Canadian Council on Animal Care
Conseil canadien de protection des animaux

ADDITIONAL INFORMATION ON EFFECTS OF EUTHANASIA METHODS ON RESEARCH RESULTS

Addendum to the *CCAC guidelines on: euthanasia of animals used in science*

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Information about various methods of killing animals and their potential effects on the scientific data being collected from the animal-based research is provided in this document. Investigators are encouraged to conduct a critical review of the information in the cited references and other relevant resources to determine if their research results will be impacted by the method of euthanasia being proposed.

Each method has been categorized as acceptable or conditionally acceptable. As noted in the *CCAC guidelines on: euthanasia of animals used in science* (2010), methods listed as conditionally acceptable require additional justification because there is greater potential for operator error or safety hazards, they might not consistently produce humane death, or they are not well documented in the scientific literature.

The CCAC encourages those involved in euthanasia to submit any relevant information they encounter to the CCAC.

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ACCEPTABLE METHODS

1. Barbiturates and Derivatives

Acceptable for most species.

1.1 Application and Cautions

1.1.1 General

- Cause deep anesthesia due to depression of the respiratory center, which progresses to apnea followed by cardiac arrest (ILAR, 1992; Close et al., 1996; EFSA, 2005; AVMA, 2007).
- Fast-acting and cause minimal discomfort following IV administration (ILAR, 1992; Close et al., 1996; EFSA, 2005; AVMA, 2007).
- IP administration is appropriate for some species but may cause irritation (Close et al., 1996; Close et al., 1997; EFSA, 2005) if concentrated solutions (i.e. barbiturates manufactured specifically for euthanasia where the concentration may be >200mg/mL) are used (Wadham, 1997); irritation can be reduced by dilution (Close et al., 1996; EFSA, 2005) and addition of a fast acting local anesthetic (Wadham, 1997; Ambrose et al., 2000).
- Intracardiac (Close et al., 1996) and intrapulmonary routes of injection are extremely painful and should not be used for any species unless the animal is fully anesthetized (Close et al., 1997; EFSA, 2005; AVMA, 2007).
- Prior sedation may be useful with excitable animals (ILAR, 1992; Close et al., 1997; Reilly, 2001; EFSA, 2005).

References

Ambrose N., Wadham J. and Morton D. (2000) Refinement of euthanasia. In: *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* (Balls M., van Zeller A.M. and Halder M.E., eds). As cited in Wadham J.J.B., Townsend P. and Morton D.B. (1997) Intraperitoneal injection of sodium pentobarbitone as a method of euthanasia for rodents. *ANZCCART News* 10(4):8-8.

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals, Part 1. *Laboratory Animals* 30(4):293-316.

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Institute of Laboratory Animal Resources – ILAR (1992) *Recognition and Alleviation of Pain and Distress in Laboratory Animals*. Washington DC: National Academy Press.

Reilly J.S. (2001) *Euthanasia of Animals Used for Scientific Purposes*, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Wadham J.J.B. (1997) Recognition and reduction of adverse effects in research on rodents. As cited in Wadham J.J.B., Townsend P. and Morton D.B. (1997) Intraperitoneal injection of sodium pentobarbitone as a method of euthanasia for rodents. *ANZCCART News* 10(4):8-8.

1.1.2 Amphibians

- IV or IP injection by experienced personnel is recommended (Close et al., 1997; EFSA, 2005).
- Subcutaneous lymph spaces may also be used in frogs and toads (AVMA, 2007).
- Time to effect may vary, with death occurring in up to 30 minutes (Andrews et al., 1993; Burns, 1995).
- Death should be ensured via pithing/decapitation (AVMA, 2007).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Andrews E.J., Bennet B.T. and Clark J.D. (1993) Report on the AVMA panel on euthanasia. As cited in American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Burns R. (1995) Considerations in the euthanasia of reptiles, fish and amphibians. American Association of Zoo Veterinarians & Association of Reptilian and Amphibian Veterinarians Joint Conference. As cited in Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D.B. and Warwick C. (1997) Recommendations for euthanasia of experimental animals, Part 2. *Laboratory Animals* 31(1):1-32.

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1.1.3 Birds

- IV and IP are acceptable (ILAR, 1992), although IP injection is often recommended (Close et al., 1997; Reilly, 2001).
- Experienced operators may inject into the foramen magnum at the base of the skull (intracephalic) (Close et al., 1996; Close et al., 1997).

References

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals, Part 1. *Laboratory Animals* 30(4):293-316.

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D.B. and Warwick C. (1997) Recommendations for euthanasia of experimental animals, Part 2. *Laboratory Animals* 31(1):1-32.

Institute of Laboratory Animal Resources – ILAR (1992) *Recognition and Alleviation of Pain and Distress in Laboratory Animals*. Washington DC: National Academy Press.

Reilly J.S. (2001) Euthanasia of Animals Used for Scientific Purposes, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

1.1.4 Cats and Dogs

- IV injection is recommended (Close et al., 1997; EFSA, 2005).
- Sodium pentobarbital is the most suitable barbiturate for cats and dogs, and should only be delivered intravenously (ILAR, 1992; Reilly, 2001).

References

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D.B. and Warwick C. (1997) Recommendations for euthanasia of experimental animals, Part 2. *Laboratory Animals* 31(1):1-32.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

Institute of Laboratory Animal Resources – ILAR (1992) *Recognition and Alleviation of Pain and Distress in Laboratory Animals*. Washington DC: National Academy Press.

Reilly J.S. (2001) Euthanasia of Animals Used for Scientific Purposes, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

1.1.5 Fish

- Removal from water causes stress and therefore other methods are preferable (Close et al., 1997; Reilly, 2001).
- IP injection is generally recommended (Close et al., 1997; EFSA, 2005).

References

Reilly J.S. (2001) Euthanasia of Animals Used for Scientific Purposes, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D.B. and Warwick C. (1997) Recommendations for euthanasia of experimental animals, Part 2. *Laboratory Animals* 31(1):1-32.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

1.1.6 Reptiles, Rodents and Rabbits

- IV administration is preferred because the effect is the most rapid and reliable (Close et al., 1996; Close et al., 1997).
- IP injection is acceptable for rodents when IV injection is difficult, but the effect is slower (ILAR, 1992; Close et al., 1997).

References

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals, Part 1. *Laboratory Animals* 30(4):293-316.

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D.B. and Warwick C. (1997) Recommendations for euthanasia of experimental animals, Part 2. *Laboratory Animals* 31(1):1-32.

Institute of Laboratory Animal Resources – ILAR (1992) *Recognition and Alleviation of Pain and Distress in Laboratory Animals*. Washington DC: National Academy Press.

1.1.7 Safety Concerns

- These drugs have addictive properties and many are controlled drugs (ILAR, 1992) requiring a license from Health Canada and appropriate storage.
- Tend to persist in the carcass and may cause sedation or even death of animals that consume the body (AVMA, 2007); contaminated carcasses must be disposed of appropriately.

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Institute of Laboratory Animal Resources – ILAR (1992) *Recognition and Alleviation of Pain and Distress in Laboratory Animals*. Washington DC: National Academy Press.

1.2 Potential Influence on Scientific Data

Observations in rodents.

1.2.1 Circulatory System

- Increased serum renin activity (Pettinger et al., 1975) (rats).
- Increased plasma aldosterone (Pettinger et al., 1975) (rats).

- Increased plasma glucose and insulin (Pénicaud et al., 1987; Bhathena, 1992) (rats).
- Decreased plasma triglycerides (Bhathena, 1992) (rats).
- Splenic enlargement (Hedenqvist and Hellebrekers, 2011) (species not specified).
- Gross/histopathology changes with pentobarbitol: spleen – emphysema, congestion (Feldman and Gupta, 1976; Iwarsson and Rehbinder, 1993) (mice, rats, guinea pigs, and rabbits).
- When combined with cervical dislocation: increased mitogen induced lymphocyte proliferation and decrease cytolytic T lymphocytes (CTL) response (Howard et al., 1990) (mice).

References

Bhathena S.J. (1992) Comparison of effects of decapitation and anesthesia on metabolic and hormonal parameters in Sprague-Dawley rats. *Life Sciences* 50(21):1649-1655.

Feldman D.B. and Gupta B.N. (1976) Histopathological changes in laboratory animals resulting from various methods of euthanasia. As cited in American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Hedenqvist P. and Hellebrekers L.J. (2011) Laboratory animal analgesia, anesthesia, and euthanasia. In: *Handbook of Laboratory Animal Science: Essential Principles and Practices* (Hau J. and Schapiro S.J., eds), Vol.1. Boca Raton FL: CRC Press.

Howard H.L., McLaughlin-Taylor E. and Hill R.L. (1990) The effect of mouse euthanasia technique on subsequent lymphocyte proliferation and cell mediated lympholysis assays. As cited in American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Iwarsson K. and Rehbinder C. (1993) A study of different euthanasia techniques in guinea pigs, rats and mice. Animal response and post-mortem findings. As cited in American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Pettinger W.A., Tanaka K., Keeton K., Campbell W.B. and Brooks S.N. (1975) Renin release, an artifact of anesthesia and its implications in rats. *Proceedings of the Society for Experimental Biology and Medicine*, Vol.148. pp.625-630.

1.2.2 Digestive System

- Gross/histopathology changes with pentobarbital: GI serosa – emphysema, congestion (Feldman and Gupta, 1976; Iwarsson and Rehbinder, 1993) (mice, rats, guinea pigs, and rabbits).
- Gross/histopathology changes with pentobarbital: peritoneal congestion, sanguineous fluid in abdominal cavity (Feldman and Gupta, 1976; Iwarsson and Rehbinder, 1993) (mice, rats, guinea pigs, and rabbits).

References

Feldman D.B. and Gupta B.N. (1976) Histopathological changes in laboratory animals resulting from various methods of euthanasia. As cited in American College of Laboratory Animal

Medicine – ACLAM (2005*) Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Iwarsson K. and Rehbinder C. (1993) A study of different euthanasia techniques in guinea pigs, rats and mice. Animal response and post-mortem findings. As cited in American College of Laboratory Animal Medicine – ACLAM (2005*) Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

1.2.3 Excretory System

- Gross/histopathology changes with pentobarbital: kidney cortex – circulatory changes (Feldman and Gupta, 1976; Iwarsson and Rehbinder, 1993) (mice, rats, guinea pigs, and rabbits).

References

Feldman D.B. and Gupta B.N. (1976) Histopathological changes in laboratory animals resulting from various methods of euthanasia. As cited in American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Iwarsson K. and Rehbinder C. (1993) A study of different euthanasia techniques in guinea pigs, rats and mice. Animal response and post-mortem findings. As cited in American College of Laboratory Animal Medicine – ACLAM (2005*) Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

1.2.4 Muscular System

- Decreased muscular contractility in isolated muscle preparations (Segel and Rendig, 1986) (rats).
- Decreased spontaneous and drug induced vascular smooth muscle contractility (Altura and Altura, 1975; Altura, 1978) (rats).
- Gross/histopathology changes with pentobarbital: cardiac muscle – acute degenerative lesions (Feldman and Gupta, 1976; Iwarsson and Rehbinder, 1993) (mice, rats, guinea pigs, and rabbits).

References

Altura B.M. (1978) Pharmacology of venular smooth muscle: new insights. As cited in Segel L.D. and Rendig S.V. (1986) Sodium pentobarbital effects on cardiac function and response to dobutamine. *Journal of Cardiovascular Pharmacology* 8(2):392-397.

Altura B.T. and Altura B.M. (1975) Pentobarbital and contraction of vascular smooth muscle. As cited in Segel L.D. and Rendig S.V. (1986) Sodium pentobarbital effects on cardiac function and response to dobutamine. *Journal of Cardiovascular Pharmacology* 8(2):392-397.

Feldman D.B. and Gupta B.N. (1976) Histopathological changes in laboratory animals resulting from various methods of euthanasia. As cited in American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Iwarsson K. and Rehbinder C. (1993) A study of different euthanasia techniques in guinea pigs, rats and mice. Animal response and post-mortem findings. As cited in American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Segel L.D. and Rendig S.V. (1986) Sodium pentobarbital effects on cardiac function and response to dobutamine. *Journal of Cardiovascular Pharmacology* 8(2):392-397.

1.2.5 Nervous System

- Compared with decapitation, pentobarbital produced a significant rise in the activity of the dopamine metabolite in selected regions of brain tissue (Zinn et al., 1989) (rats).
- When combined with decapitation: increase in acetylcholine release in the brain; and decrease in testosterone in immature and mature male rats, with increase prolactin in mature male rats (ACLAM, 2005) (male rats).

References

American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Zinn S.A., Lookingland K.J., Tucker H.A. and Moore K.E. (1989) Alterations in concentrations of dihydroxyphenylacetic acid in the median eminence of rats euthanatized with pentobarbital. As cited in Reilly J.S. (2001) *Euthanasia of Animals Used for Scientific Purposes*, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

1.2.6 Respiratory System

- Gross/histopathology changes with pentobarbital: lung – emphysema, congestion (Feldman and Gupta, 1976; Iwarsson and Rehbinder, 1993) (mice, rats, guinea pigs, and rabbits).

References

Feldman D.B. and Gupta B.N. (1976) Histopathological changes in laboratory animals resulting from various methods of euthanasia. As cited in American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Iwarsson K. and Rehbinder C. (1993) A study of different euthanasia techniques in guinea pigs, rats and mice. Animal response and post-mortem findings. As cited as American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

2. Benzocaine

Acceptable for aquatic species via immersion.

2.1 Applications and Cautions

2.1.1 General

- Central nervous system depressant used via immersion with aquatic species (Close et coll., 1996; AVMA, 2007).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals, Part 1. *Laboratory Animals* 30(4):293-316.

2.1.2 Fish and Amphibians

- Exposure via immersion in benzocaine solution (≥ 250 mg/L) (AVMA, 2007).
- Benzocaine must be dissolved in a small volume of acetone (Close et al., 1996; DeTolla et al., 1995; Close et al., 1997; Reilly, 2001; AVMA, 2007) or ethyl alcohol (AVMA, 2007; DeTolla et al., 1995) prior to final dilution in water; benzocaine hydrochloride is directly soluble in water and can be used directly for euthanasia (DeTolla et al., 1995).
- Skin irritant, neutralize to pH 7.5 before use (Brown, 1988; Summerfelt et al., 1990; Close et al., 1997).
- Continue exposure for at least 10 minutes following cessation of respiratory movements (Noga, 1996).
- Should be followed by a physical (Close et al., 1996; Reilly, 2001) or chemical method to ensure death.

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Brown L.A. (1988) Anesthesia in fish. *Veterinary Clinics of North America: Small Animal Practice*. As cited in Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals, Part 1. *Laboratory Animals* 30(4):293-316.

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DeTolla L.J., Srinivas S., Whitaker B.R., Andrews C., Hecker B., Kane A.S. and Reimschuessel R. (1995) Guidelines for the care and use of fish in research. *Institute for Laboratory Animal Research* 37(4):159-173.

Noga E.J. (1996) Fish Disease: Diagnosis and Treatment. As cited in American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Reilly J.S. (2001) Euthanasia of Animals Used for Scientific Purposes, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Summerfelt R.C., Smith L.S., Schreck C.B. and Moyle P.B. (1990) Anaesthesia, surgery and related techniques. In: Methods for Fish Biology (Schreck C.B. and Moyle P.B., eds). As cited in Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals, Part 1. *Laboratory Animals* 30(4):293-316.

2.1.3 Safety Concerns

- Powder is a respiratory irritant and should be handled with care.
- Benzocaine is not an approved formulation and therefore has no target animal or human safety data for its use as a fish anesthetic.
- Not registered for veterinary use with fish destined for food production in Canada; investigators are individually responsible for the use of agents that have not been approved for such use.

2.2 Potential Influence on Scientific Data

2.2.1 Circulatory System

- Increased hematocrit and increased cortisol, glucose, lactate and anion concentrations (Ryder and Dennison, 2005) (fish).

References

Ryder K. and Dennison N. (2005) *Harmonisation of the Care and Use of Fish in Research*. . International consensus meeting, Gardermoen, Norway, May 23-26, 2005. UK: Home Office.

2.2.2 Metabolic System

- May affect liver enzyme activity and cytochrome P450 levels (Arinç and Sen, 1994) (fish – Gilthead seabreams).

References

Arinç E. and Sen A. (1994) In vivo effects of the anesthetic, benzocaine, on liver microsomal cytochrome P450 and mixed-function oxidase activities of gilthead seabream (*Sparus*

aurata). Comparative Biochemistry and Physiology. *Pharmacology Toxicolology and Endocrinology* 107(3):399-404.

3. Clove Oil (Eugenol)

Acceptable for fish via immersion.

3.1 Application and Cautions

3.1.1 Fish

- Effectively induces hypoxia, hypercapnia, unconsciousness and death (Borski and Hodson, 2003).
- Acceptable for euthanasia via immersion at >400 mg/L (Borski and Hodson, 2003).
- Exposure should continue for at least 10 minutes following cessation of respiratory movements (Borski and Hodson, 2003).
- Should be followed by a physical or chemical method to ensure brain death.

References

Borski R.J. and Hodson R.G. (2003) Fish research and the institutional animal care and use committee. *Institute for Laboratory Animal Research Journal* 44(4):286-294.

Holloway A.C., Keene J.L., Noakes D.G. and Noccia R.D. (2004) Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Agriculture Research* 35(11):1025-1030.

3.1.2 Safety Concerns

- Not registered for veterinary use with fish in Canada; investigators are individually responsible for the use of agents that have not been approved for such use.
- Should be limited to applications in which fish will not be consumed (Borski and Hodson, 2003).

References

Borski R.J. and Hodson R.G. (2003) Fish research and the institutional animal care and use committee. *Institute for Laboratory Animal Research Journal* 44(4):286-294.

3.2 Potential Influence on Scientific Data

3.2.1 Circulatory System

- May affect biochemical blood measures (Holloway et al., 2004) (fish – rainbow trout).

Reference

Holloway A.C., Keene J.L., Noakes D.G. and Noccia R.D. (2004) Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Agriculture Research* 35(11):1025-1030.

3.2.2 Metabolic System

- May result in increased levels of cortisol, tri-iodothyronine (T3) and thyroxine (T4) (Holloway et al., 2004) (fish – rainbow trout).

References

Holloway A.C., Keene J.L., Noakes D.G. and Noccia R.D. (2004) Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Agriculture Research* 35(11):1025-1030.

4. Electrical Stunning

*Acceptable for pigs only, and requires application of a second method; conditionally acceptable for chickens. Please note: "electrocution is an adequate method of euthanasia in principle (for chickens), but the insulating characteristics of the feathers may reduce the effectiveness. Focusing electrocution on the head area of the bird may improve the technique" (*CCAC guidelines on: the care and use of farm animals in research, teaching and testing, *2009).*

4.1 Application and Cautions

4.1.1 General

- Causes unconsciousness via brain seizure, but is only temporary (~ 30 sec).
- Must be immediately followed by a fast-acting and permanent method of euthanasia, such as exsanguination or cardiac arrest stunning (cardiac fibrillation that leads to death). Cardiac arrest stunning should never be performed on conscious animals because they can remain conscious for 10-30 sec after onset of cardiac fibrillation.
- Stunning equipment and procedures must be designed for use with the particular animal to be euthanized.
- Proper training and adequate restraint of the animal are important to ensure effectiveness and sudden loss of consciousness.

4.1.2 Safety Concerns

- Risk of electrocution for personnel (EFSA, 2005).
- Electrical equipment should be well maintained and calibrated prior to application to live animals (EFSA, 2005).
- Animals should be adequately restrained (EFSA, 2005).

References

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

4.2 Potential Influence on Scientific Data

4.2.1 Muscular System

- May affect the biochemistry of tissues e.g. muscle (Grandin and Smith, 2004) (pigs).
- An acute fall of the muscle pH due to the powerful activation of the glycolysis in the muscles (Henckel, 1998) (pigs).

References

Grandin T. and Smith G.C. (2004) *Animal welfare in humane slaughter*. In: *Agricultural Mechanization and Automation* (McNully P. and Grace P.M., eds), Vol. 2, Encyclopedia of Life Support Systems (EOLSS), developed under the auspices of the UNESCO. Oxford UK : Eolss Publishers.

Henckel P. (1998) Influence of stunning method on pH-decrease and meat quality, as cited in Grandin T. and Smith G.C. (2004) *Animal welfare in humane slaughter*. In: *Agricultural Mechanization and Automation* (McNully P. and Grace P.M., eds), Vol. 2, Encyclopedia of Life Support Systems (EOLSS), developed under the auspices of the UNESCO. Oxford UK : Eolss Publishers.

4.2.2 Nervous System

- Stunning of pigs with an electric current is expected to increase the brain extracellular levels of GABA (gamma aminobutyric acid) (EFSA, 2004).

References

European Food Safety Authority – EFSA Scientific Panel on Animal Health and Welfare (2004) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. *European Food Safety Authority (EFSA) Journal* 45:1-29.

4.2.3 Skeletal System

- May cause broken bones (Pork ‘98, 1998) (pigs).

References

Pork ’98 (1998) Stunning affects pork quality, as cited in Grandin T. and Smith G.C. (2004) Animal welfare in humane slaughter. In: *Agricultural Mechanization and Automation* (McNully P. et Grace P.M., eds), Vol. 2, Encyclopedia of Life Support Systems (EOLSS), developed under the auspices of the UNESCO. Oxford UK : Eolss Publishers.

5. Etomidate and Metomidate

Acceptable for fish via immersion.

5.1 Application and Cautions

5.1.1 General

- Imidazole-based non-barbiturate hypnotic agents that act by depression of the central nervous system (Close et al., 1996) and have no analgesic properties (Close et al., 1997).
- Relatively quick acting and highly soluble in water (Brown, 1988; Close et al., 1997; Summerfelt et al., 1990).
- Acceptable for euthanizing fish by immersion (Close et al., 1997).
- Immersion must be followed by a physical or chemical method to cause brain death.
- Immersion method may be weak or ineffectual on fish which breath-hold or breathe air.
- The efficacy of etomidate is pH dependent and it has proven to be more effective in alkaline waters (Amend et al., 1982).
- Temperature influences the toxicity of etomidate, with higher temperatures rendering the drug less toxic (Limsuwan et al., 1983).

References

Amend D.F., Goven B.A. and Elliot D.G. (1982) Etomidate: effective dosages for a new fish anesthetic. *Transactions of the American Fisheries Society* 111(3):337-341.

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5.1.2 Safety Concerns

- Etomidate is not registered for veterinary use with fish in Canada; investigators are individually responsible for the use of agents that have not been approved for such use.

5.2 Potential Influence on Scientific Data

5.2.1 Endocrine System

- As a result of the lack of respiration, increases in blood concentrations of adrenaline and cortisol have been demonstrated in fish anaesthetized with metomidate (Iwama et al., 1989).
- Metomidate concentrations above 3 mg/L have been shown to block the cortisol response, and result in increases in blood lactate levels and haematocrit in Atlantic salmon (Olsen et al., 1995).

References

Iwama G.K., McGeer J.C. and Pawluk M.P. (1989) The effects of five fish anaesthetics on acid-base balance, hematocrit, cortisol and adrenaline in rainbow trout. *Canadian Journal of Zoology* 67(8):2065-2073.

Olsen Y.A., Einarsdottir I.E. and Nilssen K.J. (1995) Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress. *Aquaculture* 134(1-2):155-168.

6. Inert Gasses (Ar, N_2)

ONLY acceptable for poultry and pigs; conditionally acceptable for rodents.

6.1 Application and Cautions

6.1.1 General

- Exposure results in extreme hypoxia which leads to depression of the central nervous system followed by apnea and cardiac arrest.
- Oxygen concentration must be <2% to stun and kill (>90% Ar/N2) (AVMA, 2007; EFSA, 2005) and it should be monitored using an appropriate oxygen analyzer.
- Resistance to hypoxia and efficacy as a euthanasia method varies significantly between species (AVMA, 2007), therefore use in any other species requires special justification.
- Inert gases should not be used in breath-holding species.

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

6.1.2 Poultry and Pigs

- Animals should be placed in a chamber that has been pre-filled with >90% Ar or N_2 (AVMA, 2007).
- Gradual-filling of an empty chamber will result in an excessive time to loss of consciousness due to the high concentration of gas required.

- Day-old chicks should not be euthanized with argon or nitrogen because of their ability to withstand low concentrations of oxygen.

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

6.1.3 Safety Concerns

- Safety concerns for personnel exposed to unscavenged gases.

6.2 Potential Influence on Scientific Data

6.2.1 Circulatory System

- Can affect blood and muscle biochemistry (EFSA, 2005) (not species specific).

References

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

6.2.2 Metabolic System

- Hypoxia and anoxia can alter brain neurotransmitter and metabolite levels (EFSA, 2005) (not species specific).

References

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

6.2.3 Muscular System

- Hypoxia and anoxia can alter brain neurotransmitter and metabolite levels (EFSA, 2005) (not species specific).

References

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

7. Inhalant Anesthetics

Acceptable for most species (except aquatic species and those capable of breath-holding).

7.1 Application and Cautions

7.1.1 General

- Not recommended for diving birds and mammals that are capable of breath holding (AVMA, 2007).
- Neonatal animals are resistant to hypoxia and may require prolonged exposure (Beaver et al., 2001; Close et al., 1996; Glass et al., 1944).
- Liquid state is an irritant and care must be taken to ensure the animal cannot come in contact with the liquid chemical (AVMA, 2007; Close et al., 1996).
- Irritant to mucous membranes at high concentrations, and shown to be aversive in many species; therefore, a gas-specific vaporizer should be used to deliver an appropriate level of anesthetic in a controlled manner (AVMA, 2007; EFSA, 2005).
- Air or oxygen should be provided during the induction period to prevent hypoxia (Close et al., 1996; UFAW, 1988).
- May produce hypersalivation and excitation during induction.
- Should be followed by a physical or chemical method to ensure brain death.
- It is important to regularly review the literature on aversion in different species and strains.

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Beaver B.V., Reed W., Leary S., McKiernan B., Bain F., Schultz R., Bennett B.T., Pascoe P., Shull E., Cork L.C., Francis-Floyd R., Amass K.D., Johnson R., Schmidt R.H., Underwood W., Thornton G.W. and Kohn B. (2001) 2000 Report of the AVMA panel on euthanasia. *Journal of the American Veterinary Medical Association* 218(5):669-696.

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Glass H.G., Snyder F.F. and Webster E. (1944) The rate of decline in resistance to anoxia of rabbits, dogs, and guinea pigs from the onset of viability to adult life. *American Journal of Physiology* 140(5):609-615.

Universities Federation for Animal Welfare – UFAW (1988) *Humane Killing of Animals*. Preprint of 4th ed. Potters Bar UK: UFAW.

7.1.2 Amphibians, Fish, and Reptiles

- An expert on reptile and amphibian anesthesia should be consulted to determine whether the use of these agents is appropriate for a particular species.
- Delivery in aquatic species via bubbling into the tank is not recommended due to slow action and irritation of the skin.
- Delivery in reptile and amphibian species, including chelonians, that are capable of holding their breath and converting to anaerobic metabolism is unacceptable as they can survive long periods of anoxia (Brannian et al., 1987; Calderwood, 1971; Jackson and Cooper, 1981; Johlin and Moreland, 1933; Close et al., 1996, Close et al., 1997) (up to 27 hours for some species); death in these species may not occur even after prolonged inhalant exposure.

References

Brannian R.E., Kirk E. and Williams D. (1987) Anesthetic induction of kinosternid turtles with halothane. *Journal of Zoo Animal Medicine* 18(2-3):115-117.

Calderwood H.W. (1971) Anesthesia for reptiles. *Journal of the American Veterinary Medical Association* 159(11):1618-1625.

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Close B., Banister K., Baumans V., Bernoth E.-V.., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1997) Recommendations for euthanasia of experimental animals: Part 2. *Laboratory Animals* 31(1):1-32.

Jackson O.F. and Cooper J.E. (1981) Anesthesia and surgery. In: *Diseases of the Reptilia*, Vol. 2. (Cooper J. E. and Jackson O. F., eds.) New York NY: Academic Press Inc.

Johlin J.M. and Moreland F.B. (1933) Studies of the blood picture of the turtle after complete anoxia. *Journal of Biological Chemistry* 103(1):107-114.

7.1.3 Rodents

- Exposure in the home cage minimizes stress (EFSA, 2005).

References

- European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

7.1.4 Safety Concerns

- Appropriate gas scavenging equipment needs to be used to prevent operator exposure (Close et al., 1996; Close et al., 1997).

References

Close B., Banister K., Baumans V., Bernoth E.-V., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals: Part 1. *Laboratory Animals* 30(4):293-316.

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7.2 Potential Influence on Scientific Data

7.2.1 Circulatory System

- Consistent low-level stress with the use of these gases may contribute to a reduction in variance for any subsequent tissue analysis (EFSA, 2005).
- If tissues are to be used for in vitro work, some validation may be necessary to compare with previous data (EFSA, 2005).
- A wash-out period may be required to remove residual anesthetic gas (EFSA, 2005).

References

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

8. Maceration

ONLY acceptable for very small fish and chicks within two days of hatching.

8.1 Application and Cautions

8.1.1 General

- Must only be performed using a purpose-built maceration unit that is properly maintained (Close et al., 1996; Close et al., 1997).

References

Close B., Banister K., Baumans V., Bernoth E.-V., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals: Part 1. *Laboratory Animals* 30(4):293-316.

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8.1.2 Birds

- Not acceptable for chicks under laboratory conditions where other methods can be used; however, it may be considered conditionally acceptable for chicks within two days of

hatching if sufficient justification is provided to the animal care committee (AVMA, 2007; Close et al., 1996).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Close B., Banister K., Baumans V., Bernoth E.-V., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals: Part 1. *Laboratory Animals* 30(4):293-316.

8.1.3 Fish

- Acceptable for fish <2 cm long, using a purpose built unit (Close et al., 1996; EFSA, 2005); use with larger fish is not acceptable due to the potential for animal suffering.

References

Close B., Banister K., Baumans V., Bernoth E.-V., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals: Part 1. *Laboratory Animals* 30(4):293-316.

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8.2 Potential Influence on Scientific Data

8.2.1 Tissue Analysis

- Generally not useful except for whole body analysis as it destroys all body tissues including the brain (EFSA, 2005).

References

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

9. Penetrating Captive Bolt

Acceptable for ruminants, pigs, horses, large rabbits, large reptiles and poultry.

9.1 Application and Cautions

9.1.1 General

- Penetration of the skull and disruption of the brain results in rapid loss of consciousness and death.

- Acceptable for use in ruminants, swine and horses (Close et al., 1996; Close et al., 1997; Dennis et al., 1985; EFSA, 2005; Reilly, 2001), some species of reptiles (Close et al., 1997), larger rabbits (AVMA, 2007; Close et al., 1996; EFSA, 2005), and poultry.
- Due to variation in skull morphology, captive bolts must be designed for use with the particular species to be euthanized (Close et al., 1996; Close et al., 1997).
- Proper training and adequate restraint of the animal are important to ensure proper placement and penetration into the brain (AVMA, 2007; Close et al., 1996; Close et al., 1997, Reilly, 2001).
- A cerebral hemisphere and the brainstem must be sufficiently disrupted by the projectile to induce sudden loss of consciousness and subsequent death (Blackmore, 1985); this can only be accomplished by a penetrating captive bolt.
- Use of a non-penetrating captive bolt results in only temporary stunning, and must be immediately followed by a fast-acting and permanent method of euthanasia, such as exsanguination (AVMA, 2007).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Blackmore D.K. (1985) Energy requirements for the penetration of heads of domestic stock and the development of a multiple projectile. *The Veterinary Record* 116(2):36-40.

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals, Part 1. *Laboratory Animals* 30(4):293-316.

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Dennis M.B., Dong W.K., Weisbrod K.A. and Elchlepp C.A. (1998) Use of captive bolt as a method of euthanasia in larger laboratory animal species. *Laboratory Animal Science* 38(4):459-462.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

Reilly J.S. (2001) *Euthanasia of Animals Used for Scientific Purposes*, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

9.2 Potential Influence on Scientific Data

9.2.1 Circulatory System

- Brain particles are found in the blood, lungs, heart and muscle after penetrative stunning methods (Anil et al., 1999; Schmidt, 1999) (cattle).

References

Anil M.H., Love S., Williams S., Shand A., McKinstry J.L., Helps C.R., Waterman-Pearson A., Saghatchian J. and Harbour D.A. (1999) Potential contamination of beef carcases with brain tissue at slaughter. *The Veterinary Record* 145(16):460-462.

Eichbaum F.W., Slewer O. and Yasaka W.J. (1975) Postdecapitation convulsions and their inhibition by drugs. *Experimental Neurology* 49(3):802-812.

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Reilly J.S. (2001) Euthanasia of Animals Used for Scientific Purposes, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Schmidt G.R., Hossner K.L., Yemm R.S. and Gould D.H. (1999) Potential for disruption of central nervous system (CNS) tissue in beef cattle by different types of captive bolt stunners. *Journal of Food Protection* 62(4):390-393.

9.2.2 Digestive System

- Can cause shedding of enterocytes from the gut wall (Reilly, 2001).

References

Reilly J.S. (2001) *Euthanasia of Animals Used for Scientific Purposes*, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

9.2.3 Muscular System

- Removal of inhibitory influences from higher centres of the brain (e.g. damage by captive bolt), before the spinal cord becomes anoxic, results in convulsive activity and enhancement of some spinal reflexes (Eichbaum, 1975; Reilly, 2001), which may affect research on muscle and brain (EFSA, 2005).

References

Eichbaum F.W., Slewer O. and Yasaka W.J. (1975) Postdecapitation convulsions and their inhibition by drugs. *Experimental Neurology* 49(3):802-812.

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Reilly J.S. (2001) Euthanasia of Animals Used for Scientific Purposes, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

9.2.4 Nervous System

- Removal of inhibitory influences from higher centres of the brain (e.g. damage by captive bolt), before the spinal cord becomes anoxic, results in convulsive activity and enhancement of some spinal reflexes (Eichbaum, 1975; Reilly, 2001), which may affect research on muscle and brain (EFSA, 2005).

10. TMS (MS222, Tricaine)

Acceptable for fish and amphibians.

10.1 Application and Cautions

10.1.1 General

- Central nervous system depressant recommended for euthanasia of aquatic species (AVMA, 2007; Close et al., 1996; Close et al., 1997).
- TMS is acidic and must be buffered.
- A second method must be used to ensure brain death following immersion.
- Stock solutions are unstable in sunlight and should therefore be stored in opaque containers in a refrigerator or preferably in a freezer, and replaced monthly or when a brown colour is observed (Close et al., 1996; Stoskopf, 1993; Torreilles, 2009).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Close B., Banister K., Baumans V., Bernoth E.-V., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals: Part 1. *Laboratory Animals* 30(4):293-316.

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10.1.2 Fish and Amphibians

- Exposure via immersion in TMS solution (≥250 mg/L) for at least 10 minutes following cessation of respiratory movements (Noga, 1996).
- May also be injected into lymph spaces and pleuroperitoneal cavities (Brown, 1988).
- Skin irritant, neutralize with sodium bicarbonate (Brown, 1988; Close et al., 1997) to pH 7.0-7.5 before use (AVMA, 2007).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Brown L.A. (1988) Anesthesia in fish, as cited in American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

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Noga E.J. (1996) Fish Disease: Diagnosis and Treatment, as cited in American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Torreilles S.T., McClure D.E. and Green S.L. (2009) Evaluation and refinement of euthanasia methods for *Xenopus laevis*. *Journal of the American Association of Laboratory Animal Science* 48(5):512-516.

Stoskopf M.K. (1993) Anaesthesia. In: *Aquaculture for Veterinarians: Fish Husbandry and Medicine* (Brown L.A., ed.), as cited in American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

10.1.3 Safety Concerns

- TMS may cause retinal toxicity after inhalation or chronic cutaneous exposure, therefore the solution should be prepared in a fume hood with gloves, mask with eye protection worn at all times (Torreilles, 2009).
- Light sensitive, discard if brownish colour develops (Close et al., 1996; Stoskopf, 1993) as it becomes toxic (Kreiberg, 2000).
- Contaminated carcasses must be discarded appropriately.
- Not to be used on animals intended for food (AVMA, 2007).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Close B., Banister K., Baumans V., Bernoth E.-V., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals: Part 1. *Laboratory Animals* 30(4):293-316.

Kreiberg H. (2000) Stress and anesthesia. In: *The Laboratory Fish: The Handbook of Experimental Animals* (Ostrander G.K., ed.) Baltimore, MD: Academic Press, pp.503-511.

Torreilles S.T., McClure D.E. and Green S.L. (2009) Evaluation and refinement of euthanasia methods for *Xenopus laevis*. *Journal of the American Association of Laboratory Animal Science* 48(5):512-516.

Stoskopf M.K. (1993) Anaesthesia. In: *Aquaculture for Veterinarians: Fish Husbandry and Medicine* (Brown L.A., ed.) Oxford UK: Pergamon Press, pp.161-167.

10.2 Potential Influence on Scientific Data

10.2.1 Circulatory System

- As a result of the lack of respiration, increases in blood concentrations of adrenaline and cortisol have been demonstrated in fish anaesthetized with buffered TMS (Iwama et al., 1989; Molinero and Gonzalez, 1995).
- May have an effect on fish physiology and blood properties (Brown, 1993), pharmacokinetics, genotoxicity, immune response, and a potential interference with hepatic cytochrome P450 spectra (Popovic et al., 2012), while others studies have shown no evidence of histopathologic changes (Baier, 2006; Wilson et al., 2009; Wright, 2001).

References

Baier J. (2006) Amphibians, as cited in Conroy C.J., Papenfuss T., Parker J. and Hahn N.E. (2009) Use of tricaine methanesulfonate (MS222) for euthanasia of reptiles. *Journal of the American Association of Laboratory Animal Science* 48(1):28-32.

Brown L.A. (1993) Anesthesia and restraint. In: *Fish Medicine*. (Stoskopf M. K., ed.). Philadelphia PA: W. B. Saunders Co, pp.79-90.

Iwama G.K., McGeer J.C. and Pawluk M.P. (1989) The effects of five fish anaesthetics on acid-base balance, hematocrit, cortisol and adrenaline in rainbow trout. *Canadian Journal of Zoology* 67(8):2065-2073.

Molinero A. and Gonzalez J. (1995) Comparative effects of MS 222 and 2-phenoxyethanol on gilthead sea bream (*Sparus aurata L.*) during confinement. *Comparative Biochemistry & Physiology A-Comparative Physiology* 111A(3):405-414.

Popovic N.T., Strunjak-Perovic I., Coz-Rakovac R., Barisic J., Jadan M., Berakovic A.P. and Klobucar R.S. (2012) Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *Journal of Applied Ichthyology* 28(4):553-564.

Wright K.M. (2001) Restraint techniques and euthanasia, as cited in Conroy C.J., Papenfuss T., Parker J. and Hahn N.E. (2009) Use of tricaine methanesulfonate (MS222) for euthanasia of reptiles. *Journal of the American Association of Laboratory Animal Science* 48(1):28-32.

CONDITIONALLY ACCEPTABLE METHODS

1. Carbon dioxide (CO_2)

Conditionally acceptable for birds, rodents and pigs; NOT an ideal method of euthanasia for any species and alternate methods should be used wherever possible

1.1 Application and Cautions

1.1.1 General

- "Induces acidosis and inhibition of neurons that leads to loss of consciousness, insensibility and finally death" (EFSA, 2005).
- Aversion to CO_2 has been reported for a variety of species (e.g., rats, mice, mink, pigs, broiler chickens, turkeys); therefore alternate methods should be used wherever possible (Raj and Gregory, 1995, 1996; Raj, 1996; Lambooij et al., 1999; Leach et al., 2002a, b, 2004; Raj et al., 2004; Kirkden et al., 2005; Niel et al., 2005). Where CO_2 is used, best practice is to anesthetize prior to administration of CO_2 to avoid distress.
- Not acceptable for use in animals that are resistant to hypoxia (e.g., neonates, breath-holding species) (Close et al., 1996).
- The literature on adding oxygen to CO_2 is currently inconclusive (see Section 5.1 of the *CCAC guidelines on: euthanasia of animals used in science*).
- If used, CO_2 must only be delivered from a compressed gas cylinder because the inflow to the chamber can be regulated precisely (AVMA, 2007).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

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Lambooij E., Gerritzen M.A., Engel B., Hillebrand S.J.W., Lankhaar L. and Pieterse C. (1999) Behavioural responses during exposure of broiler chickens to different gas mixtures. *Applied Animal Behaviour Science* 62(2):255-265.

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Leach M.C., Bowell V.A., Allan T.F. and Morton D.B. (2004) Measurement of aversion to determine humane methods of anaesthesia and euthanasia. *Animal Welfare* 13(Suppl.1):S77-S86.

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Raj A.B.M. (1996) Aversive reactions of turkeys to argon, carbon dioxide and a mixture of carbon dioxide and argon. *Veterinary Record* 138(24):592-593.

Raj A.B.M. and Gregory N.G. (1995) Welfare implications of the gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. *Animal Welfare* 4(4):273-280.

Raj A.B.M. and Gregory N.G. (1996) Welfare implications of the gas stunning of pigs 2. Stress of induction of anaesthesia. *Animal Welfare* 5(1):71-78.

Raj A.B., Leach M.C. and Morton D.B. (2004) Carbon dioxide for euthanasia of laboratory animals. *Comparative Medicine* 54(5):470-471.

1.1.2 Birds

- When the use of CO_2 is justified, concentrations <60% should be used as behavioural results at 60% carbon dioxide have been shown to cause an undue amount of stress (Ambrose et al., 2000).
- CO_2 is not acceptable for use with diving species due to the excessive time taken for effectiveness (EFSA, 2005; UK Home Office, 1986).

References

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United Kingdom Home Office (1986) *Code of Practice for the Humane Killing of Animals under Schedule 1 to the Animals (in Scientific Procedures) Act 1986*. London UK: United Kingdom Home Office.

1.1.3 Fish and Amphibians

- Should not be used in aquatic species (Close et al., 1997; Reilly, 2001).
- CO_2 forms carbonic acid when it combines with water, which can cause pain in aquatic species due to changes in water pH; rainbow trout show strong aversive behavior for 30 s to

3 min. after immersion in CO_2 (Kestin et al., 1995; Marx et al., 1997), and time to loss of consciousness in Atlantic salmon is approximately 6 min (Robb et al., 2000).

References

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Reilly J.S. (2001) *Euthanasia of Animals Used for Scientific Purposes*, 2nd ed. Adelaide, Australia: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART).

Kestin S., Wotton S. and Adams S. (1995) *The effect of CO_2 concussion or electrical stunning of rainbow trout (*Oncorhynchus Mykiss*) on fish welfare* [Poster]. International Conference Aquaculture Europe '95, Trondheim, Norway, 9-12 August 1995. Gent, Belgium: European Aquaculture Society (EAS), pp.380-381.

Marx H., Brunner B., Weinzierl W., Hoffmann R. and Stolle A. (1997) Methods of stunning freshwater fish: Impact on meat quality and aspects of animal welfare. *Z Lebensm Unters Forsch A* 204(4):282-286.

Robb D.H.F., Wotton S.B., McKinstry J.L., Sorensen N.K. and Kestin S.C. (2000) *Commercial slaughter methods used on Atlantic salmon: determination of the onset of brain failure by electroencephalography*. As cited in Algers B., Blokhuis H.J., Bøtner A., Broom D.M., Costa P., Domingo M., Greiner M., Hartung J., Koenen F., Müller-Graf C., Morton D.B., Osterhaus A., Pfeiffer D.U., Raj M., Roberts R., Sanaa M., Salman M., Sharp J.M., Vannier P. and Wierup M. (2000) Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission related to "Species-specific welfare aspects of the main systems of stunning and killing of farmed fish: rainbow trout". *The European Food Safety Authority Journal* 1013(1):1-55.

1.1.4 Reptiles and Lagomorphs

- Should not be used in breath-holding species such as reptiles and rabbits due to the excessive time taken to take effect (Ewbank, 1983; Close et al., 1997; Reilly, 2001).

References

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D.B. and Warwick C. (1997) Recommendations for euthanasia of experimental animals, Part 2. *Laboratory Animals* 31(1):1-32.

Ewbank R. (1983) Is CO_2 euthanasia humane? *Nature* 305:268-268.

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1.1.5 Rodents

- Where the use of CO_2 is justified, current best practice is filling an empty chamber at a gas flow rate of 20-30% volume per minute (Hornett and Haynes, 1984; Ambrose et al., 2000; Hawkins et al., 2006) (see sample calculation of flow rate)[1].
- CO_2 should be preceded by less aversive inhalant anesthetic gases appropriate to the species and strain (Leach et al., 2002a, 2004; Raj et al., 2004; Conlee et al., 2005; EFSA, 2005; Makowska and Weary, 2009).
- Maintaining animals in the home cage can help reduce stress due to novel environments (Hackbarth et al., 2000; Maguire and Arthur, 2003; Raj et al., 2004; ACLAM, 2005; EFSA, 2005).

References

Ambrose N., Wadham J. and Morton D. (2000) Refinement of euthanasia. In: *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* (Balls M., van Zeller A.M. and Halder M.E., eds). Amsterdam: Elsevier Science, pp.1159-1169.

American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Conlee K., Stephens M.L., Rowan A.N. and King L.A. (2005) Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. *Laboratory Animals* 39(2):137-161.

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Hackbarth H., Kuppers N. and Bohnet W. (2000) Euthanasia of rats with carbon dioxide - animal welfare aspects. *Laboratory Animals* 34(1):91-96.

Hawkins P., Playle L., Golledge H., Leach M., Banzett R., Coenen A., Cooper J., Danneman P., Flecknell P., Kirkden R., Niel L. and Raj M. (2006) *Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals*. Newcastle, Australia, February 27-28, 2006.

Hornett T.D. and Haynes A.R. (1984) Comparison of carbon dioxide/air mixture and nitrogen/air mixture for the euthanasia of rodents. Design of a system for inhalation euthanasia. *Animal Technology* 35:93-99.

Leach M.C., Bowell V.A., Allan T.F. and Morton D.B. (2002a) Aversion to gaseous euthanasia agents in rats and mice. *Comparative Medicine* 52(3):249-257.

Leach M.C., Bowell V.A., Allan T.F. and Morton D.B. (2004) Measurement of aversion to determine humane methods of anaesthesia and euthanasia. *Animal Welfare* 13(Suppl.1):S77-S86.

[1] An example of how the flow rate can be calculated for a particular CO_2 chamber for a fill rate of 20% of chamber volume per minute (adapted from Hawkins et al., 2006). Measure the chamber's internal length, width, and height in centimeters. Multiply those three numbers (length x height x width) to determine the chamber's volume in cubic centimeters (e.g., 20 x 15 x 30 cm tank = 9000 cm^3 in volume). Divide by 1000 to convert the volume to liters (9000÷1000 = 9 liters). Then multiply 9 by 0.20 because you want only 20% of the tank to fill per minute, i.e. 1.8 liters.

Maguire S. and Arthur S. (2003) *Influence of exposure cage, method of carbon dioxide exposure and pairing on cardiovascular parameters, activity, and stress-related plasma values to carbon dioxide anesthesia and euthanasia in rats [PS54]*. Proceedings of the American Association of Laboratory Animal Science Conference, Seattle, WA, pp.81-82.

Makowska I.J. and Weary D.M. (2009) Rat aversion to induction with inhalant anaesthetics. *Applied Animal Behaviour Science* 119(3):229-235.

Raj A.B., Leach M.C. and Morton D.B. (2004) Carbon dioxide for euthanasia of laboratory animals. *Comparative Medicine* 54(5):470-471.

1.1.6 Safety Concerns

- Those performing the procedure should be in a well-ventilated area (ILAR, 1992).

References

Institute of Laboratory Animal Resources – ILAR (1992) *Recognition and Alleviation of Pain and Distress in Laboratory Animals*. Washington DC: National Academy Press.

1.2 Potential Influence on Scientific Data

1.2.1 Circulatory System

- Rapid-fill: increased total leukocytes, lymphocyte counts and glucose values and decreased aspartate aminotransferase (AST), creatine kinase (CK) and calcium values (Walter, 1999) (rats).
- Reduction in blood pH (Angus et al., 2008; Traslavina et al., 2010) (mice and rats).
- Inaccurate serum potassium levels (Traslavina et al., 2010) (mice).
- Lung hemorrhage may affect histological studies, although affected areas may be avoided by judicious sampling (EFSA, 2005) (not species specific).
- In comparison with isoflurane and cervical dislocation, results in increased drug concentrations in plasma during pharmacokinetic testing (Angus et al., 2008) (mice and rats).

References

Angus D.W., Baker J.A., Mason R. and Martin I.J. (2008) The potential influence of CO_2, as an agent for euthanasia, on the pharmacokinetics of basic compounds in rodents. *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 36(2):375-379.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

Traslavina R.P., King E.J., Loar A.S., Riedel E.R., Garvey M.S., Ricart-Arbona R., Wolf F.R. and Couto S.S. (2010) Euthanasia by CO_2 inhalation affects potassium levels in mice. *Journal of the American Association for Laboratory Animal Science* 49(3):316-322.

Walter G.L. (1999) Effects of carbon dioxide inhalation on hematology, coagulation, and serum clinical chemistry values in rats. *Toxicologic Pathology* 27(2):217-225.

1.2.2 Metabolic System

- Pre-fill: decreased liver glycogen, pyruvate, ATP (Brooks et al., 1999) (rats).
- CO_2 inhibits muscle glycolytic enzymes and retard onset of rigor mortis (EFSA, 2005) (not species specific).

References

Brooks S.P.J., Lampi B.J. and Bihun C.G. (1999) The influence of euthanasia methods on rat liver metabolism. *Contemporary Topics in Laboratory Animal Science* 38(6):19-24.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

1.2.3 Nervous System

- Inhalation of CO_2 leads to altered neurotransmitters in the brain (EFSA, 2005) (not species specific).
- Faster flow rates cause greater increases in glutamate in hippocampus and cerebellum (Gos et al., 2002) (rats).
- CO_2 may cause activation of the Hypothalamic-Pituitary-Adrenal Cortex system and cause a release of corticosteroids prior to death (EFSA, 2005) (not species specific).

References

Gos T., Hauser R. and Krzyzanowski M. (2002) Regional distribution of glutamate in the central nervous system of rat terminated by carbon dioxide euthanasia. *Laboratory Animals* 36(2):127-133.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

1.2.4 Respiratory System

- Gross/histopathology changes in: lungs – congestion (Feldman and Gupta, 1976) (mice, rats, guinea pigs, and rabbits), hemorrhage (seen in mice with the addition of oxygen and the level of hemorrhage moderated by strain differences) (Ambrose et al., 2000), emphysema, atelectasis; cardiac muscle – variable degenerative changes (influenced by time of exposure to CO_2 causing acidosis, hypoxia) (ACLAM, 2005) (observed in some rodent species).

References

Ambrose N., Wadham J. and Morton D. (2000) Refinement of euthanasia. In: *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* (Balls M., van Zeller A.M. and Halder M.E., eds). Amsterdam: Elsevier Science, pp.1159-1169.

American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

Feldman D.B. and Gupta B.N. (1976) Histopathological changes in laboratory animals resulting from various methods of euthanasia. As cited in American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

2. Cervical Dislocation

Conditionally acceptable and when justified, should only be used on birds, rodents, or rabbits that meet the size criteria.

2.1 Applications and Cautions

2.1.1 General

- Causes death via brain stem damage resulting from separation of the spinal cord from the brain.
- High potential for operator error, which might result in animal suffering.
- When use is justified, it is only acceptable for species with anatomy that allows this procedure to be performed quickly and effectively (for manual cervical dislocation: birds <3kg, mice, rodents <200g, rabbits <1kg (requires strength and expertise); commercial cervical dislocators must be used on heavier rats and rabbits) (AVMA, 2007; Close et al., 1996).
- Animals should be anesthetized prior to cervical dislocation (EFSA, 2005).
- It is essential to check that the neck is broken at the end of the procedure by palpation of the vertebrae. If adequate separation is not observed, a backup method, such as decapitation or exposure to high concentrations of CO_2, should be used immediately.

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals Part 1. *Laboratory Animals* 30(4):293-316.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

2.1.2 Safety Concerns

- Should only be performed by highly trained and competent individuals (AVMA, 2007; Close et al., 1996).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals Part 1. *Laboratory Animals* 30(4):293-316.

2.2 Potential Influence on Scientific Data

2.2.1 Nervous System

- Neuropeptide levels and brain histology may be affected due to tissue damage to the central nervous system or induced neuronal discharge (EFSA, 2005) (not species specific).
- Decreased coronary flow; decreased contractile function in isolated perfused heart preparations (ACLAM, 2005) (not species specific).
- High levels of serotonin in lung (Yamamoto, 1988) (mice).
- Increase in granulocyte and macrophage colony forming cell counts in murine bone marrow cultures (Varki, 1979) (mice).

References

American College of Laboratory Animal Medicine – ACLAM (2005) *Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia*. ACLAM.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

Varki A.P., Fritz J.L. and Davis R.B. (1979) Effects of cervical dislocation on colony-forming cells in murine marrow cultures. *Experimental Hematology* 7(8):397-400.

Yamamoto Y., Hasegawa H., Ikeda K. and Ichlyama A. (1988) Cervical dislocation of mice induces rapid accumulation of platelet serotonin in the lung. *Agents and Actions* 25(1-2):49-56.

3. Concussion

Conditionally acceptable for fish and only acceptable for emergency killing in other species (newborn piglets).

3.1 Application and Cautions

3.1.1 General

- High potential for operator error, which might result in animal suffering.
- If incorrectly performed the animal may be injured and not either stunned or killed (EFSA, 2005).
- Concussion should be followed by the physical destruction of brain tissue by pithing or crushing the brain (CCAC, 2005).
- Usually preceded by anesthesia to quiet the fish (Kreiberg, 2000).

3.1.2 Safety Concerns

- The procedure must be performed by someone with appropriate training, and should be conducted in an area beyond the sensory range of other animals.

References

Canadian Council on Animal Care – CCAC (2005) *CCAC guidelines on: the care and use of fish in research, teaching and testing*. Ottawa ON: CCAC.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

Kreiberg H. (2000) Stress and anesthesia. In: *The Laboratory Fish.* (Ostrander G., ed.). San Diego CA: Academic Press, pp.503-511.

3.2 Potential Influence on Scientific Data

3.2.1 Nervous System

- Damage to brain tissues.

4. Decapitation

Conditionally acceptable for rodents and birds.

4.1 Application and Cautions

4.1.1 General

- Decapitation causes death through anoxia of the central nervous system due to blood loss (EFSA, 2005).
- Anesthetizing animals before decapitation will minimize distress and any subsequent pain (EFSA, 2005); the use of anesthesia prior to decapitation would make this an acceptable method of euthanasia.
- A purpose built mechanical device with a sharp blade should be used for decapitation (EFSA, 2005).
- When use is justified, it is only acceptable for species with anatomy that allows this procedure to be performed quickly and effectively.
- This method is used in small rodents as well as in small and young birds (<250 g) followed by destruction of the brain (EFSA, 2005).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

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4.1.2 Safety Concerns

- Personnel performing decapitation must be trained in the proper and safe use of the equipment.
- The operator using decapitation should be aware of the danger of this apparatus and should take adequate precautions to prevent personal injury (EFSA, 2005).

References

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

4.2 Potential Influence on Scientific Data

4.2.1 Circulatory System

- Increased plasma electrolyte calcium, magnesium, sodium, and potassium (Conahan, 1985; Schriefer, 1989) (rats).
- Elevated levels of some plasma amino acids and other related compounds (Milakofsky, 1984) (rats).
- Hemoglobin present in plasma, suggesting hemolysis (Schriefer, 1989) (rats).

References

Conahan S.T., Narayan S. and Vogel W.H. (1985). Effect of decapitation and stress on some plasma electrolyte levels in rats. *Pharmacological Biochemistry and Behavior* 23(1):147-149.

Milakofsky L., Hare T.A., Miller J.M. and Vogel W.H. (1984) Comparison of amino acid levels in rat blood obtained by catheterization and decapitation. *Life Science* 34(14):1333-1340.

Schriefer J.A., Plunkett W.C. and Hassen A.H. (1989) Decapitation increases plasma sodium and potassium in the rat. *Journal of Pharmacological Methods* 21(2):155-159.

4.2.2 Nervous System

- Neuropeptide levels and brain histology may be affected by tissue damage to the central nervous system or induced neuronal discharge (EFSA, 2005) (not species specific).
- Abnormally high levels of noradrenaline (Depocas, 1977) (rats).
- Associated with a 10-fold increase in circulating norepinephrine and an 80-fold increase in circulating levels of epinephrine (Popper, 1977) (rats).

References

Depocas F. and Behrens W.A. (1977). Effects of handling, decapitation, anaesthesia, and surgery on plasma noradrenaline levels in the white rat. *Canadian Journal of Physiology and Pharmacology* 55(2):212-219.

European Food Safety Authority Panel on Animal Health and Welfare – EFSA (2005) Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to "Aspects of the biology and welfare of animals used for experimental and other scientific purposes". *European Food Safety Authority Journal* 3(12):1-183.

Popper C.W., Chiueh C.C. and Kopin I.J. (1977) Plasma catecholamine concentrations in unanesthetized rats during sleep, wakefulness, immobilization and after decapitation. *The Journal of Pharmacological and Experimental Therapeutics* 202(1):144-8.

5. Gunshot

Conditionally acceptable for cattle, sheep, goats, horses, donkeys and for use in field settings.

5.1 Applications and Cautions

5.1.1 General

- Not listed as an acceptable method of euthanasia under laboratory conditions due to greater potential for operator error or safety hazard, which might result in animal suffering.
- May be useful to end animal suffering where other methods are not practically available and the operator has appropriate skills and experience (Longair et al., 1991).
- The operator must take into account differences in brain position and skull conformation between species, as well as the energy requirement for skull bone penetration (Blackmore, 1985; Blackmore et al., 1995a; Blackmore et al., 1995b; Finnie, 1994; Longair et al., 1991).
- Loss of consciousness is instantaneous if the projectile destroys most of the brain (AVMA, 2007).

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Blackmore D.K. (1985) Energy requirements for the penetration of heads of domestic stock and the development of a multiple projectile. *The Veterinary Record* 116(2):36-40.

Blackmore D.K., Bowling M.C., Madie P., Nutman A., Barnes G.R., Davies A.S., Donoghue M. and Kirk E.J. (1995a) The use of a shotgun for emergency slaughter or euthanasia of large mature pigs. *New Zealand Veterinary Journal* 43(4):134-137.

Blackmore D.K., Madie P., Bowling M.C., Nutman A., Davies A.S., McLeod W.R., Taylor J. and Degen M. (1995b). The use of a shotgun for euthanasia of stranded cetaceans. *New Zealand Veterinary Journal* 43(4):158-159.

Finnie J.W. (1994) Neuroradiological aspects of experimental traumatic missile injury in sheep. *New Zealand Veterinary Journal* 42(2):54-57.

Longair J.A., Finley G.G., Laniel M.-A., MacKay C., Mould K., Olfert E.D., Rowsell H. and Preston A. (1991) Guidelines for euthanasia of domestic animals by firearms. *Canadian Veterinary Journal* 32(12):724-726.

5.1.2 Safety Concerns

- Dangers to the operator and a high potential for operator error.

5.2 Potential Influence on Scientific Data

5.2.1 Nervous System

- Destruction of brain tissue.
- May prevent proper post-mortem analysis. This is particularly important if animals are to be tested for rabies (CCAC, 2003).

References

Canadian Council on Animal Care – CCAC (2003) *CCAC guidelines on: the care and use of wildlife*. Ottawa ON: CCAC.

6. T-61™

Conditionally acceptable for rabbits, cats and dogs.

6.1 Applications and Cautions

6.1.1 General

- An injectable, nonbarbiturate, non-narcotic mixture of three drugs (AVMA, 2007; Close et al., 1996; Morgan Morrow, 2005).
- Provides a combination of general anesthetic, and local anesthetic actions (AVMA, 2007; Close et al., 1996).
- Acts quickly but must only be injected IV very slowly – perivenous injection or too rapid IV injection causes pain (Close et al., 1996; Close et al., 1997); other routes of injection on conscious animals are not acceptable.
- Does not appear to cross the placental barrier, so should not be used for pregnant females greater than two-thirds of the way through gestation.

References

American Veterinary Medical Association – AVMA (2007) *AVMA Guidelines on Euthanasia*. Schaumburg IL: AVMA.

Close B., Banister K., Baumans V., Bernoth E.M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D. and Warwick C. (1996) Recommendations for euthanasia of experimental animals Part 1. *Laboratory Animals* 30(4):293-316.

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Morgan Morrow W.E. (2005) *Euthanasia Methods and Decisions*. Forty-Ninth Annual North Carolina Pork Conference. Greenville NC, February 16-17, 2005.

6.1.2 Rabbits, Cats, and Dogs

- Where use is justified, very slow intravenous injection only.
- Where possible a sedative should be administered prior to use of T-61™ to assist with restraint during injection and to protect the animal from any adverse effects that may be associated with the accidental failure of the procedure.

6.2 Potential Influence on Scientific Data

6.2.1 Respiratory System

- Doses larger than recommended may cause pulmonary edema and other tissue lesions (Morgan Morrow, 2005) (pigs).
- The use of T-61™ results in histopathological findings such as endothelial lesions, pulmonary congestion, pulmonary edema and hemolysis (Merck Animal Health, 2012) (not species specific).

References

Merck Animal Health (2012) T-61™ euthanasia solution. In: *Compendium of Veterinary Products* [online database]. North American Compendiums (NAC).

Morgan Morrow W.E. (2005) *Euthanasia Methods and Decisions*. Forty-Ninth Annual North Carolina Pork Conference. Greenville NC, February 16-17, 2005.