## 実験動物の安楽死処置法英語版 Euthanasia Procedures

When disposing of laboratory animals on completion of the experiment in accordance with the animal experiment protocol or due to the laboratory animals being subjected to severe pain and suffering during the course of the experiment when anesthetics and analgesics can not be used in the research, the researcher(s) should conduct euthanasia.

Selection of the agent and method used for the euthanasia procedure depends on the animal species and the objective of the experiment. In general, a chemical method (overdose of a barbiturate anesthetic, administration of a non-explosive inhalation of anesthetic or carbon dioxide gas) or a physical method (cervical dislocation, decapitation, exsanguination under anesthesia, etc.) is used. However, from the standpoint of animal welfare, the principal investigator should seek the advice and guidance of a laboratory animal specialist as required since there are slight international differences on what are judged to be appropriate methods of euthanasia for laboratory animals.

\* Euthanasia procedures refer to procedures resulting in the rapid loss of consciousness and then death of a laboratory animal not associated with pain or suffering. In addition to Guidelines on Methods of Sacrificing Animals (Notice No.40 of the Prime Minister's Office, July 4, 1995), international guidelines should be taken into consideration.

\* Euthanasia should be performed by methods that do not cause distress to other animals in the laboratory. This requires careful attention because until animals lose consciousness they can vocalize and release pheromones.

\* A person who has acquired the skills required for handling a particular animal species should conduct euthanasia procedures, and the death of the animal should always be verified.

\* Confirmation of death is very important. A mistake of dose is occasionally occurred.

## A chemical method;

\* Overdose administration of Barbituric acid derivatives is applicable to many species of animals such as mice, rats, rabbits, dogs, pigs, monkey so on when administered IV, intraperitoneal or intracoelomic. Sodium pentobarbital (100-150mg/kg) best fits a rapid onset of action, and loss of consciousness in minimal or transient pain associated with venipuncture. However, Pentobarbital is no longer manufactured or sold and cannot be used. As a substitute for Pentobarbital, Rabonal (sodium thiopental) 100-150 mg/kg, Ional (sodium secobarbital) 150 mg/kg, or Isosol (thiamylal sodium) 150-200 mg/kg might be administered intravenously or intraperitoneally. Due to the high alkalinity (pH around 10) of barbiturate anesthetics, routes other than intravenous administration are conditionally acceptable.

\* Overdose administration of a non-explosive inhalation of anesthetic gas can be carried out with isoflurane or sevoflurane. As rabbits will struggle and breath-hold when confronted with any unpleasant odors of halogenated anesthetics, it is necessary to preanesthetize the animal with a sedative. Rabbits are usually sacrificed with excessive amounts of barbiturate anesthetics.

\* Carbon dioxide is also commonly used for euthanasia. An optimal flow rate for CO2 euthanasia systems should displace 30% to 70% of the chamber or cage volume/min using a 100 % CO<sub>2</sub>. Whenever gradual displacement methods are used, CO2 flow should be maintained for at least 1 minute after respiratory arrest to ensure that they are dead.

Carbon dioxide has low anesthetic effect and animals finally die with suffocation in 100% CO<sub>2</sub>. Direct exposure with 100% CO<sub>2</sub> suffocates animals with cruelty. Therefore, animals should be anesthetized with low dose of CO<sub>2</sub> at first stage and gradually raised to high dose, and finally reached 100% CO<sub>2</sub> for suffocation.

## A physical method;

\* Cervical dislocation is carried out by stretching the animal and rotating the neck. The spinal cord is disrupted and nerve impulses to the vital organs such as the respiratory system and the heart are no longer transmitted. This method is an applicable in mice, rats, hamsters, gerbils, kittens an

d small birds, but not in larger animals. If it is done quickly and expertly it is a painless method.

\* Decapitation can be performed by using scissors or a guillotine.

\* Exsanguination from a cut through the carotid arteries under anesthesia is applicable to large animal such as dogs and pigs.

Reference: AVMA Guidelines for the Euthanasia of Animals: 2020 Edition