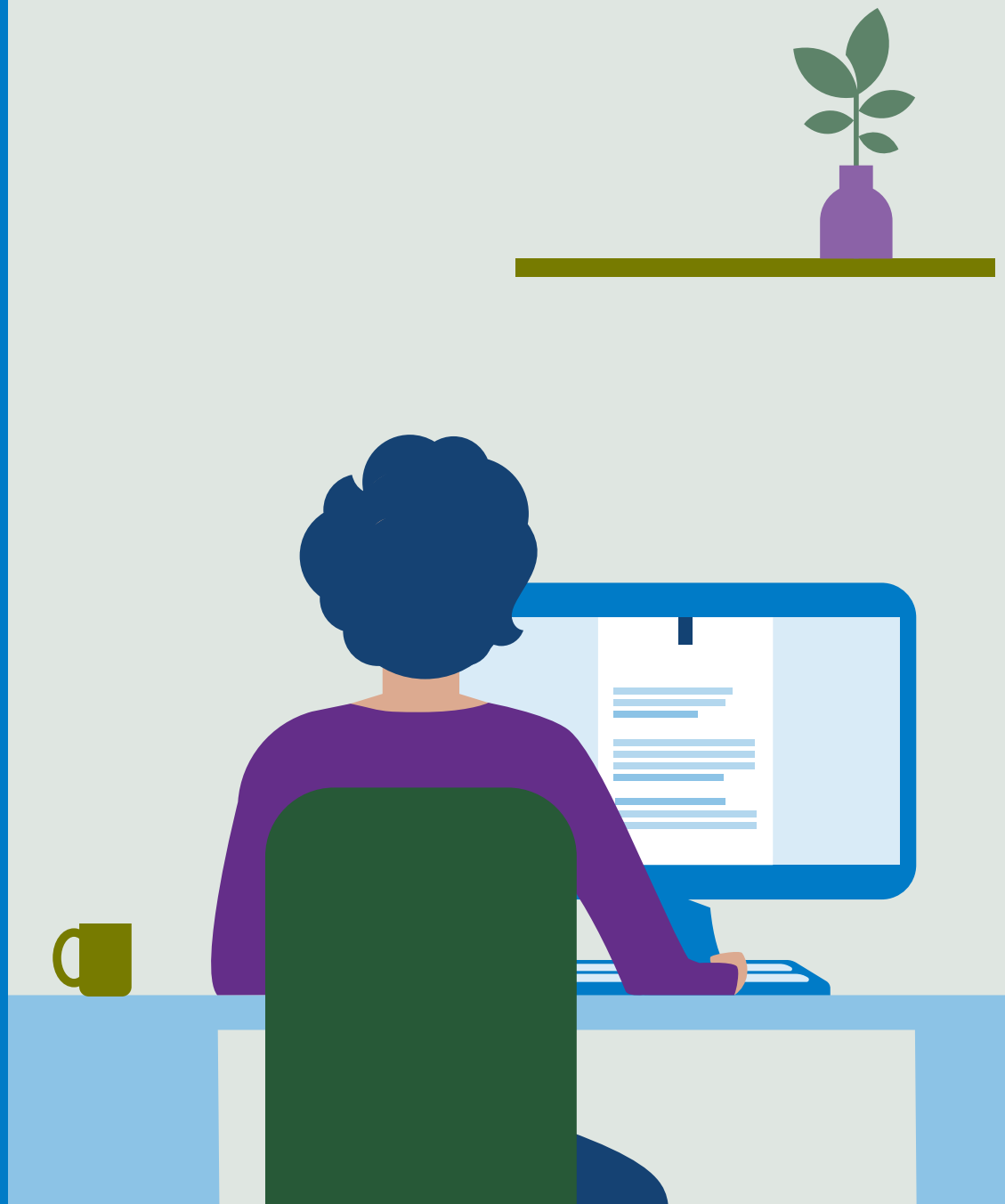
Central Authority for Scientific Procedures on Animals

What constitutes an animal procedure under the experiments on animals act?

Guideline issued by the Central Authority for Scientific Procedures on Animals



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1. Introduction

This guideline sets out the frameworks that determine whether a procedure is considered to be an animal procedure. In other words, it clarifies whether an activity is subject to a project licence requirement and, consequently, to registration requirements. The framework is based on the national Animal Experiments Act (Wet op de dierproeven, Wod) and Directive 2010/63/EU.

Following the revision of the Animal Experiments Act in 2014, the first version of this guideline was published. At that time, a working group was established with broad representation from the laboratory animal science field. In 2025, the revised Animal Experiments Act had been in force for ten years. The CCD considered this an appropriate moment to evaluate and revise the guideline published in 2015.

Two elements of the Act fall outside the scope of this guideline. For the field of research involving wild animals in their biotope, the CCD has issued a separate guideline entitled *Experiments involving wild animals in their biotope*.

In addition, the generation, breeding, genotyping and housing of genetically altered animals fall outside the scope of this guideline. For these activities, the CCD has published a separate guideline entitled *Generating, breeding, genotyping, monitoring and maintaining genetically altered animals*.

This guideline sets out, step by step, the decision points that ultimately determine whether an activity performed on an animal is to be regarded as an animal procedure. The document also includes a decision tree (Appendix 1) and an extensive table with examples (Appendix 2) to support the decision-making process.

Disclaimer: *This document may contain translations of Dutch legal texts. These are unofficial and for informational purposes only. In case of discrepancies, the original Dutch legislation prevails.*

2. Legal framework

The Animal Experiments Act (Wet op de dierproeven) asks three questions to determine whether a given activity constitutes an animal procedure:

1. **Does the procedure involve a laboratory animal as described in the Act?** The Act applies to certain animal species and developmental stages.
2. **Is the experiment carried out for one of the objectives specified in the Act?** This relates to both excluded and included objectives.
3. **Does the expected level of discomfort exceed the threshold of severity?** Does the anticipated pain, suffering, distress or lasting harm equal or exceed that caused by the introduction of a needle in accordance with good veterinary practice?

These three framework-defining questions are discussed below, with reference to the Experiments on Animals Act.

2.1 Does the procedure involve a laboratory animal?

To determine whether a procedure performed on an animal constitutes an animal procedure, it makes sense first to consider the animal in question. Only if the animal qualifies as a laboratory animal within the meaning of the Act can it then be assessed whether the activity to be carried out on that animal falls within the other criteria of an animal procedure (i.e. objective and discomfort).

Section 1b paragraphs 5 and 6

5. This Act applies to:
   a. live non-human vertebrate animals, including:
      - independently feeding larval forms; and
      - foetal forms of mammals from the last third of their normal development;
   b. live cephalopods and other invertebrate species designated by Order in Council for which it may reasonably be assumed that they can experience discomfort as a result of an animal procedure.

6. By way of derogation from paragraph 5, this Act applies to animals used in animal procedures that are at an earlier stage of development than those referred to in paragraph 5, where those animals are intended to be allowed to live beyond that developmental stage and, as a result of the animal procedure carried out, are likely, after reaching that developmental stage, to experience pain, suffering, distress or lasting harm.

Section 1(b), paragraphs 5 and 6, specify the animals and developmental stages to which the Act applies:

- Live vertebrates (mammals, birds, reptiles, amphibians, fish and lampreys);
- Foetal live mammals from the third trimester of pregnancy;
- Live reptiles, amphibians and fish at the larval stage (independently feeding larval forms);
- Animals used at an earlier developmental stage than described above, but which remain alive after the procedure and, as a result, may experience pain, suffering, distress or lasting harm at later developmental stages;
- Live cephalopods

Section 10e(1)
It is prohibited to perform an animal procedure involving the following species:

- chimpanzee (*Pan troglodytes*)
- bonobo (*Pan paniscus*)
- orangutan (*Pongo pygmaeus*)
- gorilla (*Gorilla gorilla*)

Section 10e(1) of the Act states that it is prohibited to perform an animal procedure involving Chimpanzees, Bonobos, Orangutans or Gorillas.

2.2 Is the experiment carried out for one of the objectives specified in the Act?

The sum of all procedures performed on a laboratory animal can only constitute an animal procedure if the objective for which the procedures are performed is one of the objectives described in Section 1c of the Act:

Section 1c
Notwithstanding Section 2(2–3), animal procedures may be carried out for the following purposes only:

a. fundamental research;
b. translational or applied research with any of the following aims:
   - the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in human beings, animals or plants,
   - the assessment, detection, regulation or modification of physiological conditions in human beings, animals or plants, or
   - the welfare of animals and the improvement of the production conditions for animals reared for agricultural purposes;
c. for any of the aims in point (b) in the development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feed-stuffs and other substances or products;
d. protection of the natural environment in the interests of the health or welfare of humans or animals;
e. research aimed at preservation of the species;
f. higher education, or training for the acquisition, maintenance or improvement of professional skills;
g. forensic research.

It is prohibited to perform animal procedures for the development, safety and efficacy of new and existing cosmetic products (Section 10d).

Section 10d
It is prohibited to perform an animal procedure for the development of new cosmetic products or for the testing of existing cosmetic products for which rules have been laid down pursuant to the Commodities Act.

Finally, Section 1b(7) lists objectives to which the Act explicitly does not apply.

Section 1b(7)
This Act shall not apply to:

a. non-experimental agricultural practices;
b. non-experimental clinical veterinary practices;
c. veterinary clinical trials required for the marketing authorisation of a veterinary medicinal product;
d. practices undertaken for the purposes of routine animal husbandry;
e. practices whose primary purpose is to identify an animal;
f. practices not likely to cause a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.

2.3 Does the expected level of discomfort exceed the threshold of severity?

Where a procedure, performed in the context of one of the seven specified objectives (see Section 1b (7) a–g above), causes a laboratory animals' discomfort equal to or greater than that caused by the introduction of a needle, that procedure constitutes an animal procedure. The following is set out in Section 1(1)(a) of the Animal Experiments Act:

Section 1
For the purposes of the provisions of or pursuant to this Act, the following definitions apply:

a. *animal procedure*: any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or for educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.

This definition means that the field of laboratory animal science must make difficult comparisons. First, it requires professionals to estimate whether and at what point, for example, stress and discomfort resulting from a behavioural test would result in a level of pain, suffering, distress or lasting harm equivalent to, or higher than, *the introduction of a needle in accordance with good veterinary practice*. In view of the different kinds of discomfort that could involve pain, suffering, fear, stress, etc., a direct comparison with the legal reference point ("*the introduction of a needle in accordance with good veterinary practice*") is in many cases neither straightforward nor unambiguous.

Second, cumulative effects must be explicitly taken into account when assessing the level of discomfort. An EU consensus document on Directive 2010/63/EU states the following: '*In addition it is important to note that a series or combination of "below threshold" techniques together may have the effect of causing an animal pain, suffering, distress or lasting harm*.'[1] In other words, when an individual procedure is below the threshold on its own, repeated application in the same animal may lead to cumulative discomfort that exceeds the threshold and must therefore be considered an animal procedure.

1 Consensus document - Working document on specific sections in Directive 2010/63/EU, Brussels, 6-7 October 2011

Please note
In the Netherlands, unlike in other EU Member States, killing an animal without prior procedures in order to harvest organs, tissues or bodily fluids is considered an animal procedure (Section 1(1a)) and therefore requires a licence. The associated level of discomfort may be no greater than mild. The Act requires that the competence and expertise of the persons performing the killing are ensured.

3. European framework

The European Union provides several sources of guidance, including legislation and a guidance document, which further clarify how to interpret the assessment of discomfort.

Guidance on this issue can be found in the following documents:

- EU Directive 2010/63/EU (Annex VIII)
- Severity assessment framework

To determine whether activities involving animals are above or below the threshold, and in other words whether they require authorisation, it is advisable to consult the definition and examples of procedures in the Directive that fall within the discomfort category 'mild'. The relevant parts of Directive 2010/63/EU are presented below.

Directive 2010/63/EU Annex VIII – Section I Severity categories
The severity of a procedure shall be determined by the degree of pain, suffering, distress or lasting harm expected to be experienced by an individual animal during the course of the procedure.

Mild:
Procedures on animals as a result of which the animals are likely to experience short-term mild pain, suffering or distress, as well as procedures with no significant impairment of the well-being or general condition of the animals shall be classified as 'mild'.

Directive 2010/63/EU also sets out classification criteria, such as the nature and duration of the procedure, which are relevant when determining whether the discomfort threshold is exceeded.

Directive 2010/63/EU Annex VIII - Section II Assignment criteria

When assigning a procedure to a particular category, the type of procedure and a number of other factors shall be taken into account. All these factors shall be considered on a case-by-case basis.

The factors related to the procedure shall include:

- type of manipulation, handling;
- nature of pain, suffering, distress or lasting harm caused by (all elements of) the procedure, and its intensity, the duration, frequency and multiplicity of techniques employed;
- cumulative suffering within a procedure;
- prevention from expressing natural behaviour including restrictions on the housing, husbandry and care standards.

Directive 2010/63/EU Annex VIII - Section III

Examples of different types of procedure assigned to each of the severity categories on the basis of factors related to the type of the procedure

1. Mild:

a. administration of anaesthesia except for the sole purpose of killing;
b. pharmacokinetic study where a single dose is administered and a limited number of blood samples are taken (totalling < 10 % of circulating volume) and the substance is not expected to cause any detectable adverse effect;
c. non-invasive imaging of animals (e.g. MRI) with appropriate sedation or anaesthesia;
d. superficial procedures, e.g. ear and tail biopsies, non-surgical subcutaneous implantation of mini-pumps and transponders;
e. application of external telemetry devices that cause only minor impairment to the animals or minor interference with normal activity and behaviour;
f. administration of substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal;
g. induction of tumours, or spontaneous tumours, that cause no detectable clinical adverse effects (e.g. small, subcutaneous, non-invasive nodules);
h. breeding of genetically altered animals, which is expected to result in a phenotype with mild effects;
i. feeding of modified diets, that do not meet all of the animals' nutritional needs and are expected to cause mild clinical abnormality within the time-scale of the study;
j. short-term (< 24h) restraint in metabolic cages;
k. studies involving short-term deprivation of social partners, short-term solitary caging of adult rats or mice of sociable strains;
l. models which expose animals to noxious stimuli which are briefly associated with mild pain, suffering or distress, and which the animals can successfully avoid;
m. a combination or accumulation of the following examples may result in classification as 'mild':
   i. assessing body composition by non-invasive measures and with minimal restraint;
   ii. monitoring ECG with non-invasive techniques with minimal or no restraint of habituated animals;
   iii. application of external telemetry devices that are expected to cause no impairment to socially adapted animals and do not interfere with normal activity and behaviour;
   iv. breeding genetically altered animals which are expected to have no clinically detectable adverse phenotype;
   v. adding inert markers in the diet to follow passage of digesta;
   vi. withdrawal of food for < 24h in adult rats;
   vii. open field testing.

The examples of procedures that fall within the 'mild' severity category (see Directive 2010/63/EU, Annex VIII – Section III above) are also helpful in gaining a better understanding of the considerations involved in assessing whether or not an activity constitutes an animal procedure.

4. Assessing discomfort: above threshold or not

As indicated in the previous chapters, factors such as the nature of the procedure, its duration, habituation and the animal species play a role in assessing the cumulative level of discomfort. The following paragraphs further elaborate on several of these aspects.

4.1 Only real and expected discomfort is taken into account

Only the realistically expected discomfort associated with the animal procedure is taken into account. Unforeseen situations that are not directly related to the experiment or the model are not included. For example, all animals have a chance of becoming ill for reasons unrelated to the experiment. The discomfort resulting from such situations is not taken into account when determining whether, on the basis of the welfare impact, an activity constitutes an animal procedure. In the event of unforeseen situations, the Animal Welfare Body (AWB) should always be consulted.

Example
A new type of feed is being tested that meets the nutritional requirements of the target animal. The experiment includes no elements that could cause above-threshold discomfort. During this experiment, a single animal dies for reasons not directly related to the feed. This incident does not in itself classify the experiment as an animal procedure or require a licence, even though the deceased animal experienced above-threshold discomfort. However, this is not the case if mortality increases over time and to a level well above expectations: substantially more animals die than can be expected in the course of the usual animal husbandry of the species in question. If the same experiment is repeated, for example to study the cause of the mortality, it can reasonably be expected that animals will die under the experimental conditions; as this is a clear case of above-threshold discomfort, it constitutes an animal procedure and requires a licence.

4.2 The relative nature of discomfort

Differences between species, differences between individuals of the same species, and the animal's environment can all influence the discomfort experienced as a result of the sum of all procedures performed on the animal. As it is not possible to standardise all aspects of the environment, relative assessments of discomfort may be used instead of absolute assessments when determining whether a procedure constitutes an animal procedure.

Once it becomes clear that procedures will be performed on an animal as part of an experiment, three aspects can contribute to the discomfort caused to the animal as a result of that experiment:

1. Discomfort resulting from the capture, handling and restraint of animals, and the subsequent stress caused by these activities.
2. Discomfort associated with carrying out the experimental procedures.
3. Discomfort resulting from the experimental procedures.

Discomfort resulting from capturing, handling and restraining an animal is not considered when deciding whether or not a procedure exceeds the 'discomfort threshold', as a veterinary professional would also need to capture, handle and restrain an animal in order to introduce a needle.
For this reason, discomfort resulting from capturing, handling and restraining does not form part of the frame of reference used to determine whether the discomfort threshold has been exceeded. Only the second and third aspects determine whether the discomfort threshold has been exceeded.

Example
Shaving a lock of hair from an unmanageable bull, in the context of answering a scientific question, does not constitute an animal procedure because it does not exceed the discomfort threshold. This is still the case even if the bull must first be captured and restrained, causing the animal severe stress and discomfort (for example, more than the introduction of a needle in a tame bull). Even if the responsible expert decides that sedation is the least burdensome way to capture the animal in order to shave a lock of hair, the introduction of the needle forms part of the capture of the animal, and not of the experimental procedure itself. The bull would also have had to be captured and restrained in order to introduce a needle.

It is clear that this perspective has a particularly significant impact on research involving wild animals in their biotope. Capturing and restraining these animals often constitute the aspect that causes them by far the greatest degree of discomfort. For this reason, as in the previous example, it is only the discomfort that results from the procedures performed after the animal is captured that determines whether those procedures constitute an animal procedure.

4.3 Interpretation of 'the introduction of a needle'

Section 1
For the purposes of the provisions of or pursuant to this Act, the following definitions apply:
a. *animal procedure*: any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or for educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.

In Chapter 2 (Section 2.3), we already examined the definition of an animal procedure as described in Section 1 of the Animal Experiments Act. In this definition, the level of discomfort is compared to that caused by the introduction of a needle in accordance with good veterinary practice.

Because there are various ways to introduce a needle (subcutaneously, intramuscularly, intraperitoneally, etc.), the discomfort caused may also vary. The frame of reference that should be used here is puncturing the dermis, although it is important to highlight that some procedures that do not involve puncturing the dermis can still cause equal or even greater discomfort.
It is important to note that this frame of reference – the introduction of a needle – also involves only very short-term discomfort; where discomfort lasts longer, it is likely to constitute an animal procedure. For further illustration, see the examples provided in the table in Appendix 2.

5. Assessment of discomfort in the ethical review and in reporting

There is a difference between the discomfort that determines whether or not an activity constitutes an animal procedure, the discomfort that is taken into account in the ethical review, and the discomfort that is recorded in the registration of animal procedures. As indicated earlier, the capture, handling and restraint of animals are not considered when determining whether an activity constitutes an animal procedure. However, once it has been determined that an animal procedure is involved, all activities contributing to the total discomfort experienced by the animal must be taken into account in the ethical review. The capture, handling and restraint of the animal, together with all other procedures performed on the animal, are therefore included in the ethical review.

The discomfort caused by the capture, handling and restraint of the animal must also be included in the project licence application.

In the registration of animal procedures, the actual discomfort experienced by the animal is recorded. This means that the discomfort resulting from capture, handling and restraint, as well as the subsequent discomfort caused by these procedures, must also be reflected in the severity classification.

6. No animal procedure, but a laboratory animal

Where procedures are carried out on animals that, according to this guideline, do not qualify as animal procedures within the meaning of the Act and therefore do not require a project licence, the animals may nevertheless be regarded as laboratory animals. The Act states the following:

Section 1b
1. This Act applies to animals that:
   a. are used for scientific or educational purposes; or
   b. are used or intended to be used in animal procedures, or are specially bred in order for their organs or tissues to be used for scientific purposes.

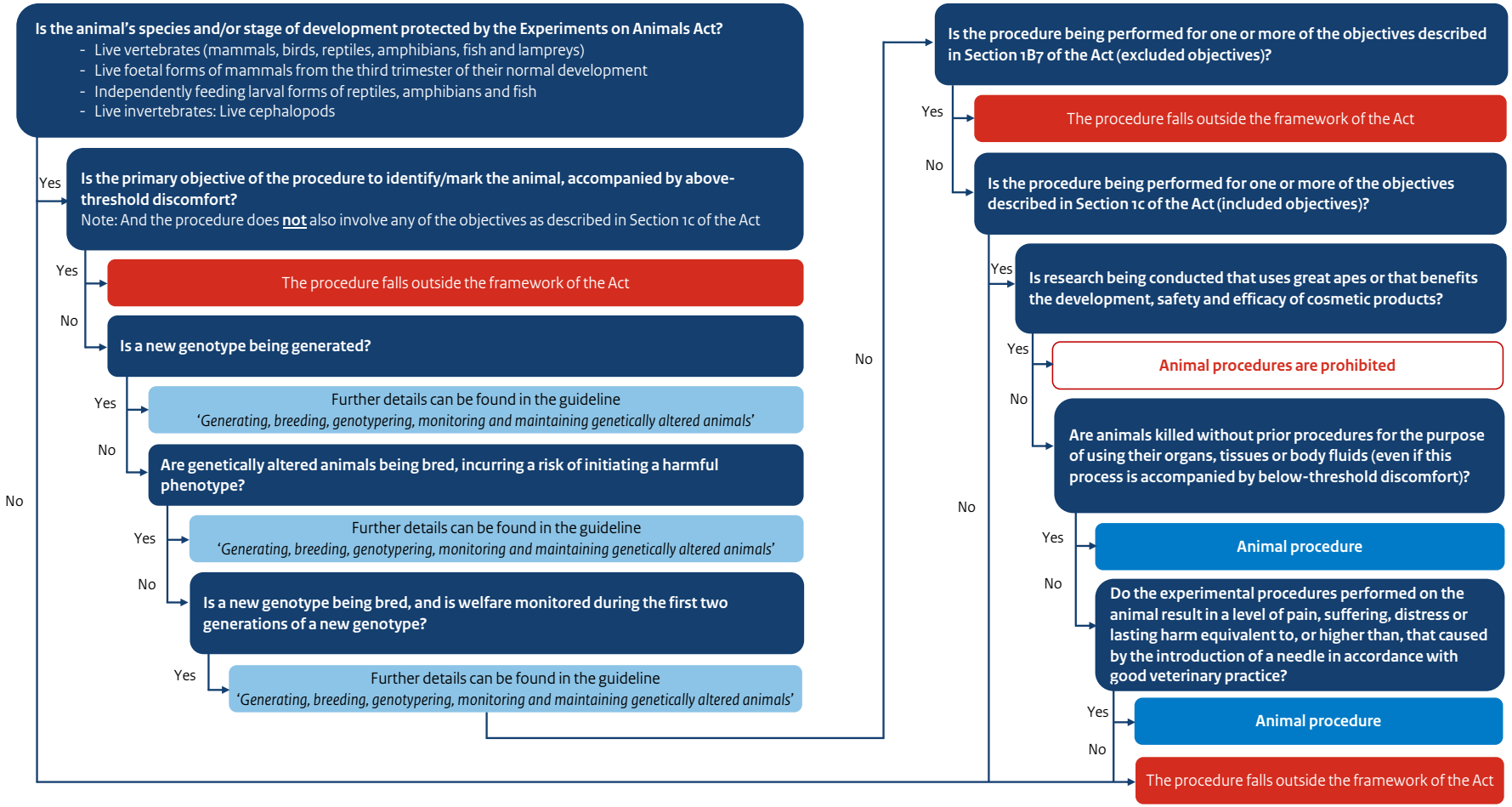
The use of the word 'intended' means that animals that are specially bred or purchased to be used for the above objectives are by definition laboratory animals. It is therefore possible to perform below-threshold procedures on laboratory animals without those procedures themselves constituting animal procedures. **An establishment licence holder is responsible for all laboratory animals**. The licence holder must ensure proper housing and supervision, in accordance with the Act, for all laboratory animals, whether or not those animals are used in animal procedures.

Even procedures that are below threshold, do not constitute animal procedures and do not involve any laboratory animals should still be subject to proper supervision. After all, the decision as to whether a procedure performed on an animal constitutes an animal procedure is a complex one, based on a correct interpretation of legislation as well as on aspects relating to laboratory animal science and welfare. **The establishment licence holder must ensure that the AWB is involved at all stages of the process determining whether a procedure performed on an animal constitutes an animal procedure**.

Appendix 1 Decision tree

The decision tree below summarises the relevant provisions of the Animal Experiments Act. The decision tree provides answers, or guidance, as to whether or not the sum of the procedures described constitutes an animal procedure under the Act.

Figure 1: Decision tree to determine whether an activity constitutes an animal procedure



Appendix 2 Table of examples

This chapter includes a table with examples grouped into the following categories:
- Procedures performed on the animal;
- Modifications to the animal's environment;
- Modifications to the animal's diet.

These examples are drawn from Directive 2010/63/EU and from the experiences of the working group members and the people they represent.

For each example, it is indicated whether or not it constitutes an animal procedure and on what grounds this conclusion has been reached.

It is challenging to properly assess discomfort in cephalopods, due to the lack of knowledge about how these animals experience discomfort. The research paper Guidelines for the Care and Welfare of Cephalopods in Research (Laboratory Animals 49(S2): 1–90, 2015) goes into more detail about the proper handling and assessment of cephalopods in experimental situations.

For other animal species as well, much remains unknown about the level of discomfort they experience and about the measures needed to reduce or mitigate this discomfort. It is therefore important that new insights in this area are taken into account when reassessing discomfort. For this reason, discomfort should always be assessed in close consultation with the AWB.

Table 1: Examples of activities (animal procedure or not)

Situation	Animal procedure?	Reasoning
Puncturing the dermis in a non-anaesthetised animal.	Yes	Discomfort equivalent to the discomfort threshold.
The insertion of a feeding tube or a gavage needle (oral gavage).	Yes	Due to impact on the animal and associated risk of above-threshold discomfort.
Harmful stimuli associated with short-term, mild pain, suffering or distress.	Yes	Discomfort at least equivalent to the discomfort threshold.
Emergence of small tumours during the course of the experiment. Not expected to cause the animal any harm.	Yes	Due to risk of above-threshold discomfort.
A procedure whose primary objective is identification (even if this is associated with above-threshold discomfort).	No	Identification as primary objective falls outside the scope of the Act. NB: This means that **none** of the objectives described in Section 1c of the Act may apply at the same time.
A procedure performed on a laboratory animal in the context of animal husbandry (resulting in above-threshold discomfort) that is standard practice in the part of the livestock farming sector or business activities relevant to this animal.	No	Procedure that is necessary to care for the animal; no extra procedures performed.
Short-term handling.	No	Procedure that forms part of regular housing and care. No above-threshold discomfort.
A non-invasive body condition score.	No	No above-threshold discomfort.
Brief restraint, within the range of normal housing and husbandry-related disturbances, taking into account the individual animal's response and the predictability and controllability of the situation for that animal.	No	Procedure that forms part of regular housing and care, taking the specific animal into consideration.
Application, **in a controlled setting**, of external telemetry devices that are expected to cause no impairment to socially adapted animals and do not interfere with normal activity and behaviour. The animal is trained with or allowed to grow habituated to the equipment using a training programme that prioritises minimising fear and stress in the specific animal.	No	No above-threshold discomfort. This applies only to kept animals. It is precisely the control over the animal that makes it possible to remove the instrument immediately if the behaviour is disturbed and above-threshold discomfort is likely to occur. NB: The case is not the same when applying external instruments to wild animals in their biotope.

Table 2: Examples of modifications to feeding regimes (animal procedure or not)

Situation	Animal procedure?	Reasoning
A diet in which, during the course of the study, the animals receive feed that is different from standard feed. This diet could negatively affect behaviour (deviation from normal behavioural patterns) or health (excess weight, nutrient deficiency).	Yes	The risk of such negative effects is viewed as above-threshold discomfort.
Adding inert markers in the diet to follow passage of digesta.	No	No above-threshold discomfort.The procedure does not add to the discomfort resulting from regular housing conditions.
A diet that fully satisfies the animal's nutritional needs. The diet is not expected to have a negative effect on behaviour (deviation from normal behavioural patterns) or health (excess weight, nutrient deficiency).	No	No above-threshold discomfort.Forms part of 'regular housing conditions'.
Food deprivation (for less than 24 hours) in adult rats. It should be noted that this applies only to healthy adult rats without other pathology. In addition, researchers and AWBs should critically assess the necessity of longer fasting periods.	No	No above-threshold discomfort.
A feeding level of at least 90% of this specific animal's expected weight with ad libitum food intake.	No	No above-threshold discomfort is involved, provided that the physiological health of the animal is safeguarded.
Housing in a metabolic cage.	Yes	Above-threshold discomfort.

Table 3: Examples of modifications to the environment (animal procedure or not)

Situation	Animal procedure?	Reasoning
Individual housing of social species for more than 24 hours, while maintaining the possibility for animals to hear, see or smell conspecifics. In this context, due consideration must be given to the animal's life stage and the natural needs of the species.	Yes	Above-threshold discomfort example given in the Directive.
A single (non-invasive) behavioural test, such as an open field test.	No	No above-threshold discomfort.
A comprehensive series of various (non-invasive) behavioural tests or one frequently recurring (non-invasive) behavioural test.	Yes	This is an example of cumulative below-threshold discomfort that results in above-threshold discomfort.
Performing one or more behavioural tests that cause the animal more stress or anxiety than an open field test.	Yes	Due to risk of above-threshold discomfort.
A non-invasive observation of the animal's normal behaviour.	No	No above-threshold discomfort.Forms part of 'regular housing conditions'.
Social enrichment / a positive experience for the animals.	No	Due to the enrichment in the animal's environment, even though the animal may initially experience the enrichment as highly stressful.

This is a publication of the Central Authority for Scientific Procedures on Animals (CCD).

The CCD is the sole competent authority in the Netherlands authorised to grant and issue project licences for conducting animal procedures.

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Publication date: april 2026