



The aim of the animal welfare science update is to keep you informed of developments in animal welfare science relating to the work of the RSPCA. The update provides summaries of the most relevant scientific papers and reports received by the RSPCA Australia office in the past quarter. Click [here](#) to subscribe.

COMPANION ANIMALS

Friendlier cats have shorter stays in shelters

The length of stay (LOS) of cats in rescue shelters varies depending on a variety of factors. Understanding and addressing these factors may improve animal welfare by increasing adoption rates and reducing LOS.

This study examined how perceptions of cat behaviour (as a proxy for socialisation) affected LOS in shelter cats of different ages. Staff at 343 United States shelters were invited to participate and 31 responded. Shelter staff were asked to classify the behaviour of the 25 most recently adopted cats from their shelter. Cats were omitted from the analyses if other factors, such as health conditions, could have affected LOS. A total of 645 cats were included in the final analyses. Cats were grouped into three behavioural categories: interactive, approachable or unapproachable. Interactive cats approached people at initial meeting, vocalised, played and accepted contact and handling. Approachable cats did not solicit contact but did not move away. Unapproachable cats hid or moved away preventing contact.

The mean LOS varied significantly between behavioural categories. On average, cats classified as unapproachable spent three times as long in the shelter compared to interactive cats. Mean LOS of interactive cats was 36.9 days, 50.8 days for approachable cats and 118.7 days for unapproachable cats. While LOS increased for older approachable and unapproachable cats, age had no effect on the LOS of interactive cats. While behavioural categorisation may have been inconsistent between different shelter staff and the authors acknowledge their sampling may not be representative, the results suggest that behavioural modification could decrease LOS.

Brown WP, Stephan VL (2021) The influence of degree of socialisation and age on length of stay of shelter cats. *Journal of Applied Animal Welfare Science* 24(3):238-245.



Love your lizard – the emotional attachment to pet reptiles

Reptiles are popular pets but commonly suffer as the result of poor husbandry, poor nutrition, poor health and high mortality. It has been suggested that owners' lack of emotional attachment to pet reptiles may be the underlying cause of these animal welfare issues. The assumption is that highly attached owners provide better care and resources to their pets whereas less attached owners provide less care and fewer resources.

This study aimed to investigate the extent of reptile owners' emotional attachment to their pet lizards, snakes and tortoises. A Lexington Attachment to Pets Scale (LAPS) survey was conducted online. Participants were asked to rate each item on a scale ranging from 'strongly agree' to 'strongly disagree'. Survey participants who answered at least 80% of the items were included in the analyses (n=2381). The majority of participants (n=1859) had both pet dog(s) and reptile(s) and completed LAPS on both.

The study found evidence that owners feel emotionally attached to their pet reptiles, particularly lizards. The authors suggest lizards were rated higher than tortoises and snakes possibly due to lizards' 'cute' facial features or greater interaction between owners and lizards. Of the participants who had both dog(s) and reptile(s), owners were more emotionally attached to their dogs. While the online survey participants may not be representative, this study suggests that poor welfare in pet reptiles is not necessarily the result of lack of emotional attachment. Rather, poor welfare may be the result of lack of knowledge, lack of knowledge application or resource limitations.

Haddon C, Burman OHP, Assheton P et al. (2021) Love in cold blood: Are reptile owners emotionally attached to their pets? *Anthrozoös* 34(5):739-749.



Pre-existing conditions as barriers to dog adoption

It can be difficult for rescue shelters to find homes for dogs with pre-existing health and behavioural issues. Shelters may be able to improve the rehoming rates of dogs with pre-existing issues if they are able to characterise and address potential adopters' concerns.

This study, conducted in the United States, investigated attitudes towards adopting dogs with pre-existing health and behavioural issues. In an online survey, participants (n=752) were shown stock images of six different dogs of a range of sizes and breeds. Each picture was randomly



allocated a description ranging from minor to major medical or behaviour issues. The descriptions included: (1) a dog who was friendly with people, dogs and cats, (2) a dog with diabetes, (3) a dog with allergies, (4) a dog with separation anxiety, (4) a dog who pulls on the leash and jumps up on people and (5) a dog with a history of abuse. To evaluate attitudes towards these dogs, participants were asked how appealing the dog was to adopt and their perception of resource demands. Demographic information such as participant age and marital status was also collected.

Overall, participants viewed dogs with pre-existing medical or behavioural issues as less appealing to adopt. In order of most to least appealing to adopt was the friendly dog, abused dog, dog with allergies, dogs that jumped/pulled, dog with separation anxiety and dog with diabetes. Women appeared to have a better appreciation for the resource demands of caring for a dog with behavioural problems compared to men. Younger people, married/cohabiting people and those who currently had a dog or grew up with a dog, were more interested in adopting. Using this information, shelters may be able to address barriers to adoption.

King CA, Smith TJ, Holman E et al (2021) Medical, behavioural and abuse status characteristics: predictors of perceived adoptability, appeal, and resource demands of shelter dogs. *Anthrozoös* 34(4):507-524.

Flat-faced cats at risk of breathing, eye and dental issues

Brachycephalic (flat-faced) animals are at risk of a range of health problems. For example, protruding eyes (exophthalmos) predispose to corneal ulceration and inflammation of the cornea (keratitis). Distorted tear ducts result in chronically weepy eyes (epiphora) and tear staining. Excessive facial folds are prone to skin infections (skin fold dermatitis). Misaligned teeth affect ability to chew and increase the risk of dental disease. Narrow nasal passages make breathing difficult.

The majority of studies on health problems associated with brachycephaly focus on dogs. This study, conducted in Germany, characterises abnormalities seen in Persian cats, the most brachycephalic of the cat breeds. A total of 69 Persian cats (38 males, 31 females) were recruited from breeding clubs. They were compared with healthy Domestic Shorthair (DSH) cats (n=10). All cats underwent clinical examination and a Computed Tomography (CT) scan to obtain three-dimensional skull reconstructions and measurements.

Skull measurements confirmed that Persian cats have a shorter skull, narrower airways and more mis-aligned teeth compared to DSH cats. In over a third of the Persian cats, the frontal sinuses were completely absent or reduced to almost nothing. The degree of brachycephaly correlated significantly with the extent to which the eyeball was sitting outside the bony eye socket (i.e., exophthalmos). On clinical examination, 21/69 Persian cats had tear staining, 12/69 had respiratory noise at rest and 8/69 had dental disease. While none of the Persian cats showed clinical indicators of corneal ulcers at the time of examination, the authors acknowledge that breeders selected which cats to present for the study which may have introduced bias. Recommendations are made for further investigations (e.g., computational flow dynamic simulation of nasal airflow) and 'outbreeding' of Persian cats to reduce brachycephaly.

Sieslack J, Farke D, Failing K et al (2021) Correlation of brachycephaly grade with level of exophthalmos, reduced airway passages and degree of dental malalignment' in Persian cats. *PLoS ONE* 16(7), e0254420.

FARM ANIMALS

6-week-old calves are also sensitive to the pain of castration

Castration is routinely performed on calves without anaesthesia or pain-relief (analgesia). In the United States, administration of analgesia at castration is voluntary. Surveys of veterinarians in the United States over the past decade found that only 32.5 to 47.4% administer analgesia most of the time or always when performing castration on calves. Producers used pain mitigation 13.1% of the time.

This study, conducted in the United States, aimed to test the assumption that younger calves are less sensitive to the pain of castration than older calves. They used electroencephalography (EEG) and substance P (SP) as pain indicators. EEG has been used to measure pain responses in humans and animals. SP is a neuropeptide that plays a critical role in pain transmission pathways. Thirty male Holstein calves aged 6-weeks (n=10), 3 months (n=10) and 6 months (n=10) were subject to sham castration followed 24 hours later by surgical castration. EEG recordings and blood samples for SP were taken before, during and after the sham and actual surgical castration.

The results indicate that calves, including those aged 6 weeks, experience pain when surgically castrated without anaesthesia or analgesia. At surgical castration, 6-week-old calves showed increased beta waves on EEG which is indicative of pain perception

(nociception). These EEG results were not seen during the sham procedure. Unexpectedly, SP concentrations were lower after surgical castration compared to after the simulated procedure. One possible explanation may be increased breakdown (catabolism) of SP with post-castration inflammation. Nevertheless, the EEG findings show that even young calves experience pain associated with castration and the study supports the use of pain mitigation strategies for routine procedures like castration regardless of age.

Bergamasco L, Edwards-Callaway LN, Bello NM et al (2021) Unmitigated surgical castration in calves of different ages: Electroencephalographic and neurohormonal findings. *Animals* 11, 1791.



An effective alternative to beak trimming for turkeys

Turkeys routinely have their beaks trimmed with the aim to reduce feather pecking and cannibalism. Beak trimming is an animal welfare concern because it is painful and can affect turkeys' ability to use, close and move their beaks normally. Blunting discs are a potential alternative to beak trimming. The discs mimic the natural blunting of the beak tip that would occur if turkeys were pecking at rough sand, grit and rock.

This study, conducted in Germany, investigated whether blunting discs could be used as alternatives to beak trimming turkeys. Half the study population (n=300) were infrared beak trimmed and the other half (n=300) were not beak-trimmed but instead provided blunting discs at the bottom of their feed pan. Performance indicators (e.g., weight, mortality, rejected parts at slaughter), animal welfare indicators (e.g., plumage quality, skin lesions) and beak measurements were recorded. The structure of the beaks was also examined microscopically (histology) from beak tissue samples collected post-mortem.

Beak-trimmed turkeys showed histological evidence of disordered nerve tissue and scar tissue indicative of pain and altered function. Non-beak trimmed turkeys had no histological changes, except one turkey with beak tip inflammation. There was no significant difference in injury rate or performance indicators between beak-trimmed and non-beak trimmed turkeys. Overall, this study demonstrated that blunting discs are an effective, more humane alternative to beak trimming in preventing injuries that occur due to feather pecking. The authors recommend that their use be further investigated to improve turkey welfare.

Grün S, Damme K, Müller M et al (2021) Welfare and performance of three turkey breeds—comparison between infrared beak treatment and natural beak abrasion by pecking on a screed grinding wheel. *Animals* 11, 2395.

Early piglet socialisation reduces the incidence of injuries post-weaning

Weaning is a stressful time for piglets. At three to four weeks of age, they are suddenly separated from their mothers and find themselves in an unknown environment with unfamiliar piglets, experience a change in diet, and are at risk of aggressive interactions from other piglets which can result in injuries (e.g., skin and tail lesions). Tail docking is a painful husbandry procedure that is routinely practiced to reduce the risk of tail biting injuries. More humane and better alternatives are currently being researched to replace conventional weaning and tail docking methods.

This study, conducted in Germany, compared the risk of skin and tail lesions in piglets in relation to the farrowing system, weaning system and tail docking. In the same building, piglets were housed in either conventional single-housing in farrowing crates (FC) (n=349), single-housing in free farrowing pens (FF) (n=340) or group housing (GH) (n=417). Piglets were then weaned using conventional weaning methods

(Conv) (n=486) or allowed to remain with their mother and littermates in the farrowing system (Reaf) (n=620). Tails were docked or left undocked by batch. Skin and tail lesions were compared across all groups of piglets.

The results indicated that early socialisation in group housing resulted in lower incidence of skin lesions post-weaning without reducing overall piglet performance. Reduced regrouping (for piglets who were allowed to remain with their mother and littermates) appeared to reduce the incidence of tail lesions and losses in undocked piglets. The authors surmise that group housing piglets appears to be an effective strategy to reduce skin lesions possibly due to decreasing the amount and time piglets spend fighting between each other.

Lange A, Hahne M, Lambertz C et al (2021) Effects of different housing systems during suckling and rearing period on skin and tail lesions, tail losses and performance of growing and finishing pigs. *Animals* 11, 2184.



Hens prefer light similar to natural sunlight

Light intensity and UV radiation can influence how hens use outdoor environments. Standard light intensity for poultry housed indoors is approximately 10 to 20 lux. In contrast, sunlight intensity can be up to 130,000 lux. Little is known about hens' preference for different light intensities and levels of UV radiation.

This study, conducted at the University of New England in New South Wales, investigated hens' light and UV preferences. The study consisted of laying hens (n=108) approximately 50 weeks old who had been kept in indoor caged systems and never been outside or seen natural sunlight before. Six Light Preference Testing Apparatus (LPTA) boxes were set up in a testing room, with half of them illuminated by standard indoor lighting (~20 lux at bird height) in the room and the other half, in addition to the lighting in the room, illuminated with three different treatments within the human/poultry visible spectrum including either: infrared, UVA or, UVA and UVB. Each treatment was applied at either low, medium and high light intensity. Hen preference testing involved habituating hens to

move freely about the LPTA for three hours. The time hens spent in the standard versus treatment light and their behaviour was recorded.

Even though the hens had never seen sunlight before, in general they exhibited preferences for the treatment light groups that approximated natural sunlight in comparison to the control group standard indoor lighting. Hens were observed to perform more foraging, pecking and preening behaviours at low light intensity UVA and UVB. When given the choice between standard indoor lighting versus UVA and UVB, hens preferred UVA and UVB except when at high light intensity. These preference findings suggest hens may benefit from sunlight access but protection (e.g., shade and shelter) is also important for when sunlight is very intense such as during the peak of the day.

Rana MS, Cohen-Barnhouse AM, Lee C et al (2021) Preference testing for UV light spectrum and intensity in laying hens. *Poultry Science* 100(6), 101063.

Improvements needed in farm animal pain management

Cattle, sheep and pigs are routinely subjected to painful procedures without any anaesthetic or pain relief (analgesia). Painful procedures include ear tagging, nose ring placement, branding, dehorning, castration, tail docking and mulesing. There is a widespread misconception that farm animals, particularly the very young, do not feel pain as other animals do. Failure to recognise, prevent and treat pain in farm animals results in significant physical and mental suffering.

This review paper brings together the literature on pain in farm animals. The authors discuss pain assessment, analgesic options and cost-benefit analyses. Pain in farm animals can be assessed in several ways including facial expressions and behaviour scoring. Validated pain scoring systems include the Sheep Grimace Scale, Piglet Grimace Scale, Cow Pain Scale and UNESP-Botucatu Unidimensional Bovine Composite Pain Scale. Technology such as accelerometers and pedometers can be used in pain assessment e.g., lameness. In the future, these systems may be automated using artificial intelligence.

There are a range of anaesthetic and analgesic protocols for use in farm animals including local anaesthetics applied topically or injected (e.g., nerve blocks) and non-steroidal anti-inflammatories (NSAIDs) which may also be delivered in various ways. In some cases, non-invasive or less-invasive alternatives to painful husbandry procedures are available such as

genetic selection or improved housing conditions. Recommendations include the need for better pain mitigation strategies, use of less-invasive techniques, pain assessment, stronger laws about minimising pain in farm animals and improved farm animal pain education and training for vets and farmers.

Steagall PV, Bustamante H, Johnson CB et al (2021) Pain management in farm animals: Focus on cattle, sheep and pigs. *Animals* 11, 1483.



ANIMALS IN SPORT, ENTERTAINMENT, PERFORMANCE RECREATION AND WORK

A simple Five Domains infographic to assess horse welfare

The Five Domains model incorporates physical and mental factors that contribute to animal welfare covering nutrition, environment, health, behaviour and emotional state. Understanding and adopting The Five Domains can help improve animal welfare.

This study evaluated horse owners' understanding of The Five Domains model and tested their response to a simple educational infographic. Using an online questionnaire, information was collected from UK horse owners (n=259) including demographics, level of equine education, current management practices, knowledge of equine welfare, and perceptions of animal welfare including emotional well-being. Participants were then shown an intervention in the form of a simple one-page infographic explaining The Five Domains in the context of horse welfare. Immediately after the intervention, participants were asked the same questions to see if the infographic had influenced their perceptions of animal welfare.

The majority (60%) of participants were not aware of The Five Domains. The intervention appeared to have a significant impact on their assessment of horse welfare. Post-intervention, owners scored their horses lower on health, behaviour/human interactions and overall welfare. This suggests that the infographic prompted horse owners to consider animal welfare differently. However, post-intervention scores for horse emotional well-being were significantly higher compared to pre-intervention. Despite almost all participants (98%) agreeing that horses can experience emotions, these together with previous findings, suggest that objective assessment of horses' emotional state remains a challenge for owners.

Fletcher KA, Cameron LJ, Freeman M (2021) Contemplating the Five Domains model of animal welfare assessment: UK horse owner perceptions of equine well-being. *Animals* 30:259-268.

Links between the diet of working horses and abnormal oral behaviours

Horses may express abnormal behaviours due to frustration and stress. These behaviours can develop in response to suboptimal conditions and an inability to express natural behaviours. Abnormal behaviours include repetitive patterns (stereotypies) and redirected behaviours. Examples of oral stereotypies include sham chewing, licking, tongue-rolling and crib-biting. Examples of redirected oral behaviours include eating bedding and eating faeces (coprophagy).

This study, conducted on working horses in Malaysia, investigated the links between diet and abnormal oral behaviours. The horses (n=207) were housed individually at seven different facilities. They were used for leisure, equestrian sports, patrolling and endurance events. Three to five times a day, the horses were fed a diet mainly consisting of concentrates (4-6 kg/day) with a small amount of hay (2-3 kg/day). Behavioural observations were recorded as well as dietary analyses to quantify key components including dry matter (DM), crude protein (CP), crude fibre (CF) and ether extract (EE).

All horses in the study performed more than one abnormal oral behaviour. Sham chewing was the most common oral stereotypy. Eating bedding was the most common redirected oral behaviour. All horses in the study were fed lower than the minimum DM stipulated by the National Research Council (2007). Dietary components varied between the different types of working horses. Lower CP, CF and EE were found to be associated with higher probability of abnormal oral behaviours. This confirms previous findings that diets high in concentrate and low in roughage put horses at risk of abnormal behaviours. Other stressors such as long-work hours, infrequent feeding, hunger and housing may also have contributed to abnormal behaviours.

Hanis F, Chung ELT, Kamalludin MH et al (2021) Do nutrient composition of feedstuffs affect the proportion of oral stereotypies and redirected behaviours among horse working groups? *Journal of Veterinary Behaviour* 46:7-14.

What is normal? Factors influencing views on horse welfare

Horses commonly experience compromised welfare. A range of human factors contribute to these welfare issues. Issues can arise when people differ in their understanding of what constitutes 'normal' horse health and behaviour.

This review paper examines the human factors that contribute to horse welfare issues including anthropomorphism, cultural biases, social norms, beliefs and interpretations. Anthropomorphism involves assumptions that non-human animals feel the same way about things as humans. For example, humans may feel more secure when sheltered and housed. However, being kept in confinement compromises horse welfare. People may assume that horses like tactile contact due to humans' desire for touch but horses may react differently. Cultural views can also affect horse welfare. For example, the cultural view that equates feeding with caring may lead to overfeeding, obesity and associated health issues. Social norms and long-standing horse husbandry beliefs can pose a risk to animal welfare. For example,

the routine use of bits can lead to pain in the head, jaw and neck. These norms, beliefs and practices are rarely questioned.

It can be difficult to interpret behavioural signs of stress in horses. For example, depending on the context, yawning and play behaviour may be indicative of a horse trying to cope with chronic stress. Owners may also struggle to detect subtle indicators of chronic pain in horses. For example, aggression or reluctance to work may be misinterpreted as a temperament issue rather than pain associated with digestive issues, back problems or lameness. There are also concerns that people who spend a lot of time around horses with compromised welfare may see their state as normal due to over-exposure. In addition, there may be reluctance to recognise welfare issues. The authors recommend the use of animal welfare indicators that are less open to misinterpretation.

Hausberger M, Lesimple C, Henry S (2021) Detecting welfare in a non-verbal species: social/cultural biases and difficulties in horse welfare assessment. *Animals* 11, 2249.



Stronger codes and laws needed to protect animals used in film and television

Animals often feature in film and television. However, little information is available about the welfare of animals used in the Australian film and television industries.

This review examines animal welfare incidents in the Australian film and television industries. In the absence of a standardised reporting system and no official reports, the authors searched media articles. Articles documented incidents of animals being dropped, thrown, handled roughly, crushed, chased, hunted, overcrowded, scared, killed inhumanely and eaten. There are numerous deficiencies and inconsistencies in codes of practices and legislation pertaining to use of animals in media. For example, there is no national animal welfare legislation in Australia and only New South Wales (NSW), the Australian Capital Territory (ACT) and Victoria (VIC) have specific codes of practice for the use of animals in the media. In the US, the industry funded American Humane Association (AHA) monitors over 1000 Screen Actors Guild (SAG) productions per year, but monitoring is at the discretion of the production. In the UK, companies keeping or training animals for exhibition must be licensed but licensing conditions are not comprehensive.

The authors make several recommendations to reduce animal welfare risks in film and television. All animals must be legally protected, productions must be required to notify authorities of animal use, on-set and off-set monitoring must take place, veterinary oversight is essential and comprehensive codes of practice must

stipulate minimum requirements for animal care and management. In addition, the portrayal of animals in film and television should consider potential animal welfare implications such as driving wildlife trade or inappropriate pet choice. Overall, given the risks of using live animals in film and television, the authors recommend that producers use alternatives such as Computer-Generated Images (CGI).

Hitchens PL, Booth RH, Stevens K et al (2021) The welfare of animals in Australian filmed media. *Animals* 11, 1986. [Author B Jones is from RSPCA Australia]



Forming habitual behaviours to improve equine welfare

Many equine care tasks represent routinely performed behaviours. Behaviours that are routinely performed can become habitual behaviours or habits (i.e., automatic responses to particular cues). Habits can be either beneficial for animal welfare (e.g., positive human-animal interaction) or harmful (e.g., habitual hitting). Human behaviour change psychology suggests that effective behaviour change can occur via the forming of many simple, easy 'tiny habits' that by repetition and 'cue-reward-routine' become a 'habit loop'.

This study, conducted in the United Kingdom, investigates whether a pro-animal welfare habitual behaviour intervention (PAWHBInt) could develop and maintain behavioural change. For 30 days, a target group of 48 equine (horses or donkeys) carers (46 females, 2 males) were asked to scratch the equines in their care and link the scratching to a routine daily task (cue). A simple action plan, reminders and a daily log were provided. Participants were interviewed

immediately after the PAWHBInt and a month later to gauge whether the scratching had become habitual.

After the PAWHBInt, scratching their equines became habitual for the majority of participants. Scratching was seen as a positive experience for some participants and their equines. Some participants commented that their equines "all demand it...whoever I am next to wants a cuddle, wants a scratch". Other participants commented that their equine "made it very plain that scratching is something she and the other horses do and she and I interact differently." This study indicates the potential for habit formation to develop and maintain pro-animal welfare behaviours. It also highlights the importance of linking the desired behaviour to existing routine behaviours, repetition and positive reward.

White J, Sims R (2021) Improving equine welfare through human habit formation. *Animals* 11, 2156.

ANIMALS IN RESEARCH AND TEACHING

Using human skin removed during elective surgery as an alternative to animal testing

The 3Rs (replacement, reduction and refinement) provide a framework for animal research. The 3Rs have been incorporated into animal research legislation in some countries. In Brazil, alternatives to animal testing have been mandated by law since 2019. As per this legislation, the Brazilian cosmetic industry must use validated alternative methods to test the safety of products including those that are to be applied to human skin (topical).

This proof-of-concept study, conducted in Brazil, investigates the use of ex vivo human skin removed during elective plastic surgery (skin explants) for topical cosmetic safety testing. Skin of standardised thickness was collected from 17 donors. The skin explants were processed aseptically and placed in culture. The skin explants were treated in triplicate with a range

of topical products to test skin corrosion and skin irritation. Tissue viability was measured post-treatment.

The results indicated that skin explants are a viable model for skin irritation and corrosion safety testing. Test results using the skin explants corresponded to test results using other testing methods (e.g., reconstructed skin models). Advantages of the explants include presence of all cell types, intact barrier function, sustainability (use of material that would otherwise be discarded) and low cost. Disadvantages include limited availability, variability and tissue viability.

Eberlin S, Facchini G, da Silva GH et al (2021) Ex vivo human skin: An alternative test system for skin irritation and corrosion assays. *Alternatives to Laboratory Animals* doi:10.1177/2F02611929211038652.

Cell and computer-based models are alternatives to animal testing

Nanoparticles are widely used in the biotechnology, agriculture and food industries. Every year, millions of animals are used in nanoparticle safety testing and the numbers are growing. As per the 3Rs (reduction, refinement and replacement), ethical research requires the pursuit of alternatives to animal use.

This review brings together the literature on alternatives to animals in the context of nanoparticle (NP) safety testing. Cell-based (in vitro) and computer-based (in silico) models represent alternatives to animal testing. There are many cell lines commonly used in safety and toxicity testing including different types of stem cells and somatic cells. Tissue engineering offers different models such as 2D and 3D scaffold-based techniques, cell spheroids and scaffold-free cell cultures in suspension. In silico models based on bioinformatics and computer simulations, can be used to assess a wide variety of NPs. For example, molecular docking studies simulate complex interactions between small molecules such as NP and large molecules such as proteins or enzymes. Computer-based Quantitative Structure-Activity Relationship (QSAR) assays are frequently used to predict the biological activity and toxicity of substances using machine learning. Some countries use QSAR instead of animal testing to predict toxic hazards. Molecular Dynamics (MD) simulation is also widely used in nanotoxicology.

In vitro and in silico models offer a range of advantages over animal testing. In addition to ethical advantages, they are faster and less expensive. However, there are currently limitations including lack of an intrinsic circulatory system, difficulties corresponding cells to whole organs and assessing accuracy. Nevertheless, there are numerous validated alternatives to animal testing and further developments are required to reduce the number of animals used.

Huang, H-J, Lee Y-H, Hsu Y-H et al (2021) Current strategies in assessment of nanotoxicity: Alternatives to in vivo animal testing. *International Journal of Molecular Science* 22, 4216.

WILD ANIMALS

Standardised testing needed for wildlife shooting and darting

Ballistic methods such as shooting and darting are widely used in wildlife management. For example, shooting is commonly used for culling, hunting and euthanasia of injured wildlife. Shooting poses animal welfare risks including animals being 'struck-and-lost', traumatic injury and delayed time to death. Darting is used to mark wildlife and for the remote delivery of immobilising drugs, medications and fertility control agents. Darting poses animal welfare risks such as traumatic injuries and infection. Risks increase when sub-optimal ballistic methods are used.

This review proposes a standardised evidence-based testing protocol to reduce the animal welfare risks of wildlife shooting and darting. The protocol considers human factors (e.g., shooter proficiency and position, ability to predict animal behaviour), kinetic energy of the projectile, projectile behaviour and animal-based indicators (e.g., proportion of animals rendered insensible within a specified time period, proportion of animals displaying injuries). The proposed protocol draws on learnings from standardised protocols for kill-trap testing and ballistic method case studies. The

authors recommend integrating pre-animal testing and animal testing with clear cut-off points to ensure unacceptable methods do not proceed. The shooting of adult harp seals in Canada, which had undesirable animal welfare outcomes, is used as a case study to illustrate 'how not to' apply untested or unfamiliar ballistic methods, i.e., no bench top or other assessments conducted.

The proposed standardised testing protocol for wildlife shooting and darting consists of: (1) pre-animal testing of accuracy, kinetic energy, projectile behaviour, equipment and personnel under field conditions, (2) testing on cadavers, (3) small-scale pilot studies on animals with clear cut-off points, and (4) broad-scale use with reporting of adverse animal welfare outcomes.

Hampton JO, Arnemo JM, Barnsley R et al (2021) Animal welfare testing for shooting and darting free-ranging wildlife: A review and recommendations. *Wildlife Research* doi:10.1071/WR20107.

Camera traps used to assess the welfare of free-roaming wild horses for the first time

Assessing the welfare of free-roaming wildlife is challenging. Challenges include difficulty locating and observing wild animals, particularly in remote and inaccessible areas.

This is the first study to use remote camera traps to non-invasively assess the welfare of free-roaming horses. A total of 47 cameras were deployed in the Blue Mountains National Park, New South Wales. The area is known to be home to a small number of free-roaming wild horses. For 15 months, cameras were deployed across grasslands (n=23), woodlands (n=17), riparian habitat (n=13) and disturbed open woodland (n=5). Still images and videos were collected. Observation events were assessed for a stratified-random subset of camera days. As per the Five Domains model of animal welfare, assessment included indicators of nutrition (e.g., body condition score), physical environment (e.g., sweating), health (e.g., coat condition) and behaviour (e.g., Qualitative Behavioural Assessment QBA).

The study assessed animal welfare indicators in 16 individual free-roaming horses in open grassland

habitat. On both still images and video, the most frequently assessable indicators included body condition score, body posture, coat condition and the presence/absence of excessive sweating. On video, the most frequently assessable indicators included presence or absence of weakness, QBA, presence or absence of shivering and gait at walk. Limitations included the need for a clear line of sight and distance from the camera. The images and video footage represent snapshots in time. These snapshots may not reflect overall time budgets or the motivations underlying certain behaviours. Nevertheless, this study demonstrates how the welfare of free-roaming wild animals can be assessed using camera traps. The authors recommend extensive field surveys for strategic camera placement and optimisation of camera settings.

Harvey AM, Morton JM, Mellor DJ et al (2021) Use of remote camera traps to evaluate animal-based welfare indicators in individual free-roaming wild horses. *Animals* 11, 2101.

A new tool to assess camel welfare

Globally, a growing number of camels are kept for agricultural, cultural and recreational purposes. Camels are renowned for their adaptations to deal with harsh conditions which may lead to erroneous conclusions that harsh conditions do not negatively affect their welfare. For example, camels commonly suffer from welfare issues including restricted feed access, insufficient mineral salts, overstocking, disease, heat stress, extreme confinement by hobbles, painful husbandry procedures such as the fitting of nose pegs. Currently, little guidance is available on how to assess and improve camel welfare.

This review considers available literature and proposes a system for assessing camel welfare in intensive and semi-intensive systems. The authors draw on well-recognised models of animal welfare including the Five Freedoms and Five Domains and incorporate aspects of the European Animal Welfare Indicators Project (AWIN) and Welfare Quality® protocols. They selected animal, resource and management-based animal welfare

indicators using their experience and taking into account validity, reliability and feasibility. The proposed system for assessing camel welfare considers feeding, housing, health and behaviour. Animal welfare indicators fall into three categories: caretaker, herd and individual animal indicators.

Caretaker level indicators are measured via interview and on-farm observation. Herd and individual animal level indicators are measured via a range of proposed tests, some have been validated in other species but still require validation in camels (e.g., the 'bucket test' for thirst which was developed in horses). Further work is required to validate animal welfare indicators for camels and their relationship to emotional states.

Padalino B, Menchetti L (2021) The first protocol for assessing welfare of camels. *Frontiers in Veterinary Science* 7, 631876.



Animal welfare is a key concern in wildlife trade

Worldwide, billions of animals are harmed and killed in the legal and illegal wildlife trades. The literature about wildlife trade generally focuses on conservation implications. Wildlife trade is rarely viewed through an animal welfare lens.

This review argues that animal welfare must be considered in the context of wildlife trade. Trade involves capture, transport, injury, insufficient food and water, confinement, and direct and indirect killing. For example, birds are stuffed into packages to be smuggled, snakes are starved and skinned alive for their skin and rhinoceros are shot for their horns. Animals continue to suffer once they reach their destination. For example, in the European Union, 75% of pet reptiles and amphibians suffer poor welfare and die within the first year. The wildlife trade compromises all Five Domains of animal welfare including health, function, environmental conditions and behavioural expression.

The authors recommend ways to improve the welfare of animals in wildlife trade. Recommendations include acknowledging the value of wild animals as sentient individuals, and strengthening policy, legislation and law enforcement. They recommend that animal welfare regulations be included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). As highlighted by the COVID-19 pandemic, the capture, transport, keeping, marketing and slaughter of wild animals poses direct zoonotic disease risks to people. Hence, improving the welfare of wild animals will also help safeguard human health.

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TRANSPORTATION OF ANIMALS

Skin tent test, faecal soiling and abnormal oral behaviours are potential animal welfare indicators in young dairy calves in lairage

Over two million dairy calves, aged four to seven days, are sent for slaughter in New Zealand annually. Animal welfare concerns include transport, handling and mixing of such young, vulnerable animals. Calves find themselves in unfamiliar environments, exposed to stressors including pathogens and noxious agents. At present, animal welfare indicators are not routinely monitored in young calves sent to slaughter. Mortality rates are recorded but these are insufficient to assess calf welfare.

This study, conducted across 12 New Zealand meat processing facilities, sought to identify potential animal welfare indicators to incorporate into future assessment systems. Across 102 pens, calves were assessed at a distance at a group level (n=5910) and individually up close (1m away) (n=504). Data recorded included animal behaviours, the presence/absence and severity of injuries, respiratory rate and a skin tent test for dehydration.

The majority of calves in lairage were Friesian or Jersey crossbred males (291/504, 69%). When observed up close, many (22%) had an elevated respiratory

rate (>36 breaths/minute) possibly indicating stress. Over 20% of calves had: a delayed skin tent test (≥3 seconds) indicating dehydration, faecal soiling indicating diarrhoea possibly of infectious origin and runny noses and eyes possibly due to exposure to wind, irritants and/or noxious agents. Close to 30% of calves exhibited abnormal oral behaviours such as sucking or licking objects or other calves (cross-sucking). These behaviours reflect poor animal welfare including hunger, boredom, frustration and lack of opportunity to engage in natural suckling behaviour. Indicators appeared to be affected by time in lairage and time since the calving season started.

The authors recommend these animal welfare indicators be incorporated into future assessment systems. Further research is required to understand the influence of farm management and transport on the health and welfare of young calves sent for slaughter.

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HUMANE KILLING

Can hundreds of millions of male layer chicks be humanely killed?

Annually hundreds of millions of male layer chicks are killed. Maceration (crushing) is a method commonly used, however, alternative killing methods are sought. Possible alternatives currently include carbon dioxide (CO₂), nitrogen (N₂) and low atmospheric pressure stunning (LAPS™).

This study, conducted at Texas A&M University in the United States, compared the use of CO₂, N₂ and LAPS™ for the killing of male layer chicks. Male chicks (n=480) at one day old were randomly allocated into 16 groups with 30 chicks in each group. Chicks were then exposed to either: normal atmospheric air and then killed by decapitation (control), CO₂, N₂, or LAPS™. Video observations of chicks in the slaughter chamber were collected to assess movement, vocalisations, loss of balance (ataxia), loss of posture, convulsions, time to unconsciousness and time to death. Blood samples were also taken after death and once chicks had stopped moving for one minute. Blood samples were analysed for stress hormones (corticosterone) and hormones associated with decreased fear-related behaviour in poultry (serotonin).

The authors found CO₂ and LAPS™ to be effective alternative killing methods for male layer chicks. N₂ was found to be unsuitable as it took too long to reach lethal concentrations resulting in delayed time to unconsciousness and death (631 seconds). Whereas, in comparison, chicks began to lose posture within 50.8 seconds in CO₂ and 58.8 seconds in LAPS™ and death was achieved in 341.0 to 356.5 seconds respectively. Corticosterone in chicks exposed to CO₂ and LAPS™ did not vary from the controls. Corticosterone was highest in chicks exposed to N₂ possibly indicative of stress, anxiety and discomfort. Chicks exposed to CO₂ and N₂ were observed convulsing with severe wing flapping which may indicate some form of consciousness and distress. Chicks exposed to CO₂ had the highest serotonin levels, but further research is required to understand the welfare implications.

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RSPCA 

ANIMAL WELFARE IN A CHANGING CLIMATE

Animal Welfare Seminar 2022
16-17 February (online)



ARTICLES OF INTEREST

COMPANION ANIMALS

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