7. Mice CO₂ euthanasia _ Support doc_01

Animals must not be left unattended until death is confirmed.

7.1 Material

- 7.1.1 Compressed CO₂ is the only acceptable source of CO₂ to euthanize rodents.
- 7.1.2 Euthanasia station
 - Automated CO2 Euthanasia station
 - Scissors
 - Records

7.2 Species

Mice of the genus Mus.

7.3 Procedure

- 7.3.1 Start the laminar flow hood.
- 7.3.2 Open the CO2 tank
- 7.3.3 Dock the cage in the automated station by sliding it in between the guiding rails onto the cage adapters. Secure locking of the IVC produces a distinct clicking noise.
 - 7.3.3.1 mice are euthanized in their home cage.
 - 7.3.3.2 Pooling of animals from different cages is not allowed
 - 7.3.3.3 Following the manufacturer's user manual, a maximum of 6 animals per cage must not be exceeded to ensure a safe euthanasia.
- 7.3.4 If the light on the IVC adapters light up red, the station is in operating mode and CO2 is flowing in the IVC
- 7.3.5 Wait for the automated euthanasia program to be completed, indicated by the signal lights turning off.
 - 7.3.5.1 Mice must stay under observation during the euthanasia process
- 7.3.6 Check death (no breathing, recumbency).
- 7.3.7 Remove the IVC from the station. This will automatically start the "after run control" mode of the station. The start of this mode is indicated by flashin of the left and right adapters. Remaining CO2 is flushed out of the machine during this process.
- 7.3.8 When the mode is completed, the station automatically goes in standby mode, indicated by a faint red glow of both cage adaptors.
- 7.3.9 Confirm death
 - 7.3.9.1 Decapitation, exsanguination, perfusion, organ harvesting, bilateral pneumothorax, rigor mortis
- 7.3.10 Close the CO2 tank
- 7.3.11 Clean the working place and dispose the cadavers
- 7.3.12 Record the euthanized animals on the Euthanasia Form and in Pyrat

7.4 Mechanism of action

Inhalation of CO_2 at concentrations of 30% or higher causes respiratory acidosis and produces a reversible anaesthetic state by rapidly decreasing intracellular pH; this induces an allosteric change in ion channels with cellular dysfunction. Death occurs with prolonged exposgre because of acidosis, hypoxia and secondary cardiovascular failure with heart arrest. Nevertheless, if animals are prematurely removed from the chamber prior to death they can recover to consciousness (AVMA). Therefore, all animals being euthanized with CO2 overdose must also receive a confirmatory method of euthanasia to ensure death.

CO₂ has the potential to cause distress via three different mechanisms: (1) pain due to formation of carbonic acid on respiratory and ocular membranes, (2) production of a feeling of "breathlessness" and (3) direct stimulation of ion channels within the amygdala, believed to be associated with the fear response. The discomfort associated with CO₂ is believed to occur starting at approximately a 15% inhaled concentration (AVMA). Potential pain and distress caused by CO₂ inhalation can be mitigated if the animal loses consciousness before the chamber is at 15% concentration. Therefore, a gradual displacement of room air with carbon dioxide into the chamber will cause the animal to lose consciousness before the CO₂ is aversive. A gradual fill rate of 30% chamber volume per minute displacement is expected at all rodent euthanasia locations. (AVMA) With the GasDocUnit, CO2 and room air are mixed within the operating unit and then introduced into the docked IVC. The GasDocUnit ensures a gradientless distribution of CO2 within the IVC. The animals are euthanized compliance with the AVMA Guidelines of 2020, the German Animal Welfare Ordinance (TierSchG) and the Ordinance on the Protection of Laboratory Animals (TierSchVersV). After the animals have become unconscious, the flow rate can be increased to 100% replacement rate/min to minimize the time of death.

7.5 Calculation

To calculate the flow rate of gas for a 30% displacement per minute:

- Chamber Volume (in L) = ((height in cm) x (width in cm) x (length in cm))/1000
- Acceptable flow rate (in L/min) = (Chamber Volume in L) X 0.30 / min

7.6 Neonates

Resistance to hypoxia results in a prolonged time to unconsciousness when CO_2 inhalation is used as a euthanasia agent in neonatal rodents. Therefore, CO_2 is considered distressing and ineffective and is not admissible for euthanizing neonates and pups (\leq 14 days)

7.7 Signage

Laboratory staff post pictorial instructions at the site of the euthanasia station on how to operate the equipment and ensure death of animals.

7.7.1	Start the laminar flow hood.	
7.7.2	Open the CO ₂ tank valve. Don't change the settings of the pressure control unit!	
A- B- C-	CO₂ tank Valve Pressure control unit (4 bar)	
7.7.3	 Dock the cage in the automated station. Secure locking of the IVC produces a distinct clicking noise. 7.7.3.1 mice are euthanized in their home cage. 7.7.3.2 Pooling of animals from different cages is not allowed 7.7.3.3 Following the manufacturer's user manual, a maximum of 6 animals per cage must not be exceeded to ensure a safe euthanasia. 	
7.7.4	If the light on the IVC adapters light up red, the station is in operating mode and CO2 is flowing in the IVC	

7.7.5 7.7.6 7.7.7	Wait for the automated euthanasia program to be completed, indicated by the signal lights turning off. 7.7.5.1 Mice must stay under observation during the euthanasia process Check death (no breathing, recumber Remove the IVC from the station. Thi	http://www.intercontent/action.com/intercontent/action
	start of this mode is indicated by flash machine during this process.	nin of the left and right adapters. Remaining CO2 is flushed out of the
7.7.8	When the mode is completed, the station automatically goes in standby mode, indicated by a faint red glow of both cage adaptors.	
7.7.9	Confirm death 7.7.9.1 Decapitation , exsanguination, perfusion, organ harvesting, bilateral pneumothorax , rigor mortis	
7.7.10	Close just the valve on the tank.	
7.7.11	Record the euthanized animals on the	e Euthanasia Form and in Pyrat
7.7.12	Clean the working place, and dispose the cadavers	