

Pain and Distress and the Use of Analgesics in Animal Research

Introduction

All personnel involved in the use of animals in research have an ethical and legal obligation to reduce or eliminate pain and distress in research animals whenever such actions do not interfere with the research objectives. The Institutional Animal Care and Use Committee (IACUC) has the responsibility and accountability for ensuring that all animals under its oversight are used humanely and in accordance with Federal Regulations and University guidelines.

Regulatory Requirements

The IACUC must assure that all aspects of an animal research program that may cause more than transient pain and/or distress are addressed in the Animal Study Protocol (ASP). Specifically, the ASP must include assurance of the following:

- alternatives to painful or distressful procedures have been considered,
- methods, anesthetics and analgesics to minimize or eliminate pain and distress are included when these methods do not interfere with the research objectives, and
- humane end points have been established for all situations where more than transient pain and distress cannot be avoided or eliminated. ***Note: Whenever possible, severe pain, distress, and death should be avoided as endpoints.***

The ASP requires justification for any procedure that results in unrelieved or more than transient pain or distress and that preemptive measures should be taken to minimize or prevent the development of pain or distress. In addition, animals must be monitored to ensure the efficacy of the preemptive or palliative measures taken, and determine if or when additional measures will be necessary. The extent and frequency of monitoring will depend on the level of post-surgical/procedural pain and/or distress anticipated and the chosen intervention strategy(s).

If unexpected pain or distress occurs, the PI should immediately contact the attending veterinarian (AV). If the unexpected pain or distress is more than an isolated incident, the PI must submit an amendment delineating the problem and stating their proposed resolution to the issue (i.e. administration of analgesics, lowering the dose of a drug that was administered, etc.). Alternatively, the PI could justify the need for unrelieved pain or distress in the amendment, or in the case of regulated species as a Column E justification.

Monitoring and Recognition of Pain and Distress

Animals should be monitored by trained individuals for pain and distress as appropriate for the species, condition and procedure. Critical to the assessment of the presence or absence of pain or distress is having the ability to distinguish between normal and abnormal animal behavior. This is especially true when dealing with species that often exhibit pain and distress with only subtle changes in their behavior. ***Note: it should be assumed that a procedure that would be expected to cause pain in humans would also cause pain in animals.***

It is ultimately the responsibility of the PI and the personnel conducting the procedure to ensure adequate identification, timely monitoring, and complete documentation of the animals undergoing potentially painful or distressful procedures. Animals should be observed a

minimum of once daily but may require more frequent observation, depending on the type of research being conducted and/or the professional judgment of the PI or AV. The animals should be monitored for expected and unexpected signs of more than transient pain or distress and, if observed, appropriate intervention strategies implemented unless the withholding of treatment is scientific justified. Observations and actions taken to relieve pain or distress must be documented.

Assessing Pain in Research Animals

A variety of clinical signs may be used as general indicators of pain or distress in research animals. These include, but are not necessarily limited to the list below. ***Note: Assessment of the degree of pain or discomfort experienced by an animal can be challenging.*** While the physiologic mechanisms of pain perception are similar in all mammals, the ability to tolerate and cope with pain may be vastly different from species to species. For example, prey species such as rodents have adapted to hide overt signs of pain to avoid signaling to a predator that they are ill and would be an easy meal. A rodent experiencing mild to moderate pain may display only subtle behavioral signs associated with its discomfort.

Moderate to severe pain in rodents leads to more obvious changes in normal physiology and behavior. Accurate recognition of these changes requires that research personnel have some knowledge of normal behavior and physiology for the species they are using.

This [online tutorial](#) produced by AHWLA provides an introduction to the recognition of postoperative pain in animals.

Common Clinical Signs Associated with Pain in Small Mammals – General

Absence of normal behavior

Weight loss

Attraction to the area of pain

Production of fewer, smaller, or no fecal pellets

Anorexia

Head tucked into abdomen

Pushing abdomen on the floor

Strained facial expression, bulging eyes

Half-closed, unfocused eyes

Aggression

Increased frequency and depth of respiration or shallow breathing

Changes in blood pressure and heart rate

Changes in skeletal muscle tone

Lameness/ataxia

Stiff movements

Polyuria/polydipsia (especially with GI pain)

Reluctance to move

Lethargy

Isolation

Head extended and elevated

Overgrooming/lack of grooming

Pacing/restlessness

Piloerection

Vocalization (especially on movement or palpation of the painful part)

Porphyrin secretion

Stretching with back arched

Self-mutilation

Stinting on palpation

Squinting

Hunched posture

Teeth grinding

Altered electroencephalogram response

Pupillary dilation

Licking

Biting

Sweating

Change in personality

Changes in eye brightness

Common Clinical Signs Associated with Pain – By Species

Cattle - Depressed; little interest in surroundings; weight loss; sudden drop in milk yield; lack of grooming; rapid, shallow respiration; increased aggression; rigid posture; grunting and grinding of teeth.

Guinea pigs - Eyes sunken and dull; changes in respiration; increased or decreased timidity; increased sleepiness; arched back; increased vocalization; weight loss; hair loss; scaly skin; dehydration; unresponsive; excessive salivation (oral problems); increased barbering; loss of righting reflex; decreased vocalization; and hypothermia.

Hamster - Ocular discharge; increased aggression; hunched posture; reluctance to move; loss of coat and body condition; increased depression; extended daytime sleep periods; lateral recumbency; hypothermia; and sores on lips, paws.

Horse - Interrupted feeding; pupillary dilation; glassy eyes; increased respiration; increased heart rate; profuse sweating; rigid stance; restlessness; depression; biting; kicking; circling; self-mutilation; and reluctance to be handled.

Mouse - Eyelids partially closed; changes in respiration; rough hair coat; increased or decreased vibrissae movement; unusually apprehensive or aggressive; scratching; biting; self-mutilation; hunched posture; sudden running; aggressive vocalization; guarding; weight loss; dehydration; incontinence; soiled hair coat; eyes sunken; wasting of muscles on back; sunken or distended abdomen; unresponsive; separates from group; hunched posture; ataxia; circling; hypothermia; and decreased vocalization.

Pig - Changes in gait or posture; increased efforts to avoid handling; increased squealing when approached or handled; depression; unwillingness to move; attempts to hide; withdrawal from penmates; and anorexia.

Rabbits - Ocular discharge; constipation or diarrhea; depression; facing back of cage; excessive self-grooming; stretched posture; early failure to eat and drink; dull attitude or increased aggression when handled; possible vocalization when handled; tooth grinding; increased respiratory rate; tooth grinding; apparent sleepiness; dehydration; weight loss; fecal staining; wasting of lower back muscles; decreased production of night feces; and unresponsive.

Rats - Aggressive vocalization; licking, biting, scratching; guarding; rough hair coat; hair loss; reduced exploratory behavior, eyes closed, porphyrin production piloerection; ungroomed appearance; dehydration; weight loss; incontinence; soiled hair coat; self-mutilation; recumbent position with head tucked into abdomen; decreased vocalization; hypothermia; staggering/falling; squirming; poor gait; and poor posture.

Ferrets - Lethargy; decreased grooming; squinting; loss of appetite; reluctance to curl when sleeping.

Birds - Escape reactions; increased vocalization; excessive movement; increase in heart and respiratory rates; inactivity with a drooping appearance; tonic immobility when handled; reluctance to perch; aggression; mouth breathing; attempts to escape; anxiety; restlessness; struggling or aggression with handling; anorexia; crouch-like stance, head pulled into body; isolation; foot lifting; jumping; decreased head movements; increased apprehension or swallowing type behavior; vocalization; wing flapping; wing droop; "mouth breathing"; ruffled feathers; lack of preening or excessive preening; localized or generalized feather loss; weight loss; decreased activity; decreased social interaction; decreased appetite or water intake; decreased feces or urine production; limping; reduced egg production; and changes in preening.

Fish - Excessive movement of fins; weight loss; anorexia; reflexive responses such as muscular and behavioral avoidance; rapid or labored respirations (gill or mouth movements); color changes; posture and orientation changes; water column utilization; decreased activity, lessened swimming; agitated swimming; activity at bottom of tank consistently; secretion accumulation around eyes, mouth, gills; skin changes and growths; strong muscular and behavioral avoidance; and strong reflexive responses.

Bats – Sweating; changes in respiratory rate; reluctant to move when stimulated; weight loss; abnormal flight pattern and anorexia.

Reptiles and Amphibians – Anorexia; hunched posture; stillness or immobility; aggression in normally passive animals; stinging on palpation or touch; blepharospasm; flight responses or startling easily; aerophagia; dysphagia; elevated head and/or extended neck position; color changes; constant dull color; avoidance and withdrawal reactions; biting at affected areas; lethargy; isolation; lameness; ataxia; absence of normal behaviors; body less coiled at site of pain; foot or digit flicking; rapid respirations; repetitive pulling head into shell and then extending the neck out and up.

Intervention Strategies

Strategies for the management of pain and distress may include non-pharmacological considerations (e.g. modified housing and husbandry practices, dietary modifications,

desensitization and acclimation strategies, etc.), pharmacological interventions, or euthanasia. The chosen strategy will vary with the species, the procedure(s) being performed, duration of action needed, route of administration preferred, degree and type of analgesia required and research being conducted. PIs should consult with the facility veterinarian during the development of the ASP, prior to its submission to the IACUC, for guidance on appropriate interventions and treatment strategies.

Preemptive Measures Certain preemptive measure may be taken before pain starts rather than treating pain after its onset. Therefore, analgesics should be administered before the animal recovers from anesthesia. Other steps that can be taken to minimize or prevent pain or distress may include the preemptive administration of low-dose opioids, which have been shown to prevent the development of some forms of pain. In addition, a single dose of a non-steroidal anti-inflammatory agent or other analgesic agent can have a positive effect on the speed with which animals return to normal behavior.

Pain Management There are many pharmacological intervention strategies for the management of pain and distress. The choices range from the use of a local/regional anesthetic to the use of potent narcotics. Mild pain may be successfully treated with the application of a local anesthetic or with the administration of a single dose of a non-steroidal anti-inflammatory agent or mixed narcotic agonist/antagonist, whereas moderate pain may require the repeated administration of the agent. The treatment of severe pain often requires the frequent administration of pure narcotic agonists and may also benefit from a multimodal approach to pain management. A multimodal approach often has the advantage of lowering the required dose of more powerful and potentially toxic agents, while still promoting the animal's well-being.

Specific Recommendations

- Animals that undergo a painful procedure such as surgery and are likely to experience post procedural pain, should receive preemptive analgesic medication (before the animals recover from anesthesia).
- Additional dosing of analgesic medication may be predetermined or based on a subjective assessment of the degree of pain experienced by the animal.
- A predetermined analgesic regimen should be incorporated into the experimental design based on a prediction of how much pain will occur after an experimental procedure. All animals in the experiment are treated with the same amount and type of drug, with administration usually beginning immediately after surgery before the animal awakens. Treatments may be repeated for a prescribed period of time. This type of regimen insures that all animals will receive some type of analgesic treatment, but it does not relieve research personnel of their responsibility to observe and evaluate each animal after surgery. Animals that continue to show signs of pain or have other complicating problems after surgery need to be assessed individually and treated appropriately.

Analgesic Drugs and Delivery Methods

There are many drugs and routes of administration available for alleviation of pain. Some drugs, such as the opiates (example: buprenorphine or morphine), provide pain relief through their action on the central nervous system. Non-steroidal anti-inflammatory drugs (NSAIDs) relieve

pain by decreasing inflammation and, in some cases, direct analgesic action. Long acting local anesthetics such as bupivacaine, provide postoperative analgesia by blocking nerve conduction.

In most cases, analgesics should be administered by injection. Analgesic drugs may be placed in drinking water or food for oral delivery, but these methods run the risk of inaccurate dosing, lack of consumption due to palatability and degradation of the drug over time as it sits in the water bottle or food product. The specific recommended dosages for analgesics are provided below, by species.

Dosage Rates for Analgesic Drugs in Birds

Drug	Dosage
Butorpanol	1–3 mg/kg IM Q 4-6 hrs
Carprofen	1 mg/kg SQ every 8-24 hrs
Flunixin meglumine	1-10 mg/kg IM every 24 hrs
Ketoprofen	1-2 mg/kg IM every 8-12 hrs
Meloxicam	0.5 mg/kg IV, IM or PO every 24 hrs

Dosage Rates for Analgesic Drugs in Bats

Drug	Dosage
Meloxicam	0.3 mg/kg IM or PO every 12 hrs

Dosage Rates for Analgesic Drugs in Laboratory Animals

Drug	Mouse	Rat	Rabbit
Bupivacaine	4 mg/kg max IM or dripped on incision	4 mg/kg max IM or dripped on incision	4 mg/kg max IM or dripped on incision
Buprenorphine*	0.05-0.1 mg/kg SC every 6-12	0.05-0.1 mg/kg SC every 6-12 hrs	0.01-0.05 mg/kg SC every 6-12 hrs
Butorphenol	1.0-5.0 mg/kg SQ every 4 hrs	2.0 mg/kg SQ every 4 hours	0.1-0.5 mg/kg IM or IV every 4 hours
Carprofen**	5 mg/kg SQ every 24 hours	5 mg/kg SQ q 24 hours	4 mg/kg SQ or 1.5 mg/kg PO every 12-24 hours
Flunixin meglumine	2.5 mg/kg SQ q 12 hours	2.5 mg/kg SC every 12 hrs	1.1 mg/kg SC every 12 hrs
Ketoprofen	5 mg/kg SQ q 12-24 hours	5 mg/kg SC every 12-24 hrs	3 mg/kg SC every 12-24 hrs
Meloxicam		1-2 mg/kg SC every 24 hrs	0.2 mg/kg SC every 24 hrs

*Buprenorphine - dilute 0.1 ml of 0.3 mg/ml product in 0.9 ml sterile normal saline; administer 0.1 ml SC to a 25 gm mouse

**Carprofen – dilute 0.1 ml of 50 mg/ml product in 3.9 ml injectable water; administer 0.1 ml SC to a 25 gm mouse

Dosage Rates for Analgesic Drugs in Agricultural Research Animals

Drug	Cattle	Sheep	Horse	Swine
Aspirin	480-960 grains PO every 12 hrs adult animal			
Buprenorphine		0.005-0.01 mg/kg IV or IM every 4 hrs		0.005-0.02 mg/kg IV or IM every 6-12 hrs
Butorphanol	0.01 mg/kg IV or IM every 4-6 hrs	0.01 mg/kg IV or IM every 4-6 hrs	0.01 mg/kg IV or IM every 4-6 hrs	
Flunixin meglumine	0.5-1.1 mg/kg IV every 24 hrs	1.1 mg/kg IV every 24 hrs	1.1 mg/kg IV or PO every 24 hrs	1.1 mg/kg IV or SC every 24 hrs
Ketoprofen			0.5 mg/kg IV every 24 hrs	
Phenylbutazone			2.2-4.4 mg/kg IV or PO every 12- 24 hrs	

Common Analgesic Drugs and Mechanism of Action

BUPHRENORPHINE (BUPRENEX) is a narcotic partial agonist analgesic intended for intravenous, subcutaneous or intramuscular administration. Each ml contains 0.3 mg of buprenorphine.

Mechanism of Action: The exact mechanism of action of buprenorphine has not been established, but it exerts its analgesic effect by high affinity binding to the Mu and perhaps other opiate receptors in the CNS. Its slow dissociation from the receptor is believed to contribute to its long duration of action.

Physiological effects: The effects of buprenorphine may be potentiated by other centrally-acting agents such as other narcotic analgesics, benzodiazepines, general anesthetics, phenothiazines, or other tranquilizers. Buprenorphine does not increase pulmonary artery pressure or systemic vascular resistance like other narcotic agonist/antagonists. It provides analgesia without significant respiratory or cardiovascular depression with a wide margin of safety.

Indications/Contraindications: Buprenorphine is indicated for the relief of moderate pain with a single dose lasting from 6-12 hours. Because of the potential problems of respiratory depression, buprenorphine should not be administered to animals with preexisting respiratory depression. Naloxone, doxapram, and/or controlled ventilation may be needed if respiratory depression

becomes severe. Buprenorphine, like most opioids, may result in increased CSF pressure if the animal hypoventilates. Therefore, use on animals with intracranial lesions or other conditions that increase intracranial pressure *may* be contraindicated. Because buprenorphine is metabolized by the liver, its activity may be increased or extended in animals having hepatic dysfunction.

BUTOPHANOLIS a synthetic narcotic agonist-antagonist. It is a member of the phenanthrene series.

Mechanism of Action:: Butorphanol has little effect at mu receptors which initiate analgesia, sedation, and depression of the cardiopulmonary system and body temperature. It is active at kappa receptors which are responsible for analgesia and sedation without depression of the cardiopulmonary system or body temperature. Butorphanol has agonist activity at sigma receptors. This allows autonomic stimulation and dysphoria. Although butorphanol is reported to have no effect at mu receptors, some antagonistic activity of unknown mechanism is observed.

Physiological effects: Butorphanol provides a level of analgesia and sedation without significant depression of the cardiopulmonary system or body temperature. It has analgesic effects 4 times that of morphine. Autonomic stimulation and dysphoria occur along with a strong antitussive effect. A moderate cardiovascular depression and panting may be observed in dogs.

Indications/Contraindications: Butorphanol may be used for restraint, for induction of anesthesia, in combination with IM anesthetics, and as a mild analgesic. Sedation with butorphanol alone is variable and of short duration. Butorphanol combined with acepromazine provides good chemical restraint. In cats and horses, butorphanol induces better visceral than somatic analgesia. Moderate to marked cardiopulmonary depression occurs with IV infusions given at a rapid rate (0.2 mg/kg/min). Female rats tend to show an increased nervousness and decreased care for the newborn after parenteral use.

CARPROFEN (RIMADYL) is a nonsteroidal anti-inflammatory agent. It is used for analgesia in certain species to control post-operative pain, and is approved in cats and dogs for use prior to surgery. It is typically given prior to surgery in other animals as well. Onset of analgesia after injection is about 1 hour.

Mechanism of Action: Nonsteroidal anti-inflammatory agent that is somewhat COX-2 selective. However, its mechanism of action is not well understood.

Indications/Contraindications: Gastric ulceration and significant kidney dysfunction are less likely with this NSAID than with most other NSAIDs. In addition, carprofen does not cause excessive bleeding during surgery. It has been reported to cause idiosyncratic hepatocellular toxicosis in dogs, especially in Labrador dogs; this may or may not be reversible.

MELOXICAM (METACAM, MOBIC) is a nonsteroidal anti-inflammatory agent. It is used for analgesia in certain species to control post-operative pain. It is typically given prior to surgery. Onset of analgesia is about 1 hour.

Mechanism of Action: Nonsteroidal anti-inflammatory agent that is COX-2 preferential. However, additional mechanisms for analgesia are likely.

Indications/Contraindications: Gastric ulceration and significant kidney dysfunction are less likely with this NSAID than with most other NSAIDs. Availability as an injection and in a palatable, honey-flavored liquid simplifies administration.

KETOPROFEN is a nonsteroidal anti-inflammatory agent. It is used for analgesia in certain species to control post-operative pain.

Mechanism of Action: Nonsteroidal anti-inflammatory agent that provides good anti-inflammatory and analgesic action. Inhibits COX-1 and COX-2 enzymes.

Indications/Contraindications: As with most NSAIDs, gastric ulceration, post-surgical bleeding and kidney dysfunction may occur. Usually minimal likelihood of toxicity when used for short periods of time in normotensive animals. Ketoprofen generally should not be given prior to surgery, since it may cause bleeding and renal compromise. Onset of analgesia is about 1 hour.