EUTHANASIA IN AVIANS

DRUGS/SUBSTANCE USED	DOSAGE	ROUTE	NOTES
CARBON DIOXIDE (CO_2) (Acceptable with conditions)	10% to 30% of the chamber or cage volume/min	INHALATION	- Birds will be placed in a non-precharged chamber and 100% CO_2 will be introduced at the rate of 10-30% of the chamber volume per minute as regulated by a flow meter attached to the CO_2 canister. Death will be confirmed post-exposure. Eggs, embryos, and neonates will be placed in a non-precharged chamber and 100% CO_2 will be introduced at the rate of 10-30% of the chamber volume per minute as regulated by a flow meter attached to the CO_2 canister. They will remain in the CO_2 chamber for >20 minutes to ensure euthanasia.
PENTOBARBITAL SODIUM (Acceptable method)	120 mg/kg for the first 4.5 kg of body weight 60 mg/kg per 4.5 kg of body weight thereafter	IV	-Administered IV may be given alone as the sole agent of euthanasia or as the second step after sedation or general anaesthesia.
POTASSIUM CHLORIDE (Adjunctive method)	75-150 mg/kg	IV/IC	May only be used when animals are under general anaesthesia or otherwise unconscious. Use of potassium chloride in awake animals is unacceptable.
CERVICAL DISLOCATION (Acceptable with conditions)	Not applicable	PHYSICAL METHOD	-Cervical dislocation has generally been used for small birds (<200 g) when no other method is available, but the procedure has been performed on birds as large as 2.3 kg. -When performed on conscious poultry, cervical dislocation must result in luxation of the cervical vertebrae without primary crushing of the vertebrae and spinal cord. Manual or mechanical cervical dislocation may be used for poultry of an appropriate size and species when performed by competent personnel who correctly apply the technique. In some classes of poultry there is evidence that cervical dislocation may not cause immediate

			unconsciousness. The legs of the birds should be grasped (or wings if grasped at the base) and the neck stretched by pulling on the head while applying a ventrodorsal rotational force to the skull. Crushing of cervical vertebrae and spinal cord is not acceptable unless the bird is first rendered unconscious.
DECAPITATION (Acceptable with conditions)	NOT APPLICABLE	PHYSICAL METHOD	Decapitation is considered to be acceptable with conditions for euthanasia of small (<200 g) birds. -Birds will be euthanized via decapitation with a sharp instrument. A bleeding cone may be utilized to assist with restraint of the birds.
PENETRATING CAPTIVE BOLT (Acceptable with conditions)	NOT APPLICABLE	PHYSICAL METHOD	-Penetrating captive bolts are acceptable with conditions for euthanasia of large poultry (turkeys, broiler breeders, ratites waterfowl, etc) when performed by competent personnel. The captive bolt pistol must be used in accordance with the manufacturer's recommendations and the bird should be appropriately restrained to avoid injury to personnel. Birds should be observed following captive bolt administration to ensure that death occurs. Any bird showing signs of recovery must receive a second shot or be killed by some other means that is acceptable for a conscious bird.
COOLING OR FREEZING (Acceptable with conditions)	<40˚F (4˚C) FOR 4 + hours	PHYSICAL METHOD	-Embryonated eggs less than 50% incubation will be euthanized by being placed in a less than 40˚F (4˚C) environment for 4+ hours.
MACERATION (Acceptable with conditions)	NOT APPLICABLE	PHYSICAL METHOD	Newly hatched poultry and embryonated eggs will be placed into a specially designed macerator, following manufacture recommendations of the equipment, to be euthanized.
ADDLING (Acceptable with conditions)	NOT APPLICABLE	PHYSICAL METHOD	Embryonated eggs less than 50% incubation will be vigorously

			shaken to disrupt the embryo and cause euthanasia.
EXSANGUINATION (Adjunctive method)	NOT APPLICABLE	PHYSICAL METHOD	Once the birds are fully unconscious or anesthetized as determined by loss of pedal withdraw reflex, they will be euthanized via severing of a major blood vessel.

Foot note:

- Wild, fearful, or excited birds may require a sedative or anaesthesia before IV injection can be performed. When IV injection is impossible, injectable euthanasia agents can be administered via intracoelomic, intracardiac, or intraosseous routes only if a bird is unconscious or anaesthetized. If the intracoelomic route is used for birds, injection into the air sacs must be avoided, because of the potential for respiratory compromise, irritation of the respiratory system, and delayed absorption via the air sacs. Euthanasia agents should also not be administered via the intraosseous route into the humerus or femur because of the potential for drowning or irritation to the respiratory system.

- Bird embryos that have attained >50% incubation have developed a neural tube sufficient for pain perception, thus they should be euthanized by similar methods used in avian neonates such as anaesthetic overdose, decapitation, or prolonged exposure to CO_2 (>20 mins).

EUTHANASIA IN CAPTIVE AMPHIBIANS AND REPTILES

DRUG/SUBSTANCE USED	DOSAGE	ROUTE	NOTES
SODIUM PENTOBARBITAL **(Acceptable method)**	60-100 mg/kg	IV, intracoelomic, in the subcutaneous lymph spaces, or in the lymph sacs	-
SODIUM PENTOBARBITAL + SODIUM PHENYTOIN **(Acceptable method)**	1,100 mg/kg	IV, intracoelomic	-Concentration : Pen: 390 mg/ml Phe : 50 mg/ml -Doses as high as 1,100 mg/kg of sodium pentobarbital with sodium phenytoin administered intracoelomically may be required for euthanasia of some species such as *Xenopus laevis*
TRICAINE METHASULFONATE (MS222) **(Acceptable method)**	5-10g/L	IMMERSION	-Concentration : 99.5% -The solution must be buffered to a neutral pH with sodium bicarbonate. A 10 g/L stock solution can be made, and sodium bicarbonateadded to saturation, resulting in a pH between 7.0 and 7.5 for the solution. -Frogs will be placed into a buffered solution of MS222. They will remain in the solution for at least 60 mins. This procedure will be followed by a physical adjunctive method to ensure euthanasia.
BENZOCAINE HYDROCHLORIDE **(Acceptable method)**	182 mg/kg	TOPICAL	-Concentration : 200 mg/ml -Frogs will be manually restrained and 20% gel benzocaine hydrochloride will be applied to the frogs ventrum at calculated dose. Frogs will be placed in a wet bucket without water till deep anaesthesia or euthanasia is ensured. This procedure will be followed by a physical

			adjunctive method to ensure euthanasia.
DECAPITATION + PITHING **(Adjunctive method)**	NOT APPLICABLE	NOT APPLICABLE	-Frogs will be anesthetized first. Once there is no response to pinching the rear foot webbing, frogs will be decapitated and then brain tissue will be destroyed via pithing to ensure euthanasia.
PITHING **(Adjunctive method)**	NOT APPLICABLE	NOT APPLICABLE	- Frogs will be anesthetized first. Once there is no response to pinching the rear foot webbing, frogs will be decapitated and then brain tissue will be destroyed via pithing to ensure euthanasia. -the pithing site in frogs is the foramen magnum and it is identified by a slight midline skin depression posterior to the skull, midline between the eyes, with the neck flexed.
RAPID FREEZING **(Acceptable with conditions method)**	< 4 g (0.1 oz) in liquid N_2	NOT APPLICABLE	-Reptiles and amphibians can be euthanized by rapid freezing when it results in immediate death. -The technique should not be used for species that have adapted freeze tolerance strategies, as this method may not result in instant death. -Placement of animals ≥4 g in liquid N_2 or other uses of hypothermia are not acceptable.

Foot note:

- Intracardiac administration of euthanasia agents is acceptable for captive amphibians and reptiles that are unresponsive to stimuli because of disease or the application of other euthanasia methods, or in cases where other routes are not possible.
- Many reptiles and amphibians are capable of breath holding and shunting of their blood, which permits conversion to anaerobic metabolism for survival during prolonged periods of anoxia 9up to 27 hours for some species). Because of this, induction of anaesthesia and time to loss of consciousness may be greatly prolonged when inhaled agents are used. Death may not occur even with prolonged exposure. Lizards and most snakes do not hold their breath to the same

extent as some of the chelonian, and therefore more likely to have a clinical response to inhaled agents. Carbon dioxide may be considered for euthanasia of amphibians and reptiles if alternate methods are not practical and where the limitations of this method are understood and addressed. Due to the potential lack of response to this method by many species and the requirement for a prolonged exposure time, other methods are preferable. Death by CO_2 must be verified, and preferably, assured by application of a secondary lethal procedure.

- Destruction of viable eggs:
    - Little information is available on the sensory capacity of amphibians and reptiles at the egg stage of development.
    - Freezing is likely appropriate for newly oviposited eggs, as would be methods of maceration that results in instantaneous death. Later stages may be destroyed using methods that are acceptable for adult animals.

EUTHANASIA IN FISHES

DRUG/SUBSTANCE USED	DOSAGE	ROUTE	NOTES
TRICAINE METHASULFONATE (MS222) **(Acceptable method)**	250-500 mg/L	IMMERSION	-The solution must be buffered to a neutral pH with sodium bicarbonate. A buffered solution (~7.0-7.5 pH) of MS222 will be made. Fish will be immersed in the solution for at least 10 minutes to allow for adequate contact time and to ensure euthanasia. Zebrafish embryos < 3 dpf: A buffered solution (~7.0-7.5 pH) of MS222 will be made. Embryos will be immersed in the solution for at least 10 minutes, and then a sodium hypochlorite solution (6.15%) 500mg/L is added to the solution. Embryos remain in this solution for at least 5 minutes to ensure euthanasia.
BENZOCAINE HYDROCHLORIDE **(Acceptable method)**	≥250 mg/L	IMMERSION	-A buffered solution (~7.0-7.5 pH) of benzocaine hydrochloride will be made. Fish will be immersed in the solution for at least 10 minutes to allow for adequate contact time and to ensure euthanasia.
SODIUM HYPOCHLORITE (BLEACH) **(Acceptable with conditions)**	500 mg/L	IMMERSION	-For zebrafish ≤ 3dpf to 7dpf. Addition of bleach solution (sodium hypochlorite 6.15%) to the culture system water at 1 part bleach to 5 parts water can be used for euthanasia. Exposure to sodium hypochlorite (bleach) is not recommended for samples in which DNA, RNA, or protein are required. -Embryos/ fry remain in this solution for at least 5 minutes to ensure euthanasia.
RAPID CHILLING (HYPOTHERMIC SHOCK) **(Acceptable with conditions)**	NOT APPLICABLE	IMMERSION	-A well insulated container is used to make a slurry of ice and water (target temperature 2-4°C). A depression is made in the ice to maximize contact of fish with the

			chilled water. Fish are transferred with a minimal amount of home-tank water into the depression. Adult fish are exposed for a minimum of 10 minutes and fry 4 to 7 dpf for at least 20 minutes following loss of operculum movement. Zebrafish embryos < 3 dpf: Embryos are exposed to the slurry for at least 20 minutes and then a sodium hypochlorite solution 500mg/L is added to the solution. Embryos remain in this solution for at least 5 minutes to ensure euthanasia.
DECAPITATION + PITHING **(Acceptable with conditions)**	NOT APPLICABLE	PHYSICAL METHOD	-Rapid severance of the head and brain from the spinal cord, followed by pithing of the brain, will cause rapid death and unconsciousness.
ISOFLURANE	>5 to 20 mL/L (10 times the upper range for anaesthesia)	IMMERSION	-These concentrated liquid anaesthetics can be added to water, although they are generally not very water soluble. Injecting the solution through a syringe and fine needle gauge needle under the water in the container used for euthanizing is helpful in ensuring dispersal in the water. -However, because both anaesthetics are highly volatile, human safety is of concern and use in a well-ventilated area is imperative.
SODIUM PENTOBARBITAL	60-100 mg/kg	IV, intracardiac, or intracoelomic	-As for intracardiac injection, the animals need to be anaesthetized first followed by an injection of a lethal dose of pentobarbital. Death usually occurs within 30 mins.

Foot note:

- Loss of movement, loss of reactivity to any stimulus, and initial flaccidity (prior to rigor mortis) may serve as indicators of death for finfish and aquatic invertebrates. More useful indicators for many finfish include respiratory arrest (cessation of rhythmic opercular activity) for a minimum 10 mins and loss of eyeroll (vestibule-ocular reflex, the movement of the eye when the finfish is rocked from side to side). The heart can continue to contract even after brain death or removal from the bodies of finfish, so the presence of a heartbeat is not a reliable indicator of life, but sustained absence of heartbeat is a strong indicator of death.

- Flushing of animals into sewer, septic, or other types of outflow systems is unacceptable for many reasons. Water chemistry and quality may delay time to death and result in exposure to noxious compounds. Slow chilling or freezing of unanaesthetized animals, including placing animals into a freezer without prior anaesthesia is also an unacceptable method. Similarly death by anoxia and dessication after removal from the water or by anoxia in water; any death due to exposure caustic chemicals; and death including prolonged traumatic injury prior to unconsciousness are unacceptable. While metomidate has been used for euthanasia of some finfish species, its listing in the Index of Legally marketed unapproved new Animal Drugs for Minor Species by the FDA (with a specific use for sedation and anaesthesia) means that its extralabel use for euthanasia is currently illegal.

- Early stages in the lives of finfish, including embryos and larvae, may require higher concentration of immersion anaesthetic or a longer duration of exposure. For example, immersion in a buffered MS222 solution having a concentration >1 g/L is not a reliable method for killing some finfish in younger life stages. For some species and in some situation, adjunctive method such as immersion in a dilute sodium hypochlorite or calcium hypochlorite solution is acceptable for zebrafish embryos and larvae as a second step method and is also acceptable with conditions as a second step for destruction of other (nonzebrafish) species' embryos and larvae.

REFERENCES:

- **AVMA GUIDELINES FOR EUTHANASIA OF ANIMALS: 2013 EDITION**