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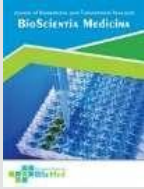
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## **Euthanasia Procedure of Animal Model in Biomedical Research**

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### **ABSTRACT**

Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful. Criteria that have been considered in recommending the methods of euthanasia include: time required to produce unconsciousness, time required to produce death, purposes, research results and compliance with the AVMA Guidelines on Euthanasia. There are special considerations for euthanizing rodent embryos, fetuses and neonates.

### **1. Introduction**

The act of inducing painless death. Selection of the method of euthanasia is dependent upon the animal species involved, objective of the procedure and skill of personnel. It is essential that proper physical control over the animal be maintained prior to euthanasia and that fear and apprehension be minimized. Noxious stimuli induce various responses including: vocalization, struggling, escape, aggression, salivation, urination, defecation, pupillary dilation, tachycardia, sweating, shivering, tremors and spasms. Not only are these responses undesirable from an aesthetic and humane point of view, they are usually undesirable complications of research where variation in baseline levels of cellular or extracellular biological values must

be minimized. Euthanizing agents terminate life by one of three basic methods: direct or indirect hypoxia, depression of vital neurons, or physical damage of brain tissue.

Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful. Criteria that have been considered in recommending the methods of euthanasia include: time required to produce unconsciousness, time required to produce death, purposes, research results and compliance with the AVMA Guidelines on Euthanasia. There are special considerations for euthanizing rodent embryos, fetuses and neonates.

The use of injectable euthanasia agents is one of the

most rapid and reliable methods of performing euthanasia. It is usually the most desirable method when it can be performed without causing fear or distress in the animal. When appropriately administered, acceptable injectable euthanasia agents result in smooth loss of consciousness prior to cessation of cardiac and/or respiratory function, minimizing pain and distress to the animal. However, heightened awareness for personnel safety is imperative when using injectable euthanasia agents because needlestick injuries involving these drugs have been shown to result in adverse effects (41.6% of the time); 17% of these adverse effects were systemic and severe. Intravenous injections deliver euthanasia agents directly into the vascular system, allowing for rapid distribution of the agent to the brain or neural centers, resulting in rapid loss of consciousness (for some invertebrates with closed circulatory systems, intrahemolymph injection is considered analogous to IV injection). When the restraint necessary for giving an animal an IV injection is likely to impart added distress to the animal or pose undue risk to the operator, sedation, anesthesia, or an acceptable alternate route or method of administration should be used. Aggressive or fearful animals should be sedated prior to restraint for IV administration of the euthanasia agent. Paralytic immobilizing agents (eg, neuromuscular blocking agents) are unacceptable as a sole means of euthanasia, because animals under their influence remain awake and able to feel pain. Having said this, there may be select circumstances (eg, for wild or feral animals) where the administration of paralytic agents (eg, neuromuscular blocking agents) may be the most rapid and humane means of restraint prior to euthanasia due to their more rapid onset compared with other immobilizing agents. In such situations, paralytic immobilizing agents may only be used if the chosen method of euthanasia (eg, captive bolt, IV injection of euthanasia solution) can be applied immediately following immobilization. Paralytic immobilizing agents must never be used as a sole means of euthanasia, nor should they be used if delay is expected between immobilization and euthanasia. When intravascular administration is considered

impractical or impossible, IP or intracoelomic administration of a nonirritating barbiturate or other approved solution is acceptable. In laboratory rats, addition of lidocaine or bupivacaine to pentobarbital reduced abdominal writhing following intraperitoneal injection. Intracoelomic administration of buffered MS 222 is acceptable for some poikilotherms. When injectable euthanasia agents are administered into the peritoneal or coelomic cavities, vertebrates may be slow to pass through stages I and II of anesthesia. Accordingly, they should be placed in small enclosures in quiet areas to minimize excitement and trauma. Intra-abdominal administration of euthanasia agents is an acceptable means of delivery in invertebrates with open circulatory systems. In anesthetized mice, retrobulbar injection of no more than 200  $\mu$ L of injectable anesthetic solution (ketamine:xylazine) is acceptable with conditions, resulting in death within 5 seconds of cessation of injection. Intraosseous administration of some euthanasia solutions to awake animals may cause pain due to the viscosity of the agent, chemical irritation, or other reasons. Administration of analgesics, slower injection of euthanasia agent, and other strategies that may reduce discomfort should be used where possible when administering euthanasia agents through preexisting intraosseous catheters. Placement of intraosseous (greater trochanter of the femur, greater tubercle of the humerus, medial aspect of the proximal tibia) catheters for administration of euthanasia agents and intracardiac, intrahepatic, intrasplenic, or intrarenal injections are acceptable only when performed on anesthetized or unconscious animals (with the exception of intrahepatic injections in cats as discussed in the Companion Animals section of the text). These routes are not acceptable in awake mammals and birds due to the difficulty and unpredictability of performing the techniques accurately with minimal discomfort. In some poikilotherms for which intracardiac puncture is the standard means of vascular access (eg, some snakes and other reptiles), intracardiac administration of euthanasia solutions in awake animals is acceptable. With the exceptions of IM delivery of ultrapotent opioids (ie, etorphine and carfentanil) and IM delivery of select

injectable anesthetics, IM, SC, intrathoracic, intrapulmonary, intrathecal, and other nonvascular injections are not acceptable routes of administration for injectable euthanasia agents in awake animals.

Physical methods of euthanasia include captive bolt, gunshot, cervical dislocation, decapitation, electrocution, focused beam microwave irradiation, exsanguination, maceration, stunning, and pithing. When properly used by skilled personnel with well maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of euthanasia. Exsanguination, stunning, and pithing are not recommended as a sole means of euthanasia, but may be considered as adjuncts to other agents or methods. Some consider physical methods of euthanasia aesthetically displeasing. There are occasions, however, when what is perceived as aesthetic and what is most humane are in conflict. Despite their aesthetic challenges, in certain situations physical methods may be the most appropriate choice for euthanasia and rapid relief of pain and suffering. Personnel using physical methods of euthanasia must be well trained and monitored for each type of physical method performed to ensure euthanasia is conducted appropriately. They must also be sensitive to the aesthetic implications of the method and convey to onlookers what they should expect to observe when at all possible. Since most physical methods involve trauma, there is inherent risk for animals and people. If the method is not performed correctly, personnel may be injured or the animal may not be effectively euthanized; personnel skill and experience are essential. Inexperienced persons should be trained by experienced persons and should practice on euthanized animals or anesthetized animals to be euthanized until they are proficient in performing the method properly and humanely. After the method has been applied, death must be confirmed before disposal of the remains.

Cervical dislocation has been used for many years for euthanasia and, when performed by welltrained individuals on appropriate animals, appears to be

humane. However, there are few scientific studies available to confirm this observation. The method has been used to euthanize small birds, poultry, mice, immature rats (< 200 g [7.1 oz]), and rabbits. For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. For immature rabbits, the head is held in one hand and the hind limbs in the other. The animal is stretched and the neck is hyperextended and dorsally twisted to separate the first cervical vertebra from the skull. For poultry and other birds, the legs of the bird should be grasped (or wings if grasped at the base) and the neck stretched by pulling on the head while applying a ventrodorsal rotational force to the skull. Crushing of cervical vertebrae and spinal cord is not acceptable unless the bird is first rendered unconscious. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency. Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation in rats, and unlike decapitation, rapid exsanguination does not contribute to loss of consciousness. For some classes of poultry there is evidence that cervical dislocation may not cause immediate unconsciousness. Advantages—(1) Cervical dislocation is a method that may induce rapid loss of consciousness. (2) It does not chemically contaminate tissue. (3) It is rapidly accomplished. Disadvantages—(1) Cervical dislocation may be aesthetically displeasing to personnel performing or observing the method. (2) Cervical dislocation requires mastering technical skills to ensure loss of consciousness is rapidly induced. (3) Its use for euthanasia is limited to small birds, poultry, mice, immature rats (< 200 g), and rabbits. General recommendations—Manual cervical dislocation is acceptable with conditions for euthanasia of small birds, poultry, mice, rats weighing < 200 g, and rabbits when performed by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical

dislocation. For heavy rats and rabbits, the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult. When performed on poultry, cervical dislocation must result in luxation of the cervical vertebrae without primary crushing of the vertebrae and spinal cord. In some classes of poultry, there is evidence that cervical dislocation may not cause immediate unconsciousness. In these cases, other physical methods such as blunt force trauma or decapitation may be more humane and should be employed when available or practicable. Those responsible for the use of this method must ensure that personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively.

Decapitation can be used to euthanize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study. Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation, more recent studies and reports indicate this activity does not imply that pain is perceived, and in fact conclude that loss of consciousness develops rapidly. Visually evoked potentials in mice were reduced more quickly after cervical dislocation compared with decapitation. Guillotines designed to accomplish decapitation of adult rodents and small rabbits in a uniformly instantaneous manner are commercially available. Guillotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose. Advantages—(1) Decapitation appears to induce rapid loss of consciousness. (2) It does not chemically contaminate tissues. (3) It is rapidly accomplished. Disadvantages—(1) Handling and restraint required to perform decapitation may be distressful for animals. (2) The interpretation of the presence of electrical activity in the brain following decapitation has created controversy, and its importance may still be open to debate. (3) Personnel performing this method should recognize the inherent danger of the guillotine and take

precautions to prevent personal injury. (4) Decapitation may be aesthetically displeasing to personnel performing or observing the method. General recommendations—This method is acceptable with conditions if performed correctly, and it may be used in research settings when its use is required by the experimental design and approved by the IACUC. Decapitation is justified for studies where undamaged and uncontaminated brain tissue is required. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal. Decapitation of amphibians, fish, and reptiles is addressed elsewhere in the Guidelines. Those responsible for the use of this method must ensure that personnel who perform decapitation have been properly trained to do so and are monitored for competence.

#### **Approved euthanasia dosage and techniques**

##### **Rodents**

1. Sodium Pentobarbital 100 mg/kg IV or IP
2. Carbon Dioxide Inhalation Chamber followed by secondary physical method (i.e. pneumothorax, cervical dislocation for rodents under 200 grams, decapitation, perfusion of a histological fixative via the major blood vessels or complete severing of the spine just below the base of the skull using a dorsal approach)
3. Cervical dislocation for rats weighing less than 200 grams and all mice after sedation (unless otherwise scientifically justified to U.C.A.R.)
4. Decapitation with guillotine only after the animal has been sedated (unless otherwise justified to U.C.A.R.)
5. Cardiac perfusion or exsanguination under deep plane of surgical anesthesia.

##### **Rabbits, nonhuman primates, dogs, cats, swine**

1. Sodium Pentobarbital 100 mg/kg IV
2. Cardiac perfusion or exsanguination under deep

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Dear author(s),

Rachmat Hidayat\*, Patricia Wulandari has submitted the manuscript "Euthanasia Procedure of Animal Model in Biomedical Research" to Bioscientia Medicina: Journal of Biomedicine and Translational Research. The paper will be screened by editor and reviewed by peer review.

Cordially,

A handwritten signature in black ink, appearing to be 'P. Magnano', with a small dot at the end.

Prof. Paula Magnano, PhD

Editor



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***(\*) Corresponding author***



**Bioscientia Medicina**

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**Peer Review Results**

Dear author(s),

Rachmat Hidayat\*, Patricia Wulandari has submitted the manuscript  
"Euthanasia Procedure of Animal Model in Biomedical Research" to  
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Research. The decision : Revision Required.

Cordially,

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Prof. Paula Magnano, PhD

Editor



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## Euthanasia Procedure of Animal Model in Biomedical Research → 1

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#### ABSTRACT → 3

Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful. Criteria that have been considered in recommending the methods of euthanasia include: time required to produce unconsciousness, time required to produce death, purposes, research results and compliance with the AVMA Guidelines on Euthanasia. There are special considerations for euthanizing rodent embryos, fetuses and neonates.

### 1. Introduction → 4

The act of inducing painless death. Selection of the method of euthanasia is dependent upon the animal species involved, objective of the procedure and skill of personnel. It is essential that proper physical control over the animal be maintained prior to euthanasia and that fear and apprehension be minimized. Noxious stimuli induce various responses including: vocalization, struggling, escape, aggression, salivation, urination, defecation, pupillary dilation, tachycardia, sweating, shivering, tremors and spasms. Not only are these responses undesirable from an aesthetic and humane point of view, they are usually undesirable complications of research where variation in baseline

levels of cellular or extracellular biological values must be minimized. Euthanizing agents terminate life by one of three basic methods: direct or indirect hypoxia, depression of vital neurons, or physical damage of brain tissue.

Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful. Criteria that have been considered in recommending the methods of euthanasia include: time required to produce unconsciousness, time required to produce death, purposes, research results and compliance with the AVMA Guidelines on Euthanasia. There are special considerations for euthanizing rodent embryos, fetuses

and neonates.

The use of injectable euthanasia agents is one of the most rapid and reliable methods of performing euthanasia. It is usually the most desirable method when it can be performed without causing fear or distress in the animal. When appropriately administered, acceptable injectable euthanasia agents result in smooth loss of consciousness prior to cessation of cardiac and/or respiratory function, minimizing pain and distress to the animal. However, heightened awareness for personnel safety is imperative when using injectable euthanasia agents because needlestick injuries involving these drugs have been shown to result in adverse effects (41.6% of the time); 17% of these adverse effects were systemic and severe. Intravenous injections deliver euthanasia agents directly into the vascular system, allowing for rapid distribution of the agent to the brain or neural centers, resulting in rapid loss of consciousness (for some invertebrates with closed circulatory systems, intrahemolymph injection is considered analogous to IV injection). When the restraint necessary for giving an animal an IV injection is likely to impart added distress to the animal or pose undue risk to the operator, sedation, anesthesia, or an acceptable alternate route or method of administration should be used. Aggressive or fearful animals should be sedated prior to restraint for IV administration of the euthanasia agent. Paralytic immobilizing agents (eg, neuromuscular blocking agents) are unacceptable as a sole means of euthanasia, because animals under their influence remain awake and able to feel pain. Having said this, there may be select circumstances (eg, for wild or feral animals) where the administration of paralytic agents (eg, neuromuscular blocking agents) may be the most rapid and humane means of restraint prior to euthanasia due to their more rapid onset compared with other immobilizing agents. In such situations, paralytic immobilizing agents may only be used if the chosen method of euthanasia (eg, captive bolt, IV injection of euthanasia solution) can be applied immediately following immobilization. Paralytic immobilizing agents must never be used as a sole

means of euthanasia, nor should they be used if delay is expected between immobilization and euthanasia. When intravascular administration is considered impractical or impossible, IP or intracoelomic administration of a nonirritating barbiturate or other approved solution is acceptable. In laboratory rats, addition of lidocaine or bupivacaine to pentobarbital reduced abdominal writhing following intraperitoneal injection. Intracoelomic administration of buffered MS 222 is acceptable for some poikilotherms. When injectable euthanasia agents are administered into the peritoneal or coelomic cavities, vertebrates may be slow to pass through stages I and II of anesthesia. Accordingly, they should be placed in small enclosures in quiet areas to minimize excitement and trauma. Intra-abdominal administration of euthanasia agents is an acceptable means of delivery in invertebrates with open circulatory systems. In anesthetized mice, retrobulbar injection of no more than 200  $\mu$ L of injectable anesthetic solution (ketamine:xylazine) is acceptable with conditions, resulting in death within 5 seconds of cessation of injection. Intraosseous administration of some euthanasia solutions to awake animals may cause pain due to the viscosity of the agent, chemical irritation, or other reasons. Administration of analgesics, slower injection of euthanasia agent, and other strategies that may reduce discomfort should be used where possible when administering euthanasia agents through preexisting intraosseous catheters. Placement of intraosseous (greater trochanter of the femur, greater tubercle of the humerus, medial aspect of the proximal tibia) catheters for administration of euthanasia agents and intracardiac, intrahepatic, intrasplenic, or intrarenal injections are acceptable only when performed on anesthetized or unconscious animals (with the exception of intrahepatic injections in cats as discussed in the Companion Animals section of the text). These routes are not acceptable in awake mammals and birds due to the difficulty and unpredictability of performing the techniques accurately with minimal discomfort. In some poikilotherms for which intracardiac puncture is the standard means of vascular access (eg, some snakes and other reptiles), intracardiac administration

of euthanasia solutions in awake animals is acceptable. With the exceptions of IM delivery of ultrapotent opioids (ie, etorphine and carfentanil) and IM delivery of select injectable anesthetics, IM, SC, intrathoracic, intrapulmonary, intrathecal, and other nonvascular injections are not acceptable routes of administration for injectable euthanasia agents in awake animals.

Physical methods of euthanasia include captive bolt, gunshot, cervical dislocation, decapitation, electrocution, focused beam microwave irradiation, exsanguination, maceration, stunning, and pithing. When properly used by skilled personnel with well maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of euthanasia. Exsanguination, stunning, and pithing are not recommended as a sole means of euthanasia, but may be considered as adjuncts to other agents or methods. Some consider physical methods of euthanasia aesthetically displeasing. There are occasions, however, when what is perceived as aesthetic and what is most humane are in conflict. Despite their aesthetic challenges, in certain situations physical methods may be the most appropriate choice for euthanasia and rapid relief of pain and suffering. Personnel using physical methods of euthanasia must be well trained and monitored for each type of physical method performed to ensure euthanasia is conducted appropriately. They must also be sensitive to the aesthetic implications of the method and convey to onlookers what they should expect to observe when at all possible. Since most physical methods involve trauma, there is inherent risk for animals and people. If the method is not performed correctly, personnel may be injured or the animal may not be effectively euthanized; personnel skill and experience are essential. Inexperienced persons should be trained by experienced persons and should practice on euthanized animals or anesthetized animals to be euthanized until they are proficient in performing the method properly and humanely. After the method has been applied, death must be confirmed before disposal of the remains.

Cervical dislocation has been used for many years for euthanasia and, when performed by welltrained individuals on appropriate animals, appears to be humane. However, there are few scientific studies available to confirm this observation. The method has been used to euthanize small birds, poultry, mice, immature rats (< 200 g [7.1 oz]), and rabbits. For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. For immature rabbits, the head is held in one hand and the hind limbs in the other. The animal is stretched and the neck is hyperextended and dorsally twisted to separate the first cervical vertebra from the skull. For poultry and other birds, the legs of the bird should be grasped (or wings if grasped at the base) and the neck stretched by pulling on the head while applying a ventrodorsal rotational force to the skull. Crushing of cervical vertebrae and spinal cord is not acceptable unless the bird is first rendered unconscious. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency. Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation in rats, and unlike decapitation, rapid exsanguination does not contribute to loss of consciousness. For some classes of poultry there is evidence that cervical dislocation may not cause immediate unconsciousness. Advantages—(1) Cervical dislocation is a method that may induce rapid loss of consciousness. (2) It does not chemically contaminate tissue. (3) It is rapidly accomplished. Disadvantages—(1) Cervical dislocation may be aesthetically displeasing to personnel performing or observing the method. (2) Cervical dislocation requires mastering technical skills to ensure loss of consciousness is rapidly induced. (3) Its use for euthanasia is limited to small birds, poultry, mice, immature rats (< 200 g), and rabbits. General recommendations—Manual cervical dislocation is acceptable with conditions for euthanasia of small birds, poultry, mice, rats weighing < 200 g, and rabbits when performed by individuals with a demonstrated

high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical dislocation. For heavy rats and rabbits, the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult. When performed on poultry, cervical dislocation must result in luxation of the cervical vertebrae without primary crushing of the vertebrae and spinal cord. In some classes of poultry, there is evidence that cervical dislocation may not cause immediate unconsciousness. In these cases, other physical methods such as blunt force trauma or decapitation may be more humane and should be employed when available or practicable. Those responsible for the use of this method must ensure that personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively.

Decapitation can be used to euthanize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study. Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation, more recent studies and reports indicate this activity does not imply that pain is perceived, and in fact conclude that loss of consciousness develops rapidly. Visually evoked potentials in mice were reduced more quickly after cervical dislocation compared with decapitation. Guillotines designed to accomplish decapitation of adult rodents and small rabbits in a uniformly instantaneous manner are commercially available. Guillotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose. Advantages—(1) Decapitation appears to induce rapid loss of consciousness. (2) It does not chemically contaminate tissues. (3) It is rapidly accomplished. Disadvantages—(1) Handling and restraint required to perform decapitation may be distressful for animals. (2) The interpretation of the presence of electrical activity in the brain following decapitation has created

controversy, and its importance may still be open to debate. (3) Personnel performing this method should recognize the inherent danger of the guillotine and take precautions to prevent personal injury. (4) Decapitation may be aesthetically displeasing to personnel performing or observing the method. General recommendations—This method is acceptable with conditions if performed correctly, and it may be used in research settings when its use is required by the experimental design and approved by the IACUC. Decapitation is justified for studies where undamaged and uncontaminated brain tissue is required. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal. Decapitation of amphibians, fish, and reptiles is addressed elsewhere in the Guidelines. Those responsible for the use of this method must ensure that personnel who perform decapitation have been properly trained to do so and are monitored for competence.

## **Approved euthanasia dosage and techniques**

### **Rodents**

1. Sodium Pentobarbital 100 mg/kg IV or IP
2. Carbon Dioxide Inhalation Chamber followed by secondary physical method (i.e. pneumothorax, cervical dislocation for rodents under 200 grams, decapitation, perfusion of a histological fixative via the major blood vessels or complete severing of the spine just below the base of the skull using a dorsal approach)
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## **Rabbits, nonhuman primates, dogs, cats, swine**

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## **Reviewer Comment:**

1→ Title of Manuscripts should be explained main review and declared type of literature review: narrative or systematic review.

2→ Keywords should be showed the main words of the study, the authors can use MeSH to develop keywords.

3→ Abstract should be showed the main of background, main of review and conclusion of study.

4→Introduction should be showed the urgency of study (epidemiology data), biological plausibility concept, and lack of knowledge in the study.

5→ Authors must check the references for make update references. References should no more than 10 years.



## Euthanasia Procedure of Animal Model in Biomedical Research

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### ABSTRACT A

Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful. Criteria that have been considered in recommending the methods of euthanasia include: time required to produce unconsciousness, time required to produce death, purposes, research results and compliance with the AVMA Guidelines on Euthanasia. There are special considerations for euthanizing rodent embryos, fetuses and neonates.

### 1. Introduction B

The act of inducing painless death. Selection of the method of euthanasia is dependent upon the animal species involved, objective of the procedure and skill of personnel. It is essential that proper physical control over the animal be maintained prior to euthanasia and that fear and apprehension be minimized. Noxious stimuli induce various responses including: vocalization, struggling, escape, aggression, salivation, urination, defecation, pupillary dilation, tachycardia, sweating, shivering, tremors and spasms. Not only are these responses undesirable from an aesthetic and humane point of view, they are usually undesirable complications of research where variation in baseline levels of cellular or extracellular biological values must be minimized. Euthanizing agents terminate life by one

of three basic methods: direct or indirect hypoxia, depression of vital neurons, or physical damage of brain tissue.

Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful. Criteria that have been considered in recommending the methods of euthanasia include: time required to produce unconsciousness, time required to produce death, purposes, research results and compliance with the AVMA Guidelines on Euthanasia. There are special considerations for euthanizing rodent embryos, fetuses and neonates.

The use of injectable euthanasia agents is one of the most rapid and reliable methods of performing



euthanasia. It is usually the most desirable method when it can be performed without causing fear or distress in the animal. When appropriately administered, acceptable injectable euthanasia agents result in smooth loss of consciousness prior to cessation of cardiac and/or respiratory function, minimizing pain and distress to the animal. However, heightened awareness for personnel safety is imperative when using injectable euthanasia agents because needlestick injuries involving these drugs have been shown to result in adverse effects (41.6% of the time); 17% of these adverse effects were systemic and severe. Intravenous injections deliver euthanasia agents directly into the vascular system, allowing for rapid distribution of the agent to the brain or neural centers, resulting in rapid loss of consciousness (for some invertebrates with closed circulatory systems, intrahemolymph injection is considered analogous to IV injection). When the restraint necessary for giving an animal an IV injection is likely to impart added distress to the animal or pose undue risk to the operator, sedation, anesthesia, or an acceptable alternate route or method of administration should be used. Aggressive or fearful animals should be sedated prior to restraint for IV administration of the euthanasia agent. Paralytic immobilizing agents (eg, neuromuscular blocking agents) are unacceptable as a sole means of euthanasia, because animals under their influence remain awake and able to feel pain. Having said this, there may be select circumstances (eg, for wild or feral animals) where the administration of paralytic agents (eg, neuromuscular blocking agents) may be the most rapid and humane means of restraint prior to euthanasia due to their more rapid onset compared with other immobilizing agents. In such situations, paralytic immobilizing agents may only be used if the chosen method of euthanasia (eg, captive bolt, IV injection of euthanasia solution) can be applied immediately following immobilization. Paralytic immobilizing agents must never be used as a sole means of euthanasia, nor should they be used if delay is expected between immobilization and euthanasia. When intravascular administration is considered impractical or impossible, IP or intracoelomic

administration of a nonirritating barbiturate or other approved solution is acceptable. In laboratory rats, addition of lidocaine or bupivacaine to pentobarbital reduced abdominal writhing following intraperitoneal injection. Intracoelomic administration of buffered MS 222 is acceptable for some poikilotherms. When injectable euthanasia agents are administered into the peritoneal or coelomic cavities, vertebrates may be slow to pass through stages I and II of anesthesia. Accordingly, they should be placed in small enclosures in quiet areas to minimize excitement and trauma. Intra-abdominal administration of euthanasia agents is an acceptable means of delivery in invertebrates with open circulatory systems. In anesthetized mice, retrobulbar injection of no more than 200  $\mu$ L of injectable anesthetic solution (ketamine:xylozine) is acceptable with conditions, resulting in death within 5 seconds of cessation of injection. Intraosseous administration of some euthanasia solutions to awake animals may cause pain due to the viscosity of the agent, chemical irritation, or other reasons. Administration of analgesics, slower injection of euthanasia agent, and other strategies that may reduce discomfort should be used where possible when administering euthanasia agents through preexisting intraosseous catheters. Placement of intraosseous (greater trochanter of the femur, greater tubercle of the humerus, medial aspect of the proximal tibia) catheters for administration of euthanasia agents and intracardiac, intrahepatic, intrasplenic, or intrarenal injections are acceptable only when performed on anesthetized or unconscious animals (with the exception of intrahepatic injections in cats as discussed in the Companion Animals section of the text). These routes are not acceptable in awake mammals and birds due to the difficulty and unpredictability of performing the techniques accurately with minimal discomfort. In some poikilotherms for which intracardiac puncture is the standard means of vascular access (eg, some snakes and other reptiles), intracardiac administration of euthanasia solutions in awake animals is acceptable. With the exceptions of IM delivery of ultrapotent opioids (ie, etorphine and carfentanil) and IM delivery of select injectable anesthetics, IM, SC, intrathoracic,

intrapulmonary, intrathecal, and other nonvascular injections are not acceptable routes of administration for injectable euthanasia agents in awake animals.

Physical methods of euthanasia include captive bolt, gunshot, cervical dislocation, decapitation, electrocution, focused beam microwave irradiation, exsanguination, maceration, stunning, and pithing. When properly used by skilled personnel with well maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of euthanasia. Exsanguination, stunning, and pithing are not recommended as a sole means of euthanasia, but may be considered as adjuncts to other agents or methods. Some consider physical methods of euthanasia aesthetically displeasing. There are occasions, however, when what is perceived as aesthetic and what is most humane are in conflict. Despite their aesthetic challenges, in certain situations physical methods may be the most appropriate choice for euthanasia and rapid relief of pain and suffering. Personnel using physical methods of euthanasia must be well trained and monitored for each type of physical method performed to ensure euthanasia is conducted appropriately. They must also be sensitive to the aesthetic implications of the method and convey to onlookers what they should expect to observe when at all possible. Since most physical methods involve trauma, there is inherent risk for animals and people. If the method is not performed correctly, personnel may be injured or the animal may not be effectively euthanized; personnel skill and experience are essential. Inexperienced persons should be trained by experienced persons and should practice on euthanized animals or anesthetized animals to be euthanized until they are proficient in performing the method properly and humanely. After the method has been applied, death must be confirmed before disposal of the remains.

Cervical dislocation has been used for many years for euthanasia and, when performed by welltrained individuals on appropriate animals, appears to be humane. However, there are few scientific studies

available to confirm this observation. The method has been used to euthanize small birds, poultry, mice, immature rats (< 200 g [7.1 oz]), and rabbits. For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. For immature rabbits, the head is held in one hand and the hind limbs in the other. The animal is stretched and the neck is hyperextended and dorsally twisted to separate the first cervical vertebra from the skull. For poultry and other birds, the legs of the bird should be grasped (or wings if grasped at the base) and the neck stretched by pulling on the head while applying a ventrodorsal rotational force to the skull. Crushing of cervical vertebrae and spinal cord is not acceptable unless the bird is first rendered unconscious. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency. Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation in rats, and unlike decapitation, rapid exsanguination does not contribute to loss of consciousness. For some classes of poultry there is evidence that cervical dislocation may not cause immediate unconsciousness. Advantages—(1) Cervical dislocation is a method that may induce rapid loss of consciousness. (2) It does not chemically contaminate tissue. (3) It is rapidly accomplished. Disadvantages—(1) Cervical dislocation may be aesthetically displeasing to personnel performing or observing the method. (2) Cervical dislocation requires mastering technical skills to ensure loss of consciousness is rapidly induced. (3) Its use for euthanasia is limited to small birds, poultry, mice, immature rats (< 200 g), and rabbits. General recommendations—Manual cervical dislocation is acceptable with conditions for euthanasia of small birds, poultry, mice, rats weighing < 200 g, and rabbits when performed by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical dislocation. For heavy rats and rabbits, the large

muscle mass in the cervical region makes manual cervical dislocation physically more difficult. When performed on poultry, cervical dislocation must result in luxation of the cervical vertebrae without primary crushing of the vertebrae and spinal cord. In some classes of poultry, there is evidence that cervical dislocation may not cause immediate unconsciousness. In these cases, other physical methods such as blunt force trauma or decapitation may be more humane and should be employed when available or practicable. Those responsible for the use of this method must ensure that personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively.

Decapitation can be used to euthanize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study. Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation, more recent studies and reports indicate this activity does not imply that pain is perceived, and in fact conclude that loss of consciousness develops rapidly. Visually evoked potentials in mice were reduced more quickly after cervical dislocation compared with decapitation. Guillotines designed to accomplish decapitation of adult rodents and small rabbits in a uniformly instantaneous manner are commercially available. Guillotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose. Advantages—(1) Decapitation appears to induce rapid loss of consciousness. (2) It does not chemically contaminate tissues. (3) It is rapidly accomplished. Disadvantages—(1) Handling and restraint required to perform decapitation may be distressful for animals. (2) The interpretation of the presence of electrical activity in the brain following decapitation has created controversy, and its importance may still be open to debate. (3) Personnel performing this method should recognize the inherent danger of the guillotine and take precautions to prevent personal injury. (4) Decapitation

may be aesthetically displeasing to personnel performing or observing the method. General recommendations—This method is acceptable with conditions if performed correctly, and it may be used in research settings when its use is required by the experimental design and approved by the IACUC. Decapitation is justified for studies where undamaged and uncontaminated brain tissue is required. The equipment used to perform decapitation must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal. Decapitation of amphibians, fish, and reptiles is addressed elsewhere in the Guidelines. Those responsible for the use of this method must ensure that personnel who perform decapitation have been properly trained to do so and are monitored for competence.

## **Approved euthanasia dosage and techniques**

### **Rodents**

1. Sodium Pentobarbital 100 mg/kg IV or IP
2. Carbon Dioxide Inhalation Chamber followed by secondary physical method (i.e. pneumothorax, cervical dislocation for rodents under 200 grams, decapitation, perfusion of a histological fixative via the major blood vessels or complete severing of the spine just below the base of the skull using a dorsal approach)
3. Cervical dislocation for rats weighing less than 200 grams and all mice after sedation (unless otherwise scientifically justified to U.C.A.R.)
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## Reviewer Comment:

- a. Authors should make more specific and attractive. Authors also should be declared the type of study clearly.
- b. Authors should make abstract more specific and try to show the main concept of the review.
- c. Authors should develop introduction more attractive. Authors should be tried to show the urgency by epidemiology data. Authors also should be developed main sub title of review in systematic, constructive and specific. Authors not only showed the standar review and no focus, try to focused in your review.

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#### ABSTRACT

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#### Introduction

The act of inducing painless death. Selection of the method of euthanasia is dependent upon the animal species involved, objective of the procedure and skill of personnel. It is essential that proper physical control over the animal be maintained prior to euthanasia and that fear and apprehension be minimized. Noxious stimuli induce various responses including: vocalization, struggling, escape, aggression, salivation, urination, defecation, pupillary dilation, tachycardia, sweating, shivering, tremors and spasms. Not only are these responses undesirable from an aesthetic and humane point of view, they are usually undesirable complications of research where variation in baseline levels of cellular or extracellular biological values must be minimized. Euthanizing agents terminate life by one of three basic methods: direct or indirect hypoxia, depression of vital neurons, or physical damage of

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Physical methods of euthanasia include captive

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Cervical dislocation has been used for many years for euthanasia and, when performed by welltrained individuals on appropriate animals, appears to be humane. However, there are few scientific studies available to confirm this observation. The method has been used to euthanize small birds, poultry, mice, immature rats (< 200 g [7.1 oz]), and rabbits. For mice and rats, the thumb and index finger are placed on

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3. Cervical dislocation for rats weighing less than 200 grams and all mice after sedation (unless otherwise scientifically justified to U.C.A.R.)
4. Decapitation with guillotine only after the animal has been sedated (unless otherwise justified to U.C.A.R.)
5. Cardiac perfusion or exsanguination under deep plane of surgical anesthesia.

#### **Rabbits, nonhuman primates, dogs, cats, swine**

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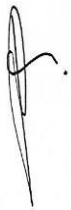
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**Letter of Acceptance**

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Cordially,



Prof. Paula Magnano, PhD

Editor



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### Euthanasia Procedure of Animal Model in Biomedical Research

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#### ABSTRACT

Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful. Criteria that have been considered in recommending the methods of euthanasia include: time required to produce unconsciousness, time required to produce death, purposes, research results and compliance with the AVMA Guidelines on Euthanasia. There are special considerations for euthanizing rodent embryos, fetuses and neonates.

#### 1. Introduction

The act of inducing painless death. Selection of the method of euthanasia is dependent upon the animal species involved, objective of the procedure and skill of personnel. It is essential that proper physical control over the animal be maintained prior to euthanasia and that fear and apprehension be minimized. Noxious stimuli induce various responses including: vocalization, struggling, escape, aggression, salivation, urination, defecation, pupillary dilation, tachycardia, sweating, shivering, tremors and spasms. Not only are these responses undesirable from an aesthetic and humane point of view, they are usually undesirable complications of research where variation in baseline levels of cellular or extracellular biological values must be minimized. Euthanizing agents terminate life by one of three basic methods: direct or indirect hypoxia, depression of vital neurons, or physical damage of

brain tissue.

Regardless of the method, it is essential to induce unconsciousness as rapidly as possible if euthanasia is to be aesthetically and scientifically successful. Criteria that have been considered in recommending the methods of euthanasia include: time required to produce unconsciousness, time required to produce death, purposes, research results and compliance with the AVMA Guidelines on Euthanasia. There are special considerations for euthanizing rodent embryos, fetuses and neonates.

The use of injectable euthanasia agents is one of the most rapid and reliable methods of performing euthanasia. It is usually the most desirable method when it can be performed without causing fear or distress in the animal. When appropriately administered, acceptable injectable euthanasia agents result in smooth loss of consciousness prior to

cessation of cardiac and/or respiratory function, minimizing pain and distress to the animal. However, heightened awareness for personnel safety is imperative when using injectable euthanasia agents because needlestick injuries involving these drugs have been shown to result in adverse effects (41.6% of the time); 17% of these adverse effects were systemic and severe. Intravenous injections deliver euthanasia agents directly into the vascular system, allowing for rapid distribution of the agent to the brain or neural centers, resulting in rapid loss of consciousness (for some invertebrates with closed circulatory systems, intrahemolymph injection is considered analogous to IV injection). When the restraint necessary for giving an animal an IV injection is likely to impart added distress to the animal or pose undue risk to the operator, sedation, anesthesia, or an acceptable alternative route or method of administration should be used. Aggressive or fearful animals should be sedated prior to restraint for IV administration of the euthanasia agent. Paralytic immobilizing agents (eg, neuromuscular blocking agents) are unacceptable as a sole means of euthanasia, because animals under their influence remain awake and able to feel pain. Having said this, there may be select circumstances (eg, for wild or feral animals) where the administration of paralytic agents (eg, neuromuscular blocking agents) may be the most rapid and humane means of restraint prior to euthanasia due to their more rapid onset compared with other immobilizing agents. In such situations, paralytic immobilizing agents may only be used if the chosen method of euthanasia (eg, captive bolt, IV injection of euthanasia solution) can be applied immediately following immobilization. Paralytic immobilizing agents must never be used as a sole means of euthanasia, nor should they be used if delay is expected between immobilization and euthanasia. When intravascular administration is considered impractical or impossible, IP or intracoelomic administration of a nonirritating barbiturate or other approved solution is acceptable. In laboratory rats, addition of lidocaine or bupivacaine to pentobarbital reduced abdominal writhing following intraperitoneal injection. Intracoelomic administration of buffered MS

222 is acceptable for some poikilotherms. When injectable euthanasia agents are administered into the peritoneal or coelomic cavities, vertebrates may be slow to pass through stages I and II of anesthesia. Accordingly, they should be placed in small enclosures in quiet areas to minimize excitement and trauma. Intra-abdominal administration of euthanasia agents is an acceptable means of delivery in invertebrates with open circulatory systems. In anesthetized mice, retrobulbar injection of no more than 200  $\mu$ L of injectable anesthetic solution (ketamine:xylazine) is acceptable with conditions resulting in death within 5 seconds of cessation of injection. Intraosseous administration of some euthanasia solutions to awake animals may cause pain due to the viscosity of the agent, chemical irritation, or other reasons. Administration of analgesics, slower injection of euthanasia agent, and other strategies that may reduce discomfort should be used where possible when administering euthanasia agents through preexisting intraosseous catheters. Placement of intraosseous (greater trochanter of the femur, greater tubercle of the humerus, medial aspect of the proximal tibia) catheters for administration of euthanasia agents and intracardiac, intrahepatic, intrasplenic, or intrarenal injections are acceptable only when performed on anesthetized or unconscious animals (with the exception of intrahepatic injections in cats as discussed in the Companion Animals section of the text). These routes are not acceptable in awake mammals and birds due to the difficulty and unpredictability of performing the techniques accurately with minimal discomfort. In some poikilotherms for which intracardiac puncture is the standard means of vascular access (eg, some snakes and other reptiles), intracardiac administration of euthanasia solutions in awake animals is acceptable. With the exceptions of IM delivery of ultrapotent opioids (ie, etorphine and carfentanil) and IM delivery of select injectable anesthetics, IM, SC, intrathoracic, intrapulmonary, intrathecal, and other nonvascular injections are not acceptable routes of administration for injectable euthanasia agents in awake animals.

Physical methods of euthanasia include captive

bolt, gunshot, cervical dislocation, decapitation, electrocution, focused beam microwave irradiation, exsanguination, maceration, stunning, and pithing. When properly used by skilled personnel with well maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of euthanasia. Exsanguination, stunning, and pithing are not recommended as a sole means of euthanasia, but may be considered as adjuncts to other agents or methods. Some consider physical methods of euthanasia aesthetically displeasing. There are occasions, however, when what is perceived as aesthetic and what is most humane are in conflict. Despite their aesthetic challenges, in certain situations physical methods may be the most appropriate choice for euthanasia and rapid relief of pain and suffering. Personnel using physical methods of euthanasia must be well trained and monitored for each type of physical method performed to ensure euthanasia is conducted appropriately. They must also be sensitive to the aesthetic implications of the method and convey to onlookers what they should expect to observe when at all possible. Since most physical methods involve trauma, there is inherent risk for animals and people. If the method is not performed correctly, personnel may be injured or the animal may not be effectively euthanized; personnel skill and experience are essential. Inexperienced persons should be trained by experienced persons and should practice on euthanized animals or anesthetized animals to be euthanized until they are proficient in performing the method properly and humanely. After the method has been applied, death must be confirmed before disposal of the remains.

Cervical dislocation has been used for many years for euthanasia and, when performed by welltrained individuals on appropriate animals, appears to be humane. However, there are few scientific studies available to confirm this observation. The method has been used to euthanize small birds, poultry, mice, immature rats (< 200 g [7.1 oz]), and rabbits. For mice and rats, the thumb and index finger are placed on

either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. For immature rabbits, the head is held in one hand and the hind limbs in the other. The animal is stretched and the neck is hyperextended and dorsally twisted to separate the first cervical vertebra from the skull. For poultry and other birds, the legs of the bird should be grasped (or wings if grasped at the base) and the neck stretched by pulling on the head while applying a ventrodorsal rotational force to the skull. Crushing of cervical vertebrae and spinal cord is not acceptable unless the bird is first rendered unconscious. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency. Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation in rats, and unlike decapitation, rapid exsanguination does not contribute to loss of consciousness. For some classes of poultry there is evidence that cervical dislocation may not cause immediate unconsciousness. Advantages—(1) Cervical dislocation is a method that may induce rapid loss of consciousness. (2) It does not chemically contaminate tissue. (3) It is rapidly accomplished. Disadvantages—(1) Cervical dislocation may be aesthetically displeasing to personnel performing or observing the method. (2) Cervical dislocation requires mastering technical skills to ensure loss of consciousness is rapidly induced. (3) Its use for euthanasia is limited to small birds, poultry, mice, immature rats (< 200 g), and rabbits. General recommendations—Manual cervical dislocation is acceptable with conditions for euthanasia of small birds, poultry, mice, rats weighing < 200 g, and rabbits when performed by individuals with a demonstrated high degree of technical proficiency. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical dislocation. For heavy rats and rabbits, the large muscle mass in the cervical region makes manual cervical dislocation physically more difficult. When performed on poultry, cervical dislocation must result in luxation of the cervical vertebrae without primary

crushing of the vertebrae and spinal cord. In some classes of poultry, there is evidence that cervical dislocation may not cause immediate unconsciousness. In these cases, other physical methods such as blunt force trauma or decapitation may be more humane and should be employed when available or practicable. Those responsible for the use of this method must ensure that personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively.

Decapitation can be used to euthanize rodents and small rabbits in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study. Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation, more recent studies and reports indicate this activity does not imply that pain is perceived, and in fact conclude that loss of consciousness develops rapidly. Usually evoked potentials in mice were reduced more quickly after cervical dislocation compared with decapitation. Guillotines designed to accomplish decapitation of adult rodents and small rabbits in a uniformly instantaneous manner are commercially available. Guillotines are not commercially available for neonatal rodents, but sharp blades can be used for this purpose. Advantages—(1) Decapitation appears to induce rapid loss of consciousness. (2) It does not chemically contaminate tissues. (3) It is rapidly accomplished. Disadvantages—(1) Handling and restraint required to perform decapitation may be distressful for animals. (2) The interpretation of the presence of electrical activity in the brain following decapitation has created controversy, and its importance may still be open to debate. (3) Personnel performing this method should recognize the inherent danger of the guillotine and take precautions to prevent personal injury. (4) Decapitation may be aesthetically displeasing to personnel performing or observing the method. General recommendations—This method is acceptable with conditions if performed correctly, and it may be used in

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## Approved euthanasia dosage and techniques

### Rodents

1. Sodium Pentobarbital 100 mg/kg IV or IP
2. Carbon Dioxide Inhalation Chamber followed by secondary physical method (i.e. pneumothorax, cervical dislocation for rodents under 200 grams, decapitation, perfusion of a histological fixative via the major blood vessels or complete severing of the spine just below the base of the skull using a dorsal approach)
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# CERTIFICATE

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