

# Appendix 1: Code of Practice ‘Prevention, recognition, and management of pain in laboratory animals’

## Summary

### 1 Ambition

This Code of Practice (CoP) ‘Prevention, recognition, and management of pain’ is intended for all persons involved in the design, assessment, conduct and supervision of animal procedures: the establishment licensee, Animal Welfare Body (IvD), designated veterinarian, researcher, biotechnician, zoological analyst and the laboratory animal care taker.

### 2 Explanation

This CoP is concerned with all forms of pain and not only focused on post-surgical pain. It aim to:

- a.) make pain prevention, pain recognition and pain management an inseparable part of the design, assessment, conduct and supervision of animal procedures;
- b.) further optimise pain prevention, pain recognition and pain management;
- c.) reduce the percentage of animal procedures involving pain where pain management is not incorporated as part of the experiment.

The overarching ambition is to limit all forms of pain in animal procedures to a minimum.

### 3 Best-efforts obligation

All parties involved have an obligation within their specific roles to keep the distress suffered by the animal, including in terms of pain, to a minimum. This requires the best efforts of all those involved.

### 4 Analogy/precaution

In cases where it is unclear whether an animal has pain, the design and assessment of animal procedures must be guided by the analogy principle: animals share the same experiences as humans, unless there is demonstrable evidence available to the contrary. In cases in which the analogy principle is insufficient, the precautionary principle may also be applied. According to the precautionary principle, in the absence of any scientific consensus as to the presence or absence of pain, it will be assumed that the animal has pain.

### 5 Pain management: when to use/not use?

Moral and statutory reasoning: underlying principles are the intrinsic value of the animal and the obligation not to let the individual animal suffer unnecessarily, or to limit its amount of pain, suffering, fear and ongoing injury to a minimum.

Scientific reasoning: pain can lead to patho-physiological changes that influence experiments in a complex and uncontrolled way. This applies in respect of acute pain, pain immediately following an intervention and chronic pain. Pain can have a greater influence on the outcomes of an experiment than where controlled pain relief is

administered. Where pain management is to be withheld despite the presence of pain, specific arguments to support this are necessary, and must be agreed to, in both the work protocol (by the Animal Welfare Committee [IvD]) and in the project proposal (by the IvD, Animal Experiment Committee [DEC] and Central Authority for Scientific Procedures on Animals [CCD]).

## 6 Pain recognition

A step-by-step pain recognition process, properly prepared, is necessary: knowledge of species specific factors, standardised observation process, appropriate to the respective phases of the animal procedure and with minimal effect on the behaviour of the animal. The use of scoring lists and the assessment system are as described in this CoP.

## 7 Pain prevention and management: more than just the administering of analgesics

In its widest sense, pain management involves not only the use of painkillers (analgesics), but also other medication and other measures such as the use of aseptic and antiseptic processes and adapted animal housing. Optimal pain management is achieved firstly through the preventive and perioperative, multimodal administering of pain relief, according to the expected extent and duration of the pain, and secondly through knowledge and expertise in aseptic, refined surgical techniques and the recognition and management of pain.

## 8 Education

All persons engaged in the prevention, recognition and management of pain should have the most up-to-date knowledge in this field and the relevant skills. The licensee must ensure that staff receive a sufficient level of continuing education.

## 9 A humane endpoint (HEP)

A HEP will be delivered to prevent or bring an end to pain. A HEP should be delivered:

- a.) if an upper threshold for pain for a procedure is exceeded;
- b.) if the scientific endpoint is reached; or
- c.) if the scientific endpoint can no longer be achieved whilst the animal is suffering pain/distress.

**If in doubt as to compliance with this code, you should contact the Animal Welfare Body (IvD).**

## Code of Practice ‘Prevention, recognition, and management of pain in laboratory animals’

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This CoP is intended as document that sets out at any point in time to be a reflection of actual knowledge and experience in the field of prevention, recognition and management of pain.

The most recent version of the CoP should be consulted here:

<http://english.ncadierproevenbeleid.nl/> In various parts of this CoP there are references to digital appendices. These appendices can also be found at <http://english.ncadierproevenbeleid.nl/>. The doses and dosing intervals specified in this CoP are recommendations based on current knowledge. When drawing up an anaesthesia and pain relief protocol, it is therefore always important to consult with the Animal Welfare Body (IvD) and/or the designated veterinarian.

## 1. Ambition

### Ambition and target groups

The possibility that animals used in animal procedures could suffer distress is an inherent part of such procedures. ‘Distress’ is a wider term and is not always caused by pain. It can also be caused by acute or chronic stress, fear or the dysfunction of organs. This Code of Practice (CoP) focuses only with the prevention, recognition and management of pain in laboratory animals.

The CoP ‘Prevention, recognition, and management of pain in laboratory animals’ offers practical recommendations to all persons involved in the design, assessment, conduct and supervision of animal procedures for prevention, recognition and management of pain in laboratory animals. These recommendations are in line with current laws and regulations and based on the latest opinions of experts.

The ambition of the CoP is:

- to make pain prevention, pain recognition and pain management an inseparable part of the design, assessment, conduct and supervision of animal procedures;
- to significantly improve pain prevention, pain recognition and pain management; and
- to reduce the percentage of animal procedures involving pain where pain management is withheld as part of the experiment.

The overall ambition is to limit all forms of pain in animal procedures to a minimum.

## 2. Explanation of terms

Pain is often defined as ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage’. (References: 1) The inability to communicate verbally and the absence of behavioural changes do not exclude the possibility that an individual can suffer pain.

### Types of pain

The impact of pain on animal welfare is closely connected with the duration and intensity of the pain sensation. This impact may also be affected by factors such as stress and fear. Humans tolerate pain much better where the pain is controllable and predictable, or if the pain is ultimately rewarded (such as the birth of a child). There are indications that this also applies to animals (References: 2).

A categorisation of pain is possible according to cause, duration and intensity. ‘Cause’ would include smaller or larger invasive or non-invasive procedures, infectious diseases, growth of a tumour and auto-immune diseases.

Under the heading ‘duration,’ the following categories may be identified:

- **Short-duration pain** (from a few seconds to tens of seconds), for instance the insertion of a needle for an injection.
- **Mid-duration pain** (from minutes up to a week), for instance post-procedural pain, usually combined with greater tissue damage.

- **Chronic pain** is for a long duration (weeks, months, years) and is usually a by-product of non-pain related research into, for example, disease models for diseases related to ageing or arthritis. Chronic pain is associated with tissue degeneration and destructive illnesses (osteoarthritis, cancer) and does not improve over the long term.

The *intensity* of the pain sensation will affect the nervous system in four different locations, i.e. the peripheral pain receptor (nociceptor), the spinal cord, the subcortical cerebrum, such as the brain stem and thalamus, and lastly the cortex. By shutting down the cortex, such as through general anaesthetic, an animal may be free of any pain during an invasive procedure because it is unconscious. However, because during this invasive procedure there is nociception (processing of pain stimuli) at the three other said locations, an animal that recovers from anaesthesia will experience a more intense post-procedural pain as if this nociception had been prevented with painkillers for the purposes of the procedure. This fact underlines the importance of preventive pain relief (=blockage of the nociception before the pain is induced) and the use of multimodal pain relief (=different groups of painkillers combined to dampen the nociception at various locations) in procedures involving a high intensity of pain. In the case of other vertebrate animals such as fish and birds, the presence of nociceptors comparable with those of mammals has been demonstrated. Whether fish experience pain in the same way as humans and mammals is still the subject of debate. However, various types of fish have been shown to behave differently and/or exhibit unusual physiological reactions in response to the potential sensation of pain.

In laboratory animal research, a categorisation of seriousness of the research is usually based on distress, which is a much broader category than one based only on pain. Some examples in the grading of distress in the case of painful procedures are set out in the digital appendix to this CoP, in *which the pain is managed where necessary by use of analgesics and/or anaesthetics*. Many more examples (including examples of distress not caused by pain) are set out on pages 23, 24 and 25 of the Explanatory Memorandum to the registration of laboratory animals and animal procedures 2014 of the Netherlands Food and Consumer Product Safety Authority (NVWA [References: 3]) or Annex VIII of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Text with EEA relevance) (References: 4).

The cause, duration and intensity of the pain, in combination with the pain prevention, pain recognition and pain management applied are important points to consider when estimating the degree of distress in the application for the project licence, the drafting of the work protocol and the subsequent registration of the actual distress suffered during the animal experiment.

### 3. Best-efforts obligation

All parties involved – the establishment licensee, the Animal Welfare Body (IvD), designated veterinarian, researcher, biotechnician, zoological analyst and laboratory animal care taker – have within their specific roles a best-efforts obligation to keep the distress suffered by the animal, including in terms of pain, to a minimum.

## Statutory framework

The Experiments on Animals Act (*Wet op de dierproeven* [Wod]) has the primary purpose of protecting animals used for scientific or educational purposes. The Experiments on Animals Act therefore sets out clear rules aimed at preventing and minimising pain for laboratory animals (digital appendix of the Experiments on Animals Act, Part B). The definition of an animal procedure includes a threshold value for degree of pain: each use of an animal for one of the purposes listed in the Experiments on Animals act ‘that can cause as much as, or more, pain, suffering, fear, or long-term damage as the injection of a needle in accordance with good veterinary science practice’ is an animal procedure (References: 5). The elimination of pain through the efficient application of anaesthetics, analgesics, or other method, however, does not mean that such a procedure using an animal is no longer an animal procedure.

The Experiments on Animals Act also prescribes (appendix Wod, Part B) that the establishment licensee should have at its disposal one or more persons who:

- care for the animals and conduct the animal procedures;
- ensure that unnecessary pain, suffering, fear or long-term damage in laboratory animals is ended in a timely manner;
- ensure that a project is conducted in accordance with the project proposal;
- supervise the welfare of the laboratory animals.

It should also have at its disposal a designated veterinarian or other sufficiently qualified expert responsible for advising on the welfare and treatment of the animals.

An adequate basic education for each person involved and regular continuing education should ensure that these persons have the most up-to-date knowledge and skills.

### Attention to pain during the various phases of the research:

#### 1. Designing the work protocol

Before conducting an animal procedure, the responsible researcher/performer should design a work protocol. The work protocol should be coordinated with the Animal Welfare Body (IvD) and the designated veterinary physician. The work protocol should focus sufficient attention on minimising the pain resulting from the treatment and interventions. For types of animal procedures that are conducted in a standard way, this method should preferably be described in a Standard Operating Procedure (SOP) in order to ensure an optimal, standardised pain relief. In the case of new types of animal procedures that could be conducted in non-standard ways, the method must be described in detail in the work protocol.

The work protocol should also clearly describe:

- what processes need to be conducted and when;
- who has what responsibilities, including the use of humane endpoints;
- which clinical effects to expect and when, and the expected progress of pain;
- how and when to prevent or manage pain (methods, dosage, points in time, methods of administration, care, animal housing);
- the method by which symptoms must be checked and frequency (e.g. every six, eight, twelve or 24 hours), as well as how such

- frequency should be revised if the degree of pain increases and/or the criteria by which the humane endpoint draws near apply;
- what the criteria for applying the human endpoints are.

## 2 Conduct of the research

The treatment and/or procedures carried out on the laboratory animals and the welfare effects thereof are to be recorded in the welfare logbook in accordance with the CoP ‘Welfare Monitoring’ (the original version is available in the digital appendix on the NCad website. It is expected that the CoP ‘Welfare Monitoring’ will be updated in the near future). It is important that this welfare logbook provides sufficient support to adequately safeguard animal welfare. In the context of painful interventions, this means that pain scores and procedures must be clearly recorded– with the aid, for example, of pain scoring lists – in combination with the administration of pain relief, so that the effectiveness can be subsequently determined.

## 3 Evaluation

After completion of an animal procedure, the evaluation of the welfare logbook and the clinical scoring lists will establish for all involved parties whether the procedure was optimally conducted and whether the animal welfare and/or research can be improved in the future.

In addition, a periodic evaluation of procedural SOPs will also contribute to further improvement. In research involving new laboratory animal models and where problems occur during the procedure, it is recommended that a laboratory-animal pathologist be asked to research further, to find out more about the nature,

degree and duration of the distress and possibly also the possible causes of intercurrent problems, and to propose steps for prevention thereof.

## Parties responsible for pain recognition and pain management

### *The establishment licensee and management of the animal facility*

- provide a climate within which all persons are expert and engaged, and take responsibility for the animals’ welfare (Culture of Care);
- Facilitate staff in enabling them to carry out their roles and responsibilities to the optimum: expertise, training, planning tasks, conduct of animal procedures and analyses.

### *The Animal Welfare Body (IvD)*

- obtains advice on anaesthesia and pain management from the designated veterinarian;
- reaches agreement with the researcher on the project proposal and the work protocols. A special focus for attention here is the prevention, recognition and management of pain, as well as the justification hereof in cases where pain management is withheld because this is not considered reconcilable with the aim of the procedure;
- supervises the conduct of the procedure, obtains information during and after the procedure about its progression and on the basis thereof discusses potential adjustments thereto with the responsible/performing researcher.

#### *The responsible researcher by virtue of Article 9 (project licence<sup>a</sup>)*

- is responsible for ensuring that the description and conduct of the animal procedures is in accordance with the statutory requirements and best practices;
- the responsible or performing researcher reaches agreement with the Animal Welfare Body (IvD) and the designated veterinary veterinarian on the design of the types of animal procedures and the work protocols, including the choice of animal species, the application of the 3Rs and the application or withholding of anaesthesia and pain relief depending on the aim of the procedure.

#### *The performing researcher by virtue of Article 9 (work protocol/study plan<sup>b</sup>)*

- is knowledgeable about the use of anaesthesia and pain relief in animal species and the type of animal procedure covered by the project licence;
- consults with the Animal Welfare Body (IvD) and/or the designated veterinary veterinarian regarding the methods to monitor welfare specific to animal species and type of animal procedure, the prevention and management of pain and the formulation of suitable humane endpoint criteria;
- agrees the terms of the work protocol for the conduct of the research with all parties involved, such as the laboratory animal care takers, biotechnicians, researchers and the management of the animal facility

#### *The designated veterinary physician*

- has expertise in administering anaesthesia and pain relief to the animal species and in the types of animal procedures used within his establishment;
- advises the responsible researcher, performing researcher, Animal Welfare Body (IvD), biotechnician, zoological analyst, and animal care taker regarding the administering of anaesthesia and pain relief in order to safeguard the quality thereof;
- where more in-depth expert knowledge is required, consults with other (veterinarian) specialists, such as diplomates from the European College of Laboratory Animal Medicine (ECLAM) or European College of Veterinary Anaesthesia and Analgesia (ECVAA).

#### *The animal welfare supervisor pursuant to Article 13.f, section 3a*

- has expert knowledge concerning the recognition of distress, including pain recognition, as well as knowledge in the field of pain management, use of humane endpoints and euthanasia comparable to that of the designated veterinary veterinarian or seeks advice on these matters from this veterinary physician;
- advises the responsible researcher, performing researcher, biotechnician, zoological analyst and animal care taker about recognition of distress, including pain, to enable an optimal refinement of the animal procedure.

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<sup>a</sup> As indicated in the application to the CCD for a project licence

<sup>b</sup> As indicated in the work protocol or study plan

*The laboratory animal care taker/biotechnician/zoological analyst/other persons conducting an animal procedure (persons listed in Article 13f, sections 2a+b)*

- is responsible for the conduct of the intervention in line with best practices and the administration in such interventions of anaesthesia and pain management in accordance with the work protocol;
- the biotechnician, zoological analyst and laboratory animal care taker are responsible for the recognition of pain within the relevant animal species and type of animal procedure, the recording of findings such as pain scores, the provision of appropriate care, the taking of action if the analgesia is insufficiently effective (including the administering of additional pain relief), providing information to the responsible or performing researcher pursuant to Article 9 of the Experiments on Animals Act and/or designated veterinary physician and then ending the distress by reaching the humane endpoint in accordance with the work protocol.

In addition, the Central Authority for Scientific Procedures on Animals (CCD) and the Animal Experiments Committees (DECs) play a role in testing animal procedures. If the applicant believes that pain management cannot be reconciled with the purpose of the procedure, the arguments in support of this belief must be convincing. If the DEC and/or the CCD is/are not convinced by these arguments, the researchers should preferably provide additional information from, for example, a further literature study, and/or a pilot study or by adding extra groups receiving pain relief to the first experiment or first experiments. The results must be retrospectively assessed at the end of the experiment. It may be established, for example, that in

respect of future experiments – within the same project and for new projects – pain relief is reconcilable after all with this type of animal procedure and this information may be shared with all those working with laboratory animals. In addition, the DECs and the CCD have a signalling function and may decide in the case of potential problems to obtain additional advice from an expert group.

#### **4. Analogy/precautionary principles**

The question whether vertebrate and other animals are able to experience pain has been a source of debate for a long time. However, the results of research conducted by various research groups over the last two decades provides growing evidence that animals can experience pain (References: 6).

Where it is unclear whether an animal has pain, the assessment and design of animal procedures must be guided by the analogy principle: animals have the same experiences as humans unless there is demonstrable evidence available to the contrary. In cases in which the analogy principle is insufficient to determine whether, and if so, how, pain management is to be used, for ethical and scientific considerations the precautionary principle should also be applied. According to the precautionary principle, in the absence of any scientific consensus as to the presence or absence of pain, an assumption will be made that the animal experiences pain. It should be emphasised here that the precautionary principle also applies to other vertebrate animals such as fish and birds. The same principles also apply to animals in the wild.

## 5. Pain management: reasons to administer/not administer?

Optimal pain prevention, recognition and management in respect of laboratory animals is important for various reasons.

Firstly, moral considerations play a role. Every animal procedure involves an intrinsic conflict between the interests of human beings (or other animals) and those of the animals used in the procedure. The underlying principles are the intrinsic value of the animal and the duty to subject the animal to as little pain, suffering, fear and long-term injury as possible. The procedure must be sufficiently important to justify the distress – including pain – of the animal. There is a best-efforts obligation to keep the ‘cost’ to the animal, in terms of pain and otherwise, to the minimum possible.

For scientific reasons also, it is important to have a carefully considered policy on pain prevention, pain recognition and pain management. Pain – acute pain, pain immediately following an intervention and chronic pain – can all influence experiments in a complex and uncontrolled way.<sup>c</sup>

### *What influence does pain have on the research results?*

Pain can influence various physiological processes and can be the underlying cause of pathophysiological changes. Pain affects the endocrine and neuroendocrine system (various hormones, endorphins), the sympathetic nervous system (including vascularisation,

intestinal motility, kidney function), the immune system (including increased risk of infection, slowdown in wound recovery), blood composition (changes in blood count), respiration (abnormal breathing patterns), the cardiovascular system (increased heart rate and blood pressure) and muscles (weakness, cramp). Furthermore, behavioural changes due to pain can influence the physiology of the animal as a result, for example, of a disrupted sleeping pattern, loss of appetite, loss of mobility, aggression and self-harm (automutilation).

All of this can result in an animal procedure being influenced in an uncontrolled and perhaps more significant way than if controlled pain relief had been administered.

### *What influence does pain relief have on the research results?*

Pain relief can itself affect the research results.

The literature reports that the administration of opiates – depending on the dosage – can have side effects such as depression, reduced heart rate, reduced intestinal motility and nausea. These side effects are identified particularly for morphine and fentanyl, but much less for buprenorphine.

The literature reports that the administration of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) – depending on the dosage – can have side effects such as gastrointestinal haemorrhages, kidney damage (which increases with dehydration) and an inhibition of the immune

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<sup>c</sup> Research into the phenomenon of pain has a special place as a research subject. When this involves the testing of various analgesics, a necessary precondition is that in all circumstances it is possible to revert to the conventional therapy and that validated pain recognition methods and humane endpoints are set out in the research protocol.

system. These side effects are described especially in older, non-selectively acting medication, but much less for carprofen and meloxicam. It is clear that the degree to which the research results are influenced depends heavily on the chosen medication, the dose administered and the period for which it is administered. This means that even in complex animal models such as the healing of bones and wounds, arthritis and immunological research, with the right choices providing full or partial pain relief can be realized whilst the desired research result can be obtained. A detailed description of the possible effects of analgesics on research results can be found at GV-Solas 2015 (References:7).

**The withholding of pain relief/pain management may be appropriate in circumstances such as:**

- Research into the phenomenon of pain, including the assessment of the efficacy of new pain relief;
- Research in which short-term pain is administered (e.g. in the form of an electric shock) to condition the animals;
- Where the administration of pain relief would cause greater harm to the welfare of the animal than the pain itself;
- Where the pharmacological properties of the pain relief would have a greater effect on the relevant readout parameters than the pain itself. In this case, the influence per research application must be documented and where necessary supported by data from additional studies.

*Note: the withholding of pain relief does not affect the obligation for the maximum use at all times of non-medicinal pain management.*

Where pain management is withheld despite the presence of pain, this needs to be specifically argued for, and agreed in, both the work protocol (by the Animal Welfare Body [IVD]) and the project proposal (by the IVD, DEC and CCD).

## 6. Pain recognition

Pain can mostly be recognised through clinical symptoms, posture and behaviour. These are generally speaking a specific characteristics, but in the context of the animal procedure or pathology they may be indications that the animal has pain. Clinical symptoms could be: an increased heart rate, faster breathing and dilated pupils.

During behavioural observations one can observe an abnormal posture (e.g. bent back, expanded stomach, begging posture) or abnormal movements (decrease or increase in movement; restlessness; rolling over; lethargy). In addition, different form of response to humans or other animals (more aggressive or more apathetic); the people best able to identify this are those who work with the animals on a daily basis.

It is important that clinical evaluations and assessments are conducted by people with a good knowledge of the normal and abnormal behaviour of the relevant animal type. The digital appendices to this CoP contain examples of pain score lists (such as grimace scales) for mice, rats and rabbits. These scales offer help in recognising pain in these animal species.

Different animal species can respond differently to pain, but even within one type of animal, different strains or even individuals can respond differently to pain. Symptoms of pain specific to animal species will be discussed in the digital appendix on the NCad website.

#### *Pain recognition: step-by-step plan*

A process to recognise pain in laboratory animals undergoing a specific animal experiment could involve the following steps:

1. Describe the expected course of the pain throughout the experiment and based on this determine the frequency of observation and the symptoms to look out for;
2. Ensure that all parties involved are familiar with this protocol;
3. The assessment should be conducted as much as possible by the same person. If this is not practically feasible, then the transfer of this task must be carefully considered. Inexperienced persons should receive training before performing this task;
4. Use a pain score list (examples of this can be found in the digital appendix on the NCad website);
5. First observe the animals and their environment without disturbing them. Observation of animals that are prey may be assisted by use of a camera, given that the behaviour of such an animal may change in the presence of the observer;
6. Assess how the animals respond to each other and to the observer. This can best be done by the person who regularly cares for the animals;
7. Inspect the animal for response to gentle palpation or to the touching of possibly painful areas, where this is practically possible;
8. Weigh the animal, record its food and liquid intake where possible and note any normal or abnormal urination (frequency) and defecation in the cage or other housing;
9. Administer pain relief where necessary and repeat the above steps 30-60 minutes following treatment to determine whether the drug and the dose are effective. Even where there is uncertainty as to the existence of pain, an assessment of the response to pain relief may be useful;
10. During the experiment, adjust the frequency of observation and the method of pain management where the findings so dictate;
11. Make a retrospective evaluation and discuss this with the relevant parties and the Animal Welfare body (IvD);
12. Review the protocols regularly and update them where the findings so dictate;
13. Ensure there is regular continuing education and/or case reviews to maintain and build on the knowledge and skills in this field.

#### **7. Preventing and managing pain: more than just administering analgesics**

##### *Pain management indicator*

The pain management indicator available in the digital appendix to the CoP is an aid to standardising the use of pain prevention and pain management in the case of procedures. The pain management indicator can generally be applied to all animal species. For specific questions, such as the issues of pain and pain management in types of animals other than rodents, the Animal Welfare Body (IvD) can offer good advice.

The underlying principle is to prevent and manage pain through firstly the preventive and multimodal (see below for explanation) application of analgesics, according to the expected level and duration of the pain, and secondly through the optimal technical and general performance of the procedures, to prevent unnecessary trauma, the administration of aseptics and antiseptics and the provision of appropriate care and animal housing.

The use of both non-pharmaceutical measures and painkillers can contribute to the prevention and management of pain in laboratory animals.

#### *Non-pharmaceutical measures*

- Adjustments to housing, such as deeper/softer bedding, more/different nest-building materials, warmer/cooler environment, dimmed lighting, easily accessible food and liquids, liquidised foods, drinking vessels with extended nozzle;
- Modifications to care, such as intensive monitoring (to enable quicker intervention) and administration of liquids;
- Optimal conduct of experimental processes, such as minimally invasive and sterile operations, reduction in the length of interventions, the refinement of the method of administration of medication (through food or drinking water instead of injections), the least invasive technique for taking samples, the use of concentrated substances (small volume vs dilution). Consult the Animal Welfare Body (IvD) for options for the refining of experimental conditions.

#### *Painkillers*

Preventive application is the administration of analgesics before the sensation of pain occurs. Effective pain relief before and during a painful intervention limits the extent of the pain following the intervention. By administering painkillers with different, complementary working mechanisms (i.e. 'multimodal'), the pain relief is more effective (Figure 1). In general, the dosage of the painkillers remains the same where there is multimodal administration. The complexity of an anaesthesia and analgesia protocol (one or more groups of painkillers, method of administration, dosage and duration of treatment) must be decided on the basis of the expected nature, level and duration of the pain. Some examples of this principle are included in the digital appendix on the NCad website.

The preventive and perioperative administration of pain relief (Figure 2) can be applied especially in the case of operative procedures and infection models because here the occurrence of pain is predictable. It is necessary to take account of the time needed to achieve an effective level of pain relief: for example, in the case of buprenorphine, there is a slow onset of action from 30 to 60 minutes.

Therapeutic administration of painkillers is also possible in animal models for which, during the procedure, less predictable acute or chronic pain occurs as a result of evolving inflammatory processes (for example, osteoarthritis or peritonitis), ageing, or tumours. The early and adequate recognition of pain is the key to the optimal therapeutic administration of pain relief.

### Categories of painkillers (see Figure 1)

Local anaesthetics temporarily block the nervous system, so that pain sensations do not reach the central nervous system. In the case of rodents, especially small rodents, and high regular dosage, cardiac arrest is possible. The duration of the effect is usually short (one to two hours in the case of lidocaine), but could be significantly longer (six to eight hours in the case of bupivacaine). Administration is usually via local infiltration or by targeted nerve blockage. This category is often used multimodally with NSAIDs (nonsteroidal anti-inflammatory drugs) and sometimes with drugs that affect the central nervous system such as opiates and pain-killing injections of anaesthetics.

Opiates are powerful painkillers that work on the central nervous system but, in the case of repeated administration, could produce side effects such as respiratory depression, constipation and abnormal behaviour such as pica (eating disorder, appetite for non-nutritive substances). Buprenorphine is used a lot (with the dosage in mice amounting to 0.05-0.1mg/kg and in rats to 0.01-0.05 mg/kg) due to its slower onset of action and the limited effect of respiratory depression. The interval between administering doses of is six to twelve hours.

Sustained-release formulations are ideal for administering pain relief over longer periods of time without the need for repeated injections. A sustained-release veterinary formulation of buprenorphine is not available at the present time on the Dutch market, although it is available in the United States. The efficacy of the sustained release human specific formulation in laboratory animals has not been validated. This category is often used multimodally with NSAIDs and sometimes with local anaesthetics.

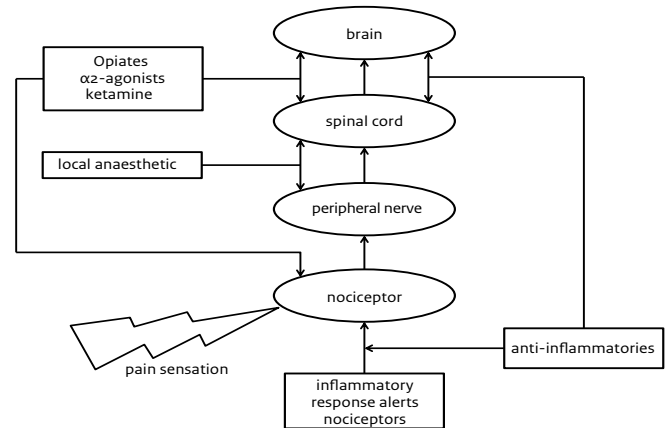


Figure 1 The different working mechanisms of painkiller categories in the pain-processing routes

NSAIDs are painkillers with a fever-reducing, painkilling and inflammation-reducing effect but which, depending on the chosen drug, dose and duration of treatment, could lead to stomach and intestinal bleeding and kidney failure. NSAIDs that are often used and safe are carprofen (dosage 4-5 mg/kg) and meloxicam (dosage in mice 1 mg/kg and in rats 0.2-1 mg/kg). The interval between doses is twelve to twenty four hours. NSAIDs can often be administered both parenterally and orally. For multimodal use: see other three categories.

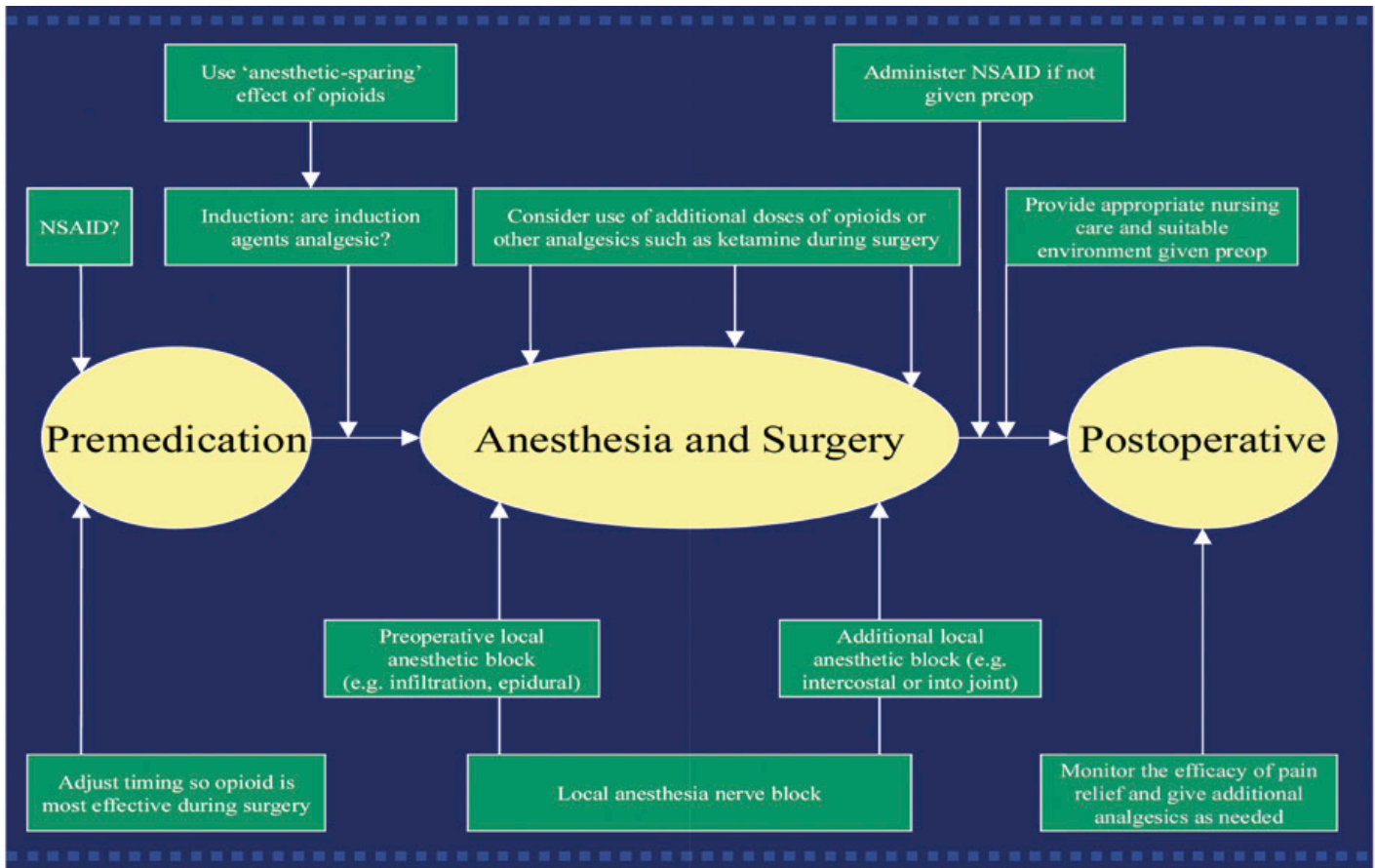


Figure 2 Points to consider in the perioperatively-effective administration of painkiller categories (adapted on the basis of Figure 5.1 of the Pain Management in Animals, References 8.)

**Dissociatives** (such as ketamine or tiletamine) and **alpha-2 adrenergic agonists** (such as xylazine, medetomidine or dexmedetomidine) are both pain-relieving groups of anaesthetics and are mostly used in combination as injection anaesthesia. Often, this anaesthesia injection is used multimodally in combination with NSAIDs and sometimes with local anaesthetics.

The dosages and dosage intervals indicated above are recommendations based on current knowledge (References: 8-11) and are often based on experiences in strictly-defined models with specific strains of animals, but sometimes using one sex, and of a certain age or weight, etc. It therefore remains important that the advice of an expert, such as a designated veterinarian, is sought in designing an anaesthesia and pain-relief protocol.

The Animal Welfare Body (IvD) can give advice in the designing of a pain management protocol. It is also possible that the recommendations can be updated on the basis of new information. It is therefore necessary to always consult the latest version of this document (available on the NCad website) before submitting a new project proposal. The digital appendix to this Code contains additional advice on pain management.

## 8. Education

An adequate basic education and regular continuing education in the field of prevention of pain, recognition of pain and the management of pain should ensure that personnel possess the most up-to-date knowledge and are adequately skilled. The licensee should facilitate the provision of adequate continuing education for personnel. At the

time of publication of this CoP there are no specific teaching materials or e-learning modules available.

## 9. A humane endpoint (HEP)

Although pain cannot be avoided, or kept to a minimum, in all animal procedures, as a general principle unnecessary pain cannot be justified. To prevent or end unnecessary pain it is necessary to determine humane endpoints. A humane endpoint can be defined as *'the earliest indicator in an animal experiment of (potential) pain and/or distress that, within the context of and scientific endpoints to be met, can be used to avoid or limit pain and/or distress by taking actions such as humane killing or terminating or alleviating the pain and distress'* (References: 12).

A humane endpoint should be administered if:

- an upper threshold for pain for a procedure is exceeded; or
- the scientific endpoint is reached; or
- the scientific endpoint cannot/can no longer be achieved whilst the animal is suffering pain/distress.

Reaching a human endpoint a humane endpoint does not therefore mean by definition that the relevant animal will be killed. It could also mean that the animal will be removed from the experiment, that the cause of the pain/distress will be removed or that the pain will be reduced.

**If in doubt as to compliance with this code, you should contact the Animal Welfare Body (IvD).**

## References

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