

## Refresher Training on Inhalational Methods of Euthanasia in Rodents

All rodent euthanasia must be performed by trained and certified personnel listed on the IACUC protocol. All euthanasia procedures must be continuously monitored by the person(s) performing the procedure, until death is confirmed. **Only those methods** of euthanasia listed and **approved** on the IACUC-approved protocol to which the animal is assigned may be used.

### Isoflurane Euthanasia in Rodents

Animals **must not be euthanatized in animal housing rooms** except during special circumstances such as during quarantine and/or exposure to infectious agents in biocontainment.

The isoflurane vaporizer must be in-date and the anesthesia machine must have a gas-scavenging system that is appropriately maintained (e.g., scavenging cannisters have their weights checked at appropriate intervals and recorded).

For quad-style anesthesia machines, adjust the isoflurane **concentration to 5%** or greater and the oxygen flow rate to **~0.5 liter/minute per chamber**. Place animal in the quad-style anesthesia chamber, observe for loss of consciousness.

**Isoflurane flow should be maintained for at least one minute after cessation of cardiovascular and respiratory movements.**

**Death must be verified** by the assurance of the cessation of respiratory and cardiovascular movements by employing a **secondary method** of euthanasia such as cervical dislocation, decapitation, or bilateral thoracotomy prior to carcass disposal.

### Carbon Dioxide Euthanasia in Rodents

To reduce stress, rodents should be euthanatized by CO<sub>2</sub> in their **home cage** whenever possible. This can be accomplished by delivering CO<sub>2</sub> inside a Euthanex machine chamber, via a Euthanex lid over the home cage after removing the filter and wire tops, or via the air supply port of an IVC cage.

CO<sub>2</sub> delivery to the home cage is accomplished by turning the CO<sub>2</sub> cylinder valve and flow meter on so that animal(s) are **slowly exposed to increasing levels of CO<sub>2</sub>** which will displace 30-70% of the cage volume/minute.

**CO<sub>2</sub> flow should be maintained for at least one minute after cessation of cardiovascular and respiratory movements.**

**Death must be verified** by the assurance of the cessation of respiratory and cardiovascular movements by employing a **secondary method** of euthanasia such as cervical dislocation, decapitation, or bilateral thoracotomy prior to carcass disposal.

Chambers/lids must be cleaned/dried between animals or groups of animals.