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# **2007 ANZCCART Conference**

## **Programme**

### **Tuesday 10<sup>th</sup> July**

9.00 – 10.00am Registration Desk Opens to Delegates

#### ***10.00 – 10.30am Morning Tea***

- |                   |                    |  |
|-------------------|--------------------|--|
| 10.30am – 10.45am | Sir Gustav Nossal  | Official Opening of Conference   |
| 10.45am – 11.15am | David A Taylor     | “Alcohol & Drug Dependency Studies”  |
| 11.15am – 11.45am | Philip Davies      | “Animal Models of Epilepsy”  |
| 11.45am – 12.15pm | Kathrine Handasyde | “Working with Wildlife: Understanding the Issues and Ensuring Best Practice” |
| 12.15pm – 12.30pm | Barrie Wells       | “Degrading Links and Telemetry Equipment on Small Animals”                   |

#### ***Lunch: (12.30 – 1.30pm)***

- |                 |                   |  |
|-----------------|-------------------|--|
| 1.30pm – 2.30pm | Jonathan Balcombe | “Rodents in Laboratories: Thinking Outside the Cage”     |
| 2.30pm – 3.00pm | Kerry Fowler      | “Using Transgenic Animals to Advance Human Health Care.” |
| 3.00pm – 3.30pm | Max Campbell      | “Why Getting it right is so important with wildlife”     |

#### ***3.30pm – 4.00pm Afternoon Tea***

4.00pm – 5.00pm Workshop Session – Divide group up by AEC categories to discuss reactions to / issues arising from talks heard so far during the conference and raise / discuss other issues relevant to your group.

5.00pm – 5.45pm Reports back from workshop groups

***6.00pm – 8.00pm: Cocktail Reception for Delegates (Hotel Rendezvous)***

## **Wednesday 11<sup>th</sup> July**

9.00am – 10.00am Paul Flecknell "Pain management in laboratory animals - are we making progress?"

10.00am – 10.30am Craig Johnson "Ways of identifying and measuring pain."

### ***10.30am – 11.00am Morning Tea***

11.00am – 11.45am Mariko Lauber "Human attitudes toward lab animals, how that impacts on interactions between animals and people, and what effect that has on research outcomes."

11.45am – 12.45pm David Mellor "Getting it right when studying the fetus and newborn - is pain relief necessary?"

### ***12.45pm – 1.45pm***

### ***Lunch***

1.45pm – 2.45pm

Workshop: Break into Quasi- AEC Groups to Consider an Initial Application looking at different types of application

2.45pm – 3.30pm

Reports back from Quasi - AEC Workshop groups

### ***3.30pm – 4.00pm Afternoon Tea***

4.00pm – 4.30pm David Adams "Synopsis of the recent AAWS Summit on pain & pain management"

4.30pm – 5.00pm Rob Gration "Bat detectors: Are they the silver bullet for applying the 3r's of animal welfare when undertaking field based surveys for bats?"

5.00pm – 5.30pm Amy R Blair "Re-evaluating the glucose tolerance test in mice: Effect of fasting duration, route of administration and dose."

### ***7.00pm – 11.45pm: Conference Dinner (Melbourne Aquarium)***

## Thursday 12<sup>th</sup> July

9.00am – 10.00am Paul Flecknell "Euthanasia of laboratory rodents, controversy and consensus"

**10.00am – 10.30am** *Morning Tea*

10.30am – 11.00am Peter Penson "Progress with External Review of Institutions and Animal Ethics Committees in Victoria"

11.00am – 11.30am Nita Harding "Reviewing Animal Ethics Committees - the NZ experience"

11.30am – 12.00noon Janine Barrett "National statistics – What’s the point?"

12.00noon – 12.30pm Leigh Ward & Ann Higgins "The AEC system in Australia. Does one size fit all?"

**12.30pm – 1.30pm** *Lunch*

1.30pm – 2.00pm Erich von Dietze "Counting animals one by one: what do we mean when we report on the animals used in science?"

2.00pm – 2.30pm Robyn Sullivan "Adverse Incident reporting"

2.30pm – 3.00pm Peter Maley Follow up discussion on adverse incident reporting presentation.

**3.00pm** *Conference Closing Followed by Afternoon Tea*

## *Alcohol & Drug Dependency Studies*

David A Taylor

Department of Pharmaceutical Biology, Victorian College of Pharmacy, Monash University

Drug addiction is a significant social problem that has adverse consequences for the addict, their family and friends and the wider community. The impact can be medical, social or a combination of both and have significant costs. An increase in our understanding of the basis of drug addiction has led to improved treatments but there is still the need for further advances. Animal models play a vital role in attempting to elucidate the basis of dependence on different drugs of abuse. They are also valuable to test the efficacy of pharmacotherapies.

Drug addiction consists of physical and psychological dependence. Physical dependence is an adaptive state whereby the tissues including the brain and neurotransmitters become accustomed to the presence of the drug. When the drug is no longer present the addict displays a withdrawal syndrome. The abstinence syndrome observed is a specific array of symptoms characteristic for the drug upon which they are dependent. The symptoms are usually an overactivity of a system that was originally suppressed. For example heroin addicts experience constipation when taking heroin but on withdrawal they suffer stomach cramps and diarrhoea.

Psychological dependence is the craving for the drug so that the user experiences the feeling of satisfaction or reinforcing (rewarding) effects induced by the drug. It is a compulsive behaviour in which the user has lost control over their drug use and continues to use despite knowing the adverse effects of doing so. Psychological dependence is more difficult to treat than physical dependence and is the reason for an addict who has become drug free relapsing.

Many species of animals are used to investigate drug addiction but the majority of work is in rats and mice. To investigate physical dependence the characteristic withdrawal syndromes may be quantified in animals made dependent on the drug. Assessing psychological dependence requires models that are indicative of the animal wanting to experience the effects of the drug. The reinforcing property of a drug can be assessed in self administration models in which animals perform a task, for example press a lever, to receive the drug, or voluntarily choose the drug when given a choice. Another model, which is less direct, uses Conditioned Place Preference. In this model the drug is administered to an animal and they are placed in an area which has distinctive environmental (visual and tactile) cues. This is repeated over several days. The animal is then given a choice of the distinctive drug treatment area or another area. Drugs of abuse will increase the time spent voluntarily in the drug area as it is suggested that the animal has associated it with the rewarding effects of the drug. Another model in alcohol studies takes advantage of the observed alcohol preference of some strains of rats. The validity of these models is exemplified by the observation that acamprosate, which is a clinically useful pharmacotherapy for alcohol dependence, reduces ethanol intake in ethanol self administration studies and in alcohol preferring rats as well as inhibiting the development of a conditioned place preference to ethanol. Consequently these models provide valuable tools to help elucidate the basis of drug addiction and identify potential pharmacotherapies.



## *Animal models of Epilepsy*

Philip J Davies

Ion Channel and Human Diseases Laboratory, The Howard Florey Institute, Parkville, Melbourne VIC.

Epilepsy is characterised by recurrent spontaneous seizures resulting from abnormal synchronized activity of neurons in the brain. It is one of the most common neurological problems afflicting at least 50 million people worldwide. For most patients, pharmacotherapy represents the main form of treatment, yet in some patients, seizures may not be controlled by antiepileptic drugs. Although epilepsy clearly has a large environmental component, it is thought that in at least half of cases, genetics is important in the onset and pathogenesis of seizures. Indeed in the last 10-15 years identification of epilepsy genes have been identified for some specific forms of epilepsy. In parallel to the identification of mutations in human genes, many investigators have identified rodent specific mutations that produce similar types of seizures. In some cases features of seizures and the mechanisms by which they are conserved show similarities between human and rodents. While it is yet to be demonstrated that these rodent epilepsy mutations exist in the human population, the mutant animal models themselves provide valuable models for epilepsy researchers to study epilepsy at both the molecular level and to characterise the pathological consequences of seizures in a whole organism. Rodent models already contribute significantly to antiepileptic drug development programs, as all currently available antiepileptic drugs were initially developed using such models and they remain a primary tool used in primary screens in the search for new drugs. However drug discovery programs rely heavily on the testing of compounds using normal “non-epileptic” rodent strains. This approach while successful in producing a range of second generation antiepileptic drugs have not changed the incidence of pharmacotherapy resistance in clinical populations, promoting the use of alternative animal models for drug development programs. More recently the potential for particular gene targeting methods such as “the knock-in approach” to introduce human epilepsy mutations into the corresponding mouse gene have been seen to have increasing value, particularly in terms of producing clinically relevant mouse models of epilepsy. Current epilepsy research using rodent models offers great promise for understanding of the molecular mechanisms that underlie epileptogenesis in humans. While rodent models are an essential part of current antiepileptic drug discovery programs, the new knock-in animal models may provide us with tools to ultimately tackle the goal of epilepsy medicine, the ability to provide treatments that prevent epilepsy or control it.

In this talk I will discuss these approaches, including some of the common methodologies used in experimental studies to better inform animal ethics committees that may assess applications involving rodent epilepsy models.

## ***Working with wildlife: understanding the issues and ensuring best practice***

Dr Kathrine A. Handasyde

Department of Zoology, The University of Melbourne, Vic, 3010

Conducting research on wild animals has numerous issues and constraints that are not generally encountered in studies using domestic or laboratory species. One set of issues can be generated by lack of information on basic biology. Knowledge of basic biology is used to establish appropriate capture, handling and sampling regimes and to understand behavioural and physiological responses that allow researchers to assess whether procedures are impacting seriously on animals. Even after appropriate capture techniques have been established there is usually some risk associated with capture of most wild animals. Another issue, in terms of designing research protocols, is a variable ability to recapture particular individuals for monitoring and sampling, and lack of ability to control external factors (such as adverse weather) that may impact negatively on the animals and therefore require researchers to alter sampling protocols. These problems often mean that researchers must try different techniques and approaches, particularly in the early stages of researching species for which there is little information available. Researchers also need to be flexible in their approach because wild populations can show enormous variation in their ecology and physiology. For example, the nutritional status and health of animals may vary between populations due to differences in food availability or quality: if animals are in poor condition it may be necessary to capture and sample them less frequently. Variation also occurs over time: for example for species that breed seasonally, such as many native mammals, females may experience high energetic loads due to lactation at certain times of the year and this may require researchers to modify sampling protocols to avoid adverse impacts on the animals during this period. Furthermore, some species are robust and tolerant of handling and sampling, others are not. The latter will need very careful attention to handling and degree of experimental manipulation to avoid serious risks for the animals. Rarity is another factor that must be considered with wildlife: if a species is rare, riskier procedures should be avoided. Conducting initial research on sister species that are more numerous is one approach that researchers use to establish safe techniques which can then be applied to rare species. However at some point it may still be necessary to conduct research on rare species. In this event, it is important to ensure that experienced and highly skilled researchers are involved in such projects. Another challenge for researchers occurs when they pursue new research questions that require trialling different or emerging technologies for which we cannot always precisely predict the impact on, or outcomes for, the animal. Many biologists trial such approaches in captivity if possible. However this cannot always be done or may simply not reflect the responses of wild animals in the field. Training students is another potential issue in wildlife research, as they will often be working in remote localities that do not provide the opportunity for a supervisor to simply step in and assist in difficult or unanticipated situations. A commitment to close supervision and careful instruction of students, in the field setting, is therefore vital. For wildlife researchers, broad experience (including willingness to network amongst colleagues for additional expertise), flexibility and specific expertise are crucial elements for successful and humane project completion.

## *Degrading Links and Telemetry Equipment on Small Animals*

Barrie Wells

Animal Welfare Officer, University of Tasmania

The ever-increasing miniaturisation of electronic devices is providing opportunities for researchers to gain unprecedented information from 'tag & release' wildlife studies and AECs are seeing an increase in applications from researchers wanting to use the latest crop of devices. Among the applications are requests to put gear on birds and small mammals that will then be released into the wild. Researchers using harnesses in particular should ensure they all have 'weak links' that will release an animal if it becomes entangled in vegetation and 'degrading or breakaway links' that will allow the harness to fall off after a specified time. Some links are being promoted as combined 'weak links' and 'degrading links' and in fact they do not seem to do these jobs well or at all.

There can be no guarantee that a wild animal will allow itself to be trapped a second time and without a degrading link there is a real possibility that some will be sentenced to carry expired equipment for the rest of their lives.

The ultimate fate of these animals needs special AEC scrutiny.

Researchers are finding that advances in radio telemetry are providing them with new opportunities to investigate the functions and activities of animals. Advances in technology, miniaturization and batteries now allow for the recording of pressure, temperature, spatial orientation, geographical position and many more parameters, limited only by imagination. With the advent of GPS technology, positions can be marked anywhere on earth with incredible accuracy and information can be uploaded to satellites and downloaded to laboratories. Automation allows information to be gathered and transmitted without the need for a researcher to be within miles. Researchers are realizing that here is a fantastic new tool to further animal research.

Miniaturization has made it possible to attach telemetry gear to amphibians, reptiles, birds, fish and all mammals, large and small. Even though it is beyond the scope of our current system, it is interesting to note that instruments have even been made that are suitable for use with insects.

AECs are seeing a growing interest in projects seeking to use this remarkable new technology and so we also need to be aware of its very real shortcomings. Telemetry gear is very efficient at collecting data but problems arise when researchers want to attach the equipment to animals and then release these animals into the wild to "live a normal life in the wild".

Many devices can be safely glued onto some animals. Equipment that is glued or tied to feathers for example will be carried by a bird for the life of the feather and ultimately shed when the feather moults. Devices glued to skin or fur will eventually be released when the skin sloughs or the fur is shed. However devices that are carried by a harness or collar are in a different category and AECs need to be aware of the possibility that we may be sentencing some animals to carry defunct equipment for the remainder of their lives.

The literature talks a lot of weak links and timed releases but there is very little discussion of degrading links. Weak links are supposed to break and set an animal free in the event that it becomes caught on something. The idea started with American hunting dogs and the device may work for dogs under hunting conditions but is not small or sensitive enough for small mammal collars or bird harnesses.

Timed releases are available for gear carried by larger animals and they operate by responding to a radio signal or a timing device that activates a release pin. They may be great for American grizzlies but don't have a place with Tasmanian Masked Owls or Devils. Models are suitable for animals down to the size of a dingo (say 25kg) but for an animal smaller than this, they are currently too heavy.

Degrading links are links that will fall apart with time. At present the development of these devices is very rudimentary. Some manufacturers have claimed their "weak links" made from cotton will release with time but this is

misleading. Cotton and other vegetable fibres will break down but there is no reliable information on their speed of breakdown and many linkages will outlive small animals.

There are many variables that affect degrading links and a device that is suitable for one situation may be unsuitable for another. The species under observation and the prevailing weather conditions are among the many variables. For example, harnesses placed on owls are quickly preened under the feathers and are relatively protected from the elements whereas collars placed on devils get some very hard wear and devils aren't much bothered by rain.

If an AEC accepts that it is unreasonable to expect an animal to be sentenced to carry a defunct device for the remainder of its life, we need to put pressure on manufacturers to spend time on R&D. To quote from a recent email from a leading telemetric manufacturer –

*“The lack of progress to date on providing a degradable link in our bird transmitter harnesses is not through lack of interest or concern on the company's part. At the moment, our R&D team are fully committed (time wise) on solving electronic and software problems with various circuits that we manufacture. We would like to devote some time to the weak-link harness issues that you have raised, but we have neither the expertise, facilities nor the time to investigate it adequately at present.”* Such an attitude leads me to think that some companies may need to rearrange their priorities.

We must never lose sight of the fact that for researchers the data is paramount.

They will all stress that the welfare of their animal subjects is of the utmost importance to them but with a few exceptions it is the data that is king. Researchers will want to gather the data if it is their interest to do so and it is up to the AEC to be aware of the problems and difficulties some animals may face as a result.

It is hard to believe that people with the brains to develop such sophisticated telemetry equipment cannot also develop an acceptable degrading link. As a suggestion, dissolving surgical suture material offers an avenue for investigation. A material such as polyglycolic acid is

used in some dissolving sutures and it breaks down by hydrolysis. Much is known about its rate of breakdown in living tissue but little is known about its rate of breakdown in air. The effects of exposure to water may be measurable in this case, but the effects of climate variations such as drought still need to be born in mind for Australian researchers. I'm assured that the required R&D work will happen but I don't know when. There will be other materials too that may warrant investigation, and that remains a hope for the future.

***Rodents in Laboratories: Thinking Outside the Cage***  
**Laboratory environments and rodents' behavioural needs: a review**

Jonathan Balcombe

Physicians Committee for Responsible Medicine, Washington, D.C.

This paper focuses on the most commonly used animals in scientific experimentation and testing—mice and rats—and presents an ethological perspective on their quality of life in the laboratory setting. Quite aside from the harmful experiments themselves, the animals' day-to-day living conditions and laboratory routines severely compromise their wellbeing. Preference studies show that both wild and captive mice and rats value opportunities to take cover, build nests, explore, forage, and gain social contact. Yet standard laboratory housing systems chronically thwart these needs, leading to physiological (e.g., stunted brain development) and behavioural symptoms (e.g., stereotypies) associated with poor welfare. Literature reviews also reveal profound and non-transient stress associated with routine laboratory procedures, including cage changing or moving, handling, blood collection, and gavage (force-feeding). Pronounced and significant changes in stress indicators (e.g., concentrations of corticosterone, heart rate, and blood pressure) occur for these procedures, indicating fear, stress, and/or distress. In sum, laboratory life for the typical rodent is marked by unrelieved frustration of basic needs, and chronic lack of stimulation and control, all exacerbated by regular stressful episodes. Rats and mice are sentient beings highly sensitive to their surroundings. They have beliefs and desires, and recent studies are revealing remarkable behavioural and social capacities. Quite aside from the scientific shortcomings of extrapolating from mouse to man, subjecting these creatures to typical laboratory regimens is fundamentally cruel.

*'Reproduced from Laboratory Animals 2006: 40: 217-235 with kind permission from Royal Society of Medicine Press, London'*

Keywords: Rats; mice; laboratory; housing; stereotypies; enrichment

### Summary

Laboratory housing conditions have significant physiological and psychological effects on rodents, raising both scientific and humane concerns. Published studies of rats, mice and other rodents were reviewed to document behavioural and psychological problems attributable to predominant laboratory housing conditions. Studies indicate that rats and mice value opportunities to take cover, build nests, explore, gain social contact, and exercise some control over

their social milieu, and that the inability to satisfy these needs is physically and psychologically detrimental, leading to impaired brain development and behavioural anomalies (e.g. stereotypies). To the extent that space is a means to gain access to such resources, spatial confinement likely exacerbates these deficits. Adding environmental 'enrichments' to small cages reduces but does not eliminate these problems, and I argue that substantial changes in housing and husbandry conditions would be needed to further reduce them.

Rodent housing conditions in laboratories represent an important potential welfare problem. Most animals used in research and testing spend their lives in small cages. Increasingly, some enrichment is provided within these cages, but there remains a significant proportion where enrichment is not provided despite consensus for needed reforms within the scientific community (Wofle 2005). It is important to not only ask whether enrichment is provided, but also if the approach of within-cage enrichment has shortcomings, because any suffering caused by inappropriate housing will typically be of greater duration than that caused by the experiments themselves (Sherwin 2002).

The number of animals affected is large, and probably increasing. Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*) comprise some 90% of all vertebrate animals used in laboratory research. While rodent use in Europe reportedly dropped from 10 to eight million between 1991 and 2002 (CEC 2005), global numbers now appear to be increasing due to a new emphasis on transgenic mice (O'Shea 2000, Fishbein 2001). For instance, one recent estimate puts the number of mice consumed by US laboratories at close to 100 million (Carbone 2004).

Minimum laboratory husbandry standards for rats and mice are prescribed by the European Community (CEC 1986) and in the UK by the British Home Office (1986), whose guidelines state that 'rats and mice should be group-housed unless a particular experiment requires otherwise' (para 3.28), and that (for animals in general) 'bedding and nesting materials should be provided, unless it is clearly inappropriate'

(para 3.60). A Council of Europe review of housing standards (nearly completed at time of writing) states that rodent 'enclosures and their enrichment should allow the animals to manifest normal behaviours' (CEC 2005, p. 20), and strongly recommends nesting materials and nestboxes, and the further addition of some form of enrichment, such as tubes, boxes and climbing racks.

In the US there are no federal regulations for laboratory husbandry of rats and mice, owing to these animals' exclusion from the Animal Welfare Act (USDA 1995). However, guidelines developed by the non-governmental organization Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) and issued by the National Research Council (NRC 1996) include recommendations for the care and use of rodents, and constitute an important resource for the exchange of information on the care and use of animals in laboratories (Howard et al. 2004). Currently, these guidelines recommend specifically for rodents only solid-bottom caging with bedding, though enrichments for all laboratory-housed animals are encouraged.

Strictly speaking, the above guidelines are recommendations and not requirements and this may be to allow room for exceptions: 'it is not appropriate for a code of practice to set mandatory requirements for housing which must be followed in all circumstances' (British Home Office 1986, para 1.13). Nevertheless, what little data are available indicate that efforts are being made to meet these recommendations. We are aware of only two current surveys of rodent enrichment. A survey of US National Institutes of Health facilities (n=22) reported that some 90% of rats and mice housed in these

facilities receive nesting materials, slightly more than 50% are provided with a structural enrichment (usually a cardboard or plastic shelter) and about 40% and 20% of rats and mice, respectively, receive manipulanda (e.g. chew toys) (Hutchinson et al. 2005). The average reported percentage of singly-housed animals was 11% for mice and 12% for rats (ibid). A recent survey assessing welfare of mice in 46 UK animal units (Leach MC, personal communication, October 2005) found that substrate (e.g. sawdust) was provided by 87% of the units and nesting material (e.g. shredded paper) was provided by 80% of units, with all units surveyed providing either one or the other.

Enrichment items were provided for mice by 63% of units, of these all provided shelters and gnawing material, 21% of units provided other enrichment items such as egg boxes, metal rings on the cage top, wheels and hammocks, and 32% of units provided additional food scattered or placed onto cage substrate (e.g. grain). In addition, 21% of the mice were found to be housed singly in 78% of the units surveyed, of these the majority were male mice (37%). A complete set of results from this survey of UK animal units will be published in early 2006 (Leach & Main 2006).

This paper reviews published empirical evidence – including studies of the animals' preferences – to examine the degree to which laboratory housing conditions may or may not meet the behavioural and psychological needs of rodents in laboratories. Preferences may not denote underlying needs; however, where preferences are expressed for commodities integral to an animal's biology – such as places to hide or nest, and space to forage, disperse, or seek

mates – denying those commodities can reasonably be assumed to be deleterious.

## **Methods**

We used an online database (PubMed) to identify studies published in English since 1966 addressing the effects of standard laboratory housing conditions on the behavioural, mental or physical status of small rodents, especially mice and rats. The following root key terms were used: animal, behaviour, caging, deprivation, distress, environmental enrichment, housing, laboratory, mouse, pathology, psychology, rat, single housing, social isolation, standard housing, stress and stereotypy. Other papers were found by scanning the cited literature sections of retrieved papers. We consulted the most recent caging standards and guidelines issued by relevant governing bodies and associations for the US (NRC 1996), the UK (British Home Office 1986) and the European Community (CEC 2003). For actual housing conditions being used in laboratories, we extracted relevant data from the methods sections of published papers. Our use of the word 'standard' as applied to housing (e.g. standard housing or standard cage) denotes a commercially produced rodent cage without enrichments (except where indicated).

## **Rats**

### *A. Social behaviour*

It has long been observed that social isolation is deleterious for rats and that so called 'isolation stress' alters physiological and behavioural characteristics (Hatch et al. 1963). Studies using adrenal weights to estimate stress levels conclude that



isolated rats are more stressed than group-housed rats (Brain & Benton 1979). Rats housed alone (n=8) were deemed more stressed than rats housed four per cage (n=8), as judged by significantly higher heart rates and arterial blood pressures recorded in the solitary rats (Sharp et al. 2002).

Rats show strong motivation for the company of others. Female Hooded Norway rats (n=6) lever-pressed an average 73 times for access to a standard cage containing three familiar rats, which was significantly higher than their demand for either a cage provisioned with novel objects and fixed furniture (average 42 lever presses) or a larger cage (average 40 lever presses) (Patterson- Kane et al. 2002). Gärtner (1968a,b) found that formerly group-housed rats actively sought the company of other rats rather than eat and sleep alone. Both male and female rats housed singly spent significantly more time performing escape-related behaviour than did rats housed in groups and this pattern persisted throughout the eight-week period of single housing (Hurst et al. 1999).

The presence of another rat appears to be reassuring in novel, potentially stressful situations. Solitary-housed rats in standard cages took nearly twice as long to enter a novel arena as did group-housed rats (n=64) kept in standard cages (Zimmermann et al. 2001). Male Wistar rats (n=12) froze and defaecated significantly less when placed in an open field environment with another (familiar) rat than when placed alone (Hughes 1969). Anti-predator vigilance – itself a possible source of stress when there is nowhere to hide – may partially account for these differences.

There is evidence that thwarting attempts to escape aggressive cage mates is stressful for rats. When unrelated rats (n=64) were housed in single-sex groups of eight in an open room (147 x 210 cm) equipped with two propylene cage bases (one inverted as a platform), low status individuals, especially females, spent more time moving around and stretching up their room walls (Hurst et al. 1996). These females had very high corticosterone levels, which the authors attributed to the frustration of attempts to leave their enclosures. The authors do not mention having provided any appropriate enrichment, which might have ameliorated these stress-like patterns. In pair-housed male Long-Evans rats (n=28), the lighter animal used a PVC conduit (15cm long x 7.5cm diameter) more than the heavier animal in 13 of 14 pairs, during both day and night, suggesting that lighter animals might use the conduit as a way to avoid heavier, presumably dominant cagemates (Galef & Sorge 2000). Females (n=28) showed no such pattern.

Social housing affords rats opportunities to play. There is a steady growth in scientific interest in animals' subjective and emotional states (e.g. De Waal 1996, Panksepp 1998, Bekoff 2000), including those of rats. Particularly when young, rats are motivated to engage in social play (Knutson et al. 1998a), and there is evidence that the activity is pleasurable. When rats play with each other, their brains secrete large amounts of dopamine into the bloodstream, and they make 50 kHz vocalizations, which have been linked to positive affect in social and sexual contexts (Knutson et al. 1998a,b, Burgdorf & Panksepp 2001). Rates of 50 kHz vocalizations were significantly higher when rats were placed in a chamber they had learned to associate with play than in a

habituated control chamber (Knutson et al. 1998a,b). A series of experiments found that rats solicited tickles and strokes from trusted human companions; the experimenters (Panksepp & Burgdorf 2003) suggest that the 50 kHz calls made during these encounters are the evolutionary antecedents of primate laughter (Panksepp 2005).

Rats also soon learn to anticipate play. Rats placed alone in a Plexiglas chamber following a week of play sessions with a fellow rat became very active, vocalizing and pacing back and forth with apparent excitement, as if anticipating play (Siviy 1998). Pharmacological dopamine blockade in these habituated rats halted all anticipatory activity (ibid).

While social housing of rats is highly desirable and strongly recommended in guidelines and regulations, it is important to recognize that not all social housing situations represent good welfare (e.g. Hurst et al. 1996). Though domesticated rats tend to coexist relatively peacefully (e.g. Schuster et al. 1993, Hurst et al. 1999), preference should be given for housing animals with prior familiarity or relatedness, and consideration given to the influences of density, sex and available resources to meet behavioural needs.

Using a conditioned place preference (CPP) study design, van den Berg et al. (1999) found that both juvenile (n=18) and adult (n=18) male Wistar rats showed a significant preference for a box containing a free moving rat compared with either an empty box or a box with a visible rat confined behind a Plexiglas barrier. Juvenile rats (n=6) also became significantly more active when anticipating 30 min of social play with another free moving rat compared with a

confined juvenile rat (van den Berg et al. 1999). The authors note that the animals' behaviour was in response to the motivational properties of rewards, such as social play and adult social contact (e.g. grooming and crawling over/under), and not aggressive or otherwise negative interactions.

### *B. Environmental complexity*

Rats are sensitive to variations in environmental complexity. Impoverished living environments can lead to impaired brain development (e.g. Bennett et al. 1969, Renner & Rosenzweig 1987). Just four days of exposure to environmental complexity (paired or group-housing in cages with wires, shelves, stairs and other playthings) can produce significant changes in wet weight of cerebral cortical samples taken from laboratory-housed rats (Ferchmin et al. 1970). Thickness of the occipital cortex increased in female rats given obstacles to food access (Diamond 1988).

Even rats raised in some enriched cages do not show the cortex development of rats housed in a semi-natural environment. Groups of 12 rats living in larger (75 x 75 x 45 cm) cages provisioned with stimulus objects that were changed daily had significantly smaller regions of the cerebral cortex than did a group of 12 rats living for 30 days in a semi-natural environment (9 x 9 x 1m outdoor enclosure with 30cm of earth, weeds, stones, branches and pieces of wood, variable food provision and wire mesh lid) (Rosenzweig et al. 1978).

The neuroanatomical effects of spacious, more naturalistic living conditions predict a range of associated physiological and behavioural improvements, including

cognition and memory (e.g. Paylor et al. 1992, Woodcock & Richardson 2000), visual-spatial learning (e.g. Faverjon et al. 2002), recovery from brain injury (e.g. Passineau et al. 2001) and resistance to stress-induced pathology (e.g. Rockman et al. 1986). Environmental stimulation also ameliorates or eliminates prenatal environmental deficits (e.g. Hannigan & Berman 2000) and the cognitive effects of aging (e.g. Kobayashi et al. 2002) and delays the onset of behavioural stereotypies (e.g. Callard et al. 2000).

Rats raised in more complex environments appear to show less fear of novelty than do rats in standard or more impoverished environments. When raised in a large (200 x 100 x 180 cm) split-level cage with bedding, cover, tubes, wood and burrowing opportunities rats (n=24) entered a novel arena significantly earlier than did rats (n=72) raised in standard commercial (Makrolon, 33 x 55 x 19 cm) cages with or without bedding, nest-boxes and tubes, and were significantly more active (exploring) during their first time in the arena (Zimmermann et al. 2001). Male Long-Evans rats (n=68) reared in perceptually impoverished cages (complete darkness with constant white noise) explored a novel open field environment less than did rats (n=72) reared in a perceptually more stimulating environment containing mazes, ramps, sand boxes, beach balls, mirrors, toys and flashing coloured lights (Gardner et al. 1975). Female Hooded Norway rats (n=35) kept in a larger cage (two adjoining 20 x 23 x 45cm cages) with two large nest-boxes, branches, cardboard box, running wheel, several plastic containers, straw and tissue paper initially explored and habituated to a novel environment significantly sooner than did rats pair-housed in standard (20 x 23 x 45 cm) or

solitary (23 x 23 x 26 cm) cages (Patterson-Kane et al. 1999). The non-enriched rats showed no decline in fear responses in successive trials, suggesting a failure to habituate and depressed learning and memory.

Rats anticipate access to more complex housing. Male Wistar rats (n=24) engaged in significantly more arousal behaviours and explored and moved about significantly more in anticipation of being put in a more stimulating cage (higher, with extensions, a shelter, a tunnel with passages and holes with inserted wood pieces, and a bin filled with old bedding) or with a sexually receptive female than did 24 males anticipating a forced swim or being moved into a standard commercial cage (Techniplast Makrolon IV: floor area 1875cm<sup>2</sup>; height 18 cm) (van der Harst et al. 1999).

Nesting is widely acknowledged to be an important behaviour for female and male rats (Patterson-Kane et al. 2001). Twelve inbred Hooded Norway rats (6 males, 6 females) housed in standard cages in groups of four preferred a cage with nesting material to a cage without (Patterson-Kane et al. 2001). Five female Wistar rats housed in a group always showed a significant preference for a cage containing a nest-box regardless of nest-box design (Patterson-Kane 2002a). Male Sprague Dawley rats (group size of 3) preferred to spend time in nest-boxes than in other parts of their cage, and they favoured opaque or semi-opaque designs (Manser et al. 1998). Preference studies have also shown rats to prefer solid flooring to grid flooring regardless of previous experience (Manser et al. 1995), and that they will work as hard to reach a solid floor to rest on as they will to explore a novel environment (Manser et al. 1996).

Because rats are highly inquisitive, any new element introduced to their cage is a source of interest. This may help explain why, on average, the rats (n=20) spent four times longer in a ‘high’ complexity environment (highest density and diversity of chains hanging from cage roof) than in a medium or low complexity environment, engaging in significantly higher rates of ambulation and resting activity (Denny 1975). Male Wistar rats kept in a standard cage with (n=10) and without (n=10) a propylene cage insert strongly preferred the altered cage to the empty one in two-way choice tests lasting 8h for each subject (Townsend 1997). The enriched rats spent more time exploring and resting in the altered cage than did their standard-housed counterparts (ibid).

### *C. Mobility*

Depending on the animal’s weight, UK and US housing requirements and recommendations for rats provide between 0.010 and 0.080m<sup>2</sup> floor area per animal, and minimum cage height of 18cm (Table 1).

Current standards largely reflect current practice. Commercially available caging systems, in which probably most laboratory housed rodents are kept, adhere fairly closely to regulatory (minimum) standards and guidelines. Floor area for five laboratory studies on rats published between 1996 and 2002 (randomly selected from papers cited elsewhere in this review, which include enriched housing conditions) provided between 0.022 and 0.105m<sup>2</sup> floor area per animal, and cage height between 15 and 20cm (Table 1).

Rats’ natural history might predict that they will value space. In the wild, average home ranges for *R. norvegicus* have been measured from <10 to 8000m<sup>2</sup> (Jackson 1982). But there are few studies addressing the perceived value of space to rats. I was unable to find any studies specifically addressing this question in Norway rats, save the following preference studies.

Female and male rats (n=10) tested in a T-maze preferred larger cages to smaller ones both in isolation and with other cage mates (Patterson-Kane 2002b). Individually-housed rats (n=8) showed no statistically significant preference for either side of a pair of cages joined via a PVC pipe, one cage being slightly higher (23 cm) than the other (16.8cm high) (Galef & Durlach 1993). That this study found no preference may be due to the fairly negligible difference between the two compartments, or that the rats might have perceived their PVC mediated enclosure not as a choice between two cages of different heights, but as a single living space with two compartments.

## **Mice**

### *D. Social behaviour*

Notwithstanding the need for social distancing between certain individuals, mice are a highly social species and almost invariably seek the company of conspecifics (Jennings et al. 1998, Sherwin 2002). Behavioural symptoms of ‘isolation stress’ (also termed ‘isolation syndrome’) in mice include aggression, stereotypies, convulsions, nervousness and handling difficulty (see van Loo et al. 2001). Physiological symptoms include

**Table 1 Living space for rats: comparison of laboratory housing standards, laboratory practice, and home ranges reported from wild populations**

Source	Floor area (m <sup>2</sup> )	Cage height (cm)
Standards and recommendations*		
National Research Council 1996 (USA)	0.011–0.045	18
Home Office Code of Practice (UK)	0.010–0.080	18–20
Laboratory practice		
Chmiel and Noonan (1996)	41 x 25 cm=0.103	19
Galef and Sorge (2000)	33 x 30 cm=0.099	17
Hurst et al. (1996)	38 x 23 cm=0.087	15
Patterson-Kane (2002a)	48 x 38 cm=0.182	20
Zimmerman et al. (2001)	55 x 33 cm=0.182	19
Wild populations		
Jackson (1982)	3000–8000	NA
Stroud (1982)	2400	NA

\*Per animal

lower immunocompetence, higher tumour incidence, gastric ulcerations, hypersensitivity to toxins and increased pathology (e.g. ‘scaly tail’) (ibid).

Despite concerns about aggression, mice have been shown to prefer dominant company to no company at all (van Loo 2001, van Loo et al. 2001). Adult male mice (n=60) showed a significant preference for dwelling in a standard cage inhabited by another male mouse compared to dwelling in an equivalent but uninhabited cage or a barren central cage. Young mice (7–8 weeks old) showed no such preference, but did so as they became older (van Loo et al. 2004). Another cohort of subordinate male mice showed a significant preference for a cage inhabited by an unfamiliar male over a similar cage with no occupant (van Loo et al. 2001).

Mice also show preference for familiar over unfamiliar mice. Subordinate male mice (n=12) showed a significant preference for their dominant cage mate, and vice versa, compared with an unfamiliar male (van Loo et al. 2001). The authors caution that the mice used in this study had been successfully group-housed for a relatively long time before testing, and that no extreme injuries were observed during that time.

#### *E. Environmental complexity*

Increased environmental complexity for caged mice has been shown to enhance brain cell genesis (e.g. Ehninger & Kempermann 2003), slow disease progression (Hockly et al. 2002), increase neuronal metabolic activity (e.g. Turner et al. 2002), and improve behavioural expression (e.g. Marashi et al. 2003), as

well as reduce the prevalence of behavioural stereotypies (e.g. Powell et al. 2000) and ameliorate learning and memory impairments (e.g. Need et al. 2003). Male mice (n=128) housed in a standard-sized cage with bedding, nest-boxes and nesting materials, tubes, and opportunities to climb and gnaw ate more food, gained weight faster, and were heavier than cohorts housed in unenriched conditions (Van de Weerd et al. 2002). Mice (n=72) provided with nesting material ate less but weighed more than cohorts without nesting material, a result which the authors attributed in part to better thermoregulation (Van de Weerd et al. 1997a).

Several studies indicate the mouse's liking for spatial complexity provided by platforms and vertical partitions, which provide opportunities for climbing, chewing and manipulation (Jennings et al. 1998). Male BALB/c mice (n=10) to whose cages was added a polypropylene insert with two raised platforms and an in-built shelter explored significantly more and bar-gnawed significantly less than did mice (n=10) pair-housed in standard polypropylene cages (Leach et al. 2000).

Play behaviour is a reliable indicator of good psychological welfare in mammals (Broom & Johnson 1993). There is good evidence that house mice, particularly juveniles, engage in both locomotor and object play (Wolff 1981, Walker & Byers 1991). There is also evidence that greater environmental complexity and space encourage play in laboratory-housed mice. Male CS mice (n=12) housed in groups of four in spacious glass terraria (100 x 40 x 34.5 cm) containing ladders, platforms, a climbing tree and ropes, exhibited significantly higher rates of locomotor play behaviour (performing horizontal

leaps and vertical hops) than did mice (n=12) housed in standard cages (37.5 x 22 x 5 cm) with or without a plastic insert and wooden scaffolding (Marashi et al. 2003).

There is pharmacological evidence that mice housed in standard cages are more anxious or stressed than mice in more complex cages. Standard-housed female C57BL/6J mice (n=30) drank significantly more water that contained an anxiolytic (midazolam, 0.08 mg/mL) than did mice (n=18) housed in cages fitted with a PVC nest-box, a running wheel, two cardboard tubes and two sheets of absorbent paper for nesting material (Sherwin & Olsson 2004).

Even so-called 'enriched' cages may still represent an unnatural degree of monotony. While stereotyped wire-gnawing was significantly higher in adult male ICR mice (n=16) housed in barren cages, the behaviour was nevertheless abundantly present in animals (n=16) whose cages had been furnished with a toilet roll tube (Würbel et al. 1998a). At 34 days old, mice in both cohorts spent equivalent time wire gnawing, and by 80 days, gnawing time for the enriched group had nearly doubled (to 400 s per 4320 s observation period). Although the standard-housed mice in Sherwin and Olsson's (2004) study drank the most water containing an anxiolytic, mice in the other two treatment cages ('enriched' and unpredictable) still consumed more treated than untreated water, suggesting that all of the cage conditions induced anxiety and/or that they made the animals more prone to addictive behaviour.

By the same token, enrichments in some situations may not always improve welfare. For example, in a study of 66

male DBA/2J mice, inter-male aggression and plasma corticosterone levels increased when standard cages (3 mice per cage) were modified with a floor platform creating several corners and burrow-like passages beneath (Haemisch et al. 1994). The study authors believe that the animals' social structure shifted from hierarchical to territorial in the modified condition, which in turn suggests that the mice found the sub-platform space both defensible and worth defending compared with a simple open box with nowhere to hide. Marashi et al. (2003) reported higher concentrations of corticosterone in enriched-housed (n=12) than standard-housed (n=12) male CS mice, but still conclude that 'an environmental enrichment is beneficial for male mice as long as the spatial conditions are generous enough to allow coping with the increased aggression brought about by the enrichment'.

Preference studies show that mice in laboratories favour a variety of environmental features still commonly absent in laboratory housing conditions. A review of 40 studies published between 1987 and 2000 concluded that mice prefer more complex cages, and will work for nesting material, shelter, raised platforms, a running wheel and larger cages (Olsson & Dahlborn 2002). While merely adding structure to a standard cage had limited effects on behaviour, providing a considerably larger and more complex cage had significant effects, including increased activity or reduced signs of anxiety in open field trials, exploration tests and elevated plus maze trials, or a reduced latency to emerge in emergence tests (Olsson & Dahlborn 2002).

Preferences for hiding shelters and nesting materials have been repeatedly demonstrated in mice, of which 22 strains

are known to build non-breeding nests (Sherwin 1997). Mice are strongly motivated to build such nests not only for breeding, but also for temperature and light regulation (Jennings et al. 1998).

All members of a sample of 39 female TO mice built nests using paper towel and/or cellulose fibre bedding when these were provided (Sherwin 1997). When sawdust was present (for 11 days), male TO mice (n=6) preferred to use it to build nests to sleep in than to sleep in any of three available tubes. When sawdust was then removed (12 days), five of the six mice took to sleeping in the tubes and then reverted to sawdust when it was once again made available (Sherwin 1996a). Sherwin (1996b) found that male TO mice (n=6) defaecated non-randomly in their cages and preferred a substrate with sawdust to a bare plastic floor for this purpose; he concluded that conventional cage designs do not provide mice with a sufficient environment to allow selective hygienic behaviours. Mice also prefer solid to grid floors, and given a choice will use the non-favoured surface (mesh or grid) to defaecate and urinate (Jennings et al. 1998). Mice (both sexes, n=48) of two strains (C57BL/6JlcoU and BALB/cAnCrRyCpbRivU) showed clear preferences for certain types of nesting materials over others, but always preferred nesting material to no material (Van de Weerd et al. 1997b).

Mice (both sexes, n=47) of two strains (C57BL/6JlcoU and BALB/cAnCrRyCpbRivU) spent significantly more time (69%) in a cage with nesting material (two pieces of Kleenex tissues) than in a cage with an empty perforated metal nest-box (25%) (Van de Weerd et al. 1998). In a second experiment, 24 female mice (housed in

groups of 6) spent more than 67% of their time in a cage with nesting material, despite the floor being gridded (Van de Weerd et al. 1998). When mice (BALB/cAnNCrIBR, n=60) were given a choice between an inhabited standard cage and a similar uninhabited cage with nesting material, all age groups showed a strong preference for the latter option (van Loo et al. 2004).

In standard cages, mice often make their nest in the shadow of the food hopper and/or drinking bottle (Baumans et al. 1987, Sherwin 1996b), apparently making the best of a poor situation. Given a choice of four commercial cages, female Zo:WK mice (n=17) showed a significant overall preference (77%) for the one cage (Cambridge) which, unlike the others, was opaque and included a shelter (Baumans et al. 1987).

### *F. Mobility*

The small size of typical laboratory cages precludes practically any opportunities to exercise or explore. Depending on the animal's weight, UK, US and EU housing requirements and recommendations for mice provide between 0.004 and 0.020m<sup>2</sup> floor area per animal, and minimum cage height of 12cm (Table 2).

As with rats, these standards mostly reflect commercially available caging systems, and current practice. Floor area reported in five randomly selected laboratory studies on mice published between 1997 and 2002, including 'enriched' housing conditions, provided between 0.005 and 0.075m<sup>2</sup> per animal, and cage height between 12 and 18cm (Table 2).

Mice appear highly motivated to enter additional space when it is provided, and

this seems most likely attributable to a desire to patrol and explore (Sherwin & Nicol 1996, Sherwin 1996c). T/O mice (n=18) worked for access to a range of 'resources' (food, other mouse, shelter and space) including ones they did not find valuable (because they did not remain there once they got there) (Sherwin & Nicol 1996). Mice (n=18) were willing to pay a cost to access resources (food, extra space and shelter), then spent only brief periods with them (Sherwin 1996c). Female mice (n=17) placed individually in a barren central cage adjoining four different cages with resources first explored the central cage for usually 5–15 min, before visiting the test cages, which they did in quick succession (Baumans et al. 1987).

Economic demand and preference studies also indicate that mice value space. Trained female CB57 mice (n=24) housed in groups of three in standard laboratory cages continued to work by pressing a lever over a six-day period to gain access to additional space, even though it lacked food, water and other mice (Sherwin 2004). Male mice (n=7) presented with cages of various sizes showed a statistically significant preference for more floor space by making more visits to larger cages, spending more time in them, and performing more lever switch actions to gain access to them (Sherwin & Nicol 1997).

## **Behavioural stereotypes**

### *G.1. Stereotypes in *Mus musculus**

The link between abnormal or impoverished housing conditions and the development of behavioural stereotypes is well established. Stereotypic behaviours are repetitive, unvarying and apparently



functionless behaviour patterns commonly seen in animals kept in close confinement. They are believed to reflect animal suffering (Mason 1991a) and are common in some rodents caged for research, including mice, chinchillas, black rats, deer mice, field voles, bank voles and gerbils (Garner & Mason 2002). Stereotypies are highly variable in origin and expression among different species, strains and individuals (Garner & Mason 2002, Mason & Latham 2004). They can be extremely prevalent in certain species or strains; about 98% of laboratory-caged male ICR mice, for instance, are prone to stereotypic behaviours (Garner & Mason 2002), and stereotypies are estimated to afflict some 50% of all laboratory-housed mice (Mason & Latham 2004).

Several behavioural stereotypies have been described in laboratory-caged *M. musculus*, including bar gnawing, bar circling and bar jumping (Nevison et al. 1999). Wire-gnawing in male ICR mice (n=64) was observed to be extremely fast, repetitive and invariant within individuals (Würbel & Stauffacher 1996). Each individual performed it in one or two preferred spots, but the frequency of stereotypies varied considerably across individuals. Stereotypies may result in self injury (Ödberg 1986), and may disrupt maternal behaviour resulting in impaired growth and increased offspring mortality (see Garner & Mason 2002).

**Table 2 Living space for mice: comparison of laboratory housing standards, laboratory practice, and home ranges reported from wild populations**

Source	Floor area (m <sup>2</sup> )	Cage height (cm)
Standards and recommendations*		
National Research Council 1996 (USA)	0.004–0.010	13
Home Office Code of Practice (UK)	0.006–0.020	12
European Community Directive 86/609 <sup>†</sup>	0.006–0.010	NA
Laboratory practice		
Nevison et al. (1999)	45x13 cm=0.059	14
Leach et al. (2000)	45x13 cm=0.059	11.4
Sherwin and Nicol (1997)	27x10 cm=0.027	12
van de Weerd et al. (1997)	25x15 cm=0.038	18
Würbel et al. (1998a)	22x16 cm=0.035	14
Wild populations		
Lidicker (1966)	139	NA
Quadagno (1968)	365	NA
Chambers et al. (2000)	2–80000	NA

\*Per animal

<sup>†</sup>Jennings et al. (1998)

Stereotypical wire-gnawing in ICR mice develops from single bites at the cage lid during exploratory climbing (Würbel et al. 1996), while stereotypical jumping originates from exploratory rearing at the cage wall (ibid). These findings suggest that mice experience at least some degree of suffering due to thwarting of exploration imposed by confinement.

There is evidence that wire-gnawing in mice derives, in part, from attempts to escape confinement. Juvenile, sub-adult and adult male mice (n=32) directed more stereotyped gnawing towards cage bars that were occasionally removed for husbandry purposes than towards fixed bars (independent of their location on the cage top or side) (Nevison et al. 1999). Mice also chewed significantly more at bars through which airborne odour cues could pass from the outside (i.e. not covered with Perspex), further suggesting that chewing reflects a desire to pass through the barrier (Nevison et al. 1999). The authors surmise that even though bar chewing in their study never resulted directly in escape, these mice may still have associated their bar chewing with occasional 'success' given that bars are removed for husbandry (ibid).

It has been suggested that cage gnawing and jumping stereotypies may aid in coping with the stress of confinement (Cooper & Nicol 1991). Several studies have determined, however, that behavioural stereotypies fail to reduce stress indicators in mice. Corticosterone levels did not decline when male mice (n=32) chewed their cage bars (Nevison et al. 1999). Outbred male ICR mice (n=20) prevented from gnawing for 10 days had chronic stress levels (measured as serum corticosterone, tyrosinehydroxylase activity and phenylethanolamine n-

methyltransferase activity) equivalent to those of controls (n=20) who were able to continue gnawing (Würbel & Stauffacher 1996). This study therefore found no evidence that stereotypic wire-gnawing reduces chronic stress.

If wire-gnawing relieved stress, we might also expect a 'rebound' effect following frustration of stereotyped behaviours. Würbel et al. (1998b) blocked stereotypic wire-gnawing in 35 outbred male ICR mice by replacing their cage lids with closely spaced bars. When this blocking was later removed, mice did not rebound by gnawing more than before, nor was duration of blocking a factor in post-inhibitory behaviour. These findings further suggest that wire-gnawing is not a coping behaviour in ICR mice (Würbel et al. 1998b).

Wire-gnawing in recently weaned mice may reflect, in part, attempts to escape and return to the mother (Würbel & Stauffacher 1997). If so, then lower weaning age and weight might correlate with higher stereotypy rates due to increased motivation to nurse. When 32 outbred male ICR mice were weaned at the standard 20 days postpartum, mice lighter than the median weight at weaning had significantly higher wire-gnawing stereotypy levels than did heavier mice (Würbel & Stauffacher 1997). A related study found that both premature weaning (17 days instead of 20) and low weaning weight resulted in significantly higher adult wire-gnawing stereotypies (measured at 80 days) (Würbel & Stauffacher 1998).

## *G.2. Stereotypies in other rodents*

*R. norvegicus* is generally not known to exhibit stereotypies in laboratory

conditions, unless they are drug-induced. In this section I outline the occurrence of stereotypies in rodent species not discussed above, with particular reference to housing conditions. Stereotypies described for bank voles (*Clethrionomys glareolus*) include repetitive bar-mouthing (Garner & Mason 2002), jumping, looping (somersaulting from the cage top) and weaving (pacing to and fro over the same point, with frequent rears when turning) (Cooper & Nicol 1991). Both form and location of the bar-mouthing stereotypy were idiosyncratic to individuals (n=8), for whom the proportion of active time spent bar-mouthing ranged from 3.5% to 28.1% (Garner & Mason 2002).

Stereotypy in bank voles has been linked to deficits in brain structure and function. Bar-mouthing stereotypy in singly-reared, standard-housed bank voles (n=8) correlated positively with latency to extinction of a maze task; stereotypic animals persisted in responding rapidly to a previously rewarded maze arm. The least stereotypic animal extinguished in 26 maze trials, and the most stereotypic took 244 (Garner & Mason 2002). These findings are consistent with prior damage to the central nervous system (Garner & Mason 2002). The authors suggest that stereotypic animals, like human clinical patients, might feel the frustration of being unable to turn decisions and preferences into actions (ibid).

Stereotypies derive from chronic environmental deprivation (Mason 1991a,b). In a study of both lab-reared and wild-caught bank voles and their offspring (n=47), all housed singly in standard cages from the start of the experiment, stereotypies (rearing, weaving, jumping, looping, pacing and rearing, figure of eight) developed in all

but the wild-caught adult cohort. At 10 days post-weaning, adult lab-reared voles (n=12) spent significantly more time stereotyping than did pups (n=23), and after 60 days, three of nine (33%) wild and seven of 14 (50%) lab-reared pups had developed locomotor stereotypies (Cooper & Nicol 1996). Ödberg (1987) found that most (though not all) bank voles performed less stereotypy when raised in or moved from a small cage to a bigger and more complex enclosure.

Powell et al. (1999) described three behavioural stereotypies in deer mice (*Peromyscus maniculatus*): repetitive jumping, patterned running and backward somersaulting. Sixteen deer mice housed two or three per standard laboratory mouse cage developed stereotypy at a significantly faster rate than did 15 deer mice housed in larger cages fitted with a running wheel, habit trails, nesting and hiding enclosures, nesting material, and sunflower seeds (Powell et al. 1999). More than 50% of the mice in standard cages exhibited stereotypy by week 8 of the study, and 62.5% by week 16. Stereotypies developed in seven of the 15 more complex-housed animals, with patterned running exceeding that of the standard-caged animals (Powell et al. 1999).

Deer mice (n=70) housed singly in standard (29 x 18 x 13 cm) cages exhibited significantly higher levels of stereotypy (repetitive jumping, backward somersaulting and patterned running) than did deer mice (n=64) housed in large (609 x 480 x 100 cm), cages furnished with cloth dividers, bedding, nesting squares, mesh cylinders and PVC pipe fittings (Powell et al. 2000).

The opportunity to dig in sand did not prevent development of stereotypic digging behaviour, whereas provision of a burrow without digging substrate did prevent stereotypic digging in Mongolian gerbils (*Meriones unguiculatus*) (Wiedenmayer 1997). Thus, stereotypic digging in gerbils seems to arise not from a need to perform digging, but from goal-directed behaviour to attain a burrow. This finding supports a cognitive element of the motivations that underlie some behavioural stereotypies (Dawkins 1988).

Male roof rats (*Rattus rattus*; n=22), pair housed since weaning in standard wire-mesh cages (25 x 76 x 20 cm), performed an average of 3477 back flips during a 24 h period at approximately 30 days of age, more than double the rate of 28 subjects (average 1511 back flips) housed in the same sized cage provisioned with a wooden nest-box (13 x 25 x 20 cm) (Callard et al. 2000). This finding suggests that providing a nest-box improves but does not eliminate welfare concerns in this housing situation. In the same study, none of 16 pair-housed rats exhibited a back flipping stereotypy when placed in an otherwise standard wire-mesh cage whose height had been increased from 20 to 91cm (Callard et al. 2000). However, by 60 days of age, all of 10 males kept in this enlarged cage were performing a repetitive circling behaviour near the cage top. When these 10 males were returned to the standard cage, all were back flipping within two days. In these experiments cage design modifications are shown to reduce, but not eliminate the performance of repetitive behaviours widely thought to denote compromised welfare in roof rats. The authors entertain the possibility that increasing cage height made performing back flips more difficult, but add that ‘the

similar circling behaviour (albeit horizontally-oriented) suggests that the factors motivating repetitive locomotor behaviours had not been suppressed’ (ibid, p. 150).

## Discussion

### *Deprived environments*

The data reviewed here permit some general conclusions about the psychological response of rats and mice to laboratory conditions. For rats, physiological and behavioural studies indicate that social isolation is detrimental to both males and females, and that the company of others can be enriching and beneficial. Rats also value and benefit from a number of resources commonly absent in laboratory housing. Few studies have addressed the possible importance of space to rats, though limited evidence suggests they value it, too.

Like rats, mice prefer company to isolation, which has been repeatedly shown to be both physiologically and behaviourally harmful. While aggressive behaviour is a concern in especially male mice of some strains, studies reviewed here suggest that these problems might be resolved by creative husbandry improvements, rather than by isolating the offending males. In addition to consideration of prior familiarity or relatedness, and the influences of density, sex, age, strain, and available resources to meet behavioural needs, husbandry measures can also be implemented, such as transferring nesting material to the clean cage following cage cleaning, which may significantly reduce agonistic encounters (van Loo et al. 2000). Providing more space than that of typical housing would

further ameliorate such concerns, because small cages thwart opportunities for antagonists to avoid one another. In practically all studies reporting aggression in captive male mice, animals were housed in small, commercial cages.

Studies also demonstrate the desirability of various other resources to caged mice. In the case of space, a dearth of data leaves open the possibility that mice treat it only as something to explore and patrol. However, given that exploration is a natural response to an absence of needed resources (e.g. escape routes, shelter, nesting material, desirable food, gnawing substrates and mates), it may be that limited space does compromise welfare in conventional laboratory housing situations.

Studies repeatedly show that a shelter and nesting material (as distinguished from a sawdust 'bedding') are virtually indispensable resources for rats and mice. Reproductive female house mice are so highly motivated to nest-build that a ball of cotton wool makes an effective trap bait (Randall 1999).

Yet, while awareness of rodent behavioural needs is improving, the provision of basic resources is still wanting in many laboratory rodent housing systems. Systematic surveys of housing provisions for rodents in laboratories have been few, but recent efforts in the UK and parts of the US suggest that significant numbers of rats and mice are still being housed without nesting materials and/or shelters in their cages, and that at least one in 10 animals is housed alone.

To the extent that deprivations persist, laboratory conditions are compromised and may cause impairments in neural and

behavioural development in rats, and behavioural stereotypies in mice and other rodents. Available evidence suggests that specific causation of stereotypies varies among different species, but that it arises generally from the frustration of natural behaviours that the animals are highly motivated to perform, such as burrowing, foraging, hiding, nesting, escaping, exploring and gnawing (Dawkins 1988, Wiedenmayer 1997, Nevison et al. 1999). Stereotypies are virtually unknown in free-living wild animals, which indicates that laboratory conditions are an underlying cause of these abnormal behaviours (Sherwin 2002). It is widely agreed that stereotypy in human psychiatric patients is highly stressful for the sufferer (Russell 2002). Mason (1991a) lists four bases for the belief that animal stereotypies also indicate suffering: (1) the contexts in which they develop, (2) behaviour patterns from which they arise, (3) factors influencing their development and subsequent performance, and (4) the fact that some stereotypies involve self-damage. These characteristics, while not providing incontrovertible evidence that stereotypic behaviour denotes a suffering animal, are nonetheless highly suggestive.

#### *Lack of control*

Several authors have suggested that lack of control over their environments may be an important factor in the compromised welfare and abnormal behaviour of animals kept in standard laboratory conditions (Wiepkema & Koolhaas 1993, Dawkins 1998, Olsson & Dahlborn 2002, Van de Weerd et al. 2002). In the wild, rats and mice must regularly make choices and decisions, such as finding food or mates, building nests and avoiding predators. It follows that they exercise

considerable control over their lives. The laboratory environment provides scant opportunities to make natural decisions or choices (Mench 1994). Movement is restricted by close confinement, feeding regimens preclude opportunities to forage and manipulate food (activities that make up a significant portion of these species' existence), and social circumstances either preclude contact with others (solitary housing) or prevent animals from effectively ousting or avoiding incompatible cage mates. Lack of shelter further prevents animals from escaping bright lights or perceived threats. Rats have been found to be less fearful when allowed to control their own light, food and water supply (Joffe et al. 1973), and it is well established that lack of behavioural control paired with aversive stimuli can produce pathological levels of stress in animals (Selye 1974).

It is increasingly recognized among those in the zoological parks community – where enrichment issues parallel those in laboratory animal research – that the variables of complexity and variety are key to successful enrichment programmes, and that giving animals optimal opportunity to ‘earn’ a living by taking control of their lives benefits welfare (Martin 1999). Varied environments allow animals to learn how their own actions affect their environment, which fosters behavioural competence and enhances the animal's ability to cope with the challenges of captivity.

#### *Naturalistic enrichment*

The implementation of enrichment strategies for rats and mice is a welcome change which should be strongly encouraged. However, ‘enrichment’, as

currently practised, is not a complete solution to animal welfare problems (Olsson & Dahlborn 2002). Many environmental enrichment studies report that a substantial proportion of animals nevertheless develop behavioural stereotypies in the ‘enriched’ condition (e.g. Powell et al. 1999, 2000, Würbel et al. 1998b, Leach et al. 2000, Callard et al. 2000). Zimmermann et al. (2001) concluded that simple forms of enrichment do not adjust for a lack of environmental stimulation.

A more humane approach aims to be in tune with the animal's natural history in the wild or feral condition. Naturalistic environments introduce meaningful biological complexity, fulfil animals' ethological needs, and help to foster normal behavioural and brain development (Würbel 2002). Würbel (2002) has proposed that populations of mice be bred and maintained in species-typical societies in naturalistic environments. Such a ‘field-study’ approach is being used by a Swiss team of investigators working in Russia, where mice lived in large open-roofed squared outdoor pens of about 400m<sup>2</sup> with two shelters (2 x 2 x 1m) filled with hay, and several wooden boxes (Dell'Omo et al. 2000). Food (undescribed) and water are supplied *ad libitum* and an electric fence bars entry from terrestrial predators (ibid). This housing method promptly detected deficits in transgenic mice that had not been recognized in conventional laboratories (Vyssotski et al. 2002).

#### *Scientific rigour*

Because impoverished conditions constrain behaviour and retard brain development in rodents, resulting in

altered brain function (Würbel 2001, 2002, Turner et al. 2002), the potential exists that scientific rigour may be compromised in experiments performed with these animals. For example, animals with behavioural stereotypies have been characterized as poor models of normal physiological and behavioural functioning, for which they often provide highly variable and unreliable backgrounds for genetic or pharmacological manipulations (Garner & Mason 2002). The study of Crabbe et al. (1999), which found significant variability in results despite painstaking attempts to standardize protocols across three laboratories, 'clearly revealed the practical impossibility of standardization to guarantee reproducibility of results' (Würbel 2002, p.4). A retrospective analysis of a large data archive (on thermal nociception in mice) found that the experimenter performing the test was a more important source of variability than was mouse genotype (Chesler et al. 2002). When rats from the same breeding colony within the same room of the same laboratory were tested on the same equipment (elevated plus maze), results varied significantly according to the animals' familiarity with the human handler. Familiar handlers generated more consistent test results than did unfamiliar handlers (Van Driel & Talling 2005).

Other studies tend to corroborate these results. Housing complexity varied inversely with behavioural phenotypic variability in the rats studied by Zimmermann et al. (2001). In a study of 128 male mice, housing in larger cages provided with materials for nesting, hiding, climbing and gnawing did not increase variability in any parameters measured, a relationship that appears to have held true for prior studies (Van de Weerd et al. 1994, 1997a) whose data were

re-evaluated (Van de Weerd et al. 2002). A study of female mice (n=432) from two inbred strains (C57BL/6J and DBA/2) and their F1 hybrids found that environmental enrichment (a Techniplast 'Mouse House' plus twice-weekly addition of a permanent hard and a temporary soft enrichment object) did not increase variability of results across three laboratories, countering concerns that enrichment undermines standardization (Wolfer et al. 2004). This is not to say that standard-housed rodents are incapable of generating reproducible results, or that enrichments do not influence scientific outcomes (e.g. Tsai & Hackbarch 1999, Mering et al. 2001). A current review (Bayne 2005) of enrichment effects on the physical, neurological and physiological health of rodents indicates that they appear almost always to be salubrious. Thus, such effects may be viewed as desirable to the extent that enriched subjects are healthier and more normal.

## **Conclusions**

There is growing recognition of the inherent problems of depriving rodents the space and resources to carry out natural behaviours, such as exploring, foraging, running, escaping hiding and hygiene maintenance. A recent survey of animal facilities at the US National Institutes of Health indicates that a slight majority of rats and mice at these facilities are now being provided with nesting and structural (shelter) enrichment (Hutchinson et al. 2005). Other indicators that rodent housing conditions are improving include the availability of commercially produced resources for nesting, shelter, gnawing and play (Key 2004), and a sharp rise since the late 1980s in the number of citations using keywords 'environmental enrichment' and

'rodent' (Hutchinson et al. 2005). Considering that two decades ago environmental rodent enrichment was scarcely being discussed, these are laudable trends. But practically all laboratory-housed rodents continue to live in small 'shoe-box' cages, many of which afford little or no opportunity to explore, hide, forage or exercise control over their social milieu. Implementing enrichment strategies involves practical and cost associated challenges to institutions whose rodent systems are already in place. As several papers reviewed here illustrate, even in so-called 'enriched' cages, detrimental effects imposed by laboratory housing systems persist. Nonetheless, both scientific and ethical arguments support an approach more in tune with these species' living environments in the wild.

The evidence reviewed here supports the conclusion that the welfare of laboratory caged rodents is compromised to the extent that they are confined, isolated, prevented from performing highly motivated behaviours, and allowed to develop stereotypies. Adequate knowledge exists to warrant significant improvements in the housing and enrichment of rodents used in research and testing. Furthermore, if it is argued that even with such improvements the lack of a naturalistic environment causes a considerable amount of animal suffering and discomfort, then this is a significant part of the ethical argument for reducing and ultimately eliminating animal experimentation. To the extent animals are used in laboratory research, the broad incorporation of such improvements is warranted.

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## ***Working With Transgenic Animals to Advance Health Care***

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The ability to introduce defined DNA sequences or transgenes into an animal's genome has provided remarkable evidence for the role of genes in health and sickness. Transgenic DNA may be introduced into the genome of animals using strategies such as: pronuclear injection of fertilised eggs; viral infection of embryos; manipulation of embryo stem cells or nuclear transplantation. Clever use of these technologies has enabled the generation of precise animal models of human disease. Subsequently, in unremitting diseases such as Alzheimer Disease, transgenic mouse models have allowed novel pharmacological agents and therapies to be evaluated (Peskind et al., *JAMA*, 296(3): 327-329, 2006).

At the MCRI, the mouse has been the researcher's animal model of choice due to its small size and relative ease of genetic manipulation. Much of the research at the MCRI springs from relevant clinical activities at the Royal Children's Hospital (RCH). Indeed, many staff have overlapping clinical, teaching and research responsibilities. In 2005, approximately 280,000 children and adolescents were treated at the RCH for a wide variety of ailments ranging from the very rare inborn errors of metabolism to complex conditions such as childhood asthma.

In the quest to achieve better health outcomes for children, MCRI scientists have employed knockout mouse models carrying human transgenes to study conditions such as methyl malonic aciduria (Peters et al., *J Biol Chem*, 278(52): 52909-13, 2003), thalassemia (Jamsai et al, *Genomics*, 88(3): 309-315, 2006) and Friedreich ataxia (Sarsero et al., *Mammal Gen*, 15(5): 370-82, 2004). Conditional mouse mutants have been used to identify a key enzyme responsible for the degradation of cartilage in arthritis – a condition that affects 1 in 4000 children (Stanton et al., *Nature*, 434(7033): 648-52, 2005). Unexpectedly, relaxin-deficient mice have revealed a role for recombinant relaxin therapy in reversing fibrosis of the lung and airways in allergic respiratory disease (Mookerjee et al., *Endocrinology*, 147(2): 754-761, 2006). Details of these mice and other examples from the literature will be used to demonstrate the phenomenal part that transgenic animals play in advancing human health care.

***The use of standardised procedures (SOPs) for managing the risks associated with wildlife animal welfare and the promotion of best practice.***

Max Campbell

Department of Primary Industries, Victoria.

Prior AEC approval has been required to obtain a Wildlife Scientific Permit from the Department of Sustainability and Environment in Victoria since 2005. The Wildlife and Small Institutions (WSI) AEC was created by the DPI to handle ethics approval for permit applicants and small institutions. The advent of the WSI AEC uncovered deficiencies in many areas of wildlife research and survey. Environmental consultants must now address animal ethics/welfare issues to the satisfaction of an AEC which expects to see SOPs in place within a quality framework. Applications to date have demonstrated a great variety of operating systems and a relatively poor understanding of ethics and the Law. All activities need to be standardised around the requirements of animal ethics, codes of practice and prevention of cruelty legislation. Work carried out by environmental consultants is the same across many projects, so SOPs need to be clearly outlined so the AEC can make informed decisions.

Effective risk management of animal welfare was overlooked in many applications. Consequently, many fundamental animal welfare concerns were raised by the AEC as it reviewed proposals. For most proposals the AEC asked applicants to develop SOPs and operating manuals and include them with their applications. In some cases there were existing, well-developed procedures manuals that simply required improvement in animal welfare sections; others had no written procedures. Applicants frequently complained that there were no consistent guidelines or standard procedures to follow. The AEC encouraged applicants to develop their own SOPs and field manuals. All agreed that producing SOPs was an educational experience that promoted the adoption of best practice and therefore a stronger commitment to it.

A risk assessment sheet for animals based on the standard O H&S format was developed as an aid to identifying and mitigating animal welfare risks and was well received by the industry. Given reasonable time-frames, both professional and amateur organisations have produced high quality procedure manuals covering all aspects of their animal work with well-considered risk management strategies for animal welfare. SOPs are a critical, if not mandatory, component of any quality management system. For AECs to make informed decisions about the projects they are evaluating, they need to have adequate information about the procedures being undertaken.

The diversity of the industry embraces a wide range of organisational size, sophistication, resources, knowledge and primary functions. Definitions of wildlife or pest animals and the applicability of a plethora of Acts, Regulations, Codes of Practice, Licences, Permits and regulatory authorities have created confusion. Nevertheless there is a fundamental need to effectively manage animal welfare overall. A consultative approach to the development of a standardised template for writing SOPs within a risk management framework will facilitate a considerable improvement in the effective management of animal welfare issues across the industry without compromising local, operator needs.

One thing that has become abundantly clear to me as a consequence of administering animal ethics committees over the past two decades, is that there is a great deal of inconsistency in the way people and organisations do things. The standard operating procedures used for animal work vary considerably between organisations and often, vary within them. Wildlife researchers and consultants in the field frequently lament the lack of consistency of, and indeed lack of official guidance in, the methods used to undertake their core business of trapping and surveys in general. There are many good resources available but they are either poorly distributed or simply not easily accessed. In other instances, documents purporting to be SOPs are merely guidelines. The diversity of the industry embraces a wide range of organisational size, sophistication, resources, knowledge and primary functions. Definitions of wildlife, noxious or pest animals (vermin) and the applicability of a plethora of Acts, Regulations, Licences, Permits, regulatory authorities and Codes of Practice have created confusion and uncertainty. Nevertheless there remains a fundamental need to effectively manage animal welfare. The ethical treatment of animals and the maintenance and implementation of humane animal welfare standards that are acceptable to the community in general, should apply to all animals however defined in various Acts, Regulations or codes of practice.

### **The Wildlife and Small Institutions Animal Ethics Committee.**

Since 2005 in Victoria, prior animal ethics approval has been required to obtain a Wildlife Scientific Permit. The Wildlife and Small Institutions Animal Ethics Committee (WSI AEC)

was created to handle ethics approval for Wildlife Scientific Permit applicants and for institutions too small to support their own animal ethics committee. Environmental consultants are now required to address animal ethics/welfare issues to the satisfaction of a committee which expects to see standard operating procedures in place within a quality framework. During its operation the WSI AEC has uncovered deficiencies in many areas of wildlife research and survey. The applications processed by the WSI AEC to date have demonstrated, at least initially, a great variety of operating systems and in general, a relatively poor understanding of ethics and the Law. Across the industry there exists a great diversity of treatments, methods and procedures. The WSI AEC needs to be able to make informed decisions on proposals from many clients who operate in the same field, often using quite different procedures and with varying levels of quality management systems, if any, in place. They must also be able to adequately assess the animal welfare aspects of proposals from the information provided by applicants. The effective risk management of animal welfare was overlooked in many of the applications submitted to the Committee. As a consequence, many fundamental animal welfare concerns were raised during meetings. All activities need to comply with the requirements of animal ethics, codes of practice and animal welfare legislation. A common complaint from applicants was that there were no useful guidelines or standard procedures to follow. For most proposals the WSI AEC requested that applicants develop SOPs and operating manuals and include them with their applications. In some cases there were existing, well-developed procedures' manuals that simply required improvement in

animal welfare sections; others had no written procedures all. A risk assessment sheet based on the standard OH&S format was developed as an aid to identifying and mitigating animal welfare risks and was well received by the industry. Given reasonable timeframes, both professional and amateur organisations have produced high quality procedures' manuals covering all aspects of their animal work with well-considered risk management strategies for animal welfare. There has also been general agreement that the process of producing the SOPs was an educative experience and that it effectively promoted the adoption of good practice. Indeed, there appears to have been a stronger commitment to good practice since the operators knew what was expected of them. Standard procedures provide the detail for good practice. The WSI AEC now provides long overdue oversight of what has to date, been largely uncontrolled and unsupervised wildlife research and survey activity.

### **Animal welfare aspects of wildlife research.**

Wildlife research generally involves free-living animals in their natural environment. The conditions under which the work is conducted are generally outside of the control of the investigators as projects are often being affected by climatic, seasonal, physical and other environmental conditions. Laboratory-based research on the other hand, can be carefully controlled and monitored with considerable precision. Laboratory animals remain safe in their enclosures under precisely defined conditions whereas animals in the wild remain exposed to predators, disease and climatic fluctuations where the loss of experimental animals is usually due to

factors independent of the research activity itself. Monitoring of wildlife is reduced to the limited time they are held captive or available for observation. The scope for improving animal welfare outcomes is therefore constrained by the limited control that researchers have over the fate of free-living animals. It can be argued that the long-term animal welfare benefits, in terms of conservation and habitat improvement, are significant and justify the short-term risks to animal welfare. However, the effective risk management of processes under the direct control of the investigator can significantly reduce the impact of field studies on wildlife by the use of appropriate standard operating procedures, effective training and thorough risk assessment. Gott (1999) described three subjective but convenient impact levels for methods used in wildlife research. The level of impact often depends more upon species-specific attributes than the nature of the investigative methods being used. Further research will determine the level of impact that particular activities actually have.

#### 1. Low impact.

This category includes passive observation of animals, scats, tracks, signs, hair and calls. These methods require minimal training and should have minimal effect on the target animals. Bats, frogs and birds may be identified accurately without capture by recording and identifying their characteristic calls. Spotlighting is generally regarded as a passive method of observation but powerful spotlights and continued disturbance of feeding and other behaviours may have deleterious effects.

## 2. Moderate impact.

Studies in this category include one-off or infrequent capture and the luring of animals for observation. Call broadcasting to lure animals to respond and identify themselves may generate adverse effects including stress and marked behaviour modification. Fitting radio collars and similar telemetric equipment may have significant effects, depending upon the size and intrusiveness of the devices used. Lifting rocks, bark, logs and other minor habitat disturbance may have far reaching impacts dependent upon the frequency, intensity and species vulnerability. Environmental disturbance may also affect non-target species that are important in the food chain.

## 3. High impact

High impact studies include repeated mark recapture over short periods, invasive procedures such as biopsies, surgery, injections, anaesthesia, tagging, fitting of intrusive telemetric devices and biometrical procedures. Handling animals also increases the risk of transferring diseases such as Chytrid fungal infections in amphibians.

Gott (1999) indicated that simple, apparently unobtrusive procedures can have major effects on some animals. For example, coloured leg bands on male finches in North America influenced sexual selection behaviour of females; thereby affecting the reproductive success of banded males. Consequently it is important for wildlife investigators to thoroughly consider the potential ramifications of the procedures and methods they intend to use in terms of what is known about the biology and ecology of the study animals so that the risks can be

managed as far as is practicable. The principal objective of research is to increase knowledge about the species under study but there is often an existing reservoir of information already available that can be used to assist the development of improved operating procedures and methods. The moist and absorbent skin of frogs for example, facilitates the ready transfer of infectious fungi and readily absorbs domestic chemicals from the hands of investigators, so the use of sterile disposable gloves and other containment practices would reduce the risks. Emergency procedures also need to be established to offset the unpredictable nature of the environment at large. Extremes of temperature will affect the survival rate of animals held captive in traps. Small marsupials may abandon pouched young when trapped. Repeated trapping compromises animal health by reducing food intake and in the case of lactating females, may affect the survival of dependent juveniles.

### **Risk Management**

Animal welfare, like occupational health and safety, benefits from the adoption of a thorough risk management approach. Risks need to be identified, evaluated and plans for mitigation need to be developed accordingly. The adoption of a “job safety analysis” approach to animal welfare has proven to be useful and beneficial. Effective animal welfare is about managing the level of risk to which animals are exposed by the procedures and processes used to study them. The risk may involve pain, stress, injury, trauma, death, confinement, physical restraint and environmental consequences which all need to be viewed from the animal’s perspective. The risk of being prosecuted for regulatory non-

compliance is also a driver and needs to be managed in a similar and parallel manner.

The level of risk for any activity is a function of the likelihood of a deleterious event happening and the magnitude of its consequences if it occurs. Simple two-dimensional risk analysis matrices (Table 1) are often used to assist in the determination of levels of risk. A hierarchy of control measures can then be applied to mitigate the perceived risks. Originally developed for occupational health and safety and financial risk assessment, simple matrices are easily adapted for managing animal welfare risks. Similarly, Job Safety Analysis (JSA) sheets are ideally suited for use with animals (Table 2). SOPs are a critical component of the control measures used to mitigate risk. In conjunction with codes of practice and standards they are used to facilitate regulatory compliance (Figure 1). Potential animal welfare risks need to

be identified for each activity and procedures developed to minimise the potential risks, support effective risk management and ensure a consistent approach to performance.

**Invasive or non-invasive procedures – what is the risk?**

Invasiveness is often defined by convenience, politics, legality or historically accepted practices rather than animal welfare or impact outcomes. For example in 1996, venipuncture for blood sampling was declared to be a non-invasive procedure for wildlife by the Regulatory Enforcement and Animal Care (REAC) section of the Animal and Plant Health Inspection Service (APHIS) of the USA and as such, is no longer regulated in the USA. This of course has no bearing on the situation here in Australia where such practices are viewed far more realistically and subject to regulation.

	Consequences and Outcomes				
Likelihood	1.Insignificant	2.Minor	3.Moderate	4.Major	5.Catastrophic
a. (almost certain)	H	H	E	E	E
b. (likely)	M	H	H	E	E
c. (moderate)	L	M	H	E	E
d. (unlikely)	L	L	M	H	E
e. (rare)	L	L	M	H	H

**Table 1 Risk assessment matrix.**

**Key:**

**E = extreme risk (time and repetition may increase risk)**

**H = high risk**

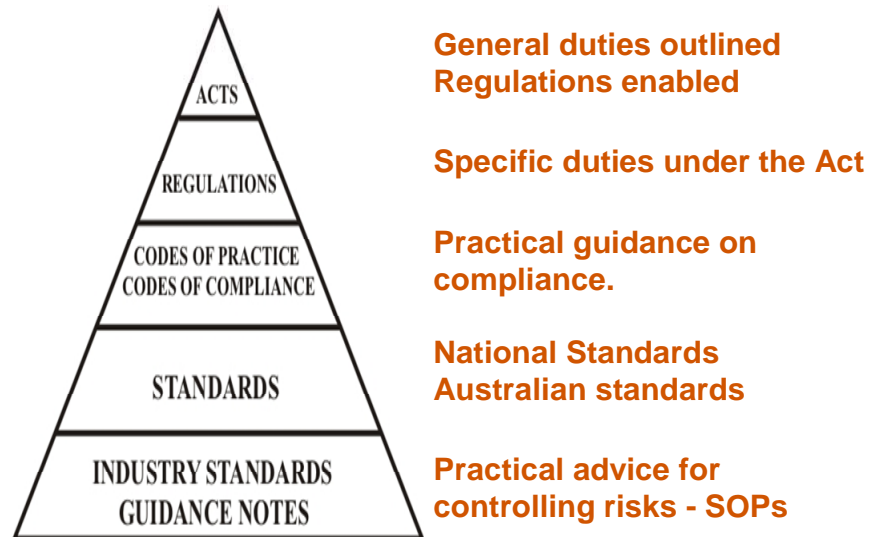
**M = moderate risk**

**L = low risk Risk is managed by appropriate SOPs**

**Table2. Animal safety analysis worksheet**

<b>Activity/Task:</b> Small mammal survey		<b>Equipment:</b> Elliot traps and cage traps		<b>Location:</b> Little Desert National Park
<b>Special Hazards/Site Conditions:</b> Hot, dry, arid conditions. Cold nights/hot days		<b>Person in charge:</b> <i>Fred Smith</i>		<b>Period of Activity:</b> 18-12-2006 to 12-01-2007
Ref.	Condition/situation Generating the Risk	Potential Hazards/Animal Welfare Issues	Risk Control Measures	
1	Weather conditions	Extremes of temperature  Rain	Provision of nesting material to conserve heat Careful location of traps under shade or provision of cover for traps and protected from weather Careful monitoring and contingency plan for cessation. Limited temperature range for conduct of activity. (4 – 26 ° C) Strict adherence to minimal time in traps. Traps cleared within 2 hours of daybreak Location away from streambeds and flooding risk Provision of cover.	
2	Handling trapped animals	Injury, stress, shock	All staff trained and accredited. Minimise or avoid handling whenever possible Emergency procedures to treat sick or injured animals.	
3	Urine, faeces and blood in traps	Disease transmission	Enforcement of standard operating procedures for cleaning and disinfection of traps and equipment.	
4	Attractive food source/bait	Attack by micro-predators such as ants or wasps	Minimise time in traps Careful site selection Monitoring Exclusion of bait components which attract ants eg. honey or high protein material.	
5	Release of animals	Risk from predators or unfit for release	Release under cover as appropriate for each species and close to the site of capture Administration of emergency treatment as indicated for sick or injured animals.	
6	Diseased animals	Transmission of disease	Cleaning and disinfecting traps and equipment. Using clean gloves to handle animals	

## The Regulatory Framework



**Figure 1. A pyramid of the regulatory framework.**

However defined, many non-invasive practices may nevertheless have significant impact on animals and thereby put animals at risk. Bird banding, marking, branding, tagging, call playback, spotlighting, hair-traps and trapping have been accepted historically as more or less safe and non-invasive and on this basis there may be some concomitant exemptions from legislation. The determination of actual impacts and risks will require research into both short term and long term effects. Practices carried out for ecotourism may be justified on economic or political outcomes without due consideration of animal welfare issues. Clearly the measurement of potential harm needs to go beyond immediate, short-term effects.

### **What are Standard Operating Procedures (SOPs)**

The International Conference on Harmonisation (ICH) defines SOPs as “detailed written instructions to achieve uniformity of the performance of a specific function”. In the simplest form they are instructions on how to do something in a consistent way with predictable outcomes. They specify what should be done as well as how, when, where and by whom it should be done. Standard operating procedures are documented instructions that enable activities to be carried out in the same predictable way every time they are undertaken. This is essential for scientific reproducibility. They are also standard methods against which performance can be measured; auditors examine the procedures and compare them to what actually occurs in the laboratory,



workshop, factory, field, farm, hospital and business in general. In particular, SOPs are critical documents for resolving issues of compliance for legal or accreditation purposes. New employees and trainees need access to SOPs to undertake their work tasks; in fact SOPs provide excellent training material. They are also a mandatory component of any quality management system. By necessity they are dynamic documents and must be updated regularly to embrace changing needs.

There is some confusion over what really constitutes an SOP. Many so-called SOPs are simply guidelines that comprise lists of possible risks and possible management strategies. The frequently seen inclusion of “should” confers a level of uncertainty and choice which reduces the effectiveness of a standard procedure. There is no room for error in experimental procedures that involve animals in particular, so in SOPs the word “must” needs to be used instead of “should”. Alternative SOPs can be developed if choice needs to be exercised.

### **SOPs and AECs**

For animal ethics committees to make informed decisions about the projects they are evaluating, they need to have adequate information about the procedures being undertaken. This is particularly important for identifying the issues relating to animal welfare and managing the associated risks. Approved industry-wide standard procedures could provide both AECs and researchers with a clearly defined benchmark. The procedures detailed in SOPs must be appropriate for and relevant to the activity being undertaken. The primary concern of animal ethics committees is animal welfare and all procedures are expected to address animal welfare issues. Simple guidelines do not

specify how a procedure will actually be done and without a quality management framework, there is no stated commitment to its execution.

### **Where are SOPs needed?**

All activities need to be covered by SOPs (Table 3). The directions or instructions that come with medicines, household chemicals and equipment serve as SOPs. They provide standard advice on how to use the product effectively and safely. If the instructions are followed the outcome should be in accordance with expectations. Environmental survey work involves diverse activities and SOPs are needed to cover all aspects of the work. In addition to the procedures for using equipment, substances and facilities, there will be procedures for managing risks relating to human and animal welfare. The adherence of wildlife consultants to a standard set of procedures or an operating manual would be of considerable benefit to those assessing proposals and would be a boon to those who are required to audit performance against a standard. There needs to be an overall management system and structure in place to be followed by all operators. Within the structure, and subordinate to it, there must be a set of SOPs for performing tasks consistently and facilitating training. The main structure must cover the legal, ethical and philosophical aspects of conducting wildlife research/consulting in addition to local processes, goals and policies.

### **Wider Adoption of Standard Operating Procedures**

Standard procedures will stand a better chance of being accepted if there is consultation with, and participation by, the intended users during the development process. Most of the clients of the WSI

AEC have expressed a willingness to participate in workshops to cooperatively develop SOPs that can be used industry-wide. However, issues of “commercial in confidence” and intellectual property have been identified for some of the procedures and survey methods used by some organisations. There is great reluctance to share information with perceived competitors. However, basic standard procedures for best practice, incorporating the core elements of approved codes of practice and legislative requirements, will be more readily adopted if there is flexibility to include some local organisational modifications. There is also a need to take into account the limitations of the industry’s current resources prior to the adoption of standard practices. Flexibility for local and specialised requirements must be embraced and incorporated. Agreement on risk management as a tool for developing SOPs and a willingness to share information across the industry are critical to success.

The actual process of developing and writing SOPs improves the skills and understanding of the users as a direct consequence of the research and consultation they need to undertake. It is an educative process which confers ownership and responsibility, assists team building, encourages the review process and empowers those involved. The users generally have sound, hands-on, practical experience and first hand knowledge of the tasks and associated risks. People are more likely to support the adoption and use of processes they help to create. Developing and writing SOPs is a waste of time and resources if they are not used.

**What should a Standard Operating Procedure comprise?**

An SOP is an important communication document and must enable a procedure to be identically reproduced by a variety of users. It is therefore essential that the text is clear, concise, precise, unambiguous, logical and easy to follow. Allowance may need to be made for other languages. Pages should be numbered and where necessary, a contents page should be provided. SOPs have a limited life and need to be regularly reviewed. They also need to be thoroughly road-tested at the time of introduction. The document must indicate the period of currency and scheduled review date. The layout of the document is important and could include the following headings or sections indicated in Table 4.

**Formats for procedures.**

The general procedures may be presented in a number of formats dependent upon the nature and complexity of the operation.

- (i) **Simple steps**  
Consecutively numbered sequential steps in a recipe - style format of up to 10 steps.
- (ii) **Hierarchical steps**  
If there are more than ten steps with repeats, the steps can be further divided into sub-steps.
- (iii) **Graphic procedure**  
If more than one major consecutive task then groups of instructions are connected by graphical representations of direction and priority.
- (iv) **Flowchart procedure**  
The steps are clearly set out within a flowchart with feedback loops and alternatives where appropriate. This may be appropriate where many decisions need to be made.

### **Table 3 Issues and activities requiring standardized procedures or resolution.**

- Euthanasia methods – species specific and must be manageable within expertise limitations.
- Treatment of vermin/pests/noxious species
- Definition of scientific/invasive procedures from a wildlife perspective based on impact on animal welfare.
- Definition of wildlife as a collective term for the purposes of animal ethics. Exclusions create uncertainty and confusion.
- Standardized methods for piscine forms
- Release strategies
- Procedures and limitations for sample or voucher specimens.
- Biometrical analysis
  - Weighing
  - Measurement
  - Restraint
  - Release
  - General handling
- Emergency procedures for sick or injured animals - species specific
  - Hypothermia
  - Release times
  - Housing
  - Physical injury
  - Disease
  - Stress/shock
- Standard for spotlighting (to minimize harmful effect on animals)
  - Wattage/luminance
  - Colour filters
  - Timing
  - Duration
  - Distance
  - Species limitations
- Monitoring regimes – based on animal welfare risk and standardized for specific survey methods.
- By-catch and management of non-target species. (Using seine nets to determine the presence of one rare fish might affect a number of other, rare non target species.)
- Emergency procedures for change in weather/environment
- Disease control – hygiene and decontamination procedures based on species of animal and its pathogen.
- Equipment procedures
- Standards for type, construction and deployment of traps and capture equipment
- Trap use/deployment and protocols (specific sites, trap type)
- Hair tube use- industry standards for hair traps adhesive.
- Standards for type and construction of traps
- Pitfall traps – construction and deployment
- Nets – as applicable
- Electro fishing devices
- Call playback
- Trip lines
- Animal handling procedures (species and task specific)
- Bats – mist nets and harp nets
- Marsupials
- Placentals other than bats
- Monotremes
- Fish
- Amphibians
- Reptiles
- Crustacea
- Birds
- Banding
- Tagging
- Micro-chipping
- Animal housing/accommodation/feeding – for long term and short term (emergency)
- Training and accreditation of operators – training standards
- Minimising environmental disturbance/impact
- OH&S for personnel
- Compliance checks/self audit
- Delegation of responsibilities/accountabilities.

**Table 4. Standard Operating Procedure format**

**Reference No :**

**Author :**

**Date commissioned :**

**Expiry date :**

**Scheduled review date :**

**Procedure Title:**

**Introduction:**

**Table of Contents:**

- 1. Purpose:**
- 2. Applicability:**
- 3. Reference: details of other procedures or relevant documents:**
- 4. Prerequisites:**
- 5. Terms and abbreviations:**
- 6. Responsibilities:**
- 7. Training/competence requirement:**
- 8. Special Equipment/materials/facilities:**
- 9. Limitations/exclusions:**
- 10. General procedure: ( include schematics, pictures and illustrations where possible)**
- 11. Safety, hazards, risks, wastes and security:**
- 12. Emergency procedures:**
- 13. Appendices:**

## **Training**

Training is a key component of any risk management system and needs to cover diverse aspects such as ethical attitudes, legislation, methodology, risk management and specific procedures. No matter how well written, SOPs require some level of training for their successful adoption. Training and competency underpin the effective implementation of any management system.

## **Summary**

Identification of animal welfare risks, their assessment and mitigation with carefully designed SOPs within a periodically

reviewed quality managed framework will improve the outcomes for wildlife. The role of the WSI AEC has been instrumental in improving animal welfare standards for wildlife in Victoria.

## **References**

**Gott, M., (1999).** Wildlife research in the Field: welfare aspects of an essential discipline. In: The Use of Wildlife for Research. Proceedings of the Conference held at the Western Plains Zoo, Dubbo, NSW, 26-27 May 1999. ANZCCART Australia

## ***Pain management in laboratory animals - are we making progress?***

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Refinement of research procedures, in order to minimise any pain or distress caused to the animals that may be used, has become a widely accepted principle. One obvious opportunity for refinement is the control of pain, particularly following experimental surgical procedures, as experience in medical and veterinary practice suggest this should be an attainable goal. Introducing refinements to reduce or prevent pain would not only meet public expectations and satisfy local and national ethical concerns, but would also improve the quality of scientific data obtained from the animals models involved.

Current practices, however, sometimes appear to fall short of what could be achieved, given our current state of knowledge of anaesthesia and analgesia. The reasons for this include concerns about the side-effects of analgesics and their potential to interact with specific research projects. One major problem, however, is our poor ability to recognize and assess pain in animals. Specific advances have been made in post-surgical pain assessment in rats and mice, and the most recent data from these species will be presented. A major obstacle to developing assessment techniques has been the time needed to analyse behavioural data. Novel computer assisted techniques are likely to assist with this process, and some preliminary results using “HomeCageScan” will be presented.

The potential for alleviation of pain is considerable, since a wide range of analgesic agents are available, most of which have been developed and evaluated in laboratory species. Despite this, recent reviews of the literature have shown that analgesics are rarely administered after experimental surgery in laboratory rodents, but are more frequently used in larger animals. This apparent bias cannot easily be justified, but some practical and other issues that may influence analgesic use will be discussed.

In order for these measures to have the greatest impact, they need to be incorporated into a package of peri-procedure care that acknowledges the multiple factors that can influence the severity of pain and distress. Taking a broad approach to the welfare of laboratory animals ensures that pain and distress are minimised, and that high quality scientific data are obtained using the minimum number of animals.

**A video of this presentation, including copies of all the slides was made available free of charge to all registered delegates at the conference.**

# *Measuring Pain in Animals: Neurophysiological Techniques and Perspectives*

Craig Johnson

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The best way to evaluate human pain appears to be to ask the patient. This presents obvious problems when assessing pain in animals resulting in increased reliance on objective indicators. Most of these can be divided into one of four categories: autonomic nervous system responses; endocrine stress responses; behavioural responses and electrophysiological indicators. These different indicators have been found to correlate to different degrees with different aspects of the response to a noxious stimulus.

Since the importance of the cerebral cortex in the perception of pain was confirmed by dynamic imaging studies in the 1990s, there has been renewed interest in the use of the electroencephalogram as an indicator of pain. This renewed interest has coincided with the development of powerful personal computers that have enabled widespread adoption of formal signal analysis techniques such as the Fast Fourier Transform.

Early studies identified a close correlation between EEG changes and the subjective perception of pain in both human volunteers and patients suffering painful conditions. Animal studies have utilised these techniques to provide information about responses to noxious stimuli in a variety of situations. In particular, the minimal anaesthesia model has been developed at Massey University (Murrell and Johnson 2006) and has currently been used in a number of mammalian species including the horse, sheep, red deer, ox, dog, rat and wallaby.

This paper will outline the development, methodology, advantages and limitations of the minimal anaesthesia model and will discuss examples of its use in applied animal pain research.

## **Introduction**

Pain has been described as an “unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP, 1979). The dual sensory and experiential aspects of pain make it the most subjective of the sensory modalities. It has been suggested that the degree of sensory stimulation is a less important factor in the degree of perceived pain than the prior state of the central nervous

system that receives the stimulation (Vossen et al. 2006). This subjectivism has made pain difficult to quantify objectively and this has led to the widespread use of a variety of subjective pain scales in human patients. Although subjective pain scoring has proved to be a very powerful tool, its use is limited to those patients that are able to describe their pain. Non-communicative patients such as very young children, adults with various forms of cognitive and communicative impairment and animals are not suitable

candidates for these methods. The need to assess pain in these groups has fuelled a continuing search for objective measures that correlate well with subjective pain scores.

Objective measures of pain and nociception can be broadly divided into four categories:

- Autonomic responses
- Endocrine stress responses
- Behavioural responses
- Neurophysiological responses

This paper will focus on the neurophysiological responses and particularly the analysis of electroencephalographic responses to noxious stimulation during controlled general anaesthesia, the so-called minimal anaesthesia model (Murrell and Johnson 2006).

### **The Relationship between Pain and the EEG**

Prior to the mid 1990s, the perception of pain was thought to be a function of the limbic structure (Lico et al. 1974). The development of functional magnetic resonance imaging allowed the areas of the brain involved in the processing of pain to be firmly identified. Cerebral structures, particularly the insula cortex and anterior cingulate gyrus were found to be specifically responsive to pain in human volunteers (Craig et al. 1996). The discovery of the inherent role of the cerebral cortex lead to a renewed interest in electroencephalographic analysis as a means of measuring pain and nociception.

### **Principles of EEG Analysis**

Electroencephalograms are often analysed using the Fast Fourier Transform (FFT). The following is a very brief explanation

of this methodology. Any signal or waveform whose statistical descriptors (mean frequency, relative frequency components etc.) do not change over time is said to be stationary. Signal analysis theory states that any stationary signal can be considered to be the sum of an infinite number of sine waves of different frequencies and strengths. Fast Fourier Transformation transposes a signal in the time domain into the frequency domain, that is it converts a conventional signal into a power spectrum, a histographic representation of the original signal (Figure 1). For a more detailed explanation of FFT analysis, see Young (2001) or Lynn (1989).

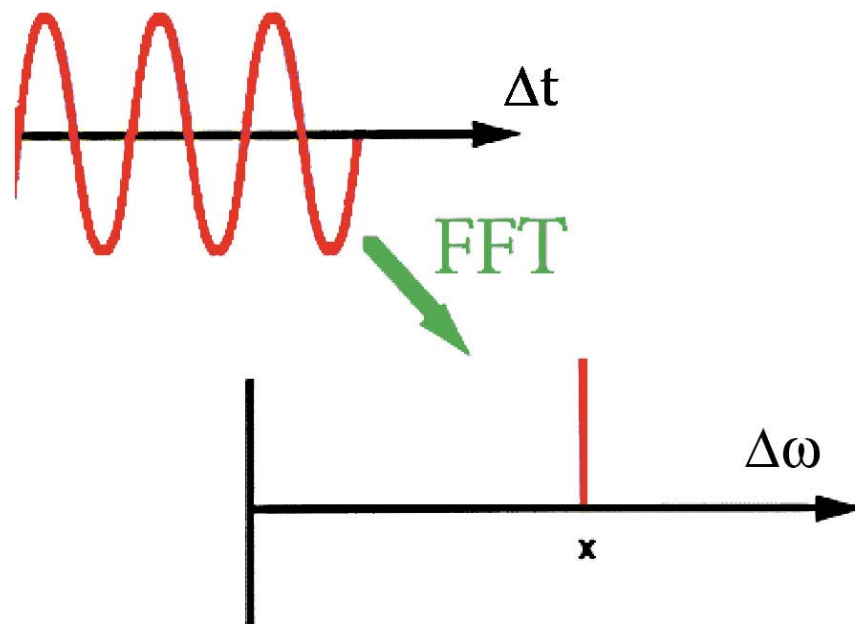
Fast Fourier transformation of one short epoch of EEG (typically one second), gives an indication of the frequencies present at that time. The power spectra of consecutive epochs can be displayed adjacent to each other to give an indication of how the frequency components change over time. This is a compressed spectral array (CSA). Typical CSAs are illustrated in Figure 2. A CSA gives a good visual representation of EEG changes, but in order to perform statistical analysis, it is necessary to derive mathematical descriptors from this waveform. The most frequently used descriptors are: median frequency (F50: the statistical median), which gives a general view of the CSA; 95% spectral edge (F95: the 95<sup>th</sup> percentile), which responds to changes in high frequencies; total EEG power (ptot: the area under the curve), which responds to the lower frequencies. For more details on these variables, see Murrell and Johnson (2006).



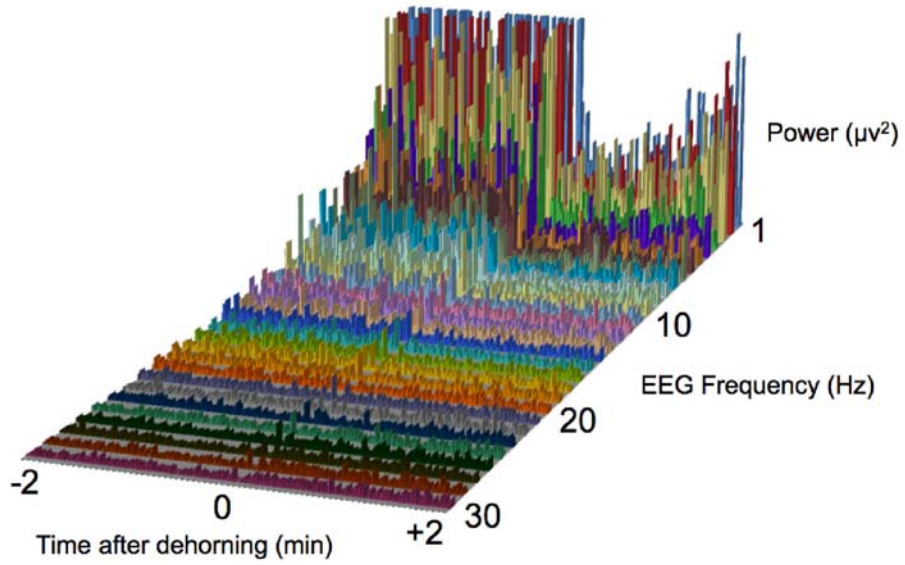
## The Minimal Anaesthesia Model

The minimal anaesthesia model takes advantage of the finding that under carefully controlled conditions of general anaesthesia, noxious stimulation can result in EEG changes (Murrell et al, 2003) that are similar to those seen in conscious animals (Ong et al. 1997). In conscious human volunteers, these changes have been shown to correlate well with subjective perception of pain (Chen et al. 1989). This means that we can compare the pain perception resulting from different noxious stimuli in animals that are anaesthetised. By definition they cannot

feel pain as they are anaesthetised, but the EEG changes give us an indication of the degree of pain that they would have perceived were they consciously aware. This gives us, for the first time, a method of investigating pain in animals that does not require us to subject experimental animals to pain. Even if animals form part of a negative control group and receive no analgesia in addition to general anaesthesia, they are not conscious throughout the study and can be given appropriate analgesia before they recover from the general anaesthetic.



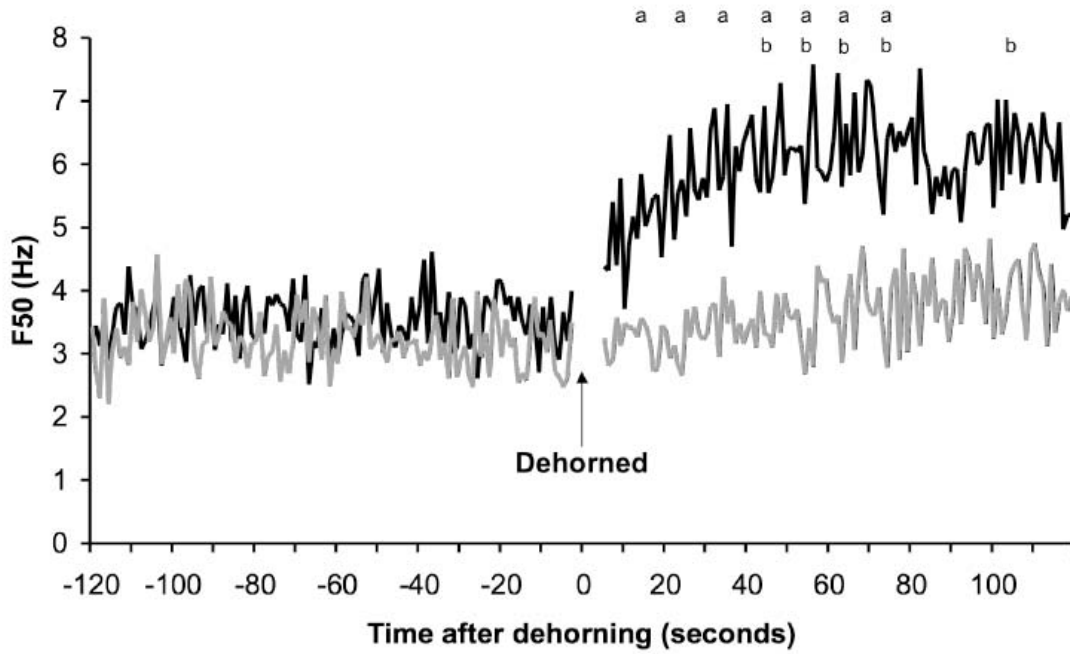
**Figure 1** Graphical representation of Fast Fourier Transformation (FFT) of a time domain signal ( $\Delta t$ ) to a frequency domain signal ( $\Delta \omega$ ). The point  $x$  in the frequency domain represents the frequency of the signal.



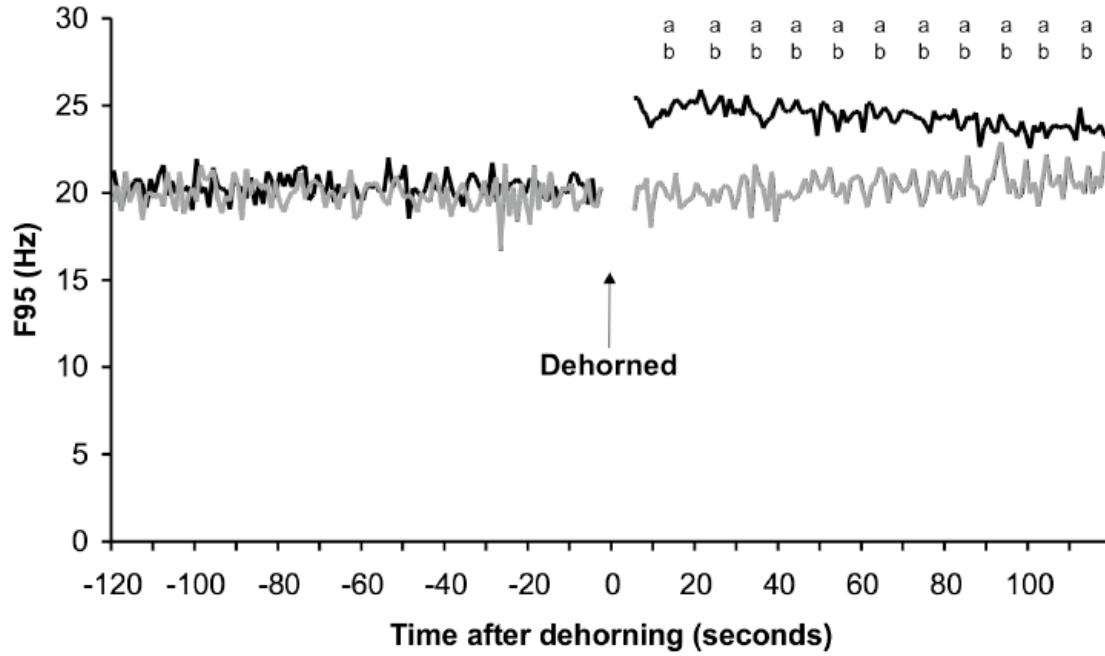
**Figure 2** Compressed spectral array of EEG during scoop dehorning. This represents the EEG response to dehorning (at time 0) in an anaesthetised heifer. An immediate reduction in low frequency power and increase in high frequency power lasting for approximately two minutes can be seen. Data from Gibson et al. (2007).

**Figure 3**

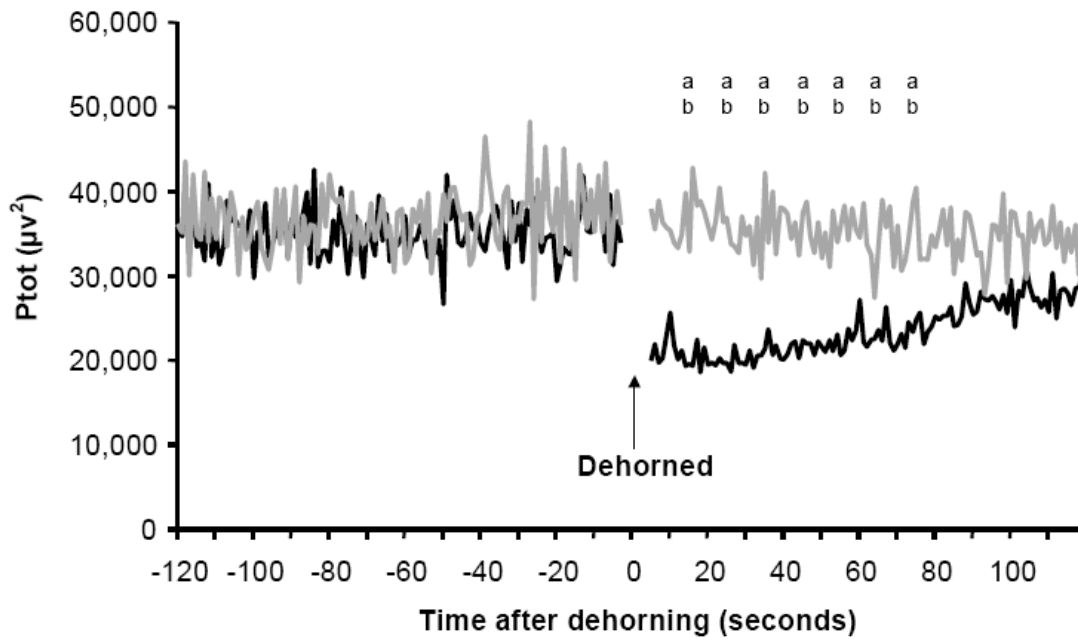
a)



b)



c)



Changes in: a) median frequency (F50); b) 95% spectral edge frequency (F95); c) total EEG power (Ptot) following scoop dehorning at time 0 in anaesthetised cattle. Data from Gibson et al. (2007).

To date the minimal anaesthesia model has been used to investigate pain in 8 species of mammal: horses (Murrell et al. 2003); sheep (Johnson et al. 2005a); red deer (Johnson et al. 2005b); cattle (Gibson et al. 2007); pigs (Haga et al. 2005); rats (Murrell et al. 2007); wallabies (Diesch et al. 2005); dogs (data in preparation). Three examples of the practical applications of this model will be discussed below:

- Scoop dehorning in calves
- Velvet antler removal in red deer
- Castration in lambs

### **Scoop Dehorning in Calves (Gibson et al. 2007)**

Changes in F50, F95 and p<sub>tot</sub> in the two minutes following scoop dehorning in cattle are illustrated in Figure 3. Dehorning resulted in an immediate increase in F95 and decrease in p<sub>tot</sub> and a more gradual increase in F50. The addition of a local anaesthetic ring block prevented these EEG responses demonstrating the effectiveness of local anaesthetic ring block as an analgesic technique for this procedure. The experiment validated the use of the minimal anaesthetic technique in cattle and has formed the bases for further studies (data under analysis).

### **Velvet Antler Removal in Red Deer (Johnson et al. 2005b)**

This study compared the use of local anaesthetic ring block or antler pedicle compression to no analgesia for the surgical removal of velvet antler in red deer. Antler pedicle compression was proposed as a method of field analgesia for velvet harvesting. This study demonstrated that antler pedicle compression was not as analgesic as local

anaesthetic ring block and in addition that the application of the compressive band was itself a significant noxious stimulus. As a result of these and other studies, the National Animal Welfare Advisory Committee declined to recommend the approval of antler pedicle compression in New Zealand and it has not been adopted for use in the field. This is an example of how results generated using the minimal anaesthesia model have been used to influence animal welfare policy at a national level in New Zealand.

### **Castration in Lambs (Johnson et al. 2004; Johnson et al. 2005a)**

These studies demonstrated that the degree to which lambs of differing ages are able to perceive the noxious stimulus of castration by rubber ring. In the first few days of post-natal life, there is very little EEG response to castration. This increases to the level that would be expected of adults by the age of seven days. These findings have stimulated further studies into the mechanisms which are responsible for the development of the perception of noxious stimuli in a variety of animals including lambs, rats, wallabies and chickens. This is an example of the minimal anaesthesia model contributing to our understanding of the basic physiological mechanisms of pain perception in the central nervous systems of mammals and other vertebrates.

### **Conclusions**

In conclusion, the minimal anaesthesia model offers significant advantages over other methodologies available to pain researchers. All animals are anaesthetised throughout the period of data collection. This means that a control group with no additional analgesia can be included into studies against which to compare the

effects of proposed techniques of analgesia. Together with the very tight degree of control afforded by the conditions of general anaesthesia, this increases the statistical power of research studies and allows significant effects to be identified using fewer animals than would be possible with other experimental techniques. Experimental animals can be given analgesia using appropriate clinical techniques after the completion of data collection, but before they recover from general anaesthesia. This ability to give better analgesia to experimental animals than they would often receive in the field means that for the first time, pain research can be carried out whilst simultaneously improving the welfare of the animals involved in the studies.

### Acknowledgements

I am indebted to all past and present members of the Comparative Analgesia Group “Team Ouch” for their enthusiasm and dedication to our studies. Research studies discussed in this paper were funded in part or in whole by: Ministry of Agriculture and Forestry (NZ); Department of the Environment, Food and Rural Affairs (UK); Velvet Antler Removal New Zealand; Palmerston North Medical Research Fund.

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## *Attitudes, human-animal interactions and research outcomes.*

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Laboratory environment (housing and husbandry) and experimental procedure are well understood contributors to variation in research outcomes between experiments and laboratories. However, a much less researched contributing factor is the experimenter. Research in both commercial and laboratory settings clearly indicate that the nature of human-animal interactions significantly impacts the stress physiology, behaviour and welfare of livestock. Studies examining variation in anxiety-related behaviours of rats and mice in controlled laboratory settings have suggested the possibility that human-animal interactions contribute to the variation in results, a possibility that is yet to be tested. Understanding the development, nature and impacts of human behaviour towards animals is essential in determining the impact of the human-animal relationship on the variation and stability of research outcomes.

### **Introduction**

The impact of animal stress responses on the stability of research results is widely discussed in the scientific literature. It is now well recognised that aspects of the laboratory environment, particularly housing and husbandry, can affect the physical and behavioural stress responses of laboratory animals (Markowitz and Timmel, 2005). There is however, an additional, less well recognised factor influencing the stress responses of animals in laboratory settings - the human-animal relationship. Research in commercial and laboratory settings has shown the human-animal relationships significantly impact on behavioural and physiological stress responses, including productivity and reproduction of livestock (Hemsworth, et al, 1989; Hemsworth et al., 1994; Hemsworth et al., 1996; Breuer et al., 2000; Hemsworth et al., 2000). Furthermore, some studies suggest the possibility of a similar link between variation in research outcomes in the laboratory, particularly behavioural responses of mice and rats, and the

experimenter; however, this relationship has not yet been fully explored (Crabbe, et al., 1999; Chesler, et al., 2002; Salome, et al., 2002). Much of the research conducted to date on human-animal interactions has focused on intensively farmed livestock in commercial and laboratory settings and companion animals. Understanding the link between the human-animal relationship, stress responses in animal subjects, and research outcome variation is essential in determining the validity and stability of research results. This paper will outline some of the human-animal interaction research conducted on livestock and discuss the antecedents of human behaviour towards animals.

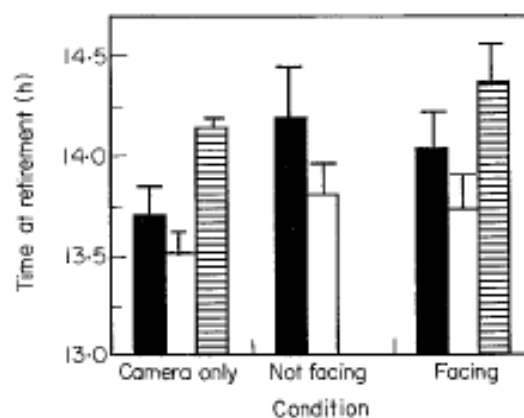
### **Human-animal relationships in laboratory settings**

In any discussion about the nature of human-animal relationships it is important to examine how the animal perceives the human (Estep and Hetts, 1992). All

relationships within and between species, involve some form of communication among the participants. Estep and Hetts (1992) suggest that the ability to interpret a signal in the intended manner may be directly correlated with how closely related the two species trying to communicate are in terms of their taxonomy, body size, sensory systems and normal intra-species communication mode. When different species' normal communication modes are similar, they are more likely to influence one another, form social bonds/attachments, and behave as symbionts or conspecifics. The less the communication systems of two species overlap, the more likely the two species will be to regard each other in a predator-prey relationship (Estep and Hetts, 1992). However, having said that, the social attachment literature is full of examples of organisms not sharing common communication systems forming attachments to one another. There is even a plethora of publications in the literature on the development of attachment relationships between animals (including humans) and inanimate objects (Estep and Hetts, 1992). How an individual perceives the communication signal may differ from how the signaller intended the signal to be interpreted (Estep and Hetts, 1992). Therefore, it is important to understand the range of interpretations of a given signal before using that signal, particularly when signalling between species.

One way to do this is to acknowledge how different species of animals may view specific human behaviours, by noting the innate reaction of a given species towards humans. Estep and Hetts (1992) discuss 5 different views animals may have of humans, particularly scientists. First, animals may view humans as predators. This is innate for many animals and not related to experience but may be modified

through handling; hence, the need for scientists to handle laboratory animals prior to conducting research. Second, animals can view scientists as prey. While this is rare in most species, it is not unheard of in wild carnivores, particularly in "wild" settings where research is observational. Third, animals may see scientists as socially insignificant in their environment. This is what most scientists aim and hope for when designing their studies, often going to considerable lengths to conceal their presence and minimize the contact between themselves and their animal subjects. However, studies have shown that experimenters who view themselves as neutral or socially insignificant (habituated to) can in fact still influence animal behaviour, particularly anti-predator behaviour (Caine, 1990). For example, Caine (1990) found that red-bellied Tamarin monkeys thought to be habituated to human observational presence delayed entering their nests at night when a human observer was present (Figure 1).



**Figure 1.** The average time ( $\pm$ SE) that the tamarins entered the nestbox. □: group 1 in the presence of the familiar observer; ■: group 2 in the presence of the familiar observer; ▨: group 1 in the presence of the unfamiliar observers. The camera-only conditions differed significantly ( $P < 0.05$ ) from the observer-present conditions.

(From: Caine, 1990)



Fourth, animals may view humans as a symbiont, where the human provides the animal with food, water, shelter, and health care, and the animal provides the human with data and perhaps, intellectual and emotional stimulation. Finally, animals may view humans as conspecifics. This is arguably the most influential of relationships when both the animal and human perceive and behave towards one another as members of the same species (Estep and Hetts, 1992).

Estep and Hetts (1992) discuss in some detail the different factors that may affect the type of relationship that develops between humans and animal research subjects, including assimilation tendency or tendency towards anthropomorphic behaviour in humans and zoomorphic behaviour in animals; similarities in communication systems, as discussed previously; sensory contact or familiarity, the basis of habituation; reinforcement and punishment; age; genetic predisposition; and fear. There is insufficient scope within this paper to discuss each of these in detail; rather, the remainder of this paper will examine one of them – fear.

### **Human-animal interactions and fear responses**

Fear is an important emotional response, eliciting a number of physiological and behavioural changes in animals that can and do impact upon research outcomes. Studies on intensively managed livestock are useful for illustrating the impact of human behaviour on fear responses in animals, particularly studies examining animal responses to routine handling. Research conducted in the Australian pig, poultry and dairy industries clearly shows that the type and frequency of negative

behaviours towards livestock significantly influences fear responses. It has been shown repeatedly that the frequent use of some routine negative behaviour by stock people can result in farm animals becoming highly fearful of humans (Table 1).

Research findings have also indicated that handling treatment directly influences the fear responses, stress physiology, and production characteristics in both the pigs and dairy cows in laboratory settings (Hemsworth et al., 1987; Breuer et al., 2000; Hemsworth et al., 2002). For example, Hemsworth et al. (1989) showed that negative and inconsistent handling of pigs, in laboratory settings, affected a range of indices of fear in their responses to humans (Table 2).

In particular, basal blood cortisol levels increased, growth rate decreased, and behavioural fear responses, as measured by time to interact with a stationary human in a standard approach test, increased significantly. Indeed, the rise in basal cortisol with a corresponding decrease in growth rate may be indicative of a chronic stress response (Hemsworth, et al., 1987).

Similarly, negative handling of dairy cows in a laboratory situation resulted in a significant decrease in milk yield and increase in flight distance during a standard approach test (Table 3). It is important to note that the behaviours referred to as “negative” in both of these studies were routine, mild to moderately negative, behaviours, such as pushing, slapping, moving quickly and unpredictably and shouting. “Positive” behaviours included slow, deliberate movement, patting, quiet talking, and placing a hand on the back of the animal when moving it.

**Table 1.** Correlations between proportion of negative behaviour by stock people and fear of humans in livestock

Species	Study	Correlation coefficient
Pigs	Hemsworth et al (1989)	0.45*
	Hemsworth et al (1994)	0.01
	Coleman et al (2000)	0.40*
Dairy Cows	Breuer et al (2000)	0.31
	Hemsworth et al (2000)	0.32**
Meat Chickens	Cransberg (1996)	0.43*
	Hemsworth et al (1996)	0.32

**Table 2. Handling, growth & stress physiology of growing pigs**

Variables	Handling Treatment			-ve
	+ve	Control	Inconsistent	
<i>Time to interact with human (s)</i>	10 <sup>a</sup>	92 <sup>b</sup>	175 <sup>c</sup>	160 <sup>c</sup>
<i>Growth rate from 7 to 13 weeks(g/day)</i>	455 <sup>b</sup>	458 <sup>b</sup>	420 <sup>ab</sup>	404 <sup>a</sup>
<i>Basal cortisol (ng/ml)</i>	1.6 <sup>x</sup>	1.7 <sup>x</sup>	2.6 <sup>y</sup>	2.5 <sup>y</sup>

Superscripts indicate  $\alpha < 0.05$ 

(From Hemsworth et al., 1987)

**Table 3. Handling, productivity & behaviour of dairy cows**

Variables	Handling		LSD
	-ve	+ve	
Milk yield (l)	16.7 <sup>a</sup>	18.0 <sup>b</sup>	1.31
Weight loss (kg)	79.9	66.1	17.9
Flight distance (m)	4.88 <sup>b</sup>	2.81 <sup>a</sup>	1.01

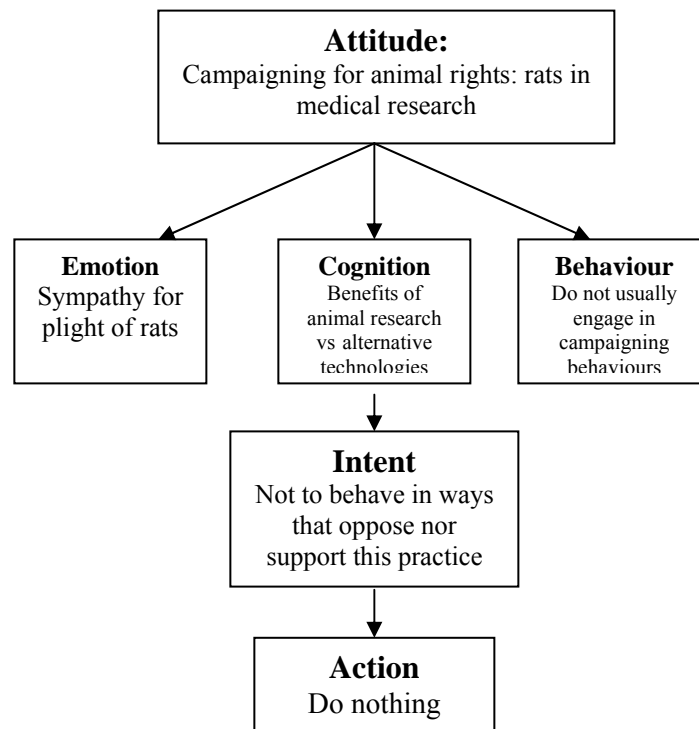
Superscripts indicate  $\alpha < 0.05$ 

(From Breuer et al., 2000)

The findings from these two studies indicate that the nature of *routine* interactions between humans and animals can influence both physiological and behavioural characteristics of the animal. While there is little or no literature of the same nature for small laboratory animals, it is not unreasonable to suppose that routine husbandry and handling practices may also affect fear responses in laboratory animals. This in turn, may influence research outcomes. If so, then understanding the nature of fear responses of the species of animals used in research and the nature of the interaction between the animal and the experimenter may be important in avoiding compromised research outcomes.

**The role of attitudes in determining human behaviour?**

Fishbein and Ajzen (1980) have argued that the immediate cause of behaviour is attitudes. Attitudes are the evaluations of events, objects, ideas, or people; the physical and social environments (Sodorow, 1990; Myers, 1998). Attitudes are generally thought to tell us something about how a person might behave in a given situation. They are thought to comprise three main components: an emotional component (our feelings), a cognitive component (our thoughts or beliefs), and a behavioural component or our tendency to behave in certain ways (Figure 1). Together these components help indicate our likely response or our intended response to a given stimulus (Sodorow, 1990; Myers, 1998). For example, I might ask you about your ‘attitude’ towards campaigning for animal rights, taking the example of using rats to test a drug thought to retard the growth of



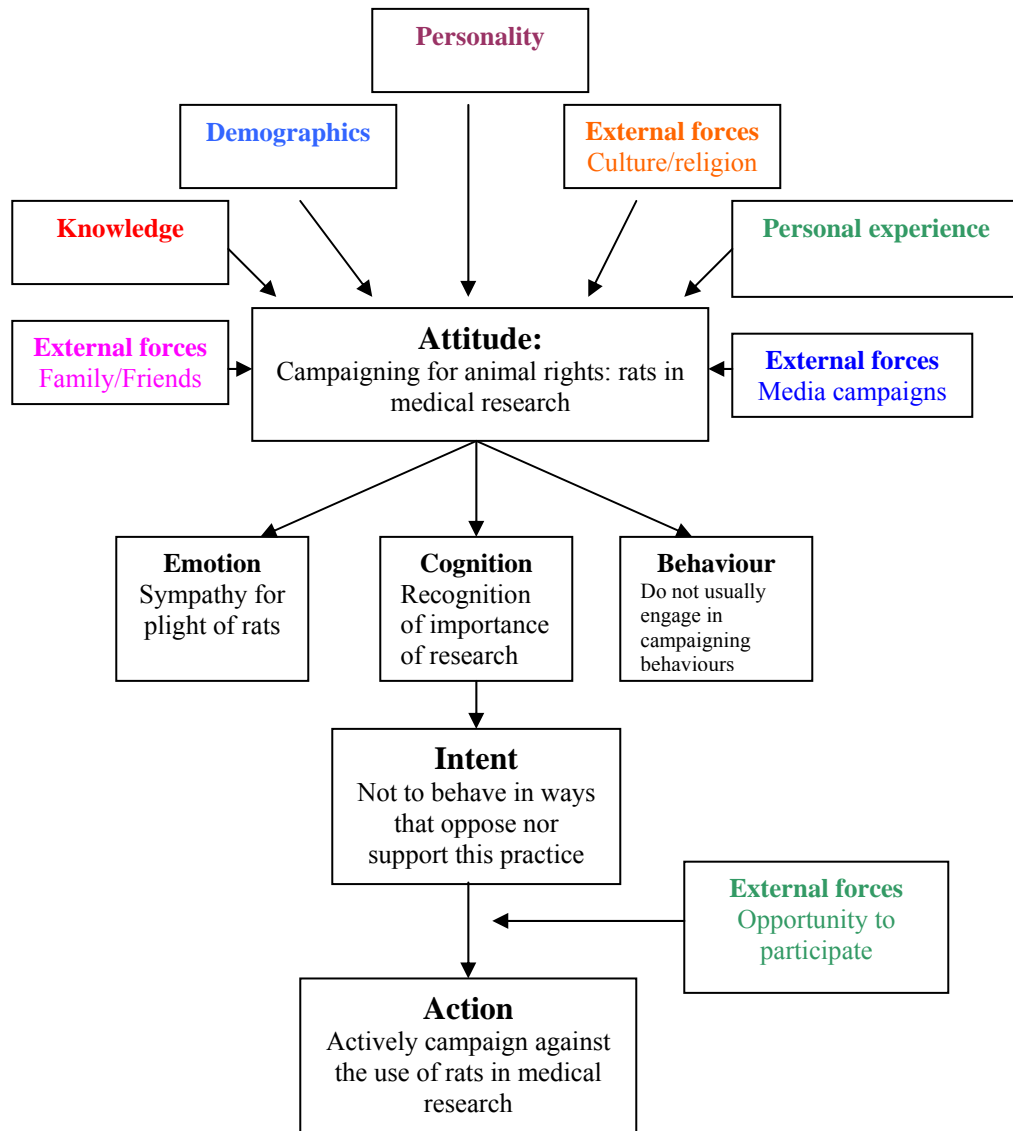
**Figure 1.** Diagrammatic representation of Attitude

tumours. Emotionally, you may respond to the plight of the rats, but also to the plight of humans suffering from tumour related illness. Cognitively you may comprehend the enormous benefits that such research could have for people with tumours, but also be aware that current technology may provide for alternatives that do not involve tests on animals. Finally, behaviour; if it is not a common behaviour of yours to participate in active campaigning for or against a view, it is likely that you will remain neutral and neither support nor oppose the practice. Therefore, your intention will be *not* to behave in ways that would oppose or support this practice, and in effect you would 'do nothing'.

Unfortunately predicting behaviour is not quite so simple as this model would suggest because there may be other external influences that can alter the pathway from intention to actual action; for example, have you engaged in such

behaviours before? What would your family and friends think of your behaviour? Or there may be other external pressures, such as exposure to a strong media campaign about the brutality of medical research on laboratory animals. Given this, if we look at our example again, we can see that your final behaviour may not always reflect your intent (Figure 2).

Fishbein and Ajzen (1980) have argued that the immediate cause of behaviour is in fact the attitude towards the stimulus in question. External factors impact on the emotional and cognitive components of our attitude to alter our intent. That is, the external factors represented in this diagram actually are some of the antecedents (or background factors) of the attitude. Other antecedents include demographic factors such as religion and culture, personality, age, gender, and personal experience. All of these factors relate directly to our target behaviour.



**Figure 2.** Factors influencing attitudes.

Theoretically therefore, attitudes are thought to be influenced by a number of internal and external factors and in turn, determine our behavioural intent and for the most part, our actions. However, is there persuasive evidence to support this view?

Research conducted over the past twenty years in the livestock industries provides some very practical examples of the influence of attitudes on behaviour. A number of studies have been conducted in

the Australian dairy and pig industries examining the relationships between attitudes and behaviour of stock people.

For example, Coleman et al. (1998) examined the attitudes of 87 piggery workers towards working with and handling pigs in commercial piggeries. They correlated the responses of the participants with observations of the piggery workers' behaviours when they were moving and handling pigs. The results indicate that the nature of the

‘beliefs’ about pigs correlated with the types of behaviours employed when handling those pigs (Table 4).

**Table 4.** Product moment correlations between attitude subscales and Stockperson behaviour for 73 stockpersons.

Subscale	Total number of negative stockperson behaviours	Proportion of negative stockperson behaviours
Negative beliefs	0.26*	0.31*
Working with pigs	0.12	0.20
Characteristics of pigs	0.06	0.12
Pigs as pets	0.24*	0.28*
Negative behaviour	0.26*	0.20
Handling non-oestrus pigs	-0.07	-0.04
<i>Handling oestrus pigs</i>	-0.12	0.02

Significance level: \* P<0.05

(From: Coleman et al., 1998)

Note:

- “*Negative beliefs*” refer to stockperson responses to statements about pigs such as “Pigs are gluttons” and “Pigs are dirty”.
- “*Working with pigs*” refer to stockperson responses to statements such as “Pigs are easy to work with” and “Pigs are friendly toward people”.
- “*Characteristics of pigs*” refers to stockperson responses to statements such as “Pigs are intelligent” and “Pigs are easily frightened”.
- “*Pigs as pets*” refer to stockperson responses to statements about pigs such as “Pigs are fun-loving” and “Pigs make good pets”.
- All of these subscales were scored so that a high score indicated a negative attitude.

The findings suggest that a stockperson with negative beliefs about the ease with which pigs can be handled and moved tended to use more negative behaviours, such as hits, slaps and shouts, when moving and handling pigs. Stockpersons that viewed pigs as pets, showed fewer negative behaviours as a proportion of the total number of physical interactions with the pigs (Coleman et al., 1998). Similar studies have been conducted in the dairy industry. Breuer et al (2000) examined the influence of stockperson attitudes towards dairy cows on the behaviour of stock people toward the cows and the fear and productivity of the dairy cows. As with Coleman et al (1998), these authors found that attitudes towards dairy cows were predictive of the behaviour towards dairy cows when handling them (Table 5).

These and other studies have consistently shown that the attitudes of stock people towards their animals are predictive of the behaviour of both the stock people and the animals (Hemsworth and Coleman, 1998; Coleman et al., 2000). While there is limited experimental data on the relationship between the experimenter’s attitude towards laboratory animals and the behaviour of the experimenter to the animals, the findings from research within livestock industries underscores the potential problems faced in laboratory research if the human-animal relationship is not taken into account.

Figure 3 summarizes a model for viewing the relationships between stockperson attitudes and behaviour and fear, welfare and productivity of animals in which attitudes are perceived to directly influence human behaviour which in turn, influences the emotional responses (such as fear) and the welfare of the animal (Figure 3).

**Table 5.** Correlation coefficients between dependent variable, stockperson attitude, and stockperson behaviour.

Independent variable	<i>Stockperson behaviour</i>							
	SPEED 50	P1	N 1	N 2	N2 %	V 1	V 2	W
<i>Stockperson attitude</i>								
ATTITUDE	-0.23	0.	0.	-	-	-	-	-
DE		17	13	0.	0.5	0.	0.	0
SCORE#				3	0*	4	2	.
				6	*	5	5	2
						*		8

Critical values for r,  $v=27$ , 0.367,  $P<0.05$ ; 0.470,  $P<0.01$

Significant correlations at \* $P<0.05$ , \*\* $P<0.01$ .

#, high score represents a positive attitude.

*Stockperson behaviour*

Speed of moving cows from pasture to milking shed (SPEED, m/s)

Num. positive tactile interactions (P1/cow/milking)

Num. negative tactile interactions (N1/cow/milking)

Num. highly negative tactile interactions (N2/cow/milking)

Percentage of negative tactile interactions (% , N2%)

Num. soft quiet vocalizations (V1/cow/milking)

Num. harsh, loud vocalizations (V2/cow/milking)

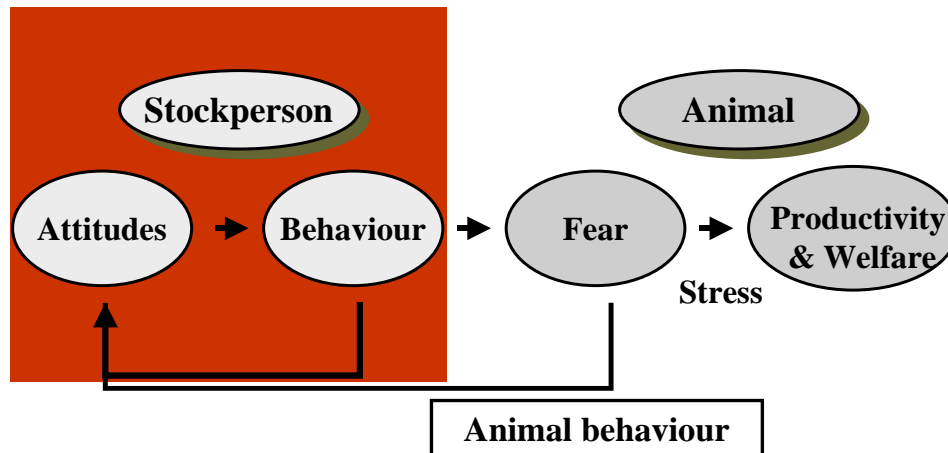
Number of waves (W/cow/milking)

*Stockperson Attitude*

Subscale derived from responses to the questionnaire such as petting, talking to cows, ease of movement and recognizing unfamiliar handlers (ATTITUDE SCORE)

(From Breuer et al., 2000)

## A model of human-animal interactions in the livestock industries



9

**Figure 3.** Model of Human-animal interactions

The importance of recognising the impact of attitudes on behaviour lies in the understanding that the relationship between humans and the animals they interact with, directly influences both the human and the animal. In terms of research outcomes, the impact of the human on the animal's emotional and physical state directly reflects the reliability and stability of research outcomes. Further research however, is required to identify and quantify the impact of routine human-animal interactions on fear responses in common laboratory species. If the outcomes of this research indicate a relationship between human attitudes and behaviour towards laboratory species, such findings could then be used to develop training programs similar to the ProHand series of professional stockperson training programs developed by the Animal Welfare Science Centre for the livestock

industries. Training researchers and animal handlers in the benefits of positive handling on animal fear responses and the flow on effects of changes in physiology and behaviour may be critical for ensuring the reliability and stability of research conducted in laboratory species.

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## ***Getting it right when studying the fetus and newborn: Is pain relief necessary?***

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It is easy to arrive at the conclusion that before birth the human fetus can feel touch and hear sounds. Indeed, it is asserted by many people that human fetuses are conscious and can *experience* noxious as well as pleasant sensations, especially during late pregnancy. These conclusions seem to be supported by the following observations: (1) the baby *in utero* will often move in response to physical pressure being applied through the abdominal wall or it will “jump” in response to a loud and unexpected sound; (2) soon after birth many babies can distinguish their mother’s voice from the voices of other women; and (3) babies born prematurely during the last 10 weeks of the usual 40-week human pregnancy are clearly capable of consciousness and respond to auditory, visual, taste, thermal, touch, painful and other stimuli. This thinking has led some clinicians to advocate that pain relieving medication (analgesics) should be given directly to the fetus during potentially painful invasive procedures, even when the mother, and therefore the fetus, is kept under general anaesthesia during the procedure.

Given that such ideas are confidently asserted about human fetuses it is understandable that similar views are held with regard to the potential for conscious experience in other mammalian fetuses. Such thinking is increasingly leading members of Animal Ethics Committees to consider requiring that analgesics be given to fetuses to protect them during and after surgical or other potentially noxious manipulations. While this trend reflects a sincere commitment to protect animals from unnecessary harm through an act of *refinement*, it is by no means clear that the actions contemplated are in fact required, nor that their anticipated outcomes will be as benign as their advocates think.

For any living animal to suffer it must have a nervous system that is functionally sophisticated enough to transduce noxious sensory inputs into experiences that are sufficiently unpleasant to cause suffering, and it must be conscious. Clearly, most ‘higher’ animals, when mature, meet these prerequisite, when conscious, and have the capacity to suffer. However, how well do we really understand the situation in mammalian fetuses and newborns? What impact does variable neurological development in different species have on the capacity to consciously experience sensations before and/or after birth? And, in light of this, how necessary is it to provide pain relief to fetuses or newborns used in invasive studies? What do we know about the impacts of fetal pain relieving agents, and are there any hazards that need to be considered? These questions are addressed in this paper.

It is concluded that in order to “get it right” with regard to the presumed necessity to provide pain relief to fetuses and newborns, we need to re-evaluate widely-held preconceptions, and adopt a cautious approach to the administration of analgesic drugs, if we are to avoid *unnecessarily* exposing fetuses and/or newborns to deleterious physiological complications which may compromise the scientific validity of the related experimental studies. Laudable moves to apply *refinement* in this context need to be tempered by reference to long established, but not well-known, scientific knowledge.

## Outline

It is easy to arrive at the conclusion that before birth, the human fetus can feel touch and hear sounds. Indeed, it is asserted by many people that human fetuses are conscious and can *experience* noxious as well as pleasant sensations, especially during late pregnancy. These conclusions seem to be supported by the following observations: (1) the baby *in utero* will often move in response to physical pressure being applied through the abdominal wall or it will “jump” in response to a loud and unexpected sound; (2) soon after birth many babies can distinguish their mother’s voice from the voices of other women; and (3) babies born prematurely during the last 10 weeks of the usual 40-week human pregnancy are clearly capable of consciousness and respond to auditory, visual, taste, thermal, touch, painful and other stimuli. This thinking has led some clinicians to advocate that pain relieving medication (analgesics) should be given directly to the fetus during potentially painful invasive procedures, even when the mother, and therefore the fetus, is kept under general anaesthesia during the procedure.

Given that such ideas are confidently asserted about human fetuses, it is understandable that similar views are held with regard to the potential for conscious experience in other mammalian fetuses. Such thinking is increasingly leading members of Animal Ethics Committees to consider requiring that analgesics be given to fetuses to protect them during and after surgical or other potentially noxious manipulations. While this trend reflects a sincere commitment to protect animals from unnecessary harm through an act of *refinement*, it is by no means clear that the actions contemplated are in fact required,

nor that their anticipated outcomes will be as benign as their advocates think.

For any living animal to suffer it must have a nervous system that is functionally sophisticated enough to transduce noxious sensory inputs into experiences that are sufficiently unpleasant to cause suffering and it must be conscious. Clearly, most ‘higher’ animals when mature meet these prerequisites when conscious and have the capacity to suffer. However, there are a number of important questions relating to the capacity of various mammalian species to sense or perceive “pain”. For example, do we really understand the situation in mammalian fetuses and newborns very well? What impact does variable neurological development in different species have on the capacity to consciously experience sensations before and/or after birth? In light of this, how necessary is it to provide pain relief to fetuses or newborns used in invasive studies? What do we know about the impacts of fetal pain relieving agents and are there any hazards that need to be considered? These questions are addressed in this paper.

It is concluded that in order to “get it right” with regard to the presumed necessity to provide pain relief to fetuses and newborns, we need to re-evaluate widely-held preconceptions and adopt a cautious approach to the administration of analgesic drugs if we are to avoid *unnecessarily* exposing fetuses and/or newborns to the deleterious physiological complications which may compromise the scientific validity of the related experimental studies. Laudable moves to apply *refinement* in this context need to be tempered by reference to long established, but not well-known scientific knowledge.

## Introduction

Most of the ideas presented here have been published in a number of relatively recent reviews which refer largely to animal studies (Mellor and Gregory, 2003; Mellor and Stafford, 2004; Lee et al., 2005; Mellor et al., 2005; Mellor and Diesch, 2006, 2007). In light of this, only the major points are summarised here and the reader is referred to these original publications.

Two main questions addressed here are: Is it necessary to provide pain relief in the form of analgesic drugs to fetuses during invasive procedures? How should we approach the provision of pain relief in the newborn?

However, first we need to consider what attributes a fetus or a newborn must have in order to experience pain and suffer as a result. There are two: (1) it must have a nervous system that is sophisticated enough to transduce potentially noxious sensory inputs (e.g. electrical impulses in pain nerve pathways) into experiences that the animal may interpret as sufficiently unpleasant to represent suffering – without such a capacity for sentience, animals cannot perceive by the senses and cannot suffer or experience good welfare; and (2), it must be conscious – an animal cannot suffer while it is unconscious.

Clearly, whether the first of these preconditions for the conscious experience of pain and suffering are met in any particular case, will depend on the pattern of development of the nervous system during pregnancy and after birth in each species of interest. The second will depend on when during neurological development; consciousness first appears in each species.

These matters are considered here.

## Developmental pattern of the mammalian nervous system

The general pattern of neurological development appears to be rather similar in most mammals, irrespective of when the capacities for sensory perception and conscious awareness first appear in relation to the timing of birth.

*Neuroanatomically*, there is a progression in all cases from rudimentary neural structures towards increasing size, complexity and maturity such that peripheral, visceral, spinal and brain nerve tracts as well as the related neural aggregations develop, proliferate, interconnect and grow. Associated with this neuroanatomical development is a progressive functional maturation, which is reflected in changes in behaviour and in the electrical activity of the brain.

*Behaviourally*, initial ‘startles’ or jerky whole-body movements progress through individual limb, neck or head movements to later apparently purposeful and eventually well-coordinated limb movements or changes in body position within the uterus or pouch (in marsupials).

*Neurophysiologically*, a progression of electrical states in the brain parallels these behavioural changes. Electrical activity in the cerebral cortex is of particular note because functional maturation of the cortex is considered to be an essential prerequisite of conscious awareness. Pre-cortical and cortical structures are electrically silent initially – i.e. there is no activity in the electroencephalogram (EEG). The EEG then exhibits sporadic spikes, which evolve into short periods of sustained activity against a background of

electrical silence. Continuous mixed sleep-like EEG activity then appears and this subsequently matures into differentiated and alternating rapid-eye-movement (REM) and non-REM sleep-like patterns. Finally, EEG patterns indicating repetitive sleep-wake cycles are the last to appear and as we shall see, this usually occurs after birth.

### **Relation between neurological development and states of unconsciousness**

During the early stages of electrical silence and sporadic short epochs of EEG activity, the cerebral cortex does not have the functional capacity to support any states resembling consciousness. Likewise, unconsciousness is likely to continue during the subsequent stage of continuous undifferentiated sleep-like EEG activity. However, once REM-non-REM differentiation occurs, the functional capacity of the brain may have matured sufficiently to support conscious awareness because it is at this stage that neural connections, which are essential for consciousness, become well established between sub-cortical brain structures and the cerebral cortex. This is indicated by the example of human infants who are born prematurely at 28-30 weeks after conception (full-term is at about 40 weeks), because they exhibit the capacity for conscious awareness during wakeful phases of their repetitive sleep-wake cycles. As we shall see however, whether or not conscious awareness appears at the stage the brain first develops the capacity to support it very much depends on when birth occurs in relation to that.

### **Onset of consciousness in relation to birth**

This general pattern of neurological development appears to be common in different mammals, but the stage when birth occurs during this developmental path depends on the species and obviously determines the neurological maturity of the young at that time (Ellingson and Rose, 1970; Tyndale-Biscoe and Janssens, 1988). We may illustrate this by comparing young that are neurologically extremely immature, moderately immature or mature at birth.

#### *Extremely immature at birth*

Newborn marsupial joeys are neurologically exceptionally immature and most development occurs postnatally while they are in their mother's pouch (Tyndale-Biscoe and Janssens, 1988). They do not appear to show clear behavioural or EEG evidence of conscious awareness for at least the first one-third to one-half of pouch life, which in the Tammar wallaby (*Macropus eugenii eugenii*) for example, has a total average duration of about 250 days (Tyndale-Biscoe and Janssens, 1988; T.J. Diesch, D.J. Mellor, C.B. Johnson and R.G. Lentle, unpublished data). Clearly the marsupial joey is insensate before birth.

#### *Moderately immature at birth*

The newborns of a number of other mammals (e.g. cat, dog, mouse, rat, rabbit) are also neurologically immature, but markedly less so than are marsupial joeys. Their EEGs variously exhibit the following characteristics at birth (Ellingson and Rose, 1970): electrical silence or very low voltage; or intermittent activity or continuous and undifferentiated activity. Only after 3-14 days does REM-non-REM differentiation occur and EEG

evidence of conscious wakefulness does not appear before this stage. The obvious conclusion to be drawn from these observations is that the young of these species are unconscious before birth and therefore cannot experience pain or any other sensations until the capacity for consciousness develops after birth.

There are implications of these observations for the acceptability or otherwise of conducting invasive procedures on such newborns without using anaesthesia or analgesia. As noted above, the absence of sensory perception during unconscious states precludes suffering and as unconsciousness in these newborns is due to neurological immaturity, potentially noxious stimulation could not arouse them to conscious wakefulness. However, once sleep-wake cycles become established, pain relief would be required if the invasive procedure is severe.

#### *Mature at birth*

Neurologically mature newborns include lambs, kids, bovine calves, fawns, foals, piglets, guinea-pig pups and human infants (Ellingson and Rose, 1970; Mellor and Gregory, 2003; Mellor and Stafford, 2004; Lee et al., 2005; Mellor et al., 2005; Mellor and Diesch, 2006, 2007). Most published information refers to fetal and newborn lambs and human infants, but sufficient is known about the other species for some cautious inferences to be made about them as well.

Using REM-non-REM differentiation and the establishment of neural connections between the sub-cortical and cortical brain regions as primary criteria, the fetal brains of animals that are neurologically mature at birth appear to develop the capacity to support conscious awareness after about

80% of pregnancy has elapsed. It is not surprising therefore, that such newborns usually become conscious within the first few minutes to hours after birth. However, even though the first appearance of consciousness may occur quite rapidly after birth (i.e. within a few minutes), it probably is not an “off-on” phenomenon like switching on a light. Rather, it appears to be more gradual, like slowly increasing the light intensity using a “dimmer” switch.

Once consciousness appears after birth, the young will perceive by their senses and will therefore be able to experience pain and potentially, suffer from it. There is some evidence however, that during the first few days after birth, the young may be less sensitive to painful stimuli. Thus, changes in the EEG that are considered to indicate the intensity of perceived pain are much lower in lambs that are castrated using rubber rings during the first day or two after birth than subsequently (CB Johnson, SP Sylvester, KJ Stafford, SL Mitchinson, RN Ward and DJ Mellor, submitted). It is suggested that this may be due to a slow waning of the pain-relieving effects of substances with known anaesthetic, sedative and analgesic actions that are synthesised before birth by the fetal brain (see below; Mellor and Diesch, 2006).

As the onset of consciousness soon after birth indicates that the capacity for consciousness is present in such newborns before birth, this raises the question of whether or not neurologically mature fetuses are in fact conscious before birth. This question will be addressed below.

## The consciousness status of fetuses

We have already seen that mammalian embryos, fetuses and newborns are most unlikely to be consciously aware when their EEG is electrically silent, intermittent or continuous with mixed sleep-like patterns. We have also seen that it is only after REM-non-REM differentiation occurs, that consciousness is likely to be possible. It has also been established that such EEG differentiation occurs before birth only in those mammals that are neurologically mature at that time. Thus, the question of whether or not prenatal or pre-hatching consciousness exists is probably relevant only to those fetuses that are neurologically mature at birth.

Three lines of evidence *taken together*, provide strong support for the view that neurologically mature mammalian fetuses do not normally exhibit conscious awareness before or during birth (Mellor and Gregory, 2003; Mellor et al., 2005, Mellor and Diesch, 2006).

First, fetal EEG patterns and fetal behaviour demonstrate that sleep-like states of unconsciousness are continuously present throughout the last half of pregnancy. This is because the continuous undifferentiated EEG patterns and the differentiated and alternating REM-non-REM patterns, which appear later and are indistinguishable from those seen during postnatal sleep, are all incompatible with consciousness. In addition, during labour there is a shift in the balance between the REM and non-REM states of unconsciousness towards the deeper non-REM state.

Second, at least eight fetal, placental and uterine factors with well-demonstrated inhibitory effects on the fetal EEG

apparently operate throughout the last half of pregnancy, as outlined below.

- *Adenosine* is a potent neuroinhibitor and sleep-inducing agent. It is present in high concentrations in the fetus and its tissue concentrations are inversely correlated with the levels of oxygen in fetal tissues. During fetal oxygen shortages the associated elevated adenosine levels can shut down cortical electrical activity and rapidly produce a silent EEG trace. This may have the protective function of decreasing the oxygen required by the cerebral cortex in such circumstances.
- *Allopregnanolone* and *pregnanolone* are neuroactive steroids with well-established and potent anaesthetic, sedative and analgesic (pain relieving) effects. They are synthesised by the fetal brain and placenta and act via a specific neuroinhibitory system in the fetus.
- *Prostaglandin D<sub>3</sub>* is a potent sleep-inducing hormone: it is also synthesised by the fetal brain and has neuroinhibitory effects.
- At least one *placental peptide* has demonstrated fetal neuroinhibitory effects.
- *Warmth, cushioned tactile stimulation and buoyancy* are demonstrably neuroinhibitory.

These observations show that mature fetal cerebrocortical function occurs in an inhibitory physiological environment which is unique to prenatal life.

Third, the neurologically mature fetus is not arousable from non-REM or REM sleep-like states to conscious wakefulness by potentially noxious stimulation such as occurs during induced hypercapnia (high carbon dioxide), exposure to sounds loud enough to cause intense auditory pain and surgical manipulations causing tissue damage. This contrasts strikingly with the



situation after birth where these are potent stimuli that arouse sleeping young to conscious wakefulness.

This non-responsiveness to potentially noxious stimulation of the fetus is a further indication of the unique inhibitory functional environment of the fetal brain. The contrasting high responsiveness of the newborn to the same stimuli suggests that expulsion from the uterus at birth would lead to a marked reduction in overall neuroinhibitory influences on the brain. In fact this does seem to occur because immediately after birth the major neuroinhibitors are substantially withdrawn and are replaced by a range of potent neuroactivators that support the onset of conscious awareness (Mellor and Diesch, 2006, 2007).

### **Implications for the use of pain relief in fetuses**

In light of the above observations, three questions are relevant to the use of analgesics during invasive fetal procedures. Is analgesic use required? Do impulse barrages in pain nerves matter during fetal procedures? What analgesics could be used?

#### *Is analgesic use required?*

With regard to preventing the fetus from consciously experiencing pain, the answer appears to be that analgesics are not required. This is in part because all fetuses that are neurologically exceptionally immature or moderately immature at birth apparently do not achieve the first prerequisite of pain experience until after birth – i.e. they do not become sentient. In addition, in fetuses that achieve much

greater neurological maturity – i.e. in those that develop the capacity for sentience – the unique inhibitory physiological environment of the fetal brain, appears to actively maintain such fetuses in sleep-like unconscious states until after birth.

Nevertheless, the fetus would in any case be protected by the usual practice of giving general anaesthesia to the dam before surgically accessing the uterus – as long as sufficient time is allowed for the general anaesthetic to cross the placenta, even neurologically mature fetuses do not respond behaviourally to very invasive surgical procedures (Mellor and Gregory, 2003). General anaesthesia therefore provides a further safeguard for fetal welfare and should help to reassure those who, despite the evidence presented above, remain sceptical about the likelihood of persistent fetal unconsciousness.

In summary, therefore, we may conclude that the use of analgesics is not required to protect fetal welfare. This is because the prenatal neurological immaturity in some species, the naturally maintained states of fetal unconsciousness in others and the use of general anaesthesia during uterine and fetal manipulations, individually and collectively ensure that the fetus is not capable of consciously perceiving pain or any other sensations.

#### *Do impulse barrages in pain nerves matter during fetal procedures?*

Although the fetus is not apparently able to *experience* pain, invasive procedures stimulate pain receptors and thereby cause impulse barrages in those pain nerve tracts that have developed by the time the procedure is conducted. These impulse barrages cause withdrawal of the

stimulated body part and other behavioural responses, stress hormone release and changes in the rates of blood flow to the brain and other organs during and shortly after invasive fetal procedures (Lee et al., 2005; Mellor et al., 2005). However, none of these responses requires an intact cerebral cortex as they are elicited by brain and other neural mechanisms below the level of the cerebral cortex (Lee et al., 2005; Mellor et al., 2005; Mellor and Diesch, 2006, 2007). Nevertheless, they raise the possibility that potentially noxious fetal stimulation might initiate responses in the developing nervous system that could make the individual more sensitive to pain in the long term, i.e. after birth. If so, this would raise the further possibility that pharmacologically blocking such sensory inputs during fetal surgeries may have longer-term benefits even though the fetus cannot *experience* those inputs as pain. However, it is important to appreciate that there are apparently no studies which have robustly tested these speculations about fetal noxious stimulation, so that there are no empirical data to demonstrate a causal relationship between such sensory inputs and the presumed potential for a subsequent greater sensitivity to pain (Mellor et al., 2005). Indeed, robust postnatal clinical studies of young human infants are increasingly suggesting that this is not an important effect (Moiniche et al., 2002).

#### What analgesics could be used?

Notwithstanding all of the evidence presented above, there may still be some people who have lingering doubts about whether or not it is necessary to use analgesics to protect fetuses against the immediate presumed noxious effects of invasive procedures and/or possible

longer-term effects. Thus, they may advocate an “If in doubt, treat” strategy. This would be most incautious. Such a strategy is based on the presumption that fetal responses to analgesics can be accurately predicted from responses of prematurely born individuals of the same post-conception age and therefore that we have enough knowledge of mechanisms of analgesic action in the fetus for this strategy to be adopted without significant hazard. Neither of these presumptions is true. First, because we are profoundly ignorant of the actions, dosage, clearance and side effects within the fetus of numerous analgesics that are efficacious in the newborn, and second, because deleterious effects on the fetus of some analgesics have been demonstrated (Bennet et al., 1986; Taylor et al., 1997; Doyle et al., 2005).

On this basis therefore, we strongly recommend that the “If in doubt, treat” strategy should be discarded, at the very least until we better understand what we are doing.

#### **Implications for the use of pain relief in newborns**

The main issue for newborn mammals is not *whether* it is necessary to apply pain relief, but *when* its use should be contemplated. For the elimination or alleviation of *perceived* pain, analgesics and/or general anaesthetics should be used when significant noxious procedures are conducted after the postnatal ages at which the young have attained the capacity for conscious awareness. As we have seen, this depends on how neurologically mature they are at birth. Thus, the postnatal age “threshold” in different species appears to range from months (marsupials), through days (moderately immature young) to

minutes or perhaps hours (mature newborns).

It is worth noting, however, that in neurologically mature young some degree of analgesic protection established *in utero* may persist for several days after birth. This is suggested by the observation in lambs castrated with tight rubber rings, where the EEG responses indicating perceived pain are very low during the first day after birth and rise thereafter (CB Johnson et al., submitted). We have suggested that this might be due to a slow postnatal decrease in brain synthesis of allopregnanolone and pregnanolone (Mellor and Diesch, 2006; CB Johnson et al., submitted), which are neuroactive steroids that have potent anaesthetic, sedative and analgesic actions, as outlined above. The implications of this for pharmacological management of pain in such newborns have yet to be assessed, but provide an interesting basis for further investigation.

### **Final comments and conclusions**

It is important to consider the status of the above observations. They are fresh insights based on recent integrative syntheses of well-demonstrated, yet not well-known findings in the scientific literature (Mellor and Gregory, 2003; Mellor et al., 2005; Mellor and Diesch, 2006, 2007), some of which date back 30-40 years. Although the literature provides a compelling case for persistent fetal unconsciousness, this proposition is nevertheless contrary to views held firmly by many people. In light of this, a major purpose of presenting this alternative view and the supporting scientific evidence is to stimulate others to challenge them experimentally. If this view survives such

challenges it may then be adopted more widely. Whatever the outcome, the evidence presented already exists in the literature and needs to be assimilated into our understanding of developmental processes in the fetus and newborn.

The starting point for the discussion enumerated above is that sentience and consciousness are preconditions for the *experience* of pain and if the pain is noxious enough, for suffering as well. Therefore, the key events for young that are developing neurologically are the stage when the brain becomes neurologically sophisticated enough to exhibit a capacity for sentience and after that, when the physiological environment of the brain enables it to support states of consciousness. Although neurological development in general, follows a fairly similar pattern in different mammalian species, the relationship of these events to the timing of birth varies, with some young being neurologically *exceptionally immature*, others being *moderately immature* and still others being *mature* at birth. Based on the assumption that consciousness is possible at the earliest when EEG activity and behaviour show that REM-non-REM differentiation has occurred, it is concluded that exceptionally and moderately immature young respectively, do not exhibit consciousness until months and days after birth. In contrast, young that are neurologically mature normally exhibit consciousness for the first time a few minutes after birth, yet they have probably developed the capacity to do so well before birth. The apparent absence of fetal consciousness in these mature species it is argued, is due to the operation of a range of well-demonstrated *in utero* neuroinhibitory mechanisms, which are withdrawn at birth.

### Implications for providing pain relief

These observations have a number of implications with regard to the provision of pain relief. First, the persistence of states of unconsciousness means that fetuses, whatever their maturity at birth, apparently do not require analgesia to prevent pain *experience*. Second, at present there is no empirical evidence to suggest a need to use analgesics to block impulse barrages during invasive fetal procedures that might otherwise increase pain sensitivity after birth. Third, use in the fetus of most analgesics known to be effective in the newborn is contraindicated because their actions, effective dose rates, clearance and side effects are unknown and because deleterious side effects have been demonstrated for some analgesics. Fourth, in any case, general anaesthesia of the dam during uterine manipulation and surgery also ensure that the fetus remains unconscious. Fifth, anaesthetic and analgesic use in young, which are exceptionally or moderately immature at birth and remain in unconscious states for some months or days thereafter, are not apparently required to prevent the *experience* of pain until these young develop the capacity for consciousness. Before that however, anaesthetics or analgesics may be needed to immobilise the young because invasive procedures do induce reflex physical withdrawal and avoidance responses that hinder delicate surgery. Sixth, neurologically mature newborns, which normally become conscious within minutes of birth, may have the benefit of some lingering *in utero* analgesic protection during the first 2-3 days after birth. Seventh, once they are conscious, all newborn and young animals should be given anaesthesia or analgesia to prevent or minimise the experience of pain

caused by significantly invasive procedures.

### Implications for euthanasia

These observations also have implications related to the impact on the fetus of killing the pregnant dam and the precautions required when killing newborn or young animals before the ages when they become conscious.

Oxygen supply to the fetus ceases with death of the dam. This usually elicits a burst of fairly vigorous physical activity in the fetus, but as such behavioural responses begin during the early stages of neurological development when the EEG is electrically silent, they are considered to be spinal or lower brain centre reflexes, which are perhaps designed to free a compressed umbilical cord and restore oxygen supply (Bennet et al., 1999, 2003). Thus, fetal movement after death of the dam is not a sign of distress or suffering in early fetuses. Nor is it in fetuses later in pregnancy. This conclusion is fully supported for all fetuses, whatever their stage of neurological development at the time of maternal death, by the EEG evidence which shows that they remain in continuous states of unconsciousness throughout pregnancy (see above). Moreover, in fetuses that are neurologically mature at birth, cessation of oxygen supply during late pregnancy after REM-non-REM differentiation has occurred causes complete suppression of the fetal EEG within 60-90 second – i.e. the EEG rapidly becomes electrically silent (Mallard et al., 1992; Watson et al., 2002; Hunter et al., 2003). This means that even after the capacity for sentience has developed in neurologically mature fetuses, the suppressive effects of a cessation of oxygen supply guarantee that

the already unconscious fetus rapidly enters a brain state that is totally incompatible with consciousness (Mellor and Gregory, 2003). On the basis of these observations, therefore, we may conclude that fetal distress and suffering do not occur after maternal death whatever the stage of fetal neurological maturity. There is one caveat for neurologically mature fetuses: when maternal death occurs close to birth, after the normal acceleration in prenatal fetal lung maturation is well advanced, exposed fetuses must be prevented from successfully breathing air because this may enable them to elevate their brain oxygen to levels that are compatible with consciousness (Mellor and Gregory, 2003; Mellor, 2003). Leaving all such fetuses in the uterus until they are dead achieves this objective, although other precautions allow earlier exposure (Mellor, 2003; van der Valk et al., 2004).

These impacts on the fetus are independent of the method used to kill the dam, assuming of course that those methods are humane. Thus, anaesthetic overdose, carbon dioxide or inert gas inhalation, effective stunning followed by transverse neck-cut exsanguination, a well executed captive bolt or free bullet head-shot, and other humane methods would pose no concerns regarding the welfare of the fetus as it dies. This is also true for fetuses during hysterectomy (spay) of the pregnant uterus.

Euthanasia of neurologically immature newborn and young animals, which have not yet achieved consciousness, would appear on the face of it to be quite straightforward as they normally cannot experience pain, distress or any other sensations. However, several factors may complicate our response to this. Such

young can locate the dam and begin to drink milk soon after birth and they may exhibit physical and vocal responses to invasive stimuli. For many people such behaviour seems to belie the notion that the young are unconscious. Also our natural, possibly inbuilt, inclination to care for and protect vulnerable young may inhibit us from killing them even if we can do so without causing pain or distress. One solution is to always employ euthanasia methods that would be acceptable if used in older conscious animals and which ensure that progress towards death is smooth and without worrisome behavioural responses (Flecknell, 2007 – *Presentation at the 2007 ANZCCART Conference in Melbourne*). Clearly, that principle also applies to the choice of euthanasia method for young that are neurologically mature at birth.

#### *Implications for legal definitions of “animal”*

A number of definitions of “animal” in welfare legislation include mammals at developmental stages from half way through pregnancy or pouch life (e.g. Anonymous, 1999). As this stipulation is evidently designed to accommodate the presumed potential for such young to suffer, these definitions of “animal” may need to be revisited if the observations outlined above survive further scientific scrutiny and experimental challenge.

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The insights outlined here regarding neurological development and the onset of consciousness clearly has implications beyond our primary focus of the appropriate use of pain relief in fetuses and newborns. To “get it right” with the fetus and newborn therefore, these wider perspectives also need to be considered.

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## *Synopsis of Australian Animal Welfare Strategy Workshop on Pain and Pain Management*

David Adams

The Australian Animal Welfare Strategy (AAWS) was endorsed as the blueprint for the future of animal welfare in Australia by our Federal Government and the Primary Industries Ministerial Council in 2004 and the following year by the National Consultative Committee, which was made up from representatives of all animal welfare, animal user and associated organizations including ANZCCART. In mid 2005, the Australian government committed \$6 million over four years to assist with the implementation of the AAWS.

In May this year, AAWS held its first Scientific Summit in Melbourne and the focus of this one day event was Pain and Pain Management. There was an excellent programme put in place for this summit, which covered a variety of related topics.

Keynote speakers at the summit meeting included the following:

Grahame Coleman	“Public perceptions of animal pain and animal welfare”
Judy MacArthur Clark	“Pain management – an international perspective”
Clive Phillips	“How does pain rank as an animal welfare issue?”
Kersti Sekel	“How pain effects animals”
Craig Johnson	“New Approaches to identifying and measuring pain”
Martin Pearson	“Practical pain management in animals”
Steve Atkinson	“Avoiding and alleviating pain in research animals”
Andrew Fisher	“New research on pain alleviating methods for farm animals”

During this brief presentation, I will touch on some of the key points that came out of these presentations and the expert panel discussion that addressed the question “Pain management – where to from here?” However, as some of the keynote speakers at that summit have also or will shortly speak at this conference, my presentation will focus more on the key points raised by others so that I may have some hope of summarizing a day long meeting in 25 minutes or less.



# ***Bat detectors: Are they the silver bullet for applying the 3Rs of animal welfare when undertaking field based surveys for bats?***

Rob Gration  
Parsons Brinckerhoff

Bats are a unique class of fauna. They are the only mammal that can truly fly. Bats are classified into two groups the megabats and the microbats. The microbats are even more unique from their close cousins due to their ability to use sound to navigate in total darkness. Donald Griffin was responsible for unlocking their secret in the late 1930's, he discovered that they use high frequency calls to gain information about their surroundings. It was he who termed the phrase *echolocation*. It would be many more decades before portable electronic equipment would be developed to record and analyse their calls. With the advent of bat detectors there is a perception that capture techniques are now obsolete. This presentation will provide a historical overview of capture techniques, the associated risks to both the animal and operator, and the limitations of bat detectors and why their use is not the silver bullet they are perceived to be.

## **Introduction**

The use of bat detectors is now a mandatory requirement when undertaking bat surveys for threatened species and surveys at wind farm sites (Lumsden 2007). They are also regularly utilised for general fauna surveys. There is a perception from those outside the bat research community that bat detectors now make capture techniques obsolete. It is my experience that animal ethics committees often see bat detectors as the silver bullet for applying the 3R's of animal welfare when undertaking field based bat surveys. The use of a bat detector can certainly overcome some of the animal and human welfare issues associated with capture techniques, but they are not without their limitations. They should be seen as one of many tools that should be applied in gaining a greater understanding of bat ecology and their management.

## **Discussion**

### ***Evolutionary Origin of Bats***

There is much speculation of the origin and evolution of bats due to a lack of fossil evidence. The first fossil evidence of bats dates back 60 million years (Altringham 1996; Hill and Smith 1984; Long et al 2002; Nowak 1994). These fossils were of Microchiroptera (microbats), Megachiroptera (Megabats) and records at the present time date back to 35 million years ago. The earliest complete fossil records were found in Germany (45 million years) and North America (50 million years) and these specimens resemble present day species. The greatest problem with most records is that they consist of small fragments of jaws and teeth (Altringham 1996; Hill and Smith 1984). Australia's bat fossil evidence dates back to 26 -23.5 millions year ago in

the Late Oligocene period to a member of the Hipposideridae family, the Leaf-nosed Bats (Long et al 2002).

The fossils tell little about the origins of bats and there is great debate as to whether the microbats and megabats share a common ancestor. The current belief is that they do not, it has been suggested the microbats evolved from insectivorous mammals and the megabats from the primates. The evidence used to support this theory has been published previously (Altringham 1996; Hill & Smith 1984).

Echolocation is relatively unique to the microbats, there is only one species of megabat known to use a simple form of echolocation. In colder regions the microbats have evolved a short term form of hibernation known as torpor, which allows microbats to restrict their metabolic rate when food resources are limited (Altringham 1996). There are examples of shrews using high frequency sound to communicate. Megabats and primates share common features such as; dentition, eye physiology and the central nervous system.

### ***Echolocation***

The study of bats as to ascertain whether they used sound to navigate had its origins in 1794. Lazzaro Spallanzani's experiments indicated that bats were able to use their ears to hunt insects; he was unable to draw conclusions as to how. In the late 1930's, an undergraduate student by the name of Donald Griffin, and Dutch zoologist, Sven Dijkgraf, independently discovered that bats used high frequency sound to navigate. Griffin used the very first ultrasonic microphone to record bat calls on an oscilloscope, it was Griffin

who termed the phrase "echolocation" (Griffin 1986; Nueweiler 2000).

### ***Survey techniques past and present***

#### ***Shooting***

Reardon & Flavel (1987) acknowledged shooting as a valid technique for collecting voucher specimens. In particular they state how much of the current knowledge for the distribution of South Australian bats is based on specimens that were shot in the early 1900's.

#### ***Roost Surveys***

Census surveys of cave / mine roosting species may take 2-forms, external exit surveys and internal roost surveys. Exit surveys minimise disturbance (Barlow 1999), where as internal surveys have the potential to cause disturbance (Thomas & La Val 1988).

#### ***Visual Emergence Counts***

The most basic of emergence methods is where observers position themselves at the entrance to the cave /mine in such a way that they can count the silhouette of exiting bats against the night sky, any bats looping around and returning into the entrance are noted and then deducted from the tally (Sutherland 1996). This method can provide a high degree of accuracy when using multiple observers (Thomas & La Val 1988). Allison first used this method in the United States in 1937 (McCracken).

Photography both still and motion have been used to record large numbers of bats (>1million) exiting Carlsbad Cavern in the United States. The still camera method involved shots being taken at 30 second intervals for the first 45 minutes and at 60

second intervals until the last bat had left. In conjunction with this, motion picture shots were taken to correct errors (McCracken 2003; Thomas & La Val 1988). Grant's (2004 pers comm., 23<sup>rd</sup> April) method for conducting exit surveys for Large Bent-wing Bats involves recording infra-red video footage and enumerating numbers in the laboratory. Infrared footage has the advantage that mammals do not see in the infrared spectrum (Marks et al 2003) and as a consequence it does not affect their behaviour. Infrared thermal imaging is a relative new method based on computerised processing of the visual field (Kunz 2003).

Trip-beams and motion detectors have inherent problems in that they do not provide accurate data on numbers, as they cannot interpret numbers re-entering the roost. They can however provide levels of activity (Thomas & La Val 1988). Validation of this method is required through the use of night vision methods (Kunz et al 1996b), Wilson (2001) suggests that trip-beams have a "negative bias" and under estimate numbers.

#### *Internal Hibernation Counts*

Hibernating bats may roost alone, or in groups that are made up of a few individuals or large dense clusters of a single species. It is possible to enumerate numbers through direct counts or surface area methods (Thomas & la Val 1988; Tuttle 2003). Disturbance of the roosting bats must be minimised to ensure that crucial body fat is not expended and maternity roosts are not abandoned (Hall & Richards 2003; Kunz et al 1996b). It is recommended that a census of hibernating bats and maternity roosts be undertaken no more than once a year (Kunz et al 1996b).

#### *Trip-lines*

Trip lining is the use of 3 kg monofilament line strung across a body of water to capture bats. The line is strung across the water body in a random fashion approximately 6 -10cm above the water. The bats are tripped into the water as they fly in for a drink. Bats are very capable swimmers, as they swim to the bank they are captured (Churchill 1998; Reardon & Flavel 1987). A torch or spotlight can be used to encourage the bat to swim away from the light source to a person waiting on the opposite bank.

#### *Mist-nets*

A mist-net is made of 2-ply nylon or polyester mesh; the denier and mesh size used is dependent upon the size of the fauna targeted. Mist-nets used for bat research are generally 50 -70 denier with a mesh size of between 19mm – 36mm; they come in a variety of lengths; 6, 9, and 12 metres being the standard lengths (Ecotone 2003; Barlow 1999). The advantages of mist-nets are that they are lightweight, portable and low cost, however unlike a harp trap they must be monitored at all times. They are also easily damaged and need high levels of human resources when multiple mist-nets are erected (Barlow 1999; Churchill 1998; Kunz et al 1996c; Mitchell-Jones & McLeish 1999).

#### *Constantine Trap (Harp trap)*

The Constantine, commonly referred to as a harp trap, was designed by Denny Constantine in the late 1950's as a single bank of vertically strung piano wire in a square frame to capture Mexican Free-tail bats as they left their cave roost (Constantine 1958; Kunz & Kurta 1988). There has been a range of improvements to the original design over the years. Tuttle

(1974) introduced a second bank of lines; Tidemann & Woodside (1978) re-designed Tuttle's trap into a more portable unit, Francis (1989) increased capture rates by introducing a third and fourth bank of lines. Gration (2003a; 2002) and Petit et al (1995) have successfully deployed 3-bank traps using various line configurations.

Positioning of the harp trap is important. It should be placed in the flight path bats use for commuting to and from their roosts and foraging sites and this normally takes the form of a track. Advantages of the harp trap versus the use of mist-nets are that they do not need to be constantly monitored and they have been demonstrated to have greater capture rates than mist-nets (Kunz & Anthony 1997; Tidemann & Woodside 1978). Disadvantages are the initial cost and their bulk.

#### *Animal and Human Welfare*

Under severe environmental conditions (prolonged drought), animals may already be under some considerable stress. The frequency of monitoring and clearing of a harp trap / mist net through the night needs to consider a number of variables that may occur at the site, i.e. is it breeding season, are bats lactating, level of bat activity on the night, overnight weather conditions, likelihood of public interference/access to traps. Predation of bats from within the collection bag on the harp traps is a significant risk; there have been several reported incidents of animal predation within traps. Bats have been known to bite each other when caught in high numbers. High capture rates may also extend the time needed to process bats and as consequence may place individuals under lengthy periods of stress. These

issues however can be addressed through the implementation of Standard Operating Procedures (Gration 2007).

There are also inherent risks to humans associated with conducting field surveys; disease, biting animals and injuries due to falls to name but a few. Of particular concern for those that handle bats is the risk of being bitten by a bat carrying Australian Bat Lyssa Virus anti bodies. Australian Bat Lyssa Virus is a close cousin of the rabies group, the almost certain outcome of contracting this disease will be death. Simple preventative measures can overcome the risk of being bitten, the most obvious is don't handle a bat if you don't need to. If handling of bats is necessary, the handler must have undertaken a course of pre-rabies shots, and be experienced with the correct handling techniques. Those that are likely to come into contact on a regular basis with bats during internal surveys of caves and mines present their own specific range of hazards; toxic gases, flooding, collapsing of substrate and vertical shafts (Kunz et al 1996a; Tuttle 2003). Histoplasmosis is of particular concern, fungal spores inhaled into the lungs can cause flu like symptoms (Churchill 1998) and death can result from infection. Armstrong & Higgs (2002) provide excellent guidelines for those intending to work underground.

#### *Bat Detectors*

Acoustic surveys are now undertaken with a purpose built unit known as a bat detector. This piece of electronic equipment processes the ultrasonic calls of the bat and converts the calls to the audible range of humans. When used in conjunction with spectral analysis software they may be used to quantify levels of activity and in some cases, identify to a

genus or species level (Barlow 1999; Churchill 1998; de Oliveira 1998; Fenton et al 1988; Gannon et al 2004; Kunz et al 1996b; Parsons & Obrist 2004). The Anabat system (Titley Electronics; Ballina NSW) is the most widely used system in Australia.

Advantages with the use of bat detectors are: they can operate remotely for a week or more gathering large data sets, can be used where capture techniques are inappropriate or cannot be deployed, utilised as a precursor to targeted surveys and do not require the capture of bats.

#### *Bat Detector Limitations*

The use of bat detectors has their limitations when used to record the presence of bats. These limitations include variation in call intensity (5-metres to 25-metres), weather conditions, placing the detector at a height where activity is likely to occur and the technical competence of the operator. The use of a bat detector can only provide a measure of activity and does not provide evidence of the number of individual bats present. Bats will vary their call depending on their surroundings and the activity mode they are flying e.g. foraging vs commuting. Some species are known to vary the frequency of their call from region to region. As a consequence a large number of voucher / reference calls are required for each species in a given region; this requires the capture of bats to record their calls.

In many instances, the quality and length of the bat call may not provide the diagnostic features required to identify to a species level. A number of species have similar call features that can only be identified to a genus or species complex level if characteristic features are not present e.g. *Nyctophilus sp* (Long eared

Bats); *Miniopterus schreibersii ssp* (Large Bent-wing Bat), *Vespadelus vulturnus* (Little Forest Bat and *Chalinolobus morio* (Chocolate Wattled Bat). Three species of threatened bats have calls that are extremely similar to non-threatened species and cannot be positively identified. Capture techniques in these circumstances are the way of positively confirming their presence.

Government agencies, industry and community expectations are that an EIA will be thorough and testable in a court or court like scenario. Bat detectors have limited scope for providing the sort of information required for Environmental Impact Assessments (EIA) and gaining greater understanding of bat ecology. As a consequence, the survey approach needs to be matched to the surveys objectives /outcomes; in most instances this will require an integrated approach through the use of a range of survey techniques. Bat detectors alone are not the silver they are often perceived to be!

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## ***Re-evaluating the Glucose Tolerance Test in Mice: Effect of Fasting Duration, Route of Administration and Dose.***

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Impaired glucose tolerance, a characteristic of type 2 diabetes, is due to insulin resistance and reduced insulin secretion. Classically, glucose tolerance in patients is assessed using an oral load following an overnight fast. In mice the glucose tolerance test is also performed following an overnight fast with the glucose bolus given intraperitoneally. We know that mice are primarily nocturnal feeders and consume ~75% of their daily caloric intake during the night. An overnight fast represents a proportionately large deprivation of calories and is therefore much longer compared to humans. These differences have prompted us to re-evaluate the glucose tolerance test in mice and to assess the effect of fasting duration, route of administration and dosage of glucose in chow and high fat fed C57BL/6 mice. To determine an appropriate level of fasting, mice were deprived of food from 8am for 0, 3, 6, 18 and 24hrs at which time an Intraperitoneal Glucose Tolerance Test (IPGTT) was performed. For the second study, mice were subjected to either an IPGTT or Oral Glucose Tolerance Test (OGTT) to determine the most suitable route of glucose administration. Dosage of glucose was determined by administering 2g/kg, 1g/kg or 0.5g/kg of glucose. Basal glucose concentrations were increased in high fat fed mice compared to chow fed mice following 6hrs ( $9.1 \pm 0.3$  vs.  $7.9 \pm 0.4$ mmol p=0.01) of fasting. Glucose intolerance was most different and therefore significant (p=0.001) in the high fat fed mice after 6hrs of fasting ( $1973 \pm 96$  vs.  $1248 \pm 83$ ) and 24hrs ( $1633 \pm 92$  vs.  $1299 \pm 70$ mmolx120min).

The difference in glucose tolerance was greater following an OGTT (142%), in contrast to an IPGTT with a 127% difference between the high fat and chow. We also found that administering 2g/kg of glucose resulted in a greater level of significance (p=0.0008) in glucose intolerance in high fat fed compared to chow fed mice.

We conclude that a, 2g/kg of glucose administered orally following 6hrs of fasting will result in maximal detection of glucose intolerance under these experimental conditions.

## *Euthanasia of laboratory rodents, controversy and consensus*

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Every year, very large numbers of laboratory rodents are euthanased either at the end of research protocols, or because they are surplus to the production needs of breeding colonies. The increased use of transgenic animals has exacerbated this problem, as the breeding programs needed to maintain many of these rodents results in the production of some heterozygote and wild-type animals that cannot be used for research. Current methods of euthanasia for relatively small groups of laboratory rodents are physical techniques, or overdose of anaesthetic. However euthanasia of large groups of animals has almost always involved the use of carbon dioxide. For well over a decade controversy has been increasing regarding the use of this agent. In addition, use of physical methods, and euthanasia using an overdose of pentobarbitone have also raised concerns and require re-evaluation.

The issues surrounding the use of carbon dioxide are complex. They have been extensively reviewed and a summary of recent reviews and source literature can be downloaded from [www.nc3rs.org](http://www.nc3rs.org) and [www.lal.org](http://www.lal.org). Initially, the major concern associated with the use of carbon dioxide was that it could cause pain, by the production of carbonic acid on the animals' mucus membranes. If this occurred before loss of consciousness, then the animals' welfare would be seriously compromised. Recent work suggests that carbon dioxide can be delivered in a way that avoids this problem; however carbon dioxide has been shown to be aversive to rodents at concentrations below those likely to cause pain. In addition to any inherent aversion to the gas, carbon dioxide may also cause dyspnoea ("air hunger") and this may cause significant distress.

It has been suggested that argon could represent a more humane alternative, based on data in farm animals, however initial work in rats has shown that argon is aversive. This seminar will attempt to summarise our current state of knowledge, indicate the decisions that need to be taken in individual institutions regarding current euthanasia techniques, and discuss the scientific information that is needed in order to advance the debate. Decisions regarding euthanasia require difficult judgements involving animal welfare, research, economic and practical considerations. It is perhaps therefore not surprising that at present there remains considerable controversy. Nevertheless, there is still the opportunity for reaching some consensus!

**A video of this presentation, including copies of all the slides was made available free of charge to all registered delegates at the conference.**

## ***Progress with External Review of Institutions and Animal Ethics Committees in Victoria***

Peter J. Penson

The process used to review welfare standards of animals in licenced institutions and the activity of affiliated Animal Ethics Committees in Victoria involves four principal steps:

- Review of AEC records and procedures.
- Inspection of premises.
- Review of content and clarity of projects.
- Audit of animal use.

Selected animal use projects are used to track progress through the Committee to the use of animals in a project.

The External Review Process has amongst its objectives:-

- ❖ Provision of an external view to build on self-regulation by the AEC.
- ❖ Provision of feed-back to the regulating body, the Bureau of Animal Welfare.
- ❖ To ascertain broad trends for improvement across the State.
- ❖ To build on communication in institutes.

The most recently completed “triennium” of external reviews conducted in Victoria has demonstrated improvement in several significant areas:

- ✓ Improved analysis and management of the welfare impacts on animals.
- ✓ Improved adoption of refinement issues.
- ✓ Improved record keeping of animal welfare measurements.
- ✓ Deeper empathy shown by researchers.

## ***Reviewing Animal Ethics Committees – the New Zealand experience***

Nita (AB) Harding BVSc, MRCVS, MACVSc

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### **Background**

Animal welfare legislation in NZ took a big step forward at the end of 1999, when the Animal Welfare Act 1999 was passed. Up until 1999 the requirements for AECs were covered by regulations. The new Act contains a whole section to replace these regulations. The new Animal Welfare Act also introduced the requirement for independent reviews of code holders and AECs. These reviews must occur within 2 years of the approval of a new Code of Ethical Conduct, and prior to renewal for a code holder who has an existing code – in essence every 5 years. The Director-General of MAF must specifically appoint the people who carry out the reviews as accredited reviewers.

### **Conduct of Reviews**

It is up to the code holder to arrange for the review of the CEC and AEC. The timing of the review is by agreement between the code holder and the reviewer, provided the reports will be available in time for NAEAC to assess these before expiry of the code holder's CEC. The first reviews under this system occurred in 2002.

### **Review Outcomes**

The reviewers are required to detail their findings in the reports, and include any recommendations, and list minor and major non-compliances. At the end of each year MAF summarise the issues that appeared most commonly in the review reports and notify code holders. MAF has held several telephone conference calls with the reviewers and members of NAEAC, and a number of workshops for AEC members. These workshops have given the opportunity for AEC members to hear presentations on specific topics, discuss issues from reviews, and to share ideas for best practice.

### **A Reviewer's Perspective**

I became involved in reviewing AECs because I have a background in auditing, experience as an external member of an AEC, and an interest in animal welfare. My overwhelming impression from the reviews I have conducted is the commitment that everyone involved in the use and care of the animals has to the well being of these animals. The code holders, AEC members and animal care staff have always been very ready to discuss any issues with me. I see the review process not just as a means of checking on compliance, but also as an opportunity to share ideas or promote discussion about how things may be done in a better way. The Animal Welfare Group in MAF has provided a great deal of support to both the institutions and the reviewers during the implementation of the AEC review system in NZ. It is a credit to those involved that this significant change to the operation of AECs in NZ has gone very smoothly, and is seen as a positive move by most people using animals for research, testing or teaching in NZ.

## Background

Animal welfare legislation in NZ took a big step forward at the end of 1999, when the Animal Welfare Act 1999 was passed. This act replaced the previous Animals Protection Act of 1960. The new act has a focus on the duty of care for animals, and incorporates the 5 freedoms as a core component, as well a requirement for treatment or euthanasia of animals that are ill or injured.

Up until 1999 the requirements for Animal Ethics Committees were covered by regulations. The new Act contains a whole section to replace these regulations, with the purpose of ensuring that the use of animals in research, testing and teaching is confined to cases where there is good reason to believe:

- The findings or results will enhance the understanding, maintenance of, or management of humans, animals and/or ecosystems, the production and productivity of animals, or the achievement of educational objectives.
- The benefits derived are not outweighed by the likely harm to the animals.
- If the work involves the use of non-human hominids this work is in the best interests of the species.

Other purposes are to ensure that the animals are properly cared for, any pain or distress is relieved where practicable, or reduced to the minimum possible. This section also promotes the concept of the 3Rs.

Any organisation wishing to use animals for research, teaching or testing must first draw up a Code of Ethical Conduct (CEC), and have this approved by MAF. The CEC covers the establishment and operation of the Animal Ethics Committee

(AEC), the monitoring of compliance with conditions of project approvals, the collection of statistical information, specifications for animal management practices and facilities, and the method of dealing with complaints. Approvals remain current for a maximum of 5 years, and are personal to the code holder.

The Animal Welfare Act also introduced the requirement for independent reviews of code holders and AECs. These reviews must occur within 2 years of the approval of a new CEC, and prior to renewal for a code holder who has an existing code – in essence every 5 years. A satisfactory review report from an accredited reviewer is required as part of the application for renewal of a CEC.

The Director-General of MAF must specifically appoint the people who carry out the reviews as accredited reviewers. To gain accreditation the DG must be satisfied that the person has:

- The relevant competencies.
- They are of good character and reputation – a police check is done.
- They are able to maintain an appropriate degree of impartiality and independence when conducting reviews.

Applications must be made in writing. Accreditation is for 5 years, at which time the reviewers must apply for reaccreditation if they wish to continue to carry out reviews. At three yearly intervals the accredited reviewers have their performance audited by the MAF compliance group.

## Conduct of Reviews

It is up to the code holder to arrange for the review of the CEC and AEC. MAF do send out reminders, but do not make the

arrangements for the review to occur. Currently there are 6 accredited reviewers whose names and contact details are available on the MAF website and in various publications.

The code holder contacts a reviewer of their choice and if the reviewer agrees to carry out the review, this is confirmed in writing and MAF notified. The reviewer is required to carry out the review in accordance with standard auditing practices, and charges the code holder for the review. The timing of the review is by agreement between the code holder and the reviewer, provided the reports will be available in time for the National Animal Ethics Advisory Committee (NAEAC) to assess these before expiry of the code holder's CEC.

The reviewer must provide the code holder with the Terms of Reference, the review checklist, and also get agreement for the code holder to provide ready access to all records and staff required for the conduct of the review.

The reviewer will usually start the review by requesting copies of documentation to be supplied so that these can be studied prior to the on-site review. Typically minutes of AEC meetings, policies and procedures for the AEC, lists of approved projects, and details of animal facilities are requested. The reviewers are expected to select a number of projects off the list of approved projects to review in detail. A 10% sample size is suggested with at least 5 projects, and a maximum of 15 projects selected. These projects should cover all grades of severity. In NZ the grading is O, A, B, C and X. Where O is no affect on the animals, and X is extreme severity of manipulation. Not all AECs have projects covering the entire range.

Reviewing this documentation prior to the on-site review gives the reviewer a feel for how the organisation goes about meeting the requirements of the Animal Welfare Act, its CEC, and the operation of the AEC. This also gives useful guidance for areas to concentrate on during the review process itself.

The review is carried out on-site. In some cases this will involve a visit to more than one location if the organisation has more than one AEC. The review involves:

- Discussion of points arising from the review of documentation prior to the on-site review.
- Discussion of remaining points on the review check sheet with appropriate personnel, usually the chair of the AEC or the Animal Welfare Officer.
- Review of records relating to the operation of the AEC and animal facilities.
- Attendance at a meeting of the AEC if at all possible.
- Interview of AEC members, particularly the external members.
- Visits to selected animal facilities, and interviews of the managers and staff in the animal facilities.
- Review of the activities of parented organisations.

Once the review is complete a draft report is prepared and sent to the code holder for review. The code holder has 15 working days to review the report and provide comments to the reviewer. The final report is then prepared and this is sent to the code holder, the Director-General of MAF, and to NAEAC.

The final report is reviewed by NAEAC, and the DG then notifies the code holder if a satisfactory level of compliance has been achieved. If so the code holder will be able to use the report to support an

application for renewal of their CEC. If a satisfactory level of compliance has not been attained, the DG will inform the code holder of the actions that must be taken to achieve a satisfactory level of compliance. This will usually require another review after a period of time to rectify any non-compliance. Failure to achieve a satisfactory level of compliance will mean

that the application to renew the CEC will be declined, or an existing CEC may be revoked.

The first reviews under this system occurred in 2002. The following table gives a summary of the reviews since 2002:

Review Type	2002	2003	2004	2005	2006	2007 (due)
Renewals	8	11	9	0	0	8
2 year reviews	0	2	1	0	1	1
Non compliance	Nil	1	3	2	2	0

Information sourced from NAEAC Annual Reports

In 2002 all CECs that were originally approved on or before 31 December 1990 had to be renewed before the end of the year. In 2003 all CECs initially approved between 1 January 1991 and 31 December 1994 had to be renewed before the end of the year. All remaining CECs expired on 31 December 2004, and therefore needed reviewing in 2004. The reviews due in 2007 are for those organisations who were originally reviewed in 2002, and whose codes expire on 31 December 2007, plus one new organisation whose code expired for the first time.

As at the 31 December 2006 there were 30 organisations with an approved CEC. Between them these organisations had 34 AECs, there are 2 organisations that have more than one AEC operating under their CEC. There are another 81 organisations with approval to use another

organisation's AEC. These arrangements are known as parenting arrangements.

### Review Outcomes

The reviewers are required to detail their findings in the reports and include any recommendations. They must also list minor and major non-compliances. Recommendations are generally observations that may improve the efficiency or effectiveness of the operation of the AEC. If during a review, a critical situation is identified, this must be notified to the DG immediately and may mean immediate suspension of project work or revocation of the CEC.

At the end of each year MAF summarises the issues that appeared most commonly in the review reports. The issues that have arisen are:

- A need for an animal welfare officer on either a full or part-time basis especially in

the bigger institutions. This is to provide professional support to the institution for animal care and management.

- An increasing trend for institutions to reimburse the external members for their time and expenses in preparing for and attending AEC meetings.
- An increasing trend for AECs to request a report from researchers at the completion of project work. This is seen as part of the monitoring process.
- The need for AECs to be adequately resourced for their routine operational activity. This is so the AEC can carry out its role properly.
- The need for the institution to give appropriate priority to requests for repair and maintenance, and capital expenditure recommended by the AEC.
- The requirement for a well written lay summary in project applications. This is necessary so that external members can adequately assess the impacts of the proposed work.
- The need for AEC approval for manipulations involving animals carried out in schools, particularly senior secondary school and for science fairs. The Ministry of Education has a process to ensure schools can comply, but in some cases schools are parented by another local institution such as a university.
- The overwhelmingly positive comment was the commitment shown by animal care, veterinary and scientific staff to the welfare of the animals used in the institutions. Where there had been problems these were usually to do with processes and procedures and had not generally impacted on the welfare of the animals.

MAF has written to all code holders advising them of the common issues arising from the reviews carried out. This feedback was intended to give all AECs an

indication of issues to consider for their own operations.

MAF has also held several telephone conference calls with the reviewers and members of NAEAC. This has been a very useful way of sharing information and discussing ideas without the expense of travelling to one central location.

NAEAC has also held a number of workshops for AEC members over the past 5 years. Some of these have been evening workshops in regional centres to which local AEC members have been invited. There have also been two full day workshops to which all AEC members have been invited. These workshops have given the opportunity for AEC members to hear presentations on specific topics, discuss issues arising from reviews, and to share ideas for best practice.

### **A Reviewer's Perspective**

I became involved in reviewing AECs because I have a background in auditing, experience as an external member of an AEC, and an interest in animal welfare. I have found this work very rewarding. It has also given me the opportunity to visit a number of institutions, both large and small, and to see a wide range of animal based work.

I have been questioned about how I manage when the institution uses animals that I may not have much experience with, or carries out a range of work with which I am not very familiar. My role is to review the code holder and the AEC against the standards set by MAF that are based on the Animal Welfare Act. I am primarily reviewing the AEC's decision-making process and compliance with the Animal



Welfare Act. Using first principles of animal welfare allows me to judge the acceptability of the care for almost all species of animal, and I have consulted colleagues at times for advice in this area. Being methodical is the best way of reviewing processes and procedures, and this applies regardless of the subject of the review.

My overwhelming impression from the reviews I have conducted is the commitment that everyone involved in the use and care of the animals has to the well being of these animals. I have seen some very innovative ideas for housing, provision of enrichment and care during surgery and other manipulations, and attention to the 3Rs.

The code holders, AEC members and animal care staff have always been very ready to discuss any issues with me. I have seen the review process not just as a means of checking on compliance, but also as an opportunity to share ideas or promote discussion about how things may be done in a better way. At times I have asked permission from one code holder to share an idea for a process with another code holder eg. formats for reporting at the end of project work.

I have not come across any situation where I felt that the welfare of the animals was compromised, but I have struck situations where the processes and procedures needed to be tidied up so that there was clear evidence of compliance. This is particularly so in relation to monitoring of both project work, and animal facilities. There is a clear requirement for this in the Animal Welfare Act. The bottom line is that the AEC must be confident that it knows what is going in all animal facilities

under its jurisdiction, including those of parented organisations.

The Animal Welfare Group in MAF has provided a great deal of support to both the institutions and the reviewers during the implementation of the AEC review system in NZ. A number of guides have been published, conference calls have been held for reviewers, institutions have been advised of issues arising from reviews, workshops for AEC members and other interested people have been held, and throughout the whole process members of the MAF staff have been available to provide support and answer questions. It is a credit to those involved that this significant change to the operation of AECs in NZ has gone very smoothly, and is seen as a positive move by most people using animals for research, testing or teaching in NZ.

### **Useful publications**

MAF (2000, May). The use of animals in research, testing and teaching. Users guide to part 6 of the Animal Welfare Act 1999. MAF Policy Information Paper 33. Wellington, NZ. Ministry of Agriculture and Forestry.

NAEAC (2002, September). Good practice guide for the use of animals in research, testing and teaching. Wellington, NZ. Ministry of Agriculture and Forestry.

NAEAC (2006, September). Guide to the preparation of codes of ethical conduct. Wellington, NZ. Ministry of Agriculture and Forestry.

NAEAC Annual Reports, published annually by the Ministry of Agriculture and Forestry.

NAEAC News. Official Newsletter of the National Animal Ethics Advisory Committee, published twice a year.

## *National statistics – What’s the point?*

Dr Janine Barrett

Senior Project Officer Ethics Subprogram – Animal Welfare Unit DPI&F (Queensland)

Investigators, AECs and/or institutions count animals used for scientific purposes for different purposes.

1. Investigators report the numbers of animals used to the AEC to meet the monitoring requirements of the Code and to allow the AEC to monitor that the numbers used are consistent with those approved as necessary and justified.
2. Some institutions report the numbers of animals used to the NHMRC (as per *their* guidelines)
3. AECs/licensee/registrants/institutions report the number of animals used per calendar year to the jurisdictional regulator, *usually* as required by legislation. The statistics from each jurisdiction are collated to form the national statistics.

What to count, how to count, and/or how to report *differs* for each purpose. This presentation will focus on the requirements for national statistics.

National statistics are collected to demonstrate that animal use is monitored in a way that reflects community expectations. Accurate national data allows for monitoring of the number, type, purpose and extent (impact) of animal use, and can be analysed to monitor trends, risks and issues arising from scientific use. Data is collected in accordance with legislation, guidelines, procedures, and reporting formats that have been developed more or less independently by each jurisdiction. There is a national agreement via the Animal Welfare Working Group (AWWG) that jurisdictions collect and submit jurisdictional data, however, inconsistencies between jurisdictions in what data is collected is limiting its value once collated nationally.

The ‘regulators’ are looking at how these discrepancies can be addressed. The objective is to develop nationally consistent legislation, guidelines and formats that will lead to the publication of meaningful national statistics. Recent developments (including a nationally approved revision of the “Type of Animal” categories), common reporting errors (such as over-reporting of “death as an endpoint”) and other current issues will be discussed.

## ***The AEC system in Australia. Does one size fit all?***

Ann Higgins and Leigh Ward

University of Queensland, Brisbane, Australia

The use of animals in research and teaching is governed in Australia by legislation. Enactment of the necessary legislation is a responsibility of the individual states but is broadly similar and all give legal force to the Australian Code of Practice for the care and use of animals for scientific purposes (7th Edition, NHMRC, 2004). The Code requires the establishment of Animal Ethics Committees to verify that use of animals is justified, to ensure adherence with the principles of replacement, reduction and refinement, to promote best practice in animal welfare, and to ensure compliance of by those who use animals with the Code and all appropriate legislation. In turn, state legislatures provide a further level of compliance assurance through regular audits of the AECs within their purview.

The legislative and regulatory framework and Code take no account of local conditions pertaining to the operation of any given AEC. To take an example. One of the strengths of the AEC system is the diversity of expertise, opinions and ethical viewpoints of its members, enshrined in the quorum category system. Yet this may be an Achilles heel for a large and diverse institution such as a research-intensive university compared to a largely single focus wildlife park. While the underlying principles of welfare are the same for both is it reasonable to expect the same system of operation? Could a single AEC suffice for each?

This paper addresses how one particular large and diverse institution, the University of Queensland, meets the challenge of ensuring the highest standards in the humane use of animals for scientific purposes and fulfilling the responsibilities of AECs under the Code while ensuring compliance with applicable legislation yet not providing undue impediment to scientific research. It further raises the question: "Has the AEC system become more about process and paperwork and less about promoting ethical standards in the use of animals?"

***Counting animals one by one:  
What do we mean when we report on the animals used in science?***

Dr Erich von Dietze  
Manager, Research Ethics, Murdoch University  
Adjunct Assoc Prof, Centre for Applied Ethics & Philosophy, Curtin University

Each year we report on the number of animals used in teaching and research at our institutions. The intention is to ensure that we are meeting the 3R standards (Replace, Reduce, Refine). I assume that 'Reduce' implies that we should at least aspire to work continuously to reduce the overall numbers of animals used in scientific endeavours, as well as reducing the impact of research or teaching activities on individual animals. Refinements and replacements are implemented, in part, to assist this goal. To give an effective measure of achievement in this area, each institution reports annually the numbers of animals used.

This practice of counting, however, hides numerous difficulties. It is only when you have to count the animals yourself that you begin to become aware of some of the issues. I begin by exploring some of the issues more generally; it is often easier to discuss the concepts related to people first. Then I will relate them to the specific issue of counting animals used for scientific purposes and the reporting of these numbers as required by the Code and legislation.

## ***Reporting Adverse Incidents***

Robyn Sullivan and Peter Maley

When using animals in scientific and medical research it is not uncommon for things to turn belly up and it is then the scientist is called to account; the Adverse Incident is on the agenda “big time”. What is an Adverse Incident; is it more than equipment breakdown, over-grown teeth or human negligence?

Adverse Incidents go beyond raising the blood pressure of researchers and AEC members; they initiate an enquiry process with enormous consequences for everyone involved. Adverse incidents can result in significant financial penalties, even loss of Licence for the institute or governing body - catastrophic is a good way in which to describe a potential outcome.

When things go belly up who has ultimate legal responsibility, is there also a moral responsibility?

When “it” hits the fan what does an AEC need to do as it addresses the issues of animal welfare/husbandry, negligence/inadequate training/human frailty? What detail does the researcher need to provide in written report to the AEC and, subsequently, to various state government bureau’s of animal welfare?

While all of the above is fine in terms of legal responsibility, does the AEC have any duty of care for the people involved in an “Incident” - is there an appropriate system of arbitration upon grievance?

Has the time come for the development of a national Adverse Incident Reporting Code to be adopted at a Commonwealth level to override what, at best, can only be currently described as little more than “ad hoc”?

In this session, not only will you will be given the prompts, both scientist and lay person will have an opportunity to determine (in session) a Statement on definitive animal welfare when things really do go belly up.

### **It is possible to do better ....**

I would like to thank my co-author Peter Maley for his contribution to this presentation and his enthusiasm for this important issue which receives too little attention. Our collaboration has been largely at long distance and as the last

person to edit our working document I take full responsibility for the final view expressed in this paper. Peter will clarify any matters and make further comments in the next segment of this afternoon’s program.

To begin, a quotation: *“For most of us, making a mistake at work might result in a bit of heartache, some inconvenience, embarrassment, perhaps even anger. Our mistakes rarely result in serious physical injury. But for some professionals ... a simple mistake can have drastic repercussions. Which is why the struggle for those professionals to perform, to do better, is so important. ... It is possible to do better. And it doesn’t take genius – it takes diligence, moral clarity, ingenuity and, above all, a willingness to try.”*

These are the words of leading US surgeon, Dr Atul Gawande, who has researched and written extensively on process improvement in the delivery of health services and particularly on the identification, analysis and management of adverse incidents in hospitals.

While Gawande’s quotation referred to medical professionals, his message is applicable to the research environment – there is enormous potential for the infliction of unnecessary pain and suffering on animals from errors and mistakes during the research process.

To reiterate, a simple mistake can have drastic repercussions – for the animals you are legally and ethically responsible for, for the personnel involved and for the research institution. While humans are fallible beings, our diligence, moral clarity, ingenuity and willingness to continually do better is the standard against which our performance should be assessed.

When considering adverse incident management we should keep at the forefront of our minds Gawande’s objective - always *“to do better”* and his

finding - that it is possible if, above all, there is a *“a willingness to try”*.

## **A brief case study**

It may be helpful to stimulate discussion later in the session to take a short walk through an actual adverse incident – names and locations omitted.

The experiment involved serial anaesthetic events to permit time course monitoring using a non-invasive technique while under anaesthesia. The aim of the experiment was to clinically assess mice of different genetic lines at three different time points.

At the outset the Principal Investigator advised that each session of non-invasive monitoring required an anaesthetic duration of 30-40 minutes. The choice of anaesthetic was restricted to agents which would minimise interference with test results. Animal Ethics Committee (AEC) approval was sought and granted for an anaesthetic to be administered i.p. three times over a three month period.

Due to the duration of anaesthesia required, a reasonably high dose of anaesthetic was administered on each occasion and the research team experienced the loss of some animals under anaesthesia. The loss rate was running around 4%. However, in September 2005, the Principal Investigator became aware that several mice had been found dead and other mice were culled as they looked unwell. The loss rate had escalated to 12%.

Although the pattern of losses had escalated and those losses were animals which had recovered from one or more anaesthetic events, the Principal Investigator did not file an Adverse Incident Report. From later communication, the Principal Investigator was waiting to be directed by Animal Care staff if an Adverse Incident Report should be made. The refrain “no-one told me to .....” is not uncommon in the context of adverse incidents.

The deaths continued to escalate and the Principal Investigator was prompted to provide an Adverse Incident Report. That report disclosed that of 37 animals (19 found dead and 18 culled) only 1 or 2 were autopsied. From subsequent communication it transpired the Principal Investigator did not perform or arrange for autopsies but assumed all animals were autopsied by someone in the Animal House. “I just assumed ....” is another frequent refrain associated with adverse incidents.

Outcomes of the review by the AEC of the Adverse Incident Report included retraining of staff in i.p. injection technique; a standardised system of injection site rotation to be used by all members of the team; a requirement that all mice found dead or culled due to ill health be autopsied in future; and an undertaking from the Principal Investigator to accept full responsibility for adverse incident reporting and to maintain records to promptly detect future changes in the rate of morbidity and mortality to expedite response times in the event of further problems.

Now we can fast forward to December 2006, to the same project, when an increase in the number of mouse deaths

was mentioned in the report from the Animal Care Manager to the AEC. In compliance with the earlier AEC review and with Section 3.3.24 of the “Australian code of practice for the care and use of animals for scientific purposes” (the Code of Practice), autopsies were conducted but the results were inconclusive.

At an AEC meeting in late February 2007, the AEC became aware from the Animal Care Manager’s report of further deaths for the month of January representing a loss rate of around 12%. Autopsy results showed all mice with inflamed intestines or intestines full of air. There was no Adverse Incident Report from the Principal Investigator but an e-mail to the Chair of the AEC on the morning of the meeting advising a report would be forthcoming and that i.p. anaesthesia had been suspended several days earlier.

In early March 2007, the AEC Executive received out of session a request to modify another arm of the experiment. The AEC Executive declined to consider any further matters concerning this project until an Adverse Incident Report was lodged.

The latest Adverse Incident Report circulated out of session was scant on detail and analysis. It was not possible to discern the number of animals for which failure of injection technique may have been a plausible contributing cause of death, nor whether there was a correlation between this postulated cause and the individual i.p. operators involved with the project. The AEC requested a meeting between the AEC Executive and the Principal Investigator.

During that meeting, the Principal Investigator was unclear what constituted an adverse incident. An acceptance that



some animals would be lost under anaesthesia and possibly from peritoneal adhesions from successive i.p. anaesthesia led to an unquestioning attitude as the loss rate escalated and a failure to recognise an adverse incident. There was an avoidance of the responsibility to analyse and control contributing factors and manage morbidity or mortality to the lowest possible level.

The role of the Principal Investigator as a risk manager was discussed during the meeting with the AEC Executive. It was agreed the Principal Investigator would make a close examination of all their methods and materials. The Principal Investigator would review the autopsy results and analyse the animal losses by i.p. operator. The research team would for the first time introduce quality controls for the injectible anaesthetic, taking responsibility to prepare fresh stock of the anaesthetic on a weekly basis. Prior to each use, the anaesthetic solution would be checked for any signs of degradation and appropriate records maintained. A revised Adverse Incident Report would be submitted to the AEC.

In the revised version of the latest Adverse Incident Report, the AEC was informed that during the four months from late 2006 to early 2007, 12% of mice were either found dead or euthanased on welfare grounds. Of the four operators administering i.p. injections, loss rates of 23% and 17% were associated with two of the i.p. operators. This compared with a loss rate of 2% and 5% for the other operators. It was subsequently agreed that only the two most competent operators would perform i.p. injections in future. The Principal Investigator is also proposing a reduction in the duration of the anaesthesia to enable a lower dose of anaesthetic to be administered.

From an AEC perspective, this was a very frustrating process and represented project management failures on a number of levels. There was a failure to understand and accept the full responsibilities required of a Principal Investigator – in fact, an avoidance of management responsibility. Untested and unverified assumptions were made about the responsibilities and actions of others. As a result, critical information was lost due to the failure to autopsy nearly 37 animals in the first incident. There was a failure to detect and promptly manage the escalation in animal morbidity in both incidents. While the Principal Investigator was supportive that any unwell animals should be euthanased, managing the risks with a view to prevention was not initially perceived as the Principal Investigator's highest priority.

## **Additional Observations**

The following comments are general observations from our experiences with a range of adverse incidents.

As external members of an AEC we have often been disappointed by the generally poor calibre of adverse incident reports submitted to AECs. It is difficult, as an external member, to determine whether the paucity of “diligence” and “ingenuity” reflected in adverse incident analysis reflects a lack of willingness or a lack of analytical capability.

The incident described in some detail earlier involved a Principal Investigator who was relatively closely involved with the project. We have, however, been exposed to a number of adverse incidents in which the Principal Investigator was

many steps removed from the laboratory. Original hand-overs and instructions from the Principal Investigator to members of the research team become diluted over time with growth in the size of the team and the departure and recruitment of staff. In our experience, the phenomenon of the ‘absentee’ Principal Investigator relying on non-existent or weak management operating systems yet responsible for monitoring a large research team greatly increases the risk of adverse incidents.

So, we are starting from a low base, some researchers are unclear what constitutes an adverse incident. Many researchers are unprepared when things go wrong or are unaware of their management responsibilities. We hope this presentation starts a dialogue on what is needed to lift our game in the management and reporting of adverse incidents.

### **What is an Adverse Incident?**

The definition of an adverse incident actually has its genesis in the planning and design stages of an experiment. Thoughtful and informed reflection of the animal welfare impact of each individual procedure and the cumulative welfare impact of any series of procedures is fundamental to experimental design. Fundamental, not only in the iterative process of experimental refinement, but also in the formulation of the risk management and monitoring plans which should form an integral part of any application submitted to an AEC.

Details of the animal welfare impact on each cohort of animals at each stage of an experiment are required by Sections 2.2.16 (ix) and (x) of the Code of Practice.

Researchers are required to consider, document and manage the known or reasonably expected impact of a procedure on animals. For example, i.p. injection of a chemical should involve an understanding of the likely effect on the animal of that agent by that route. For example, what is the pH of the substance? What is the potential for an inflammatory response? What are the risks of an acute or chronic reaction or an individual adverse reaction to the chemical agent? How will animals be monitored to minimise the risk of an acute or chronic reaction?

Researchers also need to consider animal welfare impacts which could occur if a specific aspect of the experimental activity does not go according to plan such as a failure of execution. What are the risks from an error in dosage or a failure of injection technique? How will these factors be controlled and/or managed? Will injection concentrations and volumes be independently checked? Will monitoring for general signs of welfare be supplemented by tailored monitoring specific to the procedure and experiment in question?

Applications to AECs should identify specific risks and how these are to be minimised and managed. Of course, the project will remain subject to unforeseen risks which cannot be envisaged or anticipated at the time of experimental design.

When detection and management of risks are incorporated into the original application to the AEC, the application has an in-built “early warning system” which ensures consideration of an AEC application is conducted in the light of full information. It also provides a framework

for managing an incident from an identified risk in the event things go wrong. For example, a researcher may disclose in an AEC application that a surgical procedure is novel or extremely complex and that deaths under anaesthesia or morbidity in the post-surgical recovery period are expected. If such disclosure is made in the original application, then all parties to the review process can consider whether a pilot would be more appropriate to stabilise the model. They can also consider the adequacy of post-procedure monitoring, pain and welfare management in the context of the researcher disclosure and assessment of the risks. The objective of a detailed experimental design which identifies and addresses risks is to prevent, to the best of our ability, unexpected clinical outcomes which fall outside the approved boundaries.

We propose, as a working definition of an Adverse Incident, the occurrence of animal morbidity or mortality which exceeds the expected and approved parameters of the protocol.

If a researcher is unsure about what constitutes an Adverse Incident, the investigator should consider whether the clinical condition of animals is consistent with the expectations established during the approval process. If the welfare of animals is not in accord with the AEC approval, then management of the situation as an Adverse Incident is recommended.

### **Proper use of Management Reporting by Investigators**

Investigator reporting to their AEC involves a simple hierarchy of management reports. Investigators are

required to report within 3 time frames according to the nature of the information being reported. The Adverse Incident Report is for the *ad hoc* reporting of issues with immediate animal welfare implications. The Annual Report provides for periodic progress review and reporting of issues which are not time sensitive. The Final Report details the scientific and animal welfare outcomes of the project on completion and provides a consolidated summary of reportable issues during the life of the project.

To remove any “grey areas” concerning reporting, guidelines for reporting specific risk scenarios can be agreed at the outset. For example, if deaths under anaesthesia are within the expectation of the original protocol, have involved no pain or distress to animals and in the view of the investigator do not involve any systematic failure, reporting through the Annual Reporting process or other pre-arranged periodic report could be agreed as standard procedure with the AEC. Whereas, failures that involve some unexpected animal welfare impact or stem from a systematic cause should be brought to the attention of the AEC promptly.

### **Why report adverse incidents?**

Adverse incidents are reported because the standard of animal welfare has departed from that approved by the AEC. The AEC is legally required to critically investigate such incidents as a basis for future prevention strategies and to determine no breach of approvals has occurred.

### **Who is responsible for reporting adverse incidents?**

Let there be no doubt, the primary responsibility for reporting an adverse incident is unequivocally that of the Principal Investigator. You need only refer to Section 1.4 of the Code of Practice which sets out responsibilities: *“Investigators and teachers who use animals for scientific purposes have personal responsibility for all matters relating to the welfare of these animals.”* The key words are, “personal responsibility for all matters”. It could not be clearer.

However, as with other systems of self-regulation, there is an inherent conflict of interest involved with self-reporting. Self-regulation relies heavily on the professional and ethical integrity of the participants.

### **Who else has responsibility?**

Of course, all investigators associated with a project are bound by the requirements of the Code of Practice and all institutional staff are bound by a code of ethics to uphold the highest professional standards.

From a practical perspective, adverse incident reporting of morbidity and mortality which stem from non-experimental activities such as colony management or facility-wide equipment failure are more appropriately the reporting responsibility of the Animal Facility Manager.

### **The dynamic of adverse incident reviews**

How are AECs informed of adverse incidents? In a well functioning system, prompt notification from the Principal Investigator followed by a properly researched and prepared Adverse Incident Report is the expectation. It is sad reflection on the workings of our current system that in many instances AECs learn of adverse incidents from sources other than the Principal Investigator. Adverse incidents may come to the attention of an AEC indirectly from inconsistencies in reporting, from applications for amendment, from subsequent applications for approval, from annual reports, from Animal Care Manager reports or from concerned peers.

The manner and the speed with which an AEC learns of an adverse incident will influence the review of that incident. Investigators who delay or withhold information from their AEC commit a breach of professional ethics and a breach of the trust on which the current system of self-regulation relies. When things go wrong, an investigator’s only means of meeting their legal and ethical obligations and maintaining the confidence of their AEC is to demonstrate the highest priority for animal welfare, to fully disclose the nature and scope of the problem to their AEC and to rigorously analyse and address the causes of the problem. More than ever this is the time for “walking the talk” that experimenting on animals is a privilege not a right.

Similarly, disclosure of material information during an adverse incident review that was known to the researcher but not disclosed at the time of the original application for AEC approval will also influence AEC review of an incident.

In circumstances in which the Principal Investigator has been open and honest with the AEC and demonstrated commitment to analysing and resolving the problem, then the Adverse Incident Report and its review can be a significant management tool for continual improvement for institutions and the regulatory agencies. Under these circumstances, it should not be a weapon to be used against a researcher. In fact, apart from the exchange of information which occurs during AEC consideration of an application, there is no more crucial time for researchers and the AEC to work together than when considering an adverse incident. This provides all parties with an opportunity to work through the issue, using their best endeavours to establish causality to ensure there is no “encore” performance. It is a time when our principle purpose must be paramount – that of animal welfare.

However, the spirit of collaborative resolution can be seriously undermined if the AEC forms the view that full disclosure has not occurred or that there is a lack of engagement on the part of the Principal Investigator to accept responsibility, analyse the causes and make meaningful recommendations for future prevention. This is the point at which the dynamic of the AEC investigation will change. By adopting a less than fully engaged role, a researcher forces the AEC to operate in compliance mode.

The key message for researchers is to work with the process not against it. Researchers have the opportunity at the outset to design and plan for contingencies and, throughout the life of the project, the option to report early and often on matters of animal welfare concern. In the final

analysis, the researcher’s response to an adverse incident and the degree of participation and engagement in the process – their degree of willingness - will determine the climate within which an AEC assesses an Adverse Incident. The ball is quite simply in the researcher’s court, the researcher can choose whether or not to be proactive.

### **When an adverse incident occurs, what steps should be taken?**

Certain risks are known and, ideally, have been provided for in the risk management and monitoring plans within the protocol. However, the incidence of these known risks may exceed the levels contemplated by the AEC approval or other risks may eventuate for which prior provision has not been made. It is important for the researcher to be familiar with the conditions and limitations of their AEC approval and not succumb to “boundary creep” over time.

When welfare is no longer consistent with the AEC approval either in scale or nature, there are a series of clear steps expected of the Principal Investigator or their delegate. Each AEC should formalise its own procedures but this generic set of recommended steps will have relevance for most situations:

- Experimentation on affected animals must cease and all issues of animal welfare must be addressed without delay. This may involve euthanasia or treatment of the animals involved. Advice from Animal Care personnel or the institutional veterinarian may be necessary.
- Prompt attention to the alleviation of pain and suffering is the over-riding requirement; the welfare interests of the

individual animal must take precedence over continuation or completion of the research protocol.

- Potential risks to animals in other cohorts or arms of this or related projects must be assessed and a risk management plan instituted.

Once immediate animal welfare issues have been addressed, it is recommended the Principal Investigator should take the following steps:

- Verbally advise the AEC Chair or Secretary of the emergence of an adverse incident. The Animal House Manager should also be advised personally if not already aware of the situation.
- Arrange post mortems to clearly establish the cause of death, preferably with the involvement of the AEC appointed veterinarian or other competent personnel.
- Conduct a review with all personnel involved in the research and animal care team to gather full details of the incident.
- Meet with the AEC Chair and secretary to provide a progress report.
- Conduct a rigorous analysis to identify causes or factors contributing to the adverse incident.
- Prepare and submit an Adverse Incident Report to the AEC including recommendations to prevent recurrence.

If other cohorts or arms of a project are possibly implicated, the Principal Investigator should seek the counsel of the AEC Chair and Executive to clearly delineate which areas of work are suspended or will need to be modified pending finalization and review of the Adverse Incident Report.

### **What is expected in an Adverse Incident Report**

Incident reporting is a well established method of obtaining information about errors to assist in the identification of causal factors. Incident reports and incident interviews are used in many industries including the civil airline, mining, medicine and the military.

A basic Adverse Incident report structure would include:

- a brief lay summary of the experimentation originally approved by the AEC (including overall number of animals approved);
- a concise description of the adverse incident including location, dates and times;
- details of the animal welfare impact *by animal* (morbidity and mortality) and the total number of animals affected by the incident;
- details of personnel present and/or involved;
- identification of potential causal factors including materials, methods and environment;
- analysis and assessment of contributing factors and conclusions;
- details of any previous incidents relating to the application; and
- recommended actions to prevent future incidents.

The AEC, having considered the Report and met with the researchers involved, may determine that all necessary steps have been taken to prevent a recurrence

and allow experimentation to resume. Alternatively, the AEC may determine that experimentation be further suspended to allow training/re-training of personnel, preparation of new Operating Procedures, increased resourcing or implementation of a range of other measures relevant to prevention.

Any attempt to “fudge the figures” or play around with words can result in personnel, no matter their level in the hierarchy, being stood aside. It can also destroy reputations and trust, often irrevocably.

### **A question or two in closing**

In a study conducted by Gawande \* of 126 incidents involving surgical patients in three Massachusetts teaching hospitals, it was found that single incidents often involve multiple factors and the system factors most frequently cited as contributing to error were ‘inexperience / lack of competence’; ‘communication breakdown’; ‘inadequate staffing’ and ‘lack of supervision’. In our experience, there are certain parallels with the causal factors reported in this study and adverse incidents in the research environment.

In closing, we would like to pose a challenge by way of two questions. Is there consistency between AECs in their management of the adverse incident process? The impression is that each AEC is doing its own thing, finding its own way. Surely, a shared learning curve has appeal as a smarter and fairer way to proceed. Is it time for a national approach to establish minimum standards or best practice guidelines for managing the process of adverse incident reporting?

The following comments are those of Peter Maley in closing the joint presentation.

It is of considerable concern to me that there are no overall checks and balances within the current seemingly *ad hoc* Adverse Incident Reporting system that offers genuine and fair concern for the experimental team or individual scientist while remaining focused on animal welfare.

The Code is very specific on this matter: Operating Procedure 2.2.14 states: “Irreconcilable differences between the AEC and an investigator or teacher must be referred to the governing body of the institution for review of the due process. The ultimate decision of an AEC after such a review must not be over-ridden.” By cross-reference one is referred to Section 2.1 Responsibilities of Institutions and in particular sub-section 12 which in referring to an Institutions responsibilities reads, “*establishing, and making known, procedures for the fair resolution of disagreements between AEC members, between the AEC and investigators or teachers, or between the AEC and the institution*”.

The Code does not define “due process”, and it should. That said, my main concern is with the final sentence; “The ultimate decision of an AEC after such a review must not be over-ridden”. Put simply, it means that at the end of the day, the AEC is the jury, judge and executioner. I fail to see any evidence of fair play and natural justice in such a framework, but there can be seen some room for “old score settling”, “teaching the beggars a lesson” or “showing just who is the boss”.

If the Adverse Incident reporting system is to achieve its objectives then it must

provide, in equal measure, protection of animal welfare and protection of the name, reputation and professionalism of an individual researcher and the research team.

In my view, the Code has it wrong. In the interests of natural justice and fairness to all players the Code falls short. There needs to be a totally independent and professional “third umpire” to arbitrate for human justice if, and when, required.

I believe that the best and fairest solution rests with the state governing body, the Bureau of Animal Welfare in Victoria and its equivalents in other states. Such a government authority is totally independent of all key personnel, removed and remote from all parties.

In Victoria, the Bureau has appointed an Animal Ethics Advisory Committee. This

committee could convene an appeal committee from amongst its ranks inclusive of each AEC category; it would be absolutely and totally independent. It seems logical that a Departmental Veterinary Officer also be included on the appeal committee. We need to address this issue and consider a change to the Code.

We want you delegates at this conference to take ownership of our presentation today and work through it with your AEC. We believe it has much to offer.

### **Reference**

Gawande AA, Zinner MJ, Studdert DM, Brennan TA. Analysis of errors reported by surgeons at three teaching hospitals. *Surgery* 2003; 133:614-21.