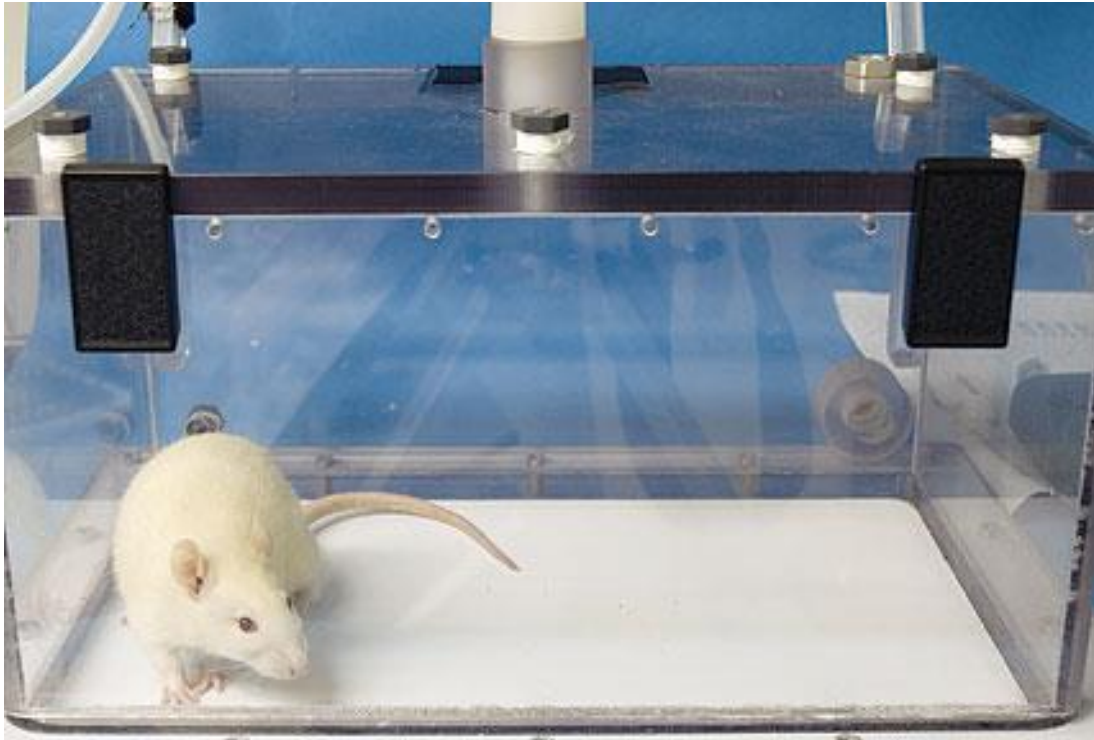# Big Wins in Humane Killing:
Replacing Carbon Dioxide with more Mumane methods for Lab Rodent Euthanasia

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Aim

Consider how we should choose the method of Euthanasia (Humane Killing) for the animals we use in research.

Give examples of rodent euthanasia in facilities where more humane methods have been adopted.

Why is the issue important?

We use large numbers of animals in the name of scientific research.

Almost all of these will have to be deliberately killed.

There is a legal/moral imperative to give these animals a 'good death'

安死术 Euthanasia
人道地终止动物生命的方法，最大限度地减少或消除动物的惊恐和痛苦，使动物安静地和快速地死亡。

A method of humanely ending the life of an animal causing peaceful and rapid death while, to the greatest extent possible, reducing or eliminating fear and suffering.

实验动物 福利伦理审查指南

Choosing a Euthanasia Method

Many methods are available including

- Physical (Cervical Dislocation, Decapitation etc).
- Chemical (Injected Drug Overdose, Inhaled anaesthetic, Carbon Dioxide)

Almost all methods have some impact on the welfare of animals

Careful consideration needs to be given to which method is used based on the scientific objects of the research and the species, age, strain etc. of the animals to be killed.
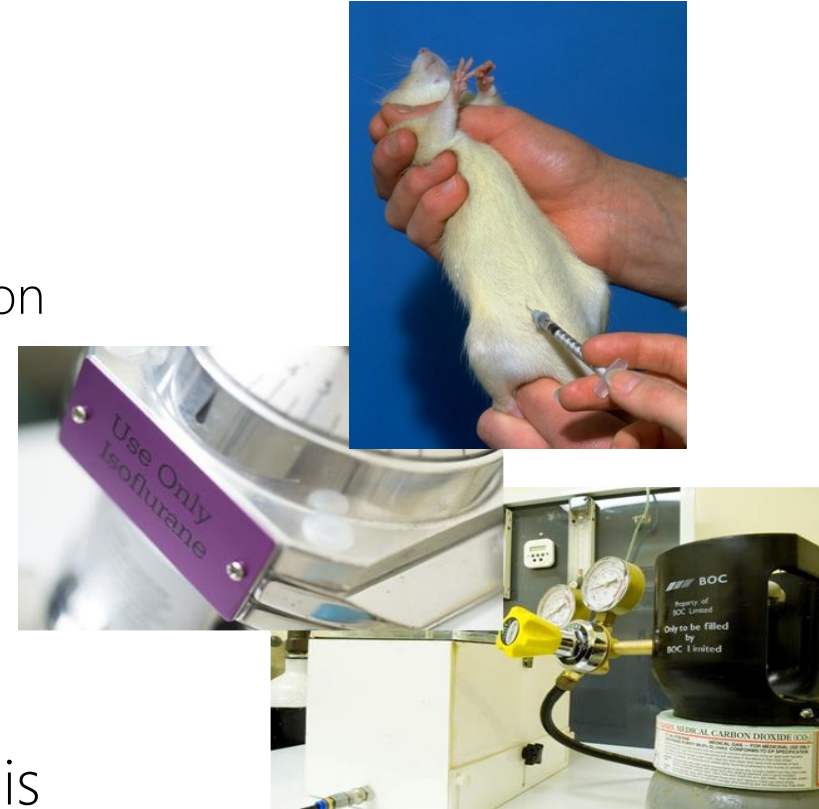
Objectives of Killing a Lab Animal

To ensure the animal has a humane death
with minimum pain or suffering

and

that the scientific purpose of using that
animal is not compromised.

Choice of Method – Welfare Constraints

The method should cause minimal pain and/or distress

If distress or pain is unavoidable the method should be as quick as possible

The method should not cause distress to other animals

Boivin, G, et al"Responses of Male C57BL/6N Mice to Observing the Euthanasia of Other Mice." *Journal of the American Association for Laboratory Animal Science* 55, no. 4 (2016): 406–11.

Choice of Method - Scientific Constraints
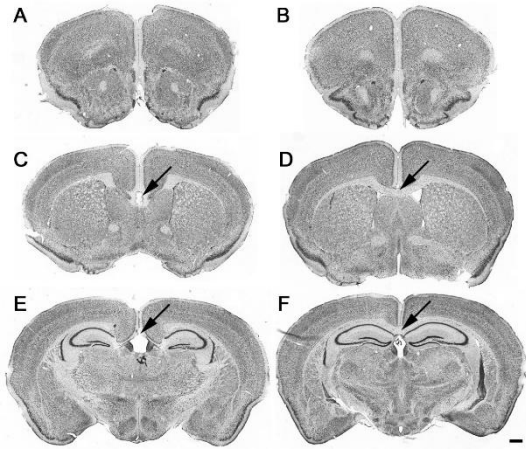
Depending on the study we may:

- need samples for post-mortem use – i.e. intact brain etc.

- need to avoid contamination of tissues/samples with drugs

- need to rapidly stop physiological processes

Choice of Method – Practical Constraints

Cost

Number of animals which must be killed

Equipment availability/safety

Use of carcasses (food, teaching specimens)

Feelings of the person carrying out the procedure

Carbon Dioxide

Most widely used method to kill lab rodents. Also widely used for poultry and pigs.

Animal breathes CO_2 in a chamber until it loses consciousness and eventually dies.

Simple, safe, effective, cheap, doesn't contaminate tissues, relatively un-distressing for operator.
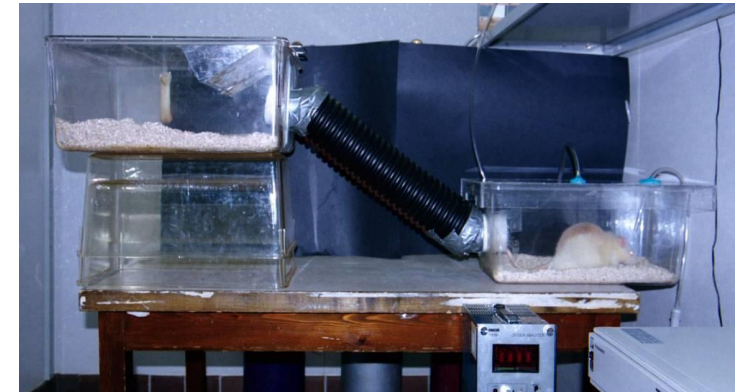
Welfare Concerns about Carbon Dioxide

Carbon dioxide causes pain in high concentrations

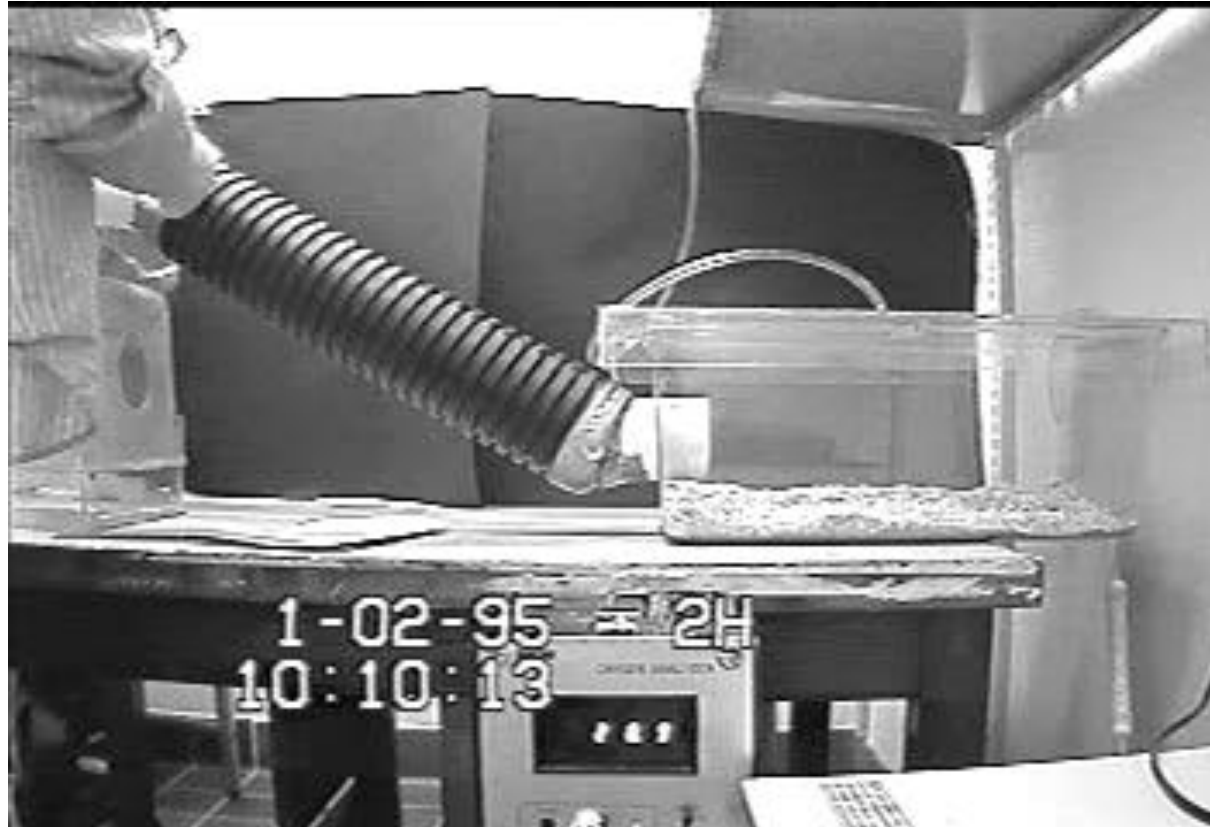$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$
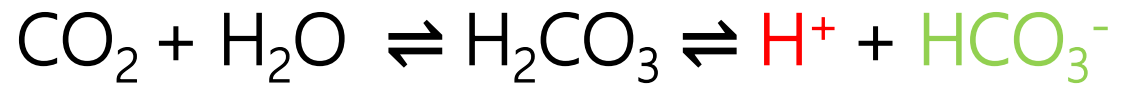
Carbon dioxide is aversive – rats and mice will avoid low concentrations of CO_2

Carbon dioxide causes panic or fear in rodents and humans. It is used as an experimental model of panic disorder.

Approach-Avoidance in CO$_2$ - Video

Kirkden RD, Niel L, et al. (2008) *Applied Animal Behaviour Science*, **114,** 216-234.
Kirkden RD, Niel L, et al. (2005), *Lab Anim*, **39,** 453-5.
Niel L, Weary D (2006), *Applied Animal Behaviour Science* **100,** 295-308.
Niel L, Weary DM (2007), *Applied Animal Behaviour Science*, **107,** 100-109.

Carbon Dioxide is Used to Model Panic Disorder

CO_2 is widely used in humans and rodents to model human panic disorder and anxiety.

Extensive evidence shows panic-, or anxiety-like reactions in response to low concentration CO_2 in both humans and rodents.

Multiple brain mechanisms for CO_2 detection and panic-like reactions have been identified.

7.5% CO$_2$ Exposure

7.5% CO$_2$ (normoxic) is anxiogenic in humans (Bailey et al. 2005).

Rating	Peak air	Peak CO$_2$
Anxious	2 (2.1)	25 (5.8)[b]
Fear	1 (2.9)	24 (4.5)[b]
Feel like leaving	3 (2.5)	23 (5.8)[b]
Happy	−10 (2.9)	−27 (4.0)[c]
Irritable[a]	2 (2.1)	16 (6.9)[d]
Nervous[a]	5 (2.3)	17 (3.4)[c]
Paralysed	4 (2.6)	15 (4.2)[c]
Relaxed	−13 (5.5)	−35 (4.9)[b]
Tense	4 (2.9)	29 (5.1)[b]
Worried[a]	1 (1.6)	18 (4.7)[c]

Values are expressed as mean (*sem*); $n = 20$.

Bailey,J.E., et al. "Behavioral and Cardiovascular Effects of 7.5% CO2 in Human Volunteers." *Depression and Anxiety* 21, no. 1 (2005): 18–25.
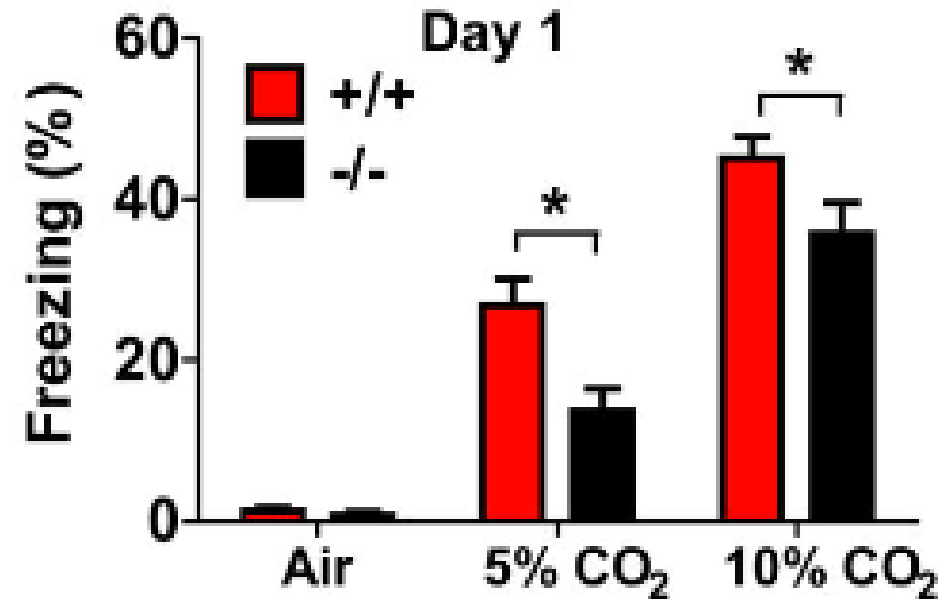
Anxiety-like behaviours in rodents exposed to CO_2

Open Field Test

Spontaneous Freezing in Mice exposed to CO_2
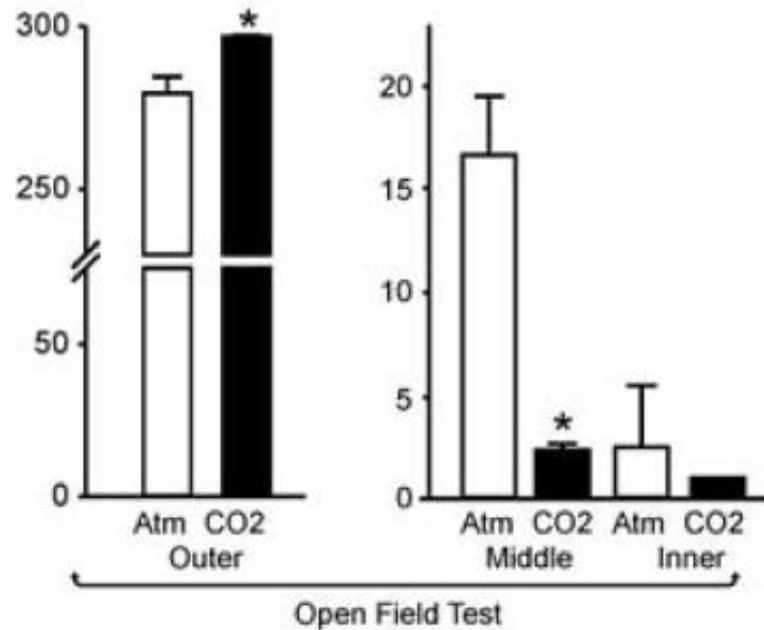
Johnson, P et al (2012). "Activation of the Orexin 1 Receptor Is a Critical Component of CO2-Mediated Anxiety and Hypertension but Not Bradycardia." *Neuropsychopharmacology* 37(8)1911–22.

Vollmer, L. et al. (2016) "Microglial Acid Sensing Regulates Carbon Dioxide Evoked Fear." Biological Psychiatry 80(7)541–51.

Science in the service of animal welfare

UFAW
Established 1926

Mechanisms of CO$_2$-Induced Anxiety

1: acid sensing ion channels (ASICs) in the amygdala,
2: orexin neurons in the hypothalamus
3: serotonergic neurons in the medullary raphe
4: T-cell death-associated gene-8 receptor in the subfornical organ (SFO)
5: hypoxia-sensitive chemosensory neurons in the periaqueductal gray (PAG).

Vollmer, L. et al. (2015). "Acid–base Dysregulation and Chemosensory Mechanisms in Panic Disorder: A Translational Update." *Translational Psychiatry* 5(5), e572. https://doi.org/10.1038/tp.2015.67.

Carbon Dioxide – Welfare Concerns

There is ample evidence that carbon dioxide is not an ideal euthanasia agent.

We should not be using an agent which is used as an experimental model for panic disorder for routine euthanasia.

More humane alternatives should be considered where feasible.

Alternatives to CO_2 for Rodent Euthanasia

Physical Methods (e.g. cervical dislocation)

Injection of Anaesthetic Overdose
(e.g. Penotbarbitone)

Overdose of Inhalation Anaesthetic
(e.g. Isoflurane/Sevoflurane)

Example 1 – Cervical Disloation

Medical Research Council (MRC) – Harwell, UK

- 250,000 mice per year
- No Carbon Dioxide Euthanasia for over 10 years
- Most killed by cervical dislocation
- Neonates killed by decapitation with scissors
- Occasional anaesthetic overdose for scientific purposes

Cervical Dislocation – Practicalities

Physically separate cervical vertebrae with a rigid rod (or fingers).

Should cause very rapid loss of consciousness and death.

Very important to confirm death.

Cervical Dislocation – Practicalities

- Physical methods are prone to failure if carried out wrongly

  (Carbone, L et al. . "Assessing Cervical Dislocation as a Humane Euthanasia Method in Mice." *Journal of the American Association for Laboratory Animal Science* 51, 352–356).

- Training is crucial.
  - Staff must be competent to carry out the procedure and ensure that the animal is instantly killed.
  - Online training:
    https://flairelearning.com/course/humane-methods-of-killing-laboratory-animals/

- The laboratory undertook an extensive X-ray study to ensure that animals were reliably killed every time.
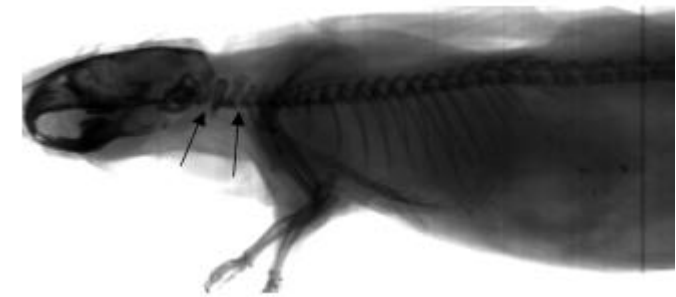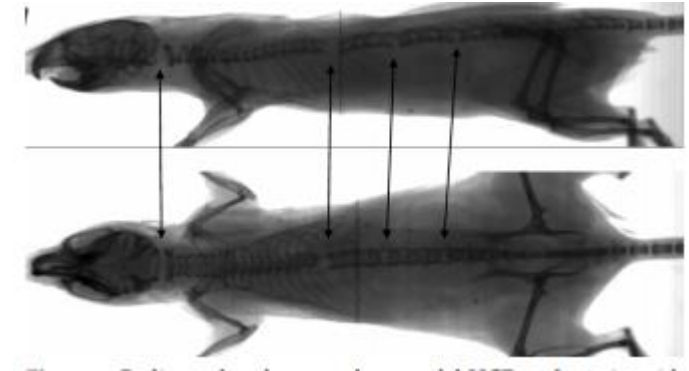
Example 2 – Replacing CO_2 with Isoflurane

Large drug company doing experimental oncology research in mice and rats.

Switched from CO_2 to induction of anaesthesia with isoflurane.

- Isoflurane has been shown to be less aversive than CO_2[1]
- Animals rendered rapidly unconscious with high concentration Isoflurane

The lab undertook research to ensure that post-mortem data obtained from animals was not affected by the change of euthanasia method and studied behaviour to show lack of distress.

1. Boulanger Bertolus ,J et al. / Applied Animal Behaviour Science 164 (2015) 73–80 , Wong, D et al. (2012) *Biology Letters* 9, no. 1 . doi:10.1098/rsbl.2012.1000.

Summary

Consider the most appropriate euthanasia method for each study rather than using the same method in all cases.

In many cases, even with large numbers of animals it is possible to avoid the use of CO_2.

Validate alternative methods if necessary.

Keep up to date on scientific advances which may affect choice of euthanasia method.

The Future?

Low Atmospheric Pressure?

Gradual reduction in atmospheric pressure over several minutes.

Recently approved for chicken slaughter in the EU.

Under test for pig slaughter and euthanasia of lab rodents.
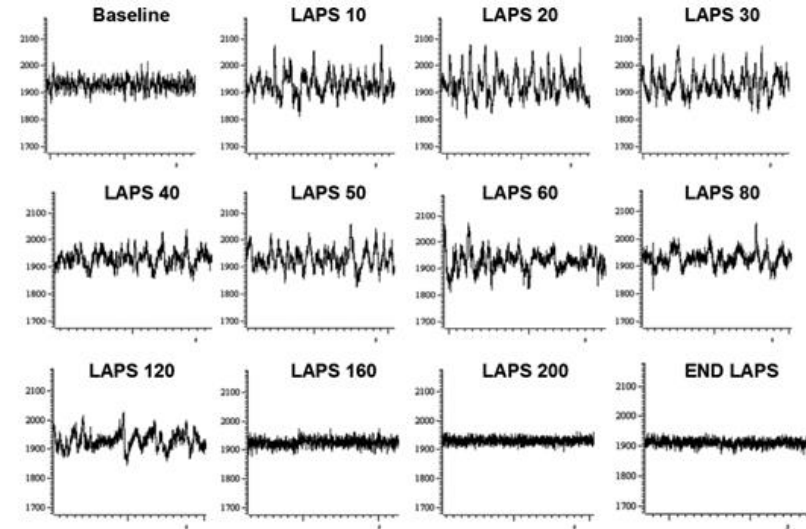
Other novel methods may emerge (new drugs, focussed microwave irradiation etc etc.)

Further Information

The American Veterinary Medical Association's Guidelines for the Euthanasia of Animals: 2013 Edition provide an exhaustive and considered overview of methods for killing most species.

https://www.avma.org/kb/policies/documents/euthanasia.pdf

A good death?: Report of the second Newcastle meeting on laboratory animal euthanasia. *Animals* 6(9), 50 (2016).

http://www.mdpi.com/2076-2615/6/9/50

Online Training: https://flairelearning.com/course/humane-methods-of-killing-laboratory-animals/

RSPCA - Good Practice for Humane Killing provides supplementary resources for members of ethical review bodies, but also more broadly useful guidance on the selection of humane killing methods for lab animals. http://tinyurl.com/GPHumKill

NC3Rs - http://www.nc3rs.org.uk/euthanasia

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AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

Conference Report

A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia

Penny Hawkins [1], Mark J. Prescott [2,*], Larry Carbone [3], Ngaire Dennison [4,†], Craig Johnson [5], I. Joanna Makowska [6], Nicole Marquardt [7], Gareth Readman [8], Daniel M. Weary [6] and Huw D. R. Golledge [9]