Report of the Workshop on Euthanasia Guidelines and Practices

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Summary — Determining ethical standards for laboratory animal euthanasia requires an assessment of the relative amounts of pain and distress caused by different methods. Animal behaviour data are an important indicator of pain and distress, but their interpretation can be controversial; moreover, behaviour is more easily assessed with some euthanasia methods than with others. While every euthanasia method requires careful study, CO_2 inhalation has come under close scrutiny both because it is so widely used for rodent euthanasia, and because it has, until recently, long been considered relatively non-aversive.

Key words: *alternatives, argon, carbon dioxide, decapitation, euthanasia, halothane, laboratory animal, rat, refinement, rodent.*

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Vera Baumans opened the workshop by asking what euthanasia methods are best for the animals and best for the experiment. Laboratory animals are euthanised because of age, illness or to minimise suffering, because organs or blood must be collected, or because animals are surplus or the experiment has ended.

Euthanasia methods have different modes of action. Some induce direct or indirect hypoxia in the brain (examples include CO and curariform drugs); some directly depress neurons vital for life function (CO_2, barbiturates); and some cause physical damage or concussion to the brain (decapitation, captive bolt). Many of the same techniques are performed either with or without prior anaesthesia.

Euthanasia methods are evaluated on several criteria (1); the ideal euthanasia method includes the following conditions:

— painless;

— rapid unconsciousness;

— rapid death;

— minimal restraint;

— minimal excitement;

— appropriate for age, species and health;

— minimal fear and psychological distress;

— reliable;

— irreversible;

— simple to administer;

— safe for operator; and

— aesthetically acceptable.

Physical methods of euthanasia include stunning, decapitation, and cervical dislocation. Chemical methods include inhalational agents (such as CO, CO_2, and volatile anaesthetics) and injected agents (such as barbiturates). Chemical methods also include drugs absorbed through skin or gills (such as tricaine methane sulphonate [MS222] and etomidate).

Assessment of the humaneness of euthanasia techniques is complicated. It can include electroencephalography (EEG), measuring either spontaneous or evoked brain potentials, and it can include study of animal behaviour. Behavioural signs of stress must be recognised, including:

— vocalisation;

— urination/defecation;

— sweating;

— struggling;

— defensive or redirected aggression;

— pupillary dilatation;

— tachycardia; and

— salivation.

Finding appropriate euthanasia techniques for neonatal animals and for ectothermic vertebrates are particularly challenging. A workshop participant called attention to recent work showing 30 minutes to loss of consciousness in decapitated eels (2).

Larry Carbone focused on two common techniques for rodent euthanasia — decapitation and CO_2 inhalation — to highlight the challenges of assessing the humaneness of very different techniques. To study the effects of CO_2, investigators have observed animal behaviour, measured corticosterone and other hormones, looked for physical evidence of damage (such as lung haemorrhage), and even asked human volunteers to report their response to single breaths of different CO_2 concentrations. Electroencephalography has not been widely used to assess the humaneness of CO_2.

In contrast, behaviour, tissue damage and reports of human subjects are not useful for assessing the potential pain of decapitation. Corticosterone measures would mostly be useful for assessing the potential distress of being handled for placement in a rodent guillotine. Thus, electroencephalography has actually been the most common tool for assessing the humaneness of decapitation, though interpretation of the observed brain waves has been highly controversial (3).

It is also important, to consider the outliers in assessing the humaneness of a euthanasia method. These may be animals who were euthanised incorrectly, or who responded slowly or aberrantly to the method. In a well-known study of brain waves in decapitated rats, the average period of EEG activation was between 13 and 14 seconds, but activation lasted over 29 seconds in one individual (4). Ethical assessment of euthanasia techniques must not just include the average animal observed under ideal conditions, but also the possibility that some individuals could suffer excessively (5). A relatively foolproof but moderately painful technique could actually be preferable to one in which most animals experience no pain while a few experience severe pain.

David Morton presented results of his colleagues' studies on aversiveness of gaseous euthanasia agents (6, 7). The animals were exposed to different inhalational agents (anaesthetics, CO_2, argon) in test chambers containing air or gas mixtures. The animals were able to enter and leave at will, and the level of aversion was assessed in terms of the initial withdrawal and total dwelling times in the chamber. CO_2 was by far the most aversive gas for both rats and mice, with the least aversive being halothane for rats, and halothane and enflurane for mice. With all the anaesthetics, the level of aversion increased as the concentration increased. The effects seen were irrespective of the strain of rat or mouse studied. They concluded that CO_2 should not be used for either euthanasia or anaesthesia of small rodents, though it may be used for euthanasia after anaesthesia with another agent (such as halothane).

Lung damage was also assessed in response to various gaseous agents. CO (which is aversive at high concentrations, but much less so at low concentrations) and CO_2 both cause lung haemorrhage before unconsciousness. Argon causes unconsciousness before there is observable lung damage.

Workshop participants expressed concern that argon has still not been studied extensively, and so should not be recommended too strongly. They were also concerned that halothane inhalation might render animals unsuitable for donations to zoos as animal food, though following halothane with CO_2 to complete the euthanasia process might also allow some halothane "wash-out".

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