Netherlands National Committee
for the protection of animals
used for scientific purposes

Prospective Assessment of Cumulative Severity

Opinion of the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes at the request of the Central Authority for Scientific Procedures on Animals

For laboratory animals of today and innovations of tomorrow

National Committee for the protection of animals used for scientific purposes

The NCad

The Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) is an independent advisory body that protects the welfare of experimental animals. The Committee does this by publishing opinions upon request and on its own initiative, by encouraging innovation and knowledge development, and by uniting stakeholders. In doing so, the NCad achieves visible improvements that are related to the Replacement, Reduction and Refinement (3Rs) of animal procedures and animal-free innovation.

NCad members
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Cover photo
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Summary and recommendations

The Central Authority for Scientific Procedures on Animals asked the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes to develop guidance to facilitate the more accurate and more consistent prospective assessment of severity as a result of any accumulation of severity (cumulative severity).

Summary

Cumulative severity
The definition of cumulative severity can be adopted from that given by the UK Animals in Science Committee[1]:

> *Actual severity must reflect the highest severity of the procedure, including any accumulation of lesser events, and not the severity at the end of the procedure or any estimate of "average" severity.*

The assessment of severity during and after the animal procedure is therefore based on the general well-being or ill-being of the animal, not focusing solely on individual activities and interventions. This means that a good prospective assessment should also include all relevant factors that have an impact on severity. The severity is not automatically the highest severity caused by a single intervention. The assessment must include all factors that potentially contribute towards the severity (or alleviating the severity). This is the only way to carry out a realistic prospective assessment of severity. It is important to bear in mind that the severity can never be less than the highest peak in severity experienced by an animal at any time during the procedure (after refinement). Knowledge of severity in previous animal procedures is important in order to assess the level of severity involved in new projects. This knowledge must therefore be readily accessible.

Classifying an animal procedure in a severity category is part of the licensing process. In order to make a substantiated prospective assessment, it is important that the parties involved in the animal procedure provide an overview of what happens to the animal, what this means for the animal, whether there are opportunities for refinement, the appropriate monitoring frequency, any humane endpoints and what

[1] Animals in Science Committee (2017) Review of harm-benefit analysis in the use of animals in research. Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group https://www.gov.uk/government/publications/arm-benefit-analysis-animals-in-science-committee-review

should then be taken into account in the prospective severity assessment. The substantiation of the severity assessment shows the CCD whether an adequate analysis has been carried out.

Factors that contribute towards cumulative severity

In addition to the obvious effects of activities and interventions, there are also other factors that have a positive or negative impact on cumulative severity:

- All suffering that can be linked to the animal procedure and life as an experimental animal must be included in the prospective severity assessment. Only suffering resulting from incidents unrelated to the animal procedure do not need to be included in the prospective severity assessment. Examples include power cuts, disease outbreak, injury due to fighting in which the animal procedure was not a contributing factor, and animals found dead whereby the death cannot be linked to the animal procedure.
- The EU Directive sets out a number of activities that remain below the severity threshold, of which a combination or accumulation may however result in a classification as 'mild'. These activities therefore also contribute towards the cumulative severity.
- Suffering as a result of life as an experimental animal, which often remains below the severity threshold, also contributes towards cumulative severity.
- The severity can never be assessed or reported as less than the highest peak in severity experienced by an animal during the animal procedure.
- Refinement, where properly applied, can reduce severity and result in a lower assessed severity.
- Well-chosen humane endpoints in the context of the scientific endpoints combined with adequate monitoring can prevent unnecessary suffering.
- Reuse of experimental animals can result in sensitisation and habituation. In that case, it must be ascertained whether this sensitisation is a severity factor in the event of reuse.
- Repetition can result in more suffering, but also habituation, depending on the situation. Conversely, training and reward can result in less suffering.
- Both positive and negative experiences at times outside of the animal procedure contribute to the general welfare of the experimental animal and to how an animal will respond to activities during the animal procedure (for example higher or lower stress levels). Whether this potentially results in a different severity assessment will need to be examined on a case-by-case basis.
- Two questions play a particularly important role in determining the contribution of lifetime experience to severity during the animal procedure:
  - Does an animal experience suffering or even increasing suffering throughout its whole life, for example due to a genetic alteration?
  - Does the animal experience additional restrictions on its natural behaviour throughout its whole life?
- Animals that are not suited to life in captivity will experience additional suffering due to capture, handling, relocation, housing and so on.

In short, the cumulative severity is primarily determined by activities and circumstances directly related to the experimental animal in question. In the case of reuse, this involves the previous procedure as well. Other previous interventions, related or unrelated to the animal procedure, can also contribute towards cumulative severity. However, this does not apply to activities and circumstances that are part of the standard housing and care of experimental animals, such as keeping experimental animals in cages and areas that meet the legal requirements and handling in the context of regular inspections and cage cleaning.

Resources

- Together with the European Commission (EC), the EU National Contact Points Working Group has developed a severity assessment model to facilitate the prospective assessment of severity and in the identification of refinement

opportunities and humane endpoints (figure 1)[2]. This model includes all events that take place during an animal procedure, but not events that occurred prior to the study (e.g. previous use of the animal).

Figure 1 *Prospective assessment and consideration of specific refinements and humane end-points*

Name of the model: *Prospective assessment and consideration of specific refinements and humane end-points*			
What does this study involve doing to the animals	What will the animals experience? How much suffering might it cause? What might make it worse?	How will suffering be reduced to a minimum?	
	Adverse effects	Methodology and interventions to minimize severity	Humane end-points

- Keeping a list of scores that is tailored to the animal procedure helps to monitor the development in severity during the animal procedure so that it is possible to intervene at the right time.
- It is important to recognise that animals do not always clearly show their suffering to humans. Training in recognising species-specific expressions of suffering and sufficient observation time are essential and the scoring system must be fairly sophisticated and continuously developed.

[2] National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012) Working document on a severity assessment framework. https://op.europa.eu/en/publication-detail/-/publication/fe448d22-282f-11e9-8d04-01aa75ed71a1/language-en/format-PDF/source-282223752

Recommendations

1. In the prospective assessment of cumulative severity, the severity of the various individual activities and how they interact (reinforcing or ameliorating) are important. The focus is not on the activities but on what the animal experiences.
2. Cumulative severity can never be lower than the highest peak in severity experienced by an animal during the animal procedure. All events that contribute towards this severity are taken into account.
3. Licence applicants must therefore provide information on all factors that can contribute towards the prospective assessment of cumulative severity.
4. If there is insufficient knowledge of how different events contribute towards the animals' suffering, it would be preferable (on the basis of the precautionary principle) to assess the severity at the upper end of the scale and to monitor the animal frequently.
5. Incidents that cannot be predicted are not taken into account when carrying out a prospective assessment of severity, however all other aspects (both reinforcing and ameliorating) that may influence the assessment are included.
6. Harmonisation of the prospective severity assessment is important. Education, further training and refresher training of all parties involved in project applications (researchers, biotechnicians, animal keepers, vets and members and supporters of the Food and Consumer Product Safety Authority [NVWA], Animal Experiments Committee [AEC], Animal Welfare Body [AWB] and Central Authority for Scientific Procedures on Animals [CCD]) must pay sufficient attention to this subject.
7. An exchange of views on similarities and differences between the severity score lists used by different animal welfare bodies and on the other factors that need to be taken into consideration alongside these lists when assessing cumulative severity also benefits harmonisation.
8. In addition to the severity assessment framework and the 2018 FELASA/ECLAM/ESLAV Working Group report, important sources of information for the prospective assessment of severity include final reports from completed projects (particularly if the prospective assessment does not correspond to the actual suffering experienced) and NVWA findings. This information must be made available and must be readily accessible.

9. The prospective severity assessment model provided in the EU severity assessment framework is a useful tool. In addition to this model, it is important to also include relevant information from the animal's previous history in the prospective severity assessment if this could influence the assessment, particularly in the case of reuse.
10. Model-specific score forms and the consistent use of score sheets should be used as much as possible in the performance of animal procedures. These tools help to limit suffering as much as possible.

Inhoud

Introduction

This opinion develops guidance for the assessment of cumulative severity in advance of an animal procedure.

Overview

In both the prospective severity assessment and the retrospective severity report, the level of severity must be classified in one of the severity categories stipulated by law. This suggests that the boundaries of these severity categories are clearly defined. In reality these boundaries are not so clear, rather there is a gradual transition. As a result, the applicable category is not always evident. Moreover, where moderate (or mild) severity was anticipated during the animal procedure, it may turn out that the threshold for severe (or moderate) severity is exceeded. It is not always easy to accurately assess severity in advance. After all, an animal procedure often consists of multiple activities that can each cause suffering. In order to assess severity as accurately as possible, the parties involved and ultimately also the licence holders need to gain insight into the combined effect of these activities. To do this, all aspects that contribute towards severity must be identified so that they can be taken into account in the final 'cumulative' severity. Classifying this severity in a category is a means of gaining further insight into the welfare of animals involved in animal procedures and communicating on this subject with those involved in the animal procedure. It also plays an important role in the harm-benefit analysis carried out by the licensing authority.

The following documents are useful for assessing severity prior to the granting of a licence and for establishing actual severity during and after an animal procedure:

- *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* (abbreviated to: the EU Directive) and specifically Annex VIII of this Directive[3];
- National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012) *Working document on*

[3] 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. Off J Union 2010; L276: 33-79.

a severity assessment framework. (Abbreviated to the severity assessment framework)[4];
- The report of a FELASA/ECLAM/ESLAV working group with the title *The reporting of clinical signs in experimental animals* (abbreviated to 2014 FELASA report)[5];
- The report of a FELASA/ECVAM/ESLAV working group with the title *Classification and reporting of severity experienced by animals used in scientific procedures* (abbreviated to 2018 FELASA report)[6].

These are valuable documents for assessing severity. The transparent and consistent way in which the case studies are handled in the latter three documents is helpful for learning how to assess severity and for harmonising this prospective severity assessment.

Request for advice

In 2019, the Central Authority for Scientific Procedures on Animals (CDD) asked the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) to develop guidance to facilitate the more accurate and more consistent assessment of any accumulation of severity (cumulative severity) in advance of animal procedures. An accurate prospective assessment of cumulative severity is important because this assessment is an essential part of the harm-benefit analysis that precedes the decision on the granting of a project licence.

Background to the request

A brief problem analysis revealed that not only the CCD, but also researchers, AECs and AWBs need more detailed guidance on how to assess cumulative severity in advance of an animal procedure. Cumulative severity could occur as a result of repeated or combined interventions, or interventions and/or events that take place more or less simultaneously and that each cause suffering. The previously mentioned documents give some indication of when cumulative severity may apply, however this indication does not provide sufficient guidance. In the United Kingdom, a number of committees and groups of researchers have explicitly addressed this issue. Support can be found in their reports. The workshops developed by the FELASA working group, which are based on case studies and have now been held on two occasions in the Netherlands (in 2018 and in 2019 during the AWB days), are a useful resource. The case studies used in these workshops can be supplemented with new case studies. This could include case studies on cumulative severity to support this opinion. These case studies will be presented to the FELASA working group, which provides a framework for severity assessment within the EU. These case studies will be added later.

Structure of this opinion

First, a definition is given of cumulative severity. The report then goes on to look at how severity can be assessed. A next chapter examines the aspects that play a role in severity and therefore also the prospective severity assessment. Finally, the report discusses tools that can be used when assessing severity prior to, and monitoring severity during, an animal procedure.

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[4] National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012) Working document on a severity assessment framework. https://op.europa.eu/en/publication-detail/-/publication/fe448d22-282f-11e9-8d04-01aa75ed71a1/language-en/format-PDF/source-282223752.

[5] Fentener van Vlissingen JM, Borrens M, Girod A et al. (2014) The reporting of clinical signs in laboratory animals: FELASA Working Group Report, Laboratory Animals 2015; 49(4) 267–283

[6] Smith D, Anderson D, DeGryse AD et al. (2018) Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group, *Laboratory Animals 2018, 52(1S) 5–57*

1. Cumulative severity

In the Netherlands, the definition of animal welfare given by Ohl and Hellebrekers (2009) is generally used: *"An individual is in a state of welfare if it is able to actively adapt to its living conditions and as a result reaches a state that the individual experiences as positive"*[7].

Severity relates to any impairment of welfare, such as pain, suffering, distress or short-term or lasting harm, experienced by an individual animal during the animal procedure. The degree of severity therefore reflects the degree of impairment of the experimental animal's welfare.

According to the applicable law, an animal procedure is when the impairment of welfare is equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. There are three different severity categories: mild, moderate, and severe. There is also a fourth category in which the animal is anaesthetised prior to the procedure and does not regain consciousness. This is referred to as non-recovery.

It is not easy to assess suffering in advance, particularly for new types of experiments: it is more difficult still to understand the accumulation of severity of repeated or multiple activities on the animal .

[7] Ohl F en Staay FJ van der (2012) Animal welfare: at the interface between science and society. In: The Veterinary Journal, 192(1) 13-19.

Proefdieren: zorg, kwaliteit en biotechniek [Experimental animals: care, quality, and animal techniques] (expected in 2022)[8]: *There are no lists or strict rules for classifying experiments on animals, however there are several examples (Annex VIII, EU Directive 2010/63/EU). The examples in each category (mild, moderate, and severe) by no means rep resent all types of activities and types of suffering in animals, but they serve as a guide. You therefore need to consider which category applies to each experiment. To do this, you must identify all elements of suffering. The compulsory categorisation thus forces you to always think about the welfare of the animals. The discussion takes place at three points in time: at the time of designing or applying for an experiment, during the experiment and after the experiment.*

Annex VIII of the EU Directiveg offers little guidance for the prospective assessment of cumulative severity. The examples given relate to the impairment of welfare in individual activities. There is one exception: several activities are listed as 'mild' severity, but when combined or repeated may result in classification as 'moderate'. The annex also refers to cumulative suffering and the lifetime experience of an animal, however neither term is specified or elaborated in examples. This suggests that it is recognised that the classification of severity not only pertains to individual activities, but that the effects of activities can accumulate and/or can reinforce each other to reach a cumulative severity. However, no further elaboration is provided on this in the EU Directive.

The severity assessment framework10 provides some guidance by demonstrating how to carry out a prospective severity assessment based on several case studies. The previously mentioned report by the FELASA working group builds on the framework and adds case studies. These case studies are used in workshops led by FELASA trainers and thus help to harmonise the assessment of severity.

Memorandum of the Central Authority for Scientific Procedures on Animals (CCD): the revised Experiments on Animals Act *(Wet op de dierproeven*): how are 'experiments on animals' defined in the Act? (2016)[11]: *As previously stated, factors such as capture, or handling do not play a role in identifying experiments on animals. However, where an experiment on animals has been identified, all factors that contribute towards the total suffering experienced by an animal, including capture, restraint and so on, must be considered in the ethical assessment and also included as such in a project licence application. The actual suffering experienced by the animal is also recorded in the animal procedures register. This means, in this case, that suffering caused by capture, handling and restraint and the resulting continued suffering must be included in the severity classification.* (p. 13).

The 'Advisory notes on actual severity'[12], the UK Government's clarification on transposition of the EU Directive into the Animal Scientific Procedures Act 1986, gives the following description of actual severity: *'Actual severity must reflect the highest severity of the procedure, including any accumulation of lesser events, and not the severity at the end of the procedure or any estimate of "average" severity.'* (p.2)

A great deal has been written about cumulative severity, particularly in the United Kingdom. In the glossary to the 'Review of harm-benefit analysis in the use of

[8] Oosten A van et al. (red.) (in preparation; expected 2022) Proefdieren: zorg, kwaliteit en biotechniek. Stichting Proefdierkundige Informatie (uitgever).

[9] 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. Off J Union 2010; L276: 33-79

[10] National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012) Working document on a severity assessment framework. https://op.europa.eu/en/publication-detail/-/publication/fe448d22-282f-11e9-8d04-01aa75ed71a1/language-en/format-PDF/source-282223752.

[11] Centrale Commissie Dierproeven (2016) Herziene Wet op de dierproeven: Wanneer is er sprake van een dierproef in de zin van de Wet? https://www.centralecommissiedierproeven.nl/onderwerpen/handreiking-wat-is-een-dierproef.

[12] Home Office (2014) Advisory notes on actual severity reporting (publishing.service.gov.uk).

animals in research' the UK Animals in Science Committee13 defines cumulative severity as follows: *'The assignment of a severity category in legislation (...) needs to take into account the potential for the intensity, duration, frequency and multiplicity of techniques to negatively affect the welfare of an animal over its lifetime i.e., to contribute to cumulative severity. Both the Home Office Inspectorate and the EU Directive 2010/63 consider cumulative suffering within a procedure as a key issue in assigning severity categories.'* (p.6).

[13] Animals in Science Committee (2017) Review of harm-benefit analysis in the use of animals in research. Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group https://www.gov.uk/government/publications/arm-benefit-analysis-animals-in-science-committee-review.

2. Assessment of severity

The Experiments on Animals Act *(Wet op de dierproeven, Wod)*[14] distinguishes between actual severity experienced during the animal procedure and assessed severity prior to the animal procedure. This assessed severity must be stated in the licence application and is an essential component of the harm-benefit analysis by the licensing authority, the Central Authority for Scientific Procedures on Animals (CCD). If the prospective assessment of severity is 'severe', the applicant will need to carry out a retrospective assessment (RA). If severity is preliminarily assessed as 'moderate' but proves to be 'severe' during the animal procedure and the model in question is to be used again, the institution's AWB will submit an amendment to the CCD. The application will be re-assessed and an RA can be added to the decision if necessary (personal communication).

The wellbeing of the animals is logged and documented during the animal procedure to establish actual severity. External observations and physiological parameters are used to record how the animal is faring and whether intervention is necessary in the form of refinement or whether a humane endpoint has been reached and the animal should be withdrawn from the study. The degree of severity because of all circumstances and interventions can also be established based on the data recorded. It should be noted here that humans will sometimes see little or no discernible signs of suffering in animals.

As the established severity, in other words the suffering the animals have experienced, is based on all circumstances and interventions, this concept comes closest to the cumulative suffering during a single animal procedure: the cumulative severity. This is because the assessment looks at the animal as a whole and not at the individual activities carried out on the animal. Past effects that are still ongoing are automatically included. Refinements can reduce the suffering caused by activities on the animal and are therefore automatically included in the welfare monitor and thus the established severity. In a trial design where activities are repeated on an ongoing

[14] Wet op de dierproeven (1977, 2014)
wetten.nl - Regeling - Wet op de dierproeven - BWBR0003081 (overheid.nl).

basis or where a series of different activities are carried out, the animal will or will not start to react differently if it knows what is going to happen. Physical, physiological, and pathophysiological causes can also result in changes in severity when an activity is repeated. The effect can be more intense (sensitisation) or less intense (habituation) and occasionally the prospect of a reward may cancel out the effect. This will all be reflected in the effects identified in the records kept from welfare observations.

However, the question posed by the CCD did not relate to establishing severity at the end of the animal procedure, but rather to assess severity prior to the animal procedure. The statements made in Annex VIII, Section II of the EU Directive15 on assessing severity prior to the animal procedure show that cumulative suffering is a factor that needs to be considered when assessing severity. The question is then how this can be assessed in advance.

The EU Directive draws a line between suffering that can and suffering that cannot be classified as being of 'mild' severity. Activities that fall below this threshold do not meet the definition of an animal procedure. Annex VIII of the EU Directive cites examples of activities of which a combination or accumulation may result in a classification as 'mild', whereas in isolation these activities are considered 'below the severity threshold' (below threshold). Examples of below-threshold suffering include:

- Assessing body composition by non-invasive activities and with minimal restraint.
- Monitoring ECG with non-invasive techniques with minimal or no restraint of habituated animals.
- 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.
- Breeding genetically altered animals that are expected to have non clinically detectable adverse phenotype.
- Adding inert markers in the diet to follow passage of digesta.
- Withdrawal of food for < 24h in adult rats.
- Open field testing.

According to the EU Directive, the combined or repeated (cumulative) effect of two or more such activities on the welfare of the animal can result in mild severity, which means that the activity is considered an animal procedure. Whether this applies will need to be examined on a case-by-case basis. The above-mentioned annex gives no specific examples of combined activities that can together cause mild severity to become moderate or moderate severity to become severe.

In summary, it can be concluded that the prospective assessment is an assessment to the best of knowledge of the maximum severity that an individual animal could experience during the animal procedure because of all circumstances and activities to which the animal is exposed, based on what is known in advance. This assessment is made based on knowledge obtained of the actual severity experienced by other animals in previous (similar) animal procedures. When assessing severity, it is therefore essential to look not just at each individual intervention during the animal procedure, but to give an assessment of what the most severe combined (cumulative) effect of the animal procedure could be on an animal. All factors that can have an impact play a role here, both positive and negative. It should be noted that, according to the EU Directive, in both the determination of the actual severity experienced and the prospective assessment of severity, the total or cumulative severity can never be lower than the highest category of severity resulting from any individual element of the animal procedure or at any time during the animal procedure. Also, that in the event of reuse, the cumulative severity can never be lower than the severity in the previous animal procedure.

[15] 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. Off J Union 2010; L276: 33-79.

The assessed severity, but also the actual severity, is not always easy to determine. That is why coordination with the parties involved in the animal procedure (researchers, biotechnicians, AWB members, veterinarians involved) is vital. This coordination on assessed severity begins with the following basic questions:

1. Does any suffering occur before the trial starts, for instance due to weaning earlier than usual?
2. Are the animals reused?
3. Do the circumstances or environment impose any additional restrictions on natural behaviour?
4. What activities are carried out and what are their consequences?
5. What is the intensity, duration and frequency of the circumstances and activity/activities?
6. What measures (including humane endpoints) have been or will be taken to reduce the severity? Does this ultimately lead to a lower assessment?
7. Are there any other influencing factors, that can for example have a positive or negative effect on the severity?

Only when these questions have been answered and it is clear what will happen to the animal and what that means for the animal, can the severity be prospectively assessed. Only when it is clear what will happen, is it possible to know what monitoring or additional monitoring needs to be carried out, what refinements can be made and when a humane endpoint will be reached. These questions already show that the severity is not determined by the activities during the animal procedure alone. There are multiple aspects that can play a role and increase or reduce the severity.

3.
Relevant aspects when prospectively assessing severity

Annex VIII of the EU Directive[16] states that several aspects must be taken into account when categorising an animal procedure according to severity:

- *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*[17].

Annex VIII also lists several *additional factors* that need to be taken into account when assessing severity, namely: type of species and genotype; maturity, age and gender; training experience; housing and care conditions; if the animal is to be reused, the actual severity of the previous procedures; refinements; and humane endpoints. According to the EU Directive, this list of additional factors should not be viewed as exhaustive.

The remainder of this opinion examines several issues that can have a positive or negative effect on cumulative severity. To this end, it is necessary to determine what is and what is not relevant to the prospective assessment of cumulative severity, however also what does not strictly affect the prospective severity assessment but does have an impact on the animal's general welfare during the course of its life. The following topics are discussed in this order:

- Legally related to the animal procedure.
- Refinement and humane endpoints.
- Reuse.
- Training and reward.
- Positive experiences.
- Lifetime experience and life as an experimental animal.

[16] 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. Off J Union 2010; L276: 33-79.

[17] Common housing and care that meet European standards will restrict natural behavior and may cause discomfort. Here is meant extra restriction such as housing without bedding or solitary housing.

Legally related to the animal procedure

It can be inferred from the EU Directive (Annex VIII, Section II) that when assessing severity in advance, suffering caused by chance events that are not related to the animal procedure should not be considered in the assessment. After all, incidents cannot be predicted. If all potential incidents were considered in advance, all animal procedures would be given the prospective classification 'severe'. At the same time, such incidents have an impact on the animals' welfare, and that the suffering caused by this type of incident can be severe and must be avoided as much as possible by means of appropriate welfare monitoring. The UK Government[18] has clearly defined the distinction between incidents that are and incidents that are not related to the procedure, citing specific examples (see Appendix 2 to this opinion).

Refinement and humane endpoints

Refinement can ameliorate suffering and can even result in classification in a lower severity category. It is therefore important to identify opportunities for refinement. This can be pain management, but also adapted care or a pleasant environment for a sick animal (examples include non-medicinal measures such as extra heat or soft bedding or social contact with animals of the same species, in other words everything that contributes to the improvement of welfare) (National Committee for the Protection of Animals Used for Scientific Purposes, 2016)[19]. Ensuring the optimal implementation of experimental activities and training animals where possible (see the section on 'Training and reward') also contribute towards refinement and can therefore reduce suffering.

Refinements are implemented where possible. The type of the animal procedure in which refinement takes place is relevant to the severity assessment. When assessing severity, cumulative severity can never be assessed as lower than the most severe effects experienced by an animal at any time during the animal procedure. Refinement in this area of the animal procedure could potentially result in a lower severity assessment. However, if the different aspects of the animal procedure combined result in a higher assessment than the most severe suffering, refinement in the different areas can also result in a lower severity assessment.

Naturally, where possible refinement is also desirable throughout the life of the animal, in other words from breeding, to husbandry, to death, and during all steps of the animal procedure. That is part of a good culture of care. It may be assumed that many of these aspects have an impact on the general welfare of the animal, and therefore also affect how animals respond during an animal procedure, which will influence welfare scores. Where this is suspected to be the case, these aspects and their refinements must also be considered in the prospective assessment of cumulative severity.

Well-chosen humane endpoints (HEP) can sometimes avoid assignment to a higher category of severity. In that case, appropriate welfare monitoring is essential. In addition, if the animal procedure does not allow a humane endpoint to be applied before a higher level of severity is reached, the suffering can in any event be shortened by killing the animal or otherwise withdrawing it from the study as soon as the animal procedure allows. This ensures that the higher severity is as brief as possible.

[18] Home Office (2014) Advisory notes on actual severity reporting (publishing.service.gov.uk).

[19] National Committee for protection of animals used for scientific procedures (2016) Prevention, recognition and management of pain in laboratory animals. NCad opinion Preventing, recognising and combating pain in laboratory animals | Publication | Netherlands National Committee for the protection of animals used for scientific purposes (ncadierproevenbeleid.nl).

Reuse

The annex to the EU Directive[20] and the severity assessment framework21 state that an animal may in principle only be reused if the actual severity of the previous procedures was 'mild' or 'moderate', the animal's general state of health and well-being has been fully restored, the further procedure is classified as 'mild', 'moderate' or 'non-recovery', and it is in accordance with veterinary advice, taking into account the lifetime experience of the animal. There is one exception to the first restriction.

D. Smith et al. of the FELASA working group22 state the following on this matter:
"Assessing the severity that an individual scientific procedure will cause to an animal can be difficult when animals undergo several multi-step procedures over prolonged periods, especially when the nature of the procedures means that the animals may also be subjected to alterations in normal housing and care practices (e.g., periods of single housing).
However, such an assessment is necessary to allow re-use, and this needs to take account of the animal's lifetime experience. This introduces a further area for consideration as now not only does the direct pain, suffering or distress caused by the various steps in the procedure need to be taken into account, but also some consideration is needed of any contingent suffering due to the animal's husbandry and care environment throughout its lifetime.
Lifetime or cumulative suffering can be considered as the combination of direct suffering (the application of scientific procedures), any clinical conditions from which the animal has suffered (which may or may not be due to the procedure being carried out, e.g. intercurrent disease or surgical wound) and contingent suffering (housing, husbandry, transport etc.); the duration of these events must be taken into account". (p. 15)

[20] 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. Off J Union 2010; L276: 33-79.

[21] National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012) Working document on a severity assessment framework. https://op.europa.eu/en/publication-detail/-/publication/fe448d22-282f-11e9-8d04-01aa75ed71a1/language-en/format-PDF/source-282223752.

[22] Smith D, Anderson D, DeGryse AD et al. (2018) Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group, Laboratory Animals 2018, 52(1S) 5–57.

It is difficult to assess whether and when an animal is fully restored to health. Depending on the species and nature of the activity, once an animal is fully restored to health it will experience the new animal procedure as an entirely new event, or recognise what is going to happen. This is comparable to the fear of 'white coats' that can develop in children, or a dog that behaves in a fearful manner at a veterinary practice. To know if this type of response is possible, it is essential to know how a species or even a breeding line responds in similar situations. It is difficult to assess in advance, as it can also depend on the animal's previous individual experiences.
In addition, there can also be physical, physiological, or pathophysiological causes that result in an increase or decrease in severity when an activity is repeated.

According to the UK Animals in Science Committee (ASC)23, it is therefore relevant to verify in the case of both complex animal procedures and reuse whether the animal is becoming habituated or sensitised. Both should be established empirically and objectively if possible. If the animal is becoming sensitised, it must then be determined whether the severity should be classed in a higher category. However, the ASC does not yet have any accurate indicators to establish this cumulative severity at an early stage.

For reuse, the precautionary principle could therefore apply as a rule: if it is not known whether the animal will experience an animal procedure as a completely new event, it is wise to assume that the suffering the animal experiences as a result of the two studies cannot be considered in isolation. In that case, there could be a strong reluctance to reuse animals. The severity of the previous animal procedure must in any event be taken into consideration in the new animal procedure, as the lifetime experience must be taken into account.

[23] Animals in Science Committee (2017) Review of harm-benefit analysis in the use of animals in research. Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group https://www.gov.uk/government/publications/harm-benefit-analysis-animals-in-science-committee-review.

Repetition, training and reward

Repetition can lead to stress, fear and aversion, particularly if an animal procedure is accompanied by a repeated series of more or less stressful events, such as handling, restraint, and subsequent pain. The animals then recognise what is going to happen and the repetition results in an accumulation of severity. If the animal procedure permits additional recovery time, a situation in which an intervention becomes increasingly stressful can sometimes be avoided, however this is not always the case. Such additional time is also not always possible based on the trial design. How the animal responds depends on factors including the species, the nature of the activity, the genotype and the learning capacity of the animal.

In the event of reuse, cumulative severity can also be lower in the subsequent study due to factors such as a training effect. Training can make an intervention less stressful. Some species can be trained to cooperate with the activity or intervention, making it less burdensome or stressful for them. In that case, training and habituation reduces the severity for the animal. Habituation through training makes the most sense for species that have a reasonable learning capacity and whereby the investment in training is proportionate to the intervention or interventions in the study. If an animal regularly undergoes a minor intervention, a reward can also contribute towards refinement (for example stroking a dog after administering an injection). The severity is then less than if the animals do not receive a reward.

Positive experiences

Positive incentives that are *directly* aimed at mitigating the effects of an intervention are generally referred to as 'refinement'. This is therefore not necessarily just pain management. For instance, social animals that live socially in the post-operative period experience a faster recovery than animals kept in solitary housing. And this therefore also means that solitary housing during recovery must be viewed as an additional burden for these animals.
The situation is different if positive incentives are provided at another point in time and are designed to compensate for the suffering the animals experience during the animal procedure. For the animals, it will make a difference whether or not they still establish a link to the animal procedure. If so, positive incentives can be classed as reducing the burden. However, if the compensation takes place at a completely different time in the animal's life it does of course contribute to the animal's general welfare but does not, in principle, alleviate the suffering during the animal procedure. Although, in view of Ohl and Hellebreker's definition of welfare[24], positive experiences at a different time in an animal's life can contribute to an animal's ability to better cope with suffering. After all, a lifetime of miserable experiences has a different impact on an animal's mental state to a good life. This argues in favour of ensuring that an animal's general welfare is as good as possible, however it is difficult to say to what extent this can be taken into account in the severity assessment. We have previously seen that the EU Directive argues that the final severity classification cannot be lower than the most severe effects experienced by an animal.

Lifetime experience and life as an experimental animal

In line with recital 31 of the EU Directive[25] efforts must be made to offer the animals the best possible lifetime experience, in other words not only when animals are reused (recital 25). The severity assessment framework[26] also states (p. 9) that each animal's whole-life experience must be taken into account within a procedure that involves a number of interventions. The severity assessment framework points, for example, to restrictions on the ability to refine housing and the need for frequent capture, handling, and restraint. The severity assessment must therefore take this lifetime experience into account. How this should be done is not straightforward.
In addition to the question of whether animals are to be reused, there are two questions that must always be asked when determining severity in relation to

[24] Ohl F en Staay FJ van der (2012) Animal welfare: at the interface between science and society. In: *The Veterinary Journal*, 192(1) 13-19.

[25] 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. Off J Union 2010; L276: 33-79.

[26] National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012) Working document on a severity assessment framework. https://op.europa.eu/en/publication-detail/-/publication/fe448d22-282f-11e9-8d04-01aa75ed71a1/language-en/format-PDF/source-282223752.

lifetime experience:

- Are the effects intrinsic, for example do the effects continue throughout the animal's life or increase over its lifetime? Consider, for instance, a disease model.
- Do the circumstances and environment impose any additional restrictions on natural behaviour that do not occur under normal circumstances? Examples include solitary housing or housing without bedding.

Lifetime experience is therefore relevant, but it is not always clear how events in the lifetime experience affect the assessment of severity during the animal procedure. The assessment of lifetime experience is based on the kind of life an animal has or has had on the whole. Is an animal only living through common lab experiences and will it then undergo an animal procedure that involves suffering, or does it also go through periods of positive life experiences? This may play a minor role in the prospective or retrospective severity assessment for an animal procedure but could certainly be taken into account when assessing whether an animal can be reused. In this context, it is also appropriate that the EU Directive stipulates that lifetime experience must also be taken into account in considering the reuse of animals.

Wild animals

The above factors must be explicitly taken into account when using animals in or from the wild. The Dutch Central Authority for Scientific Procedures on Animals has published a guide to Experiments on wild animals in their habitat in relation to animals caught in the wild[27]. This guide states: *When determining whether effects exceed the threshold of severity (in other words whether an activity is considered a procedure), only the effects caused by activities after capture and any continued suffering resulting from these activities should be taken into account. (...) The law presumes that a pre-anaesthetised state (for example due to capture) is not included when determining whether subsequent activities result in effects that exceed the threshold of severity.* (p. 5).

(...)
If an activity is identified as a procedure requiring a licence, all factors contributing to the suffering experienced by an animal in the context of the experiment must then be listed in the project application. This includes suffering as a result of capture, restraint and so on. Capture procedures must be assessed by the AWB in the context of the 3Rs and the impairment of welfare resulting from capture/restraint is part of the assessments carried out by the AEC and CCD. (p. 6).

For the purpose of the prospective severity assessment, it is then relevant that animals that are not suited to life in captivity (animals captured from the wild, but also some species of captive animals) will experience additional suffering as a result of capture, restraint, handling and any relocation and temporary or permanent housing. In the case of these animals, these additional aspects must be given greater weight in the assessment of severity.

[27] Centrale Commissie Dierproeven (2018) Dierproeven met wilde dieren in hun biotoop https://www.centralecommissiedierproeven.nl/documenten/formulieren/19/1/9/handreiking-dierproeven-met-wilde-dieren-in-hun-biotoop-december-2018.

4. Tools for assessing and monitoring severity

To make an accurate prospective severity assessment, knowledge of the severity (and of refinement, replacement, and reduction) acquired in previous similar animal procedures is important for everyone involved in a licence application: researchers, AECs, the CCD and the AWBs. The competent authority (the Central Authority for Scientific Procedures on Animals, CCD) uses this knowledge in the project evaluation, which assesses several factors including whether all relevant aspects have been taken into account in the prospective severity assessment. It is therefore important that information on severity in previously conducted research is easily accessible and is part of the education and further training of those directly involved in the performance and assessment of experiments on animals. In addition to the severity assessment framework28 and the FELASA/ECLAM/ESLAV Working Group report29, potential sources of this information include final reports from completed projects (particularly if the prospective assessment does not correspond to the actual suffering experienced) and NVWA findings. This information must therefore be made broadly available.

Several AWBs have drawn up a list of specific activities and interventions with the associated severity. The advantage of this type of list is that it gives an indication of severity for each activity or intervention, helping to ensure that the prospective severity assessment model (see below) is completed accurately. In addition to this type of list, it is also essential to look at the combined effect of the various activities and also at the other aspects that, as we saw previously, affect the cumulative suffering experienced by the animal. It is important that the assessment is not based on the activities carried out on the animal, but on the animal itself, in other words what happens to the animal, how this affects the animal, whether there are

[28] National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012) Working document on a severity assessment framework. https://op.europa.eu/en/publication-detail/-/publication/fe448d22-282f-11e9-8d04-01aa75ed71a1/language-en/format-PDF/source-282223752.

[29] Smith D, Anderson D, DeGryse AD et al. (2018) Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group, Laboratory Animals 2018, 52(1S) 5–57.

opportunities for refinement and so on. It may be useful to compare these lists (anonymously) to see whether there are any differences and if so, to discuss these differences with each another. This could be done in the interest of lifelong learning and harmonisation of prospective severity assessment.

The book Proefdieren: *zorg, kwaliteit en biotechniek* [Experimental animals: care quality and animal techniques] (in preparation)[30] states the following in this regard:

> *The fact that we are required to classify a study in categories of cumulative severity forces us to talk to the team about suffering. This team can consist of animal keepers, biotechnicians, analysts and researchers. When writing the project application, you as a researcher determine whether you feel that the suffering outweighs the purpose of your research. Your project application is extensively discussed and agreed with the AWB. The AEC judges whether your assessment of cumulative severity is realistic. Information on severity is therefore part of the project application, providing the AEC with insight into what the animals are required to undergo during the experiments. This information allows the AEC to balance the cumulative severity against the purpose of the project application. The AEC then sets out its findings in a recommendation to the CCD. The CCD is the authority that ultimately grants or rejects the application.*
> *(...)*
> *Talking about severity and potential refinements challenges you to take an in-depth look at the animals' overall experience and the extent to which this affects their welfare. This means that you can look for all sorts of refinements. For each animal procedure the AWB will determine, if necessary, with advice from the appointed veterinarian, whether the activities fit within the framework of the licence and whether all conceivable refinements have been implemented.*

[30] Oosten A van et al. (red.) (in preparation; expected 2022) Proefdieren: zorg, kwaliteit en biotechniek. Publisher: Stichting Proefdierkundige Informatie.

During each animal procedure, a monitoring list tailored to the specific study must be kept gaining insight into the actual suffering experienced by the animals and to ensure that action can be taken if the animal's welfare is compromised to an unacceptable extent. The list looks at how the animal is faring and documents observations about the animal. It is therefore not about the effects of the individual activities on the animal, but about the effect of all activities and interventions involving the animal.

The severity assessment framework[31], and later also the FELASA/ECLAM/ESLAV Working Group[32], set out a working method for describing the effects, possible refinements, and humane endpoints for all events the animal experiences during the animal procedure (Figure 2).

[31] National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes (2012) Working document on a severity assessment framework. https://op.europa.eu/en/publication-detail/-/publication/fe448d22-282f-11e9-8d04-01aa75ed71a1/language-en/format-PDF/source-282223752.

[32] Smith D, Anderson D, DeGryse AD et al. (2018) Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group, *Laboratory Animals 2018, 52(1S) 5–57.*

Figure 2 *Prospective assessment and consideration of specific refinements and humane endpoints*

v			
What does this study involve doing to the animals	**What will the animals experience? How much suffering might it cause? What might make it worse?**	**How will suffering be reduced to a minimum?**	
	Adverse effects	**Methodology and interventions to minimize severity**	**Humane endpoints**

This model is still based on individual events and interventions. It also focuses on events during the animal procedure and does not include other lifetime experiences. However, it helps to produce a clear overview of what happens during all stages of the animal procedure. This provides the opportunity, based on the knowledge and experience of those involved (researchers, veterinarians, biotechnicians, AWB members), to answer the question as to whether the combination of all these events, activities and refinements may result in a higher assessment of suffering than for each of the events in isolation. This approach also sharpens thinking on potential refinements and humane endpoints that can reduce the severity. When using the model, it is therefore important to also take into account those aspects of the animal's life prior to the animal procedure that can affect how the animal will experience the suffering, as described in the previous chapter.

Both the severity assessment framework and the article by the FELASA/ECLAM/ESLAV Working Group (2018) show a scoring system for each of the detailed examples, in other words for each animal model, that can be used to assess clinical and other symptoms during the animal procedure. These scoring systems are used as an aid to determine the actual severity experienced and to determine whether additional refinements are required or a humane endpoint has been reached. In a number of these scoring systems, points are assigned to certain effects (see an example of this on the next page, Figure 3). The severity classification or whether the humane endpoint has been reached is determined on the basis of the score for each effect or the aggregate scores. This type of scoring system is produced in advance based on reasonable suspicions as to how the animal could respond to the activities or interventions. These suspicions are based on knowledge acquired in previous animal procedures and can also help to make a prospective assessment of severity. It is usually known what to expect based on previous projects and animal procedures.

Figure 3 *Example of a scoring system produced by the FELASA/ECLAM/ESLAV Working Group (2018) for: Neuropathic pain – spinal nerve ligation (spinal cord injury) p.30/31*[33]

Score 0–5 plus surgery = MODERATE
Either 2 scores of 3 in any of the categories or a total score of 12 and above = HEP
Note: that as surgical complications are generally noted in the immediate post-op recovery period, close monitoring and expert, empathetic judgement are essential during the first 24 h to ensure that adverse effects are identified and actions taken to address these. Animals are humanely killed if their suffering exceeds of the moderate category.

1. Review frequency of monitoring.
4. Provide appropriate supplementary care, e.g. mash and additional fluids. Dehydration/diarrhea: Ringer Lactate or regular serum. Abdominal dilation (ascites): draining for pressure reduction. Weight loss: soft food
5. Review progress with vet

Either 2 scores of 3 in any of the categories or a total score of 12 and above = HEP

General clinical signs	Score
Appearance	
5–10% weight loss	1
11–15 % weight loss	2
16–20% weight loss	3
20%+ weight loss	HEP
Coat slightly unkempt	1
Slight piloerection	2
Marked piloerection	3
Body function	
Rapid, slow or deep breathing – slight	1
Rapid, slow or deep breathing – moderate	2
Rapid, slow or deep breathing – marked	3
Food and water intake	
Not drinking up to 10% of body weight per 24 h	1
Not drinking at all	3
Reduced food intake	1
Anorexia	3
Behaviour	
Slightly-decreased mobility	1
Markedly decreased mobility	2
Significant mobility problems	3
Immobility >24 h	HEP
Tense and nervous on handling	2
Markedly distressed on handling, e.g. shaking, vocalising, aggressive	3
Procedure-specific indicators	Score
Wound healing	
Wound edges are smoothly closed, no sign of inflammation	0
Wound edges are slightly swollen and erythematous	1
Wound is clearly infected/partially opened	3
No improvement of wound infection to topical and systemic treatment/wound is completely opened	HEP
Status of the operated limb	
Slightly reduced weight bearing	1
Marked gait impairment (lameness)	2
Severe gait difficulty/paw is often held in a constant elevated position	3
Major motor deficit >24 h postop/signs of autotomy	HEP
TOTAL	

HEP: humane end-point

[33] Smith D, Anderson D, DeGryse AD et al. (2018) Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group, Laboratory Animals 2018, 52(1S) 5–57.

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6.
Appendix 1
List of abbreviations

ASC	=	Animals in Science Committee
CCD	=	Central Authority for Scientific Procedures on Animals (*Centrale Commissie Dierproeven*)
AEC	=	Animal Experiments Committee
ECLAM	=	European College of Laboratory Animal Medicine
ESLAV	=	European Society of Laboratory Animal Veterinarians
EU	=	European Union
FELASA	=	Federation of European Laboratory Animal Science Associations
HEP	=	humane endpoint
AWB	=	Animal Welfare Body
NCad	=	Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (*Nationaal Comité advies dierproevenbeleid*)
NVWA	=	Netherlands Food and Consumer Product Safety Authority (*Nationale Voedsel en Waren Autoriteit*)
UK	=	United Kingdom
Wod	=	Experiments on Animals Act (*Wet op de dierproeven*)

7.
Appendix 2
What is and what is not part of an animal procedure

It is not always obvious what is and what is not part of the animal procedure. The UK government provides a more detailed explanation as to how the EU Directive should be interpreted and makes the following distinction to this end (pp. 6 and 7).

"Non-procedural harms should not be included in the assessment. Non-procedural events would usually affect, or be liable to affect, animals not involved in the particular study, for example, animals in the same room, same shipment. Examples of non-procedural harms include the following.

- *Failure of environmental controls, which result in harm to or loss of animals.*
- *Major disease outbreaks affecting animal units, which affect, or could affect normal animals.*
- *Fighting injuries where these are not due to phenotype or study.*
- *Death or disease of animals relating to factors/illnesses that are unrelated to the procedure, such as tumour development in an untreated wild type control animal or where the mortality rate is similar to an untreated group or the background strain.*
- *Incidents that might occur at any time (including at the time of the procedure) which might have occurred at any time during routine husbandry, for example, a mouse catching its tail in the cage lid.*

When assessing actual severity in these cases an informed decision must be made as to what the suffering of the animals would have been without these incidents. If it is not possible to determine the procedural related component of suffering that would have occurred if there had been no harms related to non-procedural effects, then the total actual harm including the non-procedural incidents should be reported. This is to ensure that all harms from the procedure have definitely been included. If in doubt, cases should be discussed with the Home Office Inspector to determine appropriate classification.

(...)

All procedure-related suffering should be taken into account. This includes expected, unexpected and unintended adverse effects or other harm that arises directly or indirectly from an action required to gain the (...) results/outputs of the study. It should include steps that would normally be expected to be below threshold, but that did in fact cause harm. This might consist of adding inert markers to the diet, restricted food availability or behavioural testing, for example. It will also include accidents and technical failures that are specific to or unique to the procedure. Examples of procedure-related harms include the following.

- *Expected harms listed in the adverse effects section of the protocol.*
- *Harms caused by failure of the equipment used.*
- *Harms caused by misdosing.*
- *Repair of a surgical wound after breakdown, whether or not performed by the NVS. Distress from restraint.*
- *Discomfort associated with cannula or implant care, or related infection.*
- *Distress unexpectedly observed during a non-regulated behavioural test that is required for data collection from an animal on procedure.*
- *Fight injuries where the fighting is related to specific needs of the study, such as repeated mixing of groups."*

8.
Appendix 3
Composition of the expert group

This document was compiled following a literature review and in collaboration with an expert group consisting of the following members:

NCad members
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In preparing its opinions, the NCad makes grateful use of the services of experts in the Netherlands and abroad. The experts consulted are not co-authors of this NCad opinion and their views on certain matters may differ from those presented by the NCad in this opinion.

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