Laboratory Animal Euthanasia Practices and CCAC Guidelines

Laboratory Animal Euthanasia
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What is Euthanasia

- Derivation of the word “euthanasia”
  - Gr. eu = well or good; thanatos = death
  - Good death
- Definitions
  - A quiet or easy death
  - Painless death
  - Mercy killing: the act or practice of killing or permitting the death of hopelessly sick or injured individuals (as persons and animals) in a relatively painless way for reasons of mercy

What is Euthanasia

From Wikipedia:
- Animal euthanasia is the act of putting to death painlessly……an animal suffering from an incurable, especially a painful, disease or condition. Euthanasia methods are designed to cause minimal pain and distress.
- Euthanasia is distinct from animal slaughter and pest control, which are performed for purposes other than an act of mercy, although in some cases the killing procedure is the same.
What is Euthanasia

- Australian Government National Health and Medical Research Council *Australian code of practice for the care and use of animals for scientific purposes* 2004

- **Euthanasia**: the humane killing of an animal, in the interests of its own welfare, to alleviate pain and distress.

- **Humane killing**: the process of killing an animal with minimal pain and distress.

Why are laboratory animals killed?

- To collect blood, tissues for the study
- To cull animals that are no longer needed
- For non-compliance issues
- Abrupt disruption in research/research personnel (death, illness, finances, etc.)
- To prevent pain & distress when the humane endpoint is reached
- If an animal becomes injured or diseased

Humane killing of laboratory animals

- Ultimately to provide knowledge and understanding for the betterment of human- and animal-kind

- There is a greater onus on the scientific community to ensure that *when the life of an animal is taken for the betterment of society*, the animal is assured of a respectful death that is as humane as possible

- Regardless of the reason for the death, laboratory animals should experience a minimum of distress, fear, or pain when they are killed
Practical considerations

- Must consider the safety of the persons involved in the killing
- Must be performed in a way that allows for useful, appropriate information to be collected
- Must consider the sensitivities of the personnel who are involved in the killing
- Must consider the environmental impact of the method used for killing, and the disposal of the carcass
  -Secondary poisoning from barbiturates, other chemicals
  -GM animals must not enter environment or food chain

Laboratory Animals Use at the U of S (excluding large animals and free-ranging wildlife)

- Rodents (Rats, mice, guinea pigs, hamsters, gerbils)
- Rabbits
- Birds (poultry, quail, ducks)
- Amphibians (frogs, salamanders)
- Fish (zebrafish, salmonids, sturgeon)

CCAC: Acceptable Methods of Euthanasia for Fish and Amphibians

- MS 222 (TMS) immersion (or injection – amphibians); buffer at concentrations ≥ 500 mg/L
- Benzocaine immersion (or injection - amphibians)
- Etomidate (Aquacalm ®) – fish only
- Metomidate (Marinil) – fish only
- Clove oil – fish only

Above methods must be followed by a second method to ensure death such as pithing, exsanguination, a sharp blow to the head, decapitation, or severing the cervical spinal cord

- Maceration (for fish less than 2 cm in length)
CCAC: Acceptable Methods of Euthanasia for Fish and Amphibians

- Amphibians: Barbiturates injected into lymph sac
- Amphibians: Overdose of inhalant anesthetic (for species that do not breath hold)

Above methods must be followed by a second method to ensure death such as pithing, exsanguination, a sharp blow to the head, decapitation, or severing the cervical spinal cord

CCAC: Conditionally Acceptable Methods – Fish, Amphibians

- Concussion (ie blow to the head), followed immediately by physical destruction of the brain, pithing, decapitation, or freezing
- Species-specific amphibian guidelines also mention decapitation followed immediately by pithing

Methods Used at U of S

- MS 222 immersion is used on our campus for both anesthesia and humane killing of fish & amphibians
Methods Used at U of S

- MS 222 immersion is used on our campus for both anesthesia and euthanasia of fish and amphibians
- Concussion has been used in some circumstances (where chemicals would interfere with research objectives)
- Rapid freezing of larval zebrafish in liquid nitrogen has also been approved by our Animal Care Committee (although currently not listed as acceptable by CCAC or AVMA)

Current Literature – Rapid Cooling

- Recent publications support the use of rapid cooling as a humane method of killing specific types of fish:
  - Humane killing of fishes for scientific research: a comparison of two methods. Journal of Fish Biology (2016) 76, 2571 – 2577; Blessing, Marshall, Balcombe (Australia)
  - Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (Danio rerio). Journal of the American Association for Laboratory Animal Science (2009) 48(6), 785-789; Wilson, Bunce, Carty (USA)

Blessing et al (Australia)

- Compared benzocaine OD to ice-water slurry (0-2 °C) in bony bream (a small-medium sized warm-water fish)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ice-water</th>
<th>Benzocaine</th>
</tr>
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<tbody>
<tr>
<td>Loss of equilibrium</td>
<td>6 ± 1.2 sec</td>
<td>121.2 ± 78 sec</td>
</tr>
<tr>
<td>Death</td>
<td>25.4 ± 4.8 sec</td>
<td>216 ± 96 sec</td>
</tr>
<tr>
<td>Distress behaviours</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

- Provided compelling evidence favouring the use of ice-slurry over benzocaine as a method of humane killing for this specific fish
- Body size and species-specific thermal tolerance are important in determining the effectiveness of ice-slurry for the humane killing of fish
Wilson et al (USA)

- Compared immersion in ice-water (64 °C) with buffered and unbuffered MS222 overdose (250 mg/L) in zebrafish > 6 months old

<table>
<thead>
<tr>
<th></th>
<th>Ice-water</th>
<th>UMS222</th>
<th>BMS222</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of righting</td>
<td>4.6 ± 0.33 s</td>
<td>25.90 ± 2.61 s</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>7.13 ± 0.27 s</td>
<td>53.52 ± 11.32 s</td>
<td></td>
</tr>
<tr>
<td>Distress behavior</td>
<td>39%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

- Concluded that rapid cooling results in more rapid, less distressful and more effective euthanasia than MS222 in zebrafish

- All animals exposed to BMS222 displayed similar signs of distress to those treated with UMS222 at this concentration (250 mg/L)

Advantages of Ice-Water

- Safe for human exposure
- Can humanely kill many fish at once
- Minimal risk of operator error
- Readily available
- Not harmful to the environment
- Inexpensive
- Shown to be more humane than MS222 or benzocaine in some species

Implications for U of S

- MS 222 immersion will continue to be used as a method of humane killing for fish and amphibians at the U of S
  - but we should ensure a second method is used to confirm death
- Concussion and other methods will be approved if scientifically justified
  - but should stipulate brain must be destroyed immediately afterwards to confirm death
- Scientific justification and the current literature will continue to be used by the AREB to evaluate other methods, such as rapid cooling
Laboratory Animals Use at the U of S
(excluding large animals and free-ranging wildlife)

- Rodents (Rats, mice, guinea pigs, hamsters, gerbils)
- Rabbits
- Birds (poultry, quail, ducks)
- Amphibians (frogs, salamanders)
- Fish (zebrafish, salmonids, sturgeon)
- Dogs
- Cats

CCAC: Acceptable Methods of Euthanasia for Birds (chicken, pigeon, etc)

- Barbiturates injected IV or diluted, buffered and mixed with a local anesthetic and injected IP (avoiding air sacs)
- Overdose of inhalant anesthetic (for species that do not breath hold)
- Anesthesia and decapitation

Above methods must be followed by a second method to ensure death such as exsanguination, a sharp blow to the head, cervical dislocation, decapitation, or severing the cervical spinal cord

- Poultry only
  - Inert gases (Ar, N₂) with air or CO₂ <30%; as long as O₂ <2%
  - Captive bolt concussion followed by immediately by exsanguination, injection of compressed air into cranium, pithing, decapitation

CCAC: Conditionally Acceptable Methods – Birds

- CO₂ (only if there are no other practical alternatives) followed by another method to ensure death
- Cervical dislocation (birds <3 kg only) followed by immediate decapitation or high flows of CO₂ if separation of the head and spine is not complete
- Decapitation without anesthesia
- Maceration (chicks within 2 days of hatching only)
**Methods Used at U of S**
- Barbiturates injected IV
- Anesthesia and decapitation
- T61 injected IV
- Manual cervical dislocation - poultry
- Cervical dislocation using burdizzo - turkeys

**Current Literature – Use of Zephyr**
- Recent publication supports the use of a pneumatic nonpenetrating captive bolt (Zephyr — originally developed for on-farm euthanasia of meat rabbits) for on-farm euthanasia of turkeys
  - Using time to insensibility and estimated time of death to evaluate a nonpenetrating captive bolt, cervical dislocation, and blunt trauma for on-farm killing of turkeys. Poultry Science (2010) 89, 1345-1354; Erasmus, Lawlis, Duncan, Widowski (Canada).

**Erasmus et al (Canada)**
- Compared Zephyr, blunt force trauma, manual cervical dislocation, cervical dislocation using a burdizzo
- Zephyr (discharged twice) and blunt force trauma (single hit with metal pipe or bat) were similarly effective at causing immediate insensibility
- Neither method of cervical dislocation caused immediate insensibility
- Zephyr has the advantage of consistency of force independent of the operator, and is not affected by operator fatigue
Implications for U of S

- Barbiturates injected IV will continue to be used for humane killing of birds
  - but should stipulate addition of a second method to ensure death
- Anesthesia followed by decapitation will continue to be used for humane killing of birds
- T61 injected IV will no longer be used routinely
- Scientific justification and the current literature will continue to be used by the AREB to evaluate other methods, such as manual cervical dislocation, and cervical dislocation using a burdizzo
- Use of the Zephyr gun should be further evaluated

Use of Zephyr pneumatic non-penetrating captive bolt gun at U of S

- A Zephyr pneumatic stun gun and air compressor is available through the Animal Resources Centre
- Should be evaluated as a replacement for a burdizzo when euthanizing birds > 3 kg
- Appropriate training sessions will need to be developed (for safe use of the air compressor, and appropriate use and position of the stun gun using paper by Erasmus et al; cadavers etc.)

Laboratory Animals Use at the U of S

(excluding large animals and free-ranging wildlife)

- Rodents (Rats, mice, guinea pigs, hamsters, gerbils)
- Rabbits
- Birds (poultry, quail, ducks)
- Amphibians (frogs, salamanders)
- Fish (zebrafish, salmonids, sturgeon)
- Dogs
- Cats
CCAC: Acceptable Methods of Euthanasia Rabbits

- Barbiturates injected IV
- Overdose of inhalant anesthetic
- Nonpenetrating captive bolt - eg Zephyr gun

Above methods must be followed by a second method to ensure death such as opening the chest, exanguination, cervical dislocation, decapitation, or severing the cervical spinal cord.

Humane Alternative Developed for Rabbit Stunning

The Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and the University of Guelph have developed an alternative method of euthanasia called the Zephyr, for inducing rabbits at processing plants. The Zephyr is based on a pneumatic stun gun designed by Canadian Biotech Animal Care (CBAC) in the United Kingdom. The device was modified and tested by OMAFRA, and is used at a number of plants.

The Zephyr-stun gun is composed of a plastic housing, mounting plate, and forearm. It is designed to deliver a blow to the rabbit's head, causing a rapid and humane death.

Nonpenetrating Captive Bolt

Zephyr pneumatic stun gun
Developed by OMAFRA and University of Guelph

Rabbit Zinger™
Developed by therabbitwinger.com
CCAC: Conditionally Acceptable Methods – Rabbits

- Manual cervical dislocation (< 1 kg; or with small #)
- Commercial cervical dislocator (>2 kg; or if large #)

- T61 injected IV (with prior sedation if possible)

Above methods must be followed by a second method to ensure death such as opening the chest, exsanguination, cervical dislocation, decapitation, or severing the cervical spinal cord

Methods Used at U of S

- Barbiturates injected IV into ear vein
- Deep anesthesia followed by barbiturates IC
- Nonpenetrating captive bolt has been used
  - Zephyr pneumatic stun gun and air compressor is available through the Animal Resources Centre
  - Training required for use of air compressor, and use and position of stun gun (OMAFRA video, pamphlet, cadaver use)

Advantages of a nonpenetrating captive bolt

- Human safety – provided properly trained
- No need for exemption for controlled drug use as with barbiturates,
- Eliminates operator fatigue seen with manual methods
- Minimal risk of operator error
- Not harmful to the environment
- Relatively inexpensive
  - Zephyr – $500 (must also purchase an air compressor)
  - Rabbit Zinger™ ($200 USD plus shipping, duty etc)
  - No compressor needed
Implications for U of S

- Barbiturates injected IV or IC under deep anesthesia will continue to be used for humane killing of rabbits but should stipulate addition of a second method, such as exsanguination or opening the chest, to ensure death.
- Nonpenetrating captive bolt using the Zephyr gun, with appropriate training, will also be used in certain circumstances but should stipulate addition of a second method, such as exsanguination or opening the chest, to ensure death.
- Scientific justification and the current literature will be continue to be used by the AREB to evaluate other methods, such as cervical dislocation or IV T61.

Laboratory Animals Use at the U of S (excluding large animals and free-ranging wildlife)

- Rodents (rats, mice, guinea pigs, hamsters, gerbils)
- Rabbits
- Birds (poultry, quail, ducks)
- Amphibians (frogs, salamanders)

CCAC: Acceptable Methods of Euthanasia in Rodents

- Buffered, diluted barbiturates injected IP with a fast acting local anesthetic (amount not specified)
- Overdose of inhalant anesthetic (for species that do not breath hold) using calibrated equipment

Above methods must be followed by a second method to ensure death such as opening the chest, exsanguination, cervical dislocation, decapitation, or severing the cervical spinal cord.
CCAC: Conditionally Acceptable Methods – Rodents

- Gradual-fill CO₂ (only if other methods are not practical)
- 98% Argon/Nitrogen (with scientific justification)
- Cervical dislocation (with anesthesia if possible)
- Decapitation (with anesthesia if possible)

Above methods must be followed by a second method to ensure death such as opening the chest, exsanguination, or severing the cervical spinal cord

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CCAC: Conditionally Acceptable Methods – Rodents

- Gradual-fill CO₂
  - Anesthetize with inhalant first if possible; introduce CO₂ soon after loss of consciousness to ensure rapid death
  - Use gradual-fill rate of 20% to 30% of chamber volume per minute (monitored using a gas flow meter) until loss of consciousness, then increase flow for more rapid death
  - Use compressed CO₂ gas in cylinders only
  - Flush chamber with air between groups to avoid increased concentration of CO₂ at the bottom
  - Confirm death with a second method, such as opening chest or cervical dislocation

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CCAC: Conditionally Acceptable Methods – Rodents

- Gradual-fill CO₂
  - Requires thorough review by ACC
  - Must assure competency of personnel
  - Requires strict, written SOPs
  - Requires written records of use
  - Requires regular post-approval review
Methods Used at U of S

- Diluted or undiluted barbiturates injected IP
- Overdose of isoflurane anesthesia
  - in euthanasia jar in fume hood – most common
  - using anesthetic machine – less common (↑ human risk)

Methods Used at U of S

- Various methods of administering CO₂
  - Gradual-fill chamber with or without flow meter
  - Use of home cage if possible (Euthanex® lid)
Methods Used at U of S

- Manual cervical dislocation (mice)
- Deep anesthesia followed by IC barbiturates, cervical dislocation, exsanguination, decapitation or cutting open the chest
- Decapitation without anesthesia (neonates)

Implications for U of S

- A campus-wide SOP for use of IP barbiturates as stipulated in the new guidelines, i.e. correct pH, addition of lidocaine, should be developed and implemented across campus
- CO₂ use should be harmonized across campus to ensure compliance with the new CCAC guidelines
  - May require additional resources such as flow meters, etc.
  - Training session will need to be developed
- **Scientific justification** and the current literature will continue to be used by the AREB to evaluate other methods, such as cervical dislocation or decapitation

Questions?
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