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**GUIDANCE DOCUMENT ON THE RECOGNITION, ASSESSMENT, AND USE
OF CLINICAL SIGNS AS HUMANE ENDPOINTS FOR EXPERIMENTAL
ANIMALS USED IN SAFETY EVALUATION**

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**GUIDANCE DOCUMENT ON THE RECOGNITION, ASSESSMENT, AND USE OF
CLINICAL SIGNS AS HUMANE ENDPOINTS FOR EXPERIMENTAL ANIMALS
USED IN SAFETY EVALUATION**

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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The Environmental Health and Safety Programme co-operates closely with other international organisations. This document was produced within the framework of the Inter-Organisation Programme for the Sound Management of Chemicals (IOMC).

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organisations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. UNITAR joined the IOMC in 1997 to become the seventh Participating Organisation. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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or contact:

**OECD Environment Directorate,
Environmental Health and Safety Division**

**2 rue André-Pascal
75775 Paris Cedex 16
France**

Fax: (33-1) 45 24 16 75

E-mail: ehscont@oecd.org

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HISTORY OF THE DOCUMENT

1. In 1994, an *ad hoc* Working Group was formed to develop an OECD Guidance Document that would provide guidance on when laboratory animals used in toxicity testing studies should be euthanized for humane reasons. Current OECD Test Guidelines generally state that animals that are moribund or obviously in pain and showing signs of severe and enduring distress should be humanely killed. The objective of the Guidance Document is to provide useful guidance and criteria for determining when an animal is in a moribund condition, or expected to become moribund, or experiencing significant pain and distress, and should therefore be euthanised.

2. The members of the initial Working Group were: Dr. Marga Bos-Kuijpers (TNO Nutrition and Food Research Institute, The Netherlands); Prof. David B. Morton (Centre for Biomedical Ethics, University of Birmingham, UK); Dr. Eva Schlede (BgVV Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany); Dr. William S. Stokes (National Toxicology Program, NIEHS, USA)

3. The Working Group met on 14th February 1995 to discuss criteria and other guidance for defining pain/suffering of animals used in toxicity testing with the aim of harmonising the decision-making process as to how and when to humanely kill suffering animals in toxicity studies. The group used its discussion of a background document drafted by Prof. Morton which laid the groundwork for this OECD Guidance Document. The Guidance document was next circulated to the National Co-ordinators and National Experts of the Test Guidelines Programme for review on 2nd October, 1998.

4. On 19th –20th November 1998, a Nominated Expert meeting was held in Zeist, The Netherlands, to critique and redraft a guidance document taking into account comments received from member countries. A list of participants is attached to this document as Annex 1. The following represents the consensus of the Nominated Experts and includes additional comments and suggestions from national experts and the National Co-ordinators of the Test Guidelines Programme.

PREAMBLE

5. The purpose of this Guidance Document is to apply the principles of the Three Rs to the use of animals in regulatory toxicity tests. The OECD encourages the humane use of animals in regulatory toxicity and safety evaluation studies and fully endorses the principles of the 3Rs, Replacement, Reduction, Refinement, which were defined by Russell and Burch (1) as:

- Replacement – “the substitution for conscious living higher animals of insentient material.”
- Reduction – “reduction of animals used to obtain information of given amount and precision.”
- Refinement – “any decrease in the incidence or severity of inhumane procedures to those animals which still have to be used.”

6. This document specifically addresses Refinement.

7. This guidance is based on best current knowledge available from Member Countries' experts, through personal contacts with investigators, peer-reviewed literature, and presentations at meetings and symposia, and is intended to be flexible so that it can change with improved knowledge in the future. It is expected that with increasing knowledge and experience, investigators in animal research will be able to identify more specific, early humane endpoints in the form of clinical signs for impending death or severe pain and distress. This would permit international harmonisation of these humane endpoints.

8. Although the principles of the 3R's are applicable to all animal species, it is generally accepted that there are differences among species in many clinical signs of pain or distress. Variables due to the species and strain of animal involved, the type of toxicity study being performed, and the types of materials being tested, are not addressed in detail. Although there are a number of similarities between mammals and other vertebrate species, the differences among the different classes of vertebrates do not allow them to be easily addressed in a single document. The general principles contained in this Guidance document are specifically designed to be applicable for all mammalian species used in toxicity testing and other experimental studies.

DEFINITIONS, EXPLANATIONS AND EXAMPLES OF RELEVANT TERMINOLOGY

Humane Endpoint:

9. A humane endpoint can be defined as the earliest indicator in an animal experiment of severe pain, severe distress, suffering, or impending death.

10. The ultimate purpose of the application of humane endpoints to toxicology studies is to be able to accurately predict severe pain, severe distress, suffering, or impending death, before the animal experiences these effects. However, the science of toxicology is not yet to the point where such accurate predictions can be made *prior to* the onset of severe pain and distress. It is possible at this time to identify pain, distress, or suffering, very early after their onset by careful clinical examination of animals on test using well-defined endpoints and criteria. Humane endpoints for use in research and testing have been addressed in a number of publications (2)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13). These adverse conditions, once identified should be minimised or eliminated, either by humanely killing the animal or, in long-term studies by (temporary) termination of exposure, or by reduction of the test substance dose.

11. Different animal species, and animals at different stages of development, may respond differently to test conditions, and exhibit different indications of distress. The clinical signs described here should be evaluated in consideration of these potential differences. If relevant humane endpoints have been identified, they should be described when an experiment is being planned, and incorporated into the experimental protocol and all related standard operating procedures (SOPs).

Death:

12. The stages leading to death can be characterised as:

- Predictable Death: presence of clinical signs indicative of death before the planned end of the experiment; for example: inability to reach water or food.

- Impending Death: when moribund state or death is expected prior to the next planned time of observation. Signs indicative of this state in rodents could include convulsions, recumbency, and tremor.
- Moribund: being in state of dying or inability to survive, even if treated.

Pain:

13. Pain can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (14).

14. Pain can be:

- Acute nociceptive pain: pain response evoked by a brief noxious stimulus which produces no tissue damage. This form of pain is not regarded as severe.
Example: pedal reflex
- Persistent (chronic) inflammatory pain: the pain resulting from tissue damage lasting for the duration of the damage or the ensuing inflammatory process, and may persist after the local tissue damage has healed. This type of pain may be severe or distressing, particularly if long lasting or permanent.
Example: self mutilation, localised infection
- Neuropathic pain: pain as a result of compromised function or abnormal activation of the peripheral or central nervous system (14). Neuropathic pain is always considered as severe and distressing pain.
Example: the presence of a large internal tumour that compresses nerves.

15. Objective signs of pain can include vocalisation, evidence of infection, aversion or avoidance by active withdrawal from stimuli, guarding affected body parts, or self mutilation. Reduced food intake may be a sign of chronic pain.

Distress:

16. An aversive state resulting from maladaptation or inability to adapt to stressors. Physical or behavioural alterations may be signs of stress. Acute stress is not regarded as a cause of distress; it may be necessary to optimise vigilance and to reduce the risk of boredom (15). Distress is usually associated with a change in motility or locomotion, and can result in stereotype behaviour.

17. The major stressors associated with distress are situations that may give rise to marked pain, fear, or anxiety. Retreat to the corner of the cage or excessive struggling or vocalising on dosing are examples of distress in anticipation of an experimental procedure.

Suffering:

18. A negative emotional state that in human beings is produced by persistent pain and /or distress. It should be assumed that persistent pain or distress in animals leads to suffering of animals in the absence of evidence to the contrary. If something is known to cause suffering in humans, it should be assumed to cause suffering in animals.

Expert Professional Judgement:

19. All decisions related to the application of humane endpoints should be made by the Study Director, or designated responsible person, after consultation with the team of experts, which includes the Principal Investigator (if different from the Study Director), the veterinarian, and an experienced animal technician. This team of experts will consult available guidance and this Guidance Document, and exercise its professional judgement. The study protocol should clearly define the conditions under which it is necessary to immediately and humanely kill an animal. The goal of the experimenter should be to use humane endpoints to minimise pain, distress, or suffering to the extent possible without compromising the scientific objectives of the experiment.

GUIDING PRINCIPLES

20. In recognition of the fact that there is strong scientific evidence that pain, distress, and suffering (for definitions, see Annex 3) can exist in animals, the guiding principles are that:

- There is strong scientific evidence that pain and distress are present in animals in comparable situations as they occur in humans (16)(17).
- Severe pain, suffering, or death are to be avoided as endpoints.
- Studies must be designed to minimise any pain, distress or suffering experienced by the animals, consistent with the scientific objective of the study.
- The earliest possible endpoints that are indicators of distress, severe pain, or impending death that should be used as indications for humanely killing the animals should be determined prior to the animals' reaching a moribund state (12).
- Studies should be terminated prior to their anticipated termination time if the objectives of the study have been satisfied, or if it is obvious that they will not be achieved.
- Studies should build on existing knowledge about the substance to be tested. This enables better prediction of the likely signs and timing of adverse effects, and allows those conducting the study to incorporate these endpoints into the experimental protocol and the related SOPs.
- The successful application of humane endpoints is dependent on the involvement of all members of the study team who should be adequately trained and aware of their individual roles and responsibilities, e.g.,
 - the Study Director or designated responsible person (design, protocol development, study monitoring, interpretation of results).
 - the veterinarian (advice on interpretation of clinical signs)
 - the animal caretaker/technician (observation, action, husbandry, care)
 - an ethical review committee or a prescribed ethical review process.

- Study Directors, and the other responsible individuals should be free to exercise professional judgement in the design and conduct of the experiments.
- All aspects of animal studies should be subject to an ethical review process as defined by animal welfare legislation and the ethical oversight groups of the testing organisation. Where such legislation is not available, it may be necessary for the laboratory to develop its own ethical guidelines and procedures.
- Conditions under which interventions should be made to alleviate pain and distress by humane killing, and the individuals who are adequately trained and authorised to kill the animals, should be defined in the protocol or the SOP.

21. This document describes procedures that can be put in place to minimise test animal pain, distress, and suffering during regulatory toxicity testing. The considerations and recommendations presented here are applicable to all laboratory animal studies.

INITIAL CONSIDERATIONS

22. In order to meet the intended objectives while minimising pain, distress, and suffering, it is essential to collect as much information as possible about the substance to be tested prior to designing the toxicity study.

23. Possible sources of information include:

- literature searches for previous studies using the test substance or related substances
- results from physico-chemical tests
- molecular modelling
- results from *in vitro* tests
- results from prior *in vivo* tests (e.g., efficacy tests; earlier toxicity tests; dose-ranging studies; pilot studies)
- statistical review of the available data and the experimental design to identify the fewest number of animals and doses that can be used without compromising the objectives of the study.

24. This will help to:

- define the objectives of the test, and the information that will be obtained
- determine whether the results which would be generated from the study would duplicate previous work

- select the most appropriate species for the study
- determine how best to design the protocol to satisfy the objectives of the test
- identify potential clinical signs and estimate the timing and duration of their occurrences
- determine any special training needed by personnel involved in the conduct of the study

Preliminary/Pilot Studies

25. Preliminary or range-finding studies are often used to determine the appropriate dose-range to use in an experiment in the absence of other information about the test substance. The dose-range study should also be used to obtain data (using clinical, biochemical, or other parameters) that can provide information useful to the identification of early endpoints as indicators of severe pain or distress which could be used in the decision to either complete the study, to terminate the study before the animals experience severe pain or suffering (12), or to determine whether analgesia or anaesthesia will be needed and can be used. If there is no information relevant to the determination of early endpoints as indicators of pain or distress, a separate pilot study may have to be performed. If a pilot study is performed, it should use only the minimum number of animals consistent with the objectives of the study. The information collected during range-finding or pilot studies should also be used to prepare or alert the study team for the actions or activities related to humane endpoints that may be needed.

Training

26. The Principal Investigator and the responsible committees (e.g., animal care and use; ethics committee) have the obligation for assuring that all individuals involved in a study have the expertise and training necessary for them to fulfil their roles. The individuals participating in the study who have direct contact with the animals must be experienced in observation of animals so as to be able to assess the physiology, behaviour, and appearance of the animals under study, and to determine if the animals are, or will be, experiencing pain or suffering. One measure of the expertise and training is a determination of whether the investigator has:

- identified and included in the protocol the earliest possible endpoint(s) for recognising impending death, severe pain, or severe distress consistent with satisfying the data needs of the study
- assured that the animals under test will not be subjected to conditions where unjustified and unalleviated pain and suffering are allowed to proceed

27. To address these points, a sample list of questions for both the Study Director and the animal care and use or ethics committee are attached as Annex 2.

RECOGNITION AND ASSESSMENT OF PAIN, DISTRESS, AND SUFFERING AS AN APPROACH TO DETECTING CLINICAL SIGNS AND ABNORMAL CONDITIONS

28. In order to recognise clinical signs of pain and distress, it is imperative that the observer be familiar with the normal and abnormal characteristics of each of the species used in a study. This is

particularly important because some species may not show obvious physical or behavioural changes even when in severe pain and/or distress. As discussed earlier in this document, because pain and distress are known to produce suffering in humans, it should be assumed that they would also produce suffering in animals.

29. An animal's response to a test substance results from the interaction of the substance with its organs, tissues and cells. Those interactions may produce adverse effects, i.e., toxicity, that are expressed as clinical signs and physiological changes. Such information can provide valuable insights into the mechanisms of toxicity, and can serve as the basis for identifying appropriate humane endpoints. Awareness of these potential clinical signs and conditions, and the ability to identify them (18), increases the likelihood of their accurate and timely detection.

30. Careful and regular observation and examination of test animals is essential for the detection of clinical signs and abnormal conditions. General observations should be made daily while more detailed examinations should be performed at least weekly, or more often, once abnormal clinical signs have been detected. Findings of abnormal conditions must be accurately documented, including onset, duration, and severity. Such documentation provides the basis for determining the presence and severity of pain and distress. This documentation also provides the basis for identifying signs and conditions that might be used as earlier endpoints for a study, as proposed by Morton (12)(19) and described in Table 1 of this document. Such observations and measurements can also be important indicators of the condition of the animal, and used to determine if the condition of the animal is irreversible and therefore an indication of impending death. In addition, post-mortem examination can be helpful to relate post-mortem findings to previous clinical signs. Thus, for both scientific and animal welfare reasons, recognition and assessment of clinical signs and abnormal conditions is essential for all toxicology studies involving animals.

31. There are several considerations in determining humane endpoints for toxicity studies. They all require frequent objective determinations of any deviations from an animal's "normal state", followed by a correlation of these changes with the possibility and severity of pain, distress, and/or discomfort (20) (see Annex 3). These considerations include:

- making appropriate clinical observations of the animals to detect abnormal signs and conditions (behaviour, physiology, etc.), and other indicators of welfare problems
- determining when such observations are indicators of pain and distress, and determining if the pain and distress are severe
- determining, when abnormal conditions that are not necessarily considered to be indicative of severe pain and/or distress, are indicative of an irreversible condition likely to lead to further deterioration (e.g., moribund condition; impending death; inability to eat or drink)

32. The Study Director must make a determination as to whether further information useful for the purposes of the study is likely to be obtained. If not, then a decision should be made to humanely kill the animal.

33. There are a number of effects involved in the adequate evaluation of an animal to determine its condition and whether there might be evidence indicative of pain and or distress (20):

- changes in physical appearance (e.g., coat texture; hair soiled with urine or faeces)
- changes in clinical signs (e.g., respiration rate; posture)

- changes in unprovoked behaviour (e.g., self mutilation; compulsive behaviour)
- behavioural changes in response to external stimuli (e.g., excitability; righting reflex)
- changes in body weight, and related changes in food and water consumption
- changes in clinical parameters (e.g., body temperature; heart and respiration rate; clinical chemistry and haematology)

Changes in external physical appearance and other clinical signs

34. A list of commonly observed clinical signs and conditions is provided as Annex 3. This list does not encompass all of the possible observations that might be made. Each study could have a standard list of clinical signs readily available that might be observed for that particular type of study, and that are appropriate for the species used. Animals should be examined regularly by appropriately trained staff and should be removed from their cages at least once weekly for weighing and detailed clinical examinations. The frequency of such examinations will depend on the species, whether any previous abnormalities have been observed, the timing and nature of the anticipated toxic effects, and the objectives of the study. For instance, an examination should be performed at least weekly for rodent species, and at least daily if abnormal clinical signs have been detected. Any previously detected lesion or abnormality should be carefully assessed, and all findings documented with regard to time of onset and severity. It is usually convenient to weigh animals at the time of clinical examination.

Behavioural signs

35. Although animals should preferably be observed during their natural, active period, without undue disturbance of the primary cage or pen, this practice is not always feasible. Because rats and mice are nocturnal, and tend to sleep during the day when various behavioural signs are not as apparent, the animals should preferably be observed during their active period, either during the night or, more practically, under red light during the day under a reversed day/night schedule. When this is not practical, daytime observation of sleeping patterns may be indicative of an absence of pain or discomfort. The animal's appearance, posture, grooming patterns, and activity levels should be noted, and a determination made about whether the behaviour is normal or abnormal. If any abnormality is noted, then it may be appropriate to assess the animal's response to an external stimulus, for example, checking the responsiveness of an animal that is recumbent and immobile (see Annex 3).

Body weight changes

36. Significant body weight loss may be one of the most sensitive indicators that an animal's condition is deteriorating. Body weight loss is usually accompanied by a change in food and water consumption, which should also be closely monitored by animal care staff. In young animals that have not reached their adult body weight, an abnormal condition may be indicated by a reduced rate of weight gain when compared to the appropriately matched control animal, rather than an actual weight loss.

Measurable clinical parameters

37. Body Temperature: Hypothermia and hyperthermia can serve as important indicators of a deteriorating clinical condition of an animal. For example, studies have documented that a 10% decrease in body temperature may be predictive of impending death (21)(22). Thus, consideration should be given to the monitoring of body temperature and the evaluation of specific temperature decreases that could serve as appropriate endpoints for humane killing of an animal. Telemetric devices and electronic implantable

transponders which can also uniquely identify an animal are available and can facilitate efficient temperature monitoring without handling of the animal (12)(23). Hypo- and hyperthermia that may be transient effects of the test chemical should be distinguished from these effects when they result from a deteriorating clinical condition.

38. Treatment-related, significant changes in heart rate and respiration rate can also be indicative of pain and distress in animals, and consideration should be given to the use of these and other physiological parameters in monitoring animals.

39. Clinical Chemistry and Haematology: Various clinical chemistry, urinary, and haematological parameters can provide an indication of an animal's condition (12). Consideration should be given to collecting and monitoring parameters that may be useful in assessing an animal's well-being. For instance, such parameters can be used to detect and characterise the severity of various conditions, such as organ (e.g., renal; hepatic) dysfunction and/or failure, anaemia, leukaemia, and dehydration. Care must be exercised not to induce anaemia or other adverse effects when taking blood samples from small animals.

Recording an Animal's Condition

40. Observational "checklists" can be used for recording the animal's condition in a study (19), and can serve as the objective basis for decisions on humane endpoints for an animal. The clinical sign should be reduced to an observation that can be recorded as present or absent to minimise observer error. One advantage of a checklist is that specific observations that are likely to occur, or considered critical to the study, are not overlooked and are unambiguously recorded. The use of checklists may also assist in improving observational skills and staff training. However, it is important to recognise that such checklists will usually not cover all possible conditions, and thus should be designed so that other observations can be added by the observer. Computerised software programs are available that facilitate the documentation of clinical signs, and can be linked to electronic identification transponders (12)(23) and electronic weighing scales.

Frequency of Observation

41. After dosing for acute studies, animals are observed individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours. Thereafter, observations should be made at least daily on all animals (24), and should include, at a minimum, determination of a normal or abnormal status and the severity of any clinical signs. For long-term studies where the effects of the dose regimen are anticipated from earlier studies, the animals do not need increased observations immediately following dosing, but should be examined for clinical signs at least once a day. An increased frequency of detailed observations should be required for animals in toxicity studies following the onset of initial abnormal clinical signs. It is important to document when the signs occur in order to be aware of the duration of continuing and persistent effects. The combination of type of sign and its duration become important when assessing severity. The sum of all signs and their duration could be envisaged as the total pain and distress endured by the animal, or the severity and intensity of pain and distress at that point in time.

MAKING AN INFORMED DECISION TO HUMANELY KILL ANIMALS

42. The decision to humanely kill an animal must be made with appropriate clinical judgement, taking into account the severity of the condition, the amount of pain or distress, the prognosis, and the potential loss of valuable data. The majority of clinical signs (see Annex 3) are not sufficient, by themselves, to support a decision to humanely kill the animal. However, they are indications of possible pain or distress, and the animals should be carefully examined for other signs which may, in combination, be indicative of severe pain or distress.

Impending Death and Moribund Condition as Criteria for Humane Killing

43. Animals that are moribund or in a state of impending death should be humanely killed to avoid unnecessary pain or distress that they may be experiencing.

44. Impending death and/or moribund condition in laboratory animals can be indicated by various clinical signs and objective measurements (25)(26)(27)(28), some of which are shown in Table 1. Following adequate evaluation, a lesser degree of severity of these signs and measurements may also be useful indicators for predicting death, as previously defined. These signs and conditions typically include one or more of the following:

- prolonged, impaired ambulation preventing the animal from reaching food or water, or prolonged anorexia
- excessive weight loss and/or extreme emaciation and/or severe dehydration
- significant blood loss
- evidence to suggest irreversible organ failure
- prolonged absence of voluntary responses to external stimuli
- persistent, difficult laboured breathing
- prolonged inability to remain upright
- persistent convulsions
- self-mutilation
- prolonged diarrhoea
- significant and sustained decrease in body temperature
- substantial tumours
- other treatment-related effects judged to be indicative of impending death

45. Animal care staff must be adequately trained for each type of toxicity study to differentiate between clinical signs indicative of a moribund condition and similar, clinical signs that may be transient effects from acute dosing procedures.

Severe Pain and Distress As Criteria For Humane Killing

46. Information on the general signs of pain and/or distress for the various laboratory animal species used in toxicology studies are readily available (16)(28)(29)(30). The following clinical signs may indicate that an animal is experiencing significant pain and distress. Pain and distress should be alleviated with appropriate treatment if it does not interfere with the conduct or the objectives of the study, or the animal should be humanely killed if there are signs of severe pain and distress, such as:

- abnormal vocalisation
- abnormal aggressiveness
- abnormal posture
- abnormal reaction to handling
- abnormal movements
- self-induced trauma
- open wounds or skin ulceration
- difficulties in respiration
- corneal ulceration (the cornea is very sensitive to pain, and according to some, stages that precede ulceration are painful, but not the ulceration itself)
- bone fractures
- reluctance to move
- abnormal external appearance
- rapid weight loss or emaciation or severe dehydration
- significant bleeding
- or any other factor that suggests that the animal may be in pain or distress.

47. A list of severe signs and conditions that are indicators that the well-being of an experimental animal may be compromised is provided as Table 1 and Annex 4. The Annex is designed for use in animal rooms and facilities as a guide to alert staff to signs that require discussion and/or action. Emphasis is on recognition of situations where, for humane reasons, the animal should be humanely killed or the study discontinued. Ideally, maximum achievable information should be obtained from every animal used, while limiting pain and distress to an absolute minimum. The concept of humaneness focuses on using clinical

signs indicative of significant reduction in the well-being of the experimental subject as the basis for humane killing of the subject.

48. Animal tests require a team approach, and collaboration of the veterinary and animal care team with the scientific staff and those responsible for ethical review. Study Directors should work with, and co-ordinate staff to establish: the time and frequency of observations, how and when invasive measurements (e.g., blood sampling) are to be made, SOPs for checking and assessment, and standardised documentation and reporting of clinical signs. Considerations should include when animals are to be checked, taking into account such factors as predicted times that toxicity may occur. Written procedures should also describe what actions are to be taken by whom, and at what time.

49. The animal technician will generally be the first to observe the clinical signs and there should be a mechanism to bring this information to the attention of the attending veterinarian and the designated responsible person, usually the Study Director. Delegation of responsibility should be considered, as appropriate, to ensure that humane endpoints can be promptly implemented, as previously agreed, by trained individuals. Regardless of who has the responsibility for terminating an animal or a study, it is important that there be a means of reaching a responsible individual at all times (including evenings, weekends, and holidays). This individual must have the authority to take the decision to humanely kill the animal(s) based on personal observations or reports from the animal care team. Humane methods of killing must be used, and those killing the animals must be trained to do so and competent. Experiments should not be allowed to proceed longer than is necessary to achieve the purpose of the study (31).

METHODS FOR HUMANE KILLING

50. The reader is referred to several well prepared documents, including those prepared by the Canadian Council on Animal Care (19), UFAW (Universities Federation for Animal Welfare) (1987) Handbook on the Care and Management of Laboratory Animals (32), and the American Veterinary Medical Association (AVMA) and European Guidelines (33)(34).

GUIDANCE ON THE HUMANE CONDUCT OF SPECIFIC TYPES OF TOXICITY TESTING

51. Toxicity studies are conducted for safety assessment, and to determine the possible adverse effects of a test substance. At times the adverse effects of the test substance may unavoidably cause the test animals pain and/or suffering. This document provides guidance towards minimising pain and distress to the extent possible without jeopardising the purposes of these studies. This section provides additional guidance for specific types of tests. However, the guiding principles and considerations previously discussed should be followed for all types of toxicity studies.

Acute Single-Dose Studies

52. All available information should be considered before animal studies are planned. This should include, but not be limited to, the results of *in vitro* tests, structure-activity relationships, information on toxicity gained from any previous animal exposures to the test material or related substances, information on the mechanism of toxicity, and any other pertinent information. A pilot or sighting study is

recommended when it is not possible to predict reliably the dose(s) of a substance that are likely to cause adverse effects. Dosing animals sequentially may prevent exposure of more animals than necessary to the toxic effects of the substance under test.

53. Multiple observations of the animals should be made during the first few hours after dosing in initial single-dose studies. Critical clinical signs that require an informed decision on whether or not to humanely kill an animal for humane reasons shortly after dosing would include: convulsions, gasping, cyanosis, vocalisation, a conscious animal unable to move, or signs of similar significance to the animal's immediate well-being. If the animal is not conscious, it is assumed there is no pain and distress and in that case it is appropriate to observe the animal to determine if it will recover. All clinical signs must also be evaluated for severity and consideration should be given to whether and how rapidly the animal is recovering.

54. OECD Test Guidelines do not require death as an endpoint. Animals humanely killed during the test will be regarded as dosage-dependent deaths.

55. Three alternative test methods (Guidelines 420, 423, and 425) to the traditional acute oral toxicity test have been adopted by the OECD. One of these, the Fixed Dose Procedure (Guideline 420), is a refinement of the traditional acute oral test in that it requires fewer, but fixed, dosage groups to be tested, and thus fewer animals. It also employs non-lethal endpoints to determine the toxicity of the test substance. Two other methods, the Acute Toxic Class Method (Guideline 423) and the Up-and-Down Procedure (Guideline 425), use impending death as the only endpoint. These tests provide similar information as the traditional test, but require fewer animals. They similarly recommend humanely killing animals that are moribund or obviously in pain and showing signs of severe and enduring distress.

56. If there is prior information that the test material may be highly toxic, there should be strong scientific justification for further animal testing, and a step-wise testing procedure using individual animals should be followed. Acute oral toxicity testing should not be done to confirm that a material is highly toxic if this judgement can be made based on other information.

57. Table 1 provides a summary of the types of clinical signs that were observed most frequently in the international validation study of the Acute Toxic Class Method.

Ocular Irritation Studies

58. All guidance provided for acute studies should be followed. As with other types of studies, all information available should be considered before animal studies are conducted. For ocular studies this should include, but not be limited to, results of *in vitro* tests, structure-activity relationships, pH <2 or >11.5, acute dermal toxicity, dermal irritation/corrosion studies, and information on toxicity gained from any previous animal exposures to the test substance or related substances. Test Guideline 405, updated in 2000, includes a testing strategy that addresses these considerations prior to animal testing. Ocular irritation studies should not be done to confirm that a material is severely irritating if this conclusion can be made based on other information. It is recognised that this provides only general guidance and does not predict irritation for all types of materials. In particular, dermal irritation may not predict eye irritation. The pH of the test sample should also be considered in conjunction with other information such as alkaline or acid reserve and osmolarity. Both of these factors have been recognised by regulatory agencies and others to mitigate pH effects (35)(36)(37)(38). If available information strongly suggests the material may be a severe irritant, there should be strong scientific justification for animal testing, and a step-wise testing procedure using individual animals should be followed.

59. If a pronounced response is produced in one animal, the substance should be classified as a severe irritant with no further testing.

60. Critical clinical signs that require an informed decision on whether or not to humanely kill an animal shortly after dosing are those listed above for all acute studies (Table 1). For eye irritation endpoints, if no ocular lesions have developed after three full days post installation, the animals can be removed from the study because further evaluation is not required. Local anaesthetics should be considered for use wherever possible (28) keeping in mind that they may affect the extent of irritation by compromising clearing of substances by the normal blink and tearing reflexes.

Systemic Repeated-Dose Studies

61. The guiding principles and considerations previously discussed should be followed for systemic, repeat-dose toxicity studies. All available data from acute studies should be used in the design of the study so as to determine the earliest endpoints that will not jeopardise the scientific integrity of the data but will minimise pain and suffering.

62. In studies involving repeated dosing, when an animal shows clinical signs that are progressive, leading to further deterioration in condition, an informed decision as to whether or not to humanely kill the animal should be made. The decision should include consideration as to the value of the information to be gained from the continued maintenance of that animal on study relative to its overall condition. If a decision is made to leave the animal on test, the frequency of observations should be increased, as needed. It may also be possible, without adversely affecting the purpose of the test, to temporarily stop dosing if it will relieve the pain or distress, or reduce the test dose.

Reproductive Toxicity Studies

63. The general guiding principles as described for acute and systemic toxicity studies should be followed. Offspring with abnormalities that could affect their quality of life should not be used for subsequent pairings.

Sensitisation Studies

64. All general guiding principles, as described above, should be followed. In testing for immune-mediated reactivity, animals are typically challenged after preparative immunisation. If anaphylactic responses are observed in more than one animal, additional animals should not be challenged at that dose.

Chronic Toxicity and Carcinogenicity Studies

65. Apart from possible treatment-related effects, in chronic experiments, a considerable number of animals will develop spontaneous disease and other pathologies (39)(40). In full life-span experiments, in the absence of lethal treatment-related effects, all animals will eventually die of spontaneous disease (see many general and species/strain specific references). Animal care should also be directed toward reducing the discomfort caused by these spontaneous conditions. The extent of this intervention will depend on the specific nature of the experiment. In practice, in rodent studies veterinary intervention is restricted to routine animal care (e.g., cutting of overgrown incisors). In most instances timely humane killing is the only means of terminating the pain and distress when chemical analgesia cannot be used. As is currently the situation in non-rodent studies (e.g., dogs; primates), the veterinarian may need to provide a greater level of intervention for routine treatment of individual animals.

66. In general, if continuing pain and distress are apparent, if the prospect of recovery is poor, or if the condition is likely to interfere with the experiment, an informed decision as to whether or not to humanely kill the animal should be made. Should a severe health disorder develop in a group of animals, termination of the experiment or the affected dose group(s) should be considered.

67. A sensitive, objective sign of health problems and of pain and distress is the body weight of individual animals. Weight loss may point to wasting diseases (cancer, chronic renal disease, etc.), pain and distress, or inability to eat (incisor overgrowth for instance). It is therefore recommended that the animal be weighed at least weekly (rodent studies). The body weight must be compared not only with the weight of the previous week, but also with the highest weight known for that animal in order to detect chronic wasting. Additional considerations are the general appearance of the animal and the presence of any conditions that might cause weight gain, such as large tumours.

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Table 1: Summary of clinical signs observed in rats during the validation studies of the Acute Toxic Class Method*

Clinical sign	Number of rats ⁽¹⁾	Dead/Moribund rats ⁽²⁾	%
Convulsion,			
- unspecified	43	43	100
- clonic	218	207	95
- tonic	96	79	82
- tonic-clonic	125	122	98
- saltatory	10	10	100
Lateral position	223	177	79
Tremor	389	296	76
Gasping	143	108	76
Vocalisation	97	79	81

*from (Schlede et al. See reference N° 41)

⁽¹⁾ Number of rats: number of animals showing the observation out of the total number of 3942.

⁽²⁾ Dead animals: last clinical signs before found dead; Moribund animals: last clinical signs before humane killing.

ANNEX 1

**List of Nominated National Experts to the Nominated Expert Meeting on Harmonisation of Criteria
Indicative of Severe Suffering of Experimental Animals
Zeist, The Netherlands 19th-20th November 1998**

ALLEMAGNE/GERMANY	
Dr. GUBER Franz Schuetzenstrasse 14 Postfach 10 01 25 D-78462	Tel: 49 75 31 243 46 Fax: 49 41 1 422 7070 Email: altex@bluewin.ch
Dr. SCHLEDE Eva Bg VV Federal Institute for Health Protection of Consumers & Vet Medicine Postfach 330013 D 14191 Berlin	Tel: 49 30 84123296 Fax: 49 30 8412 3851 Email: e.schlede@bgvv.de
Dr. SAUER Ursula Academy for Animal Welfare Spechtstr. 1 D-85579 Neubiberg	Tel: 49 89 6002910 Fax: 49 89 60029115 Email: akademie.fuer.tierschutz@muenchen.org.de
CANADA/CANADA	
Dr. GAUTHIER Clément Executive Director Canadian Council on Animal Care 315-350 Albert Street Ottawa, ON K1R 1B1	Tel: 1 613 238 4031 Fax 1 613 238 2837 Email: Rfauteaux@bart.ccac.ca
ESPAGNE/SPAIN	
Professor CERVERO SANTIAGO Fernando Dpto de Fisiologia Edificio de Medicina Universidad de Alcala de Henares Madrid	Tel: 34 91 885 45 95 Fax: 34 91 88 54 807 Email: Fernando.Cervero@uah.es
ETATS-UNIS/UNITED STATES	
Dr. SASS Neil US Food and Drug Administration Center for food Safety & Applied Nutrition Division of Toxicological Research (HFS-505) 8301 Muirkirk Road, MOD-1 Laurel, MD 20708	Tel: 1 301 594 5800 Fax: 1 301 827 1236 Email: nls@vm.cfsan.fda.gov
Dr. STOKES William National Toxicology Programme (MD-EC17) NIEHS 11 TW Alexander Drive Research Triangle Park, NC 27709	Tel: 1 919 541 7997 Fax: 1 919 541 0947 Email: stokes@niehs.nih.gov

FRANCE/FRANCE	
Dr. LAROQUE Phillipe Head of Pathology Dept Laboratories Merck Sharp et Dohme Chibret Route de Marsat, B.P.134 63203 Riom Cedex 9	Tel: 33 4 73 63 49 97 Fax: 33 4 73 38 56 91 Email: phillipe.laroque@merck.com
Dr. SCHORSCH Frédéric INERIS Dept Toxicology-Ecotoxicology Parc Technologique ALATA B.P.2 60550 Verneuil-en Halatte	Tel: 33 3 44 55 63 13 Fax: 33 3 44 55 66 05 Email: Frederic.Schorsch@INERIS.fr
ITALIE/ITALY	
Dr. LAVIOLA Giovanni Laboratory of Organ and System Pathophysiology Istituto Supeiore di Sanita Viale Regina Elena,299 00161, Roma	Tel: 39 06 4990 2105 Fax: 39 06 4957 821 Email:laviola@iss.it
PAYS-BAS/THE NETHERLANDS	
DR. DORTLAND Paul Laboratory of Pathology and Immunology RIVM PO Box 1 3720 BA Bilthoven	Tel: 31 30 27 7426 81 Fax: 31 27 42 744 Email: p.dortant@RIVM.nl30
Dr. Van IERSEL Arthur Institute' Centre of Alt. to Animal Testing Lab. for Medicines & Medical Devices Nat. Inst.of Public Health & Environ. P.O. Box 1 3720 BA Bilthoven	Tel: 31-30 27 420 56 Fax: 31-30 27 444 21 Email: aaj.van.iersel@rivm.nl
Dr. FENTENER VAN VLISSINGEN Martje Animal Welfare Officer POB 360 3700 AJ ZEIST	Tel: 31 30 694 44 82 Fax: 39 06 4957 821 Email: Fentener@voeding.tno.nl
ROYAUME -UNI/UNITED KINGDOM	
Dr. MORTON David University of Birmingham Edgbaston Birmingham B15 2TT	Tel: 44 121 414 3616 Fax: 44 121 414 6979 Email: d.b.morton@bham.ac.uk

Dr. GREENOUGH Rick Director of Toxicology Inversk Research International Tranet Edinburg EH33 2NE Scotland	Tel: 44 1875 618 359 Fax: 44 1875 614 555 Email: Rick_Greenough@SGSgroup.com
Mrs. HOLGATE Barbara Zeneca Mereside Alderly Park Macclesfield Cheshire SK10 5TJ	Tel: 44 1625 512301 Fax: 44 1625 510111
BIAC	
Dr. STITZEL Katherine Proctor and Gamble Miami Valley Laboratory Cincinnati, Ohio 45069	Tel: 1 513 627 2965 Fax: 1 513 627 2188 Fax: STITZEL.KA@PG.com
SECRETARIAT	
Dr. KOËTER, Herman B.W.M. Environmental Health and Safety Division Environment Directorate 2, rue Andre Pascal 75775, Paris Cedex, France	Tel: +33-1 45 24 98 44 Fax: +33-1 45 24 16 75 Email: Herman.Koeter@oecd.org
Prof. GOLDBERG, Alan (consultant) Director Center for Alternatives to Animal Testing Johns Hopkins University Baltimore, MD 21212 USA	Tel: +410 223 1692 Fax: +410 223 1603 Email: Goldberg@jhsph.edu

ANNEX 2

QUESTIONS TO DETERMINE WHETHER EARLIEST POSSIBLE ENDPOINTS HAVE BEEN SOUGHT

(From: CCAC Guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching, and testing (9)).

- what are the scientific justifications for using the proposed endpoint?
- have all existing relevant data been evaluated?
- what is the expected time course for the animals from the initial treatment to first signs of pain and/or distress, to the death of the animal?
- when are the effects to the animal expected to be the most severe?
- if the course of adverse effects cannot be determined prior to the start of the study, could they be developed through the conduct of a pilot study with appropriate observations by the animal care and veterinary staff?
- have a list of observations on which the endpoint will be based been developed?
- who will monitor the animal and maintain records of observations?
- has a chain for reporting observation findings been established?
- what will be the frequency of observations during the course of the study and during those times predicted to be critical for the animals?
- do the investigators, veterinary care, and animal care staffs have the training and experience necessary to perform the observations necessary to effectively and efficiently monitor the animals?
- what steps have been implemented to attend to animals which demonstrate severe signs and symptoms?

ANNEX 3

CLINICAL SIGNS AND CONDITIONS INDICATING THE NEED FOR CLOSER OBSERVATION OR HUMANE KILLING

The following is a list of common conditions and clinical signs that may be indicative that an animal is experiencing pain and/or distress. The list is primarily based on observations in rats and mice, but many of the signs also apply to other mammals used in toxicity testing. Some of the signs are sufficient indications of severe pain, distress, or impending death, and will lead to a decision that the animal should be humanely killed. The majority of signs are not sufficient, by themselves, to support a decision to humanely kill the animal. However, they are indications of possible pain or distress, and the animals should be carefully examined for other signs which may, in combination, be indicative of severe pain or distress.

When one or more signs or conditions are observed, these should be documented with the dates of initial and subsequent observations and all treatments. If the animal is not humanely killed, a more detailed examination of the animal should be performed, the frequency of observation should be appropriately increased, and the cage or pen should be clearly marked, and the details noted in the records of the experiment.

The list is not all-encompassing because other clinical signs may occur. Animal care facilities should add other clinical signs and conditions that may be appropriate for specific studies.

Abdominal rigidity:

see Boarded abdomen. May be detected by holding a small animal up to ear and squeezing abdomen gently. If breathing stops then this is indicative of abdominal pain. Causes may be peritonitis due to leakage of gut contents into the abdomen, or an inflamed abdominal organ, which are extremely painful.

Abortion:

May be detected by foetal remains on bedding, blood on bedding, decrease in abdominal size.

Agalactia:

May be observed by no milk in stomachs of nursing rodents, or failure to express milk from the mammary gland. Young will die, and if not cross-fostered or provided with supplemental nutrition or milk, should be humanely killed.

Anaemia:

Indicates a loss of blood (through faeces, urine, reproductive tract) or poor red blood cell replenishment, to the extent that it produces clinical signs of laboured or decelerated breathing. (also discernible as pale membranes, pale ears and feet, dyspnea, hyperventilation).

Analgesia:

see Reflexes

Anuria:

No urine flow (anuria) due to renal failure (it may be reduced oligouria) but worth checking for urine retention (see below).

Apathy:

see Immobile/Inactive

Ataxia/incoordination/staggering/unbalanced:

Due to neuromuscular co-ordination, weakness, or post seizure recovery period. Observe carefully and continue to check body weight.

Bleeding from any orifice:

see Anaemia. Some internal haemorrhaging may be detectable as blood escapes from natural orifices. The seriousness will depend on the amount and frequency of the bleeding (q.v. anaemia).

Blepharospasm:

see Eyelid closure. The cause is usually some damage to the eye and this should be investigated further. If incidental to the study (particularly when only one eye is affected) then veterinary advice should be sought and the animal may be treated or withdrawn from the study.

Blood in faeces or urine:

see Anaemia.

Blood around nose and eyes:

In rodents, it is necessary to differentiate between blood and porphyrin secretion. It is often a stress-related condition in rodents, the secretions are not being removed by grooming. If it is blood, the presence in only one nostril may be a result of physical injury.

Boarded abdomen:

see Abdominal rigidity.

Body temperature, abnormal:

Any alteration in body temperature could be accompanied by a lowered activity level. Hypothermia of more than 10% from normal body temperature may be associated with impending death.

Body weight loss or emaciation:

Particularly when bodyweight has decreased by more than 20% compared with control animals, or bodyweight has decreased by more than 25% over a period of 7 days or more. Usually accompanied by reduced or absence of food intake. Body condition should be determined as well as in chronic conditions (e.g. tumour growth) as body weight may stay the same or even increase, but loss of muscle and subcutaneous fat lead to a marked loss of body condition. This is detectable through feeling the pelvis and backbone, and one may see a square tail as muscle atrophy reveals the square shape of the vertebrae.

Breathing difficulties (Dyspnea):

This can be presented in a variety of signs such as panting, hyperventilation, laboured breathing, see-saw or abdominal-thoracic breathing, grunting with each breath (this may be indicative of abdominal pain also).

Cachexia:

see Body weight loss

Chewing, persistent:

see Self-mutilation; Compulsive behaviour.

Chromodachryorrhea:

see Blood around nose and eyes

Circling:

see also Ataxia. Characterised by an animal going repeatedly round and around the cage making a track, may be accompanied by bodyweight loss. May indicate damage to the brain or to the inner ear. May be caused by a concurrent infection, but could also be caused by test substances.

Comatose:

see also Recumbency. The animal may be unarousable due to extreme lassitude, sedation etc., or toxic effects of the test substance.

Compulsive behaviour:

Such behaviours may be gnawing, biting at the substrate or even parts of their own body (e.g. feet).

Constipation:

May be indicated by lack of faeces in the cage, but must differentiate from decreased faeces due to anorexia. If prolonged the animal will become lethargic and die.

Convulsions:

see Seizures

Corneal ulceration:

May be accompanied by blepharospasm, watery eyes, and ocular and nasal discharge. The early stages can be particularly painful, and may be incidental to the study, such as drug-induced decreased tear production, or caused by the test substance. Seek veterinary advice, and if recovery is sought, treat under veterinary supervision.

Coughing/Sneezing:

If persistent, may be an intercurrent infection and veterinary advice should be sought.

Cyanosis:

Blue or dark red extremities, such as pinna, feet, mucous membranes of eye and mouth.

Dehydration:

Can be assessed by lifting and twisting the skin and observing how quickly it returns to its normal 'flat' position. Usually occurs as result of reduced water intake or inadequate water intake in the case of intestinal (diarrhoea), kidney or endocrine disease (polyuria).

Diarrhoea:

Diarrhoea can present in a variety of forms from frank watery or bloody faeces (dysentery) to soft stools. Increased frequency of defecation can indicate greater severity. Humane criteria listed for bodyweight and other diagnoses, should be considered.

Discharge, abnormal:

Animals normally keep themselves very clean. Discharge may be from any external orifice. Veterinary advice should be sought to differentiate between infectious etiologies and effects of test substances.

Dyspnoea (difficult breathing):

see Breathing difficulties. Can be a cause of severe distress.

Epistaxis (nasal bleeding):

see Anaemia.

Excitable:

see Seizures. An animal may be difficult to restrain or catch, it may throw itself around a cage in a type of fit, causing injuries. May be due to excessive fear or to neuronal change altering the animal's behaviour.

Eyelid closure:

see Blepharospasm; Corneal ulceration. Eyelids may be fully or partially closed.

Eyes fixed/sunken:

Usually observed in presence of severe bodyweight loss and dehydration. Indicates an animal is close to death, and should be humanely killed. This may also be a transient effect of drug treatment, and not an indication of pain or suffering.

Fractured bone:

May be indicated by swollen limb or lameness.

Gasping:

see Dyspnoea

Grooming - failure to do so:

In rodents, this may lead to porphyrin accumulations near the eyes and nose, and there may be soiling in the anogenital region. The animal is definitely ill, and may be in severe pain and discomfort. In dermal studies, the animal is not necessarily ill if lack of grooming is due to the taste of the substance under test.

Hunched/stiff posture:

see Boarded abdomen. Often seen in sick animals and may be due to abdominal discomfort or only be a general sign of illness.

Hyper-reflexia:

see Excitable. An exaggerated response to a stimulus such as noise or touch.

Immobile/Inactive:

This includes inactivity, lassitude, listlessness, and/or reluctance to move. Animal is ill, may be close to death if accompanied by body weight loss, dehydration, sunken or fixed eyes. The red light response test¹ should be performed.

Jaundice (icterus):

Typically observed by the presence of yellowish-coloured ears, feet and membranes. Serum clinical chemistry (bilirubin) can assist in determining the cause, such as haemolysis (pre-hepatic icterus), liver damage (hepatic icterus), bile tract blockage (post-hepatic icterus), or infection. May also be accompanied by inactivity when painful condition exists.

Joints swollen:

Painful condition may be indicated when accompanied by a strong withdrawal and vocalisation response, an inability to move around freely, relative inactivity compared to controls, or if animal (rodent) remains inactive during the red light response behaviour test¹.

¹

The red light response test is carried out by turning out the normal white lights and observing the animal in the dark or under a red light when it will carry out its nocturnal patterns of behaviour. This is normally characterised by an increase in activities such as investigation, climbing and play within 5 min.

Kyphosis:

Characterised by fixed backward curvature of the spine. This may be due to spasm of the flexor muscle of the vertebral column, and if so would be painful, and the animal should be humanely killed. If intermittent it may be a form of seizure (see Seizures).

Lateral position:

See Recumbency

Limping/Lameness:

Unable to fully bear weight on that limb due to pain in the foot, leg or one of the joints. Fractures should be considered as a possible cause.

Locomotory behaviour:

May be reduced (see Immobile) or abnormal in some way.

Lordosis:

Fixed forward curvature of the spine. This may be due to spasm of the extensor muscle of the vertebral column and if so would be painful and the animal should be humanely killed. If intermittent then it may be a form of seizure (see Seizures).

Loss of condition, body muscle:

see Body weight loss.

Mammary gland abnormalities:

A painful condition may be present if one or more mammary glands is swollen, discoloured, discharging pus or blood, or the animal is extremely sensitive to touching of the gland (vocalisation, withdrawal, and overreaction).

Moribund:

A diagnosis and decision point based on several other items of information, at which time the animal is deemed to be dying with quality life already significantly impaired, and humane killing becomes unavoidable at this point. A moribund animal will often appear comatose.

Motor excitation:

see Hyper-reflexia. An exaggerated movement or limb response to a touch.

Not eating/drinking:

see Bodyweight loss.

Oedema:

Characterised by swelling in areas such as extremities, such as below the mandible. May be indicative of insufficient heart function or low protein levels in the blood. There are numerous causes of oedema, many of which are not a cause for humane killing.

Pale mucous membranes:

see also Anaemia; Cyanosis; Dyspnoea. May be indicative of anaemia or circulatory insufficiency (e.g. cardiac or pulmonary insufficiency, or shock).

If accompanied by laboured or accelerated breathing, may be indicative of a severe or irreversible condition. A haematocrit can be conducted to quantify the severity of suspected anaemia.

Paralysis:

May occur because of action of substance on the CNS or spinal cord. Any animal dragging its limbs for any significant period of time should be humanely killed.

Paresis:

May occur because of action of substance on the CNS or spinal cord, or musculature or neuromuscular junction. Any animal showing obvious or irreversible muscle weakness that may affect its ability to eat, drink, or breathe should be humanely killed.

Piloerection:

The hairs of on animal's fur look harsh or starey as they are partially erect. This can be a sign of not grooming and general ill health.

Pinna reflex:

see Reflexes. Pinch the ear flap and normally an animal will shake its head. Absence of the reflex may be a sign of distress.

Prostrate:

see Recumbency. Usually an animal which has lost its righting reflex and has been in that condition for a few hours. May be a symptom of moribund condition.

Pruritis:

see Self-mutilation. Animal may scratch or bite at itself which may lead to superficial injury which can progress to deeper lesions and infection.

Pupillary constriction/dilation:

A light responsiveness test should be carried out to determine if the condition is fixed or if there is a pupillary response. Dilatation of the pupil together with inactivity may indicate an animal is close to death especially with a sluggish pupil response time. Dilation or constriction may also be a substance effect.

Rales, pulmonary:

see Dyspnoea. Detected by stethoscope. Rales may indicate pulmonary secretions as a result of intercurrent infection (pneumonia) or the test substance. Substances inducing bronchial and bronchiolar secretions may predispose the animal to infection.

Rectal prolapse:

see Tenesmus; Diarrhoea. Part of the rectum protrudes from the anal sphincter. The animal will have to be humanely killed as the prolapse may become infected or the animal may self-mutilate.

Recumbency, prolonged:

see Prostrate. May be lateral (on its side) or abdominal, and if the animal has lost its righting reflex that is more serious. It may be temporary or prolonged though, if for more than a few hours it is likely that the animal is close to death if it is not in any form of seizure.

Red eye(s)/nose:

see also Grooming. Refers to redness around the eyes or around the nose. Indicative of the animal failing to groom. The animal may also have a soiled anogenital region.

Reflexes:

Sluggish, slow, or abnormal responses or loss of reflexes such as corneal, pupillary, pedal, righting (ability to correct to normal posture when gently pushed or overbalanced) or responses to noise, may be due to unconsciousness or extreme lassitude.

Retention of faeces:

see Constipation.

Righting reflex:

see Reflexes.

Salivation, excessive or abnormal:

Indicative of a failure to swallow or hyper-salivation in response to the test substance. If unable to swallow a clinical examination is required to determine the aetiology as it may well affect the animal's ability to eat (see Body weight).

Seizures:

The animal may lie on its side and tremor, the muscles may be rigid or flaccid, it may last only for a few seconds or may be longer, it may be brought on by interaction with the observer. If the seizure lasts for more than one minute and is repeated for more than 5 times a day without being induced, then the animal should be humanely killed especially if due to the substance being tested. If seizures are induced and further time for study is needed then animals should be moved to a quiet area and handled minimally. Seizures in animals with broken limbs, or where a previous seizure has resulted in injury, are a cause for humane killing, irrespective of frequency.

Self-mutilation:

see Pruritis. Licking, scratching or gnawing at an area, which if persistent, may result in ulcerative dermatitis. Depending on the extent of the self-mutilation, or if whole phalanges have been removed from the digits, consider humane killing or other appropriate action.

Skin bruising/colour/crepitus:

May be due to a subcutaneous bleed, or air under the skin (if over the thorax consider lung puncture and humane killing). If due to gas forming organisms treatment is generally not an option, and the animal should be humanely killed.

Spasm:

see Seizures.

Staggering:

see ataxia.

Sunken flanks:

see Bodyweight; Dehydration. The abdominal walls of an animal may be suddenly drawn in (writhing) and can indicate abdominal pain (as in a colicky pain), or it may also be through emaciation.

Suppuration:

Indicative of infection. See Discharge, although suppuration may come from sources other than natural orifices.

Swellings:

see Joint swelling. Note the position and extent. May indicate oedema (q.v.), hernias of the inguinal or femoral rings, abscess, growth of some sort, bruising, pregnancy, etc.

Tenesmus:

Constant straining to pass faeces. Usually associated with diarrhoea (q.v.) and rectal prolapse.

Tetany:

see Seizures.

Tremor:

see also Seizures; Convulsions. The animal may show muscular twitching or rapid skin movements.

Urine retention:

see Anuria. Palpate hardened and distended bladder through the abdominal wall. Is often painful. Can be confused with renal failure.

Vaginal prolapse:

Part of the vagina protrudes from the vulva. The animal will have to be humanely killed as the prolapse may become infected or the animal may self-mutilate.

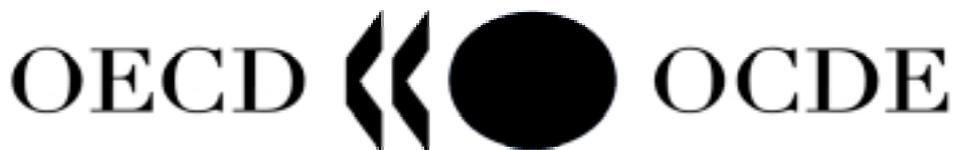
Vocalisation:

May be unprovoked, result from handling, or associated with an animal being fearful of being touched. If abnormal or persistent, may be indicative of a painful or distressful condition.

Vomiting:

Rare in rodents as they lack the physiological reflex and/or are anatomically unable to do so because of the arrangement of the diaphragmatic musculature. In other animals check on frequency and volume lost (see Body weight, and check for fluid loss; see Dehydration). If allowed to persist, animal will die through dehydration and electrolyte imbalance.

ANNEX 4



(For Display, or at Hand, in Animal Rooms and Facilities)

**CLINICAL SIGNS AND CONDITIONS OF ANIMALS REQUIRING
ACTION BY ANIMAL CARE STAFF AND STUDY DIRECTORS**

Instructions:

When any of the following conditions or clinical signs are observed, the animal technician must immediately notify the responsible study director and/or veterinarian, and appropriate action should be taken. A decision should be made as to whether to humanely kill the animal, or to take other appropriate action to alleviate the pain and distress.

If there is a scientific necessity for not humanely killing or treating the animal(s) to alleviate the pain and/or distress, a written plan must be established indicating the schedule for future observations, and the decision endpoints or schedule for treatment or humane killing.

Clinical signs and conditions where humane killing may be appropriate:

1. **Any condition resulting in a prolonged or irreversible inability to eat or drink**, e.g., prolonged immobility, obstruction of the oral cavity, missing or abnormal teeth.
2. **Diseases or conditions indicating severe pain, distress or suffering**, e.g., fractures, self-induced trauma, abnormal vocalisation, abnormal posture or movements, open wounds or ulcers.
3. **Rapid or continuing weight loss**, e.g., 20% or greater body weight over a few days, or gradual but continued weight loss.
4. **Generalised decrease in grooming and abnormal appearance over an extended time period**, e.g., rough hair coat, extensive alopecia, prolonged diarrhoea, urine-stained hair coat, swollen limbs, paralysis, or other central or peripheral nervous disturbances (convulsions, circling behaviour, prostration).
5. **Severe or continuing respiratory distress**, e.g., coughing, sneezing, nasal discharge, bloody nares or mouth.
6. **Frank bleeding**, anaemia, or unusual discharges.
7. **Evidence of microbial infections or other diseases**, including those that interfere with the experimental protocol or cause any of the above.

For further details, see OECD Guidance Document: Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation (OECD, 2000).