Euthanasia method for laboratory animals

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Euthanasia Law by Carbon Dioxide Amended

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

Change from 10-30% every minute

A gradual increase in CO_2 concentration at a replacement rate of 30-70% of vessel volume per minute is recommended. The CO_2 flow should be continued for at least 1 minute after respiratory arrest.

- Immature animals, reptiles, amphibians and some burrowing and diving animals have respiratory adaptations that require higher or longer exposures to CO_2. Alternatively, euthanasia methods other than CO_2 exposure may be required.

→ One-day-old rats need CO_2 exposure for 35 minutes and mice for 50 minutes. At 10 days of age, 5 minutes of exposure can be reliably lethal.

- Placing a conscious animal directly into a container pre-filled with 100% CO_2 is not acceptable.

→ Death is induced by the anesthetic effect of CO_2 by gradually increasing the concentration. It is not suffocation.

Takatoshi Kuhara LABIO 21 May 2020

Barbiturates as Potential Alternatives to Pentobarbital

	Pentobarbital (Nembutal)	Secobarbital (Ional)	Thiopenthal (Labonard)	Thiamiral (Isosol)
Duration of action	Short-acting type	Short-acting type	Short-acting type	Short-acting type
Formulation	Liquid (5%)	Dissolution type powder formulation before use vial bottle	Dissolution type powder formulation before use glass ampoule	Dissolution type powder formulation before use vial bottle
Liquid volume	50mL(5%)	4mL(0.2g, 5%)	20mL(0.5g, 5%)	50mL(0.5g, 5%)
Solution stability	3 years at room temperature	24 hours at room temperature: 99.5% 24 hours refrigerated: 99.4%	24 hours at room temperature: 99.1% (Out of standard for 24 hours due to increased related substances) 24 hours refrigerated: 99.9%	24 hours at room temperature: 99.7% 24 hours refrigerated: 96.1%
Additives	Ethanol, propylene glycol pH adjuster	none	Sodium carbonate, sodium chloride, pH adjuster	Sodium carbonate pH adjuster
pH	9.5(9.4)	9.7～11.0(10.2)	10.2～11.2(10.9)	10.5～11.5(11.0)
Description (vi, im)	-			Same as left
Laws and regulations	(Powerful drug, psychotropic drug (Type2), habit-forming drug)	(Powerful drug, psychotropic drug (Type1), habit-forming drug)	Powerful drug, habit-forming drug	Powerful drug, habit-forming drug
Caution			Im:2% solution	Im:2-2.5% solution

Koji Hanai, Tadashi Okamura, Tsutomu Kurosawa, Domestic regulation and ethical issues of barbituric acid derivatives used for euthanasia, LABIO 21, SEP 2020, p17-21
Sachiko Akagi, Haruko Hirayama, Katsumi Momiki, Usefulness of secobarbital in euthanasia, LABIO 21, SEP 2020, p17-21、p22-26 "LABIO21" search by：http://www.nichidokyo.or.jp/labio21.html

Domestic regulation and ethical issues of barbituric acid derivatives used for euthanasia

・Pentobarbital (PB) is a superior euthanasia drug due to its potent central nervous, cardiovascular and respiratory depressant effects and long duration of action.

→ Sales of Nembutal were discontinued in 2007, followed by Somnopentyl in 2019, making it impossible to obtain pharmaceutical-grade PB in Japan.

・Although overdose of secobarbital is also an acceptable euthanasia method without additional conditions, it is classified as a class 1 psychotropic drug in Japan (PB is a class 2 psychotropic drug).

→ Type 1 and Type 2 are classified according to medical value, and although regulations differ when importing and exporting, there is no difference in regulations when using them for research, requiring stricter control than Type 3 psychotropic drugs.

・Because pharmaceutical PB is very expensive in the US, the use of non-pharmaceutical grade PB is approved by multiple institutions.

→The use of non-pharmaceutical grade PB needs to be thoroughly discussed by the Animal Care and Use Committee, etc. There must be a reason for its use, and it is not an unconditional recommendation.

・If you choose a more regulated drug, you may be held accountable. 。

→ Other drugs are not suitable. Careful consideration should be given to the benefits, etc. from pharmacological and pharmacological effects.

Euthanasia of small rodents

Euthanasia refers to the act of inducing rapid unconsciousness and death in experimental animals without pain. Animal experimenters performing euthanasia must be properly trained and select a humane method of euthanasia according to the purpose of the experiment.

1. Cervical dislocation

If it fails, it will be unbearable pain for the experimental animal, so administer anesthetics and analgesics as much as possible. Expementors must ensure that experimenters performing cervical dislocations are well trained and that they are always performed humanely and effectively. Rats weighing 200 g or less should be tested.

2. Decapitation

The Principal experimentors must ensure that the workers are adequately trained and consider the mental stress of the workers.

3. Pentobarbital overdose

Administer 3-4 times the anesthetic dose or 100-150 mg/kg intravenously or intraperitoneally. It is a psychotropic drug, so be careful when storing it.

Euthanasia of small rodents

4. Inhalation anesthetic

Isoflurane and sevoflurane are recommended. Halothane is a fast-acting anesthetic and the most effective inhalational anesthetic for euthanasia, but is not recommended because it is highly hepatotoxic and accidental inhalation may cause liver damage. Diethyl ether is flammable and explosive and should not be used.

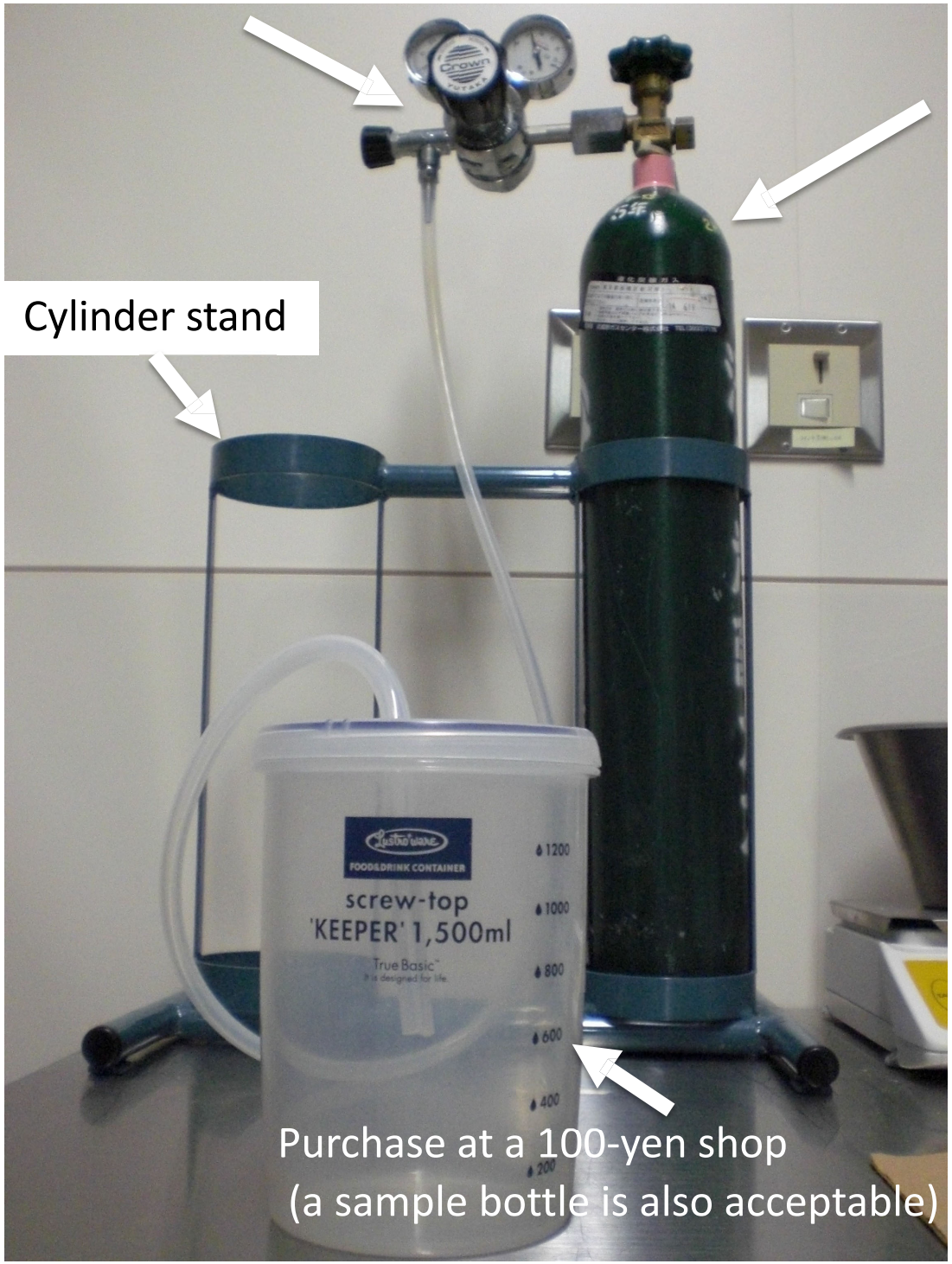
5. Carbon dioxide

Since carbon dioxide has a fast-acting anesthetic effect at high concentrations, loss of consciousness occurs first, and then death occurs due to hypoxia in the unconscious state. At this time, there are many discussions from the viewpoint of animal welfare about what conditions the carbon dioxide gas concentration in the chamber should be, and a complete conclusion has not been reached.

The optimal flow rate is one that replaces 30-70% of the chamber volume per minute, and the carbon dioxide flow continues for at least 1 minute after death is confirmed. When the animal is removed from the container, death is rechecked and other euthanasia methods are performed if the animal has not died.

Newborns are more resistant to hypoxia and therefore take longer to euthanize than mature animals. Carbon dioxide cylinders are the only recommended source of carbon dioxide; dry ice, fire extinguishers, or chemical reactions should not be used as carbon dioxide sources.

Carbon dioxide regulator



Carbon dioxide cylinder
2.5kg for mice, 7kg for rats

Silicon tube

Fetal/neonatal anesthesia in rodents

Early maturing species (guinea pigs) and late maturing species (mice, rats, hamsters, etc.) differ in the state of development of the central nervous system.

Animal species	Fetuses/Newborns under 7 days of age	Newborns over 7 days of age
Mouse, rat, hamster	Unnecessary	Necessary
Guinea pig	Necessary from fetus after 34 days of gestation	

Recommended anesthesia

1. Use of inhaled anesthetics such as isoflurane and sevoflurane
   (Consider that it takes time to reach the anesthesia stage)
2. Hypothermia without direct contact of the fetus with a cold source
3. Use of fetal injectable drugs
   (Since liver function may not be fully developed, consideration should be given to dosage and usage.)

Statement on analgesia, anesthesia and euthanasia of fetal and neonatal rodents (2nd edition, 2015)
https://jalam.jp/htdocs/index.php?key=jonyq7toz-1209#_1209

Fetal and neonatal euthanasia of rodents

(1) Pregnancy fetus

Fetuses in the first trimester of pregnancy do not have the ability to sustain life on their own, so there is no need to euthanize them. Do not take measures to prolong the time until death.

(2) Fetus in the third trimester of pregnancy

Injection anesthetic overdose and chemical or physical methods under deep anesthesia are recommended for fetal euthanasia in the third trimester of pregnancy. However, if the fetus is euthanized with the mother, it is not necessary to remove it from the mother to actively euthanize the fetus after euthanizing the mother. However, it should be noted that it takes time for the fetus to die after the death of the mother (if the mother under anesthesia is euthanized by exsanguination, the fetus should be left in the uterus for at least 5 minutes after the bleeding stops). Fetuses such as mice and rats do not perceive pain or discomfort even after removal from the mother's body, so there is no need to consider pain relief. On the other hand, guinea pig fetuses, like adults, should be euthanized under anesthesia unless scientifically necessary by the Animal Care and Use Committee.

(3) Newborn

When euthanizing neonates such as guinea pigs or neonates such as mice and rats older than the age of pain perception (7 days after birth), similar to adult animals, injection anesthetic overdose or deep anesthesia should be used. Chemical or physical methods are recommended. In neonates such as mice and rats, euthanasia with inhalation anesthetic alone is not humane, considering the time to death It should be noted that neonates less than 7 days of age, such as mice and rats, do not perceive pain or discomfort in the same way as fetuses of the same species, so there is no need to consider pain relief.

Statement on analgesia, anesthesia and euthanasia of fetal and neonatal rodents (2nd edition, 2015) https://jalam.jp/htdocs/index.php?key=jonyq7toz-1209#_1209

Compassion Fatigue(CF)

- A condition characterized by mental and physical exhaustion leading to a diminished ability to feel empathy and compassion for others, often expressed as the negative price of Compassion.
- Burnout (BO) and Secondary Traumatic Stress (STS) are included.

Nursing terminology created in the early 1990s

- Workers of various occupations may fall into this category, including veterinarians and people engaged in the management and breeding of experimental animals.
- It is generally believed that stressful behaviors such as animal euthanasia are the cause of CF.

JALAM Academic Assembly Committee

Who is improving animal welfare for?

Risk factor survey

Front. Vet. Sci.,05 March 2020
https://doi.org/10.3389/fvets.2020.00114

- surveying risk factors in US and Canadian animal research workers
- A total of 801 people responded to the online survey

Items highly related to CF(BO,STS)

- Poor social support
- Severe animal stress/distress
- Uncontrolled euthanasia practices
- Diversity and infrequent enrichment
- Desire to introduce more enrichment
- Use of physical euthanasia method (BO)
- Sex (BO)
- High human-animal interaction (STS)

Cervical dislocation or decapitation

Compassion Satisfaction

Items highly related to CS

- Abundant social support
- Less stress/pain for animals
- High human-Animal interactions

Items with little relation to CF(BO,STS)

- Type of breeding animal
- Frequency of euthanasia