

**CODE OF PRACTICE FOR
MONITORING THE WELFARE OF LABORATORY ANIMALS**

This code of practice has been translated into English under the authority of the animal welfare officer of the University of Groningen, the Netherlands, December 2007

FOREWORD

I am delighted to introduce this Code of Practice for monitoring the welfare of laboratory animals.

Article 10 of the revised version (revised in 1996) of the Experiments on Animals Act [Wet op de dierproeven] provides for explicit implementation of the 3 Rs (Replacement, Reduction and Refinement). One of the preconditions for animal experimentation is that experiments should cause laboratory animals as little distress as possible. This implies constant monitoring of their welfare during an experiment to allow prompt intervention, even in the case of unexpected abnormalities. At the end of an experiment, details of all distress actually undergone by the animals must be recorded. The findings should be used when estimating the expected level of distress in subsequent experiments. An accurate estimate of the severity of distress is particularly important to the Committee on Animal Experimentation, the body charged with weighing the interests of an experiment against the distress caused to the animals. The above-mentioned refinement to the legal framework means that distress suffered during an experiment must now be meticulously monitored, recorded and evaluated at the end of the test.

Towards the end of the 1990s, it emerged that people had a need for information on how to achieve this in practice, so the Inspectorate approached various experts from the groups involved in carrying out animal experiments in order to find a way of meeting this joint responsibility. An approach had to be found that would work in practice but which would also allow those involved to decide how best to interpret and fulfil their own responsibility. The working party's activities have resulted in the enclosed Code of Practice for monitoring welfare, which I strongly recommend is brought to the attention of researchers, animal handlers, bio-technicians, analysts and Committees on Animal Experimentation. I am convinced that the Code will become a worthwhile instrument for Committees on Animal Experimentation deliberating on proposed animal experiments.

The Code is intended to provide the tools for applying the existing legislation and to create safeguards so that all those involved in setting up, assessing and carrying out experiments on animals will receive the right information. It is based on the assumption that although safeguarding animal welfare in the practical situation is already a widely accepted practice, the structure of the information frameworks and the way data is recorded still leaves significant room for improvement. The Inspectorate assumes that the working methods laid down in Code will be followed.

The Inspectorate is obviously extremely grateful to the working party for all its hard work, and is highly impressed with the result: this Code of Practice for monitoring welfare.

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CODE OF PRACTICE FOR MONITORING THE WELFARE OF LABORATORY ANIMALS

The **aim** of this Code is to make sure that all relevant parties will at all times have full insight into every aspect of upholding and monitoring the welfare of laboratory animals. To this end, the following requirements have been set.

The following elements must be present and available before an experiment commences: the **research plan** containing the recommendations of the Committee on Animal Experimentation, at least, in the office of the laboratory animal expert, and in the direct vicinity of the animals: **a working protocol**.

During the experiment, the following elements must be present and available in the direct vicinity of the animals:
the working protocol for the staff performing the experiment and **a welfare diary**.

After the experiment, the organization must produce the following elements per experiment:
a welfare evaluation, registering any distress that may have occurred. The researcher should have a copy of this evaluation and it should also be present in the office of the laboratory animal expert.

The **intensity** and **level of detail** required in the observations made with regard to monitoring the welfare of the animals will depend on the expected level of **distress** and the level of **distress** that develops **during the testing, breeding or handling**.

The **research plan** should specify how the test will cause as little distress as possible to the animals. The nature and extent of the expected distress will be fully explained, argued and systematically recorded on a scale of 1 to 6. Estimates of the nature and extent of the distress must be based on all sources of information that can reasonably be accessed.

The **working protocol** must comprise all the details from the research plan relevant for the persons carrying out the experiment. Specific points for special attention when caring for and handling the animals must be clearly stated. The responsibilities and authority of those involved with carrying out the experiment must be laid down. The criteria and methods for applying responsible endpoints must also be determined. If the research plan contains sufficient detail, it may also serve as the working protocol.

The **welfare diary** is designed to constitute a system for recording observations and measurements relating to welfare. The measures taken for the purposes of monitoring welfare should also be recorded in the diary. It will only be possible to record the findings and measures if a uniform identification of animals or groups of animals is carried out.

The researcher is responsible for compiling a **welfare evaluation** (summarized overview) of relevant information about the continued health and welfare of the animals during the experiment, on the basis of the welfare diary and any other additional information. This should result in an estimate of the distress undergone. The evaluation should be clear to the laboratory animal expert and the controlling bodies. A welfare evaluation per research plan should be available in the institute after the experiment.

Attention must also be paid to the special requirements relating to:

- Consulting experts
- Biotechnological procedures involving animals
- Archiving

This Code is aimed at:

- a) animals used for experimentation
- b) animals bred and kept for the purposes of experimentation, with the possibility of congenital defects or disproportionate distress.

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EXPLANATION OF THE CODE

1 INTRODUCTION

Welfare is a broad concept. It is defined as 'good physical and mental health, now particularly used in relation to wellbeing in the immaterial sense'. Many of the parameters used for determining the level of welfare in humans are not properly defined and therefore difficult to quantify. There is also no clear frame of reference. This problem is even more poignant when it comes to estimating the level of welfare in animals. In the case of animals, welfare can only be estimated in relation to observations and measurements.

The level of welfare of laboratory animals is often expressed in terms of the opposite concept: the decline in or damage to welfare, or in other words, the severity of distress. The term 'distress' forms the basis of the Experiments on Animals Act [*Wet op de dierproeven*], which aims to protect laboratory animals. In this context, monitoring welfare should be seen as constantly striving to keep the level of distress to a minimum.

The need to monitor the welfare of laboratory animals is on the one hand prompted by deliberations regarding the quality of the experiment, and on the other hand by the moral obligations towards animals as established in the current regulations.

Quality in research implies the accurate estimation and evaluation of the anticipated effects of a test in relation to the actual effects felt during a test. An objective record of the observed effects on animals should therefore be kept. Decline in welfare and the effect this has on the animals will affect the way the results of the experiment are interpreted. A researcher¹ will only be in a position to evaluate the results of the experiment in the correct light if a conscious effort is made to record actual distress suffered as accurately as possible. Experiments carried out under GLP (Good Laboratory Practice) should already satisfy the stringent requirements relating to documentation, which will in many cases provide details of the continuing health and welfare of the animals.

The legislator plays a concrete role in protecting animals by issuing regulations aimed at reducing distress to an absolute and unavoidable minimum. This is explicitly expressed in Article 10 of the Experiments on Animals Act, which states that it is forbidden to carry out an experiment on animals if the test can be performed in a way that would cause less distress, and if the importance of the experiment is disproportionate to the distress it will cause. This ban implies that the researcher should inform the Committee on Animal Experimentation² as fully as possible about the expected level of distress so that the committee can weigh this against the importance of the test. Article 15 of the Experiments on Animals Act stipulates that the permit holder³ is obliged to keep (amongst other things) records regarding the distress and provide relevant information to the Minister of Health, Welfare & Sport. To this end, the researcher must create conditions that allow optimum information to be provided about the actual distress suffered. All in all, this means that when carrying out an experiment on animals, the welfare of those animals must be monitored. Insight must be available about all the elements involved and the best method for structuring adequate monitoring of animal welfare.

¹ Official as referred to in Article 9 of the Experiments on Animals Act. Determines details of the research and is responsible for preparing, carrying out and documenting the experiment.

² Committee on Animal Experimentation, approved in accordance with the Experiments on Animals Act. Makes ethical decisions on the interests based on the research plan. Makes recommendations to the permit holder, but mainly communicates directly with the researcher. The conditions laid down in a positive recommendation are binding.

³ The person inside the organization responsible for complying with the conditions of the permit (often a member of the management team, Board).

The Inspectorate for Health Protection, Commodities and Veterinary Public Health set up a working party to look into ways of achieving this. Members included: F.A.R. van den Broek, Ms. J.M. Fentener van Vlissingen, Ms. H.E. From, Ms. C.P.A.T. Klein, R.A.A. van Oosterom, Professor R. Remie (chair), Ms. L. Rijswijk and Ms. P.M. Scholten.

The working party set itself the task of compiling guidelines to enable a practical, adequate and organized system for monitoring and recording welfare. The methodology the group formulated for monitoring the welfare of laboratory animals has been incorporated into this Code. The guidelines had to be workable and practical for different species of animals in various types of institutes. For this reason, no detailed instructions per species and clinical observation have been prescribed, although the appendix includes a number of options for developing the general principles. However, the Code does insist that under the terms of the methodology, **a working protocol** should always be available, **a welfare diary** must be kept and a **welfare evaluation** should be carried out and registered. The information recorded must comply with the explicit quality criterion: 'clear to the controlling bodies and updated regularly enough to give an accurate account of the continuing health and welfare of the animals'. This methodology will enable parties to comply with the current legislation.

Although the Code formulates more detailed requirements about reporting, unnecessary administrative burden should be avoided. Thus far there has been no clear insight into the procedures during testing. Abnormal observations were almost certainly passed on by word of mouth. Nonetheless, much uncertainty remained about the extent to which certain observations resulted in action and how they affected the final distress score. It should be perfectly clear that the interpretation, application and welfare data resulting from a working protocol will always depend on the nature of the research and the degree of distress, and will therefore vary from institute to institute. This leeway exists. However, it should always be possible to monitor any changes in the health and welfare of the animals on the basis of the previously mentioned methodology. The restrictions and stipulations relating to permits as referred to in Article 6 of the Health and Welfare of Animals Act [*Gezondheid- en Welzijnswet voor dieren*] largely covers this obligation. This Code provides a practical framework in this respect.

2. FROM RESEARCH PLAN TO WORKING PROTOCOL

Before the experiment

The research plan is defined in the Experiments on Animals Act, Article 10a.2. Article 2a of the Animal Experimentation Decree [*Dierproevenbesluit* (Bulletin of Acts and Decrees 1996, 566)] elaborates on this section of the law by defining the minimum requirements of the research plan:

- The question⁴ being addressed by the research
- The importance of the research for the health or nutrition of man or animal
- The social and scientific significance of the research
- The person or committee responsible for evaluating the scientific quality of the research
- Arguments for rejecting alternatives
- The severity of distress to which the animals may be subjected
- The origins of the animals
- The expertise of the person deciding on the way the experiment will be carried out (Art. 9, Experiments on Animals Act)
- The intended treatment and care of the animals and the Article 12 official(s)
- The nature, frequency and duration of the procedures
- The use of anaesthetics or pain relief and other methods of reducing distress, including the possible implementation of humane endpoints
- Previous use of the animal
- The use and timing of euthanasia
- The final destination of the animal after the experiment.

The research plan should serve as the basis for the recommendations of the Committee on Animal Experimentation.

The research plan should focus specific attention on the scientific and social background to the research question. The technical details relating to implementation are less important here, but should be clear enough to allow the Committee on Animal Experimentation to reach a well-considered judgement. An advantage of this procedure is that it allows the Committee on Animal Experimentation to concentrate on its core task: weighing up the expected level of distress caused to the animal against the scientific and social significance of the question being addressed by the research.

One of the aspects assessed by the Committee on Animal Experimentation is whether the test complies with Article 10 of the Experiments on Animals Act, which forbids experiments to be carried out if an alternative experiment would cause less distress to the animals. The Committee on Animal Experimentation must be certain that every effort has been made to reduce all distress to a minimum, within the confines of the experiment. The Committee therefore correctly assesses all plans in the light of Article 12 of the Experiments on Animals Act, which states that assurances must be in place to guarantee the proper care and treatment of the animals.

Nonetheless, this is merely a recommendation given in advance concerning an experiment. Or as Article 10a of the Experiments on Animals Act states: an experiment with animals may not be carried out unless the Committee on Animal Experimentation has issued a (positive) recommendation *beforehand*.

It is therefore the responsibility of the Committee on Animal Experimentation to demand assurances, wherever possible, that the experiment will cause as little distress as possible and that the animals will receive due care and proper treatment.

⁴ The concrete question addressed by the research and the background (prior research, for example results of previous experiment findings, relevant literature).

Four conditions must be satisfied:

1. The Committee on Animal Experimentation must be fully informed about the distress that may be caused to the animals.
2. Measures must be taken to ensure that the technical details are properly described and correspond with the research plan, and that the research is carried out according to this description.
3. During the experiment, planned inspections must be carried out to ensure that the distress undergone by the animals is kept to a minimum. The findings must be recorded (welfare diary). This requirement regarding the recording of distress in retrospect is based on Article 15 of the Experiments on Animals Act.
4. The laboratory animal expert or researcher may notify the Committee on Animal Experimentation of any deviations from the assumptions on which it based its original recommendations after the experiment.

In order to comply with these conditions, a good estimate of the expected distress is essential. There must also be a method in place for linking specific observations to specific animals or groups of animals. This necessitates a uniform method for identifying the animals. In addition to the technical details, the working protocol must also specify how the correct observations will be made at the correct times. The duties, responsibilities and powers of authority of those involved must also be laid down in the protocol.

Estimating the level of distress

The researcher should describe the nature of the distress. The expected severity of distress is expressed as a score between 1 and 6.⁵ As the severity of distress is a vital aspect affecting the Committee on Animal Experimentation's recommendation,⁶ the estimate should be made as accurately as possible. The researcher must be able to indicate how this estimate has been arrived at. Sources that can be used for more information include:

- Experiences from previous experiments as recorded in the welfare evaluation
- Consultations between the laboratory animal expert⁷ (animal welfare officer) and other experts from within and outside the institute
- Experiences from general veterinary practice
- Human analogy from medical practice and patient experiences
- Relevant literature
- Data from other research (with laboratory animals, pathology or ethology; see also chapter 5 of this Code).

If a well-structured system of welfare control is implemented as dictated by this Code, at the end of the experiment the researcher will have sound information about his estimate of the actual

⁵ Explanatory notes to the registration of laboratory animals and animal experiments, page 54.

⁶ Document in which the Committee on Animal Experimentation reports the result of its evaluation. In most cases, this should be read alongside the research plan that has been submitted, or a note of it will have been made in the plan.

⁷ Controlling body/advisor as referred to in Art. 14 of the Experiments on Animals Act, usually also made responsible (by the permit holder) for the registration of staff, laboratory animals and animal experiments.

distress; this estimate is arrived at together with the animal handlers.⁸ The knowledge acquired will enable adjustments to be made to future estimates for similar experiments. The procedures are represented in a diagram and list of terms in Appendix 1.

The working protocol (Appendix 2)

The essential elements of the research plan have been defined at the beginning of this chapter. Some of the compulsory elements of the research plan are of particular importance in terms of carrying out the experiment correctly. During its deliberations, the Committee on Animal Experimentation does not need to go into these mainly technical details in any great depth. However, a Committee on Animal Experimentation must be able to rely on the existence of the necessary assurances and on the knowledge that it will be informed about whether everything 'went according to plan' afterwards. The working protocol contains a number of elements from the research plan that have been specifically enhanced and supplemented with all the information that an animal handler needs to comply with the obligation to 'provide the animals with due care and proper treatment'. The working protocol is also intended to ensure that the level of distress is kept to a minimum. The working protocol kept in the vicinity of the animals should clearly correspond with the relevant research plan. In the implementation phase, several working protocols can be compiled per research plan. However, it should always be easy to trace every working protocol back to a section of the relevant research plan.

In the working protocol, the researcher must indicate the 'technical' requirements and conditions he considers necessary. He must also alert the animal handlers to any points for special attention before the experiment. These are directly linked with the nature of the procedures being carried out or the disposition of the animals being used. It is possible that on the basis of his experience with a particular type of research, the researcher will not expect any specific abnormalities. In this case, he will have to indicate how often the animals should be inspected, particularly in terms of the aforementioned general parameters. This could imply a 'package' relating to a general impression (posture, level of activity, food intake, defecation, etc.). Observations such as 'no abnormalities' are only acceptable if the term has been clearly defined, or in other words, if it is clear precisely what has been checked. The findings should be recorded via a methodology that satisfies the requirements of the experiment. The intensity and degree of detail can be kept to a minimum. Specific instructions given by the researcher should be followed and a structure for recording the findings should be in place.

The working protocol must comprise:

- An unambiguous reference to **the research plan and the Committee on Animal Experimentation recommendations**.
- A clear, concise description of the **scientific background**.
- The **aim of the experiment**, briefly and clearly explained in good Dutch.
- The expected level of distress and the required specifications. The nature and degree of distress expected by the researcher provides a basis on which the animal handler can assess his findings. Where necessary, the researcher can add specifications for the particular species of animals involved in the experiment. If he is unable to make an educated estimate with regard to a specific procedure, he should share this information with the animal handlers.

⁸ This also includes the bio-technician and analysts. These are the officials referred to Art. 12 of the Experiments on Animals Act. They take care of and handle the animals. In broader terms, it also includes qualified laboratory workers and vets. A researcher who personally takes care of / handles the animals is also covered by this term.

- **Responsible endpoints.** The working protocol must (if applicable) specify the way that the principle of humane endpoints will be applied. This means that an animal will be destroyed or that measures will be taken to prevent any more distress. The guidelines on humane endpoints are as follows:
 - ‘Spontaneous’ death or more severe distress than expected may not be used as the intended endpoint or as a parameter. If the experiment warrants an endpoint of this kind, the research plan submitted to the Committee on Animal Experimentation must contain explicit arguments to back this up.
 - Euthanasia may not be put off until an animal is already at the point of dying.
 - An animal must be euthanased if it is experiencing more distress than strictly necessary for the aim of the experiment.
 - An animal must be euthanased if it is no longer suitable for the purposes of the experiment and is experiencing continuous distress.
- The name of the designated **Article 9 official**, and the names of the **Article 12 officials** involved must be included.
- The **duties, responsibilities and powers of authority** of the staff involved must be stated. The allocation of tasks to the various people involved in carrying out the animal experiment can be a structural arrangement within the institute, or an arrangement made for a specific experiment and included in the working protocol. It should be clear who is responsible for which specific duties, without the need to make further agreements (for example, the animal handler cleans the cages every week and provides fresh food; the researcher ensures that the test materials are available on time). Everyone is responsible for the smooth running of his part of the experiment, and any obstacles should be reported (example: a member of staff who is running out of time should check whether his replacement needs particular information before handing over his duties). The staff involved are also primarily responsible for the welfare of the animals: an animal handler who notices an abnormality should report this to the researcher and the laboratory animal expert if necessary. As the party with final responsibility, it is up to the researcher to take appropriate action. Duties and responsibilities should be clearly laid down. However, anyone accepting full responsibility should be given the authority to take the necessary measures. For example, if the researcher authorises a bio-technician to destroy any animal suffering serious distress, the duties have been clearly defined. The rules are also clear if the researcher insists on taking the decision himself, but he must ensure that he is always available (including outside working hours if necessary), and he must arrange for a replacement in his absence. Particular problems, in as far as they can be predicted, can be incorporated into the working plan, stating the measure that can or must be taken in such a case. The working protocol should include a stipulation stating that the welfare diary must include a record of who took a certain decision/action, and in consultation with whom.
- Information about the **animals**. All information about the animals relevant to the experiment must be included here. Appendix 1 specifies the points for special attention that should be considered. The method and specific details used to identify the animals must therefore appear in the working protocol. Identification must satisfy a number of conditions. It must be possible to make a clear link between the research plan and the ensuing working protocol. The identification must remain clearly legible on the animal or the accommodation unit for a long period, and it must comprise a number of specifications essential for identifying the place the animals have been assigned in the research plan/working protocol (all specific information important for linking the observations to certain animals or groups of animals).
- **The circumstances under which they are kept and the care regime** have an important influence on the welfare of the animals. Obviously, the research question will sometimes

require the animals to be kept under specific circumstances. In practice, it is advisable to describe the standard accommodation at the institute and refer to this, stating any deviations. The place where the animals are being kept must be referred to by name.

- **Food.** As specified in the section on 'accommodation', it is acceptable just to specify any deviation from the standard.
- **Design of the experiment, procedures/operative procedures.** Define and identify the test system. Give an overview of the design of the experiment and if possible, a test framework. This should state the allocation into experimental groups, the number of animals involved, the number of animals in the control group, the dates on which particular procedures will be carried out and the people carrying out these procedures. Special procedures, such as giving an injection, the dosage and volume to be administered, should be specifically mentioned.
- **A template for a welfare diary** with research-based points for special attention for the animal handlers, as well as a list of decision moments / responsibilities in response to certain findings. The intervals and general and specific parameters governing the inspections should be indicated, as should the method for recording the findings. If abnormalities are not expected, the minimum package of observations to be made and recorded should be specified. A 'clinical score sheet' can be used to record certain routine observations. Please refer to the chapter entitled 'Welfare diary'. Appendix 3 contains a model list of symptoms according to the organ systems. This list can be useful for deciding on a targeted 'package of observations'. All procedures / operations carried out must be noted in the welfare diary so that a link can be made with the clinical findings. In the case of different procedures or groups of procedures being carried out on different animals or groups of animals, it is best to make separate listings in the welfare diary. Information about the experiment and the procedures that require special attention from the animal handlers can be recorded here. An operation report of all major surgical operations or procedures should be added to the welfare diary per animal. A group report will suffice for 'routine' operations, as long as there is mention of individual animals showing deviant symptoms during the course of the procedure.
- **Provisions for additional testing** (chapter 5). If the researcher expects that extra tests will be needed, for example ethological or welfare-based pathomorphological tests, the working protocol must state the agreements made with the experts concerned on this point. Similar conditions apply if complications resulting from a new surgical procedure, for example, are difficult to gauge.
- A research plan that comprises the details described above can obviously serve as a working protocol. For an impression of the practical possibilities of the outlined structure, please refer to Appendices 4 and 5 of this Code.

3. THE WELFARE DIARY

During the experiment

Observations relating to the welfare of laboratory animals should be recorded with appropriate frequency. The data collected for the purposes of a working protocol constitute the welfare diary. In the same way as the working protocol, the welfare diary should be kept in the immediate vicinity of the animals. It provides a matrix for recording procedures and the associated observations and findings for the duration of the experiment.

The working protocol must specify the aspects to be controlled and the frequency of the inspections. The required information must then be recorded in the welfare diary. The registration medium is a set of standard documents or a database in which the animal handlers can record their general and/or specific findings.

Obviously each experiment will have its own specific points for special attention and so the form of the matrix and the frequency of the recordings will not always be the same. For example, it is possible that the vital functions of the animals will have to be checked during the days immediately following a major operation. This could include the colour of the mucous membranes, temperature, condition of the wound, etc. However, after the operation and assuming the animals are in good condition, it will soon become unnecessary to continue recording this information. In the working protocol, the researcher should indicate how often which specific parameters need to be checked. All animals should be inspected daily and the findings recorded. During this routine inspection for calamities, the animals should be checked by hand.

All animals must be checked individually at least once a week and any abnormalities should be recorded. The researcher can order additional inspections depending on the nature of the experiment and the expected level of distress. The researcher can also indicate in the working protocol that no specific abnormalities are expected for a certain period.

For example, the working protocol may specify that the mice should be cleaned and checked twice a week. If the animals appear normal and healthy, the animal handler need do no more than make a note that he has found no abnormalities within these limits.

‘Clinical score sheet’ (see Appendix 3)

The matrix in the welfare diary can (partly) be made up of a so-called ‘clinical score sheet’. This is a table that sets out the parameters for the general impression and/or specific parameters for the organ system in terms of time. The researcher must indicate the package of observations required in the light of the experiment. If the findings are to be recorded with a particular frequency, a sheet of this kind is recommended. Appendices 3 and 5 show an example. They also give a number of parameters that can be used to provide insight into the general welfare of the animal. Specific parameters indicating the condition of an organ system are also represented here per organ system.

These ‘clinical symptoms’ can be incorporated into the clinical score sheet as long as they remain within the framework set out by the researcher in the working protocol. In terms of animal welfare, the right selection means the most functional set of observations appropriate to the experiment that will provide the best possible impression of the distress undergone by the animals.

It should always be remembered that these specific observations are needed to provide sound (scientific) insight into the course of the experiment. In this respect, the saying ‘if you don’t look, you won’t see anything’ is worth remembering.

A welfare diary should be kept for every working protocol. An advantage of this method is that it provides a direct and clear connection with the research plan concerned. The welfare diary should be organized so that relevant information can be recorded efficiently. The aforementioned matrix will therefore not always be the same in every welfare diary, and the model in Appendix 4 is purely intended as an example. As the research progresses, terms can be added and the frequencies adjusted. If the working protocol contains a general or a research-specific model of a matrix, a copy can be added to the welfare diary every week and allowed to 'grow in the required direction'. Certain research-specific observations (such as measuring data or post-mortem reports) can also be included in the welfare diary if they provide more detailed insight into the distress.

Measures.

The researcher responsible must indicate how the responsibilities and mandates have been assigned (see working protocol). Certain procedures and activities will be carried out during the experiment or the care regime under the terms of these responsibilities. A record should always be kept of who took particular decisions / action, and in consultation with whom if applicable. The welfare diary should be specific about which measures have been taken, by whom and on what grounds.

The intended purpose of the animal should also be clear (pathological research, material for the researcher, etc.)

4. THE WELFARE EVALUATION, A SUMMARIZED OVERVIEW

After the experiment

After the experiment, a summarized overview must be made of the information from the welfare diary. The result is a welfare evaluation. Specific observations must be laid down and identical observations over a longer period of time clustered. For longer experiments in particular, it will be possible to derive a great deal of data from the working protocol. As mentioned previously, the aim of the Code is to ensure efficient (not disproportionately difficult) recording.

The information compiled during the course of the experiment must therefore be compressed and evaluated in a logical manner. Repeated, identical findings can be centralized. It goes without saying that the design of a cumulative overview of this kind, the welfare evaluation, is a matter of 'common sense'. For the sake of clarity, it should be as compact as possible. On the other hand, a welfare evaluation should not be so compact that the reader loses sight of important information. The following condition puts it very clearly: the welfare evaluation should make it easy to trace the progress of the health and welfare condition of the animals in relation to the procedures during the course of every single experiment.

As already mentioned, an optimum balance should be sought between the insight the evaluation is supposed to provide and the degree of detail.

The actual distress (Art. 15 Experiments on Animals Act)

This summarized overview (the welfare evaluation) provides the researcher responsible, in consultation with other experts, with the elements needed to score the actual level of distress and compare it with the level estimated before the experiment.

It should be noted that the scores used to represent distress (from 1 to 6) have substantial uncertainty margins. Nonetheless, a reasonable attempt should be made to allocate the most appropriate score to the various categories of animals in the welfare evaluation. Not every (minor) abnormality discovered in an individual animal or a small group of animals will necessarily change the score. The abnormalities should be noted. Substantial abnormalities should be taken into account in the score. Finally, the various animals or groups of animals are assigned a score. It should be decided on a case-to-case basis whether a 'greatest common denominator' can be found or if specific groups warrant separate codes.

All things being equal, the researcher will have indicated this 'division' in both the research plan and the working protocol.

On completion, the welfare evaluation, now a brief document containing the final distress scores, must be approved by the researcher. A copy must be sent to the laboratory animal expert and enclosed with the research plan. If the researcher and those responsible for caring for/handling the animals fail to reach a consensus about the level of distress, a record of the arguments must be compiled and brought to the explicit attention of the laboratory animal expert.

This information is an excellent supplement for the laboratory animal expert in his duties monitoring animal welfare. The welfare evaluation can be important to the Committee on Animal Experimentation when adjusting the estimate of distress if this deviates substantially from the estimate made in the assessment.

5. CONSULTING WITH EXPERTS

For the purposes of an experiment with animals, the researcher must consult with experts who can provide useful information. This applies to the phase before and during the experiment as well as afterwards. Much of this consultation will relate to the scientific question being posed. As part of this Code, it must be said that in the interests of reducing the stress caused to the animals involved and in order to improve the estimate of the distress the animals will undergo, consultation with experts is essential. It would be inappropriate to provide an exhaustive overview of the various fields of expertise that the researcher can consult, as scientific research and the questions it throws up are far too extensive.

The basic principle is that all reasonable sources must be consulted when considering ways of reducing distress and obtaining well-documented insight into the distress likely to be suffered. Once again, it must be stressed that on the basis of Article 10 of the Experiments on Animals Act, reasonable efforts must be made to adhere to the principle of the three Rs (Reduction, Refinement and Replacement) throughout the process. Welfare monitoring is mainly concerned with refinement.

Before the experiment

For adequate compliance with the do's and don'ts of Article 10 of the Experiments on Animals Act, it is essential that the researcher becomes as familiar as can be reasonably expected with the welfare aspects of the experiment. In many cases, to comply fully with this Article researchers will have to consult experts. This is one of the points that the Committee on Animal Experimentation will assess, and so the researcher must make sure he is properly informed about all the alternatives. He must seek advice from a methodologist and a statistician about whether it would be possible to answer his question using fewer animals. He must then use anaesthesiological, surgical and other expertise to refine the setup of the experiment as far as possible (with as little distress as possible). He must estimate the nature and extent of the distress by consulting clinical expertise, the laboratory animal expert, bio-technicians, etc.

The researcher must convince the Committee on Animal Experimentation that he has sought all the information he could reasonably be expected to find. In many cases, the laboratory animal expert will prove to be a good source of initial information.

During the experiment

Meticulous monitoring of the health and welfare of the animals and an adequate response to unforeseen circumstances are crucial. For example, during the experiment a microbiologist must be consulted at the first sign of intercurrent infections. The information must be interpreted by an expert before animals are removed from the experiment or treated. In the case of unexpected side-effects or intercurrent death, the pathologist will be able to provide more insight into the possible cause. The opinion of an ethological expert may be needed before destabilizing groups of animals to carry out individual tests on one of their kind. Where possible and reasonable, the researcher must consult experts for an objective analysis of the degree of deterioration in welfare, and to prevent possible deterioration of the other animals.

After the experiment

It is an unavoidable fact that many experts need to be consulted for a sound interpretation of the data collected, even after the experiment. With respect to monitoring the health and welfare of the animals and ascertaining the actual distress they have undergone, special attention must be paid to the pathomorphological and microbiological examinations. Factors that cannot directly be predicted by the researcher and which often have an unexpected, direct effect on the welfare of the animals can, for example, be assessed by the laboratory animal expert, pathologist or a specialist with pathological expertise such as a veterinarian. These experts will be able to give a better picture of the actual distress experienced. A pathologist, for example, can divert his focus

away from the desired scientific results and concentrate on judging the severity of distress undergone by the animals.

For the purposes of this welfare-based approach, the role the pathologist is expected to play must be made clear at an early stage of the experiment. The researcher must state in the working protocol exactly when and why the pathologist should be brought in to make a more detailed assessment of the distress. He must also be well informed about the background of the experiment, be given a good anamnesis (animal handlers and researcher) and have fresh (properly prepared if necessary) material. It is therefore practical if he is given a copy of the research plan and the working protocol relating to the animals or the animal material he is expected to examine.

A general formula would be impossible with regard to this Code. A correct attitude and sense of responsibility towards the animal will allow the researcher to make an adequate decision about the possibility of welfare-based pathological examination. The Code therefore only mentions a few cases that warrant calling in the services of a pathologist and/or microbiologist:

- In the case of intercurrent (unexpected) symptoms (including the death of animals) that cannot be explained by the researcher. These are symptoms of which the nature, extent and/or frequency are important for the welfare of the other animals or for obtaining more objective information relating to the actual distress being suffered. The working protocol includes provisions for the logistics needed. It cannot be too strongly emphasized that this procedure must also be followed in the case of intercurrent symptoms amongst genetically modified animals.
- Cases in which the researcher is unable to give an accurate indication of the possible degree of welfare deterioration in advance. This includes procedures (possibly involving administered substances, new surgical techniques) for which the effects on the animals' welfare cannot be adequately estimated by the researcher.

In the case of genetic modification, it is recommended that the phenotypical distress generated by the altered genetic constellation of any modified species included in the experiment be assessed as objectively as possible in advance. The pathologist should add his (welfare-based) findings to the welfare evaluation documenting the (possible) congenital distress. If the researcher can show that a decrease in distress cannot be expected, additional examination will not be necessary. If there is any doubt about consulting experts, the Code always prescribes the 'yes, unless...' principle. The researcher must be able to demonstrate why, in certain cases, he thinks that the contribution of an expert would give a better indication of the possible distress. He must indicate this in the research plan under 'the expected level of distress'. In compiling its recommendations, the Committee on Animal Experimentation must ensure that all reasonable sources have been, or will soon be, consulted.

6. POINTS FOR SPECIAL ATTENTION IN THE GENETIC MODIFICATION OF ANIMALS

The Code on monitoring the welfare of animals applies in full force when carrying out bio-technological procedures.

- **The following items must be present and available before the experiment:**
 - **the research plan**, including a specified reference to the recommendations of the Committee on Animal Experimentation, **the number of the permit to carry out bio-technological procedures and the stipulations and restrictions it contains**, certainly in the office of the laboratory animal expert
 - **a working protocol**, including **the number of the permit to carry out bio-technological procedures and the stipulations and restrictions it contains**, in the immediate vicinity of the animals.

- **The following items must be present and available in the immediate vicinity of the animals during the experiment:**
 - **the working protocol, including the number of the permit to carry out bio-technological procedures and the stipulations and restrictions it contains**, for the persons performing the procedures:
 - **a welfare diary.**

- **Per experiment, the following items must also be present within the institution after the experiment:**
 - **a welfare evaluation.**

- The **intensity** and **degree of detail** required from the observations carried out in respect of monitoring welfare depends on the **level of distress** expected and the **level of distress** that develops during the course of experimenting on, breeding or keeping the animals. It is not always easy to predict in advance the possible effects on the phenotype and the welfare when carrying out bio-technological procedures on animals. This makes it important to detect and analyse possible abnormalities.

When recording data on the genetic modification of animals, a distinction must be made between adverse effects on welfare **as a result of bio-technological procedures** and **distress as a result of follow-up procedures**, such as subjecting the animal to further tests.

The legislation states that any breeding of laboratory animals involving **a risk of distress** is considered to be an animal experiment.

The system for monitoring welfare should be organized so that it provides insight into distress resulting from biotechnological procedures as well as distress resulting from follow-up procedures. The total extent of distress should be recorded in the annual Register of Experiments on Animals (Ministry of Health, Welfare and Sport) and any unexpected effects from bio-technological procedures should be reported to the Ministry of Agriculture, Nature and Food Quality without delay.

The following section lists a number of points for special attention that could be useful for constructing a system for monitoring welfare when making transgenes and 'knock-outs':

1. It is important to keep accurate records of reproduction techniques and data. They can be used to calculate success rates, which in turn provide pointers for embryonic death or perinatal death. Dead or unviable offspring are often disposed of by adult animals directly after birth and can be missed during observations.

2. It is not always clear which abnormalities will occur and need to be recorded before the event. Terms can be added to the clinical score sheet in response to the discovery of new clinical symptoms.
3. There are specific moments after birth when targeted observations about development can be made. Clear developments such as hair growth and the opening of ears and eyes can be scored at pre-specified moments.
4. Weighing the animals can wait until they are weaned, after which they can be weighed at regular intervals. A visual comparison should be made between animals from the same brood and of the same age, and with the same genetic background.
5. Research data, in as far as it affects the estimation of distress levels, must be added to the welfare diary (blood test results or pathology, for example).
6. If striking clinical abnormalities occur, the significance to the welfare of the animals must be ascertained. Even if the deviant phenotype is not a direct indication of impaired welfare, it must be recorded.
7. In the welfare evaluation, a distinction must be made between distress as a result of biotechnological procedures and stress resulting from follow-on procedures. Attention must also be paid to whether the abnormality is similar to the disorder for which the genetically engineered animal is the model, or if it is more likely to be an unexpected side-effect.
8. At an early stage, consideration must be given to the matter of whether the welfare or the phenotype have been significantly affected. If so, an immediate decision must be made about whether the strain that has been created is of sufficient scientific importance to warrant being kept alive. Reporting the matter to the Ministry of Agriculture, Nature and Food Quality must also be considered, in accordance with the stipulations of the permit.

7. ARCHIVING

The working protocol, the welfare diary, the welfare evaluation and reports from the experts consulted must be filed with the research plan and the registration data after the experiment. This information must be accessible to the laboratory animal expert at the very least.

Article 10 of the Animal Experimentation Decree states that data relating to the number, the species and the date that the animals were acquired, the origins of the animals, the use made of the animals, the reason for and date of removing the animals must be stored for five years after the end of the calendar year to which the information relates.

For practical reasons, it is stated here that the welfare evaluation must be kept for the same period, but that the draft information noted down in the welfare diary and the consultations with experts need only be stored for two years.

8. RELEVANT LITERATURE

Canadian Council on Animal Care. Guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching and testing. 1 –29, 1998.

Olfert, E.D. Considerations for defining an acceptable endpoint in toxicological experiments. Lab Animal 38 –43, 1996.

Morton, D. B. Adverse effects in animals and their relevance to refining scientific procedures. ATLA 18: 29 –39, 1990.

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Report of FELASA Working Group on Pain and Distress. Pain and distress in laboratory rodents and lagomorphs. Laboratory Animals 28: 97 –112, 1994.

Humane Endpoints in Animal Experiments for Biomedical Research. Proceedings of the International Conference, 22-25 November 1998, C.F.M. Hendriksen and D.B. Morton (eds.) The Royal Society of Medicine Press limited, 1999.

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On the Internet: http://www.mgc.har.mrc.ac.uk/mutabase/shirpa_summary.html.

APPENDIX 1

AN OUTLINE OF THE RESPONSIBILITIES A BRIEF DESCRIPTION OF THE PROCESSES

Conducting an experiment involves four roughly defined phases: the preparation, the assessment by the Committee on Animal Experimentation, the experiment itself and the reporting. The diagram shows the connections between these phases.

In the *preparation* phase, the researcher operationalizes the research topic into an experimental design. The researcher must consult with experts. For the experimental technical aspects relevant to the welfare of the animals, he must consult with the people responsible for conducting the experiment (bio-technicians, veterinary surgeon) and other specialists in the field of animal experiments and alternatives, such as the laboratory animal expert.

For the purposes of the *assessment by the Committee on Animal Experimentation*, the researcher must draw up a research plan as specified in the Experiments on Animals Act. This **research plan** must on the one hand describe the importance of the research and on the other hand, the damage to the interests of the animals concerned. Alternatives must be paid particular attention, and a number of implementing aspects must be specified in line with the legal requirements. The research plan is not usually detailed enough to guide the implementation process. The Committee on Animal Experimentation makes recommendations, sometimes containing binding conditions.

For the actual *execution* of the research, the researcher must devise a working protocol containing all the details of how the experiment will be conducted: the schedule, the methods to be used, the identification of the animals, samples, etc., and also the way in which the required observations will be recorded. Provisions must be made to ensure that the animals' welfare is properly monitored and that the observations are recorded. Observations relating to the welfare of the animals must be noted down in the welfare diary. The researcher must be in charge during the execution of the experiment itself and is as such responsible for the welfare of the animals.

In the *reporting* phase, the researcher must correlate, interpret and record his research findings in a report or another publication. He must also ensure that the data relating to the laboratory animals and the animal experiments is included in the annual reports to the Government (Registration of Animal Experiments and Laboratory Animals). These reports require information about distress and must be drawn up by the researcher together with those responsible for caring for and handling the animals. Data on welfare (the welfare diary) must be summarized and evaluated: the welfare evaluation.

The following section examines the key tasks in more detail.

OUTLINING THE RESPONSIBILITIES

1. People and institutions

Researcher:	Functionary as defined in Art. 9 of the Experiments on Animals Act. Determines the way the experiment will be conducted and is responsible for the preparation, execution, reporting.
Animal handlers/bio-technicians:	Functionaries as defined in Art. 12 of the Experiments on Animals Act. They care for and handle the animals. In a broader sense, this also covers laboratory staff (HBO), and clinical veterinary surgeons. A researcher who cares for and/or handles the animals himself is also covered by this description.
Bio-technician:	The staff of a (centralized) laboratory animal facility, not further specified in legal terms.
Laboratory animal expert:	Advisor/supervisory official as defined in Art. 14 of the Experiments on Animals Act, permanent advisor to the Committee on Animal Experimentation, usually also made responsible (by the permit holder) for the registration of persons and the coordination of the registration of laboratory animals and animal experiments.
Committee on Animal Experimentation:	Officially recognized via the Experiments on Animals Act. On the basis of the research plan submitted by the researcher, this body weighs up the ethical interests and assesses the existence of possible alternatives (reduction, refinement, replacement). Advises the permit holder but mainly communicates directly with the researcher. The conditions set out in a positive recommendation are binding.
Permit holder:	The person within the organization responsible for complying with the permit conditions (often member of management, executive board).

2. Information relating to the research

Research question, pre-research:	<p>The concrete question being addressed by the research and the background (pre-research: for example, results from other tests, consulted works etc).</p> <p>The concrete research question must be defined specifically enough to form the basis of a research plan.</p>
Consultation (preparation phase):	<p>The researcher must consult with internal and possibly external experts when drawing up a research plan. Attention should be paid to the feasibility, optimization of the approach and alternative methods (reduction, refinement, replacement). The expected effects of the test procedures and the welfare of the animals must be explicitly mentioned. The information must be used to compile a research plan to be submitted to the Committee on Animal Experimentation.</p>
Scientific question, interest:	<p>The importance of the research in a broader context: what is the scientific / social significance of answering the concrete research question, what is the scientific quality (external opinion, credibility of the research group, technical ability)?</p> <p>This information is particularly relevant for the deliberations of the Committee on Animal Experimentation, and to a lesser extent, for the working protocol.</p>
Committee on Animal Experimentation recommendation:	<p>Document in which the Committee on Animal Experimentation reports the findings of its assessment. This should usually be read alongside the submitted research plan.</p>
Working protocol:	<p>A document that prescribes the details necessary for carrying out the research (the time schedule, the animals and their care, the staff involved, the responsibilities, administration of substances and observation, etc.) Forms or software for recording observations must be considered appendices to the working protocol, as should references to standard procedures. With a view to managing and adequately recording the distress, the working protocol must pay attention to the expected effects and the plans for intervening to prevent unnecessary suffering. The working protocol may be adjusted as the experiment progresses, but only within the confines of the research plan approved by the Committee on Animal Experimentation.</p>

Welfare diary:	<p>Observations relating to the welfare of the laboratory animals must be recorded with appropriate frequency. The information collected according to the working protocol is deemed to be the welfare diary. Agreements must be made (and laid down) about how best to do this within the specific organization or even within the bounds of a particular experiment. The nature and frequency of the observations should be specified. Results of these observations must be kept in the vicinity of the animals so that they can be consulted there. It must also be possible for those carrying out the experiment, supervisory bodies and Government inspectors to take note of the observations in the vicinity of the animals for the duration of the experiment. All people involved in carrying out the experiment must be clearly informed about dealing with certain expected or unexpected problems. There must be no unnecessary delay in making decisions. Humane endpoint (also referred to as 'responsible endpoint'): removing animals from the experiment or euthanizing them in order to prevent further (unnecessary) distress.</p>
Registration of animal experiments:	<p>As from 1998, reports of animal experiments must be sent per animal to the Government, after the experiment has finished (at the end of the reporting year). The distress must be reducible to concrete observations in the practical situation (clinical condition, conclusions on effects of the experiment procedures, pathological research, etc.). The information should also be clearly processed and evaluated (welfare evaluation).</p>
Welfare evaluation:	<p>The researcher is responsible for initiating a summary and assessment of the raw data. A proper welfare evaluation will involve input from the researcher, the staff involved in carrying out the experiment and the laboratory animal expert. There are various ways of recording the information, for example as records of an interview or a brief report. After the experiment has finished, the underlying information (observations and measurements) must remain available for at least 2 years.</p>
Reporting:	<p>This involves recording the substantive details of the implementation, the progress and the results of an experiment, in the form of a report or publication (task of researcher).</p>
Archiving:	<p>The Animal Experimentation Decree (Art. 10) includes provisions governing the periods for which data relating to laboratory animals must be stored. Information relating to the distress must remain available in a similar way, on the understanding that this may also be retained in the form of a summary (the welfare evaluation).</p>

APPENDIX 2

LEGAL FRAMEWORK

The legal basis for this code is:

Article 10.

1. It is forbidden to conduct an animal experiment with an aim:
 - a. that, according to general opinion and the opinion accepted amongst experts, can also be achieved in a way other than by means of an animal experiment, or by means of an animal experiment whereby fewer animals can be used or less distress caused than is the case in the experiment in question.

Article 12.

1. The person conducting the animal experiment is, notwithstanding his obligations to comply with the prevailing regulations relating to a permit or exemption applying to him, obliged to ensure that the animals are properly cared for and treated in accordance with or pursuant to the relevant regulations laid down by order in council.

Article 13.

1. The person conducting the animal experiment is obliged to ensure that the laboratory animals are spared distress as far as this is possible without compromising the experiment.
4. The person conducting the animal experiment is obliged to ensure that when one of the animals involved undergoes a procedure whereby if allowed to live, it will suffer distress other than for a short period, the animal will be destroyed immediately. If this compromises the experiment, the animal must be destroyed as soon as the experiment allows.

Article 15.

The person conducting the animal experiment is obliged to keep records relating to the acquisition of the laboratory animals and the experiments carried out, and to provide information to Our Minister, in accordance with the relevant regulations laid down by order in council.

Moreover, Article 10.5.h of the Animal Experimentation Decree stipulates the following:
The permit holder is also obliged to keep records per experiment of h. the risk of distress to animals involved in the experiment.

In the regulations on accommodation and care of the laboratory animals, Article 17 states:

1. The animals must be checked at least once a day. The inspections carried out and the findings must be recorded.
2. If an abnormality or suffering is discovered during the inspections, this must be remedied as soon as possible.

The conclusion is that proper insight into the distress of the animals must be obtained. This can only be achieved if a clear and concise picture of the progress and the distress being caused is made available during the experiment.

Article 1.4

For application of the provisions stated in or pursuant to articles 11-14 of this Act, with regard to the institutions of those people conducting activities as referred to in Article 1.1, the presence of animals is deemed to be the same as conducting animal experiments, unless it can be demonstrated that the animals are present for another reason.

APPENDIX 3

CLINICAL SYMPTOMS

Clinical symptoms can be ascertained in live animals without the need for special techniques. Various classification methods are possible, for example, according to the nature of the symptoms, the body part of the animal displaying the symptoms, or the organ system involved. It should be stressed that the aim of observing and recording clinical symptoms is not to make a diagnosis.

The following example is designed to clarify these terms:

An albino rat is sluggish and weak (general symptom) and its eyes are pale (observation of an anatomical body part). However, the animal does not have an eye disorder but anaemia, a disorder that can be seen in the eyes. Anaemia is a symptom of the blood-forming system (organ system). Closer examination shows that the blood contains abnormal cells that seem to indicate leucosis (this is not a clinical symptom but an additional observation). The post mortem shows the animal to have generalized leucosis (this is a diagnosis of the cause of the pale eyes and sluggish behaviour).

When drawing up lists of observations that need to be carried out (whether this involves many or few), the general symptoms, the symptoms according to anatomical body parts and the symptoms per organ system often tend to overlap. This is not necessarily a problem, as long as the list provides evidence of the most important or the most apparent symptoms.

Clinical observations per animal or per group of animals.

There are two dimensions involved in recording observations:

Observations	The time of the observations									

The following list of examples is neither limiting nor exhaustive. It merely provides a number of examples that could be used. The ultimate list depends on the type of animals and the experiment, and can comprise a small or a large number of sections. It need not only involve observations on damage to the animals' health or welfare. The birth of x number of young animals can also be considered a clinical observation.

Each term gives an English expression and a brief explanation.

GENERAL

- Found dead. The animal is found dead. Note down any special circumstances.
- Decreased appetite/ water intake. This can be measured (food/water intake) or estimated (residual food).
- Abnormal excreta. The quantity and quality of excrement products in the cage or sometimes on the animal.
- Decreased growth/ bodyweight. It is better to measure this as estimates are inaccurate. The value should be compared with the normal weight for an animal of this type, strain and age, or (at the individual level) with the last recorded weight for that animal and/or the highest known weight of that animal.
- Hypo/hyperthermia. Ascertained by physical examination of the extremities and obviously by taking the animal's temperature.
- Decreased activity. Determined by observation and then by monitoring. Observing nocturnal animals in infra-red lighting can be more sensitive than in ordinary lighting.
- Unkempt. The animal does not clean its coat, nose, etc.
- Abnormal posture. The animal walks, stands or lies in an abnormal posture (description), for example, hunched back.
- Emaciation. Serious decrease in subcutaneous fat and muscle tissue.
- Dehydration. Sunken eyes, skin does not restore itself when pinched and let go.
- Fluid accumulation. Subcutaneous (oedema), abdomen (ascites), etc.
- Piloerection. Raised hairs, often in combination with hunched back (serious symptom).
- Colour. Colour of skin, mucous membranes, eyes as indication of the quality of circulation (abnormalities: pale, blueish, yellow).

HEAD

- Expression. Abnormalities in attention and mood can often be observed in the head.
- Salivation. Profuse saliva production.
- Mouth breathing. Breathing via the mouth.
- Eating. The animal has problems taking, chewing or swallowing food or water.
- Dentition. Abnormalities in the number, the shape, the position or the structure of the teeth.
- Nasal discharge. Profuse discharge from the nose (describe the nature: mucous, pus, blood).
- Eyes. Position (e.g. sunken), discharge (nature), abnormalities of the eye itself (colour, shape), abnormal movement.
- Ears. Abnormalities in the outer ear or auditory passage.
- Deformities. Abnormalities in the symmetry or the shape of the head.
- Abnormal head position. Abnormal position in relation to the body, hanging or twisted head.

BREATHING

- Frequency. The frequency can be estimated (fast or slow) or timed (breaths per minute).
- Breathing type. The breathing quality: shallow, deep, heaving abdomen when exhaling, etc.
- Abnormal breathing sounds. Coughing, sniffing, snoring.

MOVEMENT AND BEHAVIOUR

- Hypo/hyperactivity. Animal is over/under active, and too spontaneous in response to stimuli.
- Impaired use of limbs. The animal cannot use one or more of its limbs, or avoids use because of pain.
- Weakness. Loss of strength (can be assessed by trial and by testing the reflexes).
- Ataxia. Impaired coordination of the limbs during movement.
- Avoidance. Animal avoids the observer (obviously in relation to previous observations).

- Painful reaction to handling. The animal squeals, bites or tenses its entire body when picked up.
- Stereotypical behaviour. The animal stimulates itself by repeating its actions when it is bored, or during stressful events.
- Seizures. Abnormal and unconscious generalized muscle activity. Training is needed to classify this symptom in any more detail.
- Nystagmus. Abnormal eye movement (moves slowly and shoots back) occurring as a result of brain abnormality (anaesthetic, pathology).

SKIN AND SKIN DERIVATIVES

- Coat. Abnormalities in thickness, structure, colour.
- Skin inflammation. Abscesses, redness, etc.
- Skin lesions. Wounds, scabs, scars.
- Degeneration. Necrosis, pressure marks, flaking.
- Neoplasms. Superficial tumours, including milk duct tumours.
- Nails, hoofs. Quality, soundness.

OTHER

This includes targeted observations specific to the type of animal (e.g. ruminating in ruminants), the living situation (e.g. the birth of young in breeding animals) or certain trial-based procedures (e.g. checks after surgery or extra dosage of a substance). Targeted observations can also require the use of simple instruments (taking the temperature, etc.).

APPENDIX 4

CLINICAL SCORE SHEET (OBSERVATIONS LIST)

In its simplest form, this is a table used to record clinical observations on the basis of the symptoms mentioned above.

The following table is for a single animal:

	time 1	time 2	time 3	time 4	time 5	time 6	time 7	time 8	time 9	time 10	time 11
symptom 1											
symptom 2											
symptom 3											

other (to be completed)											

The times can refer to short intervals (hours after an operation) or longer intervals (e.g. weekly), or a combination of both.

In theory, this can be compared with other methods of recording, for example a weekly observation of all animals from a certain perspective. The recent history of each animal should be easy to find at the location. This is a point that should be considered when deciding where to store the data (in a computer, for example).

APPENDIX 5

EXAMPLE OF A WORKING PROTOCOL, WELFARE DIARY AND WELFARE EVALUATION.

To illustrate the concrete requirements of this Code, this appendix contains examples of all three aspects.

EXAMPLE OF A WORKING PROTOCOL

1. Title of the research
Generating antibodies against surface antigens in E. coli
2. Simple and concise description of the scientific background
Specific antisera are needed to characterize the E. coli bacteria that can cause illness in animals. These are generated in rabbits using specimens of E. coli cell walls.
3. The Committee on Animal Experimentation number
Committee on Animal Experimentation Colilab BV, number 99-13, approved
4. Names of those involved and responsible during the experiment (Art. 9 and 12)
Researchers (Art. 9): P. Techneut, deputy Q. de Baas
Bio-technicians (Art. 12): Piet Prik, Ferdinand Freund
Animal handlers (Art. 12): Kees Konijn, Viola Voedster
Laboratory assistants, serology: Barend Bloed, Sylvia Spinster
5. Information about the animals
Rabbits, 15 New Zealand White, approx. 4 kg, m/f, SPF origins from Rabbit Services International general partnership, Lutjebroek
6. **Accommodation**
See SOP 'Housing and handling rabbits'.
7. Nutrition
Rabbit pellets standard/ adlib. Remarks: Only 40 grams of pellets will be given on the day before a bio-technical procedure.
8. Experiment setup, surgical procedures / procedures
Experiment setup
All groups will be given similar treatment: repeated immunisations and blood tests according to a schedule, five antigens, three animals per antigen.
Surgical procedures / procedures
Date, procedure and party carrying it out according to the work schedule.

Carrying out the procedures

Animal handlers take care of the animals (food and water, fresh bedding). Daily observations and notes: on form 1 for the experiment as a whole, on form 2 for each animal. The animals should be cared for as usual. Restricted food on the day preceding the day of blood draining/immunisation in connection with sedation.

The bio-technician performs the immunisation and blood testing as follows:
Antigen (labelled as 1, 2, 3, 4 or 5, noted on the syringe with the emulsion and per animal, one syringe of 1 ml per animal) to be provided by the researcher. Use 21-G needle, inject SC in the back, no more than 0.25 ml per site. Shaving not necessary.

Blood samples taken using a vacutainer system (5 or 10 ml phials), from the ear artery, using 21-G needle (disinfect the ear with 70% alcohol first).

Total blood draining via heart puncture, using an 18-G needle connected via a tube to a bottle drawn under pressure.

Anaesthetic: sedation (Hypnorm i.m.) for taking blood samples and immunisation, general anaesthetic (Nembutal i.v.) for blood drainage.

9. Welfare diary

According to the schedules in the appendix (see forms 1 and 2).

Expected distress:

Moderate, as a result of reaction to local inflammation and repeated bio-techniques.

In the event of problems:

- What to do in the event of problems:

The researcher must be alerted in the event of any problems, and otherwise the bio-technician.

Action must be taken on the same day in the event of serious distress.

- What is the relevance of the symptoms for welfare:

Inflammation of injection site: moderate

- Frequency of observation and records:

Note down abnormalities for the group as a whole on a daily basis, including climate etc. (Form 1). Note down abnormalities per animal on Form 2.

- What are the indicators (give criteria!) for removing animals from the experiment, or euthanizing them prematurely?

In the event of more than moderate distress, for example, a broken back, abnormalities in the teeth.

PLEASE NOTE: it is important to extract the serum if the animal is euthanased prematurely. Contact the laboratory assistant in plenty of time! The following people can be called at weekends:

Barend Bloed (tel.: 06-53124689) or Sylvia Spinster (tel.: 22465711 or 22465712 (at her mother's)).

10. Welfare evaluation

At the end of the experiment, consultations should be held by all parties concerned (initiated by Technaut). They will discuss how the experiment went and whether the emulsion used for immunisation caused too much inflammation.

EXAMPLE OF A WELFARE DIARY

FORMS 99-13, completed for a specified period.

FORM 1

Daily GENERAL control of animal accommodation and animals

Relates to experiment no. 99-13 in room no. Rabbit Room 1.3

Date	Time	Remarks, action	Initials
25-12-1999	1200	No abnormalities	VV
26-12-1999	930	Temperature 24 °C, alerted TD, solved	VV
27-12-1999	1000	No abnormalities	KK
28-12-1999	1100	No abnormalities	KK
29-12-1999	900	No abnormalities	VV
30-12-1999	1400	No abnormalities	PP
31-12-1999	930	No abnormalities, restricted food	FF
1-1-2000	800	No abnormalities, blood sample taken, immunisation	PP, FF
2-1-2000	1130	No abnormalities	PP
3-1-2000	1600	No abnormalities	VV
4-1-2000	1100	No abnormalities	KK

FORM 2

Observations and action PER ANIMAL.

Experiment no. 99-13 Animal no. 13

Date	Time	General (text)	Score back 0,1,2,3	Score ear 0,1,2,3	Initials
2-1-2000	1130	Nervous	2	1	PP
3-1-2000	900	Calm	1	0	VV
9-1-2000	1000	No abnormalities	1	0	KK
16-1-2000	900	No abnormalities	0	-	VV
23-1-2000	1500	No abnormalities	0	0	VV
2-2-2000	900	Sluggish, poor appetite	3	2	FF
3-2-2000	1000	Normal, good appetite	1	1	PP
9-2-2000	900	Removed from experiment (injected with wrong antigen)	0	0	PP, PT

Fill in '0' if there are no abnormalities, and '-' if no targeted observations were made. Notify the researcher in the event of a 3 in score for back or score for ear.

EXAMPLE OF A WELFARE EVALUATION

Evaluation 99-13

Consultation 21 July 2000.

Present: QB (chair), PP (report), FF, KK, VV, BB. PT arrived later.

The chair stated that the sera were good, and that it was a pity that animal 13 had been removed from the experiment (wrong dosage).

KK said that the animals had become too fat. VV thought they were calm.

The new adjuvant had worked well and leaves fewer marks than the traditional Freund's. PP comments that it should be taken out of the fridge sooner, as it is difficult to inject when cold.

No problems taking blood samples. The serum samples were good and the final harvest was 40 ml/kg. VV asks whether the amount of blood per kg is always the same. Then it is a good thing if the animals are fat.

All in all, the experiment went well (PT agrees). The syringes with serum should be more clearly labelled next time. It is easiest if one particular antigen is finished at a time.

The score for the level of distress was moderate for all animals except animal no. 13. The distress level for animal 13 was slight/moderate.