Humane Euthanasia Guide

In this guide you will find all of the information required for the humane euthanasia of “SMALL LABORATORY AND WILD-CAUGHT RODENTS (MICE, RATS, HAMSTERS, GUINEA PIGS, GERBILS, DEGUS, COTTON RATS, ETC)” according to the guidelines set out by the American Veterinary Medical Association (AVMA). The full text can be found here; relevant sections will be copied verbatim in this guide:
https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf

There are a number of methods laid out in the full guidelines list, but many of these are not possible for the average owner or breeder due to requiring restricted or prohibitively expensive equipment or drugs. The methods most accessible will be covered here with links to items that can be purchased where relevant.

CO_2 - Carbon Dioxide

In Section 2.2.2, carbon dioxide is discussed and described as follows:

> *Carbon dioxide*—Carbon dioxide, with or without premedication with halogenated anesthetics, is acceptable with conditions for euthanasia of small rodents.[58] Compressed CO_2 gas in cylinders are the recommended source of CO2 because gas inflow to the chamber can be precisely regulated. An optimal flow rate for CO_2 euthanasia systems should displace 30% to 70% of the chamber or cage volume/min, with the understanding that there is potential for increased distress due to dyspnea at lower flow rates or mucous membrane pain associated with flow rates at the high ends of this range.[31,32,54,56,59–64] However, as there is no clear evidence of a flow rate that optimally minimizes both pain and distress for all species, sexes, and genetic backgrounds, veterinarians should use their professional judgment to determine which flow rate is appropriate for their circumstances.[31,32,56] Prefilled chambers are not recommended due to the potential for significant pain upon inhalation of the gas.[60,65] If euthanasia is not conducted in the home cage, induction chambers should be emptied and cleaned between uses. Addition of O_2 to CO_2 will prolong the time to death and may complicate determination of consciousness. There appears to be no advantage to combining O_2 with CO_2 for euthanasia.[11,60]

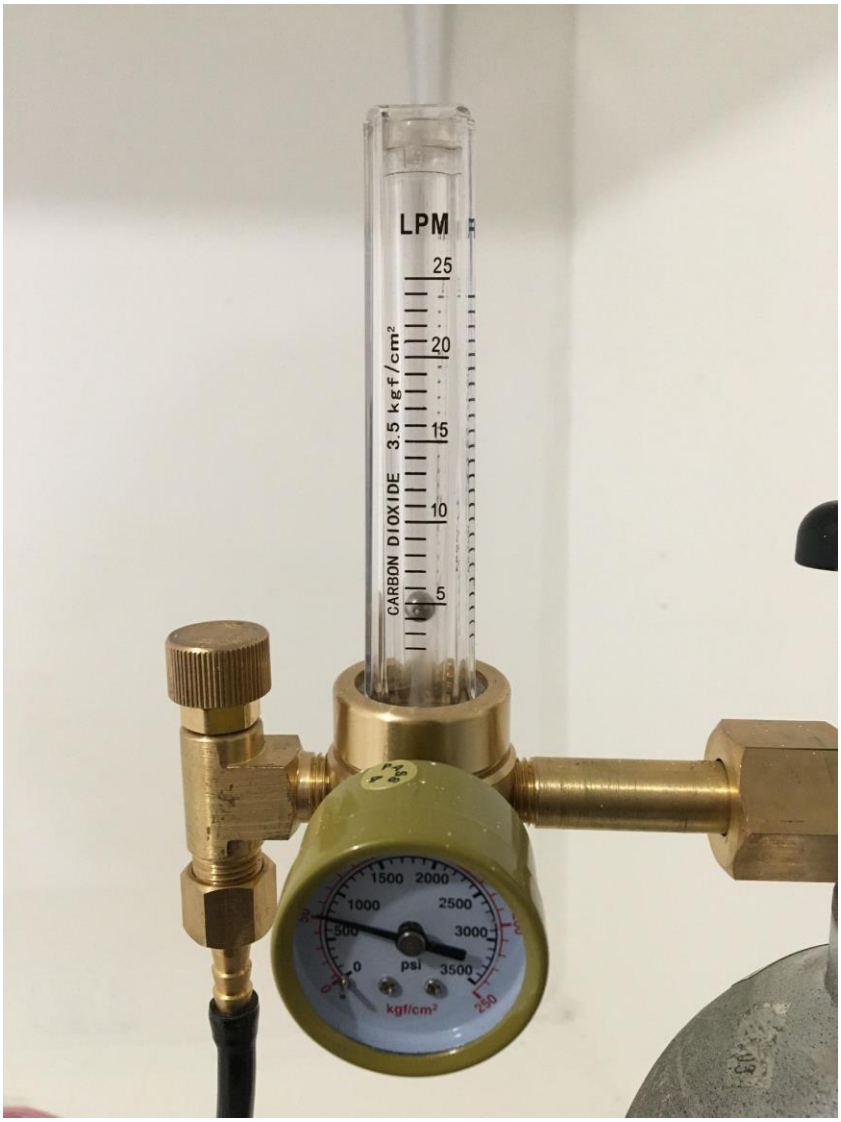
In order to be truly humane, this indicates that a regulator that provides you with the actual flow rate of CO_2 **must** be used in conjunction with the cylinder of CO_2 gas. Use of bike pumps, small CO_2 cartridges, SodaStream (or similar) conversions, or any of the chemical methods involving dry ice or baking soda/vinegar are **INHUMANE** and will not be allowed to be discussed in the group (comments will be removed and repeat offenders will be removed from the group). None of those processes can be accurately measured to provide the required 30-70% volume/minute replacement necessary to be humane. Uncontrolled release methods where the gas is released by pressing a button or fully opening a valve are very high-flow and regardless of chamber size are almost certainly out of the range for humane euthanasia.

The necessary components for a humane CO_2 chamber are the following:

1. A CO_2 tank
    a. Unless you need to euthanize on a very small scale, the price is likely cheapest to get a 5lb or larger capacity cylinder. These can be obtained cheapest from a local air supply, paintball, homebrewing, or liquor store. This is a one-time purchase item as refills for these are typically exchanged for a newer, full cylinder so the safety testing is continuously up to date at a much lower price than the initial purchase price. (Paintball canisters will need to be replaced every few years as the safety testing/inspection expires)
    b. An example from Amazon: https://www.amazon.com/lb-Aluminum-CO2-Cylinder-Valve/dp/B00UXJ9SS8/
    c. The exchange price is typically much lower than purchase (as an example, my 5lb tank cost about $90 at a local liquor store whereas exchanges for full tanks are around $18 - this will vary based on where you purchase and where you do exchanges)
2. A euthanasia chamber
    a. For this, any plastic container with sufficient size will work. Consider how many at a time you will need to euthanize and how much volume you will be using each time. Many prefer gasket-sealing containers and add a small escape hole for the O_2, others just use non-air-tight containers and let the edges of the lid allow the O_2 to escape.
3. A regulator that indicates the flow rate
    a. This is the **vital** piece of equipment that lets you determine you are in the humane range of air flow. Many also will indicate the level of gas left in the tank so you will not run out of gas partway through.
    b. **Look to ensure your CO_2 cylinder has the same type of connector as the regulator you want to purchase.** (The 5lb tank in this example and the example regulator below are both CGA320 valves - paintball tanks often have a different connector and may require an adapter to use the example regulator included here)
    c. This is a good option, the ball will float to the flow rate and you can use our **calculator** to determine the rate you should use for your container: https://www.amazon.com/gp/product/B07JNF21DK
        i. If the above is not in stock - instead a CGA580 regulator can be purchased and an adapter to CGA320 such as the following:
        ii. Adapter: https://www.amazon.com/CGA-320-CGA-580-Adapter-Dioxide-Flowmeter/dp/B06W5P767K
        iii. Regulator: https://www.amazon.com/BETOOLL-HW9003-Argon-Regulator-Welding/dp/B01I67VKFW
4. Tubing
    a. This is simply to connect your regulator to your tub. This can be acquired at a hardware store pretty cheaply - bring your regulator to find the exact fit.

b. The regulator above calls for ¼" Inner Diameter (ID) tubing - this can be friction fit over the output fitting and then inserted into a snug hole in the chamber or a barbed fitting attached to the chamber for this purpose.
c. The tube can be connected to the tub by slipping into a tight hole, by siliconing or hot gluing it into a hole, or by using hardware to create a perfect seal. This is not entirely necessary, but may help reduce any gas waste.

In assembly, you will want to place a hole or some method for the O_2 to escape at the top, as oxygen is lighter than carbon dioxide and will be replaced from the bottom up in the chamber during use. The CO_2 entry can be down low or from above, this is personal preference since the gas will sink to the bottom due to its higher molecular weight than the oxygen in the chamber to start.



The image above shows the flow meter in action - this particular meter is set to ~5 liters per minute, as indicated by the ball bearing hovering at that mark. The pressure gauge with the

needle below shows the pressure from the CO2 canister itself. The small knob on the left of the device is used to adjust the flow.

This is an example of a full CO2 setup. The CO2 canister is on the right with the attached flow meter. Then the tubing runs into the tub. It is ideal to use some form of substrate as rodents will defecate after death when their muscles around the bowels relax. This specific setup is using the oxygen tube to let the oxygen escape the airtight container; to prevent any oxygen from re-entering the container, the end of the tube can be placed into a cup of water. Note that the oxygen exit hole is at the highest point of the container!

Cervical Dislocation

In Section 2.2.2, cervical dislocation is discussed and described as follows:

> *Cervical dislocation*—Cervical dislocation is used in laboratory settings. Cervical dislocation requires neither special equipment nor transport of the animal and yields tissues uncontaminated by chemical agents. Loss of cortical function following cervical dislocation is rapid and occurs within 5 to 10 seconds as measured by a significant reduction in amplitude recordings of visual evoked responses and EEG.[76,77] Cervical

> dislocation is acceptable with conditions for mice and rats < 200 g. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency

The best way to become proficient in cervical dislocation is through being taught by an experienced mentor and to practice on pre-killed animals until you are confident you will not cause undue stress or pain to a live animal. If you cannot have a mentor come demonstrate and teach you, we highly recommend proceeding with CO_2 as your primary euthanasia method or at the very least practice yourself on pre-killed animals extensively before moving to live animals.

Freezing as Euthanasia for Rodents < 10 Days

In Section S2.2.4.2 Fetuses and Neonates, the following is given as an allowable method of euthanasia for fetuses/neonatal rats and mice; we have not found any information to support or deny whether this is suitable for ASF specifically since the section refers explicitly to mice and rats.

> *Hypothermia*—The gradual cooling of fetuses and altricial neonates (mice and rats) is acceptable with conditions. There are no data to support the use of hypothermia as a single method, and it should be followed with a secondary method following loss of movement. As cold surfaces can cause tissue damage and presumably pain, the animals should not come in direct contact with ice or precooled surfaces. Hypothermia for anesthesia is not recommended after approximately 10 days of age. [53,88–90,95–97] Therefore, it is also an unacceptable euthanasia method in animals older than this age.[98]

What this means is that until 10 days of age, babies can be euthanized humanely by being placed in a room temperature container with something insulating them from the container itself (a layer of bedding, a hand towel, etc.) so that they are cooled gradually without contact with a freezing surface. It should be noted in section S2.2.4.1 regarding inhaled anesthetics for neonates that it can take 35-50 minutes for rats and mice in this age range to pass when using CO2.