations were conducted on each animal to examine for gross pathological signs of methaemoglobinaemia. All procedures were carried out under the provisions of the Animals (Scientific Procedures) Act 1986 and with the approval of the institute's Ethical Review Panel.

### Results

Female and male rats received a mean dose of 263 (SD 6) and 199 (SD 13) mg/kg respectively. All treated rats died, with mean time to death of 67 (SD 36) and 354 (SD 158) minutes for female and male rats respectively. Treated animals showed a general cascade of signs of methaemoglobinaemia with

cyanosis of the hind feet and nose, ears became pale, whisker twitching stopped or was reduced all by 9 (SD 2) minutes post dosing. All treated animals then became lethargic (often in a prostrate posture), body movements became uncoordinated and slowed by 26 (SD 19) minutes. The animals then became unresponsive to air being blown on the back or face, followed by no response to a manual tail or ear pinch at 147 (SD 112) or 163 (SD 133) minutes respectively. When animals were close to death they became unresponsive to handling and lost their righting reflex. Immediately prior to death the corneal reflex was absent and the respiration rate dropped below 48 breaths per minute (normal respiration rate was 85-110 breaths per minute). Control animals did not show any signs of intoxication. In both the control and treated animals there were no signs of laboured breathing, excessive salivation or vocalisation. Post-mortem the blood of all treated rats was dark brown in colour with MetHb levels of 76% (SD 4) and 74% (SD 7) in female and male rats respectively, compared with controls at 0%.

## Discussion

The time to death from methaemoglobinaemia in rats was significantly shorter than that previously reported for anticoagulants (Littin et al., 2000), and was dose-related rather than sex-related. Initially following intoxication rats appeared asymptomatic, then 9 minutes post-dosing cyanosis was observed followed by lethargy and ataxia (26 minutes). In humans it has been reported that consciousness becomes increasingly depressed with MetHb concentrations of between 45-55%, and at levels of 55-70% there is circulatory failure, cardiac arrhythmias and coma (Hall et al., 1986). Initial results from a separate pilot study suggest that rats dosed with 30 mg/kg of the active compound had MetHb levels of 56% at 15 minutes, peaking to 69% at 30 minutes. Based on these findings, the behavioural observations and the human literature it was concluded that the rats experienced depressed consciousness from 26 minutes onwards. The mean time to un-responsiveness to pain was 147 minutes and total unconsciousness was achieved by 163 minutes. It appears from these results that the events leading to death from methaemoglobinaemia are relatively more humane than those from anticoagulant intoxication, based on the reduced time to death, hypoxia-induced cerebral depression and absence of obvious signs of distress or pain.

## References

- Anon 2010 Assessing the humaneness and efficacy of a new feral pig bait in domestic pigs. Report for the Australian Government Department of the Environment, Water, Heritage and the Arts, Australia, 1-11
- Dilks P, Shapiro L, Greene T, Kavermann MJ, Eason CT, Murphy EC 2011 Field evaluation of para-aminopropiophenone (PAPP) for controlling stoats (*Mustela erminea*) in New Zealand. *New Zealand Journal of Zoology* 38: 1-8
- Eason CT, Murphy EC, Hix S, MacMorran DB 2010 The development of a new humane toxin for predator control. *Integrative Zoology* 1: 443-448
- Fisher P, O'Connor C 2007 Oral toxicity of p-aminopropiophenone to ferrets. *Wildlife Research* 34: 19-24
- Fisher P, O'Connor CE, Morriss G 2008 Oral toxicity of p-aminopropiophenone to brushtail possums (*Trichosurus vulpecula*), dama wallabies (*Macropus eugenii*), and mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases* 44: 655-663
- Fisher PM, O'Connor CE, Murphy EC 2005 Acute oral toxicity of p-aminopropiophenone to stoats (*Mustela erminea*). *New Zealand Journal of Zoology* 32: 163-169
- Hall AH, Kulig KW, Rumack BH 1986 Drug- and chemical-induced methaemoglobinaemia. *Clinical features and management. Medical Toxicology and Adverse Drug Experience* 1: 253-260
- Littin KE, O'Connor CE, Eason CT 2000 Comparative effects of brodifacoum on rats and possums. *New Zealand Plant Protection* 53: 310-315
- Murphy EC, Eason CT, Hix S, MacMorran DB 2007 Developing a new toxin for potential control of feral cats, stoats, and wild dogs in New Zealand. In: Witmer GW, Pitt WC, Fagerstone KA (eds.) *Managing vertebrate invasive species*. p. 469-473, National Wildlife Research Center, Fort Collins, USA
- Scawin JW, Swanson DW, Marrs TC 1984 The acute oral and intravenous toxicity of p-aminopropiophenone (PAPP) to laboratory rodents. *Toxicology Letters* 23: 359-365
- Stolk JM, Smith RP 1966 Species differences in methemoglobin reductase activity. *Biochemical Pharmacology* 15: 343-351