CCAC
CCPA

Canadian Council on Animal Care
Conseil canadien de protection des animaux

CCAC guidelines: Scientific procedures (Part B – Analgesia, anesthesia, and surgery)

Date of Publication: March 2025

© Canadian Council on Animal Care, 2025

ISBN: 978-1-998370-03-0

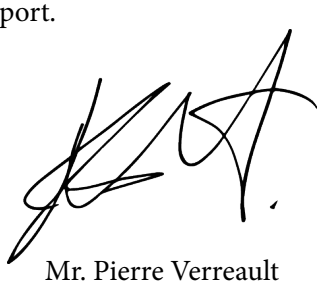
190 O'Connor St., Suite 800
Ottawa, Ontario, K2P 2R3

www.ccac.ca

ACKNOWLEDGEMENTS

The Canadian Council on Animal Care (CCAC) Board of Directors is grateful for the expertise contributed by the members of the CCAC Subcommittee on Analgesia, Anesthesia, and Surgery and for their engagement throughout the guidelines development process. In addition, the board is grateful to all those who provided critical input during the two review periods. We would also like to acknowledge the contributions of both the CCAC Standards Committee and the CCAC Assessment and Certification Committee members, who provided important guidance to the subcommittee. Finally, we would like to thank the CCAC Secretariat project team for its excellent work as well as the members of the CCAC Translation Advisory Group (Dr. Geneviève Fortin-Simard, Ms. Kiana McFadden-Houle, and Dr. Sylvie Fortier) for the review of the French translation of the document. The CCAC also acknowledges its funders, the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada. The CCAC could not continue to deliver on its current mandate without their support.

Ms. Catherine Rushton
Chair, CCAC Board of Directors



Mr. Pierre Verreault
CCAC Executive Director

ANALGESIA, ANESTHESIA, AND SURGERY SUBCOMMITTEE

Dr. Eric Troncy (Chair), Université de Montréal
Dr. Jamie Ahloy Dallaire, Université Laval
Dr. Djamal Bennamane, ITR Laboratories Canada Inc.
Dr. Danyel Bueno Dalto, Agriculture and Agri-Food Canada
Dr. Frédéric Chatigny, Cégep de Sherbrooke
Dr. Chereen Collymore, Charles River Laboratories
Ms. Kate Cooper, University of Victoria
Dr. Will Cupples, Simon Fraser University (emeritus)
Dr. Shereen Hamza, University of Alberta
Dr. Jane Harms, Government of Yukon
Dr. Hélène Héon, Centre hospitalier de l'Université de Montréal
Dr. Adam Hering, Victoria, British Columbia
Ms. Sharon McCarthy, Mississauga, Ontario
Dr. Keith Sharkey, University of Calgary
Dr. Susan Vogel, Charles River Laboratories

TABLE OF CONTENTS

Scientific procedures (Part B – Analgesia, anesthesia, and surgery)

PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer-review organization responsible for setting, maintaining, and overseeing the implementation of standards for ethical animal care and use in science throughout Canada. CCAC standards are based on professional expertise and current interpretation of scientific evidence.

The *CCAC guidelines: Scientific procedures (Part B – Analgesia, anesthesia, and surgery)* is part of a series of general guidelines documents for the ethics and care of all animals used in scientific activities, including wild animals in the field or brought into scientific facilities, and animals owned by third parties that are used in science. General guidelines streamline information for protocol authors, animal care committees, facility managers, veterinarians, technicians, and animal care personnel to help facilitate improvement in both the care given to animals and the manner in which scientific activities are carried out. More specific guidance on the application of this guidelines document for particular species or groups of animals can be found in the CCAC's types of animal guidelines documents.

This specific document describes general guidelines for analgesia, anesthesia, and surgery.

This guidelines document details the standards that are expected to be met by holders of the CCAC Certificate of GAP – Good Animal Practice®. For scientific activities conducted within Canada or outside of Canada, protocol authors based at CCAC-certified institutions are subject to these standards. Protocol authors are also subject to any relevant legislation and regulations in the jurisdiction where the scientific activity is conducted.

SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT

The following list of guideline statements serves as an executive summary covering the most important aspects of analgesia, anesthesia, and surgery. These guideline statements are included throughout this document alongside details and references that provide support and context for their implementation. Throughout this document, the term 'should' is used to indicate an obligation, for which any exceptions must be justified to, and approved by, an animal care committee. The term 'must' is used for mandatory requirements.

2. ANALGESIA

Guideline 1

Appropriate analgesia should be provided for painful or potentially painful procedures. Analgesia must only be withheld if there is definitive evidence that all available options will compromise the integrity of the scientific activity. In rare cases when analgesia is withheld, there must be increased oversight of the protocol.

Section 2 Analgesia, p.8

Guideline 2

Appropriate analgesia should be selected through a collaborative process between protocol authors and veterinarians.

Section 2.1 Decision-Making Process, p.8

Guideline 3

Persons responsible for monitoring animal pain, including the effectiveness of the analgesia, must be trained and deemed competent in this regard.

Section 2.1 Decision-Making Process, p.9

3. ANESTHESIA

Guideline 4

Appropriate anesthesia, individualized to each animal and procedure, must be provided for each procedure warranting anesthesia. The anesthetic approach must be based on the expected welfare impact that the animal will experience rather than solely on the procedure.

Section 3 Anesthesia, p.22

Guideline 5

Supplemental oxygen should be provided during anesthesia. Intubating the animal and maintaining intravenous access are best practices.

Section 3.4 Anesthetic Methods and Agents, p.27

Guideline 6

If an inhalant anesthetic is to be used, it must be delivered using a reliable and titratable source.

Section 3.5 Required Equipment for Inhalant Anesthesia, p.32

Guideline 7

When using inhalant anesthesia, the vaporizer must be calibrated and verified for correct function. The anesthesia machine must be well maintained, and leak tests must be performed before each use. A fully functional scavenging system must be in place. Personnel must be deemed competent to use the anesthesia machine and associated equipment before using them.

Section 3.5 Required Equipment for Inhalant Anesthesia, p.32

Guideline 8

Animal monitoring must start in the pre-anesthesia phase and be regularly maintained until full recovery.

Section 3.8 Animal Monitoring, p.34

Guideline 9

Adequate care must be provided to all animals during the recovery period.

Section 3.10 Recovery Considerations, p.39

4. SURGERY

Guideline 10

Aseptic technique must be maintained during all aspects of a recovery surgical procedure and is strongly encouraged for non-recovery procedures.

Section 4 Surgery, p.40

Guideline 11

Surgical procedures must only be completed by someone who has been deemed competent to perform the procedure, or by someone being directly supervised by such a person.

Section 4 Surgery, p.40

Guideline 12

Appropriate analgesia must be provided as part of post-operative care; this includes non-pharmacological and pharmacological options.

Section 4.11 Post-Surgical Recovery, p.48

1 INTRODUCTION – ANIMAL PAIN

Throughout this document, the term 'should' is used to indicate an obligation, for which any exceptions must be justified to, and approved by, an animal care committee. The term 'must' is used for mandatory requirements.

1.1 PAIN AND NOCICEPTION

This document uses the definition of pain formulated by the International Association on the Study of Pain for pain in humans: "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (IASP, n.d.; Raja et al., 2020). Thus, pain is fundamentally a subjective experience of each animal. Pain differs from nociception, which refers exclusively to the neural process of encoding noxious stimuli (IASP, n.d.; Raja et al., 2020) and does not imply any sentient processes. Generally, however, nociception underlies pain in conscious animals. This may not be true in appropriately anesthetized animals; when afferent neurons are surgically severed, preventing sensory information from reaching the central nervous system; or when certain internal organs are damaged or removed, but these instances are exceptions.

Animal pain is generally inferred based on behavioural or physiological indicators (e.g., facial grimace scale (Sotocinal et al., 2011), lameness evaluation (Shearer et al., 2013), quantitative sensory testing (Montiero et al., 2020), and measuring cytokines (Miller et al., 2014); see also Section 1.4, "Recognizing and Treating Pain"). The inability of an animal to express pain-related indicators (e.g., due to paralytics) or the inability of humans to detect pain-related indicators (e.g., due to a lack of knowledge or technology on their part) does not necessarily mean that an animal is not experiencing pain (Raja et al., 2020).

Following the precautionary principle and extensive scientific literature across species, **all animals under the CCAC mandate** (namely, nonhuman vertebrates or cephalopods; see *Requirement for submitting an animal protocol: Addendum to the CCAC policy statement on terms of reference for animal care committees* (CCAC, 2020)) **are assumed to have the capacity to feel pain**. Thus, protocol authors must maximize refinements in scientific protocols to minimize pain.

1.2 MODULATORS OF PAIN

Pain has three main determinants: sensory or discriminative, motivational or affective, and cognitive or central control (Melzack and Casey, 1968). This means that the subjective experience of pain is not solely dependent upon the specific injury or disease but can be altered by the individual's affective and cognitive states. For example, environmental enrichment, a widely regarded method to improve affective states in animals, also reduces pain-related behaviours when compared to similarly impaired animals housed without enrichment (e.g., Vachon et al., 2013; Parent-Vachon and Vachon, 2018; Wang et al., 2019). Some examples of enrichments that may help reduce pain-related behaviour include access to exercise, increased environ-

mental complexity, and access to preferred resources, including social partners (e.g., Vachon et al., 2013; Pham et al., 2010). Similarly, pain and depression-like behaviour in animals are highly correlated and may have a reciprocal causative relationship (Li, 2015).

Many cognitive activities or psychosocial factors can lessen or increase the experience of pain. For example, shifting attention away from painful stimuli can reduce pain intensity and the expression of pain-related behaviours (e.g., Gentle, 2001), and in humans, even perceived control over painful stimuli improves pain tolerance and the ability to cope with intractable pain (e.g., Salomons et al., 2004). A similar phenomenon has been seen in nonhuman animals (e.g., Schaap et al., 2013). Furthermore, previous experience and prior conditioning or training can have either a negative or positive impact on the experience of pain, depending on the nature of the learned association (Miguez et al., 2014). Finally, there are well-established sex differences in pain perception and responses to analgesics (Hurley and Adams, 2008), and the presence of male personnel may even produce stress-related analgesia in some animals (Sorge et al., 2014).

Many different factors can modulate an animal's experience of pain. This also means that there are many ways to lessen the impact of pain on animal welfare, in addition to the provision of analgesia, through measures such as environmental enrichment and positive reinforcement training.

1.3 TYPES OF PAIN

It is important to categorize different types of pain insofar as they may require different treatments or methods of evaluation (Backonja, 2003; see also Section 2, "Analgesia"). One of the main ways to differentiate types of pain is by the rapidity of onset and the timing of the course of clinical signs. Acute pain (considered to be physiological pain) is caused by a specific stimulus or injury, tends to last for a short duration (tied to the healing of the injury or removal of the stimulus), and is adaptive in that it generally motivates individuals to perform protective actions that promote recovery (Grichnik and Ferrante, 1991). Conversely, chronic pain (pathological pain) is not necessarily tied to a specific injury, lasts for extended periods, and generally does not have a protective function (Grichnik and Ferrante, 1991), though this may not be true in all cases (e.g., Lister et al., 2020). Additionally, chronic pain often increases pain sensitivity to other stimuli (e.g., Lee et al., 2010).

Another potential distinction is between somatic and visceral pain – this distinction is based on where the pain sensation originates within the body. Somatic pain results from damage to non-visceral tissue (e.g., skin, muscle, deep tissue) and is generally well-defined and easy to locate (Woolf, 1995). Visceral pain, however, originates from internal organs, typically results from stretching, ischemia, or inflammation, and can be hard to locate as the sensation is often diffuse (Cervero and Laird, 1999).

Within the two broader categories of somatic and visceral pain, there are additional types of pain. The three main types considered in this document are nociceptive, neuropathic, and inflammatory. Nociceptive pain describes either somatic or visceral pain processed by a normal, unaltered nervous system that arises from damage to non-neural tissue (Mogil, 2009; IASP, n.d.). This type of pain is often acute and commonly results from noxious stimuli such as heat or cuts, but it can also contribute to chronic pain.

Neuropathic pain is caused by a lesion or disease of the somatosensory nervous system (IASP, n.d.). This type of pain is often chronic, with pain and sensory symptoms persisting beyond the healing period (Backonja, 2003). Neuropathic pain is often connected to disease states such as cancer or diabetes, or results from direct damage to a nerve.

Inflammatory pain is typically characterized by allodynia and hyperalgesia that occur in response to tissue damage and associated inflammation (Polston and Wallace, 2017). Post-operative pain, which is a specific form of acute pain caused by surgical trauma, falls within this category (Gupta et al., 2010). For expectations regarding monitoring and treating post-surgical pain, see Section 4.11, "Post-Surgical Recovery".

More recently, a fourth type of pain has been characterized: nociplastic pain. This type of pain "arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain" (IASP, n.d.). This is an emerging area of work, and its implications on animal welfare are currently unclear.

1.4 RECOGNIZING AND TREATING PAIN

One of the main methods used to assess animal pain is through the evaluation of behavioural changes. In general, animals experiencing pain eat less, spend less time grooming, are less active, and may become more aggressive (e.g., Mellor et al., 2000; Mayer, 2007). Those assessing the behaviour of the animals should be familiar with species-typical behaviour, and where possible, each animal's baseline behaviour, as pain should be assessed based on changes from this baseline. For some species, there are well-characterized behavioural responses to acute pain (e.g., dogs and cats (Mathews et al., 2014; Epstein et al., 2015), rodents (Deuis et al., 2017)), but for many animals, there is a lack of specific, validated behavioural signs of pain (Viñuela-Fernández et al., 2007). As a general guiding principle, pain can be recognized as it elicits protective reactions, results in learned behaviour, and possibly modifies species-specific behaviour (Zimmerman, 1986). However, not all pain results in readily observable behavioural changes, and some animals may hide their pain in the presence of predators (in this case, humans) or conspecifics to mask their vulnerability (Anil et al., 2002). One option to address this issue is to video record the animal's behaviour so that it can be assessed remotely without any observer effects on the behaviour. Additional guidance on recognizing and monitoring pain can be found in CCAC types of animal guidelines, the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017), the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021), and the *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022).

There are also physiological correlates of pain, particularly acute pain, stemming from activation of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis; these include increased blood pressure or heart rate and higher levels of circulating 'stress hormones' such as epinephrine and cortisol or corticosterone (Chawla and Kochar, 1999). These indicators may be used in conjunction with behavioural indicators; however, they should not be used as the primary method of pain assessment for three main reasons: 1) these responses are more indicative of nociception than pain as they still occur in anesthetized and decerebrate animals (e.g., Sivarao et al., 2007); 2) they are not specific to pain, but rather indicate general arousal levels; and 3) they are harder to assess practically, and the assessment itself may have a negative welfare impact on the animals.

In terms of practically managing animal pain, any protocol that involves a potentially painful procedure must describe how this pain will be alleviated. If an animal is going to experience tissue damage, this should always be considered painful. Similarly, if an animal is going to experience something that would be considered painful for a human, this should also be considered painful to the animal. **The precautionary principle must always be applied to cases of potential animal pain**. If there is uncertainty around whether an animal will experience pain, treatment must be provided. Pain relief should be provided unless the protocol author can provide sufficient evidence that the treatment will compromise the integrity of the data, or that

the provision of analgesia will have a greater negative welfare impact on the animal than withholding it during low-impact procedures. When pain relief is provided, it is imperative that animal pain be monitored to ensure the efficacy and safety of the analgesia.

1.5 CONSEQUENCES OF UNMITIGATED PAIN ON SCIENTIFIC OUTCOMES

Pain affects physiological and behavioural outcomes, creating potential confounds within many types of studies (AALAS, 2019). For example, post-surgical pain along with recovery from anesthesia can result in post-operative complications such as impaired respiratory function, decreased gut motility and urinary retention, long-term changes in the central nervous system, sleep impairments, and infections (Kona-Boun et al., 2005; Richardson and Flecknell, 2005). Pain can also impair wound healing and cause dysregulation of several hormones, neurotransmitters, and enzymes (Jirkof, 2017). Immunity can be compromised by pain (Baral et al., 2019), peripherally and within the brain, which has an additional effect on pain-associated affective disorders (Barcelon et al., 2019). Another consideration is that pain can be socially modulated, potentially resulting in hyperalgesia or increased anxiety in the cage mates of animals experiencing pain (e.g., Langford et al., 2006; Baptista-de-Souza et al., 2015; Smith et al., 2016). There are also concerns about the translatability and reproducibility of rodent studies that do not employ pain mitigation (e.g., Peterson et al., 2017).

Untreated pain can have a serious effect on scientific outcomes and should thus be controlled as part of good experimental design (Richardson and Flecknell, 2005). In some cases, however, certain types of analgesia may be known to interfere with scientific objectives. If this is the case, the first step must be to consider alternative types of analgesia (e.g., Lilley et al., 2015). The next step should be to acknowledge and compare the scientific impacts of analgesia provision versus untreated pain, possibly using a pilot study. Only in cases where providing pain relief would irrevocably corrupt the data, where no alternatives exist, and where the research is deemed to be of significant enough value should pain relief be withheld. If pain relief is withheld, these protocols must receive increased oversight, including rigorous humane intervention point monitoring (see Section 2.1, "Decision-Making Process").

2 ANALGESIA

Guideline 1

Appropriate analgesia should be provided for painful or potentially painful procedures. Analgesia must only be withheld if there is definitive evidence that all available options will compromise the integrity of the scientific activity. In rare cases when analgesia is withheld, there must be increased oversight of the protocol.

2.1 DECISION-MAKING PROCESS

This section describes principles and practices that should guide decision-making around the use of analgesia.

Guideline 2

Appropriate analgesia should be selected through a collaborative process between protocol authors and veterinarians.

Institutions are encouraged to create high-level standard operating procedures (SOPs) that describe pain management practices related to scientific procedures. Ideally, these SOPs would be tailored to the species of animal, the expected severities (e.g., mild, moderate, severe) and durations (e.g., short, medium, long) of pain, and should form the basis for a shared understanding of pain management expectations among all stakeholders. Thus, these SOPs would also describe pain management principles and steps to limit negative animal welfare impacts. The SOPs could then be adapted by protocol authors, following veterinary consultation where appropriate (see below). Using SOPs in this manner would help standardize pain management and promote transparency while minimizing the burden on animal care committees to create and maintain SOPs for many different situations. Alternatively, procedure-specific pain management practices could be described within procedural SOPs at the animal care committee's discretion.

The most important principle is that all decisions regarding the use of analgesia are made in a collaborative process between everyone involved in the scientific activity and animal care. Protocol authors and veterinarians must collaborate during the protocol-writing stage so that expectations are clear, and animals receive appropriate care. One potential framework to facilitate the implementation of a maximally refined analgesic regimen is to use a goal-setting approach: the protocol author should make the specific scientific goals clear and, if applicable, explain why the standard approach to pain management is not possible. The veterinarian should suggest alternative analgesics that may not interfere with the scientific goals but are still effective in managing any expected pain (see Section 2.3, "Types of Analgesics"). This approach allows the goals of effective pain management and sound research to be met (see also Section 1.5, "Consequences of Unmitigated Pain on Scientific Outcomes"). It is important to note that practices, experiences, and even ethical decision-making frameworks regarding pain management may vary over time and between institutions; as a result,

even experienced personnel (i.e., both protocol authors and veterinarians) must engage in a collaborative approach and seek continuing education on a regular basis.

Guideline 3

Persons responsible for monitoring animal pain, including the effectiveness of the analgesia, must be trained and deemed competent in this regard.

It is expected that each person involved in a scientific activity is trained and competent to perform the procedures described in the animal use protocol (*CCAC policy statement for: senior administrators responsible for animal care and use programs* (CCAC, 2008); *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015)). However, those newer to the procedures may not be skilled at dealing with unexpected events. Assistance and supervision must be provided for those who have not yet mastered the required techniques.

In addition, those responsible for monitoring animal pain, including the effectiveness of the analgesia, must also be trained and competent in their work: an SOP must not replace competent pain assessment and close monitoring of animal welfare. Registered veterinary technologists and technicians are an invaluable resource in this area and should be included in the monitoring process. They often have considerable expertise in assessing pain and welfare in animals.

Institutions should strive to maintain a culture of care while taking steps to ensure that those responsible for monitoring pain do not experience burnout or compassion fatigue – see Figley and Roop (2006) and Mitchener and Ogilvie (2002) for examples of how to accomplish this.

Procedures requiring general anesthesia tend to be obvious and less controversial than those where analgesia alone is indicated. However, if there is any uncertainty regarding the requirement for general anesthesia, these principles can similarly be applied.

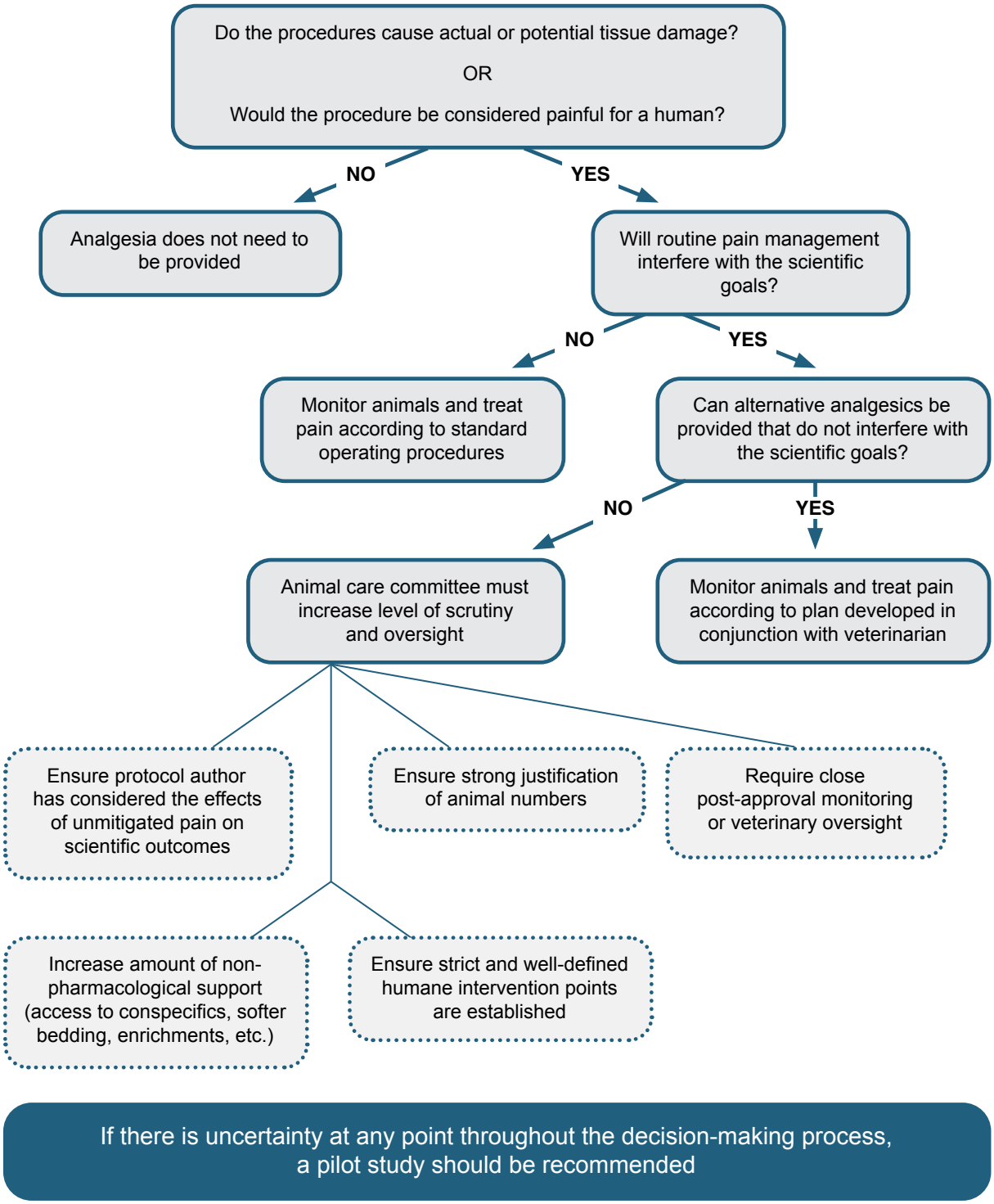
2.1.1 Withholding Pharmacologic Analgesia

Analgesia should never be withheld except in rare cases where unambiguous scientific justification is provided by the protocol author and accepted by the animal care committee. The animal care committee must closely scrutinize all protocols where there is likely to be animal pain, but analgesia must be withheld. This review should ensure that there is a strong justification of animal numbers and focus on refining the procedures to minimize the impact on each animal (see the CCAC principles for the ethical use of animals in science). Note: Analgesia can only be withheld if there is strong scientific evidence provided to the animal care committee that all available analgesics will compromise the data and that the impact of the analgesia will be greater than the impact of unmitigated pain. If it is unknown whether suggested analgesics will interfere with the data, pilot studies should be conducted to determine their effects, rather than assuming the analgesics will compromise the scientific activity.

Animal care committees must also ensure that protocols withholding analgesia receive more monitoring oversight in the form of increased post-approval monitoring or direct supervision by the veterinarian. Increased non-pharmacological support should also be provided to these animals; for example, access to conspecifics, softer bedding, and enrichment items can help to lessen the impact or experience of pain (see Section 1.2, "Modulators of Pain"). Finally, animals not provided with analgesics must have stringent and

well-defined humane intervention points (see the *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)).

2.1.2 Analgesia Decision-Making Tree



2.2 CONSIDERATIONS WHEN SELECTING ANALGESICS

Analgesics are drugs that reduce or eliminate the experience of pain. As a group, they are the primary method for managing animal pain. There are many different types of drugs, each with their mechanisms of action and varying effectiveness in treating different types of pain. This section provides an introductory guide to selecting analgesics, but veterinarians must always be consulted for new protocols that require analgesia. Consultation with a veterinarian is typically not necessary when the same protocol is renewed unless something has changed. Some examples of potential methods that may help streamline analgesic selection include:

- instituting a process of veterinary pre-review for all protocols requiring analgesia before they go to the animal care committee
- adding a checkbox to the animal use protocol form asking if the analgesia regimen has been reviewed by a veterinarian
- creating and using general pain-management SOPs, or SOPs for common or repeated procedures, which can continually be referenced if none of the variables are changed

When selecting appropriate analgesics for the protocol, several questions should be considered, such as:

- What types of pain are the animals going to experience?
- What is the expected amount of time that the animals will be experiencing pain?
- How severe is the pain expected to be?
- What is the drug's mechanism of action?
- What is the planned or available route of drug administration?
- Have the animals been habituated to the method of drug administration?
- How often does the drug need to be administered to be effective?
- What are the side effects of the drug?
- What information is known about species-typical (age, sex, etc.) responses to the drug?
- Is validated dosing information available for the species in question?
- How might the drug affect the interpretation of data?
- What alternative or additional drugs can be used if the initial selection is ineffective?

Protocol authors and veterinarians should seek to answer these questions when determining the optimal strategy to treat animal pain. Analgesia must never be withheld because of uncertainty regarding the questions listed above. If alternative drugs need to be considered, literature searches should be conducted, other experts consulted, and further validation work done, including pilot studies.

Analgesic doses should be tailored to the relevant characteristics of the individual animal and the dosage of the drug. A common risk is under-dosing animals and thus providing inadequate levels of analgesia (Simon et al., 2017). Thus, in cases of uncertainty, there should always be a preference to provide more rather than less analgesia. Furthermore, pre-emptive analgesia, in addition to the standard practice of providing it after a procedure, should be employed whenever possible (e.g., Kaka et al., 2018).

Multimodal analgesia – providing two or more drugs that act via different and ideally complementary mechanisms – should also be employed when indicated and when the drugs can be safely combined (e.g., Foley et

al., 2019; Kona-Boun et al., 2006). Long-lasting analgesics should always be preferred over short-term counterparts, to reduce the amount of animal handling needed. Finally, close monitoring of the animals must be done to ensure that pain is being effectively managed, even if one is following a well-established analgesic regimen (Flecknell, 2018). Scheduling concerns and personnel convenience must not take precedence over animal welfare.

2.3 TYPES OF ANALGESICS

Analgesia is a rapidly evolving area of animal medicine. New drugs and technologies for managing animal pain are emerging, and they should be considered, even if they are not explicitly mentioned in this document.

This section provides introductory information on different classes of analgesic drugs. For more in-depth information regarding pharmacology or species-specific drug use, readers are encouraged to see the relevant CCAC types of animal guidelines document, conduct a literature search, and consult experts.

Note: Many of the drugs listed below are not licensed for use in animals intended for human consumption or may have required withdrawal times; this applies to commercial animals and any wild animals that may be hunted.

2.3.1 Non-steroidal Anti-Inflammatory Drugs (NSAIDs)

Mechanism of Action

NSAIDs primarily work by inhibiting cyclooxygenase (COX-1 or COX-2 depending on the specific drug selectivity) enzymatic activity. There are different family series of NSAIDs, ranging from non-selective to COX-2-specific. However, in general, NSAIDS exhibit anti-hyperalgesic, anti-inflammatory, anti-pyretic, and anti-thrombotic activity (Burian and Geisslinger, 2005). Of all the possible drug options in this class, COX-2 inhibitors should be preferred as they have lower toxicity.

There is also a newer class of NSAIDs: the piprant class (e.g., Shaw et al., 2015). These are prostaglandin E_2 receptor 4 (EP_4) antagonists, and they are promising as they have fewer expected side effects and decreased toxicity. While the piprant class of NSAIDs are currently only commercially available for dogs, they may become more widely available in the future.

Indication

NSAIDs are primarily used to treat inflammatory pain, especially surgical pain. They are also used to treat both acute and chronic nociceptive pain, and musculoskeletal pain.

Potential Disadvantages and Side Effects

There are some considerations to be aware of when using NSAIDs:

- The best NSAID to use is species- and possibly strain-dependent.
- Pharmacokinetics vary between species; thus, dosing cannot be easily extrapolated.
- NSAIDs may cause gastrointestinal distress or ulceration (this is species-specific), though this can be well controlled with additional supportive therapies.

- NSAIDs may cause renal injury, particularly when given to dehydrated animals, as well as liver toxicity and articular cartilage degradation.
- NSAIDs are contraindicated in animal models of gastrointestinal inflammation and in animals with damaged kidneys.
- NSAIDs should not be mixed with corticosteroids; a washout period is necessary if both drugs are to be given.

Common NSAIDs for Animal Use

- carprofen
- cimicoxib
- deracoxib
- firocoxib
- flunixin
- grapiprant
- ketoprofen
- meloxicam
- robenacoxib
- tolfenamic acid

Note: While not an actual NSAID, acetaminophen is not advised for use in animals. It has poor analgesic effects and has the potential to be hepatotoxic to the animals (e.g., Maruyama and Williams, 1988; McConkey et al., 2009; Jaeschke et al., 2014). Acetaminophen should be used with caution and only when it has demonstrated efficacy. Ideally, alternative analgesics should be used (Flecknell, 2018).

2.3.2 Opioids

Mechanism of Action

Opioids are powerful analgesics that work by activating one or more of the mu, kappa, and delta receptors. Activating these receptors reduces afferent neuronal excitability and transmitter release while also acting on the spinal cord dorsal horn to prevent central sensitization by hyperpolarizing cells (Ruscheweyh and Sandkühler, 2005). Opioids have three major benefits: 1) their effects are actively reversible; 2) adverse side effects are uncommon and are largely dose- and patient-dependent; and 3) they have a sparing effect with other analgesics (primarily alpha-2-adrenoceptor agonists) and anesthetic drugs, which allows for decreased doses when used in combination. For these reasons, opioids are often the safest and preferred choice of analgesics to use for animals.

Indication

Opioids are often used to treat acute nociceptive pain and are especially effective in **preventing** severe pain. Animals do not have to be in severe pain for opioids to be given, as they are also effective in treating mild to moderate pain, depending on the opioid and the dose.

Potential Disadvantages and Side Effects

While opioids are one of the safest analgesic options, there are a few considerations to be aware of:

- They are controlled drugs, so inventories and access to opioids must be strictly monitored.
- Partial and full mu agonists should not be mixed as the advantages of both would be reduced. Mixing these agents may be appropriate in specific cases, but this must be done cautiously and in consultation with a veterinarian.
- Opioids may cause respiratory depression.
- Opioids may cause constipation due to decreased gut motility in some species (e.g., horses, pigs, sheep).
- Opioids may be mood-altering and can cause changes to normal behaviour, especially in high doses (e.g., pica in rats). They may have a sedative effect, and dysphoria is possible with potent opioids like fentanyl. Thus, changes in behaviour during this time can be hard to interpret as they may mimic pain-related behaviour.
- If animals have been treated with opioids over a long period, there must be a transition or weaning process to end the treatment.

Common Opioids for Animal Use

- buprenorphine
- fentanyl
- hydromorphone
- methadone (also an N-methyl-D-aspartate (NMDA) receptor antagonist)
- morphine

Note: Butorphanol should be used only as a sedative, not as an analgesic, as it is ineffective in managing pain. Some species appear to receive no analgesia from it at all (e.g., Sladky et al., 2007; Davis et al., 2006).

2.3.3 Local Anesthetics

Mechanism of Action

Local anesthetics inhibit the propagation of action potentials along nerves by blocking sodium voltage-dependent channels. These drugs block the transmission of pain impulses in all sensory nerve fibres within a confined area. This results not only in a loss of pain, but also of temperature, touch, and response to pressure, depending on the types of nerves that have been blocked.

Local anesthetics tend to be very cost-effective, and improved local anesthetics are a promising new research area. For example, a 72-hour local anesthetic is now available in the United States (NOCITA® (bupivacaine liposome injectable suspension)), and even longer-lasting drugs may become available. These are promising new tools that should be adopted as they become available in Canada. Furthermore, lidocaine is a relatively underused analgesic for fish (Chatigny et al., 2017), but it is effective and can be used more frequently (e.g., Schroeder and Sneddon, 2017).

Indication

Local anesthetics are effective in preventing and treating acute pain and are frequently combined with other analgesics as part of a multimodal analgesic regime. They are also used as epidurals or blocks for orthopedic surgeries.

Potential Disadvantages and Side Effects

Local anesthetics have some considerations to take note of:

- Knowledge of anatomical landmarks is often critical.
- Currently available drugs are only short-acting, though there are some strategies to extend their duration (e.g., fenestrated irrigation catheter).
- Overdosing a local anesthetic can induce depression of the central nervous system and lead to bradycardia and cardiac arrest – this occurs more commonly after inadvertent intravenous administration of bupivacaine or a high dose of intravenous lidocaine.
- Some species have a limited dose range (e.g., horses, cats).

Common Local Anesthetics for Animal Use

- bupivacaine
- lidocaine

Note: Although bupivacaine and lidocaine are sometimes administered together, there is currently no evidence that they work better when mixed. The best practice is to choose the most appropriate option.

2.3.4 N-Methyl-D-Aspartate (NMDA) Receptor Antagonists

Mechanism of Action

NMDA receptor antagonists reduce the induction of pain-associated central sensitization and have primary anti-hyperalgesic effects and, therefore, an analgesic component. However, NMDA receptor antagonists should be combined with other analgesics (e.g., opioids) in a multimodal way to be fully effective in controlling pain, though ketamine alone may be effective in treating chronic pain (Yang et al., 2020). Dissociative agents such as ketamine have the potential to induce a catatonic state, so it is often necessary to combine them with other sedatives, benzodiazepines, or alpha-2-adrenoceptor agonists. Ketamine does not cause catalepsy in nonhuman primates, so it may be administered alone for these animals.

Indication

NMDA receptor antagonists are often used to treat surgical pain; they are also helpful in treating opioid-resistant and pathological pain (Visser and Schug, 2006).

Potential Disadvantages and Side Effects

There are some considerations for the use of NMDA receptor antagonists:

- They are controlled drugs, so inventories and access to NMDA receptor antagonists must be strictly monitored.
- They can have highly species-specific effects.
- They are short-acting.
- Higher doses can cause significant behavioural disturbances.

Common NMDA Receptor Antagonists for Animal Use

- amantadine
- ketamine
- methadone (also an opioid)
- tiletamine[1]

2.3.5 Alpha-2-Adrenoceptor Agonists

Mechanism of Action

Peripherally, alpha-2-adrenoceptor agonists activate alpha-2-adrenoceptors to inhibit nociceptor excitability. In the spinal cord, these drugs inhibit afferent nociceptors and pain-relay neurons, while at the cerebral level, they reinforce inhibitory control and have a sedative effect. Alpha-2-adrenoceptor agonists combine very well with opioids, and also reduce the necessary dose of anesthetic required. It should be noted that this type of analgesic can also be antagonized with atipamezole for a fast recovery.

Indication

Alpha-2-adrenoceptor agonists are effective at treating both visceral and somatic acute pain. They are also effective at mitigating surgical pain, but their efficacy in treating chronic pain is not currently well characterized.

Potential Disadvantages and Side Effects

There are some considerations for using alpha-2-adrenoceptor agonists, particularly when given in higher doses:

- They cause disturbances in cardiovascular function.
- They reduce gut motility to a similar or greater extent than opioids.
- It is hard to separate their sedative, cardiovascular, and analgesic effects.

1 Tiletamine requires additional paperwork to acquire in Canada (emergency drug release for veterinarians). It is only sold as a combination drug, and comes combined with a benzodiazepine (zolazepam), which is a controlled drug. Tiletamine may also act more as an anesthetic than an analgesic.

Common Drug Options for Animal Use

- (dex)medetomidine

Note: Xylazine has a higher risk of major cardiovascular side effects (Lemke et al., 1995; Pettifer et al., 1996; Rankin, 2015) and since alternative drugs with lower risks are available, xylazine should not be used alone as an analgesic.

2.3.6 Gabapentinoids

Mechanism of Action

Gabapentinoids, also known as alpha-2-delta ligands, are a class of drugs that are derivatives of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA, i.e., GABA analogues) which block alpha-2-delta-subunit-containing voltage-dependent calcium channels and lead to an inhibition of excitatory neurotransmitter release (Rahimzadeh, 2013). They also reduce central sensitization.

Indication

Gabapentinoids are particularly effective at treating neuropathic pain and pain derived from nerve injuries, when combined with classic analgesics. They can also serve as a potential analgesic in chronic conditions such as osteoarthritis in cats (Klinck et al., 2018).

Potential Disadvantages and Side Effects

There are some considerations for using gabapentinoids:

- They should be administered frequently, usually more than once per day.
- They can cause behavioural changes, either mania or sedation.
- Their exact effectiveness and administration protocols may be unclear for certain species.

Common Drug Options for Animal Use

- gabapentin
- pregabalin

2.4 NON-PHARMACOLOGICAL ANALGESIA

Non-pharmacological interventions that may reduce pain should be implemented whenever possible as an adjunct to pharmacological agents, to enhance pain prevention, management, and treatment. **Non-pharmacological methods of analgesia should supplement but must never replace pharmacological methods.** The general principles outlined below may not be applicable for all species or for all scientific contexts. However, they can be considered broadly useful for a wide range of animals, and when used appropriately, they have the potential to improve animal welfare (Mosley et al., 2022).

2.4.1 Environment Modifications

Simple environmental accommodations can benefit animals and prevent or reduce discomfort. These include, but are not limited to:

- easy-to-access food and water dishes (raised, lowered, etc.)
- non-slip floors
- additional or specialized bedding
- access to conspecifics (e.g., Detillion et al., 2004; Jirkof, 2015)
- supplemental temperature regulation
- decreased visual and auditory stimulation
- a box or similar structure to provide a hiding place

2.4.2 Additional Care

Additional considerations for the care of animals in pain include, but are not limited to (Shearer, 2009):

- minimizing the duration of any required procedures or handling
- using gentle and respectful animal-handling techniques – when restraint is necessary, using conservative restraint to minimize breathing problems and pain from handling the painful body part
- quickly tending to all secondary disease symptoms, such as nausea and vomiting
- preventing a dry, sore mouth by providing additional moisture
- preventing dry, sore eyes by using eye lubricant or artificial tears
- providing animals with opportunities to urinate and defecate frequently, using assisted standing devices if needed
- keeping the animal clean, particularly around the genitals, anus, feet, eyes, and mouth
- providing mobility aids such as specially designed slings and carts
- if animals have had a procedure impacting their mouth, jaw, or surrounding tissues, providing a soft diet until the animal is healed
- providing attention to satisfy the animal's emotional needs, depending on the species

2.4.3 Physiotherapy and Rehabilitation

Physical modalities may also be useful in the treatment and management of acute and chronic pain. Some available physical modalities are described below. However, this is an emerging area of animal care knowledge, and evidence is limited on the safety and efficacy of some of these practices in some species. These practices should only be used when the treatments themselves will not have a negative welfare impact on the animals.

2.4.3.1 Thermal Modification

Cold therapy (e.g., applying cold compresses) can be used, depending on the chronicity of the injury. Cold therapy reduces inflammation, edema, and pain, and is best applied during the acute inflammatory phase

of tissue healing, and after exercise. It is effective in reducing pain, particularly acute post-operative pain. Cold therapy decreases blood flow by causing vasoconstriction and by reducing tissue metabolism, oxygen use, and muscle spasms. Some precautions should be taken when cold is applied in cases such as on open wounds, in very small animals, or to animals with compromised thermoregulation capacity (Millis and Levine, 2014). For acute injuries, including surgical areas, cold compression has a demonstrable benefit in reducing pain and inflammation, enhancing the return to normal function.

Heat application, or thermotherapy, increases tissue temperature, blood flow, and metabolism. It is useful for patients with chronic pain, especially pain associated with muscle spasms, acting through various mechanisms. Thermotherapy contributes to muscle relaxation and reduced stiffness. Some precautions should be taken when heat is applied on very small animals or animals with compromised thermoregulation capacity. Thermotherapy is contraindicated during acute inflammation, as it may worsen inflammation and edema because of increased circulation. Caution must be taken to prevent tissue burns (Millis and Levine, 2014).

2.4.3.2 Physical Therapy, Massages, and Therapeutic Exercises

Physical therapy may be implemented to aid in returning an animal to normal function following surgery, trauma, or as a part of a long-term pain management strategy. Physical therapy reduces pain, facilitates healing, increases or maintains muscle strength, restores normal joint movement, increases general condition, and restores normal functionality (Samoy et al., 2016).

Massage relieves pain, aids in relaxation, and promotes lymphatic drainage, circulation, and tissue movement. Soft tissue massage is also thought to improve symptomatic pain relief. Suggested mechanisms of action are muscle relaxation and increasing the pain threshold through the release of endorphins. Massage may be used in patients after surgery to help maintain mobility and control pain; it also helps to reduce edema. Massage is also useful after exercise to reduce muscle soreness and reduce tension in animals with osteoarthritis (Millis and Levine, 2014).

Therapeutic exercises may enhance strength and endurance and manage obesity (Millis and Levine, 2014).

2.4.3.3 Transcutaneous Electrical Nerve Stimulation

Transcutaneous electrical nerve stimulation involves the delivery of an electrical current to the skin by surface electrodes, and it provides analgesia through several mechanisms of action. It is used primarily in chronic conditions but may be useful for treating acute pain. Some precautions should be taken as the electrodes should not be applied to animals with seizure disorders, or on certain body areas (Millis and Levine, 2014; Samoy et al., 2016).

2.4.3.4 Low-Level Laser

Several studies in humans and animals have demonstrated that laser therapy reduces pain sensation (Pryor and Millis, 2015). Although the exact mechanism is still unclear, several mechanisms are postulated, such as a release of endogenous opioids (e.g., endorphins and enkephalins), and the induction of an inhibitory effect on the conduction of peripheral nerves by inhibiting peripheral nociceptors. Low-level laser therapy can be useful for treating chronic pain and muscle spasms. It may have benefits on tissues such as tendons and ligaments, and in the treatment of osteoarthritis. Precautions for human and animal safety are needed, and the treatment should be used by trained and competent personnel (Millis and Levine, 2014; Samoy et al., 2016).

2.4.3.5 Static Magnet Therapy and Pulsed Electromagnetic Field Therapy

Static magnet therapy provides a continuous magnetic field that is thought to alter physiological processes, including increasing local blood flow (Millis and Levine, 2014). It is primarily useful for treating osteoarthritic pain.

Pulsed electromagnetic field therapy is a non-invasive, non-thermal treatment that involves pulsing electromagnetic fields in tissue to promote healing. It may be used to treat non-union fractures, post-operative pain, osteoarthritis, and plantar fasciitis (Gaynor et al., 2018).

2.4.3.6 Therapeutic Ultrasound

Therapeutic ultrasound uses the energy created by the vibration of a piezoelectric crystal. Due to an electrical current, the crystal starts to vibrate and creates ultrasonic sound waves. One modality is 'continuous wave', which promotes tissue heating. Tissue heating is claimed to have an influence on a variety of conditions; these influences include increased collagen extensibility, blood flow, nerve conduction, and enzyme activity, and a decrease in pain sensation.

The other modality of therapeutic ultrasound is 'pulsed wave'. The aim of pulsed waves is not to heat the tissue but to deliver energy to the deeper tissues. Pulsed wave ultrasonography aids in the modulation of the inflammatory process (Samoy et al., 2016); it also contributes to analgesia by producing and releasing growth factors and stimulating nociceptors that may inhibit afferent pain signals. In veterinary medicine, therapeutic ultrasound is used to treat conditions such as tendinitis, spondylosis, non-union fractures, and osteoarthritis (Millis and Levine, 2014).

2.4.3.7 Acupuncture

Acupuncture is commonly used in modern veterinary medicine to treat pain, both acute and chronic, and many specific painful conditions. In acupuncture, a thin, sterile acupuncture needle is inserted into a series of acupuncture points to create a comprehensive treatment intended to address discomfort and disease via multiple pathways and mechanisms. Other forms of acupuncture point stimulation exist, such as low-level laser therapy, heat, and electro-acupuncture. Electro-acupuncture is a common adjunct to acupuncture treatments, to enhance the treatment outcome and prolong the benefit of the treatment. An electrical current is applied to several acupuncture points to gain more vigorous and prolonged stimulation through the needles (Fry et al., 2014).

There is diversity in the types of acupuncture points; some are associated with specific anatomic locations near major nerves, blood vessels, or lymphatic vessels. Commonly used acupuncture points include richly innervated locations and regions that have autonomic nervous associations. Additionally, many acupuncture point locations are closely associated with regions that generate muscular dysfunction and pain, such as myofascial trigger points, musculotendinous junctions, and muscle motor points (Robinson, 2009).

There is evidence in both basic science and clinical research that supports acupuncture's safety and efficacy. Therefore, acupuncture can be accepted as part of a multimodal approach to the treatment of a wide variety of painful conditions when used by a well-trained and competent practitioner (Fry et al., 2014).

2.5 FUTURE CONSIDERATIONS

The constantly increasing body of knowledge on pain physiology is leading to a greater understanding of how to intervene and reduce the experience of pain. One treatment that may become available to veterinary medicine in the near future is that of anti-cytokine therapy. Cytokines are a broad class of small regulatory proteins that modulate cell-to-cell interactions and are particularly implicated in inflammation and immune responses (Shubayev et al., 2010). Thus, pain can be reduced by introducing anti-inflammatory cytokines or inhibiting pro-inflammatory ones. Indeed, there are cytokine-related therapies currently available for painful human diseases such as rheumatoid arthritis, sciatica, and herniated discs (Shubayev et al., 2010). There is also some evidence that anti-inflammatory cytokines are effective in treating neuropathic pain (e.g., Hao et al., 2006; Vale et al., 2003). Calcium channel blockers and gene therapy are also showing promise (e.g., Snutch and Zamponi, 2018; Guo et al., 2019). Species-specific monoclonal antibody therapies that target a cytokine or other major factor in the pain process may also soon be available.

Additional methods of pain management may be developed over time. Protocol authors and veterinarians working with animals in pain should stay up to date with these technologies and adopt them as they are validated.

3 ANESTHESIA

Guideline 4

Appropriate anesthesia, individualized to each animal and procedure, must be provided for each procedure warranting anesthesia. The anesthetic approach must be based on the expected welfare impact that the animal will experience rather than solely on the procedure.

Within this section, no distinction is being made between what are traditionally known as 'major' and 'minor' surgical procedures. This is because the division does not necessarily correlate with the actual welfare impact experienced by the animal and may serve to conceal the welfare impact of some 'minor' procedures. Instead, due consideration should be given to each animal, and the anesthetic treatment must be based on the expected welfare impact that will be experienced, rather than on the procedure alone. Furthermore, the guidance provided in this section applies to the use of anesthesia for both non-survival and survival procedures, with the exception that some anesthetics can only be used for non-survival procedures, and that post-operative care must be provided for survival procedures.

Unless otherwise noted, the guidance in this section is based on Flecknell (2016). Readers are encouraged to consult this book for more detailed information.

3.1 PRE-ANESTHESIA PREPARATION

3.1.1 Animal Training

Animals should be desensitized to as much of the anesthesia process as possible prior to being anesthetized, regardless of whether the procedure is recovery or non-recovery. Training and habituation are useful, not only for calming animals and protecting their welfare, but also for ensuring the effectiveness of the anesthesia. This can be done by exposing animals to sham parts of the procedure and then giving them palatable food rewards (Laule, 2010). For example, the following process can be repeated until animals no longer show anxious or fearful behaviour: the animals can be handled, ideally using non-aversive handling techniques (e.g., Hurst and West, 2010), moved to the induction area, allowed to explore briefly, then given a food reward prior to being returned to their home cage. It may be more challenging to do this with larger animals, but it is still desirable (Grandin and Shivley, 2015). Ideally, this practice will reduce the welfare impact associated with that part of the procedure. Ensuring that animals have pre-exposure to these treats may also reduce neophobia to post-operative food rewards, thus ensuring they begin eating again sooner, to limit the metabolic cost associated with the anesthesia or surgical procedure.

Note: Animals may not become desensitized to the anesthetics themselves. In fact, there is some evidence that animals can develop a learned aversion to inhalational anesthesia through repeated exposure (e.g., mice develop a learned aversion to isoflurane (Moody and Weary, 2014); rats develop a learned aversion to

sevoflurane and isoflurane (Bertolus et al., 2015)). Furthermore, this learned aversion may generalize to all halogenated compounds (Hawkins et al., 2016), so rotating between similar anesthetics is not an effective approach to avoid a learned aversion. Thus, repeatedly anesthetizing the same animal should only be done in exceptional circumstances or when required for scientific reasons. It is currently unknown whether this learned aversion can be overcome through desensitization training but providing post-procedural food rewards should be encouraged nonetheless, as long as it is safe to do so.

3.1.2 Animal Assessment

Each animal must be assessed before anesthesia and surgery to ensure their fitness and health for the procedure. This assessment should include:

- a physical examination, including measuring body weight, which should be used to calculate the appropriate dose
- other relevant tests (e.g., complete blood counts)
- consideration of the animal's life stage and history
- noting any concerns related to the animal's genotype, phenotype, or experimental model that may influence anesthesia and recovery

If there is the potential that an animal will not respond well to the planned anesthesia or make a full recovery – assuming recovery from the procedure is expected – the animal must not be anesthetized nor undergo the procedure until an acceptable alternative anesthetic solution can be found. In such cases, pilot studies should be used to find a solution, and the veterinarian and protocol author should work together closely on this.

In the case of free-ranging wildlife, there is often little or no opportunity to conduct an assessment before immobilizing an animal. Targeted animals should be assessed as much as possible before or during the pursuit of the animal for body condition and overall health issues (e.g., injuries, physical abnormalities). If there is concern that the animal does not appear to be in optimal health, the animal should not be anesthetized. More information regarding wildlife anesthesia can be found in the *CCAC guidelines: Wildlife* (CCAC, 2023).

3.1.3 Fasting

For some animals, fasting before the administration of anesthesia is an important practice, to avoid vomiting and subsequent aspiration during the peri-anesthesia period. The following are general guidelines that should be modified based on the animal's condition (e.g., age, health status, body condition, pregnancy), the chosen anesthetic, and the procedures being performed:

- Larger, monogastric animals (e.g., cats, dogs, nonhuman primates, pigs) should be fasted for 4-6 hours; animals that are susceptible to gastric ulceration (e.g., pigs) should not be over-fasted, and treatment of such animals with gastroprotectants and antacids may be considered (e.g., Friendship et al., 2000).
- Ruminants can be fasted for 12 hours off grain and 24 hours off hay to reduce bloat, though maintaining rumen health is an equally important consideration (*CCAC guidelines on: the care and use of farm animals in research, teaching and testing* (CCAC, 2009)).
- Guinea pigs and rabbits can be fasted for 3-4 hours, if necessary, to ensure they are not retaining food in their pharynx.

- Larger birds (e.g., ducks, chickens) can be fasted for 6-12 hours, but small birds should not be fasted longer than 2 hours; in species with a crop, crop palpation can help identify if the fasting period was sufficient.
- Fish should be fasted for 12-24 hours: they can vomit, which negatively affects water quality (Stetter, 2001).
- Small rodents do not need to be fasted since they cannot vomit, but one should ensure their mouths are clear of food before intubating the trachea.
- Amphibians should typically be fasted for 4-48 hours, depending on species or size, but species that consume whole vertebrate prey should be fasted for one week.
- Reptiles should be fasted, with the length of time based on the species; in general, animals should miss one feeding cycle.

Further information on each animal type can be found in the relevant CCAC guidelines document. To reduce the welfare impact, fasting should occur during the sleep phase of the animal's circadian cycle.

Water does not need to be restricted for any animals except ruminants, which should be water-deprived for 1-2 hours before the induction of anesthesia.

3.1.4 Antiemetics

Antiemetic drugs should be considered for all fasted species noted above. These drugs are intended to prevent nausea and vomiting post-operation, thus minimizing fluid loss – which can trigger dehydration – and allowing the animals to begin oral feeding sooner. Maropitant, a neurokinin-1 receptor antagonist, is a commonly used drug for this purpose in cats and dogs (e.g., Chi and Hay Kraus, 2020; Yalcin and Keser, 2017) and works best when administered shortly before anesthesia (e.g., Lorenzutti et al., 2016). However, maropitant may not consistently reduce nausea and the incidence of gastroesophageal reflux, depending on the emetic properties of other drugs used concomitantly. Additionally, subcutaneous injections of maropitant can be painful, so it must be administered according to the manufacturer's instructions (i.e., chilled, and given slowly intravenously or when animals are already sedated (Narishetty et al., 2009)). Additionally, the maropitant may reduce the amount of isoflurane or sevoflurane required for anesthesia (Swallow et al., 2017; Alvillar et al., 2012).

Ondansetron, a selective serotonin 5-HT3 receptor antagonist, is another drug that is an effective antiemetic in cats (Quimby et al., 2013), dogs (Yalcin and Keser, 2017), and pigs (Szelenyi et al., 1994). Whenever possible, antiemetic drugs should be provided to animals to improve functional recovery from anesthesia.

3.1.5 Anticholinergics

Historically, anticholinergics were commonly administered to all animals, but there is generally no need for this practice if animals are healthy and the suggested anesthetics are used. If, during the pre-anesthetic assessment, animals are found to have excessive salivation or tend to develop bradycardia, consideration should be given to providing an anticholinergic at the veterinarian's discretion. When necessary, anticholinergic drugs (e.g., atropine, glycopyrrolate) are given as a pre-medication to reduce bronchial and salivary secretions that may otherwise occlude airways during anesthesia. Anticholinergics also serve to maintain normal heart rate when other drugs administered concomitantly, such as mu opioid agonists, are bradyarrhythmic. However, anticholinergics are not inert and are contraindicated if other drugs (e.g., alpha-2-adrenergic agonists) are concurrently administered (Plumb, 2018; Sinclair, 2003).

3.2 TRANQUILIZERS AND SEDATIVES

Tranquilizers and sedatives are very useful in multimodal analgesia and are a valuable consideration in many anesthetic protocols. They are not analgesic drugs and cannot be used to manage animal pain, but they may allow other analgesic drugs to work more effectively by reducing stress.

Sedatives produce drowsiness and reduce fear and anxiety in animals. These drugs should not be given post-operatively unless given alongside analgesia, as they make pain monitoring more difficult. Animals given tranquilizers become much less alert but can be readily roused by many stimuli (e.g., noise, physical contact, light), including pain, since these drugs have no analgesic properties. Thus, care should be taken to avoid potential reactions to stimuli by the animals so that the effects of tranquilizers and sedatives are not overridden by central nervous system stimulation.

There is considerable overlap in the actions of many agents, along with species variation, making definitive classifications between tranquilizers and sedatives difficult. Introductory information on commonly used tranquilizers and sedatives is provided below.

3.2.1 Phenothiazines (e.g., acepromazine)

Phenothiazines have been used for a long time and are efficient. They produce sedation and are also useful to reduce the required dose of anesthetic required for surgical anesthesia. Body temperature should be monitored when phenothiazines are administered. Phenothiazines' slow onset of action could be a disadvantage, and they should not be given to animals with fluid deficits (e.g., dehydration). Older published dose ranges are higher than the ranges now used, and recent texts or literature should be used to obtain the appropriate dosage ranges. Exceeding the appropriate dose leads to the increased intensity and occurrence of side effects (e.g., cardiovascular, esophageal reflux, metabolic) and a longer duration of sedation.

3.2.2 Butyrophenones (e.g., azaperone)

Butyrophenones have similar effects as phenothiazines, as explained above, but are generally more potent. Azaperone is also included in the drug mixture butorphanol-azaperone-medetomidine, which is commonly used for wildlife (see Section 3.3.2, "Butorphanol-Azaperone-Medetomidine").

3.2.3 Benzodiazepines (e.g., diazepam, midazolam)

Benzodiazepines act as both a tranquilizer and sedative, though the effects are quite variable between species. These drugs potentiate the action of most anesthetics and produce good skeletal muscle relaxation. Generally, benzodiazepines are good anxiolytics and may be useful as an appetite stimulant.

3.2.4 Alpha-2-Adrenergic Agonists (e.g., (dex)medetomidine, xylazine)

Alpha-2-adrenergic agonists are potent sedatives and markedly potentiate the action of most anesthetics and opioid analgesia. For example, xylazine is commonly combined with ketamine to produce surgical anesthesia. However, (dex)medetomidine has similar effects to xylazine, but a much lower incidence of cardiovascular side effects (Sinclair, 2003; Rankin, 2015) and requires much smaller doses; it should be preferred over xylazine for these reasons. The sedative effect of alpha-2-adrenergic agonists can be easily reversed using the specific antagonist atipamezole, though xylazine is usually short-lasting and does not need to be

reversed. Alpha-2-adrenergic agonists are easily overridden by adrenaline, so additional calming measures may be required (e.g., limiting visual and auditory stimulation, positive reinforcement desensitization training), especially for wild or more dangerous animals.

3.3 CHEMICAL AGENTS FOR WILDLIFE CAPTURE AND TRANSPORT

This section contains a list of drugs that can be used for wildlife sedation and immobilization, but this list is not exhaustive. These drugs should not be preferred for use in domesticated species, and any such use should take place only upon the advice of a veterinarian. Before using any drugs in wildlife and zoo or exotic species, consultation with professionals experienced with wildlife, current literature, the *CCAC guidelines: Wildlife* (2023), and other relevant resources should be undertaken. Some of these drugs have significant human health implications – even drugs used in regular veterinary medicine, such as (dex)medetomidine, are often highly concentrated for use in wildlife and are thus more dangerous to humans. Finally, there are several drugs used commonly in wildlife (e.g., ketamine) that are covered later in this section because they can also be used for domesticated animals.

3.3.1 Telazol

Telazol is a combination of a dissociative anesthetic, tiletamine, and a benzodiazepine, zolazepam. Although it is not reversible, telazol alone provides anesthesia in many carnivore species but must be combined with an alpha-2-adrenergic agonist such as (dex)medetomidine (or xylazine if necessary) for use in ungulates (e.g., deer). This combination can produce surgical levels of anesthesia in many species for short periods. It is used extensively in Canada for wildlife immobilization, but a veterinarian must complete an emergency drug release request to obtain the drug.

3.3.2 Butorphanol-Azaperone-Medetomidine

Butorphanol-azaperone-medetomidine is a combination of butorphanol (opioid), azaperone (butyrophenone) and medetomidine (alpha-2-adrenergic agonist). Premade butorphanol-azaperone-medetomidine is available in Canada, and this combination can be used in many species of wildlife and zoo or exotic animals to produce surgical levels of anesthesia for short periods (e.g., Siegal-Willott et al., 2009; Butler et al., 2017). Medetomidine is the primary agent in this combination, so attention to the reduction of external stimuli is necessary, and the ability of this class of drugs to be overridden by adrenaline should be considered when selecting chemical immobilization agents in potentially dangerous predator species. Due to the high likelihood of regurgitation and hyper-salivation with this drug combination, the animal must be carefully positioned. Reversal of this drug mixture with atipamezole is always necessary when used at wildlife dosages.

3.3.3 Ultra-potent Opioid Agonists

Etorphine, thiafentanil, and carfentanil are the three ultra-potent opioids most used for the chemical immobilization of wildlife. They are usually combined with an alpha-2-adrenergic agonist for use in ungulate wildlife species. When used as a primary agent, these agonists induce heavy sedation resulting in the loss of reflexes without causing total loss of consciousness. They are reversed with naltrexone or diprenorphine. The availability of these ultra-potent opioid agonists in Canada is variable (e.g., carfentanil is currently unavailable in Canada) because of their extreme danger to humans, and incidents where drugs with abuse

potential (i.e., fentanyl) were contaminated with carfentanil. Ultra-potent opioid agonists must be handled using appropriate personal protective equipment and safety protocols.

3.3.4 Long-Acting Neuroleptics

This class of drug is generally used as long-acting tranquilizers in wildlife to reduce the stress associated with periods of prolonged confinement during transport or captivity. Zuclopenthixol, perphenazine, and haloperidol are three of the most used drugs for this purpose. Attention should be paid to the drug preparation selected, which can alter the drug's persistence and duration of action from hours to weeks (decanoate is long-lasting while acetate is shorter-acting). The desired duration of effect must be balanced with the potential consequences of undesirable side effects, which commonly include anorexia. When developing drug protocols, a wildlife veterinarian or anesthesiologist with experience using the desired drugs in the target species should be consulted.

3.4 ANESTHETIC METHODS AND AGENTS

Guideline 5

Supplemental oxygen should be provided during anesthesia. Intubating the animal and maintaining intravenous access are best practices.

The following section briefly describes commonly used anesthetic agents. It provides general guidance only. Species-specific information can be found in the corresponding CCAC types of animal guidelines document. A veterinarian should always be consulted regarding all anesthesia decisions.

This section is divided into three subsections: methods of anesthesia that should be preferred and used whenever possible; methods that should not be used – typically for animal welfare or human safety reasons – and should only be used if other options are not available or there is scientific justification for their use that is accepted by the animal care committee; and methods that must not be used as they are outdated, dangerous, or have too high a welfare impact. In all cases, overdosing must be avoided, and only pharmaceutical-grade products should be used.

Tracheal intubation should be preferred for all general anesthesia cases, and the dead space associated with this method should be minimized. The patient's end-of-breathing circuits are a common source of dead space (Grubb et al., 2020). For species where intubation may be challenging, the necessity of intubation should be discussed with the veterinarian; this may also take the form of following an approved SOP. Animals receiving anesthesia should receive oxygen with a face mask (or equivalent apparatus), even if injectable drugs are used.

3.4.1 Methods of Anesthesia that Should be Used

3.4.1.1 Isoflurane

Isoflurane is an excellent anesthetic that produces rapid induction and recovery. It is non-irritating, and animals tend not to find it aversive when exposed for the first time, though they may develop a learned aversion

after the first experience; see Section 3.1.1, "Animal Training"). Inhalant anesthetics are very well suited to prolonged procedures, though an isoflurane-specific precision vaporizer is required.

3.4.1.2 Sevoflurane

Sevoflurane produces faster induction and recovery than isoflurane. The main advantages of sevoflurane over isoflurane are that the depth of anesthesia can usually be more precisely controlled, and many animals recover without a period of involuntary excitement. However, sevoflurane is more expensive and requires a sevoflurane-specific precision vaporizer, different from the one required for isoflurane.

3.4.1.3 Propofol

Propofol is a GABA agonist used for a wide range of species. Given intravenously, it induces rapid induction of anesthesia and animals typically have a smooth and rapid recovery, even if additional doses are administered. It is best used for short periods of anesthesia, alone or in combination with other anesthetics for longer procedures. If inhalant anesthesia is unavailable, propofol is a good alternative, though in these cases, it is best paired with other drugs, such as ketamine or opioids, to provide multimodal anesthesia. There is also the potential for transient apnea when using propofol, so supplementary oxygen should be available.

3.4.1.4 Alfaxalone (Alfaxan®)

Alfaxan® is the brand name of the current formulation of alfaxalone. Alfaxan®'s predecessor, Saffan®, was withdrawn from the market due to severe adverse effects likely caused by the solubilizing agent Cremophor EL®, a derivative of castor oil. However, the current formulation of alfaxalone is a water-soluble neuroanesthetic that is rapidly metabolized by the liver and does not cause histamine release. The anesthetic action of alfaxalone is due to its binding to GABA receptors.

Similar to propofol, alfaxalone has a rapid onset, short duration, and minimal side effects. It also has very similar clinical uses and properties to propofol. Nevertheless, alfaxalone has few cardiovascular effects when given within the normal dose range.

3.4.1.5 Ketamine

Ketamine is a dissociative agent that can be combined with many other drugs (e.g., medetomidine, xylazine, diazepam) to produce surgical levels of anesthesia for short periods in many different species.

3.4.1.6 Tricaine Methane Sulfonate (TMS, aka MS-222)

TMS is the most widely used anesthetic for fish[2] and amphibians. Only the pharmacologic-grade product should be used. TMS is usually very effective for rapid induction and deep anesthesia. Variations among fish and amphibian species, size, maturity, environment (e.g., temperature), and between individuals should be considered when determining the anesthetic dosage. TMS usually requires buffering to ensure an appropriate pH for the animals.

2 More information on fish anesthesia can be found in *Anesthetics* (Ackerman et al., 2005).

3.4.2 Methods of Anesthesia That Should Not Be Used

The methods described in this section should only be used if there are no viable alternatives or if there is scientific justification for their use that is accepted by the animal care committee. In the future, these methods may be moved into the "methods that must not be used" section.

3.4.2.1 Tribromoethanol (Avertin®)

Tribromoethanol is an anesthetic for mice that can also be used for rats. It produces short periods of anesthesia (15-20 minutes), and animals typically have a rapid recovery. However, stored solutions decompose rapidly, and this agent can cause severe irritation and peritoneal adhesions following its use. Even freshly prepared tribromoethanol solutions can have unpredictable efficacy and adverse side effects (e.g., low-grade peritonitis to post-anesthetic mortality), and re-administration at a later date has been associated with high mortality in some species (e.g., gerbils; Norris and Turner, 1983). Those using this method are strongly encouraged to look for alternatives that have a lesser impact on animal welfare.

3.4.2.2 Urethane

Urethane produces long-lasting, stable anesthesia, and can be used for small rodents. However, it causes vascular leakage, which leads to decreased blood volume and potentially compromised cardiovascular function (Severs et al., 1981). Furthermore, urethane is carcinogenic and thus dangerous for humans. Its use should be avoided whenever possible. When it is used, additional precautions should be put in place for human safety – the local health and safety authority should be consulted. Urethane can only be used for non-recovery procedures.

3.4.2.3 Thiobutabarbital (Inactin™)

The product Inactin™ is no longer available. However its compound, thiobutabarbital (also referred to as ethyl-(1-methylpropyl) malonyl-thiourea or EMTU), continues to be used for its prolonged and stable anesthetic state in rats for renal studies (e.g., Buelke-Sam et al., 1978; Cupples et al., 1982). Reagent-grade thiobutabarbital can be obtained from chemical suppliers, though this method of procurement should not be used (Dyson et al., 2023).

EMTU produces a smooth induction of anesthesia after intravenous administration and has a prolonged duration of action, particularly in rats following intraperitoneal (Buelke-Sam et al., 1978) or intravenous (Walker et al., 1983) administration. While it appears to be a satisfactory induction agent when given intravenously, resembling thiopental in its effects, its effects when given by the intraperitoneal route may vary, so it should not be considered reliable in producing longer anesthesia when administered intraperitoneally. Its analgesic efficacy is variable.

Thiobutabarbital may be combined with ketamine in separate intraperitoneal injections to utilize the initial deeper period of anesthesia to place invasive monitoring equipment for non-survival studies such as cardiac catheterization (Lorenz, 2002). The combination of ketamine plus thiobutabarbital results in a longer duration of action in rats than in mice, and supplementation with additional thiobutabarbital may be needed for both species during a prolonged procedure (Lorenz, 2002).

3.4.2.4 Nitrous Oxide

Nitrous oxide does not have sufficient anesthetic potency to produce anesthesia or even unconsciousness in most animals. Thus, it must not be used for this purpose. However, it is beneficial in reducing the required concentrations of other anesthetics, thus mitigating the effect those agents have on blood pressure and respiration at any given depth of anesthesia.

There are some safety concerns for humans and animals with the chronic use of nitrous oxide if scavenging is not used. Following cessation of nitrous oxide, 100% oxygen must be supplied to the animal for five minutes to prevent hypoxia. Furthermore, because of the occupational hazard of nitrous oxide to humans, and the fact that it is not absorbed by activated charcoal, the gas must be actively scavenged directly into the room ventilation extraction system.

3.4.2.5 Cryoanesthesia

This method must not be used for any animals other than mice or zebrafish. The immediate welfare impact of cooling and warming animals remains unknown, and accurately monitoring the body temperature (of mice) is very difficult. For these reasons, this method should only be used when there is scientific evidence that alternative methods will compromise the data. Cryoanesthesia is a technique that warrants further scientific investigation.

This technique must not be used once mice are older than post-natal day 7 (Lagerspetz, 1966) or have started to grow fur. Similarly, this method must not be used on embryo or larval zebrafish older than 14 days (Chen et al., 2014). Animals past these thresholds must be anesthetized with inhalant or injectable anesthetics.

In mice, cryoanesthesia, or deep hypothermia, is a method of brief anesthesia that avoids the potential neurotoxic effects that other anesthetics have on developing brains (e.g., Jevtovic-Todorovic et al., 2003). This technique relies on the fact that very young altricial mice are poikilothermic and resistant to the brain damage associated with cephalic circulatory arrest. Practically, cryoanesthesia is a simple technique and produces sufficient anesthesia for brief, minimally invasive procedures (Phifer and Terry, 1986), though the recovery time can be quite variable (e.g., Richter et al., 2014). This procedure appears to have minimal long-term effects (e.g., Richter et al., 2014; Janus and Golde, 2014). However, anesthetic depth during deep hypothermia is less controllable than during inhalant or injectable anesthesia, and this procedure should only be conducted by experienced and competent personnel. Animal care committees are also encouraged to develop SOPs for this procedure that ensure animals' extremities and skin are protected from frostbite during cooling, and that the animals warm up slowly afterwards.

For zebrafish, gradual cooling slows movements and metabolic activity and has been used for some minor procedures; however, hypothermia does not completely block nerve impulses and, therefore, should not be used for invasive procedures (Matthews and Varga, 2012).

3.4.2.6 Clove Oil or Eugenol

Clove oil is a naturally derived anesthetic that can be used for fish and amphibians (e.g., Fernandes et al., 2017). It is useful because it can be safe for users and the environment and is relatively inexpensive (Park, 2019). These features make it well suited to capture-release field studies. For some use cases, it may also be

more effective in reducing stress compared with TMS (e.g., Gullian and Villanueva, 2009), and fish may find it less aversive (e.g., Wong et al., 2014).

The naturally derived product can vary in the concentration of active ingredients eugenol and iso-eugenol, which typically comprise 90-95% of clove oil by weight. Additionally, the correct dose can vary substantially between species (Priborsky and Velisek, 2018). Thus, commercially available, standardized formulations should be used where approved and appropriate. Ensuring appropriate dosing may be more challenging when using clove oil for anesthesia.

Finally, available products may require extra precautions and mandatory withdrawal times before slaughter due to their potential ecotoxicity. Those using clove oil, or any commercial clove oil-derived products, are responsible for mitigating its potential risks.

3.4.3 Methods of Anesthesia That Must Not Be Used

3.4.3.1 Tonic Immobility, Animal Hypnosis, or Trancing

Tonic immobility is a fear-based reaction of many prey species whereby they remain motionless in the face of threats from which they cannot escape (Giannico et al., 2014). It is characterized by a lack of spontaneous movement or overt response to external stimuli for up to several minutes, yet there is evidence that animals remain aware of external events (e.g., elevated physiological stress measures during tonic immobility, though not always (McBride et al., 2006; see also Giannico et al., 2014)), and tonic immobility can be interrupted by mild tactile or auditory stimuli. Thus, not only is the induction of such a state problematic (e.g., rabbits show behavioural indicators of fear during the induction of tonic immobility; McBride et al., 2006), but tonic immobility does not induce the loss of consciousness required for surgical procedures. Furthermore, although there may be a degree of analgesia produced by tonic immobility (e.g., Leite-Panissi et al., 2001), there is considerable variation in this effect between individual animals (e.g., Flecknell et al., 2015). For these reasons, tonic immobility must never be used as a method of analgesia or anesthesia.

3.4.3.2 Neuromuscular Blocking Agents and Other Muscle Relaxants

Drugs such as succinylcholine, pancuronium, gallamine, atracurium, and vecuronium act peripherally at neuromuscular junctions, while others such as glyceryl guaiacolate – guaiacol or guaifenesin – act centrally on the spinal cord. **Neuromuscular blocking agents and other muscle relaxants produce motor paralysis only. There is no sedation or analgesia, and thus, they must never be given to conscious animals.**

If neuromuscular blocking agents or other muscle relaxants are to be used as part of an anesthetic protocol, there must be direct veterinary oversight, and the person performing the procedure must be highly competent. It is crucial that the appropriate anesthetic depth is reached before administering neuromuscular blocking agents or other muscle relaxants since they preclude the checking of reflexes required to ensure adequate anesthetic depth. Since these drugs provoke respiratory muscle paralysis, tracheal intubation and mechanical ventilation are essential. Furthermore, heart rate and, whenever possible, blood pressure must be monitored for the duration of such an anesthetic protocol, to assess the anesthetic depth. A peripheral nerve stimulator should be used to assess the efficacy of the neuromuscular blocking agent, to ensure an appropriate dosage is used.

3.4.3.3 Chloralose

Chloralose has poor analgesic properties and produces only light anesthesia. It is thus not suitable for any surgical or otherwise painful procedures. Furthermore, recovery from anesthesia with chloralose is prolonged and associated with involuntary excitement.

3.4.3.4 Diethyl Ether

Diethyl ether is an antiquated anesthetic drug. Induction is painful and stressful for the animals as diethyl ether is very irritating to mucous membranes. Furthermore, both induction and recovery are slow, prolonging the animal's state of negative welfare. Finally, the compound is dangerous: the vapours are flammable and explosive. There is no legitimate reason to use this drug as all modern alternatives are superior.

3.4.3.5 Pentobarbital and Other Barbiturates

Barbiturates such as pentobarbital are poor anesthetics. They have poor analgesic activity, cause severe respiratory depression, and only produce surgical anesthesia at dosages close to those that cause respiratory failure. Recovery can also be prolonged, and mortality can be high.

3.4.3.6 Halothane

Halothane is a potent volatile anesthetic that produces rapid induction, though its high fat solubility results in a prolonged recovery. Halothane is no longer available as a veterinary drug, and although it can be purchased from chemical suppliers – a method of procurement that is strongly discouraged in all cases – it is hepatotoxic and unsafe for humans. Isoflurane and sevoflurane have rendered this agent largely obsolete.

3.5 REQUIRED EQUIPMENT FOR INHALANT ANESTHESIA

Guideline 6

If an inhalant anesthetic is to be used, it must be delivered using a reliable and titratable source.

Guideline 7

When using inhalant anesthesia, the vaporizer must be calibrated and verified for correct function. The anesthesia machine must be well maintained, and leak tests must be performed before each use. A fully functional scavenging system must be in place. Personnel must be deemed competent to use the anesthesia machine and associated equipment before using them.

Inhalant anesthetics require the following equipment (either made-for-purpose or equivalent materials that serve the same purpose):

- an agent-specific precision vaporizer
- a source of carrier gas, typically oxygen or medical air
- a breathing system from which the anesthetic mixture is inhaled
- a face mask or endotracheal tube for connecting the breathing system to the animal
- a scavenging system to protect humans from the vapours

Every anesthesia machine that has a closed or rebreathing circuit should have the following two safety features: an in-circuit manometer and a safety pop-off valve (Grubb et al., 2020).

Appropriate gas flow must be maintained during induction and anesthesia (ILAR, 2011). Sources of anesthetic such as cotton balls soaked in volatile anesthesia put in bell jars or falcon tubes cannot provide consistent, reliable, and safe levels of anesthesia and may be extremely aversive to the animals. When not properly scavenged, these gases are also potentially harmful to humans (NIOSH, 2024) and the environment (Devlin-Hegedus et al., 2022). This method should only be considered in exceptional circumstances and should only be used before euthanasia. It must never be used for a recovery procedure.

3.6 STAGES OF ANESTHESIA

It is crucial to ensure that animals are at the proper anesthetic depth before performing any procedures. If the anesthetic depth is insufficient, the animal may experience states of negative welfare. Conversely, it is vital that animals are not over-anesthetized where the risk of mortality or critical organ depression is high. There are four classic stages of anesthesia:

- **Stage I (Induction)**: From the beginning of induction to loss of consciousness.
- **Stage II (Excitatory)**: From the loss of consciousness to the onset of automatic breathing. In this stage, most reflexes remain intact, struggling may occur, and breathing can be irregular, with breath-holding.
- **Stage III (Surgical)**: From the onset of automatic breathing to respiratory paralysis. This is the stage animals must be in before beginning a surgical procedure. In this stage, most reflexes will be lost, the muscles should be relaxed, and the animal should have regular breathing and a steady heartbeat.
- **Stage IV (Danger)**: From respiratory paralysis to death. Anesthetic overdose causes medullary paralysis with respiratory arrest and cardiovascular collapse. Animals should not reach this stage of anesthesia unless anesthetic overdose is being used as a method of euthanasia.

3.7 FLUID THERAPY

Administering fluids during an anesthetic procedure is generally a good practice. Animals undergoing very short procedures do not necessarily require fluids, yet they may still benefit from them (Grubb et al., 2020). The fluids may be administered subcutaneously, intraperitoneally, or intravenously, and should always be warmed to body temperature to prevent hypothermia in endothermic species. The administration of fluids should be discussed with and approved by the veterinarian to ensure the most appropriate methods and fluid types are used.

3.8 ANIMAL MONITORING

Guideline 8

Animal monitoring must start in the pre-anesthesia phase and be regularly maintained until full recovery.

Monitoring is an essential part of every anesthetic procedure, regardless of the drugs used. The anesthetic depth of the animals must be monitored to ensure that it is not too light – animals show active reflexes, movement, or physiological responses during surgery – or too deep, which poses a risk of death or associated comorbidities. Monitoring also ensures that normal physiological parameters are maintained and allows for a fast response should a problem – such as an animal responding to a painful stimulus – arise.

Many monitoring techniques can be used: some are simple, some are more complicated, and some require equipment. Monitoring equipment should be employed whenever possible, but **monitoring equipment must not replace direct evaluation of the animal by competent personnel** (Pachtinger, 2013). Treatment decisions should be based on information from the electronic equipment and on observational assessments of the animal (Grubb et al., 2020). The monitoring techniques should always be chosen in consultation with a veterinarian, and the personnel responsible for monitoring must be experienced and competent. SOPs are an invaluable tool to ensure consistency in monitoring.

3.8.1 Monitoring Techniques

The following is a list of possible monitoring techniques. The chosen techniques must be suitable for the scientific activity in general, the specific surgical procedure, and the specific animal being anesthetized.

3.8.1.1 Reflex Testing

The following reflexes can be used to assess anesthetic depth, as they will disappear as the animal gets deeper into anesthesia:

- **Righting**: As anesthesia progresses, the animal will lose the ability to remain upright.
- **Pharyngeal**: If the animal swallows when you gently pull its tongue out, the anesthesia is not deep enough.
- **Paw withdrawal**: A pinch on the toe or foot web is normally painful, causing animals to withdraw their paw. If animals withdraw their paw in response to this pinch, the anesthesia is not deep enough. This is the preferred reflex test for rodents. If the toe pinch cannot be used, a suitable alternative is to pinch the tail. For fish, a tail pinch should be used in the same manner. Pinching of abdominal skin is not a sensitive indicator of pain and must not be used to evaluate the pain response of the animal.
- **Palpebral**: If the animal blinks when you touch the inner or outer corner of its eyelid, the anesthesia is not deep enough. (Note: This test should not be used in rabbits, as they retain their palpebral reflex even at very deep levels of anesthesia.)
- **Corneal**: If the animal does not blink when a drop of sterile saline is administered to the corneal surface of the eye, the anesthesia is too deep. Care should be taken not to touch the animal's eyes directly.

If the reflex testing indicates that anesthesia is not deep enough, it may be necessary to wait a few minutes or administer an additional dose – care must be taken to avoid overdosing the animal.

3.8.1.2 Muscle Tone

Muscle tone refers to the amount of tension in the muscle. As anesthetic depth increases, muscle tone decreases. Muscle tone can be tested by pulling on the lower jaw or a limb: rigid tone indicates an inadequate anesthetic depth. It is important to note, however, that muscle tone can be affected by some drugs, independent of the anesthetic depth. For example, ketamine, in the absence of a sedative, can increase muscle tone, and neuromuscular blocking agents reduce muscle tone. Thus, care must be taken to use this technique only when it provides a reliable assessment of anesthetic depth.

3.8.1.3 Vital Functions

All anesthetics cause respiratory and cardiovascular depression to some degree. As an animal becomes too deeply anesthetized, respiration and cardiac output decrease, resulting in poor blood oxygenation, inadequate tissue perfusion, and decreased blood pressure and body temperature. Thus, vital functions must be monitored, but this does not necessarily require specialized equipment.

3.8.1.3.1 Cardiovascular Monitoring

Mucous Membranes

Mucous membranes are typically visible around the nose, mouth, genital area, and anus. The colour of mucous membranes makes it possible to assess the degree of hypoxia and perfusion. Pink is a healthy, normal colour, indicating adequate blood flow. A pale or pasty white colour indicates a lack of blood flow, while a blue colour (cyanosis) reflects a lack of blood oxygenation. In albino animals, the colour of the footpad, pinna of the ear, or colour of the eye can be monitored in addition to mucous membranes.

However, the use of mucous membranes has some caveats and may not be appropriate in every circumstance. Mucous membranes are not a sensitive indicator of hypoxemia because the blue colouration will not appear until hypoxia is profound (Grubb et al., 2020). Additionally, if the animal has lost a large volume of blood, the mucous membranes will remain pale even if the animal is normoxemic. Alpha-2-agonists and hypothermia also confound this monitoring method as they cause vasoconstriction, which causes mucous membranes to appear pale even when animals are not hypoxic.

Capillary Refill Time

Capillary refill time is a good indicator of cardiovascular function, and tissue perfusion in particular. It is assessed by briefly applying gentle pressure to an accessible mucous membrane or area of pale skin (e.g., gums, pinna of the ear, footpad), releasing the pressure, and then counting the time in seconds that it takes for the blanched tissue to return to its normal colour. The capillary refill time in a healthy anesthetized animal should be between 1-2 seconds. A refill time of greater than three seconds suggests cardiovascular compromise caused by fluid loss, anesthetic overdose, or hypotension.

Heart Rate and Heart Rhythm

When possible, heart rate should be assessed by auscultating the heart with a stethoscope. Auscultation also provides information on the quality and amplitude of the heart sounds. If the animal is intubated, using the esophageal stethoscope makes monitoring easier. Additionally, the use of a pulse oximeter, as explained below, helps to monitor the heart rate, but should not replace the use of the stethoscope. In smaller animals (e.g., rodents), it is possible to detect changes in heart rate, particularly decreases, by placing a finger on the animal's chest. In some large animals, it may be possible to monitor heart rate visually. Elevations in heart rate may indicate that an animal is feeling pain and is too lightly anesthetized, especially after stimulation, whereas a greatly reduced heart rate may indicate deep anesthesia.

Heart rate and rhythm can also be used to assess anesthetic depth. For each stage, the heartbeat is:

- **Stage I (Induction)**: normal, or possibly increased (stress)
- **Stage II (Excitatory)**: possibly increased or irregular
- **Stage III (Surgical)**: slow and steady
- **Stage IV (Danger)**: slow and irregular

In large animals, femoral pulse monitoring is relatively easy, though other arteries can also be used (e.g., lingual, metatarsal, facial). Monitoring the pulse is another way to assess heart rate, heart rhythm, and the strength of the heartbeat; it also provides an estimation of blood pressure. If the pulse becomes difficult to perceive, this indicates a critical problem. Alpha-2-agonists make monitoring pulse quality challenging because they cause vasoconstriction.

3.8.1.3.2 Respiratory Monitoring

Respiration can be monitored visually by observing the movement in the belly or chest of the animal and noting the depth, rate, and quality of breathing. The breathing should be deep and regular. Visual assessment of respiration also includes ensuring that the animal has a patent airway and is ventilating adequately (Pachtinger, 2013). Additionally, the chest can be auscultated with a stethoscope to monitor respiration rate and evaluate the airway adequacy and ventilation quality. Mechanical or manual ventilation support must be provided when necessary.

Visual monitoring of respiration can also be used to assess anesthetic depth. For each stage, the respiratory rate is:

- **Stage I (Induction)**: irregular; if an animal is using the whole chest to breathe, this may indicate it is in light anesthesia
- **Stage II (Excitatory)**: fast and irregular
- **Stage III (Surgical)**: slow and steady, unchanging with stimulation
- **Stage IV (Danger)**: very slow, shallow, and irregular

3.8.1.3.3 Body Temperature

Endothermic animals frequently become hypothermic during anesthesia because of the inhalation of cold gases, exposure of body cavities to room air, and loss of normal thermoregulatory mechanisms and behaviours. In particular, rodents lose body heat rapidly due to their large surface area to volume ratio. All animals must have a stable, normothermic environmental temperature for the duration of the procedure.

Hypothermia depresses most physiological functions (e.g., respiration, cardiac function, cerebral processing), slows the metabolism of anesthetics, and prolongs recovery times (Grubb et al., 2020). All these factors can contribute to anesthetic death. Thus, the anesthetized animal's body temperature should be monitored frequently using a thermometer, ideally continuously with a probe, with care taken not to let the body temperature drop below a normothermic state. It is much easier to prevent hypothermia than to treat it afterwards.

Hyperthermia is much less common in laboratory settings, but it may occur because of excessive intentional or unintentional (e.g., from heat emitting surgery lights) application of heat (see also Section 4.8, "Intra-Operative Monitoring and Nursing Care"). Hyperthermia may also be caused by pharmacological actions (e.g., due to some opioids and ketamine) and certain pathological or genetic conditions, and it is more common when conducting remote capture activities on large, wild ungulates such as bison or musk ox.

3.8.1.3.4 Specialized Equipment for Monitoring Vital Functions

The following equipment should be used whenever necessary. **However, none of these techniques should replace the above-noted methods of visually monitoring vital signs; they should be used in addition to them.** Any equipment used must be validated and calibrated for the species it is being used to monitor.

Capnography

Capnography measures the carbon dioxide (CO_2) in expired breath. The peak level or end-tidal of CO_2 is close to arterial levels of CO_2 (Pachtinger, 2013). During each expiration, the level of CO_2 rises, which the monitor displays as a graph tracing. The waveform of the graph can provide important information on the respiratory and, indirectly, the cardiovascular function of the animal. It also allows for the detection of changes in ventilation and anesthesia machine dysfunction. The end-tidal CO_2 concentration provides an indication of whether the animal is hypoventilating (increased CO_2) or hyperventilating (decreased CO_2). This measure is very useful for monitoring compromised animals or when controlled ventilation is used. If there is a limit on the available technology (e.g., due to budget constraints), capnography is currently the single most valuable technology for anesthesia monitoring.

Electrocardiogram

An electrocardiogram assesses the electrical activity of the heart, providing information about heart rate and rhythm (Pachtinger, 2013). This equipment is readily available for larger animals but can be difficult to implement for smaller animals such as rodents. Typically, electrocardiography is not needed for young, healthy animals, but should be considered for older animals and those with compromised health. Generally, specialized training is required to detect and treat the life-threatening arrhythmias that the electrocardiogram identifies, although there are some new systems that can do this automatically for some species.

Pulse Oximetry

Pulse oximetry (SpO_2) is a non-invasive, readily available method for assessing blood oxygen saturation (SaO_2) and heart rate in most animals. It is one of the primary ways of assessing the severity of hypoxemia in an animal (Pachtinger, 2013), though hypoxemia is rare when the animal is intubated and provided supplemental oxygen (Grubb et al., 2020). If hypoxemia is identified, it must be corrected immediately. Causes of hypoxemia may include inadequate oxygen supply (check anesthesia machine and airways), hy-

poventilation, hypercapnia (excessive CO_2 in the blood), and abnormal pulmonary or cardiac functioning. Additionally, the pulse oximeter displays a useful pulse wave for monitoring the animal's heart rate. Species-specific equipment should be used with this method – probes exist for rodents, though they are fragile and expensive.

Blood Pressure

Monitoring blood pressure is extremely valuable and should be performed whenever possible. Hypotension or low blood pressure is a common complication during anesthesia, which leads to compromised tissue perfusion. Conversely, hypertension or high blood pressure is much less common due to the generally negative cardiovascular effects of anesthetics, even in animals with primary hypertension (Grubb et al., 2020).

Direct measurement of arterial blood pressure is considered the gold standard as it allows for continuous monitoring (Pachtinger, 2013). However, an invasive procedure is required to insert a catheter into an artery, which is especially challenging in small animals. Alternatively, indirect blood pressure can be measured non-invasively using an inflated cuff – the most appropriate cuff placement will vary, depending on the species and procedure – or Doppler echocardiography. It is important to note that this indirect method may result in erroneous readings due to patient movement, arrhythmias, tachycardia, and inappropriate cuff placement or cuff width (Pachtinger, 2013).

Central Venous Pressure

Accurate measurement of central venous pressure requires the precise placement of a central venous catheter: peripheral venous pressure is not a reliable indicator of central venous pressure (Hasking, 2015). Although it is not routinely monitored, central venous pressure is especially useful when animals are in intensive care and volume overload must be avoided (and the instrumentation is likely already available). It may also be useful to measure central venous pressure in experimental settings where it is necessary to measure the effect of fluid therapy on the cardiovascular system.

Glucometer

Glucometers are inexpensive and require minimal amounts of blood to provide an accurate measurement of blood glucose levels. Hypoglycemia, especially in small or neonatal animals, can lead to prolonged recovery from anesthesia and other complications (e.g., seizures).

Urinary Output

Monitoring urine production is only possible in larger animals, where catheterization of the bladder is relatively easy. This is most useful for long procedures, if the animal is in critical condition, or if the animal has compromised renal function. For mammals, the administration of fluids should be adjusted to ensure a urinary output of 1-2 mL/kg/hr. Urinary catheterization must be done under strictly aseptic conditions to prevent urinary tract infections.

Blood Gas Analysis

An arterial blood gas analysis is the gold standard for the direct assessment of pulmonary function (Pachtinger, 2013). The most commonly evaluated parameters are pH, partial pressure of oxygen, partial

pressure of carbon dioxide, and bicarbonate. Advanced machines may also be able to assess electrolytes, blood urea nitrogen, creatinine, and blood lactate concentrations (Pachtinger, 2013). This method is most used when animals are already cannulated during lengthy procedures.

3.9 EMERGENCY SUPPORT

Support should be available for animals in case of any adverse events during anesthesia or recovery from anesthesia. Emergency support may include access to drugs for cardiovascular and respiratory rescue, or the ability to re-intubate if required. A veterinarian should be consulted to select the most appropriate options for each scientific context.

3.10 RECOVERY CONSIDERATIONS

Guideline 9

Adequate care must be provided to all animals during the recovery period.

During the immediate post-operative period, recovering animals are highly susceptible to the side effects of anesthesia. The following support mechanisms should be readily available and applied as necessary:

- **Temperature support**: A method of active warming (applying external heat to the patient) is preferred for endotherms, but a heating pad under half of the recovery cage for rodents or blankets for large animals, can alternatively be provided. An intravenous fluid warmer can be used if the animal is on intravenous fluids.
- **Oxygen support**: Supplemental oxygen should be available, either in a chamber or via a mask or endotracheal tube, to treat potential hypoxia.
- **Vitals monitoring**: Vitals should be checked routinely until the animal is fully recovered.
- **Hydration support**: Intravenous or subcutaneous fluids to prevent or treat dehydration are preferred; commercial gel diets on the cage floor may also be used for rodents.
- **Dietary support**: Palatable food should be offered once it is safe to do so (e.g., commercial gel diets for rodents, soft dog food for pigs). To avoid neophobia, animals should have been previously introduced to these foods.
- **Aspiration prevention**: Equipment to suction excess fluid from the airway (if needed) should be available; this is generally for larger animals such as pigs or sheep.
- **Temporary social isolation**: Animals should be kept in isolation until they regain consciousness.

Animals must not be left to recover from anesthesia unattended. Competent personnel must be available to monitor the animals in a dedicated recovery area until they have made a full recovery. Part of this monitoring must also include checking for signs of post-surgical infection or other secondary complications. A full recovery is indicated by a returned righting reflex and a stabilization of physiological parameters (e.g., body temperature) within expected limits. No additional procedures should be performed on an animal until it has made a full recovery from anesthesia, which includes a return to normal eating and drinking behaviour.

4 SURGERY

Guideline 10

Aseptic technique must be maintained during all aspects of a recovery surgical procedure and is strongly encouraged for non-recovery procedures.

Surgical procedures have the potential to greatly impact animal welfare. Good surgical technique, proper instrumentation, and competent pre-, intra-, and post-operative care are essential to minimize any such impacts. Of crucial importance is the use of aseptic technique, a series of practices and procedures that prevent contamination from pathogens and minimize the risk of infection. Aseptic technique, therefore, applies to all aspects of each surgical procedure (e.g., handwashing, donning gloves, site and animal preparation, surgery, suturing).

Guideline 11

Surgical procedures must only be completed by someone who has been deemed competent to perform the procedure, or by someone being directly supervised by such a person.

Persons performing surgery must have demonstrated competency in the specific surgical procedures being performed. In the case of inexperienced individuals, there must be direct supervision by a competent person. Additional training may be required, even for those with previous surgical experience undertaking a new procedure, or those working on a new species. Comprehensive surgical training should first take place with non-animal models, cadavers, or non-recovery procedures, before undertaking any surgical procedure. **Skilled surgeons can drastically improve animal welfare and surgical outcomes, reduce recovery times, and prevent secondary complications**. Institutions may find it helpful to utilize external training and surgical certification bodies (e.g., the Academy of Surgical Research (ASR, n.d.). Finally, experience has demonstrated that SOPs detailing how to maintain aseptic technique and others for common surgical procedures are very helpful.

The guidance in this section must be followed for all recovery surgeries. Non-recovery surgeries being performed as a training procedure should also follow the guidance below so that trainees are learning best practices, albeit with less pressure to avoid mistakes. In the case of non-recovery experimental surgeries, some of the guidance below may not be necessary (e.g., complete sterility is ideal for preventing bacterial contamination, though this may not be required). Each animal care committee should decide when protocol authors may omit certain requirements when performing non-recovery surgeries. However, clear endpoints, proper anesthesia and analgesia, and aseptic technique must always be in place as they are still very important for protecting animal welfare and ensuring good scientific outcomes. All surgical procedures must be covered by an animal-care-committee-approved protocol.

4.1 FACILITIES AND SURGICAL AREA

With the exception of field surgeries (specific guidance for performing surgical procedures in the field can be found in the *CCAC guidelines: Wildlife* (2023)), surgical procedures must be conducted in dedicated surgical facilities or in a dedicated area in a procedure room, separate from other activities (including animal holding areas). Dedicated surgical facilities should be used whenever possible. The *CCAC guidelines: Laboratory animal facilities* (CCAC, 2024) broadly describe the expectations for these facilities, and Clevenger et al. (2018) discuss the key elements that should be present in surgical facilities:

- **Animal preparation area**: Rodents and non-mammals may be prepped in the same room where the surgery will take place, as long as the preparation is done in a different physical space and the surgical area remains clear of contamination.
- **Surgeon preparation area**: This should include a separate scrub area.
- **Surgical site**: The precise location where the surgery will take place (e.g., bench top).
- **Surgical support area**: For sterilization of instruments and storage of instruments and supplies.
- **Post-operative recovery area**: Where the animal will remain immediately after surgery.
- **Adjunct surgical support areas**: Other areas that may be needed, depending on the animal use (e.g., a diagnostic laboratory).

Before any surgical procedure takes place, whether conducted in field conditions, a laboratory, or in a dedicated facility or area, the surgical area and work surfaces should be thoroughly cleaned and disinfected. All surfaces and any devices or equipment (animal restraint devices, monitoring equipment, stereotaxic devices, etc.) that will be required in the surgical field should be disinfected to reduce or eliminate potentially infectious organisms. Infection must be minimized. The surgical surface should also be prepped to facilitate thermoregulation and keep the animal dry unless the species or procedure requires a certain level of moisture.

4.2 EQUIPMENT STERILIZATION

All surgical instruments, implantable devices, and equipment that will contact the surgical site must be sterilized per one of the techniques described below. The sterilization method selected will depend on the specialized equipment available and the composition of the material to be sterilized. Proper sterilization techniques must be followed for the method employed, to obtain consistent results. Sterilization equipment should undergo regular maintenance, and a quality assurance program must be routinely used to validate sterilization techniques.

Biological indicators should be used regularly to ensure sterilization equipment is functioning properly. Chemical sterilization indicators should be included inside each surgical pack to confirm that the required conditions were met to properly sterilize its contents.

All surgical supplies and equipment must be cleaned before sterilization to remove organic material that may interfere with sterilization, and linens must be laundered before sterilization.

The following subsections contain brief descriptions of methods of sterilization that can be employed.

4.2.1 Steam Autoclaving

Autoclaves sterilize equipment by subjecting it to pressurized steam, typically at 121°C for 30 minutes (gravity cycle) or 132°C for 5 minutes (pre-vacuum cycle). Both methods should be followed by a 30-minute dry-off period. **Autoclaving is the gold standard for instrument sterilization and is the most common method.**

4.2.2 Gas Sterilization

For items that cannot withstand the high temperature, pressure, and moisture generated by steam autoclaving, the gas ethylene oxide is used to kill microorganisms and their spores by alkylation (Mendes et al., 2006). Ethylene oxide is absorbed by many materials, so all items should undergo aeration after sterilization to remove residual ethylene oxide. Ethylene oxide is dangerous: it is flammable and very toxic. Direct exposure must be avoided.

4.2.3 Gamma Irradiation

In gamma irradiation, high-energy photons are emitted from an isotope source, producing ionization or electron disruptions throughout a product. In living cells, these disruptions result in damage to the DNA and other cellular structures. These photon-induced changes at the molecular level cause the death of the organism or render the organism incapable of reproduction. The gamma irradiation process does not create residuals or impart radioactivity in processed products.

4.2.4 Glass Bead Sterilization or Dry Heat Sterilization

Due to its ability to reach temperatures of at least 232°C, the bead sterilizer is useful for sterilizing **instrument tips only** and is most commonly used when operating on more than one animal in a single session from a single sterile pack. The first surgery must be completed with instruments that have been properly sterilized using one of the methods above. Following the first surgery, instruments should be manually cleaned in sterile water and dried with sterile gauze. Tips of the instruments are then individually inserted into the glass bead sterilizer for 30 seconds to 2 minutes, depending on the size and quantity of instruments, and subsequently allowed to cool. **Only the portion of the instrument that has been embedded in the glass beads will be sterile: the instrument handle and, subsequently, any hands touching the instrument, are non-sterile.** Each surgical pack should only be used for a single group of animals – or until the instruments become too soiled – before requiring full re-sterilization (i.e., autoclave, gas, or gamma sterilization). Each animal care committee should define, ideally in an SOP, the appropriate group size for their context.

4.2.5 Cold Sterilization (Vaporized Hydrogen Peroxide and Liquid Chemosterilants)

Hydrogen peroxide plasma sterilization is an acceptable method that works by producing free radicals within a plasma field which disrupt the metabolism of microorganisms. Cold sterilization works well for materials that cannot tolerate high temperatures and humidity, though it cannot be used for porous materials such as gauze.

Liquid chemosterilants, which are virucidal, fungicidal, and bactericidal with an exposure time and temperature per the manufacturer's instructions, **should only be used if no other option is available**. Instruments should be clean and dry before immersion. After the proper exposure time, instruments should be rinsed

thoroughly with sterile saline or sterile water to avoid damage to patient tissue. **Alcohol provides disinfection, not sterilization, and must not be used to sterilize instruments.**

4.2.6 Post-sterilization Equipment Storage

Sterilized materials must be stored in a manner that preserves the integrity of the packaging material. The date of sterilization must be recorded on the package. Properly packaged sterile materials should be used following the first in, first out approach. The quality of the packaging material, the conditions under which items are stored and transported, and the amount of handling they undergo all affect the chances that the package and its contents will remain sterile. All packages containing sterile items should be inspected before use to verify barrier integrity and dryness: if they meet these requirements, they are fit for use (CDC, 2008). Any package that is wet, torn, dropped on the floor, or damaged in any way must not be used; the instruments must be re-cleaned, packaged in a new wrap, and re-sterilized.

4.3 PRE-OPERATIVE PREPARATION

The surgeon should thoroughly plan the surgical procedure before its initiation. Experience has demonstrated that SOPs or checklists are extremely helpful in this regard. This process should ensure that all required materials, equipment, and personnel are available before the start of a surgical procedure.

Whenever possible, surgeries should not be conducted by a surgeon alone. It is very difficult for one person to perform the surgical procedure while maintaining a sterile surgical field and monitoring the anesthetized animals effectively at the same time. Strong consideration should be given to optimizing available human resources (e.g., research and animal health technicians, graduate students, and post-doctoral fellows), who, by assisting each other, can perform more surgeries in less time with improved animal welfare outcomes (Pritchett-Corning et al., 2011). If the surgeon is to perform the surgical procedure alone, every effort must be made to ensure that the pre-operative preparation facilitates the maintenance of asepsis during the surgical procedure and that the animals remain sufficiently and adequately anesthetized.

Ideally, complete sterility would be implemented in every surgery. However, if this is not possible, an acceptable compromise is the "sterile tips technique", whereby the surgeon is restricted to using **only sterile ends** of the instruments to manipulate the surgical field (Hoogstraten-Miller and Brown, 2008). Gloved but non-sterile hands should not come into contact with the surgical field or with anything that will come into direct contact with the surgical field (e.g., sutures). This allows the surgeon to manipulate non-sterile objects as required (e.g., anesthesia machine, lighting, the animal itself). If using a gas anesthetic, care must be taken to ensure that the mask does not accidentally fall off the animal's face while it is under the drape. Each animal care committee should decide if and how performing surgery alone should be accomplished, and it is best practice to describe this in an SOP.

Sterile items such as instruments, gloves, suture material, scalpel blades, and packs should be opened before handwashing and placed onto a sterile surface. Examination gloves used for handling animals and working in the laboratory are not the same as sterile surgical gloves. Sterile surgical gloves should be used for all surgical procedures, though examination gloves can be used, provided that aseptic technique is maintained (e.g., Hoogstraten-Miller and Brown, 2008). The surgical packs must be opened in such a way as to prevent contamination of the items. Selection of the type and size of suture materials and needles should be done in advance and be based on the type of surgery, the specific tissues involved, and the species of animal used – see below for more information on suturing.

The surgeon and assistants must wear a clean gown or laboratory coat and wash their hands, either with antibacterial soap or an alcohol-based gel, immediately before surgery (Gaspar et al., 2018; WHO, 2009). They must also wear gloves; additional personal protective equipment may be required depending on the context of the procedures (Villano et al., 2017). **Once the above preparations have been made, the goal of the aseptic technique is to prevent the surgeon and assistants, instruments, implantable materials, equipment utilized, and surgical site from becoming contaminated.** The surgeon and assistants must restrict their contact to the surgical site and previously sterilized equipment until the incision is closed (i.e., avoid touching or handling anything that has not been sterilized). Should a break in sterile technique occur, sterility should be regained by donning new sterile gloves and using new sterile surgical instruments. If this is not possible, an acceptable compromise is that the surgeon must spray their hands with disinfectant, and any contaminated instrument tips should be sterilized (i.e., with a glass bead sterilizer for at least 30 seconds and allowed to cool; see Section 4.2.4, "Glass Bead Sterilization or Dry Heat Sterilization") before reuse.

If animals develop infections after surgery, surgical practices must be scrutinized for potential breaks in sterility.

4.4 SURGICAL SITE PREPARATION

General pre-anesthesia animal preparation (see Section 3.1, "Pre-Anesthesia Preparation") and surgical facility requirements (see Section 4.1, "Facilities and Surgical Area") are covered in previous sections. This section will, therefore, focus on the proper preparation of the surgical site.

Proper preparation of the surgical site involves several steps. The incision site should be clearly defined, and any fur or feathers should be removed from an area larger than the planned incision. To avoid contaminating the surgical setup, fur or feather removal should be done in a location different from that used to perform surgeries (i.e., not on the surgical table). All loose debris should be vacuumed or carefully wiped away to prevent contamination of the incision.

Once the site is free of all fur or feathers, surgical preparation of the skin may commence (e.g., Del Valle et al., 2018). Skin preparation should be adapted to the animal and the procedure. In general, the area should be gently scrubbed using a surgical soap, beginning at the centre of the site and working out towards the perimeter. Following an appropriate contact time, the scrub foam should be removed by repeatedly wiping the skin, utilizing fresh, clean gauze soaked with the appropriate solution, beginning in the centre as described above. An antiseptic solution alternating with either alcohol, sterile saline, or sterile water is a commonly used combination where three wipes of each product are performed, alternating with each wipe. Products are available that combine the solution with alcohol; when using this type of solution, only three total wipes of the scrubbed area are performed. For procedures such as vascular or joint implants, where foreign material is incorporated into the animal, additional measures to ensure aseptic technique may need to be taken, such as using sterilized gauze in a sterile container for the wipes or donning sterile gloves for the final skin preparations. The manufacturer's instructions for the antiseptic solution should be followed, and the same product used consistently (i.e., don't switch between chlorhexidine and iodine for the same surgical preparation).

For fish and amphibians, surgical preparation is slightly different. These animals should be manipulated as little as possible to avoid disturbing the protective mucous layer on the skin. The incision site should be cleaned with sterile saline or iodine; chlorhexidine should not be used (Philips et al., 2015). For fish, removing scales should be avoided. If scales need to be removed, only the minimum amount required should be

removed. For additional guidance, especially for non-mammalian species, please refer to the appropriate CCAC types of animal guidelines.

Following skin preparation, the incision site must be covered with a sterile drape. The drape should be at least large enough to prevent the suture material from contacting anything else, including the surgical table surface. Ideally, the drape should be twice the width and length of the surgical field. Sterilized surgical crepe paper, sterile plastic surgical drape, or a fenestrated draping are acceptable for mammals and birds. Plastic food wrap is an alternative that is sterile, cost-effective, and allows visualization of the whole animal during the procedure (Emmer et al., 2019). Plastic food wrap should be used for fish and amphibians as it does not damage the mucous layer. The centre of the drape overlying the incision site should be cut out (i.e., a fenestration created) to visualize and access the incision site.

4.5 SURGICAL TECHNIQUE

As a general practice, those performing animal surgery should always adhere to Halsted's surgical principles (Fossum, 2019):

- **Gentle handling of tissue**: Minimal trauma reduces the amount of inflammation and improves healing; it also prevents excessive tension, kinking, and circulatory compromise.
- **Meticulous hemostasis**: This is essential to prevent blood loss and potentially life-threatening hemorrhage; it also ensures appropriate visualization of the tissues during the procedure.
- **Preservation of blood supply**: Impaired blood supply slows healing, and tissue necrosis may develop if the blood supply is insufficient.
- **Strict aseptic technique**: Contamination and infection of the surgical wound will delay healing, often requiring additional antibiotic treatments, and may cause discomfort or pain for the animal – in some cases, euthanasia may be required.
- **Minimum tension on tissues**: Apposing incision edges under too much tension lead to discomfort and pressure necrosis. Sutures may "cut out", resulting in partial or complete dehiscence. "Cutting out" occurs when the pressure on the skin within the suture loop exceeds the pressure that allows blood flow.
- **Accurate tissue apposition**: Bringing the proper tissues precisely together promotes wound healing.
- **Obliteration of dead space**: Preventing fluid accumulation in dead spaces improves wound healing as it allows reparative cells to migrate into wounds. Fluids also mechanically prevent the adhesion of flaps or grafts to the wound bed.

4.6 MULTIPLE SURGERIES IN A SINGLE SESSION

Sometimes, multiple animals are surgically manipulated during a single session from a single sterile pack. Care must be taken to avoid contaminating one animal from another. Ideally, gloves should be changed between animals, and a new surgical pack should be used each time. If this is not possible, gloves must be disinfected, and a glass bead sterilizer must be used to sterilize the tips of surgical instruments between animals (see Section 4.2.4, "Glass Bead Sterilization or Dry Heat Sterilization"). **Only the instrument tips are sterilized**. Before inserting instruments into the glass bead sterilizer, they should be washed to remove organic debris and then dried. Care must be taken to avoid touching tissues with hot instruments, instrument handles, or other non-sterile instrument parts. Because non-sterile instrument handles are held with gloved fingers, contact between the fingers and the animal tissues should be avoided. Each surgical pack should

only be used for a single group of animals – or until the instruments become too soiled – before requiring full re-sterilization (i.e., autoclave, gas, or gamma sterilization). Each animal care committee should define, ideally in an SOP, the appropriate group size for their context.

4.7 SURGERY ON IMMUNOCOMPROMISED ANIMALS

Surgeries on immunocompromised animals or animals treated with hazardous agents should be carried out in biological safety cabinets. However, using biological safety cabinets should not prevent the use of instruments that would be essential to perform an appropriate surgery (e.g., the surgical microscope). If the use of a biological safety cabinet is not possible, additional precautions should be taken to ensure complete sterility and asepsis.

4.8 INTRA-OPERATIVE MONITORING AND NURSING CARE

Vigilant intra-operative monitoring is crucial to a successful surgery. In addition to monitoring anesthesia (see Section 3.8, "Animal Monitoring"), care should be given to other aspects of an animal's surgical experience to ensure a fast and full recovery with minimal welfare impacts. Thus, the following protections should be in place:

- **Body positioning**: Animals must be positioned to ensure that breathing and regional blood flow are not inadvertently restricted during surgery. This may happen if an animal's neck is sharply bent or if objects are placed on their chest. If an animal must be restrained during surgery, this should be carefully done to ensure there are no restrictions on breathing or blood flow and that reflexes can be easily assessed. Furthermore, whenever possible, animals should be kept in a position that prevents aspiration.
- **Ocular protection**: Anesthetized animals cannot blink, yet their eyes remain open during surgical procedures. Non-antibiotic ophthalmic ointment must be applied to their eyes to prevent drying and injury to the cornea. Care must be taken to avoid scratching the cornea with drapes or other equipment.
- **Padding**: During surgery, animals must rest on a soft, padded surface to prevent the development of pressure sores. The depth of padding required depends on the animal's weight and size. Fish should be rested on a wet sponge.
- **Temperature support**: For all procedures lasting more than a few minutes, temperature support must be provided for the animal. A stable ambient temperature should be maintained for ectotherms, and heat support provided to endotherms (e.g., circulating hot water blanket, hot water bottle, heated stage, patient warming blanket). Care must be taken to ensure that the animal does not overheat, as they do not have the ability to get away from the heat source when unconscious – heat lamps are not advisable for this reason. Feedback-controlled heating blankets can help prevent overheating. The use of electric heating pads should be discouraged, and they must never be placed directly on or under unconscious animals as they are very likely to cause burns.
- **Fluid therapy**: As previously noted (see Section 3.7, "Fluid Therapy"), it is generally a good practice to administer fluids during a surgical procedure whenever possible, though the specific fluid regime should be tailored to each animal and procedure. In some cases (e.g., commonly with rodents), fluids are not given during surgery, but animals are supported with fluids before or after the procedure (habituating animals prior to the procedure to things like electrolyte oral solutions or high-energy meal replacements may be beneficial if these are going to be used to aid in recovery). For fish and amphibians,

the skin should be kept moist throughout the procedure with sterile saline; water from their tank is also acceptable, provided that it will not come into direct contact with the wound.

- **Analgesia**: Appropriate analgesia should be provided as required to manage the animal's pain before it regains consciousness from anesthesia.

4.9 SUTURING

The selection and use of appropriate suture materials are imperative for successful wound closure and healing. Since suture material is a foreign material and provides a substrate on which bacteria may grow, ideally, a new pack of suture material should be used for each patient due to the high risk of contamination and infection. However, if this is not possible, sterile packs of suture material may be used for more than one animal if the suture material for each animal is sterile at the time of use. **Extreme caution must be used during the incision closure to ensure that suture material does not come into contact with non-sterile areas.** Each animal care committee should decide how suture material is to be managed, and it is a best practice to describe this in an SOP.

Selected materials should be of the correct size and tensile strength, with appropriate absorption (if absorbable) and handling characteristics for the animal species, intended procedure, and incision placement (Kladakis, 2014). Generally, however, monofilament materials should be preferred over braided multifilament materials. Sutures may be either absorbable or non-absorbable, the choice of which depends on the type of tissue to be sutured, and the length of support needed.

For routine surgical procedures, commercial suture materials with swaged or attached needles in sterile packets are ideal. Cutting and reverse-cutting needles have sharp edges and are best used for skin suturing. Non-cutting, tapered, and round needles are commonly used for suturing easily torn tissues such as peritoneum, muscle, or intestine (Kladakis, 2014).

Proper incision closure is essential to avoid wound opening and subsequent infection. Suturing can be done in a pattern chosen by the experienced surgeon, based on the specific context, noting that the choice of suture pattern may also affect the surgical outcome (e.g., Zellner et al., 2016; Radad and El-Shazly, 2007). Based on the surgical principles outlined above in Section 4.5, "Surgical Technique", wound clips should only be used to close incisions if there is no other option. For surgeries where the body cavity is entered, the suture technique should focus on proper tissue apposition and elimination of dead space. The surgeon should decide on the best approach to achieve these goals for each situation and should always consider obtaining expert advice from the veterinarian.

It typically takes 48-72 hours for a wound to seal itself (Hanks and Spodnick, 2005). During this time, the wound should be protected from sources of infection such as licking or spraying water (for example, when hosing out the environment). Wound clips and sutures must be removed once the wound has healed, typically 7-14 days after placement, depending on the species, unless there are exceptional circumstances where the welfare impact of removing the sutures would be greater than leaving them in.

4.10 RECORD KEEPING

The protocol author must keep detailed surgery logs for each animal and make them accessible to the veterinarian, the animal care committee, and others as needed. This documentation should be kept

alongside the animals, at a minimum, until they have recovered. Where appropriate, the cage card or room log should also indicate any procedures performed and when they were performed to facilitate effective animal monitoring.

4.11 POST-SURGICAL RECOVERY

Guideline 12

Appropriate analgesia must be provided as part of post-operative care; this includes non-pharmacological and pharmacological options.

Considerations related to immediate recovery from anesthesia are described earlier in this document (see Section 3.10, "Recovery Considerations"). This section contains guidance specifically related to recovery from surgery.

Regular monitoring and care of the wound must take place until it is healed. Pain assessment should also occur in the post-surgical period, ideally using a chart or scoring sheet developed for the specific species (see also the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017)). Routine antibiotic use should not be done, and the use of any antibiotics should be done in consultation with a veterinarian and be based on the type and location of the surgery.

Additional long-term post-operative care may also be required, depending on the nature and outcome of the surgical procedure. This may include specialized food and water offerings, daily medication, physiotherapy, or other forms of specialized treatment.

Any subsequently planned scientific activities should not proceed on an animal with unresolved surgical complications. If a surgery is being conducted to induce or model a disease, the animal must still be assessed and deemed in sufficient condition to proceed with further scientific activities. Additionally, precautions in the form of humane intervention points must always be in place (*CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)).

Whenever possible, it is best practice to allow animals to fully recover from surgical events, including full tissue healing, before subsequent data collection. For example, animals may take from 2-8 weeks to return to their baseline after having a telemetry device implanted, depending on the species and individual (e.g., Baumans et al., 2001; Cools et al., 2011). Allowing animals to make a full recovery is a precaution for their welfare and ensures that accurate data can be recorded.

REFERENCES

More information about documents currently in preparation can be found in the 'Guidelines in Development' section of the CCAC website.

Academy of Surgical Research – ASR (n.d.) Academy of Surgical Research Website [Web page]. ASR (accessed on 2025-03-17).

Ackerman P.A., Morgan J.D. and Iwama G.K. (2005) *Anesthetics*. Published as a resource for the *CCAC guidelines on: the care and use of fish in research, teaching and testing*. Ottawa ON: CCAC (accessed 2025-03-17).

Alvillar B., Boscan P., Mama K., Ferriera T., Congdon J. and Twedt D. (2012) Effect of epidural and intravenous use of the neurokinin-1 (NK-1) receptor antagonist maropitant on the sevoflurane minimum alveolar concentration (MAC) in dogs. *Veterinary Anesthesia and Analgesia* 39:201-205.

Anil S., Anil L. and Deen J. (2002) Challenges of pain assessment in domestic animals. *American Veterinary Medical Association* 220:313-319.

American Association for Laboratory Animal Science – AALAS (2019) Importance of Pain and Pain Management in Rodents. *Comparative Medicine* 69(6).

Backonja M. (2003) Defining neuropathic pain. *Anesthesia & Analgesia* 97:785-790.

Baptista-de-Souza D., Nunciato A., Pereira B., Fachinni G., Zaniboni C. and Canto-de-Souza A. (2015) Mice undergoing neuropathic pain induce anxiogenic-like effects and hypernociception in cagemates. *Behavioural Pharmacology* 7:664-672.

Baral P., Udit S. and Chiu I. (2019) Pain and immunity: implications for host defence. *Nature Reviews Immunology* 19:433-447.

Barcelon E., Cho W., Jun S. and Lee S. (2019) Brain microglial activation in chronic pain-associated affective disorder. *Frontiers in Neuroscience* 13:213.

Baumans V., Bouwknecht J., Boere H., Kramer K., van Lith H., van de Weerd H. and van Herck H. (2001) Intra-abdominal transmitter implantation in mice: effects on behaviour and body weight. *Animal Welfare* 10:291-302.

Bertolus J., Nemeth G., Makowska I. and Weary D. (2015) Rat aversion to sevoflurane and isoflurane. *Applied Animal Behaviour Science* 164:73-80.

Buelke-Sam J., Holson J., Bazare J. and Young J. (1978) Comparative stability of physiological parameters during sustained anesthesia in rats. *Laboratory Animal Science* 28:157-162.

Burian M. and Geisslinger G. (2005) COX-dependent mechanisms involved in the antinociceptive action of NSAIDS at central and peripheral sites. *Pharmacology & Therapeutics* 107:139-154.

Butler Z., Gamba D., Morris S., Zahratka J. and Lehmer E. (2017) Novel use of a rapidly reversible field anesthetic in small game mammals using yellow-bellied marmots (*Marmota flaviventris*) as a model organism. *BIOS* 88:135-143.

Canadian Council on Animal Care – CCAC (2008) *CCAC policy statement for: senior administrators responsible for animal care and use programs*. Ottawa ON: CCAC (accessed on 2025-03-17).

Canadian Council on Animal Care – CCAC (2009) *CCAC guidelines on: the care and use of farm animals in research, teaching and testing*. Ottawa ON: CCAC (accessed on 2025-03-17).

Canadian Council on Animal Care – CCAC (2015) *CCAC guidelines on: training of personnel working with animals in science*. Ottawa ON: CCAC (accessed on 2025-03-17).

Canadian Council on Animal Care – CCAC (2017) *CCAC guidelines: Husbandry of animals in science*. Ottawa ON: CCAC (accessed on 2025-03-17).

Canadian Council on Animal Care – CCAC (2020) *Requirement for submitting an animal protocol: Addendum to the CCAC policy statement on terms of reference for animal care committees*. Ottawa ON: CCAC (accessed on 2025-03-17).

Canadian Council on Animal Care – CCAC (2021) *CCAC guidelines: Animal welfare assessment*. Ottawa ON: CCAC (accessed on 2025-03-17).

Canadian Council on Animal Care – CCAC (2022) *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints*. Ottawa ON: CCAC (accessed on 2025-03-17).

Canadian Council on Animal Care – CCAC (2023) *CCAC guidelines: Wildlife*. Ottawa ON: CCAC (accessed on 2025-03-17).

Canadian Council on Animal Care – CCAC (2024) *CCAC guidelines: Laboratory animal facilities*. Ottawa ON: CCAC (accessed on 2025-03-17).

Centers for Disease Control and Prevention – CDC (2008) Sterilizing practices – guideline for disinfection and sterilization in healthcare facilities. (accessed on 2025-03-17).

Cervero F. and Laird M. (1999) Visceral pain. *The Lancet* 353:2145-2148.

Chatigny F., Kamunde C., Creighton C. and Stevens D. (2017) Uses and doses of local anesthetics in fish, amphibians, and reptiles. *Journal of the American Association for Laboratory Animal Science* 56:244-253.

Chawla P. and Kochar M. (1999) Effect of pain and nonsteroidal analgesics on blood pressure. *WMJ: Official Publication of the State Medical Society of Wisconsin* 98:22-25.

Chen K., Wang C-Q., Fan Y-Q., Xie Y-S., Yin Z-F., Xu Z-J., Zhang H-L., Cao J-T., Han Z-H., Wang Y. and Song D-Q. (2014) The evaluation of rapid cooling as an anesthetic method for the zebrafish. *Zebrafish* 11: 71-75.

Chi T. and Hay Kraus B. (2020) The effect of intravenous maropitant on blood pressure in healthy awake and anesthetized dogs. *PLOS One* 15:e0299736.

Clevenger R., Bernal J., Talcott M., Gleason T., Rindfield T. and Hoyt R. (2018) Surgery. In: *Management and Care of Animal Care and Use Programs in Research, Education, and Testing*, Second Edition. Boca Raton FL: CRC Press.

Cools F., Janssens S., Vanlommel A., Teisman A., Towart R. and Gallacher D. (2011) ECG arrhythmias in non-implanted vs. telemetry-implanted dogs: need for screening before and sufficient recovery time after implantation. *Journal of Pharmacological and Toxicological Methods* 64:60-67.

Cupples W., Veress A. and Sonnenberg H. (1982) Lack of effect of barbiturate and ketamine anesthesia on renal blood flow in chronically instrumented rats prepared for micropuncture. *Canadian Journal of Physiology and Pharmacology* 60:204-207.

Davis M., Mylniczenko N., Storms T., Raymond F. and Dunn J. (2006) Evaluation of intramuscular ketoprofen and butorphanol as analgesics in chain dogfish (*Scyliorhinus retifer*). *Zoo Biology* 25:491-500.

Del Valle J., Fisk E., Noland E., Pak D., Zhang J., Crim M., Lawrence F. and Hankenson F. (2018) Comparison of aqueous and alcohol-based agents for presurgical skin preparation methods in mice. *Journal of the American Association for Laboratory Science* 57:401-414.

Deuis J., Dvorakova L. and Vetter I. (2017) Methods used to evaluate pain behaviours in rodents. *Frontiers in Molecular Neuroscience* 10:284.

Detillion C., Craft T., Glasper E., Prendergast B. and DeVries A. (2004) Social facilitation of wound healing. *Psychoneuroendocrinology* 29:1004-1011.

Devlin-Hegedus J., McGain F., Harris R. and Sherman J. (2022) Action guidance for addressing pollution from inhalational anaesthetics. *Anaesthesia* 77:1023-1029.

Dyson, M., Jirkof P., Lofgren J., Nunamaker E., and Pang D. (Eds.) (2023). *Anesthesia and Analgesia in Laboratory Animals,* 3rd Edition. Elsevier.

Emmer K., Celeste N., Bidot W., Perret-Gentil M. and Malbrue R. (2019) Evaluation of the sterility of press'n seal cling film for use in rodent surgery. *Journal of the American Association for Laboratory Animal Science* 58:235-239.

Epstein M., Rodan I., Griffenhagen G., Kadrlik J., Petty M., Robertson S. and Simpson W. (2015) 2015 AAHA/AAFP pain management guidelines for dogs and cats. *Journal of the American Animal Hospital Association* 51:67-84.

Fernandes, I., Bastos Y., Barreto D., Lourenço L. and Penha J. (2017) The efficacy of clove oil as an anesthetic and in euthanasia procedure for small-sized tropical fishes. *Brazilian Journal of Biology* 77:444-450.

Figley C. and Roop R. (2006) Compassion fatigue in the animal-care community. Washington DC: Humane Society Press.

Flecknell, P. (2016) *Laboratory animal anesthesia*. Oxford UK: Academic Press.

Flecknell, P. (2018) Rodent analgesia: assessment and therapeutics. *Veterinary Journal* 232:70-77.

Flecknell P., Lofgren J., Dyson M., Marini R., Swindle M. and Wilson R. (2015) Preanesthesia, Anesthesia, Analgesia, and Euthanasia. In: *Laboratory Animal Medicine*, 3rd Edition. San Diego CA: Elsevier.

Foley P., Kendall L. and Turner P. (2019) Clinical management of pain in rodents. *Comparative Medicine* 69:468-489.

Fossum T. (2019) *Small animal surgery*. Elsevier Canada.

Friendship R., Melnichouk S. and Dewey C. (2000) The use of omeprazole to alleviate stomach ulcers in swine during periods of feed withdrawal. *Canadian Veterinary Journal* 41:925-928.

Fry L., Neary S., Sharrock J. and Rychel J. (2014) Acupuncture for analgesia in veterinary medicine. *Topics in Companion Animal Medicine* 29:35-42.

Gaspar G., Menegueti M., Lopes A., Santos R., Araújo T., Nassiff A., Ferreira L., Eulalia M., Dallora L., Canini S. and Bellissimo-Rodrigues F. (2018) Alcohol-based surgical hand preparation: translating scientific evidence into clinical practice. *Antimicrobial Resistance and Infection Control* 7:80.

Gaynor J., Hagberg S. and Gurfein B. (2018) Veterinary applications of pulsed electromagnetic field therapy. *Research in Veterinary Science* 119:1-8.

Giannico A., Lima L., Lange R., Froes T. and Montiani-Ferreira F. (2014) Proven cardiac changes during death-feigning (tonic immobility) in rabbits (*Oryctolagus cuniculus*). *Journal of Comparative Physiology A* 200:305-310.

Gentle M. (2001) Attentional shifts alter pain perception in the chicken. *Animal Welfare* 10:187-194.

Grandin T. and Shivley C. (2015) How farm animals react and perceive stressful situations such as handling, restraint, and transport. *Animals* 5:1233-1251.

Grichnik K. and Ferrante F. (1991) The difference between acute and chronic pain. *The Mount Sinai Journal of Medicine* 58:217-220.

Gullian M. and Villanueva J. (2009) Efficacy of tricaine methanesulphonate and clove oil as anaesthetics for juvenile cobia *Rachycentron canadum*. *Aquaculture Research* 40: 852-860.

Gupta A., Kaur K., Sharma S., Goyal S., Arora S. and Murthy R. (2010) Clinical aspects of acute post-operative pain management and its assessment. *Journal of Advanced Pharmaceutical Technology & Research* 1:97-108.

Grubb T., Sager J., Gaynor J., Montgomery E., Parker J., Shafford H. and Tearney C. (2020) AAHA Anesthesia and Monitoring Guidelines for Dogs and Cats. *Journal of the American Animal Hospital Association* 56:59-82.

Guo R., Zhou Y., Long H., Shan D., Wen J., Hu H., Yang H., Wu Z. and Lai W. (2019) Transient receptor potential Vanilloid 1-based gene therapy alleviates orthodontic pain in rats. *International Journal of Oral Science* 11:11.

Hanks J. and Spodnick G. (2005) Wound healing in the veterinary rehabilitation patient. *Veterinary Clinics Small Animal Practice* 32:1453-1471.

Hao S., Mata M., Glorioso J. and Fink D. (2006) HSV-mediated expression of interleukin-4 in dorsal root ganglion neurons reduces neuropathic pain. *Molecular Pain* 2:6.

Hasking S. (2015) Monitoring anesthetized patients. In: Veterinary Anesthesia and Analgesia: the Fifth Edition of Lumb and Jones. Hoboken NJ: Wiley-Blackwell.

Hawkins P., Prescott M., Carbone L., Dennison N., Johnson C., Makowska I., Marquardt N., Readman G., Weary D. and Golledge H. (2016) A good death? Report of the second Newcastle meeting on laboratory animal euthanasia. *Animals* 6:50.

Hoogstraten-Miller S. and Brown P. (2008) Techniques in aseptic rodent surgery. *Current Protocols in Immunology* 82:1.12.1-1.12.14.

Hurley R. and Adams M. (2008) Sex, gender, and pain: an overview of a complex field. *Anesthesia & Analgesia* 107:309-317.

Hurst J., and West R. (2010) Taming anxiety in laboratory mice. *Nature Methods* 7:825-826.

Institute for Laboratory Animal Research – ILAR (2011) The Guide for the Care and Use of Laboratory Animals: Eighth Edition. National Academies Press, Washington, D.C. 247 pp.

International Association for the Study of Pain – IASP (n.d.) Terminology | International Association for the Study of Pain [Web page]. IASP (accessed on 2025-03-17).

Jaeschke H., Xie Y. and McGill M. (2014) Acetaminophen-induced liver injury: From animal models to humans. *Journal of Clinical and Translational Hepatology* 2: 153-161.

Janus C. and Golde T. (2014) The effect of neonatal cryoanesthesia on physical development and adult cognitive function in mice. *Behavioural Brain Research* 259:253-260.

Jevtovic-Todorovic V., Hartman R., Izumi Y., Benshoff N., Dikranian K., Zorumski C., Olney J. and Wozniak D. (2003) Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *The Journal of Neuroscience* 23:876-882.

Jirkof P. (2015) Effects of experimental housing conditions on recovery of laboratory mice. *Lab Animal* 44:65-70.

Jirkof P. (2017) Side effects of pain and analgesia in animal experimentation. *Lab Animal* 46:123-128.

Kaka U., Rahman N., Abubakar A., Goh Y., Fakurazi S., Omar M. and Chen H. (2018) Pre-emptive multimodal analgesia with tramadol and ketamine-lidocaine infusion for suppression of central sensitization in a dog model of ovariohysterectomy. *Journal of Pain Research* 11:743-752.

Kladakis S. (2014) Choosing sutures in small animal surgery. *Journal of Dairy, Veterinary & Animal Research* 1:00015.

Klinck M., Monteiro B., Lussier B., Guillot M., Moreau M., Otis C., Steagall P., Frank D., Martel-Pelletier J., Pelletier J., del Castillo J. and Troncy E. (2018) Refinement of the Montreal Instrument for Cat Arthritis Testing, for use by veterinarians: detection of naturally occurring osteoarthritis in laboratory cats. *Journal of Feline Medicine and Surgery* 20:728-740.

Kona-Boun J-J., Cuvelliez S. and Troncy E. (2006) Evaluation of epidural administration of morphine and bupivacaine for postoperative analgesia after premedication with an opioid analgesic and orthopedic surgery in dogs. *Journal of the American Veterinary Medical Association* 229:1103-1112.

Kona-Boun J-J., Silim A. and Troncy E. (2005) Immunologic aspects of veterinary anesthesia and analgesia. *Journal of the American Veterinary Medical Association* 226:355-363.

Lagerspetz K. (1966) Postnatal development of thermoregulation in laboratory mice. *Helgoländer wissenschaftliche Meeresuntersuchungen* 14:559-571.

Langford D., Crager S., Shehzad Z., Smith S., Sotocinal S., Levenstadt J., Chanda M., Levitin D. and Mogil J. (2006) Social modulation of pain as evidence for empathy in mice. *Science 312*:1967-1970.

Laule G. (2010) Positive reinforcement training for laboratory animals. In: *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*. Oxford UK: Wiley-Blackwell.

Lee Y., Lu B., Bathon J., Haythornthwaite J., Smith M., Page G. and Edwards R. (2010) Pain sensitivity and pain reactivity in osteoarthritis. *Arthritis Care & Research* 63:320-327.

Leite-Panissi C., Rodrigues C., Brentegani M. and Menescal-De-Oliveira L. (2001) Endogenous opiate analgesia induced by tonic immobility in guinea pigs. *Brazilian Journal of Medical and Biological Research* 34:245-250.

Lemke K., Tranquilli W., Thurmon J., Benson G. and Olson W. (1995) Influence of cholinergic blockade on the development of epinephrine-induced ventricular arrhythmias in halothane- and isoflurane-anesthetized dogs. *American Journal of Veterinary Research* 54: 2132-2138.

Li J-X. (2015) Pain and depression comorbidity: a preclinical perspective. *Behavioural Brain Research* 276:92-98.

Lilley E., Armstrong R., Clark N., Gray P., Hawkins P., Mason K., Lopez-Salesansky N., Stark A., Jackson S., Thiemermann C. and Nandi M. (2015) Refinement of animal models of sepsis and septic shock. *Shock* 43:304-316.

Lister K., Bouchard S., Markova T., Aternali A., Denecli P., Pimentel S., Majeed M., Austin J-S., de C. Williams A. and Mogil J. (2020) Chronic pain produces hypervigilance to predator odor in mice. *Current Biology* 30:R866-R867.

Lorenz J. (2002) A practical guide to evaluating cardiovascular, renal, and pulmonary function in mice. *American Journal of Physiology. Regulatory, Integrative, and Comparative Physiology* 282:R1565-1582.

Lorenzutti A., Martín-Flores M., Litterio N., Himelfarb M. and Zarazaga M. (2016) Evaluation of the antiemetic efficacy of maropitant in dogs medicated with morphine and acepromazine. *Veterinary Anesthesia and Analgesia* 43:195-198.

Mathews K., Kronen P., Lascelles D., Nolan A., Robertson S., Steagall P., Wright B. and Yamashita K. (2014) Guidelines for the recognition, assessment, and treatment of pain. *Journal of Small Animal Practice* 55:E10-E68.

Matthews M. and Varga Z. (2012) Anesthesia and euthanasia in zebrafish. *Laboratory Animal Research* 53:192-204.

Maruyama H. and Williams G. (1988) Hepatotoxicity of chronic high dose administration of acetaminophen to mice. *Archives of Toxicology* 62:465-469.

Mayer J. (2007) Use of behavior analysis to recognize pain in small mammals. *Lab Animal* 36:43-48.

McBride E., Day S., McAdie T., Meredith A., Barley J., Hickman J. and Lawes L. (2006) Trancing rabbits: Relaxed hypnosis or a state of fear? In *Proceedings of the VDWE International Congress on Companion Animal Behaviour and Welfare*. Vlaamse Dierenartsenvereniging v.z.w. pp. 135-137.

McConkey S., Grant D. and Cribb A. (2009) The role of para-aminophenol in acetaminophen-induced methemoglobinemia in dogs and cats. *Journal of Veterinary Pharmacology and Therapeutics* 32:585-595.

Mellor D., Cook C. and Stafford K. (2000) Quantifying some responses to pain as a stressor. In: *The Biology of Animal Stress – Basic Principles and Implications for Animal Welfare*. New York NY: CABI Publishing.

Melzack R. and Casey K. (1968) Sensory, motivational, and central control determinates of pain: a new conceptual model. *The Skin Senses* 1:423-435.

Mendes G., Brandão T. and Silva C. (2006) Ethylene oxide sterilization of medical devices: a review. *American Journal of Infection Control* 35:574-581.

Miguez G., Laborda M. and Miller R. (2014) Classical conditioning and pain: conditioned analgesia and hyperalgesia. *Acta Psychologica* 145:10-20.

Miller R.E., Miller R.J. and Malfait A-M. (2014) Osteoarthritis joint pain: The cytokine connection. *Cytokine* 70:185-193.

Millis D. and Levine D. (2014) *Canine rehabilitation and physical therapy*. Elsevier Canada.

Mitchener K. and Ogilvie G. (2002) Understanding compassion fatigue: Keys for the caring veterinary healthcare team. *Journal of the American Animal Hospital Association* 38:307-310.

Moody C. and Weary D. (2014) Mouse aversion to isoflurane versus carbon dioxide gas. *Applied Animal Behaviour Science* 158:95-101.

Mogil J. (2009) Animal models of pain: Progress and challenges. *Nature Reviews Neuroscience* 10:283-294.

Montiero B., Otis C., del Castillo J., Nitulescu R., Brown K., Arendt-Nielsen L. and Troncy E. (2020) Quantitative sensory testing in feline osteoarthritic pain – a systematic review and meta-analysis. *Osteoarthritis and Cartilage* 28: 885-896.

Mosley C., Edwards T., Romano L., Truchetti G., Dunbar L., Schiller T., Gibson T., Bruce C. and Troncy E. (2022) Proposed Canadian consensus guidelines on osteoarthritis treatment based on OA-COAST stages 1-4. *Frontiers in Veterinary Science* 9:8300098.

Narishetty S., Galvan B., Coscarelli E., Aleo M., Fleck T., Humphrey W. and McCall R. (2009) Effect of refrigeration of the antiemetic Cerenia (maropitant) on pain on injection. *Veterinary Therapeutics* 10:93-102.

National Institute for Occupational Safety and Health – NIOSH (2024) About Anesthetic Gases and Reproductive Health [Web page]. NIOSH (accessed on 2025-03-17).

Norris M. and Turner W. (1983) An evaluation of tribromoethanol (TBE) as an anaesthetic agent in the Mongolian gerbil (*Meriones unguiculatus*). *Laboratory Animals* 17:324-329

Pachtinger G. (2013) Monitoring of the Emergent Small Animal Patient. *Veterinary Clinics: Small Animal Practice* 43:705-720.

Parent-Vachon M. and Vachon P. (2018) Environmental enrichment relieves chronic pain in rats following a spared nerve injury to induce neuropathic pain. A preliminary study. *Veterinary Medicine* 9:69-72.

Park I-S. (2019) The anesthetic effects of clove oil and MS-222 on far eastern catfish, *Silurus asotus*. *Development & Reproduction* 23:183-191.

Peterson N., Nunamaker E. and Turner P. (2017) To treat or not to treat: The effects of pain on experimental parameters. *Comparative Medicine*: 67(6):469-482.

Pettifer G., Dyson D. and McDonell W. (1996) An evaluation of the influence of medetomidine hydrochloride and atipamezole hydrochloride on the arrhythmogenic dose of epinephrine in dogs during halothane anesthesia. *Canadian Journal of Veterinary Research* 60:1-6.

Pham T., Hagman B., Codita A., Van Loo P., Strommer L. and Baumans V. (2010) Housing environment influences the need for pain relief during post-operative recovery in mice. *Physiology & Behavior* 663-668.

Phifer C. and Terry L. (1986) Use of hypothermia for general anesthesia in preweanling rodents. *Physiology and Behavior* 38:887-890.

Philips B., Crim M., Hankenson F., Steffen E., Klein P., Brice A. and Carty A. (2015) Evaluation of presurgical skin preparation agents in African clawed frogs (*Xenopus laevis*). *Journal of the American Association for Laboratory Animal Science* 54:788-798.

Plumb D. (2018) Plumb's Veterinary Drug Handbook, 9th Edition. Hoboken, NJ: Wiley Blackwell. 1456pp.

Polston G. and Wallace M. (2017) Analgesic Agents in Rheumatic Disease. In: *Kelley and Firestein's Textbook of Rheumatology* (10th Ed.). (Firestein G.S., Budd R.C., Gabriel S.E., McInnes I.B. and O'Dell J.R., eds.). Chapter 67, pp. 1075-1095. Philadelphia PA: Elsevier.

Priborsky J. and Velisek J. (2018) A review of three commonly used fish anesthetics. *Reviews in Fisheries Science and Aquaculture* 26:417-442.

Pritchett-Corning K., Luo Y., Mulder G. and White W. (2011) Principles of rodent surgery for the new surgeon. *Journal of Visualized Experiments* 47:2586.

Pryor B. and Millis D. (2015) Therapeutic laser in veterinary medicine. *Veterinary Clinics of North America: Small Animal Practice* 45:45-56.

Quimby J., Lake R., Hansen J., Lunghofer P. and Gustafson D. (2013) Oral, subcutaneous, and intravenous pharmacokinetics of ondansetron in healthy cats. *Journal of Veterinary Pharmacology and Therapeutics* 37:348-353.

Radad K. and El-Shazly M. (2007) Clinical and pathological assessment of different suture techniques for microvascular anastomosis in rat femoral artery. *Journal of Veterinary Science* 8:269-273.

Rahimzadeh P. (2013) Gabapentinoids in pain setting. *Journal of Pain & Relief* 2:e114.

Raja S., Carr D., Cohen M., Finnerup N., Flor H., Gibson S., Keefe F., Mogil J., Ringkamp M., Sluka K., Song X., Stevens B., Sullivan M., Tutelman P., Ushida T. and Vader K. (2020) The revised International Association of the Study of Pain definition of pain. *Pain* 161:1976-1982.

Rankin D. (2015) Sedatives and Tranquilizers. In: *Veterinary Anesthesia and Analgesia*: the Fifth Edition of Lumb and Jones. Hoboken NJ: Wiley-Blackwell.

Richardson C. and Flecknell P. (2005) Anesthesia and post-operative analgesia following experimental surgery in laboratory rodents: Are we making progress? *ATLA* 33:119-127.

Richter H., Wollman E., Schmidt M., Zillman U., Hellweg R., Sprengel R. and Gass P. (2014) The effects of neonatal cryoanesthesia-induced hypothermia and adult emotional behaviour and stress markers in C57BL/6 mice. *Behavioural Brain Research* 270:300-306.

Robinson N. (2009) Complementary and alternative for pain management in veterinary patients. In: *Handbook of veterinary pain management*. St. Louis MO: Mosby.

Ruscheweyh R. and Sandkühler J. (2005) Opioids and central sensitisation: II. Induction and reversal of hyperalgesia. *European Journal of Pain* 9:149-152.

Salomons T., Johnstone T., Backonja M. and Davidson R. (2004) Perceived controllability modulates the neural response to pain. *The Journal of Neuroscience* 24:7199-7203.

Samoy Y., Van Ryssen B. and Saunders J. (2016) Physiotherapy in small animal medicine. *Fysiotherapie bij kleine huisdieren Vlaams Diergeneeskundig Tijdschrift* 85:323-334.

Schaap M., van Oostrom H., Doornenbal A., Baars A., Arndt S. and Hellebrekers L. (2013) Predictability of painful stimulation modulates the somatosensory-evoked potential in the rat. *PLOS ONE* e61487.

Schroeder P. and Sneddon L. (2017) Exploring the efficacy of immersion analgesics in zebrafish using an integrative approach. *Applied Animal Behaviour Science* 187:93-102.

Severs W., Keil L., Klase P. and Deen K. (1981) Urethane anesthesia in rats. *Pharmacology* 22:209-226.

Shaw K., Rausch-Derra L. and Rhodes L. (2015) Grapiprant: an EP4 prostaglandin receptor antagonist and novel therapy for pain and inflammation. *Veterinary Medicine and Science* 21:3-9.

Shearer J., Stock M., Van Amstel S. and Coetzee J. (2013) Assessment and management of pain associated with lameness in cattle. *Veterinary Clinics of North America: Food Animal Practice* 29:135-156.

Shearer T. (2009) Hospice and palliative care. In: *Handbook of veterinary pain management*. St. Louis MO: Mosby.

Shubayev V., Kato K. and Myers R. (2010) Cytokines in pain. In: *Translational Pain Research: From Mouse to Man*. Boca Raton FL: CRC Press/Taylor & Francis.

Siegal-Willott J., Citino S., Wade S., Elder L., Hayek L. and Lance W. (2009) Butorphanol, azaperone, and medetomidine anesthesia in free-ranging white-tailed deer (*Odocoileus virginianus*) using radiotransmitter darts. *Journal of Wildlife Diseases* 45:468-480.

Simon B., Scallan E. and Steagall P. (2017) The lack of analgesic use (oligoanalgesia) in small animal practice. *Journal of Small Animal Practice* 58:543-554.

Sinclair M. (2003) A review of the physiological effects of alpha-2-agonists related to the clinical use of medetomidine in small animal practice. *Canadian Veterinary Journal* 44: 885-897

Sivarao D., Langdon S., Bernard C. and Lodge N. (2007) Colorectal distension-induced pseudoaffective changes as indices of nociception in the anesthetized female rat: Morphine and strain effects on visceral sensitivity. *Journal of Pharmacological and Toxicological Methods* 56:43-50.

Sladky K., Miletic V., Paul-Murphy J., Kinney M., Dallwig R. and Johnson S. (2007) Analgesic efficacy and respiratory effects of butorphanol and morphine in turtles. *Journal of the American Veterinary Medical Association* 230:1356-1362.

Smith M., Hostetler C., Heinricher M. and Ryabinin A. (2016) Social transfer of pain in mice. *Science Advances* 2:e1600855.

Snutch T. and Zamponi G. (2018) Recent advances in the development of T-type calcium channel blockers for pain intervention. *British Journal of Pharmacology* 175:2375-2383.

Sorge R., Martin L., Isbester K., Sotocinal S., Rosen S., Tuttle A., Wieskopf J., Acland E., Dokova A., Kadoura B., Leger P., Mapplebeck J., McPhail M., Delaney A., Wigerblad G., Schumann A., Quinn T., Frasnelli J., Svensson C., Sternberg W. and Mogil J. (2014) Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nature Methods* 11:629-632.

Sotocinal S., Sorge R., Zaloum A., Tuttle A., Martin L., Wieskopf J., Mapplebeck J., Wei P., Zhan S., Zhang S., McDougall J., King O. and Mogil J. (2011) The rat grimace scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain* 7.

Stetter M. (2001) Fish and amphibian anesthesia. *Veterinary Clinics of North America: Exotic Animal Practice* 4:69-82.

Swallow A., Rioja E., Elmer T. and Dugdale A. (2017) The effect of maropitant on intraoperative isoflurane requirements and postoperative nausea and vomiting in dogs: A randomized clinical trial. *Veterinary Anesthesia and Analgesia* 44:785-793.

Szelenyi I., Herold H. and Göthert M. (1994) Emesis induced in domestic pigs: a new experimental tool for detection of antiemetic drugs and for evaluation of emetogenic potential of new anticancer agents. *Journal of Pharmacological and Toxicological Methods* 32:109-116.

Vachon P., Millecamps M., Low L., Pailleux F., Beaudry F., Thompson S., Bushnell M. and Stone L. (2013) Alleviation of chronic neuropathic pain in mice by environmental enrichment well after the establishment of chronic pain. *Behavioural and Brain Functions* 9:22.

Vale M., Marques J., Moreira C., Rocha F., Ferreira S., Poole S., Cunha F. and Ribeiro R. (2003) Antinociceptive effects of interleukin-4, -10, and -13 on the writhing response in mice and zymosan-induced knee joint incapacitation in rats. *The Journal of Pharmacology and Experimental Therapeutics* 304:102–108.

Villano J., Follo J., Chappell M. and Collins M. (2017) Personal protective equipment in animal research. *Comparative Medicine* 67:203-214.

Viñuela-Fernández I., Jones E., Welsh E. and Fleetwood-Walker S. (2007) Pain mechanisms and their implication for the management of pain in farm and companion animals. *The Veterinary Journal* 174:227-239.

Visser E. and Schug S. (2006) The role of ketamine in pain management. *Biomedicine & Pharmacotherapy* 60:341-348.

Walker L., Buscemi-Bergin M. and Gellai M. (1983) Renal hemodynamics in conscious rats: Effects of anesthesia, surgery, and recovery. *American Journal of Physiology* 245:F67-74.

Wang C., Wu Y., Zhang Q., Yu K. and Wang Y. (2019) An enriched environment promotes synaptic plasticity and cognitive recovery after permanent middle cerebral artery occlusion in mice. *Neural Regeneration Research* 14:462-469.

Wong D., von Keyserlink M., Richards J. and Weary D. (2014) Conditioned place avoidance of zebrafish (*Danio rerio*) to three chemicals used for euthanasia and anaesthesia. *PLOS ONE* 9: e88030.

Woolf C. (1995) Somatic pain – pathogenesis and prevention. *British Journal of Anesthesia* 75:169-176.

World Health Organization – WHO (2009) *WHO guidelines on hand hygiene in health care*. Geneva Switzerland: WHO Press.

Yalcin E. and Keser G. (2017) Comparative efficacy of metoclopramide, ondansetron and maropitant in preventing parvoviral enteritis-induced emesis in dogs. *Journal of Veterinary Pharmacology and Therapeutics* 40:599-603.

Yang Y., Maher D. and Cohen S. (2020) Emerging concepts on the use of ketamine for chronic pain. *Expert Review of Clinical Pharmacology* 13:135-146.

Zellner E., Hedlund C., Kraus K., Burton A. and Kieves N. (2016) Comparison of tensile strength among simple interrupted, cruciate, intradermal, and subdermal suture patterns for incision closure in ex vivo canine skin specimens. *Journal of the American Veterinary Medical Association* 248:1377-1382.

Zimmerman M. (1986) Physiological mechanisms of pain and its treatment. *Klinische Anesthesiol Intensivtherap* 32:1-19.

GLOSSARY

Agonist – a substance which initiates a physiological response when combined with a receptor.

Allodynia – pain caused by a stimulus that does not normally provoke pain.

Analgesia – drugs and technologies that reduce the ability to feel pain.

Anesthesia – an induced state of temporary loss of consciousness.

Antagonist – a substance which binds to, and thus blocks, a receptor to dampen a biological response.

Antiemetic – a drug that prevents vomiting.

Antiseptic – substances that prevent the growth of disease-causing microorganisms.

Cyclooxygenase – an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins. It has two isoforms, one of which is involved in the creation of prostaglandins which mediate inflammation and pain.

Disinfectant – a chemical agent that destroys bacteria.

Drape – material that provides a physical barrier to protect the surgical field from contamination.

Environmental enrichment – enhancements to an animal's environment that go beyond meeting its basic species-specific needs, and further improve overall quality of life.

Hyperalgesia – increased pain from a stimulus that normally provokes pain.

Neuroleptics – antipsychotic drugs that have an effect on cognition and behaviour.

Nociception – the neural process of encoding noxious stimuli.

Pain – an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage.

Piezoelectric crystal – a crystal, typically quartz, that produces a small amount of electricity when a force is applied that changes its shape.

Procedure – the part of the scientific activity specifically related to data collection (research and testing), or hands-on demonstration or interaction with animals (teaching and training). For example, this would not include routine husbandry activities such as cage cleaning.

Protocol author – the person who is ultimately responsible for the work performed under the protocol. Frequently, this person is the primary investigator (researcher), but may also be the course instructor, study director, or testing lead. The protocol author may delegate tasks to other members of the scientific team (e.g., graduate students, post-doctoral fellows), but must always be considered responsible for the protocol.

Reflex – an involuntary reaction.

Scientific activity – includes all aspects of any research, teaching, training, or testing activities.

Sterile – free from bacteria or other microorganisms.

Suture (noun) – a stitch, or row of stitches, holding together the edges of a surgical incision.

Suture (verb) – to stitch up an incision with a suture.