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# Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals

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## University of Newcastle upon Tyne, UK

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### Introduction

The humane killing of laboratory animals is an issue of great importance to the scientific community as a whole. It is widely recognised that animals should be euthanased with the minimum possible discomfort, pain or distress, for welfare, ethical and legal reasons. However, there are uncertainties relating to the humaneness of some techniques, including the use of carbon dioxide[*]. Although many laboratory animals, especially rodents, are killed using $CO_2$ according to a variety of protocols, there is currently no definitive guidance on whether and how $CO_2$ can be administered humanely. There is also uncertainty about the feasibility of using alternative gaseous euthanasia agents, with respect to both animal welfare and human health and safety.

The Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals was thus convened with a number of aims:

- to bring together scientists who have examined $CO_2$ as a killing technique, so as to achieve consensus views that will help to inform best practice in $CO_2$ euthanasia;
- to establish where there are areas of disagreement and identify what research needs to be done to address these;
- to identify what further research needs to be done relating to $CO_2$ euthanasia in general;
- to meet the immediate need for guidance on $CO_2$ euthanasia at a local level, that is, within animal research and testing facilities;
- to consider whether any preferable alternatives are currently available.

The meeting comprised a series of presentations that summarised the progress made to date by key researchers in the field, followed by discussion sessions that addressed how $CO_2$ should best be administered, possible alternative euthanasia agents and future research directions (see Appendix I for lists of speakers and observers). Observers were selected to ensure adequate representation of the views and opinions of those directly involved with euthanasing animals, regulating the use of euthanasia methods and co-ordinating efforts to improve animal wellbeing. Attendees included not only researchers in $CO_2$ euthanasia but also experts from relevant outside fields, representatives of the animal supply industry, animal care staff, research regulators

[*] This is not confined to its use in the laboratory; for example, the UK Farm Animal Welfare Council (FAWC) has concluded that the use of high concentrations of $CO_2$ to stun and kill pigs is not acceptable and wishes to see the practice phased out by 2008. See FAWC (2003) *Report on the Welfare of Farmed Animals at Slaughter or Killing. Part 1: Red Meat Animals.* London: Defra Publications

and animal welfare and Three Rs[**] organisations. Rats and mice were the only species to be considered, as they are the most commonly used laboratory animals to be killed using $CO_2$ and are also the subject of much research into its effects. Note that the focus of much research to date has been the rat, which is reflected in the studies cited below. There are significant differences between rodents and other mammals with respect to the effects of $CO_2$ inhalation, so the conclusions within this report cannot necessarily be applied to other species.

This report begins by summarising the points of consensus that were agreed during the meeting. Each point is then expanded upon below, with brief supporting and substantiating information as appropriate. There is a body of literature on the effects of $CO_2$ on animal physiology and behaviour, which will not be repeated here; recommended reviews are listed in Appendix II. Words underlined in the text are defined in the glossary on page 14.

## Summary consensus points

The meeting aimed to achieve a consensus view wherever possible, but there were inevitably some differences of opinion between the experts present. The key points set out below therefore represent the *majority* view of the delegates at the meeting. As it was not always possible to achieve a full consensus on every issue, any points of dissent are set out in the full report below. All of the speakers have agreed that this report represents a fair summary of the discussions on the day and of current knowledge on the topics set out below.

**Problems with CO2 killing**

1. There is no "ideal" way of killing animals with $CO_2$ - both pre-fill and rising concentrations can cause welfare problems.
2. If animals are placed into a chamber containing a high concentration of $CO_2$ (above 50 %), they will experience at least 10 to 15 seconds of pain in the mucosa of the upper airways before the loss of consciousness. This is a serious welfare problem.
3. If animals are placed into a chamber with a rising concentration of $CO_2$, they will find it aversive at a certain level and may experience "air hunger" or dyspnoea, which is unpleasant (and, in humans, is reported as highly distressing). This may also be a serious welfare problem.

**Good practice for CO2 euthanasia**

4. It was the general opinion of the participants that it is more important to avoid or minimise pain and distress than it is to ensure a rapid loss of consciousness. Or, a "gentle" death that takes longer is preferable to a more rapid, but more distressing death.
5. The optimum chamber filling rate is uncertain. Use of 100 % $CO_2$ at a flow rate of 20 % of the chamber volume per minute has been shown to produce loss of consciousness without evidence of pain, but not without evidence of dyspnoea. Reduced flow rates can be increased once animals have lost consciousness.

[**] Replacement of animal experiments with humane alternatives, reduction in animal numbers, and refinement of husbandry and procedures to improve welfare and reduce suffering.

6. It is possible that the addition of $O_2$ to carbon dioxide may reduce, but not overcome, welfare problems caused by pain or dyspnoea. It is also possible that high concentrations of oxygen would prolong consciousness, which may not be desirable. There is currently insufficient information in the literature to reach a clear conclusion on the appropriate level of $O_2$.

**Alternative gaseous euthanasia agents**

7. It is not yet possible to recommend the use of gases that cause death by producing hypoxia, such as argon, nitrogen, carbon monoxide, helium or xenon, to euthanase rats or mice. Hypoxia may be the preferred method for other, non-rodent species, but it is not possible to generalise between species and there are insufficient data on the impact of the above gases on the affective states of rodents at present.
8. Volatile anaesthetic agents may provide appropriate alternatives to $CO_2$, but the aversiveness of these gases can vary. They can either be used as the sole euthanasia agent, or they can be used to anaesthetise animals before completing euthanasia by switching to $CO_2$.

**Future research**

9. More research is needed into the physiological and affective responses to a range of gaseous agents, to identify good practice and possible alternatives to $CO_2$. This requires a multidisciplinary approach and effective communication between researchers.

## Background to summary points

### Problems with $CO_2$ killing

**1. There is no "ideal" way of killing animals with $CO_2$ - both pre-fill and rising concentrations can cause welfare problems.**

*Background*

Achieving euthanasia using any method may be complicated by potential trade-offs between achieving a rapid death (so as to minimise stress) and avoiding physical discomfort or pain. This conflict is acute in the case of carbon dioxide.

Exposing animals to carbon dioxide can cause distress because acutely sensitive $CO_2$ chemoreceptors and pH receptors have evolved in vertebrates, with the result that carbon dioxide is a potent respiratory stimulant that rapidly induces dyspnoea or breathlessness. It can also cause discomfort and pain because it is converted to carbonic acid in the mucosa of the eyes, nose and mouth, which activates polymodal nociceptors. Given a free choice, animals avoid carbon dioxide when concentrations rise above a certain threshold. When they do not have a free choice, i.e. they are confined to a chamber, animals will sometimes attempt to escape from the gas. All methods of delivering carbon dioxide with the aim of killing animals can therefore present welfare problems, because concentrations of $CO_2$ that will induce anaesthesia or cause death will inevitably cause some degree of aversion. The essential goals are thus to determine (a) whether $CO_2$ should be used at all and (b) if its use is to continue, how it can be administered so as to cause the least possible pain and distress.

Assuming that $CO_2$ is going to be used to kill animals for the foreseeable future, the majority of delegates believed that it was important to recommend the best possible administration protocol on the basis of current knowledge about the effects of the technique. This was attempted in key point (5). However, given that there is no "ideal" protocol, some also felt that each facility should arrive at a conclusion for itself, using the information presented in this report. The two key factors to consider are:

- how long does it take animals to lose consciousness?
- do animals experience adverse effects before they lose consciousness?

The remainder of this report aims to help answer these questions, to the extent of current available knowledge, so that individual facilities can define and implement best practice.

**2. If animals are placed into a chamber containing a high concentration of $CO_2$ (above 50 %), they will experience at least 10 to 15 seconds of pain in the mucosa of the upper airways before the loss of consciousness. This is a serious welfare problem.**

*Background*
All delegates agreed that placing animals into chambers pre-filled with high levels of $CO_2$ causes serious welfare problems, because of the degree of pain that the animals probably experience before the loss of consciousness.

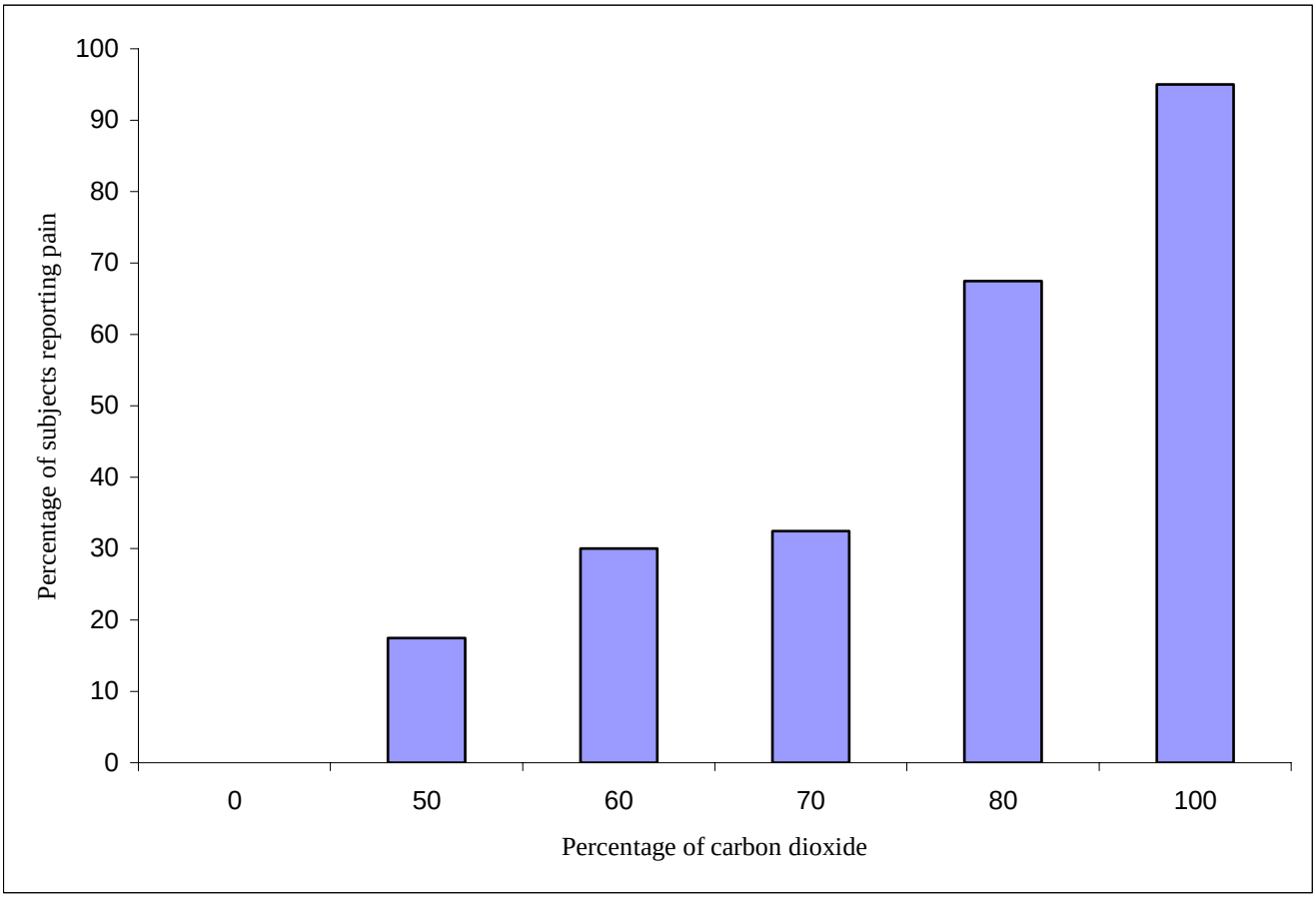
The innervation of the nasal, ocular and respiratory mucosa, and sensory processing following $CO_2$ exposure, are highly conserved between species and are very similar in rats, humans and other mammals. In particular, rats and humans share similar cerebral event-related potentials and the same response threshold for nociceptors. This suggests, but does not prove, that rats and humans perceive $CO_2$ stimulation in the same way[i].

Studies where humans have reported on their experiences of inhaling $CO_2$ can therefore help to predict concentrations that may be painful for rats and mice. For example, human volunteers breathing $CO_2$ in a single breath through the nose described a variety of unpleasant sensations (see table 1 and fig 1). All concentrations were frequently described as "burning"[i].

**Table 1: Humans subjects' ratings of different concentrations of $CO_2$ inhaled in a single breath through the nose[i]**

| Gas | Not unpleasant | Unpleasant | Uncomfortable | Painful |
|---|---|---|---|---|
| $O_2$ | 40 | | | |
| 50 % $CO_2$ | 4 | 15 | 14 | 7 |
| 60 % $CO_2$ | 3 | 7 | 18 | 12 |
| 70 % $CO_2$ | 1 | 8 | 18 | 13 |
| 80 % $CO_2$ | | | 13 | 27 |
| 100 % $CO_2$ | | | 2 | 38 |

**Figure 1: Graphical representation of Table 1**




Other human studies have reported that $CO_2$ is painful at concentrations of around 50 %[ii]. Assuming that the experience of inhaling these concentrations is similar in the rat and mouse, pain may be experienced if these animals are placed into concentrations of $CO_2$ above 50 to 60 %. Carbon dioxide has also been demonstrated to activate nociceptors in anaesthetised rats[iii] and to cause breath-holding, or slowing of respiration, and slowing of heart rate at concentrations reported as painful in man (40 to 50 %)[iv].

The duration of pain caused by high concentrations of $CO_2$ has been investigated in Charles River CD rats using EEG and cardiovascular telemetry at the University of Newcastle[v]. The study found that 100 % $CO_2$ caused:

- almost immediate bradycardia (in < 3 seconds), indicating marked nasal irritation or nociceptor activation;
- *relatively* rapid loss of consciousness (in < 15 seconds), but with the potential for at least 10 to 15 seconds of pain and distress;
- EEG silence at 38 ± 2 seconds (range 23 to 50 seconds).

To conclude, it is possible that humans, rats and mice experience the upper airway irritation caused by $CO_2$ inhalation in similar ways, and it is known that humans find concentrations of 50 % and above uncomfortable or painful. Rats remain conscious, and are likely to be experiencing pain, for at least 10 to 15 seconds in high concentrations of $CO_2$.

**3. If animals are placed into a chamber with a rising concentration of $CO_2$, they will find it aversive at a certain level and may experience "air hunger" or dyspnoea, which is unpleasant (and, in humans, is reported as highly distressing). This may also be a serious welfare problem.**

*Background*

As the concentration of atmospheric $CO_2$ rises, it will first reach a level at which it becomes aversive, presumably due to dyspnoea, and will then reach a level that causes adverse effects such as haemorrhage and seizures. As $CO_2$ is a respiratory stimulant, it causes a progressive increase in breathing frequency and depth which is followed by a decline in breathing as respiratory centres are depressed Carbon dioxide concentrations of approximately 7 % cause dyspnoea in humans, which becomes severe at around 15 %[vi,vii]. It is possible that rats and mice may experience similar levels of dyspnoea at the same concentrations, although this will be difficult to diagnose as, like pain, dyspnoea is a subjective experience. While breathing changes are commonly used as a diagnostic sign, they are not entirely reliable as dyspnoea can occur in the absence of obviously disturbed breathing[viii] and heavy breathing does not necessarily imply the presence of dyspneoa. However, the possibility that conscious rodents may be experiencing dyspnoea should be taken seriously, as it is known that dyspnoea can be highly distressing in humans[ix].

Whatever its cause, aversion is a potential welfare problem because it (a) may indicate that animals are experiencing discomfort or distress, and (b) may well lead to negative emotional states if the animal cannot escape from the aversive stimulus. The strength of aversion is also an important factor, as low levels of aversion may be tolerable whereas higher levels could cause significant distress. However, note that a lack of obvious distress behaviours, e.g. escape behaviours and/or vocalisation, during forced exposure to $CO_2$ or any other gas does not necessarily indicate that welfare is good.

An "approach-avoidance" technique can be used to quantify the aversiveness of $CO_2$ at different levels. In one such experiment rats were offered a palatable food as an incentive to remain in the gas. At static $CO_2$ levels of 5 and 10 %, the rats ate more quickly and then left the chamber but the quantity they consumed was unchanged[x]. Consumption was reduced at 15 % and animals were unwilling to remain in the chamber for the food reward at levels above 15 %. The conclusions were that $CO_2$ was aversive to rats at levels of 15 % and above, and that this was consistent with dyspnoea rather than pain. A similar study has shown that, when rats are provided with food rewards in a chamber with $CO_2$ fill rates ranging from 3 to 27% of chamber volume per minute, they find the gas aversive and leave at a mean concentration of about 15% $CO_2$[xi,xii]. When rats were deprived of food, they still left the chamber at about 15%, suggesting that CO2 is more aversive than acute hunger[xiii].

From the above studies, it appears that rats exposed to a rising concentration of $CO_2$ will find the gas aversive when the concentration reaches about 15 %. Aversion at this concentration is consistent with dyspnoea rather than pain. Discomfort and distress may persist until the animals are anaesthetised at concentrations of around 30 to 40 %, but little is known about rats' experience during this period.

Adverse physical effects, e.g. laboured breathing, seizures or haemorrhaging, may or may not be welfare problems, depending upon the level of consciousness at which

they occur. Since carbon dioxide is an anaesthetic gas that results in loss of consciousness at concentrations of 30 to 40 % for CD rats[xiv,xv,xvi], animals may be unconscious before the onset of adverse physical effects, but not necessarily before the onset of aversion and/or dyspnoea. Most available evidence suggests that consciousness has been lost before seizures occur during slow fill[xvii,xviii]. However, note that it may not be possible to judge whether animals are unconscious by inspection alone, as they may be conscious but immobile.

Difficulty in interpreting behavioural signs of loss of consciousness and clinical signs such as changes in breathing rate or depth, for the reasons outlined above, can make it hard to judge whether animal welfare is significantly compromised. Correlating such observations with EEG data can provide some further guidance on the humaneness of different exposure protocols. However, EEG data cannot provide definitive answers, as EEG is suppressed to an extent by $CO_2$. It is also not currently always clear, or easy to differentiate, which particular EEG patterns are indicators of activation by stress or pain.

A study at the University of Newcastle monitored heart rate, blood pressure, EMG (recorded from electrodes placed in the neck muscles) and EEG in Charles River CD rats exposed to a rising $CO_2$ concentration (20 % of the chamber volume per minute)[v]. This protocol resulted in:

- loss of consciousness *before* autonomic signs of nasal irritation (bradycardia) (see table 2);
- gasping and seizures, which occurred during deep anaesthesia so were not welfare concerns.

Pre-treatment of nasal mucosa with lignocaine (a local anaesthetic) or acetazolamide (which slows carbonic acid formation) had little effect on cardiovascular and EEG parameters, suggesting that acute pain due to acid formation was not significant using this filling rate and exposure method.

**Table 2: Sequence of events in Charles River CD rats during exposure to a rising concentration of $CO_2$ at a fill rate of 20 % chamber volume per minute**

| Event | Time (seconds; mean and SE) | $CO_2$ concentration (%) |
|---|---|---|
| Recumbency (N = 11) | 110 ± 6 | 30 |
| EMG silence (N = 6) | 110 ± 5 | 30 |
| Loss of consciousness (behavioural) (N = 11) | 156 ± 5 | 39 |
| Bradycardia | 242 ± 85 | 47 |
| Brain death (isoelectric EEG) (N = 6) | 327 ± 8 | 72 |

There was no evidence from heart rate or blood pressure data of marked arousal or stress in this study (table 3), but the anaesthetic and other physiological effects of $CO_2$ could have confounded this. It is also very important to note that the fill rates used in this study were considerably slower than those used in many facilities. Faster fill rates may lead to nociceptor activation before the loss of consciousness.

**Table 3: Mean arterial blood pressure (BP) and heart rate (HR) in Charles River CD rats during exposure to rising concentrations of $CO_2$ and air at a fill rate of 20 % chamber volume per minute.**

| | 10 s exposure to $CO_2$ | | 100 s exposure to $CO_2$ | |
|---|---|---|---|---|
| | **Mean BP (mmHg)** | **HR (beats/min)** | **Mean BP (mmHg)** | **HR (beats/min)** |
| Air | 141 ± 16 | 405 ± 41 | 141 ± 17 | 376 ± 68 |
| $CO_2$ | 130 ± 17 | 415 ± 48 | 137 ± 24 | 275 ± 42 |

Legend: Responses during air exposure were recorded 24 hours earlier.

## Good practice for CO2 euthanasia

**4. It was the general opinion of the participants that it is more important to avoid or minimise pain and distress than it is to ensure a rapid loss of consciousness. Or, a "gentle" death that takes longer is preferable to a more rapid, but more distressing death.**

*Background*
This is a general principle that, in the opinion of the delegates, applies to any method of euthanasia. Inevitably, this was considered from a human viewpoint, i.e. the delegates imagined what their own experiences might be in both situations and generalised their preferences to other species. Making this decision on behalf of other animals means assuming that the experience of pain and distress - and its importance and emotional impact - is sufficiently analogous between different vertebrate species. The meeting delegates believed that this was the case with respect to inhaling carbon dioxide. The key question is therefore whether a "gentle" death can be achieved using $CO_2$.

**5. The optimum filling rate is uncertain. Use of 100 % $CO_2$ at a flow rate of 20 % of the chamber volume per minute has been shown to produce loss of consciousness without evidence of pain, but not without evidence of dyspnoea. Reduced flow rates can be increased once animals have lost consciousness.**

*Background*
Table 4 below sets out the pros and cons of pre-filling chambers with 100 % $CO_2$ against introducing the gas at a flow rate of 20 % chamber volume per minute. It is known that pre-fill is probably painful but is *relatively* quick. However, it is important carefully to consider the term "relatively". Consciousness may well be lost within 15 seconds of exposure to 100 % $CO_2$, but there is a strong possibility that the experience is extremely unpleasant. Most people would actively avoid being exposed to such a stimulus for 15 seconds.

**Table 4: Comparison of the physiological effects of pre-fill vs. rising concentration of carbon dioxide**[v]

| | **Pre-fill (100 % $CO_2$)** | **Rising concentration (20 % chamber volume per minute)** |
|---|---|---|
| Adverse effects before loss of consciousness | Pain, potentially severe | No pain, but other effects unclear - possible distress, discomfort, dyspnoea |
| Time to onset of adverse effects | Instant | Ataxia at around 55 seconds |
| Time to loss of consciousness | 38 ± 2 seconds (N = 6) | 156 ± 5 seconds (N = 11)* |
| Time to cortical inactivity | 45 seconds | 5 to 6 minutes |

* This is a conservative estimate based on behavioural loss of consciousness - the true value may be 110 seconds using recumbency and EMG data as a basis. It is still not clear when animals are actually unconscious.

The rising concentration technique should not cause pain, but may cause distress by other mechanisms as described above. The aim should be to induce unconsciousness before animals become significantly distressed. The majority of delegates agreed that the potential for exposing conscious animals to noxious $CO_2$ levels can be minimised by using slow flow rates, but optimal flow rates and administration protocols have not been established. A fill rate of 20 % of the chamber volume per minute appeared to cause loss of consciousness before nociceptor activation (see above)[v]. Note that the chamber was filled from the top, which mixes the $CO_2$ more effectively than filling from below. Filling from below can produce localised high $CO_2$ concentrations, so it may be necessary to assess individual chambers. A flow rate of 20 % chamber volume per minute does not solve all the welfare problems, as can be seen from table 4; however the majority of delegates agreed that a gradual fill technique was preferable to pre-fill.

It was recognised that there may be some practical limitations to using slow flow rates, on the grounds that establishments having large numbers of rodents to kill may not be able to spend up to 8 or 10 minutes on each batch, to ensure that no animals recover. This can be overcome, either by having more euthanasia stations or by filling the chamber at 20 % of its volume per minute until the animals are unconscious, then increasing the flow rate to 100 % volume per minute or more. Gas flow meters are easily obtainable, and systems are available that can be programmed to fill chambers at different rates so that the process can be automated once the time to unconsciousness is known. It is still essential to monitor animals for signs of pain or distress, even if the euthanasia process is automated.

6. **It is possible that the addition of $O_2$ to carbon dioxide may reduce, but not overcome, welfare problems caused by pain or dyspnoea. It is also possible that high oxygen would prolong consciousness, which may not be desirable. There is currently insufficient information in the literature to reach a clear conclusion on the appropriate level of $O_2$.**

*Background*
Some studies have demonstrated that the addition of oxygen to carbon dioxide made the gas less aversive to birds. For example, a two-phase system has been developed in which birds are first placed in an atmosphere comprising 40 % $CO_2$, 30 % $N_2$ and 30 % $O_2$, which will anaesthetise them after one minute. In the second phase, a mix of 80 % $CO_2$, 15 % $N_2$ and 5% $O_2$ is used to kill the birds. Results with this "controlled atmosphere system" are promising, in particular with respect to the smooth induction of unconsciousness[xix]. However, other studies have shown that poultry avoid this gas mix and that behavioural and autonomic signs that may indicate aversion have been reported[xx,xxi].

The addition of oxygen to carbon dioxide has been shown to reduce agitation and gasping in the rat[xxii], but it has been suggested that the apparent lower aversiveness *may* have been due to the difference in $CO_2$ concentration and flow rate[xxiii]. However, one study on rats at the University of British Columbia compared $CO_2$ with a mix of $CO_2$ and $O_2$, at a uniform $CO_2$ flow rate, and found that aversion to $CO_2$ was slightly (but not completely) reduced with $O_2$ supplementation (R. Kirkden, pers. comm.).

A number of delegates expressed the view that evidence for the benefits of $O_2$ supplementation was not clear. For example, haemorrhaging has been reported with the addition of oxygen in mice, but it is not known whether this occurred before or after the loss of consciousness[xxiv]. The group did not feel able to recommend protocols for the addition of $O_2$, but there was full agreement that more studies are needed into the effects of supplementation with oxygen (see below).

## Alternative gaseous euthanasia agents

7. **It is not yet possible to recommend the use of gases that cause death by producing <u>hypoxia</u>, such as argon, nitrogen, carbon monoxide, helium or xenon, to euthanase rats or mice. Hypoxia may be the preferred method for other, non-rodent species, but it is not possible to generalise between species and there are insufficient data on the impact of the above gases on the <u>affective</u> states of rodents at present.**

8. **Volatile anaesthetic agents may provide appropriate alternatives to $CO_2$, but the aversiveness of these gases can vary. They can either be used as the sole euthanasia agent, or they can be used to anaesthetise animals before completing euthanasia by switching to $CO_2$.**

*Background*
Possible alternative inhalational agents to $CO_2$ can be divided into (a) gases that cause hypoxia and (b) volatile anaesthetic agents. Each potential alternative has its own animal welfare, practical, human safety and economic issues.

In the case of gases that cause hypoxia, such as argon, nitrogen, carbon monoxide, helium or xenon, there are a number of studies in the literature that have set out to evaluate their potential as euthanasia agents. Unfortunately, the literature is currently not sufficiently comprehensive to enable a judgement on the suitability of any of these gases for rats and mice. It is known that spontaneously breathing humans lose

consciousness without experiencing discomfort when exposed to profound hypoxia[xxv] and argon-induced hypoxia in pigs is not associated with aversion or distress[xxvi], yet studies on rodents have shown that rats are not prepared to enter chambers containing high levels of argon for reasons that are not yet understood (Niel personal communication). It is therefore not possible to extrapolate results between species.

Within the rodent studies that have been published to date, protocols have employed either pre-fill or different flow rates, and it is not always clear whether animals were conscious when adverse effects occurred. As discussed above, animals may convulse while they are conscious or unconscious, but without EEG data it is impossible to assess whether they might have been conscious at the time.

The key elements of the humane induction of anaesthesia are (i) the animal's initial perception of the anaesthetic agent and (ii) any distress associated with induction, for example due to irritancy of the vapour. Determining whether animals find volatile agents aversive can provide important information when deciding on the most humane killing technique.

Measures of aversion include locomotory responses such as initial withdrawal times from the test chamber; re-entry times if animals go back inside; and total dwelling times, or the total amount of time that animals spend in the chamber. Types of behaviour that are commonly monitored include rearing, washing, sniffing the test chamber entrance and elimination. If gases are aversive, then these behaviours should increase, re-entry times should increase, and withdrawal and total dwelling times should decrease. Using these parameters, the least aversive euthanasia agent appears to be halothane for rats, and halothane and enflurane for mice[xxvii]. These can then be followed by $CO_2$ after loss of consciousness if there is a need to save time.

There can be issues with respect to the availability of gaseous anaesthetics; for example, the production of halothane is about to cease at the time of writing. Of the alternative volatile anaesthetics currently available, sevoflurane is the closest to halothane with regard to aversion. Changing from $CO_2$ to volatile anaesthetics also involves capital outlay, as calibrated vaporisers and efficient scavenging devices are necessary due to human health and safety concerns (many national health and safety authorities set maximum exposure limits for anaesthetic gases). Equipment costs need not be prohibitive, however, as second hand equipment is often available.

## Future research

**9. More research is needed into the physiological and affective responses to a range of gaseous agents, to identify possible alternatives to $CO_2$ and define good practice for killing with carbon dioxide. This requires a multidisciplinary approach and effective communication between researchers.**

*Background*

While the meeting identified areas of consensus, it was also clear that there are significant gaps in current knowledge that need to be addressed in order to make definitive recommendations on humane euthanasia using gaseous agents. In particular, more research is needed to find an appropriate alternative to the use of

carbon dioxide as a matter of high priority. It is also important to study and evaluate the links between the chemistry of $CO_2$, its physiological effects and their significance for animal welfare, especially for mice. There is an inherent ethical dilemma in conducting studies that may cause animals pain and distress in order to improve the wellbeing of other animals. However, given the large number of rats and mice killed using $CO_2$, and the uncertainty as to what they experience, further animal studies were believed to be justified by the majority of delegates.

The group produced a list of recommended study topics, set out below and broadly divided into behavioural and physiological studies.

**Behavioural studies**

- The strength of aversion to argon and to volatile anaesthetics, and the associated welfare consequences, in rats and mice.
- Examination of different methods to evaluate strength of aversion, as some may be limited, e.g. animals will be unable to escape if they are immobile; or flawed, e.g. $CO_2$ exposure might interfere with feeding motivation in an approach-avoidance test.
- Use of conditioned place preference and aversion studies to compare a variety of gaseous killing methods.
- Effects of adding supplementary oxygen to $CO_2$.

**Physiological studies**

- The time and concentration of a range of gaseous agents, including $CO_2$, required to (a) anaesthetise and (b) kill rats and mice. This should be determined using EEG and correlated with EMG data; any occurrence of adverse effects should also be evaluated.
- Studies to evaluate the physiological effects of exposure to a rising concentration of $CO_2$, to try to infer what mice and rats could be experiencing between aversion and unconsciousness.
- More measurement in rats and mice of physiological parameters that are used to predict stress levels, such as heart rate and corticosterone levels
- Studies on ventilatory patterns and their relationship to the sensation of dyspnoea in humans.

The above list shows that a multidisciplinary approach, involving different research fields, techniques and approaches, will be necessary to gain a fuller understanding of animals' experiences of euthanasia. This will clearly require effective communication between researchers, with respect to practical aspects - such as agreeing protocols for evaluating the effects of gases and keeping in touch regarding research programmes and collaborations - and also free discussion on interpreting results and agreeing appropriate research directions, as occurred during the meeting. A particular point that should be emphasised is the relative lack of information on mice, which needs to be addressed because extrapolation of data from rats may not always be appropriate.

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## Glossary

**Affective:** Emotional, or influenced by the emotions.
**Air hunger:** The perception of insufficient breathing; of not getting enough air (see **dyspnoea**).
**Animal:** Vertebrate, terrestrial animal post-partum or post-hatch. Note that this is a more restrictive definition than in some national laws regulating experimental animal use and may also prove to be more restrictive than the revised European Union Directive 86/609. This definition is a practical one, in relation to the types of animal regularly killed using $CO_2$, and does not reflect the delegates' opinion on the appropriate scope of regulations relating to animal research and testing.
**Aversion:** A tendency to stop carrying out a behaviour, or the avoidance of an object or place, because it has become associated with a noxious or unpleasant stimulus. The assumption is that animals unable to remove themselves from such a stimulus are likely to be distressed.
**Bradycardia:** Fall in heart rate, often defined as that below resting level.
**Chemoreceptors:** Sensory nerves that respond to chemical stimuli.
**Conscious:** Awake; aware of the surroundings and capable of perceiving sensory stimuli.
**Dyspnoea:** A subjective experience of breathing discomfort. Also known as "air hunger", "breathlessness" or "shortness of breath". Not necessarily indicated by laboured breathing.
**EEG:** Electroencephalogram; record of electrical activity of the brain.
**EMG:** Electromyogram; record of electrical activity in muscle.
**Euthanasia:** Literally, a "good death", i.e. killing an animal without causing discomfort, pain or distress.
**Event-related potential:** Response by the brain to a stimulus, measured using electroencephalography (EEG).
**Hypoxia:** Decreased oxygen supply.
**Mucosa:** Mucous membranes; tissues that line all body cavities that lead to the outside such as the mouth, nose and alimentary tract. These membranes have cells and glands that secrete mucus.
**Nociceptor:** A specialised nerve cell that sends pain signals in response to harmful stimuli such as heat, chemical or mechanical damage to tissues. Some nociceptors respond to one specific stimulus such as heat, whereas **polymodal nociceptors** respond to chemical, thermal and mechanical stimuli.
**Pre-fill:** Filling a chamber with a gas before animals are placed into it.
**Response threshold:** Level of stimulation that causes a neuron (nerve cell) to transmit an impulse.
**Rising concentration:** Placing animals into a chamber containing air and then introducing a gas.
**Welfare:** Wellbeing. For welfare to be good, an animal's physical, environmental, nutritional, behavioural and social needs must be met and they must be in good health. Euthanasia will unavoidably compromise these needs but it is still possible - and essential - to address physical and behavioural requirements.

## Appendix I

### Lists of speakers and observers

**Speakers:** Prof. Robert Banzett, Harvard School of Public Health, USA; Prof. Anton Coenen, Nijmegen Institute for Cognition and Information, Netherlands; Dr. Jonathan Cooper, Department of Biological Sciences, University of Lincoln, UK; Dr. Peggy Danneman, The Jackson Laboratory, Maine, USA; Prof. Paul Flecknell, Comparative Biology Centre, University of Newcastle, UK; Dr. Huw Golledge, Comparative Biology Centre, University of Newcastle, UK; Dr. Richard Kirkden, Animal Welfare Program, University of British Columbia, Canada; Dr. Matt Leach, Comparative Biology Centre, University of Newcastle, UK; Lee Niel, Animal Welfare Program, University of British Columbia, Canada; Dr. Mohan Raj, Department of Clinical Veterinary Science, University of Bristol, UK

**Observers:** Dr. Melissa Bateson, School of Psychology, University of Newcastle, UK; Dr. Kath Conlee, The Humane Society of the United States; Brian Corning, Charles River, UK; Dr. Geoff Dandie, Australian and New Zealand Council for the Care of Animals in Research & Teaching; Dr. Colin Dunn, Editor, *Laboratory Animals*; Dr. David Farningham, Home Office Inspectorate, UK; Roger Francis, Institute of Animal Technology, UK; Geoffrey Hale, Covance Laboratories, Harrogate, UK; Dr. Penny Hawkins, Royal Society for the Prevention of Cruelty to Animals, UK: Patrick Hayes, Editor, *Animal Technology and Welfare*; Dr. James Kirkwood, Universities Federation for Animal Welfare, UK; Prof. David Morton, Biomedical Services Unit, University of Birmingham, UK; Dr. Laura Playle, National Centre for the Replacement, Refinement and Reduction of Animals in Research, UK; Terry Priest, Animal Procedures Committee, UK; Dr. Johnny Roughan, Comparative Biology Centre, University of Newcastle, UK; Dr. Kathy Ryder, Home Office Inspectorate, UK; Tim Watson, National Centre for the Replacement, Refinement and Reduction of Animals in Research, UK; Prof. Dan Weary, Animal Welfare Program, University of British Columbia, Canada; Dr. William White, American College of Laboratory Animal Medicine; Lucy Whitfield, Royal Veterinary College, UK; Siân Wright-Williams, Comparative Biology Centre, University of Newcastle, UK

## Appendix II

### Recommended reviews

Conlee KM, Stephens ML, Rowan AN & King LA (2005) Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. *Laboratory Animals* **39**, 137-161

*Extensive literature review on $CO_2$ killing with discussion and recommendations; see also further correspondence on this topic in* Laboratory Animals ***39**, 353-354 and **39**, 452-455.*

European Food Safety Authority - Animal Health and Welfare Panel (2005) Scientific Report: "Aspects of the Biology and Welfare of Animals Used for Experimental and Other Scientific Purposes" (EFSA-Q-2004-105). Annex to *EFSA Journal* **292**, 1-136 (http://www.efsa.eu.int/science/ahaw/ahaw_opinions/1286/ahaw_labanimalswelfare_report1.pdf)

*Review produced in response to request by Council of Europe DG ENV for opinion on various aspects of the revision of Directive 86/609, which regulates animal research and testing in the European Union. Gaseous euthanasia, including $CO_2$, is addressed in section 4.8.5, pp 79-100.*

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[xi] Kirkden RD, Niel L & Weary DM (2005) How aversive is gradual-fill carbon dioxide euthanasia for rats? Abstract, Canadian Association of Laboratory Animal Science Symposium

[xii] Niel L, Stewart SA & Weary DM (accepted) Does flow rate affect aversion to gradual-fill carbon dioxide exposure in rats? *Applied Animal Behaviour Science*

[xiii] Kirkden, RD, Niel, L & Weary, DM (2005) How aversive is gradual fill carbon dioxide euthanasia for rats? *CALAS/ACSAL, 44th Annual Symposium, Vancouver, BC, June 25-28, 2005*, p. 31.

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