Oppfriskningskurs
Forsøk med Dyr

2022
Material to prepare before session

Materiale til forberedelse før samling

http://org.uib.no/dyreavd/LASeducationUpdate5years.html

NORWEGIAN ANIMAL RESEARCH REGULTION

LOVGIVNING BRUK AV DYR I FORSØK

Læringsmål etter denne delen

1.1. Identify and describe the national and EU laws and guidance which regulate the scientific use of animals and in particular the activities of those carrying out scientific procedures involving them.

1.2. Identify and describe related animal welfare legislation.

1.3 Describe the authorization that is needed before acting as user, breeder or supplier of laboratory animals and especially the authorization required for projects and where applicable individuals.

1.4. List sources of information and support that are available (regarding national legislation).

1.5. Describe the role of the personnel mentioned in Article 24, 25 and 26, and their statutory duties and other responsibilities under the National Legislation.

1.6. Describe the roles and responsibilities of the local animal welfare bodies and the national committee for the protection of animals used for scientific purposes.

1.7. Indicate who is responsible for compliance at an establishment and how this responsibility may be exercised (e.g. through the local AWB).

1.8. Describe when a procedure becomes regulated under National legislation (minimum threshold of pain, suffering, distress or lasting harm).

1.9. Indicate who bears primary responsibility for the animals undergoing procedures.

1.10. List which species, including respective stages of development that are included in the scope of the Directive / National law.

1.11. Indicate the circumstances in which animals under the scope of the Directive should be humanely killed or removed from the study to receive veterinary treatment.

1.12. Describe the legislative controls over the killing of animals bred or used for scientific procedures.

Module 1: National legislation – NORWAY

Contents

National and EU laws and guidance that regulate the scientific use of animals in Norway.

Relevant laws, regulations and guidelines

- Animal Welfare Act («Lov om dyrevelferd»)
- Regulation on the Use of Animals in Research (Norw: "Forskrift om bruk av dyr i forsøk")

Norway has also agreed to adhere to the EU Laboratory Animals Protection Directive (2010-63) and the Council of Europe Convention on Laboratory Animals (ETS 123).

The Animal Welfare Act applies for all issues that affect the welfare of vertebrates, cephalopodes, cyclostomata, decapodes and hone-bees.
§ 13 cover animals used in research, education, and medical testing.

The Regulation on the Use of Animals in Research applies especially for use of animals in research, education and testing.

The following issues are covered by the Regulation on the Use of Animals in Research.

- Scope of the regulation - § 1
- Area of application - §§ 2, 3
- Definitions - § 4
- Approvals
- Approvals of institutions for animal experiments- §§ 5, 12
- Approval and Application of animal experiments - §§ 6, 7
- Project summary - § 8
- Compliance with the principles of the 3Rs - § 9
- Purpose of the study - § 10
- Methods, test strategies and endpoints - §11
- Ban of certain experiments - § 13
- Anesthesia and analgesia - § 14
- Termination of experiments - § 15
- Euthanasia and killing - § 16
- Reuse of animals - § 17
- Rehoming of animals - § 18
- Endangered species - § 19
- Primates - § 20

- Animal bred for purpose - §22
- Stray animals of domestic species - § 23
- Demands to competence - § 24
- Named persons with special responsibility for oversight - § 25
- Animal welfare body - § 26
- Named veterinarian or fish health specialist - § 27
- Responsibility of primary investigator - § 28
- Housing and care - § 29
- The physical plant and equipment - § 30
- Recordkeeping - § 31
- Records for dogs, cats and primates - § 32
- Marking of dogs, cats and primates - § 33
- Breeding plan for primates - §34
- Documentation - § 35
- Annual report - § 36
- Administrative issues - §§ 37-41

For institutions that are accredited by AAALAC International the following guidelines will also apply

- The Guide to the Care and Use of Laboratory Animals
- European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Council of Europe (ETS 123)
- Guide for the Care and Use of Agricultural Animals in Research and Teaching (if such species are relevant)

Related regulations

Other laws and regulations for animals. There are several other regulations that apply for different activities that might influence animal welfare and are therefore relevant for animals in research. Some examples are:

- Regulations on Commercial Transport of Animals («Forskrift om næringsmessig transport av dyr»)
- Regulation on Killing of Animals
- Regulation on Import and Export of Animals

- Veterinarians and Other Animal Health Personnel Act («Lov om veterinærer og annet dyrehelsepersonell»)

Other laws and regulations for animal experiments and work with animals.

There are also other laws and regulation related to animal experiments like

- Regulation of Drugs («Forskrift om legemidler»)
- Gene modified animals (GMO)
    - Gene Technology Act («Lov om framstilling og bruk av genmodifiserte organismer m.m. genteknologiloven»).
    - Regulation of Enclosed use of GM Animals («Forskrift om innesluttet bruk av genmodifiserte dyr»)
    - Regulation on Enclosed use of GM Microorganisms («Forskrift om innesluttet bruk av genmodifiserte mikroorganismer»)
    - Regulation on Marking, Transport, Import and Export of GMO («Forskrift om merking, transport, import og eksport av genmodifiserte organismer»)
- Health and safety regulations
    - Work Environment Act («Arbeidsmiljøloven»)
    - Regulation on Work Practice («Forskrift om utførelse av arbeid»)
    - Regulation for Work Places («Arbeidsplassforskriften»)
    - Regulation on Preventive Measures and Limits («Forskrift om tiltaks- og grenseverdier»)
    - Radiation Protection Act («Lov om strålevern og bruk av stråling»)
    - Regulation on Radiation Protection ("Strålevernforskriften")
    - Regulation on Use of Humane Cells and Tissues ("Forskrift om håndtering av humane celler og vev»)
- Regulations for wildlife research
    - Catching of Wildlife for Scientific Purposes
    - The Svalbard Environmental Protection Act
    - Regulation for Motor Traffic in Svalbard

        - Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES)
    - Administrative laws and regulations
        - Administration Act (Forvaltningsloven)
        - Public Transparency in Administrasjon Act (offentlighetsloven)

Authorization needed before acting as user, breeder or supplier of laboratory animals - authorization required for projects and individuals.

Institutions (User, breeder or supplier) Institutions (User, breeder or supplier of laboratory animals) must be approved by Mattilsynet (The Norwegian Food Safety Authority). Both the physical plant as wells as organization of personnel and activities must be approved.

Institutions must:

- Define an Animal welfare body (§26)
- Name persons with special responsibility for oversight ("Person med særlig kontrollansvar" – PMSK) (§26)
- Develop system and policy documents

Annual report of animal uses the previous year must be submitted within March 1 to Mattilsynet. Number of animals, field of research and actual severity must be reported.

Small video on reporting in FOTS can be found on this link:

Competence: Applicant and participants Must as a minimum fulfill training program in accordance with Appendix E of the regulation that covers (§24):

1. National regulation of the use of animals in research
2. Ethics related to relationship between humans and animals, intrinsic value of life and arguments for and against use of animals in research
3. Basic relevant biology, anatomy, physiology, reproduction and genetics for the species in question.
4. Animal welfare, care and enrichment
5. Species specific handling

6. Animal health and hygiene
7. Recognition of species specific signs of fear, pain or other harm
8. Anesthesia, analgesia and euthanasia
9. Humane endpoints
10. 3R
11. Design of experiments

Persons must maintain and document competence through continuous practice and education.

Persons designing experiments (Function B) must have received adequate training in the scientific discipline relevant to the work to be performed and have specific knowledge of the relevant animal species' biology, including their physiological and behavioral needs.

Persons who carry out procedures (function A), care of animals (Function C) or kills animals (Function D) shall be supervised when they perform tasks until they have demonstrated that they master the necessary skills. It must been documented what species and procedures the candidate has been trained in and master so that they can work independently.

Mattilsynet considers that FELASA C course recommendations for the teaching of theory meets requirements for theoretical training for function A = those who perform experiments and function B = those designing procedures and projects.

Those who have already FELASA C courses must:

- Familiarize themselves with the new Norwegian regulations of July 1 2015
- Must relate to the requirement for continuing education

Those who have a FELASA C course from another country must take a course module in national Norwegian regulations.

Persons with special oversight responsibility are to ensure that everyone working with animals in his/her project meets the requirements of education and training, and also constantly updating and training.

It is each researcher's responsibility to make sure your CV is up to date in relation to the requirements for continuous updating and training in laboratory animal science.

Individual projects: Permission to use animals in research must be obtained from Mattilsynet after application in FOTS («Forsøksdyrforvaltningens tilsyns- og søknadssystem») https://asp.gitek.no/fdu/pmws.dll/Login?RestoreSession=r4byycEd0uY28GRD

Some general requirements to projects:

- Applicant and participants competence (of all personnel involved)
- Public project summary
- Information on severity (expected pain and discomfort)
- Demonstrate compliance to 3R
- Harm-benefit assessment
- Animals to be used (number, sex, species)

In addition, applicant has to provide information on

- Funding body
- Planned start and end of experiments
- Public access of information
- Background and purpose
- Rationale for using the chosen animal model.
    - If relevant: deviant phenotype that may impact animal welfare
- Sedation, analgesia and anesthesia
- Calculating number of animals (experimental groups and group sizes)
- Methods description
    - Preparation and habituation of animals for procedures
    - Procedures to be performed

- Monitoring and sampling
- Supervision of animals
- Method for euthanasia
- Criteria for humane endpoints and follow-up actions to be taken to minimize pain, suffering and distress

Approval can be valid for maximum 4 years. For wildlife experiments max 2 years.

Sources of information and support available.

- Lovdata – all Norwegian laws and regulation are available here
  - www.lovdata.no
  - https://lovdata.no/dokument/SF/forskrift/2015-06-18-761
  - https://lovdata.no/dokument/NL/lov/2009-06-19-97
  - https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571
- Mattilsynet (the Norwegian Food Safety Authority)
  - http://www.mattilsynet.no/dyr_og_dyrehold/dyrevelferd/forsoksdyr
- norecopa – the Norwegian national consensus platform for alternatives to use of animals
  - www.norecopa.no
  - Q&A from Mattilsynet http://norecopa.no/eu-direktivet-2010/63/eu
- Institutional Animal Welfare Body – give advice on several issues
- Designated veterinarian in the institution - give advice on several issues

Other sources

- Rådet for dyreetikk (Advisory board for animal ethics, Ministry of agriculture) – disciss ethical issues with regard to animal use in general
  - http://www.radetfordyreetikk.no/
- The National Committee for Research Ethics in Science and Technology (NENT)

- https://www.etikkom.no/forskningsetiske-retningslinjer/etiske-retningslinjer-for-bruk-av-dyr-i-forskning/
- Guidelines in English
- Guidelines in Norwegian

Personnel mentioned in Article 24, 25 and 26, their statutory duties and other responsibilities under the Norwegian legislation.

Specific requirements for personnel (Article 24 in the EU Directive 2010/63)

According to §§ 24 and 25 in the regulation on animal experimentation, personnel working with animals at breeders, suppliers and users shall have the minimum competence described in Appendix E before they start to plan/design experiments, care for or euthanize animals.

The minimum demands in Appendix E are:

1. The regulation of animal experimentation
2. Ethics related to the relationship between humans and animals, the intrinsic value of life and arguments for and against use of animals for scientific purposes.
3. Basic and relevant species specific biology related to anatomy, physiology, breeding, genetics and changes in genetics
4. Animal behavior, housing, care and environmental enrichment.
5. Species specific handling methods
6. Animal health procedures and hygiene
7. Species specific signs of fear, pain or other negative impact for the most common research animals.
8. Anesthesia, analgesia and euthanasia
9. Use of humane endpoints
10. Demands to replacement, reduction and refinement
11. Design of experiments (if relevant)

Persons responsible for planning experiments (function B) shall be adequately educated in the scientific field they work within and

have specific competence in the relevant species biology, physiology and behavioral needs of the species in question.

Persons performing procedure (Function A), animal caretakers (function C) or killing animals (Function D) shall be supervised until they demonstrate that they master the necessary skills.

The institution must describe written procedures that safeguard these requirements.

Named persons with special responsibility for oversight (PMSK) shall:

- Control animal welfare and caretaking
- Make sure that all staff have access to relevant information about the species in question
- Safeguard that persons working with animals fulfill demands to competence.

Designated veterinarian (Article 25 in EU directive 2010/63)

According to § 27 in the Regulation on Animal Experimentation all institutions shall have a named veterinarian or fish-health specialist[1] with special competence in laboratory animal medicine.

The veterinarian or fish-health specialist shall:

- Give advice on animal welfare and treatment
- Evaluate if the animal is fit to live a good life after an experiment is terminated
- Evaluate if an animal can be used in another experiment after the first is terminated
- Evaluate if an animal is fit for rehoming after termination of an experiment:
    - If an animal is rehomed a veterinary journal with veterinary medicine and social issues for the animal shall follow the animal

[1] Fish-health specialists are persons who have completed an Integrated Master Programme in Aquamedicine and are authorized as by Mattilsynet. Fish-health specialists are only competent for this function as long as the institution only keeps aquatic animals but not sea ling mammals

- Give advice to the Animal Welfare Body (preferably the veterinarian should be a permanent member of the animal welfare body)
- Any deviation from housing conditions defined in the regulation in appendix F, part B "Species specific demands of animal experimentation" must be based on a veterinary-medical evaluation

Animal welfare Body (Article 26 in EU directive 2010/63)

According to § 26 in the Regulation of Animal Experimentation the institution shall have an animal welfare body ("Dyrevelferdsenhet – DVE ")

The animal welfare body shall

- Give advice in acquiring, housing, care and use of animals
- Give advice on compliance with the 3Rs (Replacement, Reduction and Refinement)
- Provide information on technical and scientific progress on replacement, reduction and refinement
- Develop and review internal operation routines to monitor, report on an follow up animal welfare issues
- Monitor how experiments impact animal welfare
- Identify and give advice on factors that contribute to further replacement, reduction and refinement
- Give advice for rehoming of animals

A recordkeeping of all advices and decisions made by the Animal welfare body shall be stored for a minimum of 3 years and be available on request from Mattilsynet.

The Animal welfare body shall as a minimum consist of the person with special responsibility for oversight (PMSK). If the institution uses animals for research, the animal welfare body shall have at least one member with relevant scientific knowledge.

The roles and responsibilities of the local animal welfare bodies and the national committee for the protection of animals used for scientific purposes.

The role and responsibility of the local Animal Welfare Body described above.

Mattilsynet (the Norwegian Food Safety Authority) is the national authority responsible for

- Approving and inspecting users, suppliers and breeders (Physical plant and organization)
- Approve applications for animal experiments
- Give permission to other people than veterinarian and fish health specialist to administer total or local anesthesia on the condition that they have received training and this must be clear from the project description and project approval.
- Withdraw or suspend approval if the trial is not conducted in accordance with the regulation or approval
- Authority to approve and exempt from the ban against cardiac puncture in anesthetized animals when not a part of a terminal procedure.
- Authority to approve other euthanasia methods than described in the regulation appendix C
- Authority to approve reuse of animals
- Authority to approve the use of animals that are not bred for experimental purposes
- Authority to approve the use of strayed domestic animals
- Authority to approve reuse of animals
- Review record from the animal welfare body
- Review animal journals
- Approve exemption for housing conditions
- Approve single housing of animals

Responsible for compliance at an establishment and how this responsibility may be exercised (e.g. through the local Animal Welfare Body.
The person responsible for compliance of the regulation should be a person with adequate influence on resources.

Procedure regulated under National legislation (minimum threshold of pain, suffering, distress or lasting harm).

The Regulation of Animal Experimentation apply for

Experiments i.e. any use of animals for scientific or educational purpose and for medical purposes that can cause pain, fear, lasting harm or other negative impact larger or equal to injection of a needle using good veterinary practice. This also applies for any interventions that cause animals to be born or hatched as well as establishing or maintaining gene modified animal colonies with similar negative impact for the animals. An experiment might also be a work program with a defined scientific aim and consisting of one or more procedures. For animals that are not bred or held for the use of organs or tissues can be used for scientific purposes, euthanasia of these animals is not considered as experiments.

Field Experiments i.e. an experiment taking place outside an approved facility.

Facility: i.e. Plant, building, group of buildings or other rooms, including those that are not enclosed and mobile installation with its interior and equipment.

Breeder: i.e. physical or judicial person that breed species listed in Appendix D with the purpose of using them, or their organs or tissues for scientific purposes.

Supplier: i.e. physical or judicial person, exempt from breeders that supply animals for use in experiments or organ/tissues for scientific purposes

User: i.e. physical or judicial person who use animals in experiments

Endangered species: i.e. species that are categorized as critically endangered or vulnerable in the Norwegian red list.

Primary responsibility for animals undergoing procedures.

Responsibility for animals in experiments. The primary investigator/project leader (project license holder) is responsible for the animals in their experiments.
He/she has to safeguard that

a. Cause of any unnecessary pain, fear, lasting harm of other negative impact is eliminated as soon as possible
b. Experiments are performed in exact accordance with the approval and decision made by Mattilsynet
c. Any deviations and compensatory actions are recorded.

Species, including respective stages of development that are included in the scope of the Directive / National law.

The Regulation apply for

- Live vertebrates
- Decapods
- Cephalopods
- Honey bees

The regulation also apply for early life stages if the animal is to be allowed to live beyond that stage of development and, as a result of the procedures performed, is likely to experience pain, suffering, distress or lasting harm after it has reached that stage of development including mammal fetuses in the last trimester and larvae of vertebrates that nurture themselves (after start feeding)

The following species must be bred for scientific purposes.

1. Mice (Mus musculus)
2. Rat (Rattus norvegicus
3. Guinea pig (Cavia porcellus)
4. Syrian/golden hamster (Mesocricetus auratus)
5. Chinese hamster (Cricetulus griseus)
6. Mongolian gerbil (Meriones unguiculatus)
7. Rabbit (Oryctolagus cuniculus)
8. Dog (Canis familiaris)

9. Cat (Felis catus)
10. All species of non-human primates
11. Frogs (Xenopus (laevis, tropicalis), Rana (temporaria, pipiens))
12. Zebrafish (Danio rerio)

Circumstances in which animals under the scope of the regulation should be humanely killed or removed from the study to receive veterinary treatment.

Animal must be killed
If unforeseen pain cannot be relieved by painkillers

Humane endpoints
Death as endpoint shall be avoided and replaced by earlier humane endpoints. If death is unavoidable, the experiment shall be designed so that

a. Death is caused for as few animals as possible
b. Duration and intensity of any harm is minimized
c. A pain-free death is safeguarded as long as possible

Any experiment with death as endpoint is classified as severe.

Rehoming of animals after experiments must only be made after veterinary evaluation of the animals.

Describe the legislative controls over the killing of animals bred or used for scientific procedures.

Killing and all handling related to the act of killing animal shall not cause unnecessary pain, fear or other harm and have to be performed taking animal welfare concerns.

Killing methods are described in annex C to the regulation
Other methods can only be used after approval from Mattilsynet

- On unconscious animals
- When purpose of experiments require a similar killing methods as used for farm animals

Death must be confirmed by either

- Confirmation of circulatory arrest
- Destruction of brain
- Dislocation of neck
- Out-bleeding
- Confirmation of rigor mortis

Animal Welfare Body

The institution shall establish an animal welfare body (AWB) (norw: "Dyrevelfe

Tasks for the Animal Welfare Body

The animal welfare body shall:

- Give advice in acquiring, housing, care and use of animals
- Give advice on compliance with the 3Rs (replacement, reduction and refinement)
- Provide information on technical and scientific progress on replacement, reduction and refinement
- Develop and review internal operation routines to monitor, report on and follow up animal welfare issues, person health and safety and impact on experiments.
- Monitor how experiments impact animal welfare, person health and safety and impact on experiments.
- Identify and give advice on factors that contribute to further replacement, reduction and refinement
- Give advice for rehoming of animals (less relevant at UiB)

A record of all advices and decisions made by the AWB shall be stored for minimum of 3 years and be available on the request from Mattilsynet

Topics discussed in AWB

- Use of painkillers after surgery
- Humane endpoints and Scoring Sheet - what do we expect!
- Microbiological health-status - ensures quality, classification and risk assessment
- Services provided by the Animal Facility for breeding
- Single housing of animals
- Aseptic procedures for surgery
- Use of and change of P2-dust masks
- Project staartup meeting - review of protocol
- Riskevaluation

2016

- Aseptic routines for surgery
- Batch anesthesia
- Cleaning of the pig house
- Testing cell cultures of human origin
- Use of facial grimace score for evaluation of pain
- Transport of animals between the units
- Training of summer-substitutes

Topics discussed in AWB

2017

- Enrichment program for pigs
- Enrichment program for rodents
- severity assessment and scoring for neurosurgical models in rodents
- Procedures for sentinel animals
- Use of cleaning and disinfection
- Procedures for unpacking animals
- Regulations for breeding at the animal facility

2018

- Infections in immundeficient animals
- Alterntil nesting material for mice
- Administrative procedures for animals use that do not need authorisation from Mattisynet
- Administrative procedures for breeding that do not need authorisation from Mattisynet
    - Level of Phenotypecharacterization
- Review of enrichment progam
- Practical training

2019

- Review of tap water quality at Vivarium
- Rodent handling made easy
- Equipment for bulk sterilization of gods – alternative to autoclaves
- Dispensing category A and B drugs for use in animals
- Evaluation of routines for PPE in barriers

2020

- Update contingency plan in light of the Corona-pandemic
- Rehoming of pigs meant for trauma course
- New organization of work task
- Improve solution for storage of pig carcasses – with focus on ergonomics
- Policies for the use of physical restraint procedures or devices
- Use of PCR for health monitoring

2021

- Acclimatization vs Single housing
- Stressfree handling of rodents
- Prescription on regulated drugs
- Water quality for rodents
- Revision of Score sheets
- Enrishment for rats
- Improve animal welfare without compromizin standardization
- Producing webinars for internal re-training

Non-technical project summaries (NTS)

Project summary

Project summary should be easily understandable to the public.

Project summary shall be anonymous and do not containnames and addresses of the user or persons involved.

The project summary shall include:

1. Purpose of the study
2. Expected harm/severity for the animals
3. Expected benefit for science or society
4. How many and what kind of animals will be used (totally in this experiment)
5. How are compliance with the requirements for replacement, reduction and refinement safeguarded

Project summary shall not contain information that is subject to confidentiality obligation under the law on public administration (Forvatningsloven)

Mattilsynet

Statens tilsyn for planter, fisk, dyr og næringsmidler

» VARSLE OSS

MAT OG VANN | DYR OG DYREHOLD | FISK OG AKVAKULTUR | PLANTER OG DYRKING

Du er her: Forside / Dyr og dyrehold / Dyrevelferd / Forsøksdyr / Forsøksdyrsøknader

Sammendrag fra godkjente søknader

Publisert 03.11.2016 **Sist endret** 29.04.2020 Skriv ut

Her finner du forsøkssammendrag fra alle søknader om forsøk på dyr som Mattilsynet har godkjent. Du finner også etterevalueringer av betydelig belastende forsøk.

2020 Alle måneder

Prosjekttittel	Etterevaluering	Godkjenningsdato	ID
Smolt production protocols and breeding strategies for synchronized smoltification		2020.12.28	25658

The first version of the ALURES NTS EU Database is launched! Non-technical project summaries (NTS) of projects authorised by the Member States since 1.1.2021 can now be searched using the EU database.

Together with statistical data, they provide a unique level of transparency on animal-based research and testing in Europe.

Animals used for scientific purposes

Non-technical Project Summaries under Article 43 of Directive 2010/63/EU on the protection of animals used for scientific purposes

For improved transparency, in addition to the provision of statistical data on the use of animals for scientific purposes, it is important that objective information on projects using live animals is made publicly available. Article 43 of the Directive establishes non-technical project summaries (NTS) to achieve this.

The detailed content of NTS can be found in Annex I of Commission Implementing Decision 2020/569/EU. Member States and key stakeholders considered additional guidance useful to help users to draft clear and appropriate NTS to promote consistency across the EU. A draft guidance was circulated for testing between January and June 2021. Feedback from the test will allow the the finalisation of the guidance document.

Until the end of 2020, Member States were required to publish NTS at national level. However, for projects authorised after January 2021, NTS are published using the open access ALURES

Animals used for scientific purposes

ALURES Statistical EU Database

This is the access page to the ALURES Statistical EU Database on the use of animals for scientific purposes. The data are collected by the Member States and submitted to the European Commission annually.

To progress towards the ultimate goal of full replacement, it is crucial to understand where, how and why animals are still required to be used for scientific purposes.

The ALURES Statistical EU Database offers free access to all interested in obtaining more information on animal use in the EU.

It is important to know that the number of reporting countries varies. 2015-2017 data is compiled of data from 28 EU Member States. From 2018 onwards, also data from Norway is included and therefore direct year to year comparisons cannot be made between 2018 and previous years.

Currently ALURES allows for datamining at EU level. **National data** are published annually by the Member States. From 2023, national data will also be accessible through ALURES.

KLASSIFISERING AV DYREFORSØK ETTER FORVENTET BELASTNING			
Metode	**Milde**	**Moderate**	**Beslatende**
Administrering av stoffer, legemidler og testsubstanser	Administrering av bedøvelsesmidler Subkutan, intramuskulær, intraperitoneal, intravenøs via overfladiske kar, der stoffet har mild innvirkning på dyret	Hyppig bruk av testsubstanser som gir moderate klinisk effekter Uttak av blodprøver >10 % på våkne dyr uten at blodtap erstattet	Toksisitets tester med død som utfall Utprøving av vaksine karakterisert av vedvarende svekkelse av dyrene eller progressiv sykdom som fører til død.
Billeddiagnostikk	Ikke invasiv billeddiagnostikk med beroligende/bedøvende midler		
Kirurgi	Overfladiske inngrep (øre/halebiopsi) subkutan implantasjon av minipumper og transpondere	Kirurgiske inngrep under generell anestesi og smertelindring assosiert med postoperativ smerte (thoracotomi, craniotomi, laparotomi, mm)	Kirurgiske inngrep som forventes å resultere i alvorlig eller vedvarende moderat postoperativ smerte, frykt eller annen lidelse eller svekkelse av dyrets tilstand.
Telemetri	Utvendig telemetrisk utstyr som ikke forstyrrer normal aktivitet og adferd		
Tumorer	Tumor som ikke forårsaker påvisbar skadelig effekt (små subkutane)	Tumorer som har moderat smerte, frykt eller påvirkning av normal adferd	Tumorsykdom som forventes å gi progressiv dødelig sykdom forbundet med langvarig moderat smerte, frykt eller annen lidelse (avmagring, invasive bentumorer, metastaser, sår/nekroser)
Genmodifiserte dyr	Mild effekt på fenotype (Nedsatt fertilitet, Tap av hår)	Forventer en moderat skadelig fenotype (Aggresjon, Hyperkolesterolemi, Nedsatt vekst, Lymfom, Osteoporoselignende tilstand, Ulcera/sår, Nedsatt immunforsvar) Etablering av GMO ved kirurgiske inngrep	Fenotype med alvorlig og vedvarende svekkelse av dyrets almenntilstand. (Cystisk Fibrose, Diabetes, Epileptiske kramper, Ileus, Økt mortalitet, Nephropati, Rectal Prolaps, Alvorlig ataxia)
Metabolismebur	<24 timer	inntil 5 dager	> 5 dager
Isolasjon	Kortvarig sosial isolasjon / enkeltoppstalling		Fullstendig isolasjon av sosiale arter over lengre periode.
Adferds studier	Kortvarig mild smerte, frykt eller annen lidelse som dyrene lett kan unngå	Fremkalling av flukt og tilbaketrekkingsreaksjoner der dyret ikke er i stand til å flykte.	Elektriske sjokk som dyret ikke kan unnvike. Immobiliseringsstress for å fremkalle magesår hos rotter Tvunget svømming eller fysisk trening som medfører utmattelse som endepunkt
Stråling		Stråling eller kjemoterapi der immunforsvaret gjennomrettes og bivirkninger varer i mindre enn 5 dager.	Stråling/kjemoterapi uten gjennomrettelse av immunsystemet
For/diett	Tilbakeholdelse av for til rotter <24 timer. Tilsetting av inerte markører i dietten for å følge tram passasjen	Tilbakeholdelse av for til rotter i 48 timer. Studier av modifiserte dietter som forventer å gi moderate kliniske symptomer	

Forskrift om bruk av dyr i forsøk

Hjemmel: Fastsatt 18.6.2015 av Landbruks- og matdepartementet og Nærings- og fiskeridepartementet med hjemmel i lov 19. juni 2009 nr. 97 om dyrevelferd § 6, § 7, § 8, § 9, § 10, § 12, § 13, § 19, § 23, § 24, § 25, § 27, § 30, § 38, jf. delegeringsvedtak 11. juni 2010 nr. 814, lov 15. juni 2001 nr. 75 om veterinærer og annet dyrehelsepersonell § 18 og lov 19. desember 2003 nr. 124 om matproduksjon og mattrygghet mv. (matloven) § 19.

Kapittel I. Innledende bestemmelser

§ 1 *Formål*

Forskriften skal bidra til å begrense bruken av dyr til vitenskapelige og utdanningsmessige formål, fremme god velferd og respekt for dyr som brukes til slike formål, og bidra til at dyrene ikke utsettes for unødige belastninger.

§ 2 *Saklig og personelt virkeområde*

Forskriften gjelder når dyr

a) blir brukt eller er ment å bli brukt i forsøk eller
b) blir oppdrettet spesielt for at deres organer eller vev kan bli brukt til vitenskapelige formål.

Forskriften gjelder levende virveldyr, tifotkreps og blekksprut. Dette omfatter også tidlige utviklingsstadier av disse dyrene hvis sanseapparatet er på et tilsvarende nivå som hos ferdig utviklede dyr, herunder fostre av pattedyr i siste tredjedel av normal utvikling og larver av virveldyr som ernærer seg selv. I tillegg gjelder forskriften når dyr på enda tidligere utviklingsstadier blir brukt i forsøk og får leve videre og sannsynligvis vil oppleve smerte, frykt, varig skade eller annen belastning etter å ha nådd utviklingsstadier som nevnt i annet punktum.

Forskriften gjelder selv om det brukes beroligende, bedøvende eller smertestillende midler, eller andre metoder slik at dyret ikke påføres smerte, frykt, varig skade eller annen belastning.

Forskriften gjelder inntil dyr som nevnt i første ledd er avlivet, omplassert eller tilbakeført til et dyrehold.

Forskriften gjelder ikke

a) ikke-eksperimentell landbruks- og akvakulturvirksomhet
b) ikke-eksperimentell klinisk veterinærvirksomhet
c) klinisk utprøving av legemidler til dyr når dette er nødvendig for å få eller beholde markedsføringstillatelse
d) prosedyrer i forbindelse med alminnelig avl og hold av dyr
e) enkel identitetsmerking av dyr
f) handlinger som det ikke er grunn til å tro vil påføre dyret smerte, frykt, varig skade eller annen belastning tilsvarende eller større enn ved å føre inn en nål etter god veterinær praksis.

Kravene i forskriften er rettet mot oppdrettere, formidlere og brukere.

§ 3 *Stedlig virkeområde*

Forskriften gjelder på norsk landterritorium, i norsk territorialfarvann, i norsk økonomisk sone, på norske fartøy og luftfartøy, på innretninger på norsk kontinentalsokkel, samt på Svalbard, Jan Mayen og bilandene. Forskriften gjelder også aktivitet som personell fra norsk fartøy utfører i nær tilknytning til fartøyet når det befinner seg i internasjonalt eller fremmed farvann.

§ 4 *Definisjoner*

a) *forsøk*: enhver bruk av dyr til vitenskapelige eller utdanningsmessige formål, og i medisinsk virksomhet, som kan påføre dyret smerte, frykt, varig skade eller annen belastning tilsvarende eller større enn ved å føre inn en nål etter god veterinær praksis. Begrepet omfatter også handlinger som har som mål, eller kan føre til at dyr fødes eller klekkes med belastninger som nevnt i første punktum. I tillegg omfatter begrepet etablering og vedlikehold av genmodifiserte dyrestammer med slike belastninger. Et forsøk kan også være et arbeidsprogram som har et definert vitenskapelig formål og som består av ett eller flere forsøk. Når dyrene ikke er avlet eller holdt spesielt for at deres organer eller vev kan bli brukt til vitenskapelige formål, regnes ikke avliving av dyrene til slik bruk som forsøk.
b) *feltforsøk*: forsøk utenfor godkjente lokaler
c) *lokaler*: anlegg, bygning, gruppe av bygninger eller andre lokaliteter, herunder steder som ikke er helt lukket eller tildekket, samt mobile anlegg. Innredning og utstyr regnes som deler av lokalet.
d) *oppdretter*: fysisk eller juridisk person som oppdretter dyr som er oppført i vedlegg D med sikte på at de skal brukes i forsøk, eller at deres organer eller vev skal brukes til vitenskapelige formål, eller oppdretter andre dyr primært til slike formål
e) *formidler*: fysisk eller juridisk person, bortsett fra oppdretter, som formidler dyr med sikte på bruk av dyrene i forsøk eller deres organer eller vev til vitenskapelige formål
f) *bruker*: fysisk eller juridisk person som bruker dyr i forsøk
g) *truede dyrearter*: dyrearter som er kategorisert som kritisk truet, sterkt truet eller sårbar i Norsk rødliste for arter eller som står oppført i vedlegg A til forordning (EF) nr. 338/97

Kapittel II. Krav om godkjenning

§ 5 *Godkjenning av oppdrettere, formidlere, brukere og lokaler*

Oppdrettere, formidlere og brukere, og lokalene de bruker, skal være godkjent av Mattilsynet. Godkjenning kan bare gis hvis kravene i denne forskriften er oppfylt. Godkjenningen kan gis en begrenset varighet.

Lokalene og driften kan ikke endres vesentlig uten ny godkjenning hvis endringen kan svekke dyrevelferden.

Oppdretteren, formidleren eller brukeren skal i søknaden oppgi hvilke dyrearter som skal brukes og spesifisere hvem som

a) har det nærmeste lederansvaret for å sikre etterlevelse av denne forskriften
b) skal ha særskilt kontrollansvar etter § 25
c) er navngitt veterinær eller fiskehelsebiolog.

Hvis nye personer overtar oppgavene som nevnt i bokstavene a – c, skal dette meldes til Mattilsynet.

Hvis det foreligger avvik fra forskriften eller godkjenningen, kan godkjenningen inndras eller suspenderes. Oppdretteren, formidleren eller brukeren skal sørge for at dyrevelferden ikke svekkes som følge av inndragningen eller suspensjonen.

§ 6 *Godkjenning av forsøk*

Dyr kan brukes i forsøk bare hvis Mattilsynet har godkjent forsøket. Kravet om godkjenning gjelder ikke forsøk som bare omfatter avliving av dyr for å bruke organer eller vev fra dem.

Hvis metodene ikke er brukt før eller det er usikkert hvor mange dyr som vil bli brukt, skal det foretas pilotforsøk.

Godkjenningen skal gis en begrenset varighet som ikke overstiger fire år. Feltforsøk kan ikke godkjennes for mer enn to år.

Forsøk kan ikke endres uten ny godkjenning hvis endringen kan svekke dyrevelferden.

Én og samme godkjenning kan omfatte flere likeartede forsøk som iverksettes av samme bruker. Dette gjelder bare hvis forsøkene skal oppfylle påbudte krav eller omfatter bruk av dyr til produksjons- eller diagnoseformål etter etablerte metoder.

Mattilsynet kan tillate at andre enn veterinærer og fiskehelsebiologer iverksetter total eller lokal bedøvelse av dyr, under forutsetning av at disse personene har gjennomført

relevant opplæring. Dette skal fremgå av godkjenningen. Dette gjelder ikke medikamentell immobilisering av vilt.

Mattilsynet kan inndra eller suspendere godkjenningen hvis forsøket ikke gjennomføres i samsvar med forskriften eller godkjenningen. Brukeren skal sørge for at inndragningen eller suspensjonen ikke svekker dyrevelferden.

§ 7 *Søknad om godkjenning av forsøk*

Søknad om godkjenning av forsøk eller endring av forsøk skal sendes inn av brukeren eller den forsøksansvarlige, og skal inneholde en beskrivelse av forsøket og et forsøkssammendrag. Søknaden skal videre inneholde nødvendige opplysninger slik det fremgår av vedlegg A og B.

I tillegg skal søknaden inneholde opplysninger om

a) brukeren
b) den eller de ansvarlige for forsøket
c) lokalene eller annet sted hvor forsøket skal gjennomføres.

§ 8 *Forsøkssammendrag*

Forsøkssammendraget skal være lett forståelig for allmennheten og skal beskrive

a) forsøkets formål
b) forventede skadevirkninger på dyrene
c) forventet vitenskapelig eller samfunnsmessig nytteverdi
d) hvor mange og hva slags dyr som skal brukes
e) hvordan kravene om erstatning, reduksjon og forbedring skal etterleves.

Forsøkssammendraget skal være anonymt og ikke inneholde navn og adresser til brukeren eller involverte personer. Forsøkssammendraget skal heller ikke inneholde opplysninger som er underlagt taushetsplikt etter forvaltningsloven.

Kapittel III. Krav til forsøkene

§ 9 *Erstatning, reduksjon og forbedring*

Levende dyr skal ikke brukes i forsøk hvis formålet kan oppnås ved å erstatte slik bruk med alternative metoder eller teststrategier.

Det skal ikke brukes flere dyr i et forsøk enn det som er nødvendig for å oppnå formålet med forsøket.

Forsøksmetodene skal stadig forbedres for å unngå, forebygge, fjerne eller minimalisere enhver mulig smerte, frykt, varig skade eller annen belastning for dyrene. Kravet om forbedring gjelder også avl, hold og stell av dyrene.

§ 10 *Formål med forsøket*

Dyr kan bare brukes i forsøk til følgende formål:

a) grunnforskning
b) anvendt forskning for å
    1. unngå, forebygge, diagnostisere eller behandle sykdom, dårlig helse eller andre unormale tilstander, eller deres virkninger, hos mennesker, dyr eller planter
    2. vurdere, påvise, justere eller endre fysiologiske tilstander hos mennesker, dyr eller planter eller
    3. bedre velferden for dyr, herunder produksjonsforholdene for produksjonsdyr
c) utvikling, tilvirkning eller kvalitets-, effekt- og sikkerhetstesting av legemidler, næringsmidler, fôr eller andre stoffer eller produkter, hvis formålet er omfattet av bokstav b
d) forskning for vern av miljøet av hensyn til helse eller velferd for mennesker eller dyr
e) forskning for å bevare dyrearten
f) yrkesutdanning eller høyere utdanning med sikte på tilegnelse, vedlikehold eller forbedring av faglige kvalifikasjoner eller
g) rettsmedisinske undersøkelser.

§ 11 *Metoder, teststrategier og endepunkter*

Levende dyr skal ikke brukes i forsøk hvis det er påbudt eller tillatt etter annet regelverk å benytte en annen metode eller teststrategi for å oppnå formålet.

Forsøksmetodene og teststrategiene skal innebære

a) bruk av så få dyr som mulig
b) bruk av dyr med minst mulig evne til å oppleve smerte, frykt og annen belastning, og til å få varig skade
c) at dyrene påføres minst mulig smerte, frykt, varig skade og annen belastning og
d) størst mulig sannsynlighet for pålitelige resultater.

Døden skal så langt det er mulig ikke være endepunkt for forsøket. Det skal i stedet benyttes tidlige og humane endepunkter. Hvis døden er uunngåelig som endepunkt, skal forsøket være utformet slik at

a) så få dyr som mulig dør
b) varigheten og intensiteten av belastningen reduseres mest mulig og
c) en smertefri død sikres så langt det er mulig.

§ 12 *Lokalisering*

Forsøk skal finne sted i godkjente lokaler hos en godkjent bruker. Mattilsynet kan gjøre unntak fra påbudet hvis det er vitenskapelig begrunnet at forsøket utføres som feltforsøk.

§ 13 *Absolutt forbud mot visse forsøk*

Forsøk som påfører dyr alvorlig smerte, frykt eller annen belastning som forventes å bli langvarig og ikke kan lindres, er forbudt.

Det er forbudt å bruke dyr i forsøk med kosmetikk etter forskrift 8. april 2013 nr. 391 om kosmetikk og kroppspleieprodukter.

§ 14 *Bedøvelse og smertebehandling*

Forsøk som påfører dyr store skader som kan medføre alvorlig smerte, skal utføres under total eller lokal bedøvelse. Hvis det ikke er uhensiktsmessig, skal også dyr som påføres mindre belastning bedøves. Ved vurderingen av om bedøvelse er uhensiktsmessig, skal det tas hensyn til om

a) bedøvelsen vil påføre dyret en større belastning enn selve forsøket og
b) bedøvelse er uforenlig med forsøkets formål.

Hvis det er nødvendig, skal det brukes smertestillende midler eller andre egnede metoder for å sikre at dyrets smerte, frykt og annen belastning begrenses mest mulig. Hvis uforutsett alvorlig smerte ikke kan lindres, skal dyret avlives umiddelbart.

Dyret skal ikke gis legemidler som helt eller delvis hindrer det i å gi uttrykk for smerte, uten at det samtidig får egnet bedøvelse eller annen smertebehandling. Det skal framlegges vitenskapelig dokumentasjon med nærmere opplysninger om bedøvelsen eller smertebehandlingen.

Dyr som kan få smerter når bedøvelsen har opphørt, skal behandles med forebyggende og postoperative smertestillende midler, eller annen egnet smertebehandling. Dette gjelder ikke hvis det kan begrunnes vitenskapelig at smertebehandling er uforenlig med forsøkets formål.

Så snart formålet med forsøket er oppnådd, skal det settes i verk tiltak for å fjerne eller minimalisere belastningen for dyret.

Blodprøvetaking fra hjertet og injeksjoner i hjertet skal foregå under total bedøvelse. I slike tilfeller skal dyret holdes totalt bedøvet inntil det avlives, hvis ikke Mattilsynet i godkjenningen av forsøket har gitt særskilt tillatelse til at dyret kan våkne av bedøvelsen.

§ 15 *Forsøkets avslutning*

Et forsøk skal anses som avsluttet når det ikke skal gjøres flere observasjoner i forbindelse med forsøket. For nye genmodifiserte dyrestammer skal forsøket anses som

avsluttet når avkommet ikke lenger viser tegn til eller forventes å oppleve smerte, frykt, varig skade eller annen belastning over grensen som er definert i § 4 bokstav a.

Ved avslutning av et forsøk skal en veterinær, fiskehelsebiolog eller annen kompetent person avgjøre om dyret kan leve videre. Dyr som forventes å ha moderat eller alvorlig smerte, frykt, varig skade eller annen belastning etter at forsøket er avsluttet, skal avlives.

Dyr som skal leve videre, skal gis nødvendig stell og oppstalling ut fra dyrets helsetilstand.

§ 16 *Avliving*

Avliving og håndtering i forbindelse med avlivingen skal ikke påføre dyret unødvendig smerte, frykt eller annen belastning, og skal skje på en dyrevelferdsmessig forsvarlig måte.

Dyr som omfattes av vedlegg C, skal avlives med metoder som er beskrevet i vedlegget.

Mattilsynet kan gjøre unntak fra kravene om avlivingsmetode og tillate en annen metode som på bakgrunn av vitenskapelig dokumentasjon vurderes som minst like skånsom. Unntak kan også gjøres hvis det er vitenskapelig begrunnet at forsøkets formål ikke kan oppnås ved bruk av den forskriftsfestede metoden.

Avliving skal skje i oppdretterens, formidlerens eller brukerens lokaler. Ved feltforsøk kan dyr avlives utenfor godkjente lokaler. Avliving skal utføres av en kompetent person. Eventuell avblødning skal foregå under total bedøvelse. Avliving i nødstilfelle skal i størst mulig grad skje i samsvar med denne paragrafen.

§ 17 *Gjenbruk av dyr*

Et dyr som allerede har vært brukt i ett eller flere forsøk, kan ikke brukes i et nytt forsøk hvis det er mulig å bruke et annet dyr som ikke har vært brukt tidligere.

Dette gjelder ikke hvis

a) den faktiske belastningsgraden i de foregående forsøkene var lett eller moderat
b) dyrets helse og velferd er fullt gjenopprettet
c) det nye forsøket er foreslått klassifisert som lett belastende, moderat belastende eller terminalt og
d) bruken er i samsvar med råd fra veterinær eller fiskehelsebiolog basert på en vurdering av den totale belastningen i dyrets levetid.

I særlige tilfeller kan Mattilsynet tillate gjenbruk av dyr som har vært brukt én gang i et betydelig belastende forsøk. Tillatelse kan gis bare hvis vilkårene i første ledd bokstavene b, c og d er oppfylt, og dyret har vært undersøkt av veterinær eller fiskehelsebiolog.

§ 18 *Omplassering og tilbakeføring av dyr*

Dyr som har vært brukt eller har vært tiltenkt brukt i forsøk, kan omplasseres eller tilbakeføres til et egnet dyrehold hvis det

a) ifølge veterinær eller fiskehelsebiolog er forsvarlig ut fra dyrets helsetilstand
b) er gjennomført hensiktsmessige tiltak for å sikre dyrets velferd og
c) ikke er fare for folkehelsen og dyrehelsen.

Oppdrettere, formidlere og brukere som skal omplassere dyr, skal utarbeide en plan som sikrer nødvendig sosialisering av dyrene.

Kapittel IV. Krav om hvilke dyr som kan brukes i forsøk

§ 19 *Truede dyrearter*

Individer av truede dyrearter som ikke er født i fangenskap, skal ikke brukes i forsøk. Dette gjelder ikke hvis forsøket har formål som beskrevet i § 10 bokstav b nummer 1, bokstav c eller bokstav e, og det er vitenskapelig begrunnet at formålet med forsøket ikke vil bli oppnådd ved bruk av andre enn truede dyrearter.

Denne paragrafen gjelder ikke primater.

§ 20 *Primater*

Primater skal ikke brukes i forsøk.

Forbudet i første ledd gjelder ikke forsøk med formål som nevnt i § 10 bokstavene a eller e. Forbudet gjelder heller ikke forsøk med formål som nevnt i § 10 bokstav b nummer 1 eller bokstav c, og som blir gjennomført for å unngå, forebygge, påvise eller behandle invalidiserende eller potensielt livstruende kliniske tilstander hos mennesker.

Det er bare tillatt å bruke primater i forsøk hvis det er vitenskapelig begrunnet at formålet med forsøket ikke kan oppnås ved bruk av andre dyrearter. Ved bruk av truede primatarter skal det i tillegg være vitenskapelig begrunnet at formålet med forsøket ikke kan oppnås ved bruk av arter som ikke er truet.

Grunnforskning på individer av truede primatarter som ikke er født i fangenskap, er uansett ikke tillatt.

Bruk av menneskeaper i forsøk er uansett ikke tillatt.

§ 21 *Viltlevende dyr i fangenskap*

(Tom)

§ 22 *Dyr som skal være avlet for forsøk*

Dyr som er oppført i vedlegg D, skal ikke brukes i forsøk med mindre de er avlet for slik bruk. Primater skal i tillegg være avkom fra dyr som er avlet i fangenskap eller kommer fra selvopprettholdende kolonier hvor dyrene er avlet bare innen kolonien, eller er hentet fra andre kolonier, men ikke fra vill tilstand, og holdes på en måte som sikrer at de er vant til mennesker.

Hvis det er vitenskapelig begrunnet, kan Mattilsynet gjøre unntak fra kravene i første ledd.

§ 23 *Eierløse og forvillede dyr av domestiserte arter*

Eierløse og forvillede dyr av domestiserte arter skal ikke brukes i forsøk.

Mattilsynet kan gjøre unntak fra forbudet i første ledd hvis det er vesentlig behov for undersøkelser av disse dyrenes helse og velferd, eller av alvorlige trusler mot miljøet, folkehelsen eller dyrehelsen. Unntak kan bare gjøres hvis det er vitenskapelig begrunnet at forsøkets formål utelukkende kan oppnås ved bruk av eierløse eller forvillede dyr av domestiserte arter.

Kapittel V. Krav om personell, kompetanse og organisering

§ 24 *Personell og kompetanse*

Oppdrettere, formidlere og brukere skal sørge for å ha tilstrekkelig personell på stedet.

Oppdrettere, formidlere og brukere skal sørge for at personene som arbeider med dyr, har tilstrekkelig utdanning og praksis i samsvar med vedlegg E før de utformer forsøk, utfører forsøk, steller dyr eller avliver dyr. Oppdrettere, formidlere og brukere skal også sørge for at personene opprettholder og dokumenterer kompetansen gjennom kontinuerlig praksis og utdanning, og at personene har relevant faglitteratur tilgjengelig.

Personer som utformer forsøk, skal ha fått tilstrekkelig opplæring innenfor det vitenskapelige fagområdet som er relevant for arbeidet som skal utføres, og ha spesifikk kunnskap om de aktuelle dyreartenes biologi, herunder deres fysiologiske og atferdsmessige behov.

Personer som utfører forsøk, steller dyr eller avliver dyr, skal veiledes når de utfører oppgavene inntil de har vist at de har den nødvendige kompetansen.

Oppdrettere, formidlere og brukere skal ha skriftlige rutiner for å sikre at kravene i denne paragrafen er oppfylt.

§ 25 *Personell med særskilt kontrollansvar*

Hver oppdretter, formidler og bruker skal peke ut én eller flere personer som skal

a) kontrollere dyrevelferden og stell av dyr
b) sikre at personer som arbeider med dyrene har tilgang til relevant informasjon om dyreartene
c) sikre at personer som arbeider med dyrene oppfyller kravene til nødvendig kompetanse.

§ 26 *Dyrevelferdsenhet*

Hver oppdretter, formidler og bruker skal ha en egen dyrevelferdsenhet.

Dyrevelferdsenheten skal som et minimum bestå av den eller de personene som har særskilt kontrollansvar etter § 25. Dyrevelferdsenheten skal motta innspill fra den navngitte veterinæren eller fiskehelsebiologen.

Dyrevelferdsenheten hos brukere skal ha minst ett medlem med relevant vitenskapelig kompetanse.

Dyrevelferdsenheten skal gi personer som arbeider med dyrene,

a) råd om dyrevelferd knyttet til anskaffelse, oppstalling, stell og bruk
b) råd om etterlevelse av kravet om erstatning, reduksjon og forbedring
c) informasjon om den tekniske og vitenskapelige utviklingen innen erstatning, reduksjon og forbedring.

Dyrevelferdsenheten skal også

a) utarbeide og revidere interne driftsrutiner for å overvåke, rapportere og følge opp velferden for dyrene
b) følge utviklingen og resultatene av forsøk når det gjelder forsøkenes virkning på dyrene
c) identifisere og gi råd om faktorer som bidrar ytterligere til erstatning, reduksjon og forbedring
d) gi råd om planer for omplassering, herunder egnet sosialisering av de dyrene som skal omplasseres, og tilbakeføring av dyr etter § 18.

Mattilsynet kan tillate at små oppdrettere, formidlere og brukere utfører oppgavene i fjerde og femte ledd på andre måter.

Oppdrettere, formidlere og brukere skal sørge for at alle råd som er gitt av dyrevelferdsenheten, og alle avgjørelser som er truffet som følge av disse, journalføres og oppbevares i minst tre år. Journalene skal på anmodning være tilgjengelige for Mattilsynet.

§ 27 *Navngitt veterinær eller fiskehelsebiolog*

Hver oppdretter, formidler og bruker skal ha en navngitt veterinær eller fiskehelsbiolog med særlig kunnskap innen forsøksdyrmedisin. Veterinæren eller fiskehelsebiologen skal gi råd om dyrenes velferd og behandling.

Fiskehelsebiologer kan bare ha denne funksjonen der det utelukkende holdes akvatiske dyr, unntatt sjøpattedyr.

§ 28 *Forsøksansvarlig*

Den eller de ansvarlige for forsøket skal sørge for at

a) årsaken til enhver unødig smerte, frykt, varig skade eller annen belastning som påføres dyr under et forsøk, blir fjernet så snart som mulig
b) forsøkene utføres i samsvar med godkjenningen og enhver beslutning som Mattilsynet har fattet
c) manglende samsvar med godkjenningen blir rettet opp med nødvendige tiltak
d) avviket og tiltaket blir protokollført.

Kapittel VI. Krav til hold av dyr

§ 29 *Levemiljø og stell*

Dyrene skal holdes i egnet levemiljø og gis fôr, vann og stell som er egnet for deres helse og velferd. Dyrenes mulighet til å få dekket sine fysiologiske og atferdsmessige behov skal begrenses minst mulig.

Miljøforholdene der dyrene holdes eller brukes, skal kontrolleres daglig. Dyrene skal ha tilsyn og stell så ofte som nødvendig, og minst én gang daglig. Tiltak skal iverksettes så snart som mulig for å rette opp mangler og gjøre slutt på unødig smerte, frykt, varig skade eller annen belastning for dyrene. Oppdrettere, formidlere og brukere skal ha nødvendig personell i beredskap for å oppfylle kravet om å tilse og stelle dyrene og iverksette tiltak også utenom ordinær arbeidstid.

Nærmere krav til levemiljø og stell, herunder dato for ikrafttredelse av artsspesifikke oppstallingskrav, er gitt i vedlegg F.

Hvis det er vitenskapelig, dyrevelferdsmessig eller dyrehelsemessig begrunnet, kan Mattilsynet gjøre unntak fra kravene i første ledd første punktum og vedlegg F.

§ 30 *Innredning og utstyr*

Oppdretteres, formidleres og brukeres lokaler skal ha innredning og utstyr som er tilpasset dyrene og forsøkene.

Innredningen og utstyret skal være utformet og konstruert og fungere slik at forsøkene kan gjennomføres så effektivt som mulig, og slik at pålitelige resultater kan oppnås ved bruk av færrest mulig dyr som påføres minst mulig smerte, frykt, varig skade eller annen belastning.

Nærmere krav til innredning og utstyr er gitt i vedlegg F.

§ 31 *Dyrejournal*

Oppdrettere, formidlere og brukere skal føre journal med opplysninger om

a) hvor mange dyr og hvilke dyrearter som oppdrettes, anskaffes, formidles, brukes, omplasseres eller tilbakeføres
b) dyrenes opprinnelse, herunder om de er oppdrettet for å bli brukt i forsøk
c) datoen da dyrene ble anskaffet, formidlet, avlivet, omplassert eller tilbakeført
d) hvem dyrene er anskaffet fra
e) navn og adresse på mottaker av dyr
f) hvor mange dyr og hvilke dyrearter som har dødd eller blitt avlivet
g) kjente dødsårsaker
h) hva slags forsøk dyrene har vært brukt i.

Opplysningene i dyrejournalen skal oppbevares i minst fem år og på anmodning være tilgjengelig for Mattilsynet.

Det skal føres kort for hvert bur eller annen oppholdsenhet med dyr som brukes i forsøk. Kortet skal angi navnet på den ansvarlige for forsøket, ankomstdato for hvert dyr, dato for forsøkets start og løpende registering av alle prosedyrer. Videre skal det angis om dyret har vært benyttet i tidligere forsøk, med angivelse av startdato for første forsøk.

§ 32 *Journal for hunder, katter og primater*

Oppdrettere, formidlere og brukere skal føre journal for hver hund, katt og primat med nødvendige opplysninger om

a) identitet
b) fødested og fødselsdato, hvis dette er kjent
c) dyret er avlet og holdt for å bli brukt i forsøk
d) primaten nedstammer fra dyr som er oppdrettet i fangenskap
e) avlsmessige, veterinærmedisinske og sosiale forhold, herunder om dyrets trivsel og om atferd overfor andre dyr og mennesker
f) de forsøkene dyret har vært brukt i.

Dyret skal følges av den individuelle journalen så lenge det holdes for formål som omfattes av denne forskriften. Journalen skal opprettes ved, eller snarest mulig etter, dyrets fødsel.

Opplysningene nevnt i første ledd skal oppbevares i minst tre år etter at dyret er dødt eller omplassert, og skal på anmodning være tilgjengelig for Mattilsynet. Ved omplassering skal relevante opplysninger om veterinærmedisinske og sosiale forhold fra den individuelle journalen følge dyret.

§ 33 *Merking av hunder, katter og primater*

Hver hund, katt og primat skal ha sitt permanente, individuelle identifikasjonsmerke. Merkingen skal skje senest ved avvenning og på en minst mulig smertefull måte.

Umerket hund, katt eller primat som overføres fra en oppdretter, formidler eller bruker til en annen før avvenning, skal følges av et dokument som særskilt identifiserer dyrets mor. Mottakeren skal beholde dokumentet fram til dyret er merket.

Umerket og avvent hund, katt eller primat som mottas av en oppdretter, formidler eller bruker, skal merkes permanent så snart som mulig og på en minst mulig smertefull måte.

Hvis et dyr ikke er merket, skal oppdrettere, formidlere og brukere på anmodning fra Mattilsynet angi årsaken.

§ 34 *Avlsplan for primater*

Oppdrettere av primater skal ha en plan for å øke andelen dyr som er avkom fra primater som er oppdrettet i fangenskap.

§ 35 *Dokumentasjon*

Oppdrettere, formidlere og brukere skal sørge for at all relevant dokumentasjon, herunder forsøksgodkjenninger og resultatet av Mattilsynets forsøksvurdering, oppbevares i minst 3 år fra forsøksgodkjenningens utløpsdato. Hvis Mattilsynet avslår søknad om godkjenning av forsøk, skal dokumentasjonen oppbevares i minst 3 år fra saksbehandlingsfristens utløpsdato. Dokumentasjonen skal være tilgjengelig for Mattilsynet.

Dokumentasjon om forsøk som skal evalueres etter at de er fullført, skal uansett oppbevares til denne evalueringen er avsluttet.

§ 36 *Årsrapport*

Godkjente brukere skal innen 1. mars sende rapport på særskilt skjema til Mattilsynet med statistisk informasjon om bruk av dyr i forsøk i foregående kalenderår, herunder informasjon om forsøkenes faktiske belastningsgrad, om dyrenes opprinnelse og om arter av primater brukt i forsøk.

Kapittel VII. Avsluttende bestemmelser

§ 37 *Tilsyn og vedtak*

Mattilsynet fører tilsyn og kan fatte nødvendige enkeltvedtak for å gjennomføre denne forskriften.

§ 38 *Dispensasjon*

Mattilsynet kan i særlige tilfeller dispensere fra bestemmelsene i denne forskriften hvis det ikke strider mot Norges forpliktelser eller EØS-avtalen eller andre internasjonale avtaler.

§ 39 *Straff*

Overtredelse av bestemmelser gitt i denne forskriften eller i enkeltvedtak gitt i medhold av forskriften er straffbart i henhold til dyrevelferdsloven § 37, dyrehelsepersonelloven § 37 og matloven § 28.

§ 40 *Overgangsbestemmelser*

§ 5 første ledd gjelder ikke oppdrettere, formidlere og brukere så lenge de har godkjenning etter forskrift 15. januar 1996 nr. 23 om forsøk med dyr.

§ 6 første ledd gjelder ikke for forsøk som er godkjent før denne forskriften trer i kraft og ikke varer lenger enn 1. januar 2018.

Forsøk som er godkjent før denne forskriften trer i kraft og varer lenger enn til 1. januar 2018, skal ha ny godkjenning innen 1. januar 2018.

§ 41 *Ikrafttredelse*

Denne forskriften trer i kraft 1. juli 2015. Samtidig oppheves forskrift 15. januar 1996 nr. 23 om forsøk med dyr og forskrift 8. juli 2010 nr. 1085 om unntak fra krav om tillatelse ved bruk av dyr i undervisning.

Plasskravene som framgår av tabellene i vedlegg F, unntatt tabell 8.5, trer i kraft 1. januar 2017.

§ 22 første ledd annet punktum trer i kraft 10. november 2022 for bruk av andre primater enn hvitøret silkeape (*Callithrix jacchus*).

Vedlegg A. Faktorer som det skal gis opplysninger om i søknaden, jf. § 7 første ledd

I tillegg til en beskrivelse av forsøket og et forsøkssammendrag skal søknad om godkjenning av forsøk eller endring av forsøk inneholde nødvendige opplysninger om

1. relevansen og berettigelsen av

   a) bruk av dyr, inkludert deres opprinnelse, anslåtte antall, art og livsstadier

   b) forsøkene

2. bruk av metoder for å erstatte, redusere og forbedre bruken av dyr i forsøkene

3. planlagt bruk av bedøvelse, smertestillende midler og andre former for smertelindring

4. tiltak for å begrense, unngå og lindre enhver form for belastning for dyrene, fra fødsel til død, når det er relevant

5. bruk av humane endepunkter

6. forsøks- eller observasjonsstrategi, statistisk design for å minimalisere antallet dyr, smerte, frykt og annen belastning, der det er relevant

7. gjentatt bruk av dyr og den samlede virkning av dette på dyret

8. den foreslåtte klassifiseringen av forsøkene etter forventet belastningsgrad, jf. vedlegg B

9. tiltak for å unngå unødvendig gjentagelse av forsøk, når det er relevant

10. de forholdene som dyrene oppstalles, holdes og stelles under

11. avlivingsmetoder

12. kompetanse hos de personene som deltar i forsøket

Vedlegg B. Klassifisering av forsøk etter forventet belastningsgrad, jf. § 7 første ledd

Forsøket skal klassifiseres etter graden av smerte, frykt, varig skade eller annen belastning som dyret forventes å oppleve i løpet av forsøket.

Del I. Klassene

Terminale forsøk

Forsøk som utelukkende gjennomføres under generell anestesi, og hvor dyret ikke skal gjenvinne bevisstheten, skal klassifiseres som "terminale".

Lett belastende forsøk

Forsøk som medfører at dyrene sannsynligvis vil oppleve kortvarig mild smerte, frykt eller annen belastning, skal klassifiseres som "lett belastende". Det samme gjelder forsøk uten noen vesentlig svekkelse av dyrenes velvære eller allmenntilstand.

Moderat belastende forsøk

Forsøk som medfører at dyrene sannsynligvis vil oppleve kortvarig moderat smerte, frykt eller annen belastning, eller langvarig mild smerte, frykt eller annen belastning, skal klassifiseres som "moderat belastende". Det samme gjelder forsøk som sannsynligvis vil forårsake moderat svekkelse av dyrenes velvære eller allmenntilstand.

Betydelig belastende forsøk

Forsøk som medfører at dyrene sannsynligvis vil oppleve alvorlig smerte, frykt eller annen belastning, eller langvarig moderat smerte, frykt eller annen belastning, skal klassifiseres som "betydelig belastende". Det samme gjelder forsøk som sannsynligvis vil forårsake alvorlig svekkelse av dyrenes velvære eller allmenntilstand.

Del II: Kriterier for klassifiseringen

Ved klassifisering skal ethvert inngrep på eller manipulering av et dyr innenfor et definert forsøk tas i betraktning. Klassifiseringen skal være basert på de mest belastende virkningene det enkelte dyret forventes å oppleve etter at alle relevante forbedringsteknikker er tatt i bruk. Type forsøk og flere andre faktorer relatert til forsøket skal tas i betraktning. Alle faktorene skal vurderes i hvert enkelt tilfelle.

Følgende faktorer relatert til forsøket skal tas i betraktning:

— type manipulasjon eller håndtering
— type smerte, frykt eller varig skade eller annen belastning forårsaket av (alle elementer av) forsøket, og belastningens intensitet, varighet og frekvens og de ulike teknikker som benyttes
— kumulativ belastning gjennom hele forsøket
— hvorvidt dyrene hindres i å utrykke naturlig atferd, herunder restriksjoner i standarden for oppstalling, hold og stell av dyrene

I del III gis eksempler på forsøk som er klassifisert på bakgrunn av faktorer relatert til selve forsøkstypen. Eksemplene skal gi den første indikasjonen på hvilken klasse som passer best for en bestemt type forsøk.

For endelig klassifisering etter belastningsgrad skal likevel følgende tilleggsfaktorer vurderes i hvert enkelt tilfelle:

— dyreart og genotype
— dyrets utviklingsgrad, alder og kjønn

— dyrets trening og erfaring med forsøket
— ved gjentatt bruk av dyr i forsøk, den faktiske belastningsgraden i forrige forsøk
— metoder som er brukt for å redusere eller eliminere smerte, frykt og annen belastning, herunder forbedring av oppstalling, hold og stell
— humane endepunkter

Del III. Eksempler
Eksempler på ulike typer forsøk som er klassifisert på bakgrunn av faktorer relatert til type forsøk:

1. Lett belastende forsøk:

   a) administrering av bedøvelsesmiddel, unntatt for avliving som eneste formål

   b) farmakokinetiske studier hvor det gis en enkelt dose av en substans og tas et begrenset antall blodprøver (totalt < 10 % av sirkulerende blodvolum), og substansen ikke forventes å gi noen påvisbar skadelig virkning

   c) ikke-invasiv billeddiagnostikk (f.eks. MRI) med egnet behandling med beroligende eller bedøvende legemidler

   d) overfladiske inngrep, eks. øre- og halebiopsier, ikke-kirurgisk subkutan implantasjon av minipumper og transpondere

   e) bruk av utvendig telemetrisk utstyr som kun forårsaker mindre svekkelse av dyrene eller mindre forstyrrelse av normal aktivitet og atferd

   f) administrering av stoffer subkutant, intramuskulært, intraperitonealt, intravenøst via overfladiske blodkar og via sonde, hvor stoffet kun har en mild innvirkning på dyret, og volumene er innenfor passende grenser med hensyn til dyrets størrelse og art

   g) modeller med fremkalling av tumorer, eller med spontane tumorer, som ikke forårsaker noen påvisbar klinisk skadelig effekt (f. eks. små subcutane, ikke-invasive knuter)

   h) avl av genetisk modifiserte dyr hvor effekten på fenotypen forventes å være mild

   i) fôring med modifiserte dietter som ikke tilfredsstiller alle dyrets næringsmessige behov og forventes å gi mild klinisk abnormalitet i løpet av studien

   j) kortvarig opphold (< 24 timer) i metabolismebur

   k) studier som innebærer kortvarig sosial isolasjon og kortvarig enkeltoppstalling av voksne rotter og mus tilhørende sosiale stammer

   l) modeller som eksponerer dyr for skadelige stimuli som gir kortvarig mild smerte, frykt eller annen belastning og som dyrene lett kan unngå

   m) en kombinasjon eller gjentakelse av følgende eksempler kan klassifiseres som “lett belastende”:

      i. vurdering av kroppssammensetning ved hjelp av ikke-invasive metoder med minimal fengsling

      ii. monitorering av EKG med ikke-invasive metoder med minimal eller ingen fengsling av tilvente dyr

      iii. bruk av utvendig telemetriutstyr som ikke forventes å ha noen påvirkning på sosialt tilpassede dyr, og som ikke påvirker normal aktivitet og atferd

      iv. avl av genetisk modifiserte dyr som ikke forventes å gi klinisk påvisbar skadelig fenotype

      v. tilsetting av inerte markører i dietten for å følge tarmpassasjen

      vi. tilbakeholdelse av fôr til voksne rotter i < 24 timer

   n) “open field testing”, dvs. vitenskapelige forsøk for å undersøke bevegelse, undersøkende atferd og fryktlignende atferd hos laboratoriedyr (rotter/mus)

2. Moderat belastende forsøk:

   a) hyppig bruk av testsubstanser som gir moderate kliniske effekter, og uttak av blodprøver (> 10 % av sirkulerende blodvolum) på bevisste dyr i løpet av få dager uten at volumtapet erstattes

   b) studier for fastsettelse av akuttoksiske doser, tester for kronisk toksisitet/carsinogenitet med ikke-dødelige endepunkter

   c) kirurgiske inngrep under generell anestesi og egnet smertelindring assosiert med postoperativ smerte, annen belastning eller svekkelse av allmenntilstanden. Dette omfatter for eksempel thorakotomi, craniotomi, laparatomi, orchidektomi, lymfadenektomi, thyroidektomi, ortopedisk kirurgi med effektiv stabilisering og sårbehandling, organtransplantasjon med effektiv avstøtningsbehandling, og kirurgisk implantasjon av katetre eller biomedisinsk utstyr (f.eks. telemetriske sendere, minipumper osv.)

   d) modeller med fremkalling av tumorer, eller med spontane tumorer, som forventes å gi moderat smerte eller frykt eller moderat påvirkning av normal atferd

   e) stråling eller kjemoterapi med subletal dose, eller med en ellers letal dose, men med gjenoppbygging av immunsystemet. Skadelige bivirkninger forventes å være milde eller moderate og kortvarige (< 5 dager)

   f) avl av genetisk modifiserte dyr, som forventes å resultere i en moderat skadelig fenotype

   g) etablering av genetisk modifiserte dyr gjennom kirurgiske inngrep

   h) bruk av metabolismebur som innebærer moderat bevegelsesbegrensning over en periode på opp til 5 dager

   i) studier med modifiserte dietter som ikke tilfredsstiller alle dyrets næringsbehov, og som forventes å gi moderat klinisk abnormalitet i løpet av studien

   j) tilbakeholdelse av fôr til voksne rotter i 48 timer

   k) fremkalling av flukt- og tilbaketrekkingsreaksjoner hvor dyret ikke er i stand til å flykte eller unngå påvirkningen, og som forventes å resultere i moderat frykt

3. Betydelig belastende forsøk:

   a) toksisitetstesting med døden som endepunkt eller hvor dødsfall må forventes og det fremkalles alvorlige patofysiologiske tilstander (f.eks. akuttoksisitetstesting av en enkelt dose (se OECDs retningslinjer for testing))

   b) testing av utstyr som ved svikt kan forårsake alvorlig smerte, frykt eller død (f.eks. hjerteassisterende utstyr)

   c) utprøving av vaksine karakterisert med vedvarende svekkelse av dyrets tilstand, progressiv sykdom som fører til døden, forbundet med langvarig moderat smerte, frykt eller annen belastning

   d) stråling eller kjemoterapi med dødelig dose uten gjenoppbygging av immunsystemet, eller gjenoppbygging med produksjon av transplantat-kontra-vertsreaksjon (GVDH = graft versus host disease)

   e) modeller med fremkalling av tumorer, eller med spontane tumorer, som forventes å gi progressiv dødelig sykdom forbundet med langvarig moderat smerte, frykt eller annen belastning (f.eks. tumorer som forårsaker avmagring, invasive bentumorer, tumorer som fører til metastatisk spredning, og tumorer som får utvikle sår)

   f) ethvert inngrep på dyr under generell anestesi, som forventes å resultere i alvorlig eller vedvarende moderat postoperativ smerte, frykt eller annen belastning eller alvorlig og vedvarende svekkelse av dyrets allmenntilstand (f.eks. fremkalling av ustabile benbrudd, thoracotomi uten adekvat smertelindring og trauma for å produsere multippel organsvikt)

   g) organtransplantasjon hvor organfrastøting sannsynligvis vil medføre alvorlig ubehag, annen belastning eller svekkelse av dyrets allmenntilstand (f.eks. xenotransplantasjon)

   h) avl av dyr med genetiske sykdommer, som forventes å oppleve alvorlig og vedvarende svekkelse av allmenntilstanden (f.eks. Huntingtons sykdom, muskeldystrofi og modeller for kronisk tilbakevendende nevritt)

   i) bruk av metabolismebur med betydelig bevegelsesbegrensning over en lengre periode

   j) elektrisk sjokk som dyret ikke kan unnvike (f.eks. for å produsere tillært hjelpeløshet)

   k) fullstendig isolasjon av sosiale arter over lengre perioder (f.eks. hunder og primater)

   l) immobiliseringsstress for å fremkalle magesår eller hjertesvikt hos rotter

   m) tvunget svømming eller annen fysisk trening med utmattelse som endepunkt

Vedlegg C. Tillatte avlivingsmetoder, jf. § 16 annet ledd

1. Ved avliving av dyr skal én av de metodene som er oppført i tabellen under benyttes.

   Andre metoder enn de som er oppført i tabellen, kan bare benyttes

   a) på bevisstløse dyr, forutsatt at dyret ikke gjenvinner bevisstheten før døden inntrer

   b) på dyr som brukes i landbruksforskning, når formålet med forsøket krever at dyret holdes på lignende måte som produksjonsdyr. Disse dyrene skal avlives i samsvar med forskrift 13. januar 2013 nr. 60 om avliving av dyr, herunder vedlegg I i forordning 1099/2009/EU om beskyttelse av dyr på avlivingstidspunktet.

2. Det skal sikres at døden er inntrådt ved én av følgende metoder:

   a) å konstatere permanent opphørt sirkulasjon

   b) å destruere hjernen

   c) å dislokere nakken

   d) å foreta avblødning

   e) å konstatere at rigor mortis har inntrådt

3. Tabell over tillatte avlivingsmetoder. Åpne felt, med eller uten merknad, indikerer at metoden er tillatt.

Dyr/anmerkninger/ metoder	Fisk	Amfibier	Krypdyr	Fugler	Gnagere	Kanin	Hund, katt, ilder og rev	Store pattedyr	Primater
Overdose bedøvelsesmiddel	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Boltepistol			(2)						
Karbondioksid					(3)				
Nakkedislokasjon				(4)	(5)	(6)			
Kraftig slag mot hodet				(7)	(8)	(9)	(10)		
Dekapitering				(11)	(12)				
Elektrisk bedøving	(13)	(13)		(13)		(13)	(13)	(13)	
Inerte gasser (Ar, N_2)								(14)	
Skyting med fritt prosjektil med egnet skytevåpen og ammunisjon			(15)				(16)	(15)	

Vilkår med referanse til tabellen:

1. skal brukes med forutgående sedering der det er hensiktsmessig
2. skal bare brukes på store krypdyr
3. skal bare brukes med gradvis påfylling. Metoden skal ikke brukes på fostre og nyfødte gnagere.
4. skal bare brukes på fugler under 1 kg. Fugler over 250 g skal sederes.
5. skal bare brukes på gnagere under 1 kg. Gnagere over 150 g skal sederes.
6. skal bare brukes på kaniner under 1 kg. Kaniner over 150 g skal sederes.
7. skal bare brukes på fugler under 5 kg
8. skal bare brukes på gnagere under 1 kg
9. skal bare brukes på kaniner under 5 kg
10. skal bare brukes på nyfødte
11. skal bare brukes på fugler under 250 g
12. skal bare brukes hvis andre metoder ikke er mulige
13. Spesialutstyr er påkrevet.
14. skal bare brukes på griser
15. skal bare brukes ved feltforsøk av erfarne skyttere
16. skal bare brukes ved feltforsøk av erfarne skyttere når andre metoder ikke er mulige.

Vedlegg D. Dyrearter som skal være avlet for forsøk, jf. § 22 første ledd første punktum

1. mus (*Mus musculus*)
2. rotte (*Rattus norvegicus*)
3. marsvin (*Cavia porcellus*)
4. gullhamster (syrisk hamster) (*Mesocricetus auratus*)
5. kinesisk hamster (*Cricetulus griseus*)
6. ørkenrotte (*Meriones unguiculatus*)
7. kanin (*Oryctolagus cuniculus*)
8. hund (*Canis familiaris*)
9. katt (*Felis catus*)
10. alle arter av primater
11. frosk (*Xenopus (laevis, tropicalis), Rana (temporaria, pipiens*))
12. sebrafisk (*Danio rerio*)

Vedlegg E. Utdanning og praksis, jf. § 24 annet ledd

Utdannings- og praksisopplegget skal minst omfatte

1. bestemmelsene i denne forskriften
2. etikk relatert til forholdet mellom mennesker og dyr, livets egenverdi og argumenter for og imot bruk av dyr til vitenskapelige formål
3. grunnleggende og relevant artsspesifikk biologi relatert til anatomi, fysiologi, avl, genetikk og genetiske endringer
4. dyreatferd, dyrehold og miljøberikning
5. artsspesifikke håndteringsmetoder og forsøk
6. dyrehelsearbeid og hygiene
7. gjenkjennelse av artsspesifikk frykt, smerte og annen belastning for de mest vanlige forsøksdyrene
8. bedøvelse, smertelindring og avliving
9. bruk av humane endepunkter
10. krav om erstatning, reduksjon og forbedring
11. design av forsøk, hvis relevant

Vedlegg F. Hold av dyr, jf. § 29 tredje ledd og § 30 siste ledd

Del A. Generelle krav

1. Fysiske anlegg

1.1 Funksjoner og generell utforming

a) Alle anlegg skal konstrueres slik at dyrene får et levemiljø som tar hensyn til dyreartens fysiologiske og atferdsmessige behov. Anleggene skal også utformes og drives slik at de hindrer adgang for uvedkommende, og at dyr kommer seg inn eller ut.

b) Oppdrettere, formidlere og brukere skal ha aktive vedlikeholdsprogrammer for å forebygge og utbedre feil og mangler ved bygninger eller utstyr.

1.2 Dyrerom

a) Dyrerommene skal rengjøres regelmessig og effektivt og ha en tilfredsstillende hygienisk standard.

b) Vegger og gulv skal ha overflatematerialer som motstår slitasjen som forårsakes av dyrene og rengjøringen. Materialene skal ikke være helseskadelige for dyrene eller være slik at dyrene kan skade seg. Alt utstyr og fast inventar skal være beskyttet slik at det ikke kan skades av dyrene eller forårsaker skade på dyrene.

c) Dyrearter som er fiendtlige mot hverandre, for eksempel rovdyr og byttedyr, eller dyr som har forskjellige krav til miljøforhold, skal ikke holdes i samme rom. Rovdyr og byttedyr skal holdes slik at de ikke kan se, lukte eller høre hverandre.

1.3 Lokaler til generelle og spesielle forsøksformål

a) Oppdrettere, formidlere og brukere skal, hvis det er relevant, ha tilgang til laboratorieutstyr for å kunne utføre enkle diagnostiske tester, obduksjoner og/eller innsamling av prøver for mer omfattende laboratorieundersøkelser et annet sted. Lokaler til generelle og spesielle forsøksformål skal være tilgjengelige for bruk i situasjoner hvor det ikke er ønskelig å gjennomføre forsøkene eller observasjonene i dyrerommene.

b) Det skal være lokaler til rådighet hvor nyanskaffede dyr kan holdes isolert til deres helsetilstand er fastslått og potensiell helserisiko for etablerte dyr er vurdert og gjort så liten som mulig.

c) Det skal være egnet plass for separat oppstalling av syke og skadde dyr.

1.4 Servicerom

a) Lagerrom skal utformes, brukes og vedlikeholdes slik at kvaliteten på fôr og strø og annet liggemateriale sikres. Rommene skal være beskyttet mot skadedyr og insektangrep så langt det er mulig. Andre materialer som kan bli kontaminert eller utgjøre en fare for dyr, skal lagres separat.

b) Rengjørings- og vaskearealer skal være store nok til å romme de installasjonene som er nødvendige for å dekontaminere og rense brukt utstyr. Rengjøringsprosessen skal tilrettelegges slik at flyten av rent og urent utstyr holdes atskilt.

c) Oppdrettere, formidlere og brukere skal sørge for hygienisk forsvarlig oppbevaring og fjerning av døde dyr og dyreavfall.

d) Der det er behov for kirurgiske forsøk under aseptiske forhold, skal det finnes ett eller flere rom som er utstyrt for dette, og egnede lokaler for postoperativ rekonvalesens.

2. Miljøet og miljøstyring

2.1 Ventilasjon og temperatur

a) Isolasjon, varme og ventilasjon i dyrerommet skal sikre at luftsirkulasjon, støvinnhold og gasskonsentrasjoner holdes innenfor grenseverdier som ikke er skadelig for dyrene.

b) Temperaturen og den relative fuktigheten i dyrerommene skal avpasses etter dyrenes art og alder. Temperaturen skal måles og registreres daglig.

c) Dyr skal ikke være henvist til utendørsarealer under værforhold som kan forårsake belastning.

2.2 Belysning

a) Dersom naturlig lys ikke gir egnet lys-mørke-syklus, skal det brukes kontrollert belysning for å imøtekomme dyrenes biologiske behov.

b) Belysningen skal være slik at dyrene kan røktes og inspiseres på en tilfredsstillende måte.

c) Daglengden og lysintensiteten skal tilpasses dyrearten.

d) Ved hold av albinodyr skal lyset tilpasses disse dyrenes lysømfintlighet.

2.3 Støy

a) Støynivået, inkludert ultralyd, skal ikke ha negativ innvirkning på dyrenes velferd.

b) Alarmsystemer skal ha lyd som ligger utenfor dyrenes sensitive høreområde, dersom dette ikke går ut over hørbarheten for mennesker.

c) Dyrerom skal ved behov være utstyrt med støyisolering og lydabsorberende materialer.

2.4 Alarmsystemer

a) Oppdrettere, formidlere og brukere som er avhengig av elektrisk eller mekanisk utstyr til styring og kontroll av levemiljøet, skal ha et reservesystem som kan opprettholde vesentlige servicefunksjoner og nødbelysning og sikre at alarmsystemene fungerer til enhver tid.

b) Varme- og ventilasjonssystemer skal være utstyrt med overvåkningsutstyr og alarm.

c) Lettfattelig instruksjon om nødprosedyrer skal være slått opp på et lett synlig sted.

3. Stell av dyr

3.1 Helse

a) Oppdrettere, formidlere og brukere skal ha en strategi for å sikre en god dyrehelse som trygger dyrevelferden og imøtekommer vitenskapelig krav. Strategien skal omfatte regelmessig helseovervåkning, et program for mikrobiologisk overvåkning, planer for håndtering av sykdomsutbrudd og en beskrivelse av helseparametere og prosedyrer ved introduksjon av nye dyr.

b) Dyrene skal kontrolleres minst én gang daglig av en kompetent person. Kontrollen skal sikre at alle syke eller skadde dyr identifiseres, og at det iverksettes hensiktsmessige tiltak.

3.2 Innfangede ville dyr

(tom)

3.3 Oppstalling og beriking

a) Oppstalling
Dyr som ikke er naturlig solitære, skal holdes i stabile sosiale grupper av individer som går godt sammen. Hvis Mattilsynet har tillatt individuell oppstalling, skal varigheten begrenses til det absolutt nødvendige. Dyrene skal hele tiden kunne se, høre, lukte og berøre hverandre. Introduksjon eller re-introduksjon av dyr i etablerte grupper skal overvåkes nøye for å unngå konflikt og oppløste sosiale relasjoner.

b) Beriking
Alle dyr skal ha tilgang til arealer med tilstrekkelig kompleksitet for å kunne utøve et bredt spekter av normal atferd. For å redusere stressindusert atferd, skal dyrene gis mulighet til å kontrollere og velge sitt miljø. Oppdrettere, formidlere og brukere skal ta i bruk egnede berikingsmetoder som øker antallet aktiviteter dyrene har tilgang til, og som gir dem større mulighet til å mestre sin situasjon. Beriking kan oppnås gjennom fysisk trening, fødesøk og finmotoriske og kognitive aktiviteter som er egnet for arten. I dyreinnhegninger skal miljøberikingen tilpasses dyrearten og dyrenes individuelle behov. Berikingsprogram skal revideres og oppdateres regelmessig.

c) Dyreinnhegninger
Dyreinnhegninger skal være laget av materialer som ikke er helseskadelige for dyrene, og utformet og konstruert slik at dyrene ikke påføres skader. De skal være laget av engangssartikler eller av materialer som tåler rengjøring og desinfeksjon. Underlaget i innhegningen skal være tilpasset dyrenes art og alder, og utformet for enkel fjerning av urin og avføring.

3.4 Fôring

a) Fôr skal ha et innhold og gis i en form og på en måte som tilfredsstiller dyrets ernæringsmessige behov og eteatferd.
b) Fôret skal være smakelig og holde god hygienisk kvalitet. Ved valg av råvarer og metode for fremstilling, tilbereding og tildeling av fôr skal det sørges for minst mulig kjemisk, fysisk og mikrobiologisk forurensning av fôret.

c) Pakking, transport og lagring skal foregå slik at forurensing, forringelse eller ødeleggelse av fôret unngås. Alle fôrbeholdere, trau og annet utstyr som brukes til fôring, skal rengjøres regelmessig og steriliseres ved behov.

d) Hvert enkelt dyr skal ha tilgang til fôret. Fôringsarealet skal være så stort at konkurransen begrenses.

3.5 Vanning

a) Dyrene skal til enhver tid ha tilgang til rent drikkevann.

b) Automatiske drikkevannsanlegg skal kontrolleres, vedlikeholdes og gjennomspyles regelmessig. Ved bruk av bur med tett bunn skal det sørges for at risikoen for oversvømmelse er så liten som mulig.

c) For akvatiske dyr skal vannforsyningen i akvarier og kar være tilpasset behovet og toleransegrensene til den enkelte dyreart.

3.6 Hvile- og soveplasser

a) Dyrene skal til enhver tid ha tilgang til liggeunderlag og soveplasser som er tilpasset dyrearten. I forplantningsperioden skal dyrene ha tilgang til egnet redemateriale eller rede.

b) I dyreinnhegninger skal det for alle dyrene være et komfortabelt hvileområde med fast underlag egnet til den aktuelle dyrearten. Alle hvilearealer skal holdes rene og tørre.

3.7 Håndtering

Oppdrettere, formidlere og brukere skal iverksette tilvennings- og treningsprogrammer som er tilpasset dyrene og forsøkenes varighet.

Del B. Artsspesifikke krav

1. Mus, rotter, ørkenrotter, hamstere og marsvin

Med oppholdsenhetens høyde menes i denne og etterfølgende tabeller for mus, rotter, ørkenrotter, hamstere og marsvin den vertikale avstanden mellom oppholdsenhetens gulv og topp, og skal være til stede over mer enn 50 % av gulvets minsteareal før berikingsobjekter settes inn.

Ved planlegging av forsøk skal eventuell vekst hos dyrene tas i betraktning for å sikre at dyrene gis tilstrekkelig plass (som angitt i tabellene 1.1 til 1.5) så lenge studien varer.

Tabell 1.1

Mus

	Kroppsvekt (g)	Minsteareal i oppholdsenhet (cm^2)	Gulvareal per dyr (cm^2)	Minste høyde i oppholdsenhet (cm)	
I besetning og under forsøk	opp til 20	330	60	12	
	fra 20 til 25	330	70	12	
	fra 25 til 30	330	80	12	
	over 30	330	100	12	
Avl		330 For et monogamt par (utavl/innavl) eller en trio (innavl). For hvert ytterligere hunndyr med kull skal det legges til 180 cm^2		12	
Besetning hos oppdrettere (*) Oppholdsenhetens størrelse 950 cm^2	mindre enn 20	950	40	12	
Oppholdsenhetens størrelse 1500 cm^2	mindre enn 20	1500	30	12	

(*) Avvente mus kan holdes ved disse høyere tetthetene for den korte perioden mellom avvenning og flytting. Dette forutsetter at dyrene blir oppstallet i større oppholdsenheter med adekvat beriking og at oppstallingsforholdene ikke forårsaker noen svekkelse av velferden som f. eks. økt nivå av aggressjon, sykdom eller dødelighet, stereotypier og andre atferdsforstyrrelser, vekttap eller andre fysiologiske eller atferdsmessige stressreaksjoner.

Tabell 1.2

Rotter

	Kroppsvekt (g)	Minsteareal i oppholdsenhet (cm^2)	Gulvareal per dyr (cm^2)	Minste høyde i oppholdsenhet (cm)
I besetning og under forsøk (*)	opp til 200	800	200	18
	fra 200 til 300	800	250	18
	fra 300 til 400	800	350	18
	fra 400 til 600	800	450	18
	over 600	1500	600	18
Avl		800 Mor med kull. For hvert ytterligere voksent dyr som settes permanent inn i oppholdsenheten, skal det legges til 400 cm^2		18
Besetning hos oppdrettere (**) Oppholdsenhetens størrelse 1500 cm^2	mindre enn 50	1500	100	18
	fra 50 til 100	1500	125	18
	fra 100 til 150	1500	150	18
	fra 150 til 200	1500	175	18
Besetning hos oppdrettere (**) Oppholdsenhetens størrelse 2500 cm^2	opp til 100	2500	100	18
	fra 100 til 150	2500	125	18
	fra 150 til 200	2500	150	18

(*) Hvis tilgjengelig areal per dyr kommer under de angitte arealkrav på slutten av langtidsstudier, skal det prioriteres å opprettholde stabile sosiale strukturer.
(**) Avvente rotter kan holdes ved disse høyere tetthetene for den korte perioden mellom avvenning og flytting. Dette forutsetter at dyrene blir oppstallet i større oppholdsenheter med adekvat beriking og at oppstallingsforholdene ikke forårsaker noen svekkelse av velferden som f. eks. økt nivå av aggressjon, sykdom eller dødelighet, stereotypier og andre atferdsforstyrrelser, vekttap eller andre fysiologiske eller atferdsmessige stressreaksjoner.

Tabell 1.3

Ørkenrotter

	Kroppsvekt (g)	Minsteareal i oppholdsenhet (cm^2)	Gulvareal per dyr (cm^2)	Minste høyde i oppholdsenhet (cm)
I besetning og under forsøk	opp til 40	1200	150	18
	over 40	1200	250	18
Avl		1200 Monogame par eller trio med avkom		18

Tabell 1.4

Hamstere

	Kroppsvekt (g)	Minsteareal i oppholdsenhet (cm^2)	Gulvareal per dyr (cm^2)	Minste høyde i oppholdsenhet (cm)
I besetning og under forsøk	opp til 60	800	150	14
	fra 60 til 100	800	200	14
	over 100	800	250	14
Avl		800 Mor eller monogame par med kull		14
I besetning hos oppdretter (*)	Mindre enn 60	1500	100	14

(*) Avvente hamstere kan holdes ved disse høyere tetthetene for den korte perioden mellom avvenning og flytting. Dette forutsetter at dyrene blir oppstallet i større oppholdsenheter med adekvat beriking og at oppstallingsforholdene ikke forårsaker noen svekkelse av velferden som f. eks. økt nivå av aggressjon, sykdom eller dødelighet, stereotypier og andre atferdsforstyrrelser, vekttap eller andre fysiologiske eller atferdsmessige stressreaksjoner.

Tabell 1.5

Marsvin

	Kroppsvekt (g)	Minsteareal i oppholdsenhet (cm^2)	Gulvareal per dyr (cm^2)	Minste høyde i oppholdsenhet (cm)
I besetning og under forsøk	opp til 200	1800	200	23
	fra 200 til 300	1800	350	23
	fra 300 til 450	1800	500	23
	fra 450 til 700	2500	700	23
	over 700	2500	900	23
Avl		2500 Par med kull. For hver ytterligere avlshunn som settes inn, skal det legges til 1000 cm^2		23

2. Kaniner

I forbindelse med landbruksrelaterte forsøk hvor formålet krever at dyrene holdes på en lignende måte som produksjonsdyr, skal dyreholdet minst tilfredsstille de kravene som er gitt i forskrift 3. juli 2006 nr. 885 om velferd for produksjonsdyr. Dette gjelder ikke i den grad Mattilsynet i godkjenningen av forsøket har tillatt avvik fra produksjonsdyr-forskriften.

Oppholdsenheten skal være utstyrt med et hevet område (hylle) som ligger høyere enn omgivelsene. Det hevede området skal være utformet slik at dyret kan ligge, sitte og uten vanskelighet bevege seg under det. Området skal ikke dekke mer enn 40 % av gulvarealet. Hvis det av vitenskapelige eller veterinærmedisinske årsaker ikke kan brukes et hevet område, skal oppholdsenheten være 33 % større for én kanin og 60 % større for to kaniner. Hvis kaniner yngre enn 10 uker gis adgang til et hevet område, skal dette være minst 55 cm x 25 cm stort, og høyden over gulvet skal være så stor at dyrene skal kunne utnytte den.

Tabell 2.1

Kaniner eldre enn 10 uker

Tabell 2.1 skal brukes for både bur og innhegninger. For hver kanin over to skal det legges til et gulvareal på minst 3000 cm^2 til og med den sjette kaninen, og deretter minst 2500 cm^2 for hver kanin over seks.

Endelig kroppsvekt (kg)	Minste gulvareal for ett eller to sosialt harmoniske dyr (cm^2)	Minste høyde (cm)
mindre enn 3	3500	45
fra 3 til 5	4200	45
over 5	5400	60

Tabell 2.2

Kaninhunn med kull

Hunnens kroppsvekt (kg)	Minsteareal i oppholdsenhet (cm^2)	Tillegg for redekasser (cm^2)	Minste høyde (cm)
mindre enn 3	3500	1000	45
fra 3 til 5	4200	1200	45
over 5	5400	1400	60

Tabell 2.3

Kaniner yngre enn 10 uker

Tabell 2.3 gjelder både bur og innhegninger.

Alder	Minsteareal i oppholdsenhet (cm^2)	Minste gulvareal per dyr (cm^2)	Minste høyde (cm)
Avvenning til 7 uker	4000	800	40
Fra 7 til 10 uker	4000	1200	40

Tabell 2.4

Kaniner: Optimal størrelse på hevet område i oppholdsenhet med størrelse som angitt i tabell 2.1

Alder i uker	Endelig kroppsvekt (kg)	Optimal størrelse (cm x cm)	Optimal høyde fra gulvet i oppholdsenheten (cm)
over 10	mindre enn 3	55 x 25	25
	fra 3 til 5	55 x 30	25
	over 5	60 x 35	30

3. Katter

Katter skal ikke holdes oppstallet enkeltvis i mer enn 24 timer av gangen. Katter som er gjentagende aggressive overfor andre katter, kan bare oppstalles enkeltvis hvis det ikke lar seg gjøre å finne en egnet partner. Hos alle individer som er oppstallet i par eller i grupper, skal sosialt stress overvåkes minst ukentlig. Hunner med kattunger som er yngre enn fire uker, og hunner i de siste to uker av drektigheten, kan oppstalles enkeltvis.

Tabell 3

Katter

Minste tillatte areal for hold av hunnkatter med unger er det samme som for en enkelt katt. Arealet skal økes gradvis slik at kullet senest ved 4 måneders alder er oppstallet etter arealkravene for voksne dyr.

Fôringsplasser og avføringskasser skal ikke være mindre enn 0,5 meter fra hverandre, og disse skal ikke bytte plass med hverandre.

	Gulv (*) (m^2)	Hyller (m^2)	Høyde (m)
Minimum for ett voksent dyr	1,5	0,5	2
For hvert dyr i tillegg	0,75	0,25	–

(*) Gulvareal utenom hyller

4. Hunder

Hunder skal så vidt mulig ha tilgang til utendørs løpegårder. Hunder skal ikke oppstalles enkeltvis i mer enn 4 timer av gangen.

Innendørs oppholdsenhet skal utgjøre minst 50 % av minstearealet som hundene gis tilgang til, jf. tabell 4.1.

Tilgjengelig areal som angitt nedenfor er basert på behovene til beaglehunder. Store raser som St. Bernhardshund eller irsk ulvehund skal gis tilgang til vesentlig større arealer enn det som er angitt i tabell 4.1. For andre raser enn beagle skal arealtilgangen bestemmes i samråd med dyrehelsepersonell.

Tabell 4.1

Hunder

Hunder som holdes i par eller grupper, kan holdes enkeltvis på halvparten av det totale arealet (2 m^2 for en hund under 20 kg, 4 m^2 for en hund over 20 kg) mens de er i forsøk, hvis denne adskillelsen er nødvendig av vitenskapelige årsaker. En hund skal ikke begrenses til dette arealet i mer enn 4 timer av gangen.

En diende tispe med valpekull skal ha samme arealtilgang som en enkelt tispe med samme vekt. Valpeenheten skal være utformet slik at tispen kan bevege seg til en annen avdeling eller et hevet område, vekk fra valpene.

Vekt (kg)	Minsteareal i oppholdsenhet (m^2)	Minste gulvareal for ett eller to dyr (m^2)	Minste tillegg for hvert ytterligere dyr (m^2)	Minste høyde (m)
opp til 20	4	4	2	2
over 20	8	8	4	2

Tabell 4.2

Hunder – avvente dyr

Hundens vekt (kg)	Minsteareal i oppholdsenhet (m^2)	Minste gulvareal/ dyr (m^2)	Minste høyde (m)
opp til 5	4	0,5	2
fra 5 til 10	4	1,0	2
fra 10 til 15	4	1,5	2
fra 15 til 20	4	2	2
over 20	8	4	2

5. Ildere

Tabell 5

Ildere

	Minsteareal i oppholdsenhet (cm^2)	Minste gulvareal per dyr (cm^2)	Minste høyde (cm)
Dyr opp til 600 g	4500	1500	50
Dyr over 600 g	4500	3000	50
Voksne hanner	6000	6000	50
Hunndyr med kull	5400	5400	50

6. Primater

Unge primater skal ikke skilles fra moren før de er mellom 6 og 12 måneder gamle, avhengig av art.

Miljøet skal tilrettelegges slik at primater kan utføre komplekse aktivitetsprogram daglig. Primater skal holdes i oppholdsenheter som gir dyrene mulighet til å ha et så bredt atferdsspekter som mulig, trygghetsfølelse og et tilstrekkelig komplekst miljø hvor dyret kan løpe, gå, klatre og hoppe.

Tabell 6.1

Silkeaper og tamariner

	Minsteareal i oppholdsenhet for ett (*) eller to dyr pluss avkom opp til 5 måneders alder (m^2)	Minste volum for hvert ytterligere dyr over 5 måneder (m^3)	Minste høyde i oppholdsenhet (m)(**)
Silkeaper	0,5	0,2	1,5
Tamariner	1,5	0,2	1,5

(*) Dyr kan holdes enkeltvis bare i unntakstilfeller.
(**) Oppholdsenhetens topp skal være minst 1,8 m fra gulvet.

Silkeaper og tamariner skal ikke skilles fra moren før de er 8 måneder gamle.

Tabell 6.2

Ekornaper

Minste gulvareal for ett (*) eller to dyr (m^2)	Minste volum for hvert ytterligere dyr over 6 måneder (m^3)	Minste høyde i oppholdsenhet (m)
2,0	0,5	1,8

(*) Dyr kan holdes enkeltvis bare i unntakstilfeller.

Ekornaper skal ikke skilles fra moren før de er 6 måneder gamle.

Tabell 6.3

Makaker og vervetaper (*)

	Minsteareal i oppholdsenhet (m^2)	Minste volum i oppholdsenhet (m^3)	Minste volum per dyr (m^3)	Minste høyde i oppholdsenhet (m)
Dyr yngre enn 3 år (**)	2,0	3,6	1,0	1,8
Dyr fra 3 års alder (***)	2,0	3,6	1,8	1,8
Avlsdyr (****)			3,5	2,0

(*) Enkeltvis oppstalling av dyr kan bare skje i unntakstilfeller.
(**) I oppholdsenhet med minstemål kan det holdes maksimum tre dyr.
(***) I oppholdsenhet med minstemål kan det holdes maksimum to dyr.
(****) I avlsgrupper kreves ikke tilleggsareal/volum for unge dyr opp til 2 års alder som holdes sammen med moren.

Makaker og vervetaper skal ikke skilles fra moren før de er 8 måneder gamle.

Tabell 6.4

Bavianer (*)

	Minsteareal i oppholdsenhet (m^2)	Minste volum i oppholdsenhet (m^3)	Minste volum per dyr (m^3)	Minste høyde i oppholdsenhet (m)
Dyr yngre enn 4 år (**)	4,0	7,2	3,0	1,8
Dyr fra 4 års alder (**)	7,0	12,6	6,0	1,8
Avlsdyr (***)			12,0	2,0

(*) Enkeltvis oppstalling av dyr kan bare skje i unntakstilfeller.
(**) I oppholdsenhet med minstemål kan det holdes maksimum to dyr.
(***) I avlsgrupper kreves ikke tilleggsareal/volum for unge dyr opp til 2 års alder som holdes sammen med moren.

Bavianer skal ikke skilles fra moren før de er 8 måneder gamle.

7. Produksjonsdyr

I forbindelse med landbruksrelaterte forsøk hvor formålet krever at dyrene holdes på en lignende måte som produksjonsdyr, skal dyreholdet minst tilfredsstille de krav som er gitt i forskrift 3. juli 2006 nr. 885 om velferd for produksjonsdyr, forskrift 18. februar 2003 nr. 175 om hold av svin, forskrift 22. april 2004 nr. 665 om hold av storfe, forskrift 18. februar 2005 nr. 160 om velferd for småfe, forskrift 2. juni 2005 nr. 505 om velferd for hest og forskrift 17. mars 2011 nr. 296 om hold av pelsdyr. Dette gjelder ikke i den grad Mattilsynet i godkjenningen av forsøket har tillatt avvik fra holdforskriftene.

Tabell 7.1

Storfe

Kroppsvekt (kg)	Minsteareal i oppholdsenhet (m^2)	Minste gulvareal per dyr (m^2 / dyr)	Eteplass til ad libitum fôring av avhornet storfe (m/dyr)	Eteplass til restriktiv fôring av avhornet storfe (m/dyr)
opp til 100	2,50	2,30	0,10	0,30
fra 100 til 200	4,25	3,40	0,15	0,50
fra 200 til 400	6,00	4,80	0,18	0,60
fra 400 til 600	9,00	7,50	0,21	0,70
fra 600 til 800	11,00	8,75	0,24	0,80
over 800	16,00	10,00	0,30	1,00

Tabell 7.2

Sauer og geiter

Kroppsvekt (kg)	Minsteareal i oppholdsenhet (m^2)	Minste gulvareal per dyr (m^2 / dyr)	Minste høyde på skillevegg (m)	Eteplass til ad libitum fôring (m/dyr)	Eteplass til restriktiv fôring (m/dyr)
mindre enn 20	1,0	0,7	1,0	0,10	0,25
fra 20 til 35	1,5	1,0	1,2	0,10	0,30
fra 35 til 60	2,0	1,5	1,2	0,12	0,40
over 60	3,0	1,8	1,5	0,12	0,50

Tabell 7.3

Griser og minigriser

Kroppsvekt (kg)	Minsteareal i oppholdsenhet (*) (m^2)	Minste gulvareal per dyr (m^2 / dyr)	Minste liggeareal per dyr (under termonøytrale forhold) (m^2 / dyr)
opp til 5	2,0	0,20	0,10
fra 5 til 10	2,0	0,25	0,11
fra 10 til 20	2,0	0,35	0,18
fra 20 til 30	2,0	0,50	0,24
fra 30 til 50	2,0	0,70	0,33
fra 50 til 70	3,0	0,80	0,41
fra 70 til 100	3,0	1,00	0,53
fra 100 til 150	4,0	1,35	0,70
over 150	5,0	2,50	0,95
voksne (konvensjonelle) råner	7,5		1,30

(*) Griser kan holdes i mindre oppholdsenheter for kortere perioder, for eksempel ved å dele opp hovedenheten med skillevegger når dette er berettiget ut fra veterinærmedisinske eller eksperimentelle grunner. For eksempel når individuelt fôropptak er påkrevd.

Tabell 7.4

Hester

Den korteste siden skal være minst 1,5 ganger dyrets mankehøyde i stangmål. Høyden i innendørs oppholdsenheter skal være så stor at dyrene kan steile i full høyde.

Mankehøyde (m)	Minste gulvareal per dyr (m^2 / dyr)			Minste høyde i oppholdsenhet (m)
	For hvert dyr oppstallet enkeltvis eller i grupper på opp til 3 dyr	For hvert dyr holdt i grupper på 4 dyr eller flere	Føllingsboks / hoppe med føll	
1,00 til 1,40	9,0	6,0	16	3,00
fra 1,40 til 1,60	12,0	9,0	20	3,00
over 1,60	16,0	$(2 \times MH)^2$ (*)	20	3,00

(*) For å sikre tilstrekkelig plass skal tilgjengelig areal for hvert enkelt dyr beregnes ut fra mankehøyden (MH - stangmål).

8. Fugler

I forbindelse med landbruksrelaterte forsøk hvor formålet krever at dyrene holdes på en lignende måte som produksjonsdyr, skal dyreholdet minst tilfredsstille de krav som er gitt i forskrift 3. juli 2006 nr. 885 om velferd for produksjonsdyr, forskrift 12. desember 2001 nr. 1494 om hold av høns og kalkun, og forskrift 2. oktober 1998 nr. 951 om hold av strutsefugl. Dette gjelder ikke i den grad Mattilsynet i godkjenningen av forsøket har tillatt avvik fra holdforskriftene.

Tabell 8.1

Tamhøns

Hvis det av vitenskapelige grunner ikke er mulig å overholde minstekravene til oppholdsenhetens størrelse, skal forsøkslederen i samråd med veterinærmedisinsk personell begrunne varigheten av dyreholdet på det begrensede arealet. I slike tilfeller kan fugler oppstalles i mindre oppholdsenheter med egnet miljøberiking og gulvareal på minst 0,75 m^2.

Kroppsvekt (g)	Minsteareal i oppholdsenhet (m^2)	Minsteareal per fugl (m^2)	Minste høyde (cm)	Minste lengde på fôrtrau per fugl (cm)
opp til 200	1,00	0,025	30	3
fra 200 til 300	1,00	0,03	30	3
fra 300 til 600	1,00	0,05	40	7
fra 600 til 1200	2,00	0,09	50	15
fra 1200 til 1800	2,00	0,11	75	15
fra 1800 til 2400	2,00	0,13	75	15
over 2400	2,00	0,21	75	15

Tabell 8.2

Tamkalkun

Alle sidene i oppholdsenheten skal minst være 1,5 m lange. Hvis det av vitenskapelige årsaker ikke er mulig å overholde minstekravene til oppholdsenehetens størrelse, skal forsøkslederen i samråd med veterinærmedisinsk personell begrunne varigheten av dyreholdet på det begrensede arealet. I slike tilfeller kan fugler oppstalles i mindre oppholdsenheter med egnet miljøberiking. Gulvarealet skal da være minst 0,75 m^2 og høyden minst 50 cm for fugler under 0,6 kg, 75 cm for fugler under 4 kg og 100 cm for fugler over 4 kg. Disse kan benyttes til oppstalling av mindre grupper av fugler i samsvar med arealkravene i tabell 8.2.

Kroppsvekt (kg)	Minsteareal i oppholdsenhet (m^2)	Minsteareal per fugl (m^2)	Minste høyde (cm)	Minste lengde på fôrtrau per fugl (cm)
opp til 0,3	2,00	0,13	50	3
fra 0,3 til 0,6	2,00	0,17	50	7
fra 0,6 til 1	2,00	0,30	100	15
fra 1 til 4	2,00	0,35	100	15
fra 4 til 8	2,00	0,40	100	15
fra 8 til 12	2,00	0,50	150	20
fra 12 til 16	2,00	0,55	150	20
fra 16 til 20	2,00	0,60	150	20
over 20	3,00	1,00	150	20

Tabell 8.3

Vaktler

Kroppsvekt (g)	Minsteareal i oppholdsenhet (m^2)	Minsteareal per fugl, parvis oppstallet (m^2)	Minsteareal per fugl i tillegg, gruppeoppstallet fugl (m^2)	Minste høyde (cm)	Minste lengde på fôrtrau per fugl (cm)
opp til 150	1,00	0,5	0,10	20	4
over 150	1,00	0,6	0,15	30	4

Tabell 8.4

Ender og gjess

Hvis det av vitenskapelige årsaker ikke er mulig å overholde minstekravene til oppholdsenhetens størrelse, skal forsøkslederen i samråd med veterinærmedisinsk personell begrunne varigheten av dyreholdet på det begrensede arealet. I slike tilfeller kan fugler oppstalles i mindre oppholdsenheter med egnet miljøberiking og gulvareal på minst 0,75 m^2. Disse kan benyttes til oppstalling av mindre grupper av fugler i samsvar med arealkravene i tabell 8.4.

Kroppsvekt (g)	Minsteareal i oppholdsenhet (m^2)	Areal per fugl (m^2) (*)	Minste høyde (cm)	Minste lengde på fôrtrau per fugl (cm)
Ender				
opp til 300	2,00	0,10	50	10
fra 300 til 1200 (**)	2,00	0,20	200	10
fra 1200 til 3500	2,00	0,25	200	15
over 3500	2,00	0,50	200	15
Gjess				
opp til 500	2,00	0,20	200	10
fra 500 til 2000	2,00	0,33	200	15
over 2000	2,00	0,50	200	15

(*) Dette skal inkludere en dam med et minsteareal på 0,5 m^2 per 2,0 m^2 oppholdsenhet og en minste dybde på 30 cm. Dammen kan utgjøre opp til 50 % av oppholdsenhetens minsteareal.
(**) Fugler som ennå ikke er flyvedyktige, kan holdes i oppholdsenheter med en minste høyde på 75 cm.

Tabell 8.5

Ender og gjess: Minste damstørrelser (*)

	Areal (m^2)	Dybde (cm)
Ender	0,5	30
Gjess	0,5	fra 10 til 30

(*) Damarealene er per 2 m^2 oppholdsenhet. Dammen kan utgjøre opp til 50 % av oppholdsenhetens minsteareal.

Tabell 8.6

Duer

Oppholdsenhetene skal være lange og smale (for eksempel 2 m x 1 m) i stedet for kvadratiske slik at fuglene kan foreta korte flyveturer.

Gruppestørrelse	Minsteareal i oppholdsenhet (m^2)	Minste høyde (cm)	Minste lengde på fôrtrau per fugl (cm)	Minste sittepinnelengde per fugl (cm)
opp til 6	2	200	5	30
fra 7 til 12	3	200	5	30
for hver fugl i tillegg over 12	0,15		5	30

Tabell 8.7

Sebrafinker

Oppholdsenhetene skal være lange og smale (for eksempel 2 m x 1 m) slik at fuglene kan foreta korte flyveturer. I avlsstudier kan par oppstalles i mindre oppholdsenheter med egnet miljøberiking. Gulvarealet skal da være minst 0,5 m^2 og høyden minst 40 cm. Forsøkslederen skal i samråd med veterinærmedisinsk personell begrunne varigheten av dyreholdet på det begrensede arealet.

Gruppestørrelse	Minsteareal i oppholdsenhet (m^2)	Minste høyde (cm)	Minste antall fôrskåler
opp til 6	1,0	100	2
7 til 12	1,5	200	2
13 til 20	2,0	200	3
for hver fugl i tillegg over 20	0,05		1 per 6 fugler

9. Amfibier

Tabell 9.1

Vannlevende salamandere

Kroppslengde (*) (cm)	Minste vannoverflateareal (cm^2)	Minste vannoverflateareal per dyr i tilegg i gruppehold (cm^2)	Minste vanndybde (cm)
opp til 10	262,5	50	13
fra 10 til 15	525	110	13
fra 15 til 20	875	200	15
fra 20 til 30	1837,5	440	15
over 30	3150	800	20

(*) Målt fra snute til kloakkåpning

Tabell 9.2

Vannlevende springpadder (*)

Kroppslengde (**) (cm)	Minste vannoverflateareal (cm^2)	Minste vannoverflateareal per dyr i tillegg i gruppehold (cm^2)	Minste vanndybde (cm)
mindre enn 6	160	40	6
fra 6 til 9	300	75	8
fra 9 til 12	600	150	10
over 12	920	230	12,5

(*) Disse kravene gjelder for oppstalling i tanker (dvs. dyrehold), og ikke for tanker som av effektiviteshensyn benyttes for naturlig paring og superovulasjon, da disse prosedyrene krever mindre individuelle tanker. Arealkravene gjelder voksne dyr i de angitte størrelseskategoriene. Ungdyr og rumpetroll skal enten ikke regnes med, eller tankmålene endres på en forholdsmessig måte.
(**) Målt fra snute til kloakkåpning

Tabell 9.3

Delvis vannlevende springpadder

Kroppslengde (*) (cm)	Minsteareal i oppholdsenhet (**) (cm^2)	Minsteareal for hvert dyr i tillegg i gruppehold (cm^2)	Minste høyde i oppholdsenhet (***) (cm)	Minste vanndybde (cm)
opp til 5,0	1500	200	20	10
fra 5,0 til 7,5	3500	500	30	10
over 7,5	4000	700	30	15

(*) Målt fra snute til kloakkåpning
(**) En tredjedel land og to tredjedeler vann, nok til at dyrene kan ligge helt under vannflaten
(***) Målt fra overflaten av landdelen opp til innersiden av terrariets øverste kant. Oppholdsenhetens høyde skal også tilpasses innredningen.

Tabell 9.4

Landlevende springpadder

Kroppslengde (*) (cm)	Minsteareal i oppholdsenhet (**) (cm^2)	Minsteareal for hvert dyr i tillegg i gruppehold (cm^2)	Minste høyde i oppholdsenhet (***) (cm)	Minste vanndybde (cm)
opp til 5,0	1500	200	20	10
fra 5,0 til 7,5	3500	500	30	10
over 7,5	4000	700	30	15

(*) Målt fra snute til kloakkåpning
(**) To tredjedeler land og en tredjedel vann, nok til at dyrene kan ligge helt under vannflaten
(***) Målt fra overflaten av landdelen opp til innersiden av terrariets øverste kant. Oppholdsenhetens høyde skal også tilpasses innredningen.

Tabell 9.5

Trelevende springpadder

Kroppslengde (*) (cm)	Minsteareal i oppholdsenhet (**) (cm^2)	Minsteareal for hvert dyr i tillegg i gruppehold (cm^2)	Minste høyde i oppholdsenhet (***) (cm)	Minste vanndybde (cm)
opp til 3,0	900	100	30	10
over 3,0	1500	200	30	10
				15

(*) Målt fra snute til kloakkåpning
(**) To tredjedeler land og en tredjedel vann, nok til at dyrene kan ligge helt under vannflaten
(***) Målt fra overflaten av landdelen opp til innersiden av terrariets øverste kant. Oppholdsenhetens høyde skal også tilpasses innredningen.

10. Krypdyr

Tabell 10.1

Vannlevende skilpadder

Kroppslengde (*) (cm)	Minste vannoverflateareal (cm^2)	Minste vannoverflateareal for hvert dyr i tillegg i gruppehold (cm^2)	Minste vanndybde (cm)
opp til 5	600	100	10
fra 5 til 10	1600	300	15
fra 10 til 15	3500	600	20
fra 15 til 20	6000	1200	30
fra 20 til 30	10000	2000	35
over 30	20000	5000	40

(*) Målt i rett linje fra fremre til bakre kant av skallet

Tabell 10.2

Landlevende slanger

Kroppslengde (*) (cm)	Minste gulvareal (cm^2)	Minste areal for hvert dyr i tillegg i gruppehold (cm^2)	Minste høyde i oppholdsenhet (**) (cm)
opp til 30	300	150	10
fra 30 til 40	400	200	12
fra 40 til 50	600	300	15
fra 50 til 75	1200	600	20
over 75	2500	1200	28

(*) Målt fra snute til halespiss
(**) Målt fra overflaten av landdelen opp til innersiden av terrariets øverste kant. Oppholdsenhetens høyde skal også tilpasses innredningen.

11. Fisk

11.1 Vanntilførsel og vannkvalitet

Det skal til enhver tid være nok vann av egnet kvalitet. Vannstrømmen i resirkuleringssystemer eller filtrering i karene skal være tilstrekkelig til å sikre at vannkvalitetsparameterne holdes innenfor akseptable nivåer. Vanntilførselen skal om nødvendig filtreres eller behandles for å fjerne stoffer som er skadelige for fisken. Vannkvalitetsparameterne skal hele tiden være innenfor akseptable grenser slik at normal aktivitet og normale fysiologiske prosesser opprettholdes for den aktuelle art og utviklingsstadium. Vannstrømmen skal avpasses slik at fisken kan svømme på normal måte og utøve normal atferd. Fisken skal gis passe tid til å akklimatisere og tilpasse seg endringer i vannkvaliteten.

11.2 Oksygen, nitrogenforbindelser, pH og salinitet

Oksygenkonsentrasjonen skal avpasses til den enkelte arts behov og forholdene fisken holdes under. Om nødvendig skal det foretas ekstra lufting av vannet i karene. Konsentrasjonen av nitrogenforbindelser skal holdes lav. pH-verdien skal tilpasses arten og holdes så stabil som mulig. Saliniteten skal tilpasses artens behov og utviklingsstadium. Endringer i saliniteten skal skje gradvis.

11.3 Temperatur, belysning, støy

Temperaturen skal holdes så stabil som mulig innenfor optimumsgrensene for den aktuelle fiskearten. Endringer i temperaturen skal skje gradvis. Fisk skal holdes i en fotoperiode som er tilpasset artens behov. Støynivået skal holdes på et minimum. Der det er mulig, skal utstyr som forårsaker støy eller vibrasjoner, så som generatorer eller filtreringsanlegg, være atskilt fra fiskekarene.

11.4 Fisketetthet og miljøkompleksitet

Tettheten av fisk skal være basert på fiskens samlede behov når det gjelder miljøforhold, helse og velferd. Fisk skal ha tilgang til et vannvolum som er stort nok til at den kan svømme normalt, tatt i betraktning fiskens størrelse, alder, helse og

fôropptak. Fisk skal tilbys egnet miljøberiking, slik som skjulesteder eller bunnsubstrat, hvis ikke atferdsmessige egenskaper indikerer at det ikke er behov for dette.

11.5 Fôring og håndtering

Fisk skal fôres med et egnet fiskefôr i riktig mengde og med intervaller tilpasset artens behov. Ved fôring av fiskelarver skal enhver overgang fra levende til tilvirket fôr vies spesiell oppmerksomhet. Fisk skal håndteres så lite som mulig.

Module 2 -

Ethics, animal welfare and the 3Rs

Contents

Introduction

This module is based on the framework for education and training by the European Commission learning outcomes for module 2 and adapted to Norwegian conditions.

The use of animals in research raises ethical questions and there are different opinions in the public if animal use can be justified. Even though there are strict regulation for animal use there are not all situations that can be addressed by regulatory compliance, and compliance with the regulations does not assure ethical behavior.

- Presentation: Introduction to research animal ethics

Curriculum to ethics module (I)

The curriculum to this module is defined as following

- all pages in this document
- all presentations in this module including PDFs
- Painful dilemmas: A study of the way the public's assessment of animal research
- The European Citizens initiative (Lenker til en ekstern side.)Lenker til en ekstern side.
- Communication from the EU commission to the "stop vivisection" initiative
- Current concepts of Harm–Benefit Analysis of Animal Experiments
- More examples: classification_of_severity_of_procedures.pdf

Ethical positions and approached to ethical reflections

Not all questions can be answered by regulations. The use of animals in research raises ethical questions and there are different opinions in the public if animal use can be justified.

Even though there are strict regulations for animal use, not all question can be addressed by regulatory compliance. Compliance with the regulations alone does not assure ethical behavior.

The aim of this module is to learn about different views, within society, relating to the scientific uses of animals and recognize the need to respect these

Kantianism and utilitarianism

In the regulations on animal research, we find duties and instructions for how to behave when animals are used in research, and these duties and instructions reflects common norm for right and wrong behavior.

Deontology or Duty Ethics

Imanuel Kant was the founder of the deontology or "duty" ethics. According to Kant there are certain rules for right behavior that are universal and inviolable ("the categorical imperative").

One of the duties according to Kant, is to never use an individual as a mean to achieve an end.

In Kant's opinion, there is a relevant distinction between man and animal in the way that man can act in accordance with reasons and rule over instincts and desires.

Tom Regan (1938-2017) is another deontologist who is famous for the book "The Case for Animal Rights" (1983). This book has significantly influenced the animal rights movement.

Tom Regan introduced the distinction between *agens* and *patiens.* By *agens* Regan means someone with moral responsibility to act in accordance with duties and can be blamed for not acting in accordance with the duties. By patiens Regan means someone which we have obligations towards, for example small children or animals.

An important difference between Regan and Kant, is that Kant stated that to have rights to be treated according to moral duties (i.e. to be a patiens) you also have to be able to act by moral duties (i.e. to be an agens). Regan state that there are many patients, which are not agens, for example, infants or animals, and they should have the right to be treated according to moral principles, even if for example an infant cannot be hold responsible, we have duties towards them. Regan rejects Kant's idea that respect is due only to rational beings. This is an important distinction between Kant and Regan.

The regulations on the use of animals reflects duty ethics as for example the duty to apply the principles of the 3Rs in planning and performing experiments involving animals.

What Kant say about using animals in research

> those “who use living animals for their experiments, certainly act cruelly, although their aim is praiseworthy, and they can justify their cruelty, since animals must be regarded as man's instruments”

Utilitarianism

As opposed to the deontology (“duty” ethics) which focus on the motive behind and action, the utilitarianism emphasizes the consequence of an action. I.e., the moral worth of an action is determined by its outcome (“the ends justify the means”) or the proper action is the one that cause the “greater good for the greatest number”.

Jeremy Bentham (1747-1832) and James Mill (1773-186) were founders of the utilitarian philosophy, and Bentham has been regarded as the earliest advocate for taking animal interests into account and argued that it is the ability to suffer, not the ability to reason that is relevant.

The question is not, Can they reason? nor, Can they talk? but, Can they suffer

> Even Jeremy Bentham, founder of utilitarian moral philosophy would not state animal research to be unethical, provided the experiment had "a determinate object, beneficial to mankind, accompanied with a fair prospect of the accomplishment of it”, thus acknowledging humans had certain precedence over other animals

Peter Singer (1946 -) is a philosopher and utilitarian who has among other subjects been a voice in the discussion of animal research. According to Singer, as also did Bentham, animals need protections against harm based on their ability to suffer not on their level of intelligence.

Singer is the author of the influential book Animal Liberation, where he criticize the way we treat animals in the industrial world which has been an inspiration for change to vegetarian or vegan life style. He also popularized the term “speciesism” referring to the privilege humans have over other animals and argues in favor of equal considerations of interest of all sentient beings (with the ability to suffer)

The utilitarian approach is reflected in the regulations as experiments in animals must be justified because of potential benefits. However, even if animal experiments are useful and ethical acceptable in some circumstances, this do not imply that animal experiments are acceptable under all circumstances. You cannot make a universal rule saying that animal experiments are useful and therefore un-problematic in general.

*Definition:

A sentient being is one that has some ability: to **evaluate the actions** of others in relation to itself and third parties, to **remember** some of its own actions and their consequences, **to assess risks and benefits,** to have some feelings and to have some degree of **awareness** (Broom 2006c, 2014).

Cognitive abilities

Many studies of cognitive ability that lead to the conclusions that:

(a) hardly any ability is uniquely human,
(b) the best bird brains allow greater cognitive ability than any mammal except man,
(c) learning by fish can be very complex, and
(d) cognition in cephalopods, jumping spiders, ants and bees is much more sophisticated than we had previously thought.

(11) (PDF) Sentience and pain in relation to animal welfare. Available from: https://www.researchgate.net/publication/289790582_Sentience_and_pain_in_relation_to_animal_welfare [accessed Aug 25 2021].

- *Peter Singer gives his key note speech at Scand-LAS Tuesday May 30 2017 in Copenhagen.*

Animal welfare versus animal rights

Another way to distinguish between different views on the use of animals is research draws a border between animal welfare or to what extent the animal can suffer and the animal's right not to be used.

An animal welfarist will say that if the animal's experienced welfare is not negative compromised there is no concern. For insentient beings or immature stages, this might not be the case. The problem with this view is that it is not always clear where is the border of animals or stages are sentient or not.

An animal right person claim that animals have the right to be protected because of their intrinsic value and cannot be used as a mean to an end so in any case it is wrong to use them whether suffering is involved or not. According to Tom Regan animals are "subject-of-life" and have moral rights and these rights apply even if they are not recognized ("*The case of animals rights*", 1983)

An example of a distinction between animal welfare and animal right is the production of GMO animals. From an animal welfare perspective this is not a problem as longs as it does not influence animal welfare in a negative way. In our legislation there is a distinction between so called harmful and non-harmful phenotypes, where the fist causes more concern than the latter that are comparable to a "normal" animal. From an animal's rights perspective changing animal's genome is under all circumstances wrong independent of the outcome because the animal's intrinsic value is compromised

Another example of the distinction between animal right and animal welfare is the use of animals for terminal procedure or for use off tissues after euthanasia. Use of animal only for harvesting organs or tissues is not even regulated in many countries including Norway. From an animal welfare perspective this cause no welfare issues if the animals is correctly treated as long as it is conscious and killed in a humane way. An animal rights advocate will however still think that this practice is wrong.

The present mainstream approach to animal use in research is predominantly utilitarian in nature, and in general greater attention is given to preventing the suffering of animals than to avoiding their killing.

Under current European legislation regulating just animal use in experiments, killing laboratory animals for harvesting organs or tissues is not considered to be a procedure.

This is consistent with the predominant welfarist view of good practice in research with animals, under which the painless killing of laboratory animals poses no ethical problem, or at least not from a welfare point a view

Anesthesia allows researchers to use great numbers of animals without further moral quandaries.

Different views on use of animals in research – other positions

If you discuss the use of animals in research with your colleagues, it likely that animal experiments are perceived as important and necessary and use of animals is therefore justified.

If you discuss this with random people you meet in the street, you likely get different answers.

A study in Denmark showed that the proportion of people against use of animals (disapprovers) was about 16-17% of the population asked. About twice that number think animal experiments are ok and do not question it too much. About half of the population asked will not give a pro or against answer but rather question of use of animals is really necessary, will the animals suffer and what is the purpose of the study.

Read the paper "Painful dilemmas: A study of the way the public's assessment of animal research balances costs to animals against human benefits"

For and against use of animals in research

Read the material on the 2 links below

- The European Citizens initiative
- Communication from the EU commission to the "stop vivisection" initiative

Task: Make a list of arguments defending and against animal experiment based on these.

Defending animal research	Against animal research

Different views on animal use – sub-graduate biology vs medical students

The use of animals in biomedical research is debated in public, within the scientific community and among students[i],[ii],[iii],[iv].

Despite increased efforts and success in developing alternative methods, they mainly reduced and refined animal experiments and only a few replaced them entirely.

Thus, animal experiments remain part of the job as a biomedical scientist[v].

Knowledge about animal experiments and alternative methods is essential for students in the biomedical field.

Although genuine efforts to improve the conditions of animals used in research and transparency, the attitude of the public towards animal experiments remains very diverse and ranges from complete abolition to strong support[vi],[vii],[viii] .

People opposed to animal experiments commonly focus on animal welfare and their suffering. In contrast, those involved in animal research tend to base their arguments on the benefits that research confers on medical care and new drug or therapy development and the lack of alternatives to animal models[ix],[x],[xi] .

Several studies showed the relationship between science and support for animal research i,[xii],[xiii]. These findings suggest a **relationship between informed knowledge and attitudes towards animal experiments**. Also, studies suggest that students of biomedical fields are more supportive of animal experimentation compared with the general public iii,[xiv],[xv].

Persons must have a university degree and adequate education and training to perform and direct animal experiments. Therefore, training courses in Laboratory Animal Science are provided. However, students become aware of this very late in their studies when decisions about their future careers have already been made.

No recommendations for the education of **undergraduate biology or medical students** regarding the theoretical principles of LAS exists, even though animal experiments and related topics may be part of their daily business as future scientists or physicians. They only meet Laboratory Animal Science when animal experiments are part of their dissertation or if they took a voluntary information course or an extracurricular.

In general, researchers must understand how animal experiments are designed and how they can be improved according to the 3Rs if they perform animal experiments on their own or evaluate them. This is also true for medical doctors. Here, basic education regarding animal experiments should be part of their studies to interpret results of animal studies in research publications and in regulatory studies, which are the basis for drug licensing and developing treatment options for patients.

Biology and medicine students report that the topic 'animal experiments' is not covered, is discussed rarely or not in enough detail. In general, biology and medical students are becoming aware of the topic of animal experiments very late when decisions about their future careers are already made. This can be a major drawback when realizing that performing animal experiments is not an option for ethical, emotional or lack of practical skills reasons.

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Humans' responsibility and importance of animal welfare

Humans' responsibility when working or caring for research animals. Recognizing the importance of having a respectful and humane attitude towards research animals.

The "Empathy model" for dilemma-like situations is based on the conviction that "one should not treat others in ways that one would not like to be treated"

Can it be ethically justified to use animals in research to obtain knowledge of how to treat diseases in man? - Or to use animals to study how to prevent or cure diseases in other animals so that animals are used as mean to an end?

There is a dilemma here. The harmful action is to make the animal ill. The good action is to maintain better understanding of the disease in advantage for other animals or man. In animal research, the animal is the inferior part and humans are superior making all decisions. It is a moral principle that the powerful or superior part has responsibilities for the weak or inferior part.

The acceptance and knowledge that animals can experience pain and fear is relatively new. It is still a debated whether fish experience pain though research has shown that some fish has cognitive capacity and learn to avoid situations they have experienced causing pain. It has been demonstrated that fish favor by use of painkillers in the similar way as man and other animals. For fish, mild procedures like simple handling can cause significant stress because handling of fish commonly includes taking them out of water - a life-threatening condition causing severe stress for the fish.

Not can only animals experience pain, they can also experience joy and pleasure. Such knowledge is gradually implemented in revised regulations for animal use, for example you need a good justification to keep social animals housed alone as this can cause stress for the animal.

"Evidence" used to decide on which animals are "sentient" and should be regulated and protected by law is based several observations like:

- complexity of life and behavior
- learning ability
- functioning of the brain and nervous system
- indications of pain or distress
- studies illustrating the biological basis of suffering and other feelings such as fear and anxiety
- indications of awareness based on observations and experimental work.

The problem is that is that the more we study different species, the more we learn that more animals are "sentient" as they meet many of the criteria listed above.

The protection of cephalopods in Europe is an example of studies on that species - have led to evidence that cephalopods are “sentient” and should protected like other advanced research animals. So, cephalopods should have the benefit of legal protections. In this case, the benefits apply to the class ‘Cephalopods’ (phylum Mollusca) as a group, rather than to the individual research animal.

«In dubio pro reo» or presumption of innocence is a principle in law that the burden of proof is on the one who declares, not on one who denies. These principles could be translated to animal research. We should always act with precaution especially when we act on the border of the regulations. The fact that we cannot be legally punished does not imply that our actions are immune against criticism, for example in media, and this can destroy a research career.

Animal research regulations are made to protect animals from unnecessary use and pain, suffering and distress. The border of the regulations is made by the lawmakers and based on common norms and current knowledge about what animals or life-forms that needs protection. However, regulations are based on past knowledge, and may therefore be out of date considering new knowledge.

Like Russel and Burch introduced the 3Rs a guiding principle for animal research Professor Carol M. Newton (1925-2014) introduced the 3Ss and tenet for responsible animal use. The 3Ss stands for good

- Science
- Sense and
- Sensibilities

appealing to a moral obligation to act with precaution and aspire for a higher level of ethical responsibility where the animals take advantage of the doubt.

*Areas in the grey zone**

- Studies in developmental stadia for example in zebrafish until start feeding
- Studies in unprotected species like Drosophila melanogaster or Caenorhabditis elegans*

*These animals are commonly used as research model but not protected by the current regulations

Our regulatory framework is based on the following assumption:

It is morally acceptable for human beings to use animals, but it is morally wrong to cause them <u>unnecessary</u> or <u>avoidable</u> suffering

This principle should apply for all species – also those that are not legally protected.

The Ethical framework reflected in laws and regulations

Harm-Benefit analysis

In the EU directive clearly state that project approval must be based on a harm-benefit analysis. That means to assess whether the harm to the animals in terms of suffering, pain and distress is justified by the expected outcome.

Harm-benefit considerations is also central in written guidelines from World Organization for Animal Health (OIE), Council for International Organizations of Medical Sciences (CIMOS), International Council for Laboratory Animal Science (ICLAS) AAALAC international and in the Guide to the care and use of laboratory animals.

The Norwegian Food Safety Authority (Mattilsynet) also apply harm-benefit considerations in their evaluation of animal experiment applications and purpose of studies (benefits) and severity for the animals (harms) must be described in the public summary that are published on the Norwegian Food Safety Authority (Mattilsynet) web page.

Justifying harmful actions because of potential benefits or weighing risk or cost against other advantages is a common way of thinking in decision-making that make resonance among most people.

It may be different opinions on what “harms” should be regarded as relevant and what “benefits” legitimize animal use.

In 1994 Mellor and Reid[2] published an assessment system for harm based on the domains of the 5 freedoms.

Mellor and Reid suggested that this approach better assured interests of the animal in a more comprehensive way than only focusing on suffering

> *"There is a danger that with focus largely on suffering we could overlook a **broader view** of welfare which may be more informative and safeguard more effectively **the interests** of the experimental animals”*

This approach was recently suggested as framework for systematic assessment evaluation of animal harm in research[3]. "Harm" is assessed based on how the experimental conditions impact on the welfare of the animal.

The current approach to “harms” is mainly based on factors that can influence the welfare of the animal, and the domains based on the 5 freedoms* has been suggested as domains to define “harm”.

- To what degree will the experiments cause pain or injury
- To what degree will the experiments cause fear or distress
- To what degree will the experiments involve hunger or thirst
- To what degree the experiments will limit the animals from expressing normal behavior
- To what degree will the experiments cause discomfort because of inappropriate husbandry

Not everyone will agree on this, especially if the advocate an animal right position and claim that animals and life have value in its own and cannot be used as a mean to an end.

The five freedoms were originally developed to assess welfare in farm animals[1] and outline five domains that affect animal welfare for animals under human control. The 5 Freedoms were developed by the UK government as a response to a report on livestock husbandry, and they were formalized by the UK Farm Animal Welfare in a press statement in 1979.

Legitimate "benefits" include health benefits for humans, animals, or environment, socioeconomic, scientific, or educational benefits as well as safety and efficacy benefits. The main criticism against benefit is that they usually are future promises - that may never be realized. However, some benefits are actual and realized as soon as the study is concluded, for example result from safety tests, new knowledge, and educational skill.

To assess benefits the following have been suggested

- *What are the (potential) benefits?*
- *Who will benefit?*
- *How will they benefit?*
- *When are benefits expected to be realized?*

Presentations

- Presentation on Harm-Benefit analysis

Further reading

Current concepts of Harm–Benefit Analysis of Animal Experiments

**Freedom from Pain or injury, fear or distress, hunger and thirst, limitation to express normal behavior and freedom from discomfort by assuring appropriate husbandry*

The Ethical framework reflected in laws and regulations - 3Rs

Russel and Burch introduced "the 3R´s" to laboratory animal science in 1959 in their book "The principal of humane experimental techniques"

The 3R stands for

- *Replacement*
- *Reduction*
- *Refinement*

Replacement means substitution for conscious living higher animals of insentient animals, or methods not involving animals (*in vitro* or simulation methods).

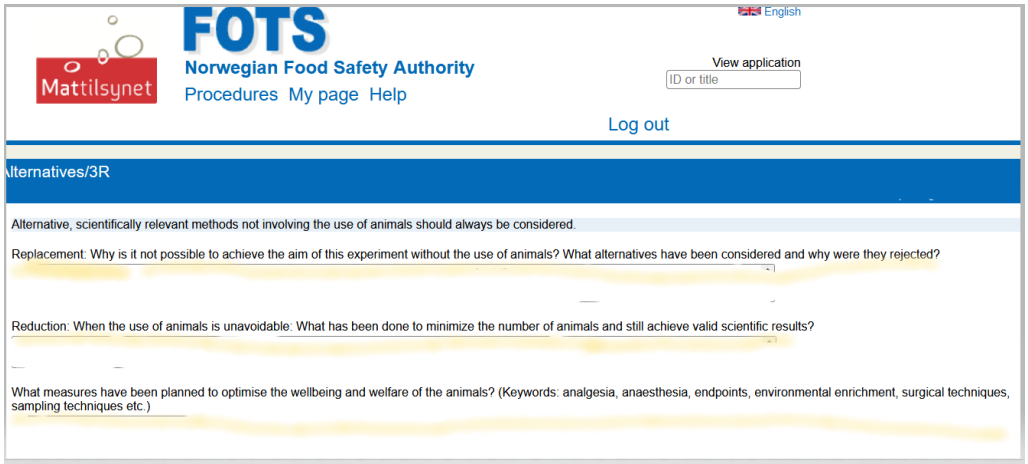
Reduction to minimize the number of animals used to obtain information of a given amount and precision

Reduction means to decrease in the incidence or severity of inhumane procedures.

The 3Rs is a mean to reduce harm. The 3Rs can be used to mitigate severity for the animals involved. By replacing animal experiments - harm to animals eliminated. The less animals used the less harm, however in some cases it might be questioned if it is better to use more animals if this cause less severity for each animal that is involved. It is also implicit in the concept of reduction to assure that the number of animals used assure statistical calculations and validity of results. A utilitarian may say that not using animals may cause more harm – as medical progress is hindered.

Refinement involves all means to mitigate negative impact on the animal, like giving painkiller if pain is involved, use least invasive procedures, easily digestible food if eating-problem is relevant etc. In the current understanding refinement also include all means to assure animals wellbeing, like ability to maintain normal behavior, a social life and access to resources to for nature behavior (enrichment)

The 3Rs are globally recognized and the 3Rs have a strong role both in modern animal research ethics and regulations. The 3Rs are explicitly mentioned as a guiding principle both in the EU directive 2010/63 and in the Norwegian Regulation on the Use of Animals in Research (Norw: "Forskrift om bruk av dyr i forsøk") (§9). In the Norwegian application for animal experiments (FOTS) as well as in the public summary, the researcher must describe and demonstrate how he/she has complied with the 3R principles in their planning and performing animal experiments.



When 3Rs are maximally applied, there will often be some “rest harm” and this harm must then be justified by the potential benefits of the study. So, Harm-benefit analysis and 3R assessment are both part of the ethical review of animal studies.

There are several good web pages to be used in search for 3R

Norwegian web pages:

- norecopa

International web pages

- NC3Rs
- Altweb
- 3R CCAC
- FRAME UK

Group discussion task

3Rs as a guiding principle in procedures

Describe and discuss the importance of the 3Rs as a guiding principle in the use of animals in the following procedures

- Handling and simple marking of a rodent versus fish
- Blood sampling in rodent versus fish
- Dietary restrictions
- Abdominal surgery
- Catching ad release for marking it with a GPS to track it

Promote good animal welfare practices

There are both ethical, legal and scientific reasons why we should pay attention to animal welfare in research. The ethical and legal reasons have been described earlier in this moduel.

There are also good scientific reasons why focus on the welfare of the animals.

- Data will be more reliable and less biased in less stressed animals.
- Pain, suffering and distress may be undiscovered, but can nevertheless have great influence on the experiments.

Housing

Single housing has been justified as a mean to standardize experiments by avoiding social interaction effects. This is a major misunderstanding. Animals may response differently to this single housing, some animals become depressed other animals become aggressive. Housing animals in stable social groups with compatible cage mates is a better choice that also meets welfare demands and natural needs for a social animal.

Pain and distress

Pain and distress cause metabolic responses in the body and involve several hormonal systems including

- hypothalamic- pituitary- adrenal axis
    - stress response, fight or flight
- renin-angiotensin-aldosterone system
    - preserve sodium, increase circulation volume to prepare for blood loss
- immune function/immunosuppression
- catabolism

Secondarily these effects can result in reduced appetite, cachexia, inadequate sleep, retarded convalescence after procedure or other adverse effect that can have major effect on experiments. In worst case animals might not at all be “fit for purpose” as an experimental model.

Maintaining good animal welfare is therefore of great importance to assure high scientific quality.

Animal welfare and good science are not in conflict with each other. Focus on animal welfare is a mean to assure good science

The five freedoms

The five freedoms are

1. Freedom from hunger and thirst	By ready access to fresh water and a diet to maintain full health and vigour
2. Freedom from discomfort	By providing an appropriate environment including shelter and a comfortable resting area
3. Freedom from pain, injury and disease	By prevention means or rapid diagnosis and treatment
4. Freedom to express (most) normal behavior	By providing sufficient space, proper facilities and company of the animal's own kind
5. Freedom from fear and distress	By ensuring conditions and treatment which avoid mental suffering

Write an essay of 500 words and explain how the Five Freedoms apply to animals in your research.

The severity classification system

Classifying experiments in project application

You will need to do a pre-evaluation of severity when are writing up your application in FOTS.

The experiment must be **classified** based on *the degree of pain, suffering, distress or lasting harm* expected to be experienced by an individual animal during the course of the experiment.

Severity assessment includes all interventions, procedures, treatments disease and euthanasia that is a part of the project and experienced by the animal. It also included assessment of transport, housing, nutrition, handling, restraint and capture.

Experiments should be classified as either:

- *Non-recovery*
- *Mild*
- *Modate*
- *Severe*

Non-recovery

Procedures, which are performed entirely under general anesthesia from which the animal shall not recover consciousness.

Mild:

Procedures on animals as a result of which the animals are likely to experience short term mild pain, suffering or distress. Procedures that cause no significant impairment of the wellbeing or general condition of the animals.

Moderate:

Procedures on animals as a result of which the animals are likely to experience short term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress. Procedures, which are likely to cause moderate impairment of the wellbeing or general condition of the animals.

Severe:

Procedures on animals as a result of which the animals are likely to experience severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress. Procedures, which are likely to cause severe impairment of the wellbeing or general condition of the animals.

All experiments that are classified as severe must undergo "retrospective assessment" so that after the project is finished you have to write a report to Mattilsynet describing the actual severity or any other information requested by Mattilsynet. Also, when you write your public summary you have to include expected severity level.

- **Presentation**

Reporting actual severity of animal experiments

Use of animals in research, education and testing must be reported to the authorities.

When severity is reported we must report the actual severity.

If for example an experiment is classified as "moderate" in the application, however some of the animals die, are in the state of dying or must be euthanized because their actual condition is more severe than predicted, then these animals must be reported as severe.

Reuse of animals

An animal that has already been used in one or more experiments, cannot be used in a new experiments if it is possible to use another animal that has not been used earlier.

This does not apply if

a) The actual severity in the previous experiment was mild or moderate

b) Animals' health and welfare is fully restored

c) The new experiment is classified as mild, moderate, or non-recovery

d) The reuse is in accordance with advice from the veterinarian - or fish-veterinarian based on judgement of the total severity in the animal's life

In exceptional cases Mattilsynet can approve reuse of animals that have been already used in a severe experiment. Such approval can only be given if conditions in b, c and d are fulfilled, and the animal has been evaluated by a veterinarian.

(§17) Regulation on the use of animals in experimentation

Harms to animals - Avoidable and unavoidable suffering

Taking the five freedoms and the current understanding of harm into consideration, experiments in animals will involve some degree of negative impact on their welfare. Some suffering will be unavoidable to achieve certain aims, and this unavoidable suffering needs to be justified.

We have an obligation however to pay attention to all avoidable or unnecessary suffering. Applying the principles of the 3Rs is one approach to achieve this, and it is the responsibility of the project leader to assure that all means are made to avoid unnecessary or avoidable suffering. This apply from the moment where the animal is defined into the experiment an include transport, housing, marking, daily care and all interventions until the animals is humanely killed.

The sum of all these interventions makes up to the **cumulative** harm and animal experience in its lifetime, and this must be taken into account when doing the harm-benefit analysis. So even if a single procedure seems mild (like injection of a needle) the experience can be severe for the animal if they are handled by an untrained person for example. Frequent handling by an untrained person can be very stressful as the animals learn from experience that this is an unpleasant situation.

This is also why **reuse of animals** is strictly regulated and should be avoided. In one way reuse could be a way to reduce the number of animals (which is according to the "reduction" principle). However, this can be a too high burden for a single animal, and to put the interest of the animal in center it is usually better to use another naive animal.

The culture of care

While regulations set formal borders for what we can do and not do with animals in our care, **culture** reflects informal norms for what we regard as right and wrong behavior when it comes to how we treat animals. Research on organizations has showed that organizational culture is just as important for behavior than formalized rules and regulations.

Culture has been defined a pattern of basic assumptions. The culture is created or developed by a group as they experience to master their problems. This strategy works appropriate to be regarded as "true" and is learned to new members of the group as the right way to understand, think and feel concerning problem solving.[1]

Culture reflects preferred behavior adequate for solving the problems and suitable for the purpose and affects values and norms as well as cultural expressions or artifacts.

With a **culture of care** in the context of animal in research, we define a preferred mode of action of caring for animal that should go beyond the (minimum) standards defined by regulations. It is based on the assumption that good animal welfare is an important prerequisite for good science.

Within a culture of care the limit of the doubt should go in favor of the animal, i.e. even if it is not proven that animals (species, life stages) might suffer, we leave the possibility open and treat the animal the best way we can to avoid unnecessary suffering.

More about the culture of care can be found on the website of the norwegian concensus platform on alternatives - norecopa

[1] Schein, E.H., *Organizational culture and leadership*. 3rd ed. The Jossey-Bass business & management series. 2004, San Francisco, Calif.: Jossey-Bass. XVI, 437 s

Systematic review – How to collect information when planning animal research?

Systematic review

Systematic review is a structured, thorough, and transparent way of doing a literature-search.

A systematic review is characterized by being

- Structured, Thorough, transparent
- Address a specific research question
- Transparent literature search and selection of papers
- Critical appraisal of papers

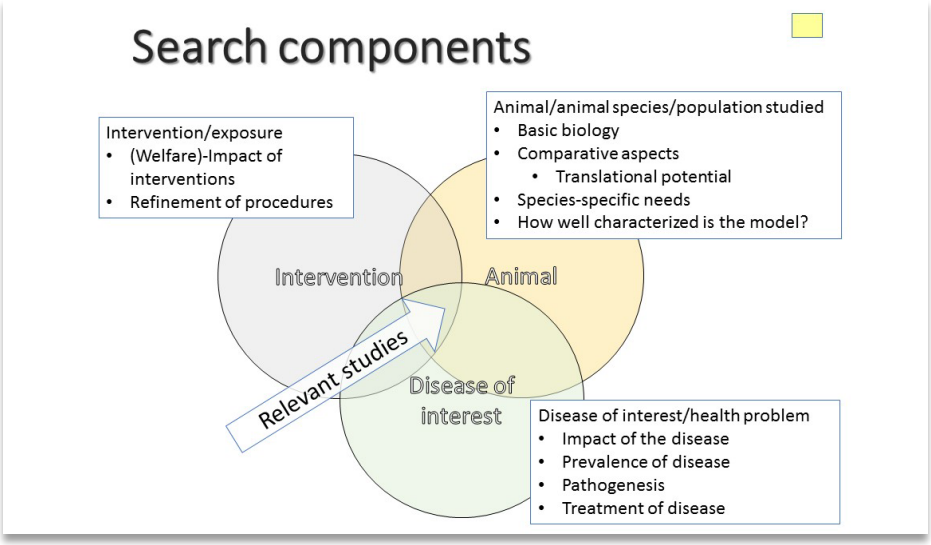
Search key words

When you are planning a study, it is common to do a literature search.

You must include more than 1 search component in your search. For biomedical research it is advised to include search for:

- **Intervention**: With focus on Welfare)-Impact of interventions and refinement of procedures
- **Animal**: With focus on basic biology, comparative aspects human vs animal and species-specific needs
- **Disease or subject of interest**: With focus on impact of the disease, prevalence of disease, pathogenesis, and treatment of disease

Several relevant sources and/or databases are included in the search that is based on relevant keywords, including commonly used synonyms («MeSH terms»). The word "mouse" can have several synonyms that you have to include. Similarly, you find some synonyms on the link for "rat".



Meta analysis

Conclusions from several relevant studies are summarized and analyzed in a meta-analysis. This way evidence from several studies are used to confirm or reject theories for example between a drug and its effect on the body, disease-mechanisms et cetera that you don't easily get from a single study.

Meta analyses are useful in planning animal experiments for example to evaluate if a model is suitable to predict an outcome or relevant effects.

If 50% of studies show an effect and the other 50% show an opposite effect, which studies shall we trust, or can we trust any of them?

Based on this analysis you can make a more qualified evaluation whether or not it is reasonable to set up new animal studies or if it's better to use other approaches to achieve more knowledge about a phenomenon.

Meta analyses are also useful and necessary in translational research from preclinical studies in animals to clinical studies in humans. Are conclusions from the animal studies so clear and reliable that they support continuous studies in patients, or do the result diverge in different directions? This can give useful information when you plan experiments both regarding using the best model and to animal welfare.

Presentations on literature search

1. Introduction
2. Literature search - key Words
3. Literature search - Systematic review and meta-analysis

Radboud University medical center in the Netherland has published a video presentations explaining the importance of doing a systematic review.

Further reading

What have we learned from Cochrane Collaboration?

- Critical review of animal experiments.
- Translational value for humans
- Choice of statistical methods and how it influence results.

Papers:

- Systematic reviews and meta-analyses of preclinical studies: publication bias in laboratory animal experiments

Sources of information relating to ethics, animal welfare and the 3Rs.

Ethics

- ANIMPACT library

Animal welfare

- Mattilsynet (Norwegian)
- animal welfare at UFAW
- UFAW handbook

3R

- Russel and Burch introduced "the 3R´s" to laboratory animal science in 1959 in their book The principles of humane experimental techniques

Norwegian web pages

- norecopa

International web pages

- NC3Rs
- FRAME UK

EURL ECVAM

European Union has established a series of European Union Reference Laboratories The European Union Reference laboratory for alternatives to animal testing formally was established in 2011

HUMANE ENDPOINTS

Module 5. Humane endpoints

Aurora Brønstad, Department of Clinical Medicine, University of Bergen, NORWAY

Contents

Humane endpoints

Planning animal studies with early, less serious endpoints is an important step against less severe animal studies. The concept of humane endpoints was introduced in Europe by OECD [1] and the European directive article 13.3 [2] state that death shall be avoided as an endpoint and be replaced by earlier, humane endpoints.

"Alternative to death" reflects a very narrow definition of humane endpoints [3] and it has been questioned if all earlier endpoints can be really considered "humane" [4]. Other authors therefore purpose a more broad definition of Humane endpoint as a **concept for continuous refinement** of animal studies [5]. This latter broad definition will be basis for the rest of his chapter as it is better aligned with the 3R principles [6-8]. Continuous improvements is embedded in the directive [2] – Article 4.3

> *Member States shall ensure refinement of breeding, accommodation, and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals.*

What is a "humane endpoint"?

Humane endpoints are defined as clear, predictable, and irreversible criteria, which can be used as a substitute for a more severe experimental outcome [5, 9].

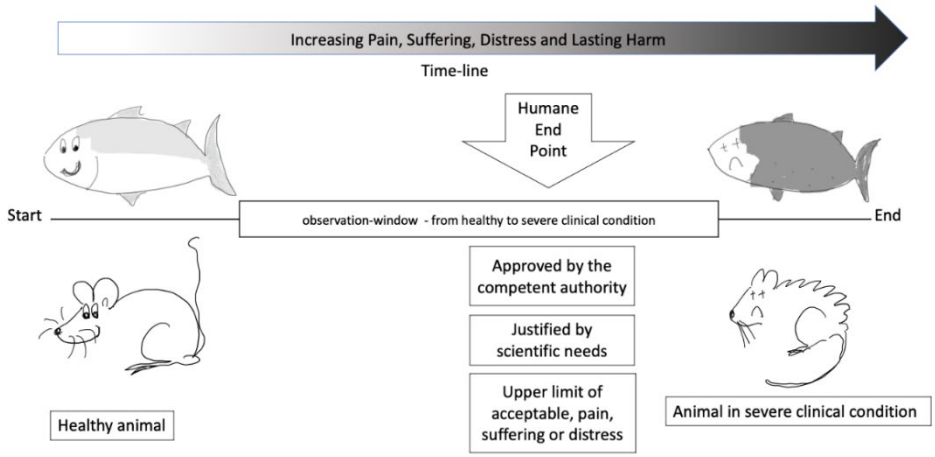
Studies to better understand diseases and hopefully develop better treatment, testing efficacy or safety of substances are the objectives for many studies in animals. The directive article 13 states that unnecessary pain, suffering, distress or lasting harm must be avoided [2]. Potential or actual pain, suffering or distress that is regarded necessary to achieve a study objective must be justified based on a harm-benefit assessment as a part of project evaluation [2, 10, 11]. That means that the negative experience of the animal must be justified by a greater benefit by doing the study. Pain, suffering and distress above what is required to achieve

legitimate scientific objectives are regarded unnecessary and inhumane [12]. Animal studies are authorized on the condition that pain, suffering and distress should not exceed the necessary, justified level. If the actual negative impact on the animal pass the level predicted in the prospective severity assessment, defining the conditions for authorization of the study [3, 12] or when the condition for the animal reaches the level of unbearable [13, 14] authorities needs to be notified and may set further conditions for continuing the study. Just as important – there must be plans in place to eliminate or minimize pain, suffering or distress for the animals.

There are also scientific reasons for applying earlier endpoints. Chronic diseases gradually progress in the level of pain, suffering and distress for the animal, it gradually become more severe (figure 1).

Figure 1

Chronic diseases gradually progress in the level of pain, suffering and distress for the animal, it gradually become more severe. The window of possibilities for humane endpoints can theoretically be set at any time between the first detectable deviation from normality to the first indication of severe suffering. The level of necessary pain, suffering or distress must be ethically justified and legally authorized be the competent authority.



Animals in advanced stages of disease are so physiologically deranged or disrupted that it does not provide reliable scientific information [12]. Pain and distress might confound animal studies and interfere with scientific outcomes such as immunological or hormonal responses. Severe clinical symptoms reflect alterations in the normal physiology and reduced ability

to adapt to maintain homeostasis. The sense of being self-threatened over time cause severe distress in the animal. The animal experience pain, loss of control, fear or anxiety, or lack of social support [14]. The serious condition of moribund or dying reflects an irreversible situation with death as the only outcome [15]. Autolytic changes in tissues take place as soon as death is imminent and if discovered by the next scheduled inspection, the carcass and tissues is already damaged or even cannibalized by cage-mates. Timely termination by euthanasia has the advantage that investigative staff were better prepared for euthanasia and tissue collection [16].

Beside the EU Directive also other internationally recognized documents like the Guide for the Care and Use of Laboratory Animals address the importance of humane endpoints which better explains the scientific importance of applying early endpoints.

> *Studies that may result in severe or chronic pain or significant alterations in the animals' ability to maintain normal physiology, or adequately respond to stressors, should include descriptions of appropriate humane endpoints* [17]

Clinical symptoms or signs of poor welfare develop when the animal cannot any longer compensate for the progressing disease. This is especially critical for pray animals – like rodents and several species of fish. Prey animals will use resources to compensate for and try to hide early signs of disease [18]. Hiding weakness for a prey animal is important to avoid attention from potential predators as this might make their lives at risk. The implication is that the animal has already suffered from coping distress for a while before staff are able to recognize the problem. The observation-window from no clinical signs to obvious, severe clinical signs can therefore be narrow for many species. Several signs humans are able to observe during ordinary inspections - reflect a serious state in several species, as for example in mice:

> *Sunken flanks, neglected grooming, piloerection, hunched back, immobility are clear evidence of severely impaired often moribund health status in mice [19]*

Therefore, we should search to define earlier predictors of pain, suffering and distress and use them to define more humane endpoints.

The concept of humane endpoints is about setting earlier endpoints and follow up with mitigating actions to minimize, reduce or eliminate unnecessary suffering is in accordance with the principle of refinement [8].

Death and near-death as an endpoint

Replacement of death as an endpoint is explicitly stated in the EU directive Article 13 and has been translated into national regulations in European countries.

> *Death as the end-point of a procedure shall be avoided as far as possible and replaced by early and humane end-points. [2]*

The EU directive also state that if death is unavoidable as an endpoint the study should be designed to result in the deaths of as few animals as possible.

The Canadian guideline [20] has also included the severe state of dying – the moribund state as – a category that should be avoided as endpoint.

> *…moribund animal is one that is close to death and may be comatose or unresponsive to stimuli, exhibit dyspnea or other severe breathing problems, hypothermia, prostration, etc. However, before the animal gets to the point of being moribund, detailed observations of the animal can help to set an earlier endpoint and thereby reduce the actual cost to the animal, in terms of pain and/or distress.*

Of animal welfare reasons also, the moribund state should be avoided as an endpoint equally as avoiding of death [4, 15]. It is questioned if the moribund stage can be regarded as a "humane" endpoint – as it is very likely that the animals has been subjected to pain, suffering and distress before it reached this stage and so it questioned if these near-death endpoints are really "humane" [4].

Define and apply appropriate humane endpoints.

Establish suitable criteria to identify when the humane endpoint has been reached.

"Harm" to research animals was defined as compromised welfare based on the impact of the 5 freedom domains [10, 11, 21, 22]. Absence of good welfare can followingly be used to assess harm and used to define endpoints that should trigger follow up action-points to restore better welfare. Such welfare indicators are often based on observations like deviating morphology, behavioral or physiological parameters [5].

Endpoints can systematically be defined based on how they impair welfare indicators and be classified as either:

- Morphological welfare indicators
- Behavioral welfare indicators
- Physiological welfare indicators

Morphological welfare indicators must be based on knowledge about the specific species and life stage. Malformations that cause disabilities and problems to maintain the animal's normal functions and responses are relevant endpoint indicators. They are especially relevant in developmental studies and characterization of new genetically lines, where malformations may be an (unexpected) outcome. Examples include skeletal deformities [23, 24], and hypoplasia of organs [25-27]. Malformation can cause critical vital malfunction such as food intake caused by jaw or teeth deformities. Changes in connective tissue may limit growth and development [28]. Lip or cleft palate deformities [29] cause problems already in the suckling stage for a neonate mammal. Protocols suggesting systematic approaches to categorization of new phenotypes of mice [30] and assessment of severity of genetic altered mice [31] are available. Malformation of vertebra column in a fish cause extra strain with swimming both for search food and escape from threats [24, 32] putting the animal under chronic coping stress. Other examples of morphological welfare indicators may include damage to skin or eyes,

ulcers, emaciation, change in body condition, body shape or fitness factor [24, 32]. The Fishwell project was made to define morphological welfare indicators in Atlantic salmon [33].

Several morphological welfare indicators are simple to register, and the optimum condition is usually well described. Others will demand more laboratory testing like CT/MR for examination of internal organs or necropsy of sentinel animals. A collaboration with an experienced comparative pathologists is strongly recommended in defining morphological [34].

A main weakness of using morphological welfare indicators is that they are **retrospective**. That means that the condition that caused morphological deviation have happen back in time and the animal might have struggled with disabilities before evidence are detected by regular observation. Also, absence of morphological deviations is not a guaranty for good welfare and presence of a morphological deviation is not necessarily representing a welfare issue either – as long as the animal cope well under the conditions provided.

Behavioral welfare indicators are detected by observation of the animal. A significant advantage is that they often reflect responses in **real time**. Changes in behavior may be the first visible response to aversive conditions and early warning. One example is response to daily husbandry practices like feeding. Animals fed at regular intervals will be hungry and come to be fed the food when it is served. Observing the animals during daily husbandry practices give valuable information of the physical condition – and such observations should be considered to define endpoints. Animals that do not respond as usual when the operator occur by the cage, pen, tank, or net should be followed up for underlying cause so appropriate actions can be applied.

Nest building is a natural behavior in mice. Healthy mice are highly motivated to build nests when they have access to nesting material [18], and incomplete or lack of nest building in mice that have access to nesting material may be an indicator of pain, suffering or distress and should be checked for underlying causes. Also reduced burrowing behavior in mice

[19, 35, 36] or the normal vertical activity (rearing) in rats [37] have been used to assess pain and distress in laboratory rodents. Other behaviors like hanging in the cage-lid is a strongly motivated, though not essential, behavior in mice and deviations from such behaviors can be used to assess pain and discomfort [38].

Not all behavioral welfare indicators reflect real-time problems. Stereotypic behavior can be a coping response to environmental distress that might have been undetected for some time [39, 40]. In case of stereotypies in individual animals, attention should also be made to other animals in the same environment, they might also be suffering distress even if stereotypies are nor manifest yet. Stereotypic behavior might also have underlying physiological causes [41] – and this might be of especially interest when defining endpoints for newly generated genetically altered animals.

The general activity is also a useful endpoint indicator. Healthy rodents show exploratory behavior rearing onto hind legs and sniffing. Activity can be reduced because the animals are weakened, exhausted or in pain. Increased activity might be a response to irritation, stress or a treat that upset the animals. Lethargy (apathy, drowsiness) is characterized by reduced sensitivity to stimuli, often occurring in the final stage of illness – a situation called moribund. Willingness to move only when stimulated will be an earlier endpoint than no willingness to move when stimulated – but still this will reflect an alarming situation for the animal.

Self-grooming is important for many species and one of the most frequently performed behavioral activities in rodents using up to 25-40% of the awake time budget [42]. Rodent fur should be smooth and dense, and ruffled fur is therefore an important early indicator of poor welfare [43, 44]. Other early indicators of pain or distress in rodents include reduced food intake and isolation from group mates [43, 44].

When resting rodents are curled up – and when housed in groups, they group together to better maintain preferred temperature and avoid thermal stress [45]. Finding a rodent alone – laying stretched out – is and indicator of impaired welfare.

Observing swimming pattern in fish give valuable information of the physical condition of the fish, however there are big differences between species and life stages. Zebrafish typically swim in the middle of the tank and swimming at the tank bottom is interpreted as an indicator of pain [46]. Salmon Parr are territorial, live in the demersal zone and show a cross-current swimming pattern in the rivers [47]. During the Parr-Smolt Transformation they reduce cross-current swimming and show tendency to move downstream against sea water [47]. In the lab in the tank salmon smolt typically swim in the same direction in a coordinated manner (they are schooling). Loss of equilibrium, position in tank or water, and gill movements (hyperventilation, hypoventilation) are useful welfare indicators in fish. Flared gills occur when fish have trouble breathing caused by low O_2 level in water or infection on the gills. Such conditions must immediately be followed up by identifying the cause and take corrective actions.

For rats [48], mice [49] and rabbits [50], and zebra-fish [51] specific pain behavior have been described. Striking painful body-part against surface has been used as an indicator of pain in rats [48] and rabbits [50] and behavior changes cause by noxious stimuli has been demonstrated in several aquatic species including cephalopods [52, 53], hermit crabs [54] and several species of fish including rainbow trout [55] and zebra-fish [46, 56]. Pain assessment by facial grimace scoring has been validated and showed to be a reliable method for pain assessment in several species [57-61] and some traits seems to be conserved across species.

Observations by humans may – depending on species and the adaption to human presence or interactions, may cause observation bias. Some animals modulate behavior to mask symptoms. This is especially a problem in prey animals [18] as predation is a strong selective force in evolution. Most species maintain a set of natural defense mechanisms (instincts) even when they are raised in captivity protected from natural predators. In some cases, natural defense mechanisms may be modulated by use of training, like with positive reinforcement or by more animal centric approaches to care and management [62]. Some animals avoid predation by occupying areas or habitats that are not suitable for their

predators or they are active in times where predators are not. For mice – the nest function as a hiding place and offering the animal a better opportunity to self-environmental control reducing the stress in the animal. For animals in other habitats like fish in water, observation and evaluation of individual animals can be difficult as animals are not easily to access. Panic response in fish reflects that the fish are stressed. It can be poor water quality, fear of a predator presence, or just the animal caretaker dressing up in other colors than they are used to.

Reversed activity cycles must also be taken into consideration in evaluation of behavioral welfare indicators, as nocturnal animal will not show their full behavioral repertoire during day time [5]. There is a risk of missing important behavioral cues of impaired welfare when animals are observed in their normal resting time. An enriched environment, providing remedies necessary to meet animals' natural needs will be necessary to be able to detect abnormal behavior.

By use of technology and remote recording like telemetry [18, 63, 64], GPS-registrations and similar automated technology for [38, 46, 59, 65-67] behavior observation can be possible without direct interfering with the animal.

A main weakness with behavioral welfare indicators is that interpretation of deviant behavior is not always clear. The use of deviant behavior assume that all personnel (researchers, technicians, veterinarians, care staff) are equally able to recognize behavioral deviations as well that all animals respond in a similar manner [5]. Basic knowledge of natural behavior for the species and life stage is a fundamental condition for using behavioral welfare criteria as endpoints.

In evaluation of animal behavior, the minimum the researcher should be able to answer are.

- How do the species naturally move?
- How do they naturally feed, what do they eat and how much?
- How do the species natural respond to disturbances, potential dangers, or interactions with conspecific as well as humans?

[68]

Assessment points that form the basis for evaluating adverse effects should include:

- Appearance
- Posture
- Spontaneous behavior
- Provoked behavior

[5]

Physiological welfare indicators and surrogate endpoints

Physiological welfare indicators and surrogate endpoints include clinical observation, like breathing pattern, hearth frequency or results from laboratory test of blood, hormone levels, saliva, urine, or other samples. They have potential as early indicators or disease markers (surrogate endpoints) predicting a cascade of events leading to a severe condition. Many of them are sensitive, have validated methods and range so comparison with the normal condition is reliable. “Surrogate endpoints” [12] are used to predict an outcome to early terminate a study before the animal develop severe distress [69]. Examples of physiological parameters

as surrogate endpoints can be a specific blood pressure measurement that predicts death, a defined tumor volume that consistently precedes painful ulceration [69] or blood glucose used as pre-lethal surrogates [12].

Subjective evaluation might be a weakness with many clinical observations. The fact that many of the physiological endpoints are quantifiable, has the advantage that they are **less vulnerable for subjective evaluation** [5, 70].

A main challenge is the collection of endpoint data. It often involves manual handling, restrain and sampling. Such procedures which can be a burden for many species, for example for fish that must be taken out of water or for animals that is not adapted to human interaction or handling. Training and habituation can be used for many species like dogs, NHP and pigs to reduce the stress related to handling and sampling [71].

New technological solutions may provide alternative less intrusive solutions for data collection. Surface hypothermia using infrared non-contact thermometers has been used as a predictor for death in infectious studies in mice [72]. Reduced body temperature measured by telemetry [63] or by infrared measurement [72] used as an early endpoint for sepsis in studies of infectious disease among others.

Method, frequency, and volume of samples must be taken into consideration in study design – as this might affect the level of stress and physiology of the animal [69].

Morphological, behavioral, or physiological endpoints.

Morphological, behavioral, and physiological endpoints as described in this section have their strength and weaknesses regarding evaluating impact on welfare or emerging pain, suffering or distress. However, by combining more of them using a triangulation approach, like combining body weight and behavioral change like burrowing, [36] or body weight/breathing patterns/activity [16] they can provide useful indicators for early humane endpoints and be a game-changer in defining earlier and more humane endpoints.

Generic, specific, or unexpected events

Another systematic way to asses endpoints is to classify as generic endpoints, project specific endpoints, or endpoints for unexpected events [3].

Generic endpoint parameters reflect unspecific welfare issues (morphologic, behavioral or physiological) like loss of appetite, lack of self-grooming [41], activity level, change in body weight or body condition [73] indicators of pain, distress or poor welfare. They give an indication that the animal is not doing well, but do not specifically relate to a study specific condition. Lack of highly motivated species specific behavior like burrowing [19, 35, 36], nest building or cage-lid hanging [38] in mice can be used to determine earlier endpoint indicators in studies as generic endpoints. Generic endpoints are relevant for all studies of gradually progressing diseases.

Using weight loss as an endpoint parameter

Weight loss, typically 10-20% is a commonly used generic endpoint parameter. There are many good reasons for using weight loss as an endpoint. It is not a risk of subjective bias, and it is also easy to measure in land living animals also without too much restrain. Some species can even be trained to walk voluntary on the weight [62]. Other species like aquatics, however, weighing is not easily performed without handling and bringing the animals out of its natural habitat that will stressful.

One criticism is that weight loss as an isolated parameter is not very sensitive for the animal´s actual condition. Maintaining body weight or weight gain can be caused by conditions like tumor growth or accumulation of fluid in the peritoneal cavity (ascites) [74]. Stable weight in an animal in a growth phase is a poor sign as they are expected to increase weight according to the weight-gain curve. Weight gain can also be a problem as obesity increase the risk of other disease, complicate self-grooming, and increase the load on the limbs and cardiac function. Also, obese animals take up more space and there might be a need to split groups in more cages or pens unless this is accounted for on from the start of the

study already. Overweight is especially a problem in long term studies. Also animals with a significant loss of body weight might be lively and have a good quality of life [12, 13].

Body weight should be evaluated together with body score evaluation [73, 75]. The body score in mice is usually defined in five categories from emaciated (score 1) to obese (score 5) and each category is well defined. However, there is a risk of subjective evaluation so body score should be combined with body weight (objective) or other objective indicators. Body condition scoring can be used more objectively by micro CT-imaging [73]. Studies relying exclusive on weight loss as the only criterion for euthanasia as an endpoint might result in an unnecessary loss of animals [76]

Weight loss is reflecting a "problem in the past", i.e., the animal might have reduced appetite or been under a catabolic process already for a while before we are able to detect it. Another complementary endpoint parameter could therefore be to measure food intake as an indication of appetite in real-time. However reduced food intake of preferred food did not seem to be a useful measure in guinea pigs [16].

Project specific endpoints are endpoints that reflect disease progression of the phenomenon of interest in the study. For a cancer study, tumor size, number of tumors, metastases, tumor's location and interaction with other functions (mobility, food intake) can be relevant project specific endpoints. For surgery – wound complications like redness, swelling, infection, broken sutures are relevant and should be included as potential endpoints with defined action-points for follow up. See example in table 2. Preclinical screening of ALS-mice showed that loss of motoric function was used as an early sensitive and rapid indicator for the initial phase of denervation of muscle-fibers [77] and reduced activity in the home cage running wheel as an early diagnostic sign [78]. For diabetes studies, examples can include blood glucose, urination, cages changes need (because of increased urination) or water consumption.

Project specific endpoints should be defined for each particular study. Any indicator used as surrogates for death must be defined for each study and one should not be tempted to reuse endpoints from other random studies or blindly rely on global endpoints [79].

An overview of indicators for study specific endpoints and alternatives to death and other severe endpoints is summarized in table 1.

Endpoints for unexpected events relate to pain, suffering or distress caused by other unrelated illness, accidents or unexpected adverse effects of the study that necessitate human intervention to avoid continued unnecessary and unjustified pain, suffering or distress [3, 5, 12]. Unwanted events might be fighting and fighting ulcers, that an animal is hurt under procedures or husbandry practices. It might also be that severity exceeds the level anticipated at the start of the experiment [5] like more serious adverse effects of a test substance than expected.

Unexpected events might include disasters like fire or flood, earthquake, technical breakdown, power supply in the building, contagious disease outbreak, a pandemic situation, or others.

The guide say [17]

> *Well-planned experiments with clearly delineated scientific and humane endpoints will help to ensure that a contingency plan is in place for problems that may arise during the study.*

Depending on the level of impact, this event might or not influence the study, and it must be made a case-by-case decision if the experiment can continue or if it should be terminated. Continuation of the study may be on the cost of animal harm and reliability of the study results. Termination and restarting the experiment will be on the cost of animal lives.

Actions to be taken when an endpoint is reached and consider possible options for refining methods to finish at an earlier endpoint.

When an animal reaches a certain defined endpoint, it must be followed up by actions to reduce or eliminate pain, suffering distress or lasting harm.

"Action points" describe the actions we are going to take to mitigate the condition and avoid further pain, suffering, distress, or lasting harm when or if an animal reaches certain stages – or endpoints - in a study.

In many cases, for many species this will be to kill the animal to avoid further suffering. However, with earlier endpoint other alternative action points should also be considered, as said in the directive [2] on care an accommodation

> *Article 33 (d) arrangements are made to ensure that any defect or avoidable pain, suffering, distress or lasting harm discovered is eliminated as quickly as possible*

That could include medical treatment to relieve pain, nausea, correct blood glucose, fluid therapy etc. An example of endpoints and action points for follow up a surgical wound after surgery (Figure 2). The humane killing in this example will only be applied if there is no effect of the treatment or mitigating actions and the animal is exposed to unnecessary pain, suffering or distress caused by the wound.

Other action points can include more frequents check, providing soft or alternative food, provide enrichment (for example for behavioral problems or fighting) or consultation by the veterinarian. The animal care staff and the designated veterinarian should be involved in planning endpoint and action points as they can provide useful information [79]. In all cases, the study director must consider whether these actions might bias the study in an unfortunate manner.

Only when the condition cannot be ameliorated, the animal must be killed as the end of a procedure as a final endpoint.

> *An animal shall be killed when it is likely to remain in moderate or severe pain, suffering, distress or lasting harm (article 17.2)*

Score sheets – clinical assessment and follow up action points.

Score sheets – endpoints and action points

A score sheet ("observation sheets" or "welfare assessment sheets") is a protocol for systematically recording of key clinical observations and other test-results of the animals [5, 12]. These observation and test results show to which degree the animals physiologically or mental state deviate from the normal and are used to determine when the animal reach predefined endpoints that trigger certain actions. The score sheet helps to identify when the condition for an animal exceeds what has been defined and approved (by the competent authority) as the humane endpoint in the light of a harm-benefit analysis (the harm to the animals must be balanced against potential benefits)

> *... a harm-benefit analysis of the project, to assess whether the harm to the animals in terms of suffering, pain and distress is justified by the expected outcome (article 28) [2]*
>
> *...the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns.[17]*

A score sheet can also be used to document when the condition for an animal exceeds what has been defined and approved by the competent authority as the humane endpoint.

When defining scores for clinical signs they are typically categorized in distinct categories from 0 – or no impact to higher numbers depending on how sever the situation is for the animal and the highest score for the most severe condition – often that cannot be relieved or scientifically justified.

The Facial Grimace Score (FGS) for pain [57, 59] for example a score 0 is no signs of pain using the FGS, a score 1 is reflecting moderately present and 2 an obviously present sign of pain.

I theory any of morphological, behavioral, or physiological welfare indicators can be used to define endpoints and action-points in a score sheet. Some might be sensitive to subjective evaluation by the observer unless very strict categories are defined. It is advisable to consider they relevance for the particular study, how feasible it is to collect data during

regular observation and to what degree can we define them in an objective manner to avoid observation bias. Ambiguous score sheets including many observation points may look impressive – but there is also a risk they are completely impractical in use. Scoresheets should therefore be regularly revised to include a limited number of relevant parameters [79].

Binary scoring

An alternative to score observations in numerous categories of increasing seriousness, as for the facial grimace score [57, 59] using 0, 1 and 2 – altogether three categories - is to use binary scoring. Observation-points are not scored and categorized of increasing seriousness – but they are scores as either present OR not present. For example, you observe:

- Indication of pain or not as +/-, 1/0 or y/n
- Normal or abnormal behavior as +/-, 1/0, or y/n
- Normal food intake or change in food intake +/-, 1/0, or y/n

The convention is that minus (or 0 or "n") indicate the normal situation while + (or 1 or "y") indicate that the animals is outside the normal range [12, 13].

Binary scoring represents a simplification that may reduce risk of misinterpretation or subjective evaluation - which can be the case when too many poorly defined score categories are used. Binary scoring is therefore less sensitive to observer bias. The principal investigator must, define how many 1/+ that defines actions to follow up and if any particular of them elicits immediate actions.

Many or few endpoint indicators in the score sheet?

Some authors stress that endpoints can rarely be generalized [12, 13]. Using too many and too general endpoints for scoring is labor intensive. Researchers might be required to evaluate variables that might appear arbitrary or unrelated to the animals condition [15]. Compliance may be increased if the measurement of the variables are related to research objectives [15]. Other authors underline that humane endpoint cannot rely

on a single variable, suggesting to use a combination of variables [36, 80] and recommend a combination of assessment parameters for a robust welfare assessment in laboratory animals [81] and early predictor of death [36]

Refinement of clinical assessment criteria and score sheets

Score-sheets should not be a static "copy-and-paste" document from study to study – but should be constantly developed and updated with further experience [12]. Hendriksen [5] propose that endpoints should be regularly reassessed as a refinement strategy to improve welfare for research animals and tailormade them to the specific study.

Conditions that have been considered crucial for validity of humane endpoints are a low frequency of both false positive and false negative predictions [70]. False positive means aborting an animal from a study (by killing) while the animal would have survived the observation period. False negative endpoints means that the animal already died before it reached the predefined endpoint [70]. False positive endpoints invalidate the potency of the study, while false negative endpoints compromise animal welfare [70].

Refinement of clinical assessment criteria should also include analysis of the performance of each single as well as combinations of multiple parameters. For example body weight reduction and reduced burrowing [36] or body weight and body temperature [82, 83] combined (e.g., the product of two parameters) of more than one physiological parameter to derive a surrogate indicator for a higher risk of death showed to be a better predictor of death than each one of them alone.

Machine learning, a technique used to identify underlying patterns from given datasets to produce reliable and repeatable predictions has been used to refine endpoints in mice [9]. By analysis of large datasets, it is possible to separate animals that are at a higher risk of death based on surrogate indicators and even identify sex differences for that risk [9]. Machine learning can also be used to set up alarms and alerts to the person

responsible for monitoring the animals, and thereby reduce the risk of failure to respond in time.

Score sheets should be regularly reassessed so that criteria that are never observed at all should be excluded [36]. Also, criteria that called for immediate euthanasia should be removed from the list of humane endpoints [9, 36] - as it might be questioned if they are really "humane" [4]. Rather criteria that call for immediate euthanasia should be listed as "emergency procedures for severe conditions and unwanted incidents". Animals dying from unexpected incidents should not contribute to refined endpoints criteria in future studies as their endpoint is irrelevant as a predictor for study specific endpoints [9].

Example of generic scoring and action points

Clinical sign	Observation	Score	Follow-up/actions
Activity and moving pattern	Normal	0	No specific actions Record Scoring and new check next day
	Reduced range of activities or reduced speed	1	• Pain treatment: s.c injection of Meloxicam 5mg/kg and check for improvement after 2 hr and do new scoring • activity should be back to normal to score 0, if not score 1 is sustained
	Reduced range of activities or reduced speed and hunchback	2	Observe 2 times per day. • Pain treatment: s.c injection of Meloxicam 5mg/kg and check for improvement after 2 hr and do new scoring • activity should improve to score 0 or 1 • if not score 2 is sustained
	Apathic. Not responding to stimuli. Going in circles, hunchback, or unsteady walking	3	Euthanasia immediately
Appearance of fur/grooming behavior	Normal (A smooth dense coat, well-groomed appearance)	0	No specific actions Record Scoring and new check next day
	Slight piloerection, ruffled fur	1	• Pain treatment: s.c injection of Meloxicam 5mg/kg and check for improvement after 2 hr and do new scoring • activity should be back to normal to score 0, if not score 1 is sustained
	Obvious piloerection, ruffled fur, secretion around eyes, nose, anus, or penis/vulva	3	Euthanasia immediately

Concern for control animals

When defining endpoints for studies, special consideration must be made to control animals. Especially negative controls (not receiving the drug candidate of interest) will not have the advantage of a potential treatment of protection effect of the test-substance. These animals will therefore likely be that first to reach the end-point state – assumed that the test substance is effective. This applies for both the generic and project specific endpoints.

Unregulated species and life stages

Regulations and guidelines define borders of which animal species and life stages that are included. Legally, humane endpoints are relevant for studies in these species and life stages. However, the regulations are based on historical knowledge and new knowledge about sentience of species and life stages is evolving. The inclusion and protection of cephalopods in the 2010 EU directive [2] is an example of a new group of non-vertebrate animals that become protected based on scientific knowledge. So, attention also to potential pain, suffering and distress for early life stages, like fish larva, before they start-feed), or drosophila may be worth considering – even if they are not currently covered by regulations.

How humane is your endpoint?

Humane endpoint is a professional concept that is defined both in regulations as well as in literature and guidelines for animal research [2, 3, 5, 17]. The most widely use, but also most narrow definition as by the directive [2] and OECD guidelines [1] promote humane endpoints as an **alternative to death** [3]. However humane endpoints as defined by Hendriksen [5] – is a **strategy to minimize pain, suffering and distress** in animal studies. Humane endpoints together with proper pain relief and animal centric practices for handling, are all **keys to design less inhumane studies** in accordance with Russell and Burch's 3Rs in "The principles of humane experimental technique" [8].

The term "humane" reflects an altruistic position identified with kindness, care, compassion or sympathy for others [84]– especially humans but also

animals. The term “humane” is also used in connection with humane killing of animals – which in veterinary practice means mercy killing performed by specially trained personnel, generally to prevent suffering from incurable or painful conditions [85].

“Humane” endpoints can similarly be perceived as studies identified with end- and action-points assuring kindness, care, compassion, or sympathy for the animals. The approach “unnecessary pain suffering and distress” cause cognitive dissonance in the context of humane endpoints – especially among non-professional, lay persons. Pain, suffering or distress might be approved necessary to reach certain scientific objectives, justified by potential legitimate benefits and when there are no alternatives. However, accepting pain, suffering or distress - even when justified - is contradictory to beliefs, ideas or values of common peoples understanding of «humane». How “humane” endpoints are - is not an absolute dimension, but relative to the context, study objectives and what alternatives that are available or that have been considered. If brining in the “humanity” dimension cause confusion and contribute to blurring communication on how animals are used in research, it might be suggested to only refer to “endpoints” –leaving out the “humanity”. However, to use “humane” to describe the process of continuously refining to aim against “more humane” practices in animal studies (compared to a less “humane” endpoint involving more pain suffering or distress), the use of “humane” still make sense.

The directives definition applies for avoiding death as an endpoint [2]. The Directive does not explicitly apply for the moribund state. However, the Canadian Council of Animal care say:

> *… moribund animal is one that is close to death and may be comatose or unresponsive to stimuli, exhibit dyspnea or other severe breathing problems, hypothermia, prostration, etc. However, before the animal gets to the point of being moribund, detailed observations of the animal can help to set an earlier endpoint and thereby reduce the actual cost to the animal, in terms of pain and/or distress.* CCAC[20]

It is therefore questioned if endpoints – like moribund that involve a serious, threatening status for the animal can be regarded as humane.

The Guide for the care and use of laboratory animals better link the experimental and humane endpoints and better connect it to refinement.

> *The experimental endpoint of a study occurs when the scientific aims and objectives have been reached. The humane endpoint is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. The use of humane endpoints contributes to refinement by providing an alternative to experimental end- points that result in unrelieved or severe animal pain and distress, including death. For many invasive experiments, the experimental and humane endpoints are closely linked.*
>
> [17]

How humane is my endpoints? - **Key check question**

- Will my endpoint be perceived as humane by the common public?
- Will the aim of the study be perceived as a legitimate justification for the endpoints defined?
- Have I room for further refinement of my study so can define more humane endpoints?

Table 1 Refinement of study specific endpoints and alternatives to death
This table shows examples of study specific endpoints and alternatives to death and other severe endpoints – and important pathway to refinement of animal studies.

INFECTIONS, IMMUNE SYSTEM

Study Objectives	Endpoint	Reference
Infectious diseases	Acute phase responses Body Temperature Weight loss	[86]
Rheumatoid arthritis in rodents	Swollen digits, knuckle, midfoot and ancle/wrist scoring, Ulceration, gait and posture analysis	[87]
Shock by cecal ligations and puncture (CLP)	Loss of ability to ambulate. Body weight and temperature	[79]
Tuberculosis vaccine studies	Bodyweight AND Breathing pattern AND activity during handling combined predict need for euthanasia 1 week ahead	[16]
Bacterial virulence	Hypotermiadose50 (HD50) replaces LD50	[88]
Sepsis	Surface temperature and sickness score at 24 hours after the first injection by machine learning	[9]
Inoculation with infectious organisms	Temperature and Body weight	[83]

CANCER, TUMOR STUDIES

Intracranial Glioma in rat	Body weight algorithm	Re[89]
Experimental liver metastases	Laparoscopy	[90]
Colon cancer in rats	Black feces or presence of fresh blood	[91]
Early Cancer detection	Ultrasound	[92]
Severe irradiations studies in NHP	Rapid decrease of body temperature for 3 consecutive days combined with general behavior score	[80]
Total-Body Irradiation in Mouse	Body weight, temperature by telemetry	[93]

MULTIPLE BODY SYSTEMS

Study Objectives	Endpoint	Reference
Murine model for cholestasis	Burrowing activity	[36]
Anti-urolithiasis activity of test compounds by zinc disc implantation	Imaging (X-ray radiographs) of the bladder deposits	[94]
Ocular Herpes virus Infection	Combined Body weight reduction (>0.05 g/day) AND Body Temperature less than 34,5°C measured by s.c. implanted temperature probe. Predict death in 98% of mice.	[82]

CENTRAL NERVOUS SYSTEM (CNS)

Amyotrophic Lateral Sclerosis (ALS)	Decline in motoric Function	[77]
	Home cage running wheel	[78]
Stroke	Weight change on the 1st and 3rd day after treatment. Core temperature change on the 3rd day after treatment (male, stroke model), or with Neuro-score, weight change, and core temperature change on the 2nd day after treatment (female, stroke model) by machine learning.	[9]

TOXICITY

Toxicity testing	Heart rate, snout–vent length, eye size, and pericardial area	[95]

SURGERY

Post laparotomy	Telemetric recording of heart rate	[18]

Table 2 Defining scores, grades and actions points.
Example of evaluation of surgical wound

	Clinical evaluation	Score	Action
WOUND	Closed wounds, No erythema	0	New observation next day
	Slight erythema around wound, no edema/swelling	3	Frequent observation 2 times per day
	Moderate erythema, edema	6	Consider painkiller or antibiotics based on veterinary recommendation
	Severe erythema, swelling/edema, open wound	8	Provide painkiller, conservative wound care or reoperation of wound
	No effect of treatment	12	Humane killing of animal

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Humane endpoints and Score sheets

Article 13 - Choice of methods

> *Death as the end-point of a procedure shall be avoided as far as possible and replaced by early and humane end-points. Where death as the end-point is unavoidable, the procedure shall be designed so as to:*

- result in the deaths of as few animals as possible; and
- reduce the duration and intensity of suffering to the animal to the minimum possible and, as far as possible, ensure a painless death.

Study of a disease to better understand it and hopefully develop better treatment is an aim for many experiments.

In such cases, there is always a risk of suffering in animals. The regulation states clearly that any unnecessary suffering must be avoided. It is therefore an obligation of the researcher to plan and perform experiments in such a way that animals do not suffer more than strictly necessary to achieve a certain research aim. Any potential or real suffering must be justified in a harm-benefit assessment, i.e. the negative experience of the animal is justified because of greater benefit.

Chronic diseases gradually progress in severity. The same apply for pain and discomfort caused by disease. Clinical symptoms or signs of suffering develop when the animal cannot any longer compensate for the progressing disease.

Severe clinical symptoms reflect severe harm for the animal. Severe clinical symptoms and death caused by disease are not acceptable as endpoints and should be replaced by earlier endpoints.

The concept of humane endpoints is about setting earlier endpoint before so that the animal is not in risk of unnecessary suffering.

Implementing humane endpoints is a 3R strategy and a tool for refining animal experiments.

Morphological welfare indicators and clinical disease markers

Morphologic welfare indicators must be based on knowledge about the specific species and life stage.

Strengths

- Simple to register
  - "look and record"
- Well known optimum
  - we (should) know how a healthy fish looks and behave
- Comparable
  - with a healthy fish
- Indicate the fish ability to master the situations
  - can they maintain barriers (against foreign substances infectious agents? toxic substances?)
  - can they maintain vital homeostatic mechanism (oxygenation, excretion)
  - can they see, (enemies? food? places to hide and escape?)
  - can they swim (escape from enemies, move to food etc.)

Weaknesses

- Retrospective
  - What we observe are consequences or responses of something happened back in time
  - Will often involve handling and restrain of animals
  - Absence of morphological deviations is not a guaranty for good welfare

Morphological welfare parameters to evaluate as humane endpoints

Skin, scales and mucus

The skin represents the fish outer barrier to the environment and protect against infectious agents (virus, bacteria, parasites), chemicals compounds and is important to maintain osmoregulation in the fish.

The skin in fish is also an important sensor organ in fish as water is a good medium for transmitting mechanical vibrations.

The surface is covered by a mucus layer that function as lubrication and reduce friction. Mechanical irritation increases mucus production as a response. The scales protect the underlying delicate structures of the skin.

Mechanical stress in connection with registrations and sampling is a likely cause of damage.

Damage to the skin represent a barrier break that can cause infections, toxic effect of chemicals, or osmotic stress in the fish.

- Scale loss means barriers (osmotic, infectious) are broken
- Oedema is one of the cardinal symptoms of inflammation - a response to tissue damage or an infection.
- Color (smoltified, moribund)
- Mucus is a part of the skin barrier. Excessive mucus can be a response to an irritation

Skin damage can be categorized based on number, size or depth of lesions on a categorical scale or as present on not present, expose subcutaneous tissues or no - on a binary scale.

Example use of Skin, scales and mucus changes in a score sheet:

Categorical scale

	Not observed	Intermediate	Maximal acceptable
Number	0	Intermediate number observed	Maximum acceptable number of skin lesions observed
Size	0	% of body surface	Max % of body surface
Depth	0	Superficial	Exposure of subcutaneous tissue

More intermediate classes can be applied to discriminate better between unique levels of severity. However, too many classes may jeopardize objectivity of scoring.

Binary Scale

	Not present	Present
Skin damage	0	1
Expose subcutaneous tissues	0	1
Signs of infections	0	1
Mucus production	0	1

Wounds and ulcers

Wound and ulcers also represent a barrier break as described for skin and scales above.

Wounds and ulcers may be secondary to damage to skin and scale, caused by mechanical impact or infections.

Wounds and ulcers also represent a breakdown of the microbiological, chemical and osmotic barrier of the fish. After surgery, wound healing of surgical wound should be evaluated.

Wounds and ulcer, and severity for the fish can be evaluated based on:

- Depth
    - are muscle or underlying tissue exposed?
- Active wounds vs wounds in healing progress
- Location of wound and potential interference with basal functions
        - Snout wounds (caused by salmon lice or bacterial infections) and interference with food uptake, ability smell etc.
- Number of wounds
- Size of wounds?
    - How much of the body surface is affected?
- Necrosis (cell death)
    - Body cannot heal the wound and the body try to reject the necrotic tissue

Based on this evaluation you must decide if wounds are acceptable for you to reach your scientific aim. Or if they occur what will you do. Can the condition be treated? When should the animals be euthanized?

Example on use of wounds and ulcers in a score sheet:

Categorical scale

	Not observed	Intermediate	Maximal acceptable
Number of wounds	0	Intermediate number observed	Maximum acceptable number of wounds observed
Size	0	% of body surface	Max % of body surface
Depth	0	Superficial	Exposure of subcutaneous tissue
Edema	0	Some swelling	Extensive swelling negatively impacting circulating
Inflammation	0	Edema (swelling), redness (rubor)	Purulent infections
Necrosis	0		Necrotic ulcer manifested

Classification of surgical wounds

Surgical wounds are usually classified as

- Class 1/Clean
  - Closed wound with no signs of inflammation
  - No involvement of internal organs (intestines, urogenital organs)
- Class 2/Clean-contaminated
  - Wound were intestines, urogenital organs are entered but under controlled conditions without unusual contamination
- Class 3/Contaminated
  - Open, fresh accidental wound and with major break of sterile technique or gross spillage from the gastro-intestinal tract.
- Class IV/Dirty-Infected
  - Old traumatic wounds with retained devitalized tissues and contaminated wounds.

The higher classes of wound should lead to corrective actions

Fin erosions and damages

The main function of the fins is to swim and for navigation and rapid propulsions in water. These are important functions for food search as well as escape from predators. Damage cause disabilities in the fish that can be stressful for the fish or put them in risk of attacks.

Fin erosions and damages to the fins can be a secondary effect of an infection an indicator of poor water quality. Damage to fins may impair the fish ability to move and navigate to find food or to escape a predator or other treats. This will be a stressful and exhausting experience for the fish.

Fin erosions and damages can also be seen as a result of aggression, because of suboptimal fish density or completion of food, territory or other resources in the environment.

- Fresh fin lesions
- Healed fin lesions

Gills

The fish gills are a richly vascularized organ of vital importance for uptake of O2, excretion of CO2, iono-regulation and excretory functions. The gills are important for osmoregulation and exchange of salts and H+ in the fish. The operculum protects the delicate mucous membrane of the gills.

Damage to the gills will imply oxygen uptake and impact vitality of the fish. Disturbance of osmoregulation will impact several vital body functions.

Gill damage can be caused by infections colonizing the gill surface. A response from the body can be increased mucus production. Damage of the delicate gill surface can cause bleeding, barrier-breakdown against pathogens and gill-dysfunction affecting oxygen uptake and reduced vitality of the fish.

Deformities operculum may impair gill function and protection. Fish with deformed opercula should not be included as research objects in experiments as they are in risk of complications.

Eyes and vision

The eyes are important for visual orientation for the fish.

They can suffer from infectious attack or mechanical damage.

Exophthalmia is a condition were the eye bulb is protruding out of the orbital cavity of the skull. In this situation the eye is in increased risk of mechanical damage. Exophthalmia can be caused of circulatory problems causing increased pressure or an underlying structure (tumor, abscess) forcing the eye build out of the orbital cavity. Unilateral exophthalmia can indicate local infection or tumor, while a bilateral exophthalmia can be caused by a systemic condition as a generalized circulatory problem

Cataract is a clouding of the lens in the eye and is usually caused by a metabolic condition causing accumulation of certain metabolites in the lens. Cataract is a progressively developing condition that cause visual impairment and blindness.

Example use of Exophthalmia in a score sheet

Grade	0 (not present)	Present	Euthanasia
Exophthalmia	Regular animal welfare check	Exclude fish from the study	Perforation of cornea and damage of deep eye structures

Body condition, Body shape and fitness factor

A good body condition of the fish reflects good welfare and absence of disease, pain suffering or distress. The most common way is to describe fitness-factor is the relation between body weight and body length. However, a deformed, shortened spine can give false positive on fitness factor. Fish come in different shapes so fitness factor must be species specific.

Body weight is an objective measure commonly used as an end-point indicator. Poor body condition reflects welfare issue over time – and is therefore a retrospective endpoint parameter. Reduced food intake can be caused of

reduced appetite (often an underlying disease) or inability to take or access the food causing emaciation.

Runts are animals that in general are smaller than they littermates.

Cachexia is an extreme emaciation with prominent skeleton and loss of muscles mass. This is indicative of severe katabolic process and/or chronic pain often associated with cancer or infectious diseases or neoplasia.

Accumulation of fluid (ascites) in the abdominal cavity will give a swelling of the abdomen and give a false impression of the body condition.

Deformities and malformations

Normal morphologic development is an indicator saying that basic needs (nutrition and environment) of the fish have been covered in the developmental phase.

Deformities and malformation occur in all species. Causes of malformation includes

- Temperature in the embryonic stage
- Maternal stress
- Nutritional deficiency

Deformities are usually congenital conditions. The impact of the severity of the deformity depends on how this impact animals' vital function.

Examples

- Deformities of the spine can affect swimming and agility of the fish – becoming a runt or a "looser" in the competition of food and recourses.
- Deformed opercula may not give adequate protection and function of the gills and suboptimal development of the fish, with increased risk of infections or other complications.

Fish with deformities should not be included in studies

More recourses on deformities in in fish

- https://nofima.no/wp-content/uploads/2016/06/Velferdsindikatorer-for-oppdrettslaks-2018.pdf
- https://nofima.no/wp-content/uploads/2020/05/Velferdsindikatorer-for-regnbue%C3%B8rret-i-oppdrett-Noble-mfl.-2020.pdf

- https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2018/standardisering-av-agd-gjellescore.enhetlig-gjellescoring-basert-pa-data-fra-eksperimentelle-forsok-og-oppdrettsanlegg-for-laks/_/attachment/download/a2076df9-a2b9-498c-9ea0-f87b578110f3:6eb3902b7574229929ababe09b5292a6187d0073/2018_19_Standardisering%20av%20AGD-gjellescore.pdf

Behavior as a welfare indicator

Use of behavior as welfare indicator and on score sheets

Strengths

- Observable (look and record)
- Reflect physiological response
- In real time
- Allow registrations without interfering with the animals

Weaknesses

- Interpretation is not always clear
  - Why is the fish doing this?
- Can be difficult to identify
  - Because fish is in water – we are on land
  - In large groups of animals

Behavioral responses to daily husbandry practices like feeding is a commonly used indicator of welfare as fish fed at regular intervals will be hungry and swim against the feed when it is served

Just observing things like swimming pattern can also give valuable information of the physical condition of the fish. Changes in behavior may be the first visible response to adverse conditions, a valuable bio-indicator and early warning sign.

Changed behavior as endpoint criteria

Monitoring appetite is usually a very sensitive, real time, parameter as healthy fish is always interested in food. This can best be observed during feeding time.

Swimming behavior is important. Counter-current swimming is important for gas-exchange (O2 uptake - CO2 excretion) so beside being and indicator it is a warning signal that vital functions are challenged.

Lethargy (apathy, drowsiness) is characterized by reduced sensitivity to stimuli, often occurring in the final stage of illness – a situation called moribund. Fish that do not respond as usual when the operator occur by the tank or net should be followed up for underlying case that should be corrected.

Other observation by the tank can include

- Swimming pattern
- Loss of equilibrium
- Position in tank
- Position in water
- Gill movements (hyperventilation, hypoventilation)
- Flaring gills
    - Flared gills occur when fish have trouble breathing caused by low O2 level in water or infection on the gills
- Panic behavior
    - Fish stressed for some reason (water quality, fear of a predator presence?)

Physiological welfare indicators

Strengths

- Can identify earlier, real time welfare issues
- Sensitive
- Simple and comparable when sampled and analyzed
- Levels of hormones and metabolites are known
- Validated for many values and analysis methods
- Often quantizable – and less vulnerable to subjective interpretation

Weaknesses

- Difficult to sample (often involve manual handling and restrain) and measure accurately
- Individual variation in optimal values or optimum is not known

- May involve handling and restrain for sampling
- Need for catching, anesthetizing and taken out of water

Example of physiological welfare indicators

Sudden changes in environment, pain, fear or aggression increase secretion of catecholamines within few minutes.

Changes in salinity, light/dark cycle and nutritional conditions will increase levels of cortisol and impact reproduction.

Adrenalin and noradrenalin
in blood increase by exhausting activity and plays an important role for circulatory adaptions.

Analysis of blood samples

- Blood-parameters (erythrocytes, white blood cells, pH)
- Metabolites (lactate, glucose)
- Vitamins and minerals
- Electrolytes
- pH
- Immunoglobulins
- Enzyme activity
- C-reactive protein (CRP)
- Hormones
    - Cortisol as a stress marker
- Other specific disease marker

Microbiological analysis

- Presence of pathogen agents

Analysis of faces

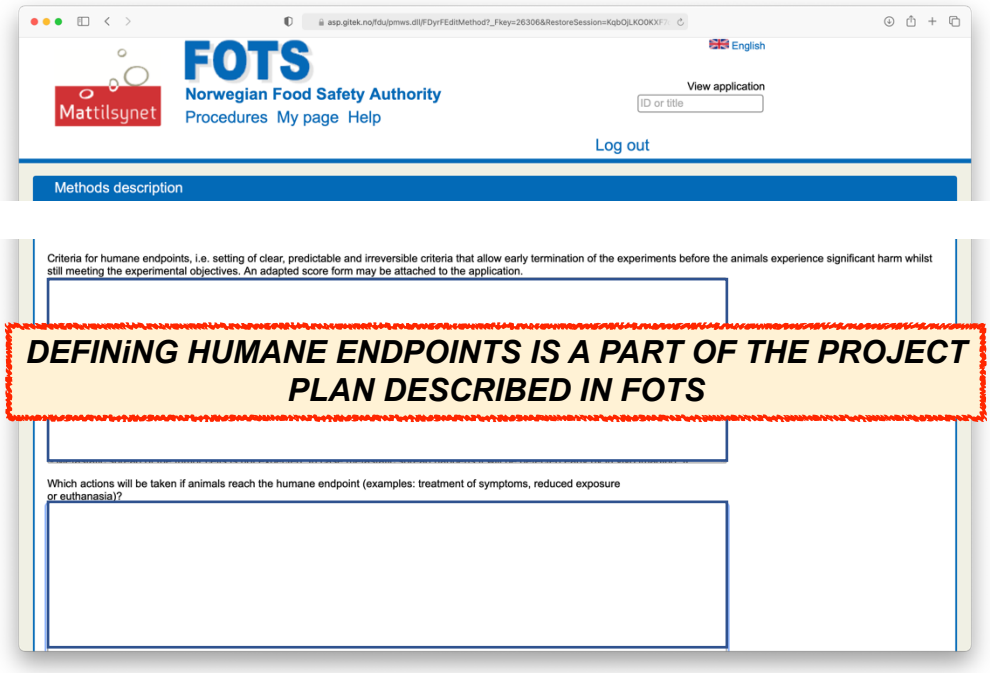
Necropsy of killed or dead fish

Analysis of environment/water

Remote observation

Implantation of telemetry devices opens for continuous recording of integrated body functions like respiration, cardiovascular functions, intestinal function etc) without direct interference by humans or the stress by restrain.

5.6 HUMANE ENDPOINTS (HEP)



5.6 HUMANE ENDPOINTS (HEP)

The EU directive 2010/63 state that death shall be avoided as an endpoint and be replaced by **earlier, humane endpoints**.

A similar statement is referred to in the Norwegian animal research regulation (*§ 11. Metoder, teststrategier og endepunkter)*

This reflects a very narrow application of humane endpoints and it has been questioned if all these earlier endpoints can be really considered "humane" *.

Other authors therefore purpose a more broad definition of Humane Endpoint as a concept for **continuous refinement** of animal studies**.

This latter broad definition will be basis for the rest of this chapter as it is better aligned with the 3R principles by Russel and Burch that are also embedded in the directive

> *Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals (Article 4.3)*

*FRANCO, N. H., CORREIA-NEVES, M. & OLSSON, I. A. S. 2012. How "Humane" Is Your Endpoint?-Refining the Science-Driven Approach for Termination of Animal Studies of Chronic Infection. *Plos Pathogens,* 8.

**HENDRIKSEN, C., MORTON, D. & CUSSLER, K. 2010. Use of Humane Endpoints to minimise Suffering. *In:* HOWARD, B., NEVALAINEN, T. & PERRETA, G. (eds.) *The COST Manual of Laboratory Animal Care and Use.* CRC Press Taylor & Francis.

5.6a Humane endpoints

Study of diseases to better understand the and hopefully develop better treatment is the objective for many studies. Animals are often used because it is impossible or regarded as unethical to study this in humans.

In such cases, there is always a risk of suffering in animals. The directive as well as the Norwegian regulation state clearly that any **unnecessary suffering must be avoided**. It is therefore an obligation of the researcher to plan and

perform the studies in such a way that animals do not suffer more than strictly necessary to achieve a certain research objective. Any inevitable suffering must be justified in a harm-benefit assessment, i.e. the negative experience of the animal is justified because of greater benefit for the majority.

Chronic diseases gradually progress in severity. The same apply for pain and discomfort caused by disease. Clinical symptoms or signs of suffering develop when the animal cannot any longer compensate for the progressing disease.

Chronic diseases gradually progress in severity and the same apply for pain and discomfort caused by disease. Clinical symptoms or signs of suffering become noticeable when the animal cannot any longer compensate for the progressing disease. Severe clinical symptoms and death caused by disease are not acceptable as endpoints and should be replaced by earlier, less severe endpoints.

Humane endpoints are relevant to apply

1. When scientific objectives are met - and there is no reason to continue the study
2. When unexpected suffering occurs
3. When anticipated suffering, described in FOTS and approved by Mattilsynet become more severe than predicted
4. When pain, suffering or distress are and inherent part of the approved study, but when mitigating factors must be initiated

The objective of <u>humane</u> endpoints is about setting early endpoints - to reduce the risk of unnecessary pain, suffering distress or lasting harm

Implementing humane endpoints is a 3R strategy and a tool for refining animal experiments.

VIDEO:
Humane endpoints - a crash course (Video 2 min 11 sek)

- https://vimeo.com/318220053/1f5fbc5a68

5.6b Humane endpoints: Understand the concept

There are several definitions of Humane endpoints. A common understanding is to

"prevent unnecessary suffering"

There are several ways of ending pain, suffering or distress in animals. Euthanasia is probably the most common outcome when an animal reaches a predefined endpoint - especially for rodents and fish. In larger animals' other strategies are also used. To reduce exposure by a pathogenic factor or to treat symptoms or any action that mitigate negative impact on the animals can be alternative endpoints in a study.

Humane endpoint is ending an experiment at the earliest possible stage and before animals experience unnecessary i.e. pain, suffering or distress that cannot be justified based on scientific objectives of the study.

Humane killing may be a possible outcome if pain or distress cannot be relieved in another way, as if the animal is found in a moribund state (dying). However, to plan a study where "dead" or "dying" (moribund) as an endpoint should be avoided and demands special scientific justification.

PRESENTATION: Humane Endpoints (HEP) 3,42 min

- https://vimeo.com/279206373/47556e38f4

5.6c Legal Anchoring and Scientific justification for Humane endpoints

Legal Anchoring

Dead or moribund (dying) are not acceptable humane endpoints.

The Norwegian regulation on the use of animals in research clearly states *(§11 - Methods, test strategies and endpoints)*

- *"*Death shall as far as possible not be the end point of an experiment
- It should instead be used early and humane end-Points*"*

A similar statement is found in the EU directive 2010/63 (Article 13 - Choice of Methods)

> *"Death as the end-point of a procedure shall be avoided as far as possible and replaced by early and humane end-points".*

The Norwegian regulation also state very clearly that the primary investigator of a project has the main responsibility to safeguard that any unnecessary pain, fear, lasting harm of other negative impact is eliminated as soon as possible (§28). So, there is a strong legal demand on the researcher to apply humane endpoints

Applying humane endpoints is in accordance with the Norwegian Animal Research Regulations with several references

- §9 - Continuous refinement and improvements of methodology to avoid, prevent, eliminate or minimize any pain, suffering or distress
- § 11 - Avoid experiments with death as an endpoint
- §14 - Use painkiller and even total anesthesia if there is risk of severe pain
- §15 - Considerations to if an animal should be kept alive when the experiments is finished must be made by a competent veterinarian or fish health specialist. This deccision has consequences for potential reuse (§17) or rehoming (§18)
- §16 - Animal must be killed by competent person an in accordance with approved methods

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- §16 - Animal must be killed by competent person an in accordance with approved methods

*The death of an animals **must** be reported as an outliers according to the ARRIVE guidelines. If it is not included in the analysis you must explain why as death can be caused by your intervention. Not doing so is violating principles of good science.*

If this animal has to be replaced to complete the experimental group it belonged to - this has ethical implications as a another animal then has to go trough similar procedures and experiences.

Moral and Social reasons

Continuing and experiment in sentient animals beyond justifiable limits of suffering as morally unacceptable.

The limits are defined by the context of the particular experiment.

Moribund is an irreversible state where death is imminent. The animal has reduced consciousness and might therefore not be aware of pain and distress; however, the concern is all the pain and distress the animal experienced before it reached this stage or died from it. Therefore, the moribund stage is not regarded as a humane endpoint of moral reasons.

Scientific Justification

There are also scientific reasons for applying humane endpoints.

Pain and distress might confound animal studies and interfere with scientific outcomes among others immunological and hormonal responses thereby causing invalid findings and conclusions.

Autolytic changes in tissues take place as soon as death is imminent and if discovered by the next scheduled inspection, the carcass and tissues is already damaged or even cannibalized by cage-mates.

The scientific advantages of applying early humane endpoint can be summarized as;

- Data more reliable in less stressed animals
- stress in itself can cause uncontrolled variation in your study
- Alternative methods may provide equally valid data
- Are there more relevant /important questions that can be addressed using EP?
- Studies with earlier endpoints are more time and cost effective

Good science and good animal welfare go hand in hand when we apply humane endpoints

Presentation: Legal Anchoring and Scientific justification for HEP (5min 17 sec)

- https://vimeo.com/279214344/9dff35fde6

5.6d Setting and Implementing Humane endpoints

Setting the endpoint

The objective of humane endpoints is to limit suffering for research animals. Consequently, the endpoint can be set at any time between the first detachable deviation from normality of a disease to the first indication of severe suffering. That means that the appearance of events of a molecular level that trigger a cascade of reactions that lead to death or severe suffering can be a potential early endpoint.

Clinical or behavioral signs deviating from normal are commonly used to define endpoints - and many opportunities are available. The "problem" in some species, especially prey animals like rodents.

Prey animals will use resources to compensate for and try to hide early signs of disease (Arras et al., 2007). Hiding weakness for a prey animal is important to avoid attention from potential predators as this might make their lives at risk, and this property has been enhanced throughout the evolution. The implication is that the animal might have suffered from coping distress for a while before humans are able to recognize the problem. The observation-window from no clinical signs to obvious clinical signs can be narrow for many species. Many of the cues we can observe are therefore not early indicators.

> *Sunken flanks, neglected grooming, piloerection, hunched back, immobility are clear evidence of severely impaired often moribund health status in mice (Jirkof et al., 2010)*

We should therefore search to define early signs of pain, suffering and distress and use them to define humane endpoints in animal studies..

Early clinical and non-clinical pathophysiological changes include deviations outside the normal physiological range for variables such as blood pressure, heart rate or body temperature. These changes may lead directly or indirectly to a severe clinical state at a later stage.

Technology innovations increases opportunity for early detection of pathophysiological changes. That include for example temperature sensitive transponders to monitor body temperature, telemetry for real-time measuring body temperature, blood pressure and heart rate. Laparoscopy allow less invasive, early diagnostic for example of liver metastasis, and imaging for a large variety of non-invasive early diagnostic.

Beside offering the opportunity for earlier diagnostic - applying non-clinical pathophysiological indicators and technology innovations is that many of them provide **quantitative** data that makes them less vulnerable for subjective interpretation - that is the case for many clinical observations that are based on judgement.

5.6di - Pilot Studies

Pilot studies

Pilot studies are small scale preliminary studies used to clarify logistical issues, severity of a procedure, need for frequent observation or follow up among others.

When the impact on the animals is not clear - because it is a new procedure or new Genetically altered animal for example - the authority can set demands that pilot experiments must be done - and going through retrospective assessment - before approval of large-scale experiments.

Pilot studies are preliminary studies suitable to clarify several aspects:

- Determine required frequency and quality of animal observation
- Define responsibility for animal observation
- When to report clinical findings?
- Who to report to clinical findings?

The regulation (§6) state that pilot studies should be used whenever the methodology has not been used before

VIDEO PILOT STUDIES (2 min 36 sek)
https://vimeo.com/472183591/726ff1c9d2

5.6d ii - Score sheets

Score sheets

"Score sheets" sheets are commonly mentioned in relation to humane endpoints.

Score Sheets are also known as "observation sheets" or "welfare assessment sheets" are lists of relevant observations for the relevant procedures, species and sometime also strain.

By convention negative signs are used to indicate normality (within the normal range), while abnormalities are indicated by a + or by a number or more depending on the degree of deviation.

Normality	Abnormality	Not certain
-	+	-/+
0	1	0/1

When no abnormalities according to the list of observation points are detected - this is usually annotated by "NAD" (Nothing Abnormal Diagnosed)

The following presentation focus on the use of score sheets to assess animal welfare or impairment of welfare by experimental conditions.

PRESENTATION: Implementing Hep I - Score Sheet (8 min 5 sek)

- https://vimeo.com/279234715/6ae310594a

5.6d iii - Implementing Humane endpoints Various topics

Critical consideration of some commonly used endpoints

There are some endpoints that are commonly used that but that should be critically reviewed.

Those are related to weight loss, tumor size or tumor ulceration. In the FOTS application it is typically stated that “Animals are killed if they loose >20% BW, tumor increase > 3 cm, or tumors are ulcerated.”

Weight change as endpoint

There are many good reasons for using weight loss as an endpoint. It is easy to measure and it is objective and there is not big a risk of subjective bias. On the other hand, weight loss, as an isolated parameter is not very sensitive for the animal’s actual condition, for example:

- Weight gain can be caused by tumor growth or ascites or accumulation of fluid in the peritoneal cavity
- Weight loss can be caused by tumor anti-cancer therapy – which often is the treatment effect we are looking for

- Stable weight in an animal in a growth phase is a poor sign – because in this case we actually want the animal to increase weight according to a weight curve
- Weight loss is reflecting a poor condition in the past, i.e. the animal may have suffered for a while before we are able to detect the weight loss.

Tumor size as endpoint

End-points related to fixed tumor size should be carefully reviewed. Tumor **location** and **aggressiveness** must be taken into account.

- A small tumor in a restricted site (cranium, eye, abdomen, muscle, footpad) can make serious problems for an animal, while a larger subcutaneous tumor on the back of a rodent can be tolerated better
    - Small tumor in mouth can interfere with food intake
    - Tumor on a limb interfere with mobility
- Tumor necrosis can be a part of healing process or a response to therapy. But an open necrotic wound can cause anemia or dehydration and represent a port for infection.

In all cases, we shall also consider the situation for untreated control animals; they are maybe the animals that bear the highest burden.

Multidisciplinary approach "build a good research team!"

Working with animals and implementing humane endpoints needs a multidisciplinary approach to succeed.

Beside the study Director/Principal investigator, who is responsible for the conduct of the experiment according to the conditions defined by the competent authority, research technical staff and graduate students the following expertise may give valuable input to the study plans

- Lab animal veterinarian
    - Advice on health and welfare for animals and recognition of clinical signs
    - Advice on severity (and if they are exceeded) and follow up on unexpected events
- Animal care staff
    - Responsible for day-to-day care

        - Through their daily routines caring for animals they observe thousand of animals -healthy and sick- and they start to develop a sense for early signs of illness – though they do not always describe them in technical terms that we are trained to use
    - Might be the first to recognize abnormalities.
    - Should be trained in daily and specific monitoring of animals
- Clinical pathologist
    - Necropsy reports from dead or killed animals give useful information about severity and interpretation of clinical signs
    - Facilitate better guidance for subsequent studies
- Statistician
    - Involved in the planning and design
    - Clarify how to deal with unexpected events statistically
- Animal welfare body
    - Sets standards for animal welfare within the institution and advice on improvements on the 3Rs

5.6 d IV - Animals found dead or in the dying (moribund) state

The Norwegian regulation on the use of animals in research clearly states (§11 - Methods, test strategies and endpoints)

- "Death shall as far as possible not be the end point of an experiment
- It should instead be used early and humane end-Points"

Still, it happens that animal are found dead, or in a condition when treatment cannot ameliorate the condition. This might happen when anticipated suffering, described in the approved project plan become more severe than predicted or when unexpected suffering occurs leading to death.

It is important to do proper investigate the reason for death and take precautions when new series of studies as planned. Necropsy by a qualified pathologist may be of great help in that investigation.

*The death of an animals **must** be reported as an outliers according to the ARRIVE guidelines. If it is not included in the analysis you must explain why as death can be caused by your intervention. Not doing so is violating principles of good science.*

If this animal has to be replaced to complete the experimental group it belonged to - this has ethical implications as a another animal then has to go trough similar procedures and experiences.

5.6d vi - End-points and Action-Points

When we define endpoints - we also have to define action-points. These describe the actions we are going to take when or if an animal reach a certain stage in an experiment.

We can define action points on several symptoms, like illustrated in the figure below - evaluating the severity of a wound. The final "end-point" can be to kill the animal humanely, but before that there are several actions including more frequent observations, considering painkillers or antibiotics or consider reoperation of the wound. The humane killing will only be applied if there is no effect of the treatment and the animal is exposed to unnecessary pain, suffering or distress caused by the wound.

	Clinical symptom/endpoint criteria	Grade	Action-point
Surgical Wound evaluation	Closed wounds, No erythema	0	New observation next day
	Slight erythema around, no edema/swelling	3	Frequent observation 2 times per day
	Moderate erythema, edema	6	Consider painkiller or antibiotics based on veterinary recommendation
	Severe erythema, swelling/edema, open wound	8	Provide painkiller, conservative wound care or reoperation of wound
	No effect of treatment	12	Humane killing of animal

Alternatives to killing as and endpoint can include

- Reduced exposure of harmful factors
- Treat symptoms of harmful factors

Other action point than this mention above can include

- providing soft or alternative food
- provide enrichment (for example for behavioral problems or fighting)
- consultation by the veterinarian
- fluid therapy
- substitution therapy

In all cases you must consider if any of these actions will have negative impact on your study.

5.6e Necessary and unnecessary Pain, Suffering or Distress

The term unnecessary harm or Pain, Suffering or Distress (PSD) is mentioned several times in the regulation (§§1, 21, 28 and 29) also in relation to humane endpoints (§11)

It says that unnecessary PSD must be avoided and this is the primary responsibility of the researcher and also housing and care must be organizes so that unnecessary PSD is avoided.

So, can PSD under any circumstances be accepted or allowed?

In studies of disease were animals are used as a model – causing harm, pain, suffering or distress is **authorized** under certain conditions and as long as unnecessary PSD is avoided.

The regulation is based on the premise that is accepted that animals experience PSD as long as this can be justified by the potential benefits.

PSD that cannot be justified by scientific reasons – cannot be ethically justified

Neccesasy Pain, Suffering or Distress

Example: Tumor Study in a rodent

In tumor studies animals will have to develop tumors and mimic the disease in of the species they are modeling. Effect of treatment-candidates is evaluated against controls.

That means that it can be justified that they develop clinical signs of tumor-disease so we can study the effect of treatment.

The consequence for animals will include the tumor and it can happen that the tumor impair body functions, including vital functions and affection of vital organs. The question is how much of these adverse or tumor associated conditions are needed for us to conclude the study.

Repeated handling for sampling for example measuring tumor size to evaluate treatment effect may also be justified

Humane endpoints and Score sheets

Article 13 - Choice of methods

> *Death as the end-point of a procedure shall be avoided as far as possible and replaced by early and humane end-points. Where death as the end-point is unavoidable, the procedure shall be designed so as to:*

- result in the deaths of as few animals as possible; and
- reduce the duration and intensity of suffering to the animal to the minimum possible and, as far as possible, ensure a painless death.

Study of a disease to better understand it and hopefully develop better treatment is an aim for many experiments.

In such cases, there is always a risk of suffering in animals. The regulation states clearly that any unnecessary suffering must be avoided. It is therefore an obligation of the researcher to plan and perform experiments in such a way that animals do not suffer more than strictly necessary to achieve a certain research aim. Any potential or real suffering must be justified in a harm-benefit assessment, i.e. the negative experience of the animal is justified because of greater benefit.

Chronic diseases gradually progress in severity. The same apply for pain and discomfort caused by disease. Clinical symptoms or signs of suffering develop when the animal cannot any longer compensate for the progressing disease.

Severe clinical symptoms reflect severe harm for the animal. Severe clinical symptoms and death caused by disease are not acceptable as endpoints and should be replaced by earlier endpoints.

The concept of humane endpoints is about setting earlier endpoint before so that the animal is not in risk of unnecessary suffering.

Implementing humane endpoints is a 3R strategy and a tool for refining animal experiments.

Morphological welfare indicators and clinical disease markers

Morphologic welfare indicators must be based on knowledge about the specific species and life stage.

Strengths

- Simple to register
  - "look and record"
- Well known optimum
  - we (should) know how a healthy fish looks and behave
- Comparable
  - with a healthy fish
- Indicate the fish ability to master the situations
  - can they maintain barriers (against foreign substances infectious agents? toxic substances?)
  - can they maintain vital homeostatic mechanism (oxygenation, excretion)
  - can they see, (enemies? food? places to hide and escape?)
  - can they swim (escape from enemies, move to food etc.)

Weaknesses

- Retrospective
  - What we observe are consequences or responses of something happened back in time
  - Will often involve handling and restrain of animals
  - Absence of morphological deviations is not a guaranty for good welfare

Morphological welfare parameters to evaluate as humane endpoints

Skin, scales and mucus

The skin represents the fish outer barrier to the environment and protect against infectious agents (virus, bacteria, parasites), chemicals compounds and is important to maintain osmoregulation in the fish.

The skin in fish is also an important sensor organ in fish as water is a good medium for transmitting mechanical vibrations.

The surface is covered by a mucus layer that function as lubrication and reduce friction. Mechanical irritation increases mucus production as a response. The scales protect the underlying delicate structures of the skin.

Mechanical stress in connection with registrations and sampling is a likely cause of damage.

Damage to the skin represent a barrier break that can cause infections, toxic effect of chemicals, or osmotic stress in the fish.

- Scale loss means barriers (osmotic, infectious) are broken
- Oedema is one of the cardinal symptoms of inflammation - a response to tissue damage or an infection.
- Color (smoltified, moribund)
- Mucus is a part of the skin barrier. Excessive mucus can be a response to an irritation

Skin damage can be categorized based on number, size or depth of lesions on a categorical scale or as present on not present, expose subcutaneous tissues or no - on a binary scale.

Example use of Skin, scales and mucus changes in a score sheet:

Categorical scale

	Not observed	Intermediate	Maximal acceptable
Number	0	Intermediate number observed	Maximum acceptable number of skin lesions observed
Size	0	% of body surface	Max % of body surface
Depth	0	Superficial	Exposure of subcutaneous tissue

More intermediate classes can be applied to discriminate better between unique levels of severity. However, too many classes may jeopardize objectivity of scoring.

Binary Scale

	Not present	Present
Skin damage	0	1
Expose subcutaneous tissues	0	1
Signs of infections	0	1
Mucus production	0	1

Wounds and ulcers

Wound and ulcers also represent a barrier break as described for skin and scales above.

Wounds and ulcers may be secondary to damage to skin and scale, caused by mechanical impact or infections.

Wounds and ulcers also represent a breakdown of the microbiological, chemical and osmotic barrier of the fish. After surgery, wound healing of surgical wound should be evaluated.

Wounds and ulcer, and severity for the fish can be evaluated based on:

- Depth
    - are muscle or underlying tissue exposed?
- Active wounds vs wounds in healing progress
- Location of wound and potential interference with basal functions
        - Snout wounds (caused by salmon lice or bacterial infections) and interference with food uptake, ability smell etc.
- Number of wounds
- Size of wounds?
    - How much of the body surface is affected?
- Necrosis (cell death)
    - Body cannot heal the wound and the body try to reject the necrotic tissue

Based on this evaluation you must decide if wounds are acceptable for you to reach your scientific aim. Or if they occur what will you do. Can the condition be treated? When should the animals be euthanized?

Example on use of wounds and ulcers in a score sheet:

Categorical scale

	Not observed	Intermediate	Maximal acceptable
Number of wounds	0	Intermediate number observed	Maximum acceptable number of wounds observed
Size	0	% of body surface	Max % of body surface
Depth	0	Superficial	Exposure of subcutaneous tissue
Edema	0	Some swelling	Extensive swelling negatively impacting circulating
Inflammation	0	Edema (swelling), redness (rubor)	Purulent infections
Necrosis	0		Necrotic ulcer manifested

Classification of surgical wounds

Surgical wounds are usually classified as

- Class 1/Clean
  - Closed wound with no signs of inflammation
  - No involvement of internal organs (intestines, urogenital organs)
- Class 2/Clean-contaminated
  - Wound were intestines, urogenital organs are entered but under controlled conditions without unusual contamination
- Class 3/Contaminated
  - Open, fresh accidental wound and with major break of sterile technique or gross spillage from the gastro-intestinal tract.
- Class IV/Dirty-Infected
  - Old traumatic wounds with retained devitalized tissues and contaminated wounds.

The higher classes of wound should lead to corrective actions

Fin erosions and damages

The main function of the fins is to swim and for navigation and rapid propulsions in water. These are important functions for food search as well as escape from predators. Damage cause disabilities in the fish that can be stressful for the fish or put them in risk of attacks.

Fin erosions and damages to the fins can be a secondary effect of an infection an indicator of poor water quality. Damage to fins may impair the fish ability to move and navigate to find food or to escape a predator or other treats. This will be a stressful and exhausting experience for the fish.

Fin erosions and damages can also be seen as a result of aggression, because of suboptimal fish density or completion of food, territory or other resources in the environment.

- Fresh fin lesions
- Healed fin lesions

Gills

The fish gills are a richly vascularized organ of vital importance for uptake of O2, excretion of CO2, iono-regulation and excretory functions. The gills are important for osmoregulation and exchange of salts and H+ in the fish. The operculum protects the delicate mucous membrane of the gills.

Damage to the gills will imply oxygen uptake and impact vitality of the fish. Disturbance of osmoregulation will impact several vital body functions.

Gill damage can be caused by infections colonizing the gill surface. A response from the body can be increased mucus production. Damage of the delicate gill surface can cause bleeding, barrier-breakdown against pathogens and gill-dysfunction affecting oxygen uptake and reduced vitality of the fish.

Deformities operculum may impair gill function and protection. Fish with deformed opercula should not be included as research objects in experiments as they are in risk of complications.

Eyes and vision

The eyes are important for visual orientation for the fish.

They can suffer from infectious attack or mechanical damage.

Exophthalmia is a condition were the eye bulb is protruding out of the orbital cavity of the skull. In this situation the eye is in increased risk of mechanical damage. Exophthalmia can be caused of circulatory problems causing increased pressure or an underlying structure (tumor, abscess) forcing the eye build out of the orbital cavity. Unilateral exophthalmia can indicate local infection or tumor, while a bilateral exophthalmia can be caused by a systemic condition as a generalized circulatory problem

Cataract is a clouding of the lens in the eye and is usually caused by a metabolic condition causing accumulation of certain metabolites in the lens. Cataract is a progressively developing condition that cause visual impairment and blindness.

Example use of Exophthalmia in a score sheet

Grade	0 (not present)	Present	Euthanasia
Exophthalmia	Regular animal welfare check	Exclude fish from the study	Perforation of cornea and damage of deep eye structures

Body condition, Body shape and fitness factor

A good body condition of the fish reflects good welfare and absence of disease, pain suffering or distress. The most common way is to describe fitness-factor is the relation between body weight and body length. However a deformed, shortened spine can give false positive on fitness factor. Fish come in different shapes so fitness factor must be species specific.

Body weight is an objective measure commonly used as an end-point indicator. Poor body condition reflects welfare issue over time – and is therefore a retrospective endpoint parameter. Reduced food intake can be caused of

reduced appetite (often an underlying disease) or inability to take or access the food causing emaciation.

Runts are animals that in general are smaller than they littermates.

Cachexia is an extreme emaciation with prominent skeleton and loss of muscles mass. This is indicative of severe katabolic process and/or chronic pain often associated with cancer or infectious diseases or neoplasia.

Accumulation of fluid (ascites) in the abdominal cavity will give a swelling of the abdomen and give a false impression of the body condition.

Deformities and malformations

Normal morphologic development is an indicator saying that basic needs (nutrition and environment) of the fish have been covered in the developmental phase.

Deformities and malformation occur in all species. Causes of malformation includes

- Temperature in the embryonic stage
- Maternal stress
- Nutritional deficiency

Deformities are usually congenital conditions. The impact of the severity of the deformity depends on how this impact animals' vital function.

Examples

- Deformities of the spine can affect swimming and agility of the fish – becoming a runt or a "looser" in the competition of food and recourses.
- Deformed opercula may not give adequate protection and function of the gills and suboptimal development of the fish, with increased risk of infections or other complications.

Fish with deformities should not be included in studies

More recourses on deformities in in fish

- https://nofima.no/wp-content/uploads/2016/06/Velferdsindikatorer-for-oppdrettslaks-2018.pdf
- https://nofima.no/wp-content/uploads/2020/05/Velferdsindikatorer-for-regnbue%C3%B8rret-i-oppdrett-Noble-mfl.-2020.pdf

- https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2018/standardisering-av-agd-gjellescore.enhetlig-gjellescoring-basert-pa-data-fra-eksperimentelle-forsok-og-oppdrettsanlegg-for-laks/_/attachment/download/a2076df9-a2b9-498c-9ea0-f87b578110f3:6eb3902b7574229929ababe09b5292a6187d0073/2018_19_Standardisering%20av%20AGD-gjellescore.pdf

Behavior as a welfare indicator

Use of behavior as welfare indicator and on score sheets

Strengths

- Observable (look and record)
- Reflect physiological response
- In real time
- Allow registrations without interfering with the animals

Weaknesses

- Interpretation is not always clear
  - Why is the fish doing this?
- Can be difficult to identify
  - Because fish is in water – we are on land
  - In large groups of animals

Behavioral responses to daily husbandry practices like feeding is a commonly used indicator of welfare as fish fed at regular intervals will be hungry and swim against the feed when it is served

Just observing things like swimming pattern can also give valuable information of the physical condition of the fish. Changes in behavior may be the first visible response to adverse conditions, a valuable bio-indicator and early warning sign.

Changed behavior as endpoint criteria

Monitoring appetite is usually a very sensitive, real time, parameter as healthy fish is always interested in food. This can best be observed during feeding time.

Swimming behavior is important. Counter-current swimming is important for gas-exchange (O2 uptake - CO2 excretion) so beside being and indicator it is a warning signal that vital functions are challenged.

Lethargy (apathy, drowsiness) is characterized by reduced sensitivity to stimuli, often occurring in the final stage of illness – a situation called moribund. Fish that do not respond as usual when the operator occur by the tank or net should be followed up for underlying case that should be corrected.

Other observation by the tank can include

- Swimming pattern
- Loss of equilibrium
- Position in tank
- Position in water
- Gill movements (hyperventilation, hypoventilation)
- Flaring gills
    - Flared gills occur when fish have trouble breathing caused by low O2 level in water or infection on the gills
- Panic behavior
    - Fish stressed for some reason (water quality, fear of a predator presence?)

Physiological welfare indicators

Strengths

- Can identify earlier, real time welfare issues
- Sensitive
- Simple and comparable when sampled and analyzed
- Levels of hormones and metabolites are known
- Validated for many values and analysis methods
- Often quantizable – and less vulnerable to subjective interpretation

Weaknesses

- Difficult to sample (often involve manual handling and restrain) and measure accurately
- Individual variation in optimal values or optimum is not known

- May involve handling and restrain for sampling
- Need for catching, anesthetizing and taken out of water

Example of physiological welfare indicators

Sudden changes in environment, pain, fear or aggression increase secretion of catecholamines within few minutes.

Changes in salinity, light/dark cycle and nutritional conditions will increase levels of cortisol and impact reproduction.

Adrenalin and noradrenalin
in blood increase by exhausting activity and plays an important role for circulatory adaptions.

Analysis of blood samples

- Blood-parameters (erythrocytes, white blood cells, pH)
- Metabolites (lactate, glucose)
- Vitamins and minerals
- Electrolytes
- pH
- Immunoglobulins
- Enzyme activity
- C-reactive protein (CRP)
- Hormones
    - Cortisol as a stress marker
- Other specific disease marker

Microbiological analysis

- Presence of pathogen agents

Analysis of faces

Necropsy of killed or dead fish

Analysis of environment/water

Remote observation

Implantation of telemetry devices opens for continuous recording of integrated body functions like respiration, cardiovascular functions, intestinal function etc) without direct interference by humans or the stress by restrain.

SEVERITY CATEGORIZATION

Severity classification

Severity of animal studies - Prospective estimate, Reporting Retrospective Assessment

Severity limits can be described by the degree of deviation from a normal, healthy, naive animal [1]. The EU directive Annex VIII has defined 3 different severity categories based on the level impact on the animal i.e. mild, moderate and severe [2]. In addition a 4th category "Non recovery" has been defined for procedures which are performed entirely under general anaesthesia from which the animal shall not recover consciousness but will be killed while it is under anaesthesia [2].

There are several good reasons of having a severity classification system. First, to continuously focus on the application of the 3R when planning studies in animals, especially for severe studies. The severity system improve transparency on actual animal welfare costs and facilitate communication between those using, caring, and monitoring research animals building a common language. All sources of pain, suffering and distress should be identified in the planning and design phase of the study and means to minimise negative effect on the animals (refinement) must be considered. Input on refinement from the Animal Welfare Body and Designated Veterinarian may be helpful to reduce severity by applying fewer aggravating alternatives. This planning phase should also uncover if there are training needs. There might be a need for specific training of those that will do the daily observation of the animals with focus on what to observe and how to report and respond. Specific assessment protocols or score sheets [3] might be helpful in the monitoring of the animals. The more severe impact on the animals – the more frequent and rigorous monitoring of animals is required [1, 4]. Factors to take into consideration when defining severity level include the procedure, the level of invasiveness, level of necessary restraint, duration of the procedure, whether treatment to reduce pain, suffering or distress can be applied and to what level the animal's normal behaviour is affected. This evaluation must also consider the species in questions, how well are they suited to the experimental conditions we provide them and how do they

cope with contact with humans. In addition, it must be evaluated if there is a need to repeat procedures during the study, will the animal learn and feel fear and will the animal be allowed to rest and recover between procedures [2]. All these factors sum up to the cumulative severity for that animal.

Upper and lower limits

The severity classification in the Directive defines lower and upper limits for what is defined as a procedure, as well as an upper limit for what should never been authorised.

The lover level is defined as pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice – Article 3.1 [2]. Similarly, an upper limit – that should be prohibited - is defined as procedures that result in severe pain, suffering or distress, which is likely to be long-lasting and that cannot be ameliorated [2].

According to Henriksen [3] the following observations reflects levels of upper limit of suffering that should not be exceeded.

- Inability to eat and/or drink.
- Rapid or continuous weight loss
    - 20% during few days
    - 15% as a chronic loss over time
- Dehydration
- General decrease in grooming behaviour
    - Recognized as dirty hair coat, red tears and nasal orifices, soiled personal and peri oral regions especially in young animals.
- Indications of pain, distress, or suffering
- Severe or continuing respiratory distress.

These conditions will lead to an unbearable [5], life threatening condition for the animal if no corrective actions are taken at an early stage [6] .

Examples of the mild, moderate and severe categories

A complete overview of procedures belonging to the different severity categories has been defined in annex VIII of the EU directive [2]. An expert working group provided examples on how to allocate animal procedures to the right category [7]. All example provided are based on the assumption that best practices and refinement measures are strictly adhered to [7].

According to the directive annex VIII **"Mild severity"** is defined as procedures on animals because of which the animals are likely to experience short term mild pain, suffering or distress. That means procedures that cause no significant impairment on the wellbeing or general condition of the animals. Mild procedures also include non-invasive imaging of animals with appropriate sedation or anaesthesia; breeding of genetically altered animals which is expected to result in a phenotype with mild effects and short term (≤24 h) restraint in metabolic cages have been defined as mild procedures.

Moderate procedures have been defined as procedures where the animals are likely to experience short term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress. Examples include surgery that involves penetration of a body cavity under general anaesthesia and appropriate analgesia, associated with postsurgical risk of pain, suffering or impairment of general condition. Moderate procedures also include breeding of genetically altered animals which are expected to result in a phenotype with moderate effect on animal welfare. The moderate severity class also includes creation of genetically altered animals through surgical procedure and use of metabolic cages involving moderate restriction of movement over a prolonged period (up to 5 days).

Severe procedures on animals are procedures likely cause severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress. That includes experiments where death is the endpoint, fatalities are to be expected and alarming pathophysiological states are induced. The severe procedures also include use of metabolic cages involving distressing

restriction of movement over a prolonged period, inescapable electric shock (e.g. to produce learned helplessness), complete isolation for prolonged periods of social species or immobilization stress to induce conditions like gastric ulcers or cardiac failure in rats or forced swim or exercise tests with exhaustion as the end point.

Also, if several mild procedures are performed in the same animal, this might cause the project to be categorized as moderate (a more severe category). The same principle applies for animals experience a series of moderate procedures – in such cases the grand score for that project can be severe.

The examples listed here is not complete but can be used as guidance and comparison when studies are planned. Guidance documents that include examples that are relevant for aquatics [8] and cephalopods [9] are available. There is also a publication and severity assessment for genetically altered mice [10].

The fourth category that is classified by the directive is the "**non recover**" class. **Non-recovery** is used for studies where animals are anesthetized without any prior intervention or procedures and the whole study is completed while the animal is in anaesthesia and animal killed without recovering from anaesthesia. In this way the animal will not have other experiences of procedures beside the injection of the needle or other method to induce anaesthesia.

Prospective severity assessment.

Studies in animals need to be assigned a severity class prospectively **before** projects are authorised by the competent authority. The prospective severity described in a project proposal is a best guess based on the guiding documents, experience and skills of the research team. [11]. This estimate of severity applies for the whole groups of animals in a study.

The actual severity experienced by each animal during a procedure shall be reported annually after the study and reported in the statistical information made publicly available.

Reporting actual severity

The actual severity of procedures will be reported by member states in the annual statistical returns. This reflects the highest severity experienced by the animal because of the procedure(s).

The actual severity does not necessarily equal the prospective severity, as experiments can show to be both less and more severe than anticipated. Experimental groups in testing a cure for a disease can advantage from that cure with less grave consequences, while control animals may have no such advantage and the outcome might be more severe for them.

The actual severity also give direction on criteria among others if an animal can be reused in another procedure as re-use depend on severity of the preceding procedure(s). Animals may only be re-used provided that the severity of the previous procedure was ‘mild’ or ‘moderate”.

A common severity classification system promotes harmonization between European countries. Sufficiently trained and competent staff are absolute requirements to assess animal welfare during the study, and to report actual severity correctly. An observational strategy and a common recording system should capture all the necessary data in a consistent format to be able to harmonize the severity category system across the European countries. A free, online training for the severity assessment has been available on the Education and Training Platform for Laboratory Animal Science [12].

Retrospective severity assessment

The prospective severity classification has impact on requirement for Retrospective Assessment (RA). The Retrospective severity assessment is helpful to identify improvements on the 3Rs as well as evaluating if the model is suitable to achieve expected objectives. All projects using non-human primates and projects involving procedures classified as ‘severe’, shall undergo retrospective assessment (article 39). The competent authority may decide that also other studies shall undergo retrospective assessment. This can be relevant for new animal models, pilots, newly

created genetically altered animals, new test-substances or classes of compounds or animal use for education and training.

The retrospective assessment provides an opportunity to review if the outcome of the study met the defined objective(s) as well as evaluated if the estimated severity prospectively matches the actual severity. These experiences should be used for consideration of 3R alternatives and best practices for planning similar studies in the future. So, the retrospective assessment is a tool for an iterative process of learning and continuous improvement.

The retrospective assessment should be carried out as soon as the experiment is completed, and both the scientific and animal care and welfare team should be involved and share experiences from the study.

More information on retrospective assessment can be found in the publication on Project Evaluation and Retrospective Assessment from the European Commission [13]

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10. Zintzsch, A., et al., *Guidelines on severity assessment and classification of genetically altered mouse and rat lines.* Lab Anim, 2017. **51**(6): p. 573-582.
11. EuropeanCommissionExpertWorkingGrouponSeverityAssessment, *Expert working group on severity classification of scientific procedures performed on animals*. 2009.
12. ETPLAS. *Education*. [cited 2021 Feb 10];

While Education and Training (E & T) under Directive 2010/63/EU is the responsibility of the Member States, harmonisation and mutual recognition of training programs are important assets for pan-European scientific exchange and mobility of personnel.

For this reason, ETPLAS was established as an information portal to enable information sharing and communication between training providers, approval/accrediting bodies, employers and Member-State authorities. It is composed of a Stakeholders' Board, an Executive Committee, and a Stakeholder Group.

The EU Education and Training Framework guidance document proposed the creation of an EU Platform and Information Portal on Education & Training, which should be established for a modular training framework, leading to the setting up of ETPLAS, which was acknowledged in the document to be an evolving process.].

13. EvaluationandRetrospectiveAssessment, E., *Project Evalaution and retrospective assessment - Expert Working group report*. 2013: Brussels.

CAREIN
Course in Animal Research in Norway

5.5 Severity classification

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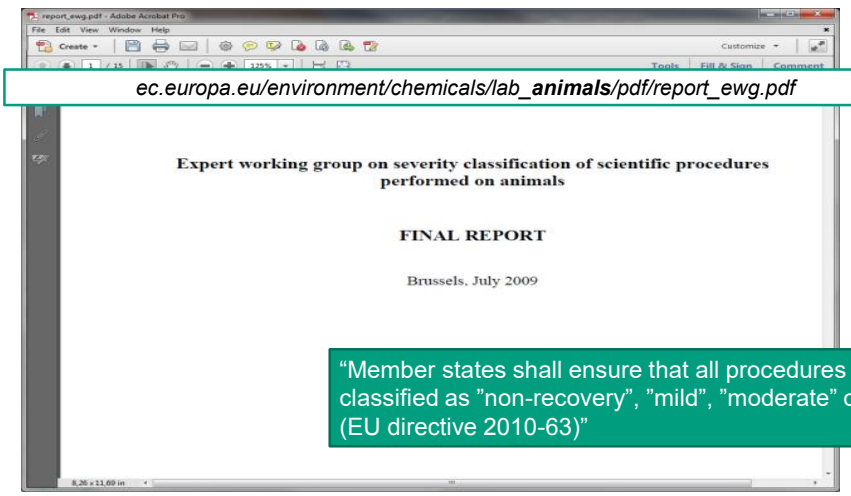
2017

Severity classification



"Member states shall ensure that all procedures are classified as "non-recovery", "mild", "moderate" or severe (EU directive 2010-63)"

2017

Severity classification

Severe: Procedures on animals as a result of which the animals are likely to experience severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress. Procedures, which are likely to cause severe impairment of the wellbeing or general condition of the animals.

Moderate: Procedures on animals as a result of which the animals are likely to experience short term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress. Procedures, which are likely to cause moderate impairment of the wellbeing or general condition of the animals.

Mild: Procedures on animals as a result of which the animals are likely to experience short term mild pain, suffering or distress. Procedures that cause no significant impairment on the wellbeing or general condition of the animals.

Non-recovery: Procedures, which are performed entirely under general anesthesia from which the animal shall not recover consciousness.

3

2017

Severity classification - Examples

Models with induction of tumors, or with spontaneous tumors, that are expected to cause progressive lethal disease
Immobilisation stress to induce gastric ulcers or cardiac failure in rats
Complete isolation for prolonged periods of social species

Models of induction of tumors, or spontaneous tumors, that are expected to cause moderate pain or distress or moderate interference with normal behavior,
Creation of genetically altered animals through surgical procedures;

Mild: Administration of substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than mild impact on the animal,

Non-recovery: Procedures, which are performed entirely under general anesthesia from which the animal shall not recover consciousness.

Sverity Classification 4

2017

More examples of mild procedures

- Pharmacokinetic study where a single dose is administered and a limited number of blood samples are taken (totalling < 10% of circulating volume) and the substance is not expected to cause any detectable adverse effect;
- Non-invasive imaging of animals (eg MRI) with appropriate sedation or anesthesia;
- Superficial procedures, e.g. ear and tail biopsies, non surgical subcutaneous implantation of mini-pumps and transponders;
- Application of external telemetry devices that cause only minor impairment to the animals or minor interference with normal activity and behavior;
- Administration of substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal;
- Induction of tumors, or spontaneous tumors, that cause no detectable clinical adverse effects (e.g. small, subcutaneous, non-invasive nodules);
- Breeding of genetically altered animals which is expected to result in a phenotype with mild effects;
- Feeding of modified diets, that do not meet all of the animals' nutritional needs and are expected to cause mild clinical abnormality within the time-scale of the study;
- Short term (<24h) restraint in metabolic cages;
- Studies involving short-term deprivation of social partners, short-term solitary caging of adult rats or mice of sociable strains;
- Models which expose animals to noxious stimuli which are briefly associated with mild pain, suffering or distress, and which the animals can successfully avoid.

European Commission Expert Working Group on Assessment of Severity 2012

2017

More examples of moderate procedures

- Frequent application of test substances which produce moderate clinical effects, and withdrawal of blood samples (>10% of circulating volume) in a conscious animal within a few days without volume replacement;
- Acute dose-range finding studies, chronic toxicity / carcinogenicity tests, with non-lethal endpoints;
- Surgery under general anesthesia and appropriate analgesia, associated with postsurgical pain, suffering or impairment of general condition. Examples include: thoracotomy, craniotomy, laparotomy, orchidectomy, lymphadenectomy, thyroidectomy, orthopedic surgery with effective stabilization and wound management, organ transplantation with effective management of rejection, surgical implantation of catheters, or biomedical devices (e.g. telemetry transmitters, minipumps, etc.);
- Models of induction of tumors, or spontaneous tumors, that are expected to cause moderate pain or distress or moderate interference with normal behavior;
- Irradiation or chemotherapy with a sublethal dose, or with an otherwise lethal dose but with reconstitution of the immune system. Adverse effects would be expected to be mild or moderate and would be short-lived (<5 days);
- Breeding of genetically altered animals which are expected to result in a phenotype with moderate effects;
- Creation of genetically altered animals through surgical procedures;
- Use of metabolic cages involving moderate restriction of movement over a prolonged period (up to 5 days);
- Studies with modified diets that do not meet all of the animals' nutritional needs and are expected to cause moderate clinical abnormality within the time-scale of the study;
- Withdrawal of food for 48 hours in adult rats;
- Evoking escape and avoidance reactions where the animal is unable to escape or avoid the stimulus, and are expected to result in moderate distress.

European Commission Expert Working Group on Assessment of Severity 2012

2017

More examples of severe procedures

- Toxicity testing where death is the end-point, or fatalities are to be expected and severe pathophysiological states are induced. For example, single dose acute toxicity testing (see OECD testing guidelines);
- Testing of a device where failure may cause severe pain, distress or death of the animal (e.g. cardiac assist devices);
- Vaccine potency testing characterized by persistent impairment of the animal's condition, progressive disease leading to death, associated with long-lasting moderate pain, distress or suffering;
- Irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or reconstitution with production of graft versus host disease;
- Models with induction of tumors, or with spontaneous tumors, that are expected to cause progressive lethal disease associated with long-lasting moderate pain, distress or suffering. For example tumors causing cachexia, invasive bone tumors, tumors resulting in metastatic spread, and tumors that are allowed to ulcerate;
- Surgical and other interventions in animals under general anesthesia which are expected to result in severe or persistent moderate postoperative pain, suffering or distress or severe and persistent impairment of the general condition of the animals. Production of unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multiple organ failure;
- Organ transplantation where organ rejection is likely to lead to severe distress or impairment of the general condition of the animals (e.g. xenotransplantation);
- Breeding animals with genetic disorders that are expected to experience severe and persistent impairment of general condition, for example Huntington's disease, Muscular dystrophy, chronic relapsing neuritis models;
- Use of metabolic cages involving severe restriction of movement over a prolonged period;
- Inescapable electric shock (e.g. to produce learned helplessness);
- Complete isolation for prolonged periods of social species e.g. dogs and non-human primates;
- Immobilization stress to induce gastric ulcers or cardiac failure in rats;
- Forced swim or exercise tests with exhaustion as the end point.

2017

Lower threshold

- The lower threshold is exceeded if the animals may experience a level of pain, suffering or distress equivalent to, or higher than that caused by the introduction of a needle.
- A number of examples are given of procedures that are considered below the threshold for regulation. It is important to note that applying several such ("below threshold") techniques together in one animal may require the procedure to be classified as mild or higher.

European Commission Expert Working Group on Assessment of Severity 2012

2017

"below threshold"

- Assessing body composition by non-invasive measures and minimal restraint;
- Monitoring ECG with non-invasive techniques with minimal or no restraint of habituated animals;
- Application of external telemetry devices that are expected to cause no impairment to socially adapted animals and do not interfere with normal activity and behavior;
- Breeding genetically altered animals which are expected to have no clinically detectable adverse phenotype;
- Feeding a diet that meets the full nutritional needs of the animals.
- Adding inert markers in the diet to follow passage of digesta
- Withdrawal of food for <24h in adult rats;
- Non-invasive observation of normal behavior without disturbing the animal;
- Open field testing.

European Commission Expert Working Group on Assessment of Severity 2012

2017

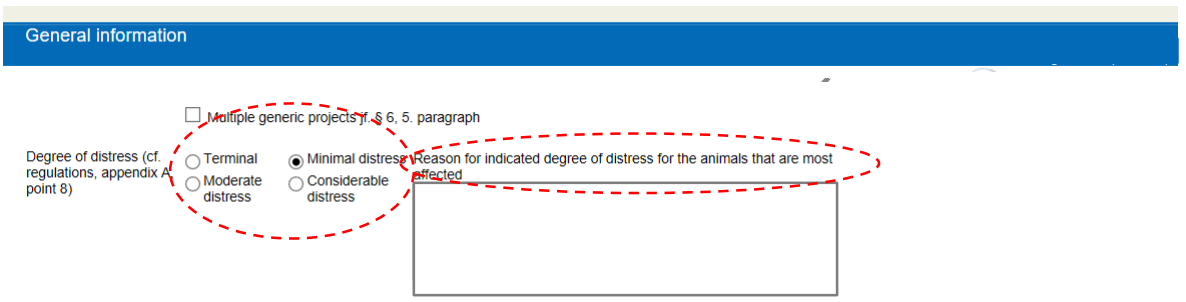
Upper threshold

- The upper threshold is exceeded if the animals may experience severe pain, suffering or distress which is likely to be long-lasting and cannot be ameliorated.
- Death as the end-point should be avoided by adopting appropriate monitoring strategies and early humane end-points wherever possible.
- Consideration should be given to the balance of the total number of animals used versus severity on the individual animal where by increasing the number of animals, the severity experienced by the individual animal may be reduced.

European Commission Expert Working Group on Assessment of Severity 2012

App B. part II – additional factors to be assessed

- animal species and phenotype
- maturity, age and gender of the animal
- adaptation of the animal with respect to the procedures
- if the animal is to be reused: the actual severity of the previous experiment
- methods employed to reduce or eliminate pain, suffering and distress, including refinement of housing, husbandry and care conditions
- the Humane End-Points.



Retrospective assessment

CAREIN

Public project summary (cf regulation § 8)

- Application summary (cf regulation § 8)
- Project summary should be easily understandable to the public.
  - 1.Purpose of the study
  - 2.Expected harm/severity for the animals
  - 3.Expected benefit for science or society
  - 4.How many and what kind of animals will be used (totally in this experiment)
  - 5.How are compliance with the requirements for replacement, reduction and refinement safeguarded

Classification of severity of procedures

Member states shall ensure that all procedures are classified as ”non-recovery”, ”mild”, ”moderate” or severe (EU directive 2010-63)

4 severity categories non-recovery, mild, moderate and severe are proposed

Non-recovery: Procedures, which are performed entirely under general anesthesia from which the animal shall not recover consciousness.

Mild: Procedures on animals as a result of which the animals are likely to experience short term mild pain, suffering or distress. Procedures that cause no significant impairment of the wellbeing or general condition of the animals.

Moderate: Procedures on animals as a result of which the animals are likely to experience short term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress. Procedures, which are likely to cause moderate impairment of the wellbeing or general condition of the animals.

Severe: Procedures on animals as a result of which the animals are likely to experience severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress. Procedures, which are likely to cause severe impairment of the wellbeing or general condition of the animals.

Examples

Mild

- Pharmacokinetic study (single dose is administered and a limited number of blood samples are taken (totally < 10% of circulating volume) The substance is not expected to cause detectable adverse effect
- Non-invasive imaging of animals (e.g. MRI) with appropriate sedation or anesthesia
- Superficial procedures, e.g. ear and tail biopsies, non surgical subcutaneous implantation of mini-pumps and transponders
- Application of external telemetry devices that cause only minor impairment to the animals or minor interference with normal activity and behavior
- Administration of substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal
- Induction of tumors, or spontaneous tumors, that cause no detectable clinical adverse effects (e.g. small, subcutaneous, noninvasive nodules)
- Breeding of genetically altered animals which is expected to result in a phenotype with mild effects
- Feeding of modified diets, that do not meet all of the animals' nutritional needs and are expected to cause mild clinical abnormality within the time-scale of the study
- Short term (<24h) restraint in metabolic cages
- Studies (<24h) involving short-term deprivation of social partners, short-term solitary caging of adult rats or mice of sociable strains
- Models, which expose animals to noxious stimuli which are briefly associated with mild pain, suffering or distress, and which the animals can successfully avoid.

Moderate

- Frequent application of test substances which produce moderate clinical effects, and withdrawal of blood samples (>10% of circulating volume) in a conscious animal within a few days without volume replacement
- Acute dose-range finding studies, chronic toxicity / carcinogenicity tests, with non-lethal endpoints
- Surgery under general anesthesia and appropriate analgesia, associated with post-surgical pain, suffering or impairment of general condition. Examples include: thoracotomy, craniotomy, laparotomy, orchidectomy, lymphadenectomy, thyroidectomy, orthopaedic surgery with effective stabilization and wound management, organ transplantation with effective management of rejection, surgical implantation of catheters, or biomedical devices (e.g. telemetry transmitters, minipumps, etc.)
- Models of induction of tumors, or Irradiation or chemotherapy with a sub lethal dose, or with an otherwise lethal dose but with reconstitution of the immune system. Adverse effects expected to be mild or moderate and short-lived (<5 days)
- Breeding of genetically altered animals expected to result in a phenotype with moderate effects
- Creation of genetically altered animals through surgical procedures spontaneous tumors, that are expected to cause moderate pain or distress or moderate interference with normal behavior
- Studies with modified diets that do not meet all of the animals' nutritional needs and are expected to cause moderate clinical abnormality within the time-scale of the study
- Withdrawal of food for 48 hours in adult rats
- Evoking escape and avoidance reactions where the animal is unable to escape or avoid the stimulus, and are expected to result in moderate distress.
- Use of metabolic cages involving moderate restriction of movement over up to 5 days

Severe

- Toxicity testing where death is the end-point, or fatalities are to be expected and severe pathophysiological states are induced. For example, single dose acute toxicity testing (see OECD testing guidelines)
- Testing of device where failure may cause severe pain, distress or death of the animal (e.g. cardiac assist devices)
- Vaccine potency testing characterized by persistent impairment of the animal's condition, progressive disease leading to death, associated with longlasting moderate pain, distress or suffering
- Irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or reconstitution with production of graft versus host disease
- Models with induction of tumors, or with spontaneous tumors, that are expected to cause progressive lethal disease associated with long-lasting moderate pain, distress or suffering. For example tumors causing cachexia, invasive bone tumors, tumors resulting in metastatic spread, and tumors that are allowed to ulcerate
- Surgical and other interventions in animals under general anesthesia which are expected to result in severe or persistent moderate postoperative pain, suffering or distress or severe and persistent impairment of the general condition of the animals.
- Production of unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multiple organ failure
- Organ transplantation where organ rejection is likely to lead to severe distress or impairment of the general condition of the animals (e.g. xenotransplantation)
- Breeding animals with genetic disorders that are expected to experience severe and persistent impairment of general condition, for example Huntington's disease, Muscular dystrophy, chronic relapsing neuritis models
- Use of metabolic cages involving severe restriction of movement over a prolonged period
- Inescapable electric shock (e.g. to produce learned helplessness)
- Complete isolation for prolonged periods of social species e.g. dogs and non-human primates
- Immobilization stress to induce gastric ulcers or cardiac failure in rats
- Forced swim or exercise tests with exhaustion as the end point.

Euthanasia

Euthanasia of animals in research

- *Animal welfare act § 12*
  - *Killing of animals ....must be performed in a properly way taking consideration to animal welfare*

- *Regulation on the Use of Animals in Research § 16*
  - *Euthanasia and handling in connection with the killing shall not cause animals unnecessary pain, fear or other stress, and shall taking consideration to animal welfare*
  - *Animals covered by Annex C, to be euthanized with methods described in the Annex.*
  - *Euthanasia must be in its breeder, Processor's or user's premises.*
  - *Euthanasia must be performed by a competent person.*
  - *Exsanguination must be conducted under total anesthesia.*

Approved methods according to EU Directive 2010/63

Recommending euthanasia methods for finfish, aquatic Invertebrates used in biomedical research is challenging due to the enormous number of species in use and variations in their biological and physiologic characteristics.

Fish	
Anesthetic overdose	Shall, where appropriate, be used with prior sedation
Electrical Stunning	Specialized equipment required.
Concussion	

EU Dir 2010/63

Reasons for killing research animal

- Humane or Scientific Endpoint of a study
  - Planned, described in FOTS, approved by Mattilsynet

Humane killing

- Get rid of surplus animals
  - From breeding (wrong sex, wrong genotype, untimely)

Humane killing

- Merciful euthanasia
  - Animal in untreatable pain, suffering or distress (§14b)

Euthanasia

Confirmation of death

a) Assure permanent circulatory arrest
b) Destruction of the brain
c) Dislocation of the neck
d) Out-bleeding
e) Confirm onset of *rigor mortis*

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*

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Chilling is not an approved method according to Annex C in the Norwegian regulation
a special justification must be provided in the FOTS application for approval

If animals are to be euthanised:
Which method of euthanasia will be used (cf. regulations § 16, part 2 and appendix C)?

Experimental animals will be euthanised by cervical dislocation, while for the non-experimental animals we'll use CO2 inhalation.

If a method of euthanasia will be used, that is not mentioned in appendix C:
Describe and give a reason for the chosen method of euthanasia (cf. regulations § 16).

S2.5 LABORATORY FISH, AMPHIBIANS, AND REPTILES

Recommending euthanasia methods for finfish, aquatic invertebrates, amphibians, and reptiles used in biomedical research is challenging due to the enormous number of species in use and variations in their biological and physiologic characteristics. In this section, only the most commonly used methods will be discussed for several frequently used species. Other methods less often used for euthanizing species used in research are discussed in detail in the relevant sections of the Guidelines. See these sections for additional information.

There are no US FDA-approved drugs for euthanasia of aquatic animals. Tricaine methanesulfate is an FDA-approved drug for temporary immobilization (sedation, anesthesia) of finfish, amphibians, and other aquatic, cold-blooded animals. Immersion of adult zebrafish in MS 222 for 10 minutes following loss of rhythmic opercular movement was previously recommended. However, because recovery of fish after exposure for this time has been shown to occur, 30 minutes is recommended as a precautionary measure until research is available to demonstrate immersion times needed to reliably cause irreversible death in zebrafish. Zebrafish show some signs of stress when immersed in MS 222, and a secondary (physical) method of euthanasia is recommended to ensure death.[108] MS 222 alone is not effective for euthanasia of zebrafish eggs, embryos, or larvae (< 14 days old), and other methods should be used for these life stages.[109]

As described in the aquatics section it is acceptable for zebrafish (*Danio rerio*) to be euthanized by rapid chilling (2° to 4°C) until loss of orientation and cessation of operculur movements. Subsequent additional exposure of the fish to chilled water for times specific to fish size and age[108,110,111] should be used to ensure death. Rapid chilling of adult zebrafish resulted in cessation of vital signs (10.6 ± 3.28 seconds) 20 times as fast as in the case of MS 222 overdose (216.3 ± 62 seconds).[112] Adult zebrafish should be exposed for a minimum of 10 additional minutes following the loss of opercular movements. Zebrafish fry 4 to 14 days after fertilization (dpf) should be exposed for at least 20 additional minutes following loss of opercular movements.[113] Rapid chilling (as well as MS 222) has been shown to be an unreliable euthanasia method for embryos < 3 dpf.[108,112,114] Immersion in diluted sodium or calcium hypochlorite solution is acceptable for embryos up to 7 days of age.[115] If necessary to ensure death of other life stages, rapid chilling may be followed by either an approved adjunctive euthanasia method or a humane killing method. Until further research is conducted, rapid chilling is acceptable with conditions for other small-bodied tropical and subtropical stenothermic species.

M2.2.2 Immersion

Euthanasia of fish and some aquatic amphibians and invertebrates must take into account the vast differences in metabolism, respiration, and tolerance to cerebral hypoxia among the various aquatic species. Because aquatic animals have diverse physiologic and anatomic characteristics, optimal methods for delivery of euthanasia agents will vary. In many situations, the immersion of aquatic animals in water containing euthanasia agents is the best way to minimize pain and distress. The response of aquatic animals to immersion agents can vary with species, concentration of agent, and quality of water; consideration of these factors should be made when selecting an appropriate euthanasia agent. Immersion agents added to water may be absorbed by multiple routes, including across the gills, via ingestion, and/or through the skin.

Ideally, immersion agents added to water will be nonirritating to skin, eyes, and oral and respiratory tissues and will result in rapid loss of consciousness (often, but not always, measured as a loss of righting response) with minimal signs of distress or avoidance behavior. Currently there are no US FDA-approved drugs for the euthanasia of aquatic animals. United States EPA–registered agents for poisoning fish (eg, rotenone, antimycin) are not recommended as euthanasia agents, because their mechanisms of action and times to death do not fit the criteria for euthanasia. Additionally, the use of these agents requires a restricted pesticide applicator's license and extralabel use of these agents is a violation of federal law. Agents approved by the FDA as tranquilizers and anesthetics for fish (eg, MS 222, metomidate) have been used extralabel as euthanasia agents for aquatic animals.

M3.8 ELECTROCUTION

Alternating current has been used to euthanize dogs, cattle, sheep, goats, swine, chickens, foxes, mink, and fish.[227,239,242,262–270] Fifty- or 60-cycle electrical current is more effective than higher frequencies.[271,272] Electrocution induces death by cardiac fibrillation, which causes cerebral hypoxia.[269,270,273] However, animals do not lose consciousness for 10 to 30 seconds or more after onset of cardiac fibrillation. It is imperative that animals be unconscious and insensible to pain before being electrocuted. Unconsciousness can be induced by any method that is acceptable or acceptable with conditions, including passing a current through the brain.[274]

Advantages—(1) Electrocution is humane if the animal is first rendered unconscious. (2) It does not chemically contaminate tissues. (3) It is economical.

Disadvantages—(1) Electrocution may be hazardous to personnel. (2) It is not useful for dangerous, intractable animals that are difficult to restrain. (3) It is aesthetically objectionable because of violent extension and stiffening of the limbs, head, and neck. (4) It may not result in death in small animals (< 5 kg [11 lb]) because ventricular fibrillation and circulatory collapse do not always persist after cessation of current flow. (5) Sometimes it is not effective in dehydrated animals.[275] (6) Personnel must be familiar with appropriate placement of electrodes and use of equipment. (7) Purpose-built equipment must be used.

S6.1.3 Preparation and Environment

As a general principle the preparations for euthanasia of fish should be very similar to the preparations for anesthesia of fish.[295-297] If possible, withholding food for 12 to 24 hours prior to euthanasia will reduce regurgitation, defecation, and nitrogenous waste production. The environment should be as quiet and nonstimulatory as possible given the circumstances. Light intensity should be reduced if possible, but with adequate lighting for personnel. This can also be achieved through use of a dark or opaque container and lid, or by use of less intense lighting (eg, red light illumination, as red light does not penetrate water well).

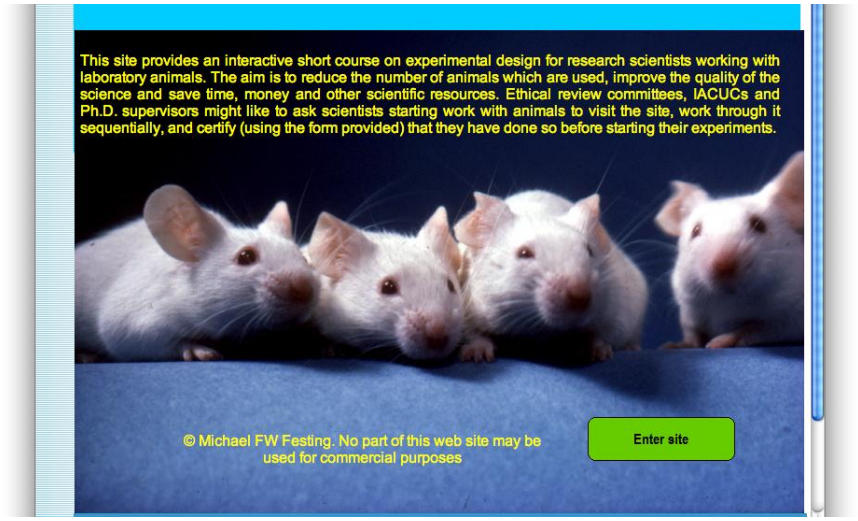
Water quality should be similar to that of the environment from which the fish originated, or optimized for that species and situation, for the duration of euthanasia. If the water is of acceptable quality for fish health, the water in which they have been housed or captured should be used, and supplemental aeration and temperature control may be necessary. Either the immersion euthanasia solution is prepared with water from the fish housing system and the fish are transferred into it or a concentrated form of the anesthetic agent as a solution (containing buffering agent if appropriate) is introduced directly into the container of fish to minimize stressors. If euthanizing a large population of fish, it is important to monitor the anesthetic bath water quality (temperature, dissolved O_2, ammonia, and organic loading, in particular). The euthanasia agent may need to be supplemented or replaced periodically because it will be removed when absorbed into the fish's bloodstream though the gills. Euthanasia methods should be tested in 1 animal or a small group of animals prior to use in a large population for an unfamiliar species, to ensure effectiveness.[298] If handling the fish is required, appropriate equipment (nets, gloves) should be used to minimize stressors.

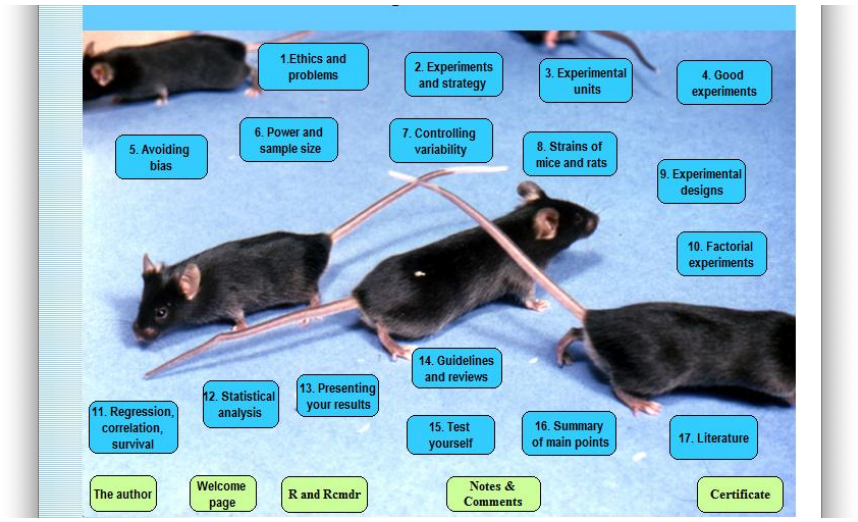
S6.1.4 Indicators of Death in Fish and Aquatic Invertebrates

Because the thousands of species of fish and aquatic invertebrates vary greatly in anatomic and physiologic characteristics, reliable indicators of death may not be available for some. However, there are some standard approaches that can be useful for many of the more commonly encountered species. Loss of movement, loss of reactivity to any stimulus, and initial flaccidity (prior to rigor mortis) may serve as indicators of death for fish and some aquatic invertebrates. More useful indicators for many fish include respiratory arrest (cessation of rhythmic opercular activity) for a minimum of 30 minutes and loss of eye-roll (vestibulo-ocular reflex, the movement of the eye when the fish is rocked from side to side). The latter is no longer present in fish that have been deeply anesthetized or euthanized.[299] The heart can continue to contract even after brain death or removal from the bodies of fish,[300] so the presence of a heartbeat is not a reliable indicator of life, but sustained absence of heartbeat is a strong indicator of death. For more sessile, less active organisms, or those with specific anatomic or physiologic adaptations that prevent use of these indicators, it may be more difficult to assess loss of consciousness and death, and consultation with species experts is recommended. Secondary methods of euthanasia are recommended, when appropriate, after the fish or aquatic invertebrate is anesthetized, to ensure euthanasia.

DESIGN OF ANIMAL STUDIES

http://www.3rs-reduction.co.uk/





DESIGN OF EXPERIMENTS

2. Types of experiment

Good experimental design will help you to:
• Improve the quality of your science
• Get published in better journals
• Save time and money
• Use fewer animals

Types of experiment

Pilot experiments are small studies (1-20 experimental subjects) used to:

- Test the logistics of a proposed larger study
- Gain familiarity with the experimental material,
- Ensure that treatments are not obviously excessively mild or severe
- Check that staff are sufficiently well trained in the necessary procedures
- Ensure that all steps in a proposed future experiment are feasible.
- Gain some information on variability, although this will not usually be sufficiently reliable to form the basis of power analysis calculations of sample size.

Exploratory experiments can be used to generate data with which to develop hypotheses for future testing. They may "work" or "not work". They may have no clearly stated hypothesis ("let's see what happens if.." is not a valid hypothesis on which to base an experiment).

Often they will measure many outcomes (characters). Picking out "interesting looking differences" (known as data snooping) and then doing a hypothesis test to see if the differences are statistically significant will lead to serious overestimation of the magnitude of a response and excessive numbers of false positive results. Such differences should always be tested in a controlled experiment where the hypothesis is stated *a priori* before the results are published.

Depending on the nature of the data, statistical analysis will often be done using an analysis of variance (ANOVA)

Confirmatory experiments are used to test some relatively simple hypothesis stated *a priori*. This is the type of experiment mainly considered in this web site.

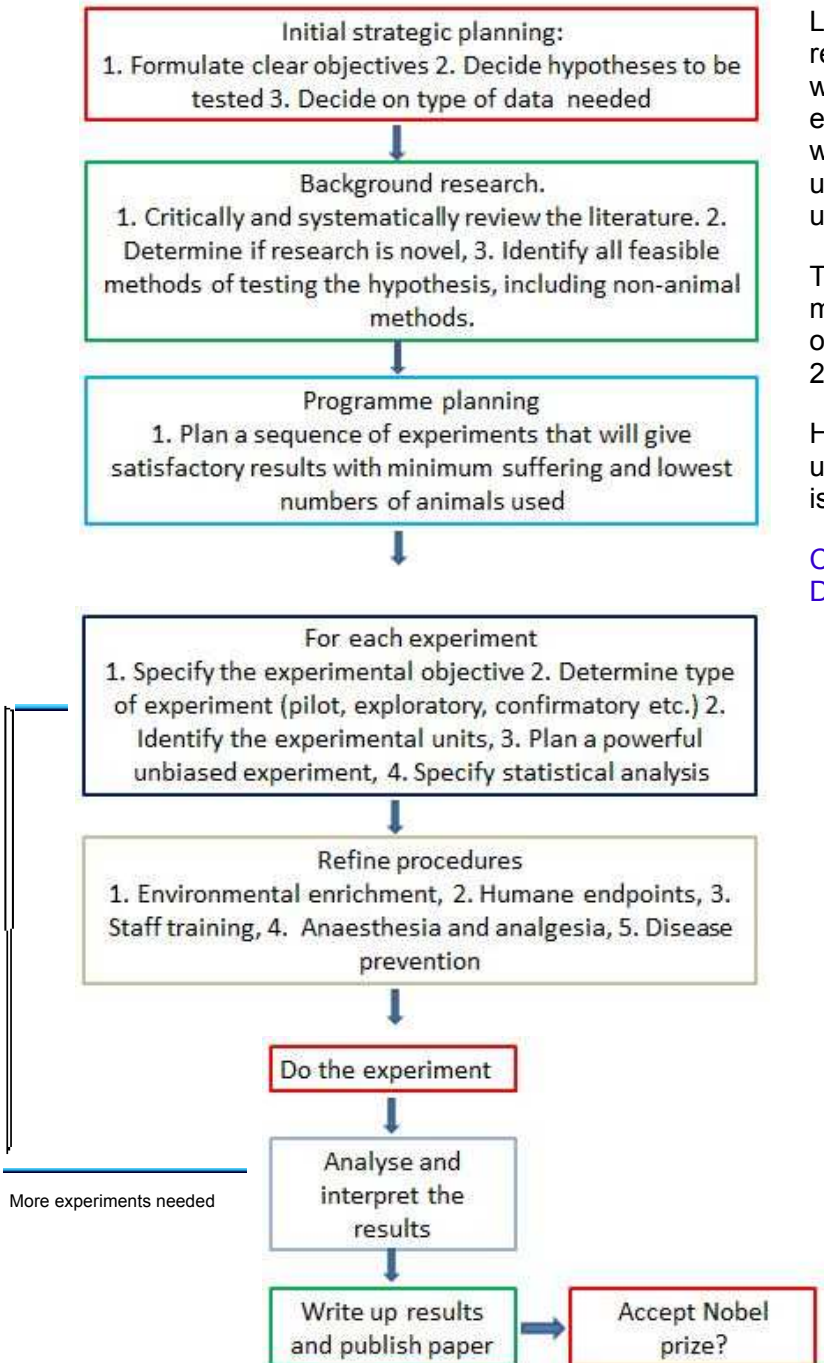
The basic principles are:

- Experiments involve *comparisons* between two or more groups
- Their aim is to *test a "null hypothesis"* that there is no difference among the groups for the specified outcome.
- If the null hypothesis is rejected at a certain level of probability (often 5%) this means that the probability of getting a result as extreme as this or more extreme in the absence of a true effect is 5% (assuming also that the experiment has been properly conducted). So it is assumed that such a difference is likely to be the result of the treatment. But, it could be a false positive resulting from sampling variation.
- Failure to reject the null hypothesis does not mean that the treatment has no effect, only that if there is a real effect this experiment failed to detect it. "Absence of evidence is not evidence of absence".
- Experimental subjects need to be *independently replicated* because individuals (of whatever type) vary. Two subjects can normally be regarded as being independent if they can theoretically receive different treatments.
- Subjects need to be assigned to groups, held in the animal house and measured *at random* in order to minimise the chance of bias (a systematic difference between groups)
- As far as possible the experimenter should be *"blind"* with respect to the treatment group in order to minimise bias.

- The experiments need to be *powerful,* i.e. they should have a high probability of detecting an effect of clinical or scientific importance if it is present.
- In many cases a *formal experimental design* such as a "completely randomised", "randomised block", "Latin square" etc. design will be used.
- In most cases it is useful if the experiment has a *wide range of applicability*. In other words the results should hold true under a range of different conditions (different strains, both sexes, different diets, different environments etc.). At least some of these factors should be explored using factorial and randomised block designs.

Experiments to explore relationships between variables. A typical example would be a growth curve or a dose-response relationship. In these experiments the aim is often to test whether the two variables are associated, and if so, what is the nature of that relationship. The typical statistical analysis involves correlation and/or regression.

Research Strategy



Little has been written on research strategy. Most projects will involve a number of experiments, possibly starting with *in-vitro* studies or those using lower organisms before using vertebrates.

The strategy presented here is a much modified version of the one proposed by Das et al 2009,ATLA 37, 27–32.

How you plan powerful and unbiased individual experiments is discussed later.

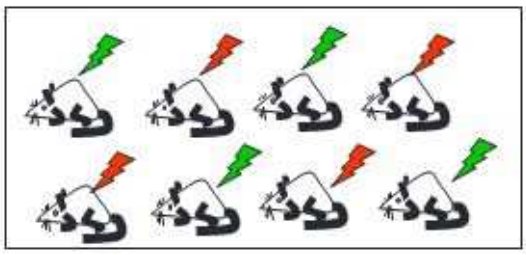
Click arrow for a pdf of paper by Das et al.

3. The experimental unit

Definition:

"The smallest division of the experimental material such that any two experimental units can receive different treatments".

It is the unit of randomisation and of statistical analysis when comparing groups

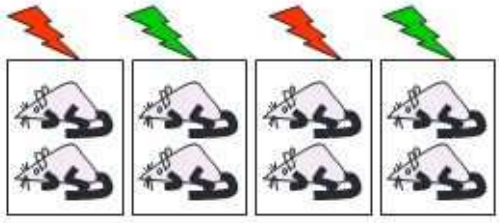


In this study the animals are all housed in one cage and the treatment is given by injection.

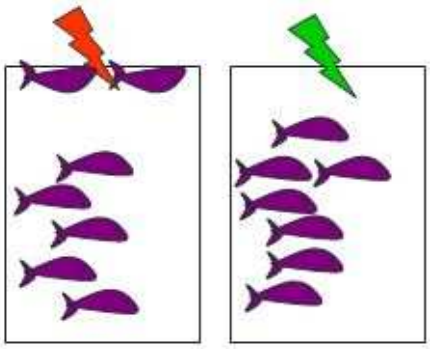
Any two animals can receive different treatments, so the animal is the experimental unit and "N" (the total number of subjects) is 8

In this study the animals are housed two per cage and the treatment is given in the food or water.

What do you think is "N", the total number of experimental units in this case?



Two Four Eight

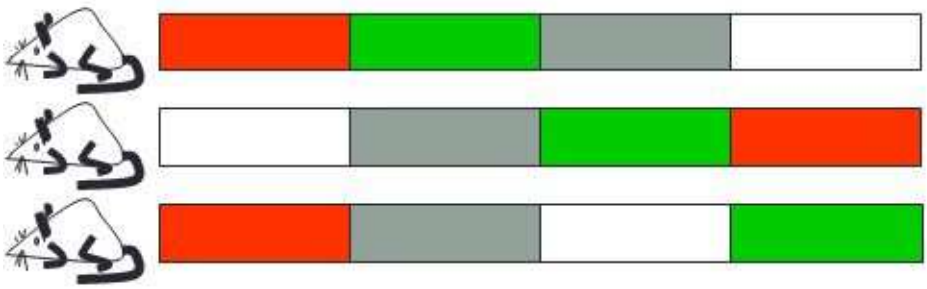


The experiment on the left has seven fish in each of two tanks. The left-hand tank has been treated with a test substance poured into the water and the right-hand has only the vehicle as a control. The aim is to measure the level of an enzyme in the fish.

What is the total number of experimental units?

Two Seven Fourteen

In a crossover experiment an animal could be given a treatment for a period, then rested and given a different treatment for a period. It is assumed that the treatment doesn't alter the animal, so it has to be very mild.



In this experiment animals are given four treatments, sequentially in random order. What do you think is N, the total number of experimental units?

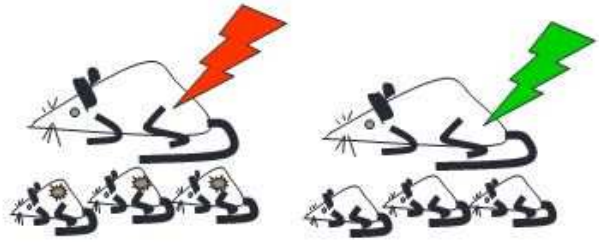
Three

Four

Twelve

A within-animal experiment could be replicated in space. One eye could be a control for the other one. Patches on the shaved back could be used to test topical applications of a test substance. Statistical analysis of the results of such a within-animal should take account of differences between animals. This is one form of a "randomised block" design described later.

In a teratology experiment (right) the pregnant female is treated with the test compound or a placebo. The pregnant females are killed at about mid-gestation and the pups are weighed, measured and studied for abnormalities.



How many experimental units are there in the experiment on the right (two females, three pups from each)

Two

Three

Six

Humans who suffer from depression seem to be more sensitive to pain. An investigator wants to know if this is also the case in rats.

Strain WKY rats are sometimes used as a model of depression, whereas Wistar rats are not depressive. So he obtains 10 rats of each strain, houses them two per cage for three weeks and tests them in random order using a standard test of pain threshold.

What is the experimental unit in this experiment?

4. A good (well designed) Experiment

Should:

1. Have a clear specification of the aims of the experiment.
The hypothesis to be tested needs to be clearly formulated *before* starting any detailed planning. It should be one which the experiment is capable of answering.
It would be a serious error to look at the results of the experiment and then adjust the hypothesis to fit them!

---

2. Be unbiased
There should be no systematic differences between the treated and control groups apart from the effects of the treatment.

Bias may result in false positive results when the effects of some other factor are confounded (mixed with) the treatment effect. It is avoided by correct identification of the experimental unit, blinding, and by randomisation

Bias is minimised by 1. correct choice of the experimental unit, 2. randomisation of the units to treatments and in the order in which subjects are housed and outcomes are measured, and 3. blinding where possible, using coded samples.

---

3. Be powerful
If the treatment really has an effect, there should be a high chance that it can be detected. Experiments which lack power will have too many false negative results.

Power is increased by 1. Larger sample sizes, 2. Good control of variability and 3. Use of sensitive subjects. However, large sample sizes cost animals and money so emphasis should be placed on the last two of these.

---

4. Have a wide range of applicability
An experiment where the results can only be replicated in some animal houses but not in others lacks generality. The range of applicability is explored using factorial and randomised block designs which can sample different situations. *See the concepts of internal and external validity, below.*

---

5. Experiments should be simple
They should not be so complex that mistakes are made, the statistical analysis is excessively complex or they are impossible to interpret.

Clearly written protocols and stand operating procedures should be used. In some cases it may be necessary to work to "Good Laboratory Practice" standards. http://en.wikipedia.org/wiki/Good_Laboratory_Practice

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6. It should be possible to statistically analyse the result of an experiment.
The statistical analysis and the experiment should be planned at the same time.

An investigator should never start an experiment without knowing how it is going to be analysed. The results of each experiment should be analysed before starting the next one so that the findings from the first experiment can be taken into account. The most powerful available statistical methods should be used, such as parametric rather than non parametric tests, where applicable..

Internal and external validity

An experiment can be said to have high *internal validity* if it has a high probability of getting the correct answer. Basically, this means that it should be unbiassed and powerful so that it is unlikely to produce either a false positive or a false negative result.

An experiment will have high *external validity* if the results can be generalised to other conditions or situations. Note that it can not have high external validity unless it first has high internal validity. The use of randomised block designs (which can sample a range of environments) and factorial experimental designs can be used to increase external validity. Repeating an experiment in another laboratory by other investigators will also increase external validity, assuming the results are repeatable.

As an example, an experiment which uses only a single strain of mice may have high internal validity, but if the same results are not seen with other strains of mice, then it will have low external validity.
It is acceptable to do an experiment with high internal validity but no exploration of its external validity, provided it is made clear that the external validity is unknown. But note that in many cases randomised block and factorial designs can be done at little or no extra cost.

6. Power and Sample Size

The power of an experiment is the probability that it can detect a treatment effect, if it is present.

The six factors listed here are intimately linked so that if we know five of them we can estimate the sixth one.

- Power
- Sample size,
- Inter-individual variability,
- The magnitude of the response to a treatment,
- The significance level and
- The alternative hypothesis

A “power analysis” is often used to determine sample size. The use of too many animals (or other experimental units) wastes animals, money, time and effort, and it is unethical. But if too few animals are used the experiment may lack power and miss a scientifically important response to the treatment. This also wastes resources and could have serious consequences, particularly in safety assessment.

The null hypothesis

In a controlled experiment the aim is usually to compare two or more means (or sometimes medians or proportions). We normally set up a “null hypothesis” that there is no difference between the means, and the aim of our experiment is to disprove that null hypothesis.

However, as a result of inter-individual variability we may make a mistake. If we fail to find a true difference, then we have a false negative result, also known as a *Type II* or beta error. Conversely, if we think that there is a difference when in fact it is just due to chance sampling variation, then we have a false positive, *Type I,* or alpha error. These are show in the table below

	Experimental conclusion	
State of nature	Accept null hypothesis	Reject null hypothesis
Null hypothesis true	Correct conclusion	Type I or α error
Null hypothesis false	Type II or β error	Correct conclusion

Type I errors are controlled by choosing the significance level. A 5% level means that on average 1/20 comparisons will be “significant” when they are just due to sampling variation

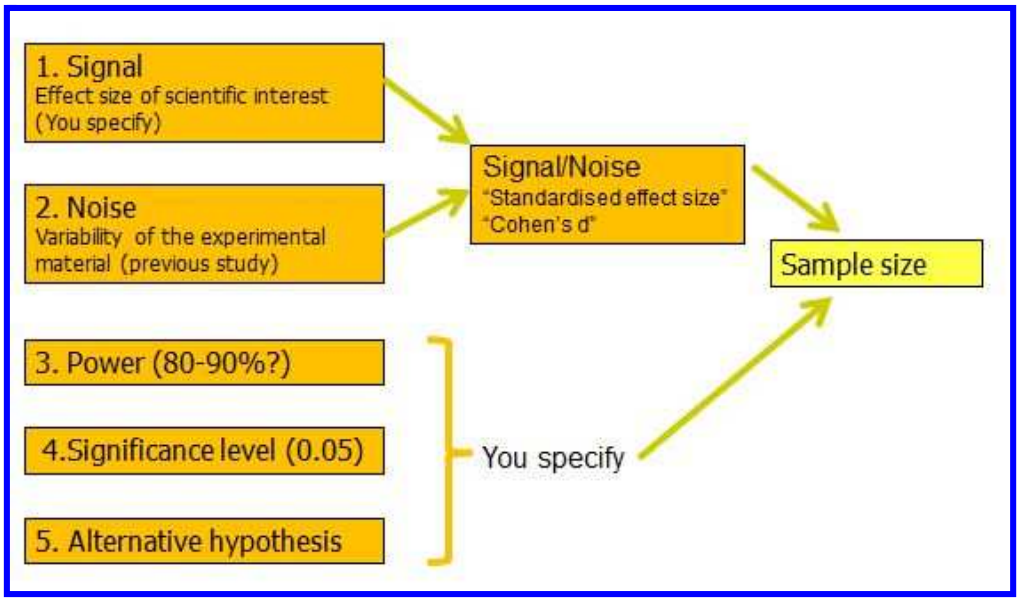
Control of Type II errors is more difficult as it depends on the relationship among several variables, the most important of which are the “signal” (difference between means of the groups), the “noise” (inter-individual variability) and the

sample size. We can often use a power analysis to estimate the required sample size as discussed below.

Power analysis

The figure shows the six variables involved in a power analysis. They are interrelated such that if any five of them are specified, the sixth one can be estimated.

Normally, the power analysis is used to estimate sample size. But if that is fixed (e.g. only 20 subjects are available) then it can be used to estimate the signal or the power of a proposed experiment.



The signal

This is the magnitude of the difference between the means of the two groups (M1-M2) which is likely to be of clinical or scientific importance. It has to be specified by the investigator.

A small difference may not be of much interest. A large one will be. What is the cutoff below which the difference is of little interest?

In applied research it should be possible to specify an effect size. but in fundamental research you may just want to know if there are *any* differences between the two groups.

In this case you will have to use another method of determining sample size such as the Resource Equation (see later). But if you have an estimate of the standard deviation it is still worth doing a power analysis to estimate *the effect size* you are likely to be able to detect for the sample size you decide to use. If you then fail to detect a statistically significant effect you will be able to say something like *"if the effect had been as large as XX standard deviations I would have had (say) a 90% chance of detecting it"*. Remember, if you specify five of the above variables you can estimate the sixth one. So in practice you can estimate sample size or effect size or power (you are less likely to want to estimate the other two variables).

The noise

This is the variation among the experimental subjects, expressed as the standard deviation (in the case of measurement characters). It needs to come from previous studies or a pilot study. If no good estimate is available it may still be worth doing a power analysis with a low and a high estimate to see what difference it makes to the estimated sample size

Noise does not need to be estimated when comparing two proportions. It is sufficient just to specify the other variables.

The signal/noise ratio

This is also known as the "standardised effect size" or "Cohen's *d*". It is sometimes used as a general indication of the magnitude of an effect. For example, Cohen in his book "Statistical power analysis for the behavioral sciences". Hillsdale N.J.: Lawrence Erlbaum Associates, 1988 suggested that values of *d* of 0.2, 0.4 or 0.8 should be considered as "small", "medium" and "large" effect sizes respectively in psychological research. However, in work with laboratory animals much larger effects are usually seen, because the noise is usually so well controlled. In this case small, medium and large effects might more realistically be set at *d*= 0.5, 1.0 and 1.5, respectively.

The other variables

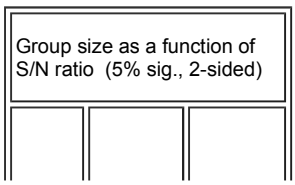
- The alternative hypothesis
  The null hypothesis is that the means of the two groups do not differ.
  The alternative hypothesis may be that they do differ (two sided), or that they differ in a particular direction, e.g. that the mean of the treated group is greater than the mean of the controls (one sided)
- The significance level
  As previously explained, this is usually set at 0.05, but this is quite arbitrary. It is the probability of a false positive result
- The power
  This is the probability that you will be able to detect the effect you specify (the signal). You will probably want a high power, so it is often set at 0.8 or 0.9 (80% or 90%). But the higher power will require a larger sample size
- The sample size
  This is the number in each group. It is usually what we want to estimate. However, we sometimes have only a fixed number of subjects in which case the power analysis can be used to estimate power or effect size.

Determining sample size by power analysis

Assume that you plan an experiment with just two groups (Treated and Control) and that you will measure a metric character.

Your null hypothesis is that there is no difference between the means of the two groups. The steps that you need to take are as follows:

- Decide on your alternative hypothesis. This will be either that the means differ (two sided) or they differ



in a particular direction (one sided). The default is two sided.
- Decide the significance level you plan to use. We will assume 5%.
- Decide what power you want (i.e. the chance of detecting a real effect if it is present).
  - If the consequences of failing to detect the effect (a Type II error) could be serious, such as in toxicity testing, you might want a relatively high power such as 90%.
  - In fundamental studies where we may only be interested in large effects a Type II error may not have such serious consequences. An 80% power may be sufficient to catch large effects and fewer subjects will be needed.
- Obtain an estimate of the noise, i.e. the standard deviation of the character of interest. This has to come from a previous study, the literature or a pilot study. If using the literature it may be best to look at several papers and take some sort of (possibly informal) average or a "guestimate". It is often helpful to do a "best" and "worst" case power analysis.
- Estimate the signal (effect size) that might interest you. How large a difference between the two means would be of scientific or clinical interest? If the difference is only small, you are probably not particularly interested in it. If it is large, then you certainly want to be able to detect it. The signal is the cutoff between these two alternatives. If the response is larger, then there will be an even greater chance of detecting it.
- Calculate the Standardised effect size (signal/noise ratio) = (Mean1-Mean2)/SD.
- The table (right) shows the S/N ratio over the range 0.2 to 3.0 and the required sample size for 80% and 90% power assuming a 5% significance level and a two-sided test.

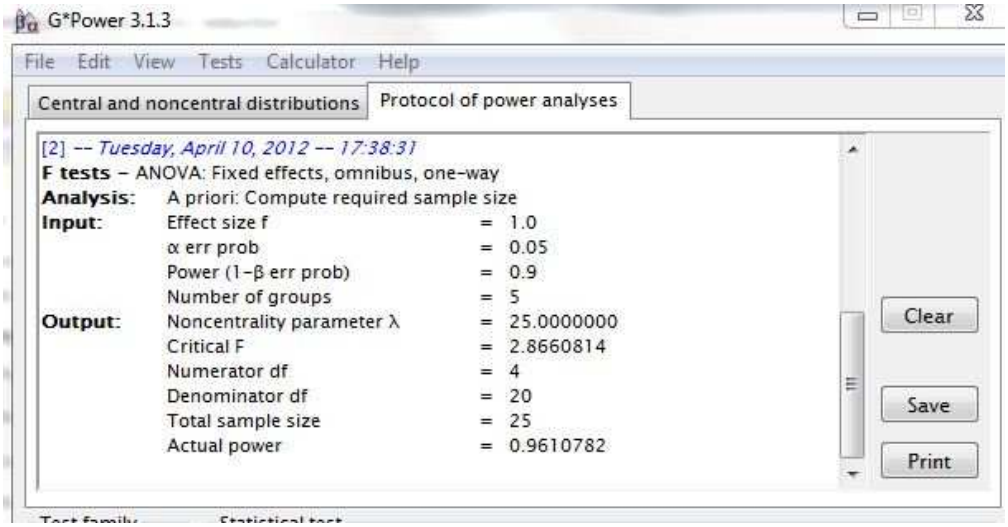
SN ratio	90% power	80% power
0.2	526	393
0.4	132	99
0.6	59	45
0.8	34	26
1.0	22	17
1.2	16	12
1.4	12	9
1.6	9	7
1.8	8	6
2.0	6	5
2.2	6	4
2.4	5	4
2.6	4	4
2.8	4	3
3.0	4	3

What if there are more than two groups?

It is technically possible to do a power analysis for an analysis of variance with several treatment groups. The problem is to specify an effect size of clinical or scientific importance when there are three or more groups. One alternative is to power the experiment assuming a t-test on the two groups likely to be most extreme such as the control and top dose (assuming there are such groups). This would mean that if the response is stronger than expected, then differences between the control and an intermediate group would become statistically significant.

Another alternative would be to specify a "small", "medium" or "large" effect size (possibly d=0.5, 1.0 or 1.5 in the case of laboratory animals) and the number of treatment groups and use the G*Power program (below) to estimate sample sizes. A screen shot of such a calculation for an experiment with five treatment groups with an effect size of 1.0, a power of 0.9 and a significance level of 0.05 is shown below. This would require 25 animals.

G*Power will also accept the estimated means of the four groups that would be of scientific interest were they to be found together with a pooled estimate of the standard deviation, and do the power analysis on that.



Power analysis for comparing two percentages (or proportions

A power analysis for comparing two proportions requires the expected control proportions, (p1) the proportion or responders in the treated group that would give a difference of clinical or scientific importance (p2), the specified power and the significance levels. The table below shows numbers needed in each group for an 80% power and 5% significance level. Note that large numbers are needed in some cases.

Sample size in each group for comparing two proportions (power=0.8, significance level=0.05)

	Percent for group 1									
% Group 2	0	10	20	30	40	50	60	70	80	90
10	74									
20	34	199								
30	21	62	293							
40	15	32	81	356						
50	11	20	39	93	387					
60	8	13	23	42	97	387				
70	6	10	14	23	42	93	356			
80	5	7	10	15	23	39	81	293		
90	4	5	7	10	14	20	32	62	199	
100	2	4	5	6	8	11	15	21	34	74

A web site that will do the calculations

Click the arrow below for a pdf paper giving more details on power analysis.

Although there is probably sufficient information given in the table above and the example below for you to estimate your required sample size, you can click below for a web site which will do the calculations for you.

Click here http://www.biomath.info

A free program for power calculations

A free program G*Power includes calculations for the t-test, F-test (one-way analysis of variance) and others. It can be downloaded from this web site

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An example comparing two means

A vet wants to compare the effect on blood pressure of two anesthetics for dogs under clinical conditions. He has published some preliminary data. The dogs were unsexed healthy animals weighing 3.8 to 42.6 kg. Mean systolic blood pressure was 141 mm Hg with a standard deviation of **36**mm, (the noise)

Assume:

1. A difference in blood pressure of **20** mmHg (the signal) or more would be of clinical importance (a clinical not a statistical decision).
2. A significance level of 0.05,
3. A power of 90%
4. And a 2-sided t-test,

Then the signal/noise ratio would be **20/36** = 0.56

From the table above the required sample size for a S/N ratio of 0.6 is about 59 dogs/group.

(Note that great accuracy is not needed as there are uncertainties in the estimates of the standard deviation and the effect size of clinical importance). However there are many statistical software packages will do the calculations. The output below is done using the R statistical package for this set of data. In this case “delta” is the signal/noise ratio and the SD is set as one, but the signal and noise could have been put in separately. Note that the sample size needs to be rounded up to a whole number. (Note that a small change in the S/N ratio from 0.6 to 0.56 makes quite a difference to the estimates: from 59 to 68 dogs per group).

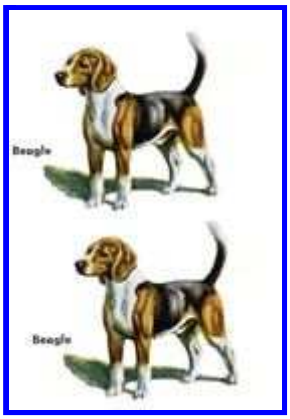
```
power.t.test(delta=0.56, sd=1, power=0.9, sig.level=0.05)

Two-sample t test power calculation
              n = 67.98649
          delta = 0.56
             sd = 1
      sig.level = 0.05
          power = 0.9
    alternative = two.sided
NOTE: n is number in *each* group
```

Sixty-eight dogs per group (132 in total) is a lot of dogs and using such animals would be time-consuming.

An alternative

In the same journal an investigator was working with male Beagles weighing 17-23 kg. These had a mean BP of 108 mm Hg. with an SD 9 mm.



Assume a 20mm difference between groups would be of clinical importance (as before). With the same assumptions as above the signal/noise ratio is 20/9 = 2.22 This is only 6/group with a 90% power (see table above).

So, by using uniform animals the number needed is reduced to 1/11th. compared with the random dogs. The table below summarises the situation. It also shows that if the vet went ahead and used the random dogs with eight dogs per group then there would only have been an 18% chance of detecting a 20mm difference in means between the two groups.

Type of dog	SDev	Signal/noise	Sample size/gp(1)	%Power (n=8) (2)
Random dogs	36	0.56	68	18
Male beagles	9	2.22	6	98

(1) Sample size: 90% power
(2) Power, Sample size 8/group (this can not be read off the graph)

Assumes α=5%, 2-sided t-test and effect size 20mmHg

This poses a problem. Can Beagles be regarded as representing “dogs”?

And is there ever any case for using genetically heterogeneous animals if all it does is increase noise and reduce the power of the experiment, leading to false negative results?

Alternative approaches

It would make no sense to go ahead and do the experiment simply using the heterogeneous dogs. But there are some obvious alternatives.

1. If each dog could be given both anaesthetics (say in random order on different days), then it would be possible to use small numbers of even quite heterogeneous dogs, assuming that there are no important breed differences in response. Technically, this would be a randomised block design (discussed later)

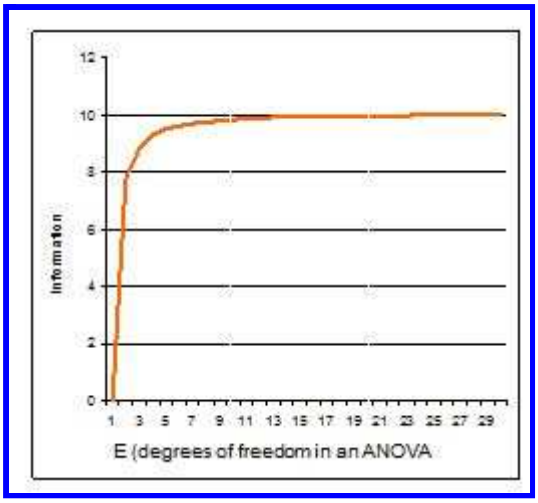
2. If it is thought that there may be breed differences in response, then the vet could restrict the study using small numbers of animals of several (say 3-4)

breeds in a "factorial" experimental design, discussed later. As far as possible there should be equal numbers in each group. This would indicate whether the two anesthetics differ over-all and whether breed differences need to be taken into account when choosing one of these anesthetics.

The Resource Equation: another method of determining sample size

A power analysis is not always possible.

- If lots of characters are being measured it may not be clear which one is the most important
- There may be no estimate of the standard deviation if the character has not previously been measured
- In fundamental research it may be impossible to specify an effect size likely to be of scientific importance
- A power analysis is difficult with complex experiments involving many treatment groups and possible interactions.



An alternative is the "Resource Equation"` method. This depends on the law of diminishing returns. It needs an estimate of E:

E= (Total number of experimental units) - (number of treatment groups)

And E should be between 10 and 20

This is not an absolute cutoff. There may be a case for E being higher if it leads to a more balanced design, the likely cost of a Type II error is high, the procedures are very mild or it is an *in-vitro* experiment with no ethical implications

E is the number of degrees of freedom in an analysis of variance (ANOVA). It is based on the need to obtain an adequate estimate of the standard deviation.

The plot above right shows the amount of information in a sample of data as a function of E. The curve rises steeply, then tails off and has almost flattened off by the time E=10, and there is little extra benefit from going on much beyond 20. However, if experimental units are inexpensive (such as tissue culture dishes) then

Suppose you decide to do an experiment with four treatment groups (a control and three dose levels) and eight animals per group. Then:

E= 32 – 4 = 28. So this is unnecessarily large.

With six animals per group E=20, which is acceptable

This method is easy to use, can be used when there are many outcomes, it does not require estimates of the effect size of clinical or scientific importance, and does not require an estimate of the standard deviation. But it is crude compared with the power analysis.

Conclusion: Use a power analysis where possible. Use the resource equation when a power analysis is not possible.

5. Avoiding Bias

Statistical bias is avoided by:

1. Correct selection of the experimental unit (as discussed previously)

2. Randomisation of the experimental units to the treatment groups in a method which depends on the experimental design. (a randomised block is different from a completely randomised design)

3. Randomisation of the order in which measurements are made and the animals are housed because there will be time and space variables which influence the results.

4. "Blinding" and the use of coded samples to ensure that the investigator or other staff can not easily influence the outcome of the experiment.

There are other types of bias which should be avoided where possible:

Selection bias occurs when an investigator manipulates the results so as to give a result which supports their hypothesis

Publication bias occurs when positive (usually) results are published but not negative ones. This might be due to journals not accepting papers with negative results (all well designed papers should be publishable), or because the authors do not bother to write up their negative results.

Randomisation

Randomisation ensures that each experimental unit has an equal probability of receiving a particular treatment. It reduces the chance of systematic differences between the treatment groups.

There will still be differences due to chance sampling errors and, by definition, in 5% of cases these differences will be "statistically significant" at the 5% level!

All good statistical packages provide ways of putting numbers or letters in random order. It can also be done using a spread sheet such as EXCEL, as shown here

Assume we want to randomise 12 subjects to three treatments A,B, & C. in a completely randomised design.

Original	=rand()	Sorted on =rand()		Animal number
A	0.527	A	0.067	1
A	0.100	A	0.100	2
A	0.067	A	0.122	3
A	0.122	C	0.210	4
B	0.665	B	0.248	5
B	0.875	C	0.265	6
B	0.478	B	0.478	7
B	0.248	A	0.527	8
C	0.210	C	0.628	9
C	0.628	B	0.665	10
C	0.265	B	0.875	11
C	0.895	C	0.895	12

The treatment designations A-C were put in the first column, 4 subjects per treatment

A random number was put in the second one (as “values” in this case, though this is not essential)

The two columns were then sorted on the random number column to give column 3, the treatments in random order. The animal numbers are then added. In this case the first three animals will be assigned to A, the 4th. To C etc. Randomisation often does not look very random. In extreme cases the subjects can be re-randomised.

Randomising a randomised block design

In a randomised block design the experiment is split up into a number of small parts or “blocks”. Typically each block has one experimental unit of each treatment. So if there are four treatments, block size is four experimental units.

Randomisation is done within each block. One way of doing this with EXCEL is as shown here. Assume the aim is to randomise four treatments: A, B, C, D, in four blocks.

Column one shows the animal number, column 2 is a random number (shown to two decimal places), and column three is the treatment assignment. The lowest number in the block is assigned to treatment A, the next to B and so on. The last column is the block number.

This randomisation can be done in the office, printed out and taken to the animal house.

Animal	"=rand()"	Treatmen	Block
1	0.75	D	1
2	0.40	A	1
3	0.73	C	1
4	0.70	B	1
5	0.02	A	2
6	0.60	D	2
7	0.08	B	2
8	0.12	C	2
9	0.07	B	3
10	0.04	A	3
11	0.54	C	3
12	0.84	D	3
13	0.94	D	4
14	0.39	A	4
15	0.80	C	4
16	0.70	B	4

Randomisation in a Latin square

In a Latin square experiment the number of rows= number of columns = number of treatments and every treatment should appear once in every row and every column.. Randomisation needs to maintain this structure.

A	B	C	D
D	A	B	C
C	D	A	B
B	C	D	A

The square on the right was written A,B,C,D on the first line, then A, B, C, D on the second line but shifted one column to the right, with the D recycled back to the first column and so on with the 3rd. and 4th. rows. Randomisation is subsequently done first by whole columns and then by whole rows (not shown). This will maintain the structure, while still allowing randomisation.

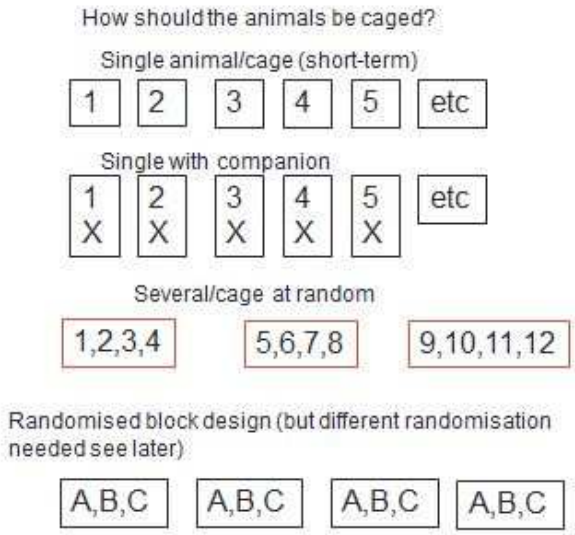
Classification variables

Some variables such as genotype, age, sex can not be randomly assigned to subjects. However, the order in which the animals (or other experimental units) are housed and measured should be randomised. If males and females are to be compared in an experiment, then they should be comparable in other ways. If old males were compared with young females it would be unclear whether any differences were due to age or sex.

How should the animals be caged?

There are various ways in which the animals can be caged (rodents are the most widely used and this section refers to them)

Treatment (randomised)	Random number now sorted	Animal number
A	0.067	1
A	0.100	2
A	0.122	3
C	0.210	4
B	0.248	5
C	0.265	6
B	0.478	7
A	0.527	8
C	0.628	9
B	0.665	10
B	0.875	11
C	0.895	12



There is no one answer to the numbers of animals housed per cage. It depends on species and the nature of the experiment.

Single housing of mice and rats may be stressful and is strongly discouraged for welfare reasons. But male mice may fight, depending on the strain and husbandry conditions.

Very valuable animals such as those fitted with telemetry apparatus, or ones with a genetic modification are sometimes housed with a companion which is not part of the experiment.

Group housing poses problems if treatment is given in the food or water as the cage is then the experimental unit unless sophisticated apparatus is used so that each animal can have a different diet. This is sometimes done with farm animals.

Group housing may also be a problem if drug treatments are involved as rats and mice are coprophageous so control animals may consume metabolites of the test compound if animals of different treatment groups are housed together.

It is not a good idea to house all the controls in one cage, all of treatment A in a second cage etc. as then the cage becomes the experimental unit. There can be “cage effects” due to social interactions which could seriously bias the results (e.g. if all the controls are fighting, but the treated animals are not).

If animals receiving different treatments (or genetically modified and wild type animals) can be housed together, then a randomised block design might be used as shown at the bottom of the figure (above).

Blinding

We usually have a vested interest in the outcome of our experiments. We might want to find “significant” differences between groups, or in some cases no significant differences (particularly if we are toxicologists). So, having done the randomisation, wherever possible use the animal numbers as codes to “blind” everyone to the treatment.

This is particularly important when making measurements, scoring histological sections or measuring behaviour. Blinding may be difficult in some cases such as when comparing two mouse strains which differ in coat colour.

Failure to randomise and blind can lead to false positive results

In this study (Bebata et al 2003 Acad. emerg. med. 10:684-687) 290 animal studies were scored for blinding, randomisation and whether the outcome was positive or negative, as defined by authors. The results are shown below:

	Odds ratio
Blind/not blind	3.4 (95% CI 1.7-6.9)
Random/not random	3.2 (95% CI 1.3-7.7)
Both/neither	5.2 (95% CI 2.0-13.5)

An odds ratio of one implies that blinding or randomisation was not associated with the outcome of an experiment. These positive odds ratios show that on average studies which were not blinded and/or randomised produced excessive numbers of (presumably false) positive results.

Studies where there was no blinding or randomisation were unreliable. >

7. Controlling Variability

If noise can be reduced the signal/noise ratio will go up. Sample size could be reduced, power could be increased or a smaller response could be detected. So control of variation is of fundamental importance when designing an experiment.

There are three ways in which inter-individual variation can be reduced.

1. By choosing animals of similar weight and age, eliminating clinical or sub-clinical infection and providing a non-stressful environment

2. By controlling the genetic variation using inbred strains (when using mice or rats).

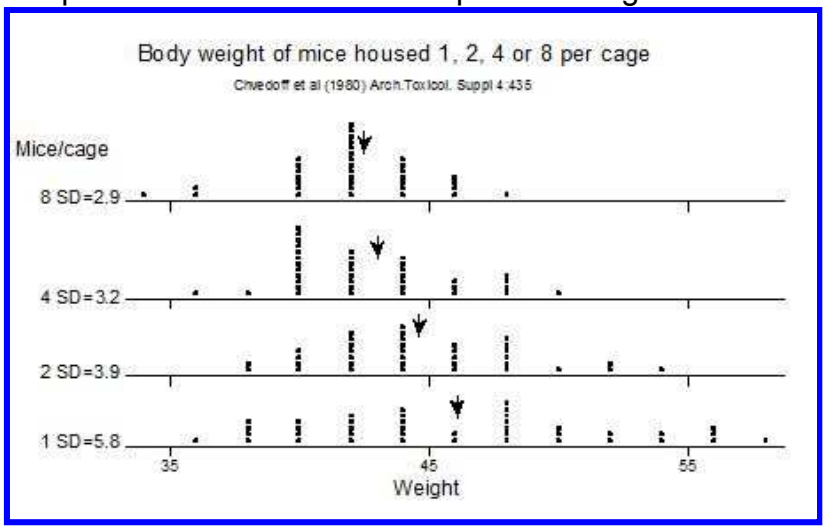
3. By using randomised block experimental designs or covariance analysis so as to remove some of the variation that can not be removed in any other way during the statistical analysis.

This page gives some examples. Both genetic variation and blocking are considered in more detail later.

Example 1

The plot shows that mice housed singly are more variable (SD=5.8) than those housed in pairs (SD=3.9) or groups, although they weigh slightly more on average. (Chvedoff M et al (1980). Effects on mice of numbers of animal per cage: an 18-month study. (preliminary results). Archives of Toxicology, Supplement 4:435-438.)

Assume you want to do an experiment to see whether a specified drug treatment affects body weight in mice, with individual mice being the experimental unit.



You plan to compare treated and control means and consider that if the two means differ by 4g or more (the signal) this would be of biological interest. You plan to use a two-sided t-test with a significance level of 0.05 and a power of 0.9. Should you house your mice singly or in pairs? (you rule out having more per cage).

Assuming that the response (signal) is not affected by number per cage you would only need half the number of animals if they were to be housed in pairs

Mice/cage	Mean	SD (noise)	Signal/noise	Number needed/group
1	46.0	5.8	4/5.8=0.86	30
2	44.7	3.9	4/3.9=1.28	14

Example 2.

Sleeping time under barbiturate anesthetic is sometimes used to indicate whether a test drug alters drug metabolising enzymes. All mice receive the barbiturate and half of them receive the test compound while the other are used as controls. A difference in sleeping time would indicate that the test substances alters drug metabolism.

The table below shows the number, mean and standard deviation of sleeping time in five inbred strains (A/N to SWR/HeN) and two outbred stocks (CFW and Swiss) of mice under hexobarbital anesthetic.

Note the much greater variability (SD) in the outbred stocks. This substantially reduces the signal/noise ratio (assuming a signal (effect size) of 4 minutes), so much larger sample (group) sizes are needed. The last column shows the power that an experiment would have if group size were fixed at 20 mice.

Strain	N	Mean	SD	No needed*	Power**
A/N	25	48	4	23	86
BALB/c	63	41	2	7	>99
C57BL/HeN	29	33	3	13	98
C3H/He	30	22	3	13	98
SWR/HeN	38	18	4	23	86
CFW	47	48	12	191	17
Swiss	47	43	15	297	13

* Power analysis: number needed in a two-sample t-test to detect a 4 min. change in the mean (2-sided) with a=0.05 and a power of 90%

** power of an experiment to detect a 4 min. change in the mean if the sample size is fixed at 20 mice/group

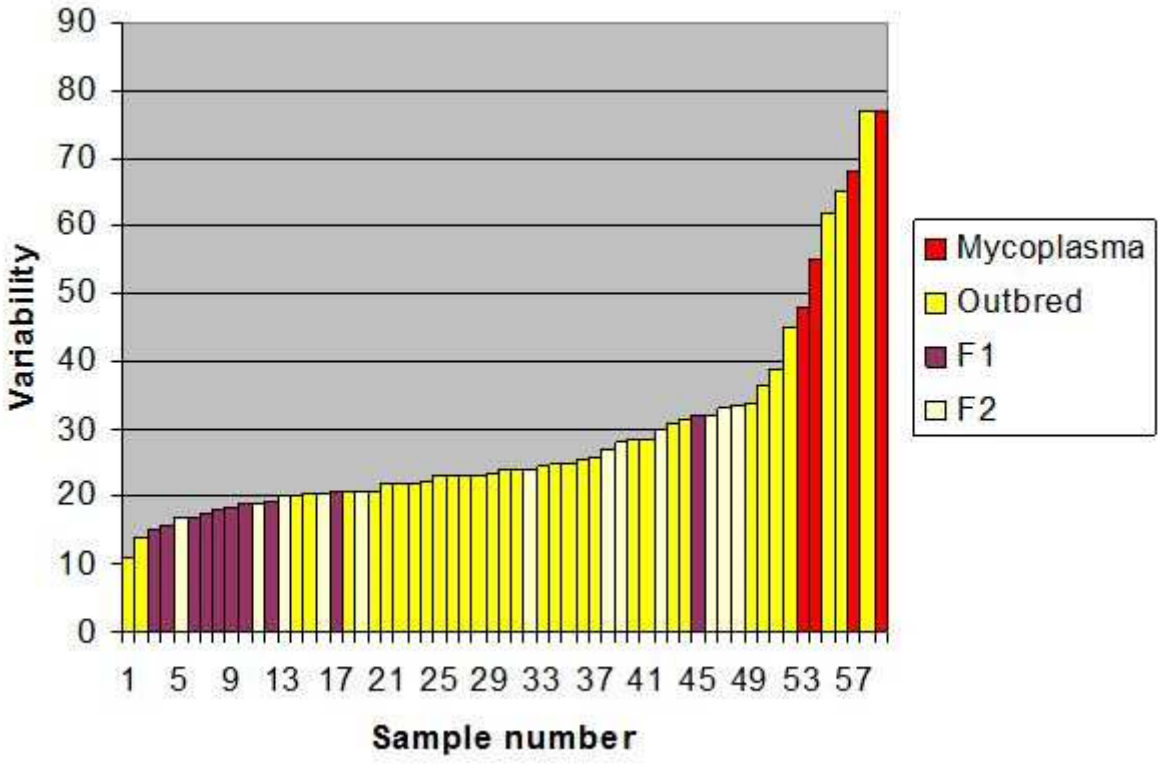
Data from Jay 1955 Proc Soc. Exp Biol Med 90:378

Controlling the genetic variation by using inbred strains resulted in this case in either a much smaller sample size being needed or a substantial increase in power if sample size was fixed at 20/group.

Example 3

This study shows the variability of kidney weight in 58 groups of rats (N=approx 30 in each group). Groups have been ranked in order of variability which is expressed as a percentage. Some groups were affected with *Mycoplasma pulmonis* causing chronic respiratory disease (in red), some were outbred, some F1 hybrid and some F2 hybrids.

The *Mycoplasma*-infected rats are clearly highly variable. Samples of outbred rats are both the most uniform and the most variable, but tend towards variability while the F1 hybrids (which are isogenic) tend to be uniform, with one exception.



(Data redrawn from Gartner,K. (1990), Laboratory Animals, 24:71-77.)

Suppose the aim of an experiment is to find out whether a drug affects the weight of the kidneys in rats. We can use a power analysis to find out how many rats of each type shown on the previous page would be needed.

Assume that we want to be able to detect a 20% change in kidney weight (either way), we want a power of 80%, a significance level of 5%, and we have data on the variability of each group. The results are shown in the table below.

Factor	Type	Std.Dev	Signal/ noise*	Sample size	Power**
Genetics	Isogenic (F1 hybrid)	19	1.05	16	60
	Non-isogenic (F2 hybrid & outbred)	28	0.71	32	32
Disease	Mycoplasma free	27	0.75	29	35
	With Mycoplasma	62	0.32	155	9

Note that twice as many animals (32 vs 16) would be needed to do the experiment with non-isogenic (outbred and F2 hybrid) rather than isogenic (F1 hybrid) rats, and five times more Mycoplasma-infected rats (155) than healthy rats (29, averaging across genotypes) would need to be used. If sample size is fixed at 10 animals/group then power would be 60% using the isogenic rats but only 32% using the non-isogenic ones.

Four examples:

1. The random dogs versus beagles in the previous section
2. Housing mice singly or in groups,
3. Sleeping time under anesthetics,
4. Kidney weight in rats of various types

All show that *uncontrolled* variability reduces the signal/noise ratio so larger sample sizes are needed to detect the effect of a treatment. This will cost money, time and effort, and the lives of animals.

But *controlled* variation can be deliberately introduced by, for example, using several inbred strains, by using both sexes, by sampling different environments or by using different diets without increasing the total number of animals, using factorial and randomised block designs (discussed later).

8. Strains of Mice and Rats

76% of animals used in research in the UK in 2008 were mice or rats, as sown below, and the use of mice continues to grow. But there are lots of types of these species. What are they all and what are their properties?

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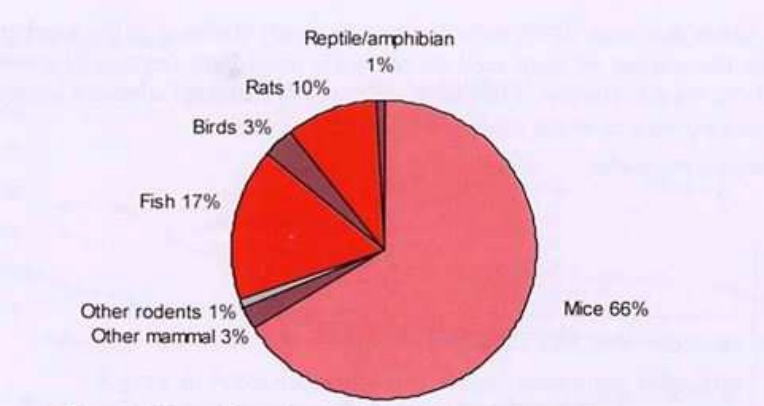


Figure 2: Procedures by species of animal, 2008 (Table 1)

The main types are

- Inbred strains (inbred lines are called “strains”)
- Outbred stocks (outbred lines are called “stocks”)
- Mutants Genetically modified strains (not discussed here)

Outbred stocks

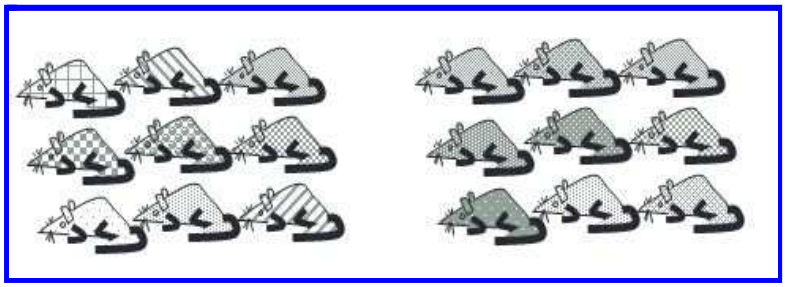
They are vigorous, cheap and prolific and are widely (and probably wrongly) used in research. They are usually maintained as large breeding colonies within which there is inter-individual genetic variation. They are maintained by random (or haphazard) mating systems. Each animal will be genetically different, but the extent of genetic variation depends on the history of the colony. Genetic bottlenecks such as when a new colony is established or the stock is hysterectomy-re-derived to eliminate disease, will tend to reduce the genetic variation, while an outcross to a different stock (sometimes by mistake) will increase it.

As research models they have some disadvantages:

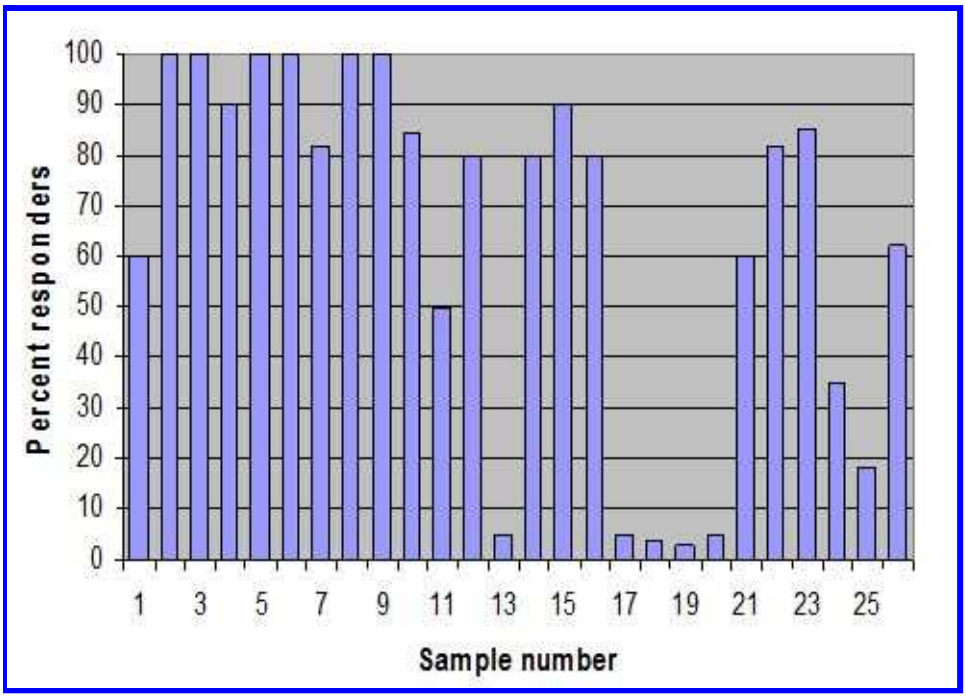
- They can change rapidly in characteristics due to selection, inbreeding and random genetic drift. The latter two can be minimised by maintaining large populations and ensuring as far as possible that each breeding male and female contributes to the next generation.
- Outbred animals are usually much heavier than inbred ones as a result of many generations of selection for fast growth rate and large litter sizes.
- They are “genetically undefined”. Nothing is known about the genotype of any individual in the colony unless it is specifically genotyped
- Stock names such as “Sprague-Dawley”, “Wistar” or “Swiss” have little genetic meaning. There are no genetic markers to define them. Sprague-Dawley rats from different breeders will be genetically different. This means that historic data collected on such stocks may be unreliable.

- There is no practical method of quality control. It is not even possible to distinguish between Wistar and Spreague-Dawley rats, the two most widely outbred rat stocks, although any stock of Wistar rats will differ from a stock of Sprague-Dawleys.

The figure shows diagrammatically that each rat within an outbred stock is genetically distinct and also that two outbred stocks will be different due to genetic sampling and selection.

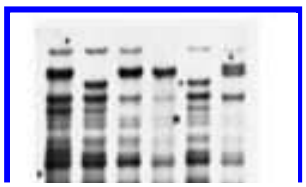


Samples of outbred rats will be different due to sampling from a genetically heterogeneous population. The figure below shows the percent responders to a synthetic polypeptide in sequential samples of Sprague-Dawley rats from the same breeder over a period of about 18 months. Sample size was about 30 animals per group. Some of the variation (e.g. in samples 1-10) is what would be expected if response depended on a single genetic locus such as the major histocompatibility complex, where there is a high proportion of responders. However, this could not account for the low response in samples 17-20 which must have come from a different colony. An investigator would not normally be aware of such variation unless they were investigating single gene markers. Seven inbred strains were also typed and these were either 100% responders or 100% non-responders.

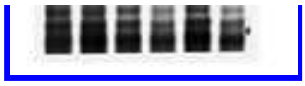


Data from Kunz HW, Gill TJ, III, Borland B. The genetic linkage of the immune response of poly (Glu52Lys33Tyr15) to the major histocompatibility locus in inbred rats. Journal of Immunogenetics 1974;1:277-287.

Genetic heterogeneity can be seen in DNA fingerprints, as shown here, although this technique has been superseded by PCR of individual loci.



When should outbred stocks be used in research?



It is difficult to think of any controlled experiment where outbred stocks would be better than inbred strains except possibly where a particular stock happens to have some characteristic of interest not found in an inbred strain. The almost universal use of outbred stocks in toxicity testing has arisen by historical accident and has never been scientifically justified.

Geneticists use outbred stocks only when they have no alternative, or for a few genetic studies. For example:

- An outbred stock can be used as a base population for a selective breeding experiment.
- They are sometimes used in genetic mapping and gene association experiments where the genotypes of many individuals is recorded at many gene loci to see if there are associations with a disease or response to an experimental treatment. But these are specialised (and expensive) studies.

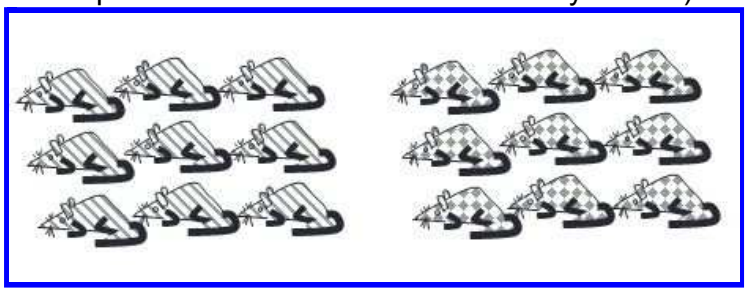
For the vast majority of work the genetic background needs to be controlled by using inbred strains (or F1 hybrids) in order to minimise inter-individual variation. This was illustrated by several examples in section *6. Power and Sample size.* The uniformity of the beagles compared with the random dogs meant that far fewer were needed to detect a specified signal. Far fewer inbred than outbred mice would be needed to detect differences in sleeping time under barbiturate anesthetic and the kidney weight was less variable in the F1 hybrid rats than in the outbred stocks.

As they are cheap to buy, outbred stock should be used if an experiment requires large amounts of a particular organ in order to extract a protein. They could also be used in classroom dissection.

Some scientists attempt to justify the use of outbred stocks on the grounds that “humans are outbred” which should make it easier to “extrapolate” to humans. But this is a fallacy. Humans and animals differ in many ways. We don’t insist on using animals weighing 70kg on the grounds that humans weight about that. And even if it were true that in some unspecified way it was easier to extrapolate to humans, what would be extrapolated would be a larger number of false negative results because the phenotypic variability inevitable leads to lower powered experiments.

Inbred strains

These are produced by >20 generations of brother x sister mating with all individuals tracing back to a single pair in the 20th. or subsequent generations. They are genetically stable and can not be changed by selective breeding. However sublines have arisen in most of the commonly used strains as a result of “residual heterozygosity” (the sublines were separated before the strain was fully inbred) and new mutations (relatively rare).



The figure illustrates the within-strain homogeneity and the between-strain differences.

There are >400 inbred strains of mice and 150 inbred strains of rats

Geneticists have recognised their value for many years:

“ In experimental medicine today....the use of in-bred genetic material...is just as necessary as the use of aseptic and anti-septic precautions in surgery" C.C. Little 1936

“The introduction of inbred strains into biology is probably comparable in importance with that of the analytical balance into chemistry.” H. Grüneberg 1952

. "...the development of inbred strains has constituted probably the greatest advance in all cancer research." Heston (1963)

These DNA fingerprints (right) of four individuals of two rat strains show the isogenicity (each individual genetically identical) of inbred strains, but also that each strain is different

The key properties of inbred strains are:

Isogenicity All individuals within a strain are genetically identical. The same genotype can be produced repeatedly.

Homozygosity: Animals are homozygous at virtually all genetic loci. This leads to immortality of the genotype because offspring are identically, genetically identical to their parents.

Phenotypic uniformity: Genetic uniformity leads to phenotypic uniformity. This means either that fewer animals can be used or the power of experiments using inbred strains will be higher than if an outbred stock had been used.

Long-term stability: Inbred strains can not be changed by selective breeding once they have become fully inbred. New mutations will lead to gradual genetic drift so it is important for investigators to specify the sub-strains which they use.

Identifiability: Each inbred strain has a unique set of genetic markers which can be used for genetic quality control. Strains do occasionally become genetically contaminated by a non-strain mating, but this can be recognised using such markers. Investigators are advised to save some tissue or DNA from the animals they use so that if they get unexpected results they can check that the animals were what they were supposed to be.

Individuality. Each strain is unique and will be different from other strains in many ways which are likely to be of interest to research scientists. Strains differ in life-span and types of spontaneous disease, there are physiological and biochemical differences between them, and they will respond differently to drugs and chemicals.

It is a perfectly acceptable scientific strategy to work on, say, C57BL/6 strain mice, or F344 rats provided it is clearly understood that the results only apply to that strain and may not apply to other strains. In many cases it is possible to do an experiment using several strains without increasing the total number of animals by using a factorial experimental design

Background data. Many thousands of papers are published each year involving inbred mouse and rat strains. Background data on strain characteristics and mouse genetics

accumulates rapidly. There are now extensive databases on mouse, and to a lesser extent rat genetics. These include:

The mouse phenome database. This has data on a wide range of strain charactersitics, searchable by subject area (e.g. behaviour, blood, bone, development etc.), strain, intervention, study design etc.

Mouse genome informatics. This has data on genes, phenotypes, disease models, gene expression, gene function, pathways, recombinases, strans and SNPs, tumours and orthology.

International mouse strain resources (IMSR), a searchable database of mouse stocks and strains available world wide.

The JAX mice database. This provides extensive information on mouse genetics, specifically relating to mice maintained by the Jackson Laboratory, Bar Harbor, Maine, USA.

The Rat Genome Database. This provides a comprehensive database on rat genetics.

When should inbred strains be used in research?

Inbred strains should be used in all experiments using mice or rats unless the use of an outbred stock is specifically justified for a particular project..

Derived inbred strains (Only brief details are given here)

There a number of more specialised strains derived from straight inbred strains. These include:

Coisogenic strains: A pair of strains which differ at only a single genetic locus (the differential locus) as a result of a mutation. “Knockout” strains usually fall into this category. Any differences between a pair of coisogenic strains will be due to the effect of the differential gene.

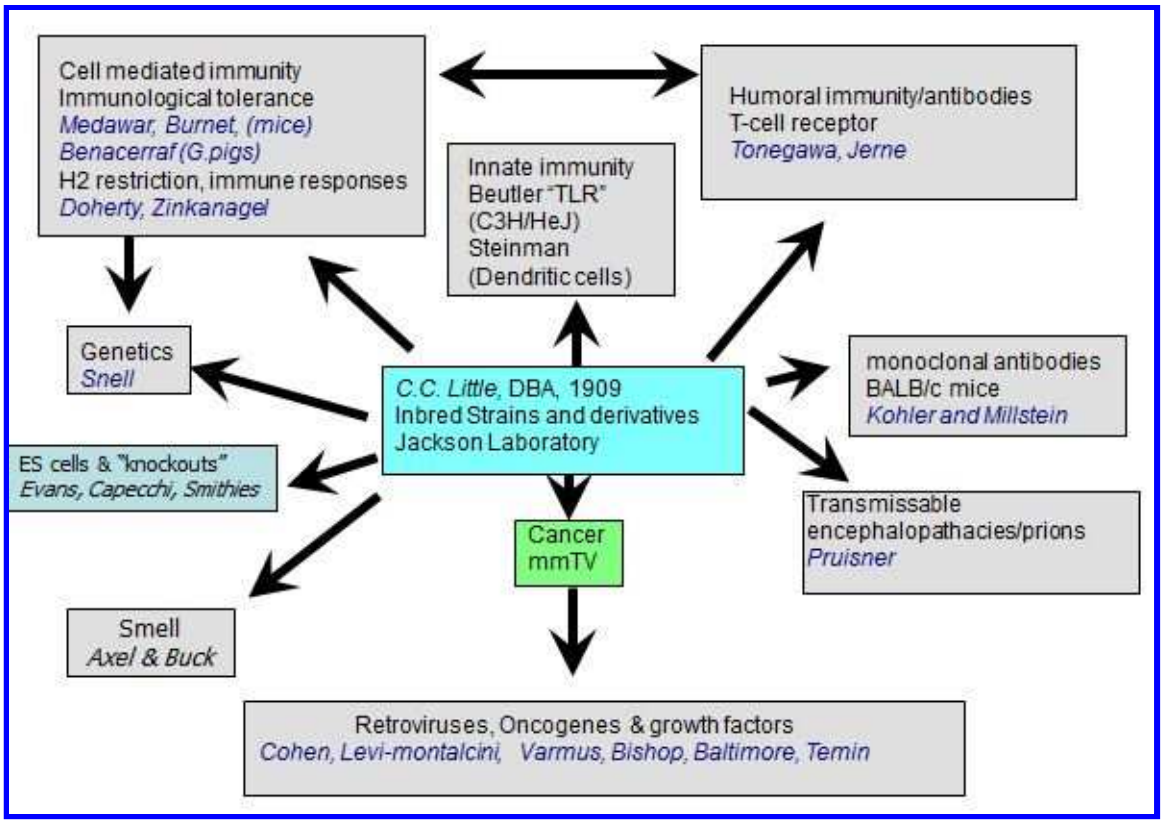
Congenic strains: A pair of strains which differ at a single genetic locus plus a section of chromosome. These are produced by back crossing a mutation or polymorphism to an inbred strain. The length of associated chromosome depends on the number of back crossing generations.

Recombinant inbred (RI) strains: These are sets of inbred strains developed from an F1 cross between two standard inbred strains. They are used to determine the mode of inheritance of some measured phenotype.

Recombinant congenic (RC) strains. Like RI strains except they are produced from a back cross generation of a cross between two inbred strains.

Chromosome substitution strains. A full set of these strains would include a genetic background strain and 20 strains in which a single chromosome has been substituted from a donor strain. They can be used to show whether there are any genes on a particular chromosome which influence a particular trait.

Twenty-four Nobel prizes since 1960 which depended on the use of inbred strains



The first inbred strain of mice was DBA, developed in about 1909 by Dr. C.C. Little, then a graduate student at Harvard University. He went on to found the Jackson Laboratory which is now the main repository in the World for genetically defined mice. These are made available to research workers throughout the World.

The properties of inbred strains which were essential in most of these studies was the genetic uniformity (isogenicity) and stability of the strains so that an identical genotype could be obtained over a long period of time, and the differences between the strains.

However, some of the Nobel prizes depended on the properties of an individual strain. BALB/c, for example, develops myelomas if injected i.p. with mineral oil. Myeloma cell lines were used in the development of monoclonal antibodies by Kohler and Millstein. Similarly, strain 129 was used in the development of embryonic stem cells by Martin Evens, and these have been central in the development of knockout strains by Smithies and Capecci. Only recently have good ES cell lines been developed from other strains.

9. Experimental Designs

This section only discusses the principles of experimental design. The statistical analysis of these designs is discussed in a later section.

Definitions

A *factor* is a discrete variable used to classify experimental units. For example, ”Gender” might be a factor with two levels “male” and “female” and “Diet” might be a factor with three levels “low”, “medium” and “high” protein. The levels within each factor can be discrete, such as “Drug A” and “Drug B”, or they may be quantitative such as 0, 10, 20 and 30 mg/kg.

Fixed effects factors, are variables which can be controlled by the investigator. These include gender, dose, diet, genotype (in the case of genetically defined strains) and any treatment which can be administered to the animals. Most experiments are designed to study the fixed effects.

Random effects factors are variables which can not be controlled by the investigator. They include inter-individual differences, litter effects, time effects and environmental effects like barometric pressure and batch differences in diet and bedding. These effects are responsible for noise (variation) *which is of little scientific interest to the investigator.* So the aim of some experimental designs, such as randomised blocks is to partition these effects out so that they do not obscure the effects of the fixed effects.

The main experimental designs are:

1. The completely randomised design. It has one or more fixed effect factor(s), often called the treatment. Subjects assigned to treatments at random regardless of any characteristics or natural structure to of the experimental material. This is the commonest design. It is simple and tolerates unequal numbers in each group.

2. The randomised block design. This is also known as a “within-subject”, “crossover” or “matched subjects”. Note that the term “repeated measures” design is sometimes used for a design where an individual receives different treatments over time (i.e. just like a crossover design). However, the term “repeated measures” is used here for a design where an experimental unit is measured several times *without* receiving different treatments.

All these designs have *one random effect variable* which is of *no interest* and *one or more fixed effect factors* (treatments) which *are* of interest. The design is used to:

- Increase power by better control of variation (eliminating some random effect variation such as height from the floor in the animal house).
- Provide a convenient way of breaking the experiment up into smaller, more convenient, parts.
- Take account of some natural structure of the experimental material, such as litter differences when studying pre-weaning animals
- Increase the generality by sampling slightly different environments.

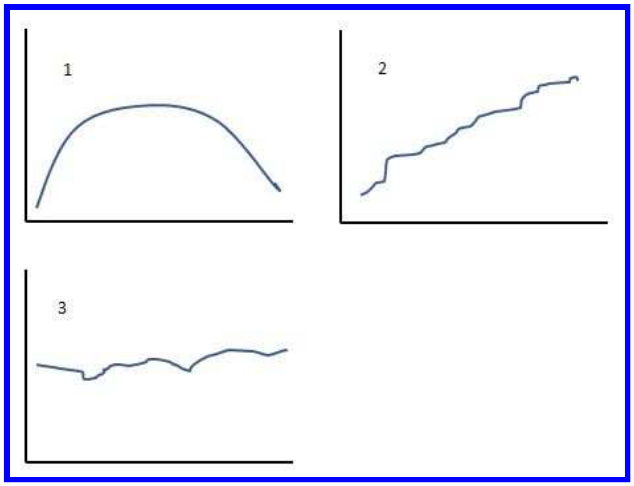
3. Latin square designs. These have two random effect variables (often designated rows and columns) and one or more fixed effects. They are used to further increase power in special situations taking account of the two sources of random effects.

4. Factorial designs. These have two or more fixed effect factors and in view of their importance they are discussed separately. Strictly they are *arrangements of the treatments* rather than *designs*, so it is possible to have a factorial treatment structure in a completely randomised, randomised block or Latin square design.

5. Split plot designs. These are randomised block designs with a factorial. treatment structure in which a main effect is confounded with blocks. It may sometimes be possible to design such an experiment by accident because in some circumstances they make good use of experimental subjects. For example, a within-animal experiment is a type of randomised block design. But suppose half the available animals are male and half female. The gender differences would be assessed using whole animals while the treatment differences would be assessed within the animals. This would be a split plot design. Such designs are discussed with factorial designs.

6. Repeated measures designs in which each experimental unit is measured several times *without* different treatments being applied and time effects *are* of interest. Note that some authors use the term "repeated measures designs" for crossover experiments in which a subject receives different treatments over a period of time.. Two cases need to be considered:

A. If there are just a few measurements on each individual, then one approach is to reduce the observations to a single number for each experimental unit.



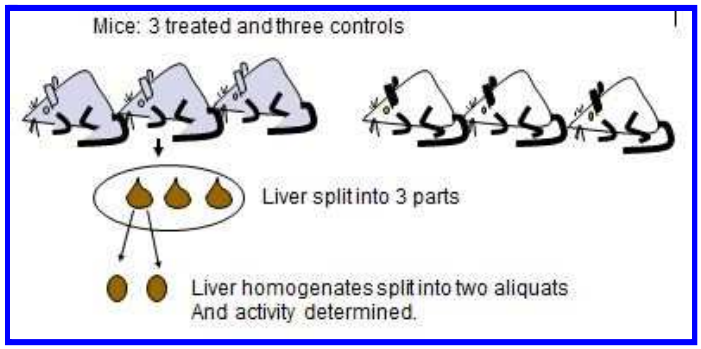
This could be the area under the curve or time to peak if response is like plot 1 on the right. Or the slope of the line or difference between the first and last few measurements it response is like plot 2 on the right. Or simply the mean of the measurements if there is no apparent trend (like plot 3 on the right).

The design can then be analysed as a completely randomised design using the single number for each subject.

B. If there are lots of measurements on each individual where the shape of the curve is of interest, such as a growth curve, then specialised methods may need to be used which are beyond the scope of this web site.

7. Hierarchical designs. In these designs more than one sample is taken from each experimental unit, and in some case the samples are sub-sampled, as illustrated below, where the liver of each individual is split into three parts, homogenised and then determinations done on two aliquats from each part. The

usual aim is to increase power by reducing measurement error. Sometimes the terms “technical replication” and “biological replication” are used. The former refers to replication of measurements on the same experimental unit.



These designs help to answer questions such as whether it is better to do more measurements on each experimental unit (which could be relatively inexpensive) or use more experimental units, if the aim is to increase power. In general if the measurements on each experimental unit are variable, then that is where there should be more replication. If they are similar, then more experimental units should be used (ethical considerations being taken into account). These designs are not discussed in any more detail here.

8. Designs measuring association: correlation and regression. In this type of experiment the aim is to see whether there is any association between two variables, and if so what is its nature. If the variables are associated but one does not cause the other, then the association can be studied and quantified using correlation. However, if altering one variable, such as dose rate (an independent variable), may cause some other variable (a dependent variable), such as red blood cell count to change, then this is studied using regression analysis. These designs are considered in a separate section.

9. Other less commonly used designs. These include: *incomplete block* designs where there is a natural structure to the experimental material but the number of treatments exceeds the natural block size and *sequential designs* where the experiment continues until certain criteria of success are achieved. These designs are rare (although important in some special situations) and are not described here.

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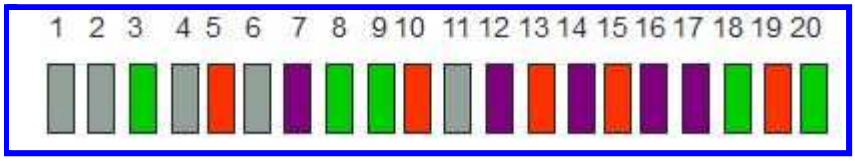
1. The completely randomised design



This is the simplest design. Each experimental unit is assigned to a treatment strictly at random without taking account of any individual characteristics. It is best used when relatively homogeneous experimental units are available. It can tolerate unequal numbers in each group and is perfectly adequate in many experimental situations. Following treatment investigators should (where possible) be blinded by using only the animal numbers when making measurements

In the figure above relatively homogeneous experimental units (animals, cages of animals etc.) were assigned at random (using EXCEL as previously

described) to treatments gray, green, red and purple. The subjects can be housed, treated and measured in any order.

The fact that 4/5 of the gray treatment are in the first ten and 4/5 purple treatments are in the last ten would not matter in most cases, although if, for example, surgery is involved skill may increase, leading to a bias against gray.

If the experiment needs to be split up, (e.g. if applying the treatments or if making the measurements takes several hours or days) then this can be done in any way as the subjects have already been randomised. However, if splitting the experiment up in this way is likely to introduce an unknown source of variation, then the design loses power. In such circumstances a randomised block design might be preferable.

This design will normally be analysed using a one-way analysis of variance or a t-test if there are only two groups.

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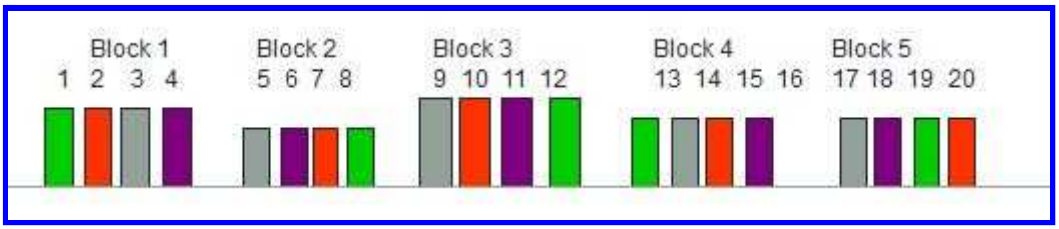
2. The randomised block design

A randomised block design is used to control a source of random variation which might otherwise obscure the effect of a treatment.



In this design the experimental material is split up into a number of “mini-experiments”, typically with one subject on each treatment. It is assumed that differences between treatments *are* of interest while differences between blocks, which are random effects are of *no* interest.

Subjects are matched using any criteria available at the time the experiment is started. This might be on size (as above), space (e.g. location within the animal house such as shelf level) or time (as in within-litter experiments, where litters are infrequent). Blocks can differ in several ways at the same time. For example, block 1 might be large animals held on the top shelf and processed on day 1..

Although it is usual to have only a single experimental unit of each treatment in a block, it is possible to have two or more. In that case there will be two error terms. One will be calculated from the differences among individuals within a block and the other from the block times treatment interaction. If these do not differ significantly, they can be combined (see statistical analysis section)

Randomisation in a randomised block design

It could be tedious to randomise each block separately so here is an alternative, assuming six treatments A,B,C,D,E,F are to be assigned in

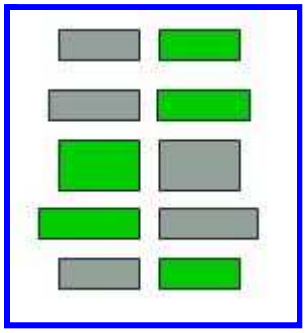
Subject	"=rand()"	Treatment	Block
1	0.852	F	1
2	0.306	C	1
3	0.425	D	1

three blocks (but it can be adjusted to any number of blocks and treatments)

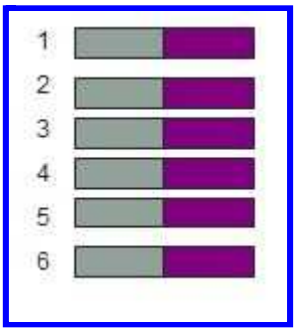
The first column has the animal number. Second column is a random number expressed to three decimal places. The third column is the treatment assignment. Within each block treatment A is assigned to the lowest number, treatment B to the next one etc.

4	0.635	E	1
5	0.010	B	1
6	0.000	A	1
7	0.977	F	2
8	0.033	A	2
9	0.393	E	2
10	0.043	B	2
11	0.048	C	2
12	0.137	D	2
13	0.921	F	3
14	0.079	A	3
15	0.425	C	3
16	0.733	D	3
17	0.852	E	3
18	0.212	B	3

Variants of the randomised block design



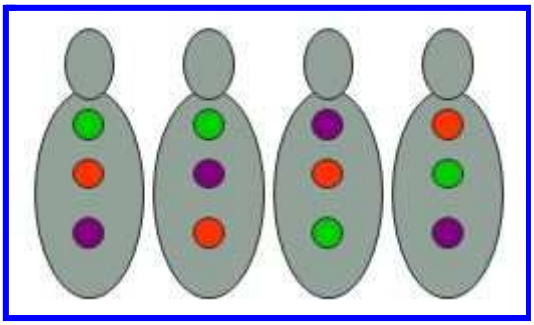
A matched-pairs design. This will normally be analysed as a paired t-test or a two-way ANOVA *without* interaction. Matched pairs of subjects have been assigned at random to the gray or green treatments.



This represents a "Before and after" experiment. While this can be regarded as a randomised block design, true randomisation is not possible. You can not have an "after" before a "before". The assumption must be made that measuring the subject before applying the treatment does not alter the subject.

A "crossover" design in which the experimental unit is the animal (or other entity) for a period of time.

Each subject receives different treatments sequentially and it is assumed that the treatment does not permanently alter the subject. The blocking factor is time, with all animals being measured at each time.



Individual animals can be “blocks”. In this case different treatments are applied to the shaved back of an animal. The experimental unit is an area of skin and it is assumed that the treatments do not interact with each other.

Blocks can be set up at different times (even weeks apart) and/or housed in different locations.

The main advantages of the RB design are that:

- It can deal with heterogeneous material by matching subjects in each block (increasing power).
- It can take account of any natural structure in the experimental material (e.g. litters)
- It is often more convenient to break the experiment down into smaller bits which can then be handled and measured more carefully in the available time.

The main disadvantages of the RB design are

- It is not very tolerant of missing observations
- It should not be done with very small experiments (say less than about 16 experimental units total) because there may be a loss of power.

---

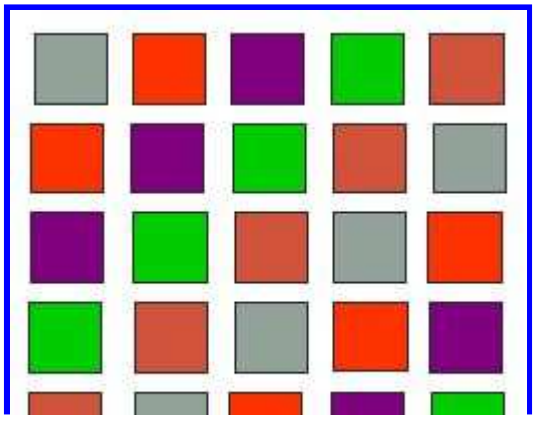
3. The Latin square design

The number of subjects is the number of treatments squared.

This is a 5x5 Latin square. It has five rows, five columns and five treatments (Gray, Red, Purple, Green, Brick). Note that there is one of each treatment in each row and in each column. It can be written by writing out the first line, then starting the second line with the second treatment from the first line (red in this case), with the first from the first line (gray) going on the end. And so on for the rest of the lines.

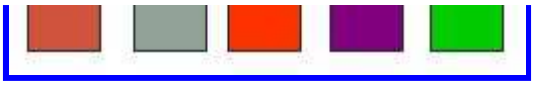
It has not yet been randomised. To maintain the layout we randomise whole rows and then whole columns.

It has one fixed factor (Treatment) and two random factors (Rows and Columns). We would use it if there are two factors such as day of the week (represented as columns) and time of the day (rows) which may influence the outcome, and we want these balanced out.



Latin squares with more than 7 treatments can become too large to be managed easily, and those with fewer

than four are too small. However, small ones (as small as 2x2) can be replicated.



4 & 5. Factorial and split-plot designs. These involve two or more fixed effect factors. Factorial designs are of great importance so are discussed in a separate section

10. Factorial Experiments

A *factor* is a discrete variable used to classify experimental units. For example, ”Gender” might be a factor with two levels “male” and “female” and “Diet” might be a factor with three levels “low”, “medium” and “high” protein. The levels within each factor can be discrete, such as “Drug A” and “Drug B”, or they may be quantitative such as 0, 10, 20 and 30 mg/kg.

A *factorial design* is one involving two or more factors in a single experiment. Such designs are classified by the number of levels of each factor and the number of factors. So a 2x2 factorial will have two levels or two factors and a 2x3 factorial will have three factors each at two levels.

Typically, there are many factors such as gender, genotype, diet, housing conditions, experimental protocols, social interactions and age which can influence the outcome of an experiment. These often need to be investigated in order to determine the *generality* of a response. It may be important to know whether a response is only seen in, say, females but not males. One way to do this would be to do separate experiments in each sex. This “OVAT” or “One Variable at A Time” approach is, however, very wasteful of scientific resources. A much better alternative is to include both sexes or more than one strain etc. in a single “factorial” experiment. Such designs can include several factors without using excessive numbers of experimental subjects.

Factorial designs are efficient and provide extra information (the interactions between the factors), which can not be obtained when using single factor designs.

Split plot designs are considered at the end of this section. They are like a cross between a factorial and a randomised block design. They were derived from agricultural research in which is was sometimes impossible to irrigate, say, a small plot without affecting the adjacent plots. So irrigated plots were large and covered several smaller plots comparing, say, planting distance.

According to RA Fisher

“*If the investigator confines his attention to any single factor we may infer either that he is the unfortunate victim of a doctrinaire theory as to how experimentation should proceed, or that the time, material or equipment at his disposal is too limited to allow him to give attention to more than one aspect of his problem.....*

.... Indeed in a wide class of cases (by using factorial designs) an experimental investigation, at the same time as it is made more comprehensive, may also be made more efficient if by more efficient we mean that more knowledge and a higher degree of precision are obtainable by the same number of observations.”

(Fisher RA. 1960. The design of experiments. New York: Hafner Publishing Company, Inc. 248 p.)

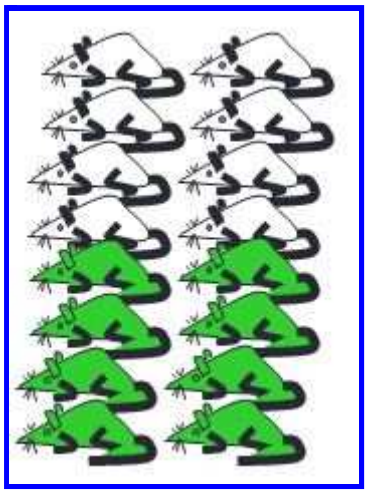
Unfortunately, although such designs are widely used, they are often incorrectly analysed. A survey found the following:

Number of studies	513
Factorial designs	153 (30%)
Correctly analysed	78 (50%)

(Niewenhuis et al., 2011, Nature Neurosci. 14:1105)

Examples

Assuming that the animal is the experimental unit, the experiment on the right has two factors, the treatment (Control versus Treated represented by the two columns) and the colour (White versus Green). This might represent the two sexes, or two strains or two diets or any other factor of possible interest.



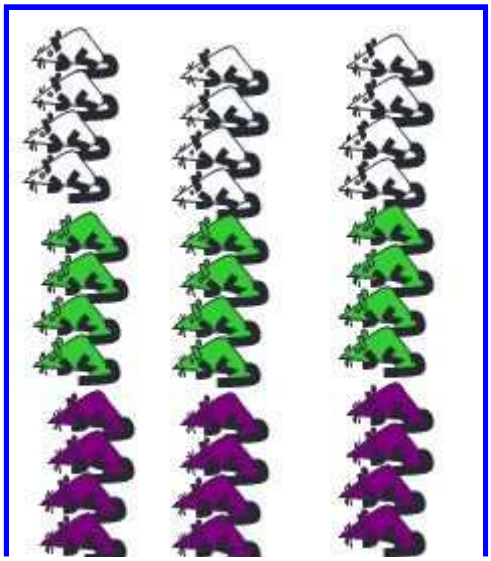
The aim is usually to see whether two factors are independent.

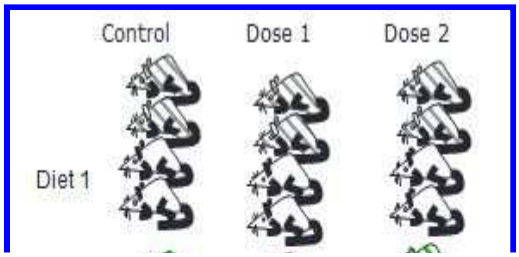
This is a 2x2 factorial because there are two factors each at two levels. Using the Resource Equation method of sample size determination there are 16 animals and 4 groups, so E=16-4=12, which, though small, is satisfactory.

Factorial designs are powerful because differences among the levels of each factor are determined by averaging across all other factors. This, if columns in the figure on the right represent “Treated” and “Control” the means are estimated by averaging across the two colours which might represent males and females. This assumes that the males and females respond in the same way to the treatment, an assumption that is tested in the statistical analysis using a *two-way analysis of variance with interaction*.

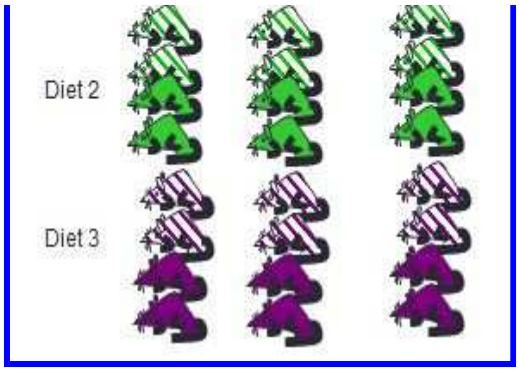
If the two sexes do not respond in the same way then this is known as an “interaction” and the differences will need to be looked at separately for each sex. However, this would be useful information which could not be obtained by doing separate experiments on each sex.

A 3x3 Factorial design (3 factors each at 3 levels) is shown below. . This might be, for example, a “Drug treatment” with levels Control, Low high doses (columns) and “Diet” with three levels of a food additive represented by the three colours





A 3x3x2 factorial is shown on the right.



Here the three factors are “Dose” at three levels, “Diet” at three levels and “Strain” (stripes versus solid) at two levels. So it is a 3x3x2 factorial design.

In this case there are 36 experimental units (animals) and 18 treatment groups so using the Resource Equation method of determining sample size, E=36-18 =18. As E is between 10 and 20 it is probably an appropriate number of experimental units.

Note that with factorial designs the concept of “group size” needs to be reconsidered. In this case each treatment and diet mean will be based on 12 subjects, averaged across the other factors. Strain means will be based on 18 animals averaged across both diets and treatments. So although there are subgroups consisting of just two animals, the means are based on much larger numbers.

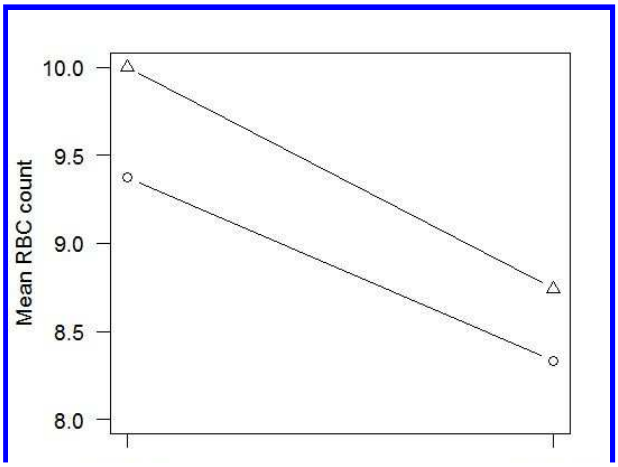
A real example.

Strain	Control	Treated	Strain means
BALB/c	10.10	8.95	
	10.08	8.45	
	9.73	8.68	
	10.09	8.89	9.37
C57BL	9.60	8.82	
	9.56	8.24	
	9.14	8.18	
	9.20	8.10	8.86
Treatment Mean	9.69	8.54	

In this study mice of two strains (BALB/c and C57BL) were dosed with a vehicle or with chloramphenicol at 2000mg/kg. This is a 2(strains) x 2(dose levels) factorial design. We want to know:

- does treatment have an effect on RBC counts
- do strains differ in RBC counts
- do strains differ in their response to chloramphenicol (the interaction).

The treatment appears to reduce red blood cell (RBC) counts. There is no overlap between treated and control individuals. Also, C57BL seems to have lower counts than BALB/c. Whether or not there is an interaction can best be seen graphically



This plot shows that BALB/c (triangles) mice have higher red blood counts than the C57BL (circles) both in the controls and in the treated group and the reduction due to the chloramphenicol was the same in both strains. So there is no interaction between strain and chloramphenicol in this case.

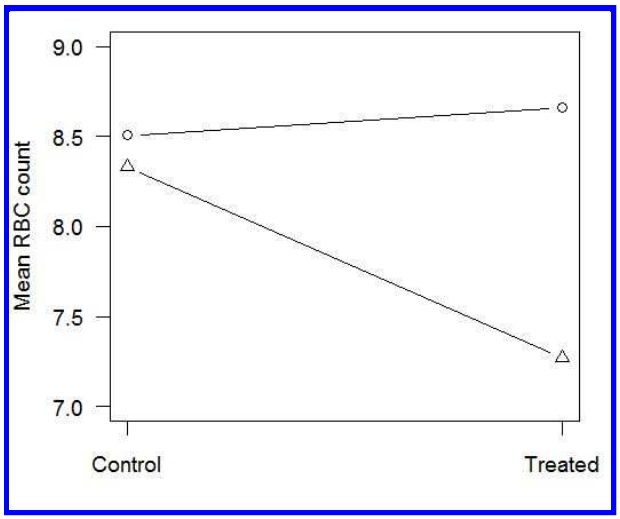
This should, of course, be confirmed by a two-way analysis of variance with interaction as described in section 11.

In contrast, here are the results with two different strains (C3H and outbred CD-1). Chloramphenicol seems to reduce red blood cell counts and CD-1 seems to have higher counts than C3H. However, plotting the means (below) also shows that there is an interaction.

Strain	Control	Treated	Strain means
C3H	7.85	7.81	
	8.77	7.21	
	8.48	6.96	
	8.22	7.10	7.80
CD-1	9.01	9.18	
	7.76	8.31	
	8.42	8.47	
	8.83	8.67	8.58
Treatment means	8.42	7.96	

Strain C3H (triangles) responded to chloraphenicol by a reduction in red blood cell counts, but in CD-1 (circles) there was no response.

The data should be analysed by a two-way ANOVA with interaction to see whether the interaction effect is statistically significant, as shown in section 11.

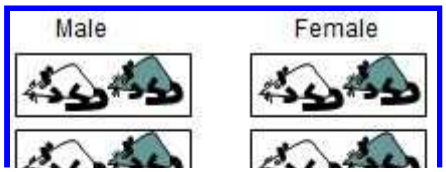


Implications of strain x treatment interactions

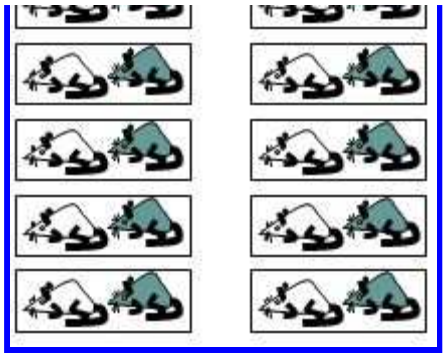
Strain by treatment interactions are almost universal. This means that results based on a single strain (or outbred stock) can not necessarily be generalised.

It is often highly desirable to replicate over several strains using a factorial experimental design, particularly in toxicity testing where the aim is to “prove a negative”. A good example is the response to bisphenol A (BPA) which is an endocrine disruptor in most strains and stocks of mice and rats, causing a range of developmental and other defects when administered at doses below the “safe” human exposure level. However in none of 13 studies were any effects observed when the CD:SD stock of rats was used. (Richter CA, Birnbaum LS, Farabollini F et al. In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol 2007;24:199-224.).

Split plot designs



These are randomised block designs with a factorial treatment structure in which a main



effect is confounded with blocks. They are worth knowing about because in some situations they may make efficient use of resources.

Suppose the aim is to compare two or more treatments using a randomised block design. For example, the experiment on the right has two animals in a cage, each receiving a different treatment. They could be two genotypes. Or it could be a within-animal experiment where the same animal is given two treatments sequentially or as a topical application to the skin on the left or right side.

Suppose it was decided that 12 cages would be sufficient (by the resource equation E=24-2=22, which is acceptable). Outcome measurements would be done on the animals, one cage at a time. This could be analysed as a randomised block design.

But suppose half the cages had males and half females? In that case an estimate of sex differences for the outcome of interest could be obtained averaging across the two animals within a cage and a sex x treatment effect could also be estimated. This would indicate whether the two sexes responded similarly to the treatments.

Given the need to use both sexes and the need to group house rodents, this might be quite a useful design in some cases.

A “split-plot” has two different experimental units (in this case *animal* (for comparing the treatments) and *cage (for comparing the sexes)* in one experiment. Technically, it is a factorial design with a main effect confounded with blocks. The statistical analysis will be discussed in the statistical analysis pages.

14. Guidelines, systematic reviews and meta analysis

Guidelines

Click arrow for a pdf of "Guidelines for "The Design and Statistical Analysis of Experiments Using Laboratory Animals"

Important information which is essential should the work need to be repeated, or if it is to be included in a systematic review or meta-analysis is often omitted.
The "ARRIVE" guidelines and GSPC (Gold standard Publication Checklist), which overlap to a large extent, provide checklists of information which the authors should consider **when designing their experiment and preparing their manuscript**. Not all the items will be relevant to every paper, but all should be considered.

Main table from The ARRIVE guidelines

Or click arrow for a pdf of the paper

Main table from The GSPC

Or click arrow for a pdf of the paper

Systematic reviews and meta-analysis

Scientists doing applied research should consider doing a systematic review of the literature, i.e. one which aims to be based on all qualifying published (and in some cases un-published) papers. In some cases this might be extended to a meta analysis. The publications below will be of interest to anyone considering this approach:

A step-by-step guide to systematically identify all relevant animal studies. Leenaars M, Hooijmans CR, van Veggel N, ter Riet G, Leeflang M, Hooft L, van der Wilt GJ, Tillema A, Ritskes-Hoitinga M.Lab Anim. 2012 Jan;46(1):24-31

A search filter for increasing the retrieval of animal studies in EMBASE is also available (de Vries et al 2011) Laboratory Animals 2011; 45: 268–270. DOI: 10.1258/la.2011.011056. and a similar filter for studies in PubMed is available from Hooijmans CR, et al (Lab Anim 2010;44:170–5)

A meta analysis is the statistical analysis of a collection of a large number of individual studies in order to reach an over-all consensus. An introduction to the techniques involved is given by Ellis, P.D. "The essential guide to effect sizes", Cambridge University Press, 2010. The magnitude of the response to a treatment is usually assessed using the standardised effect size described in section 6 (as a signal/noise ratio).

A meta analysis of ischemic preconditioning in the animal kidney, provides an example of the sort of study which can be done (Wever, KE 2012, www.plosone.org, 2012 | Volume 7 | Issue 2 | e32296)

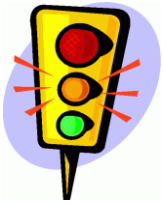
3Rs

ALTERNATIVES TO USE OF ANIMALS

Aurora Brønstad - Veterinarian – PhD

The three R's - Russell & Burch - The principal of humane experimental techniques - 1959

- Replacement
- Reduction
- Refinement



3R are globally adopted

The three R's

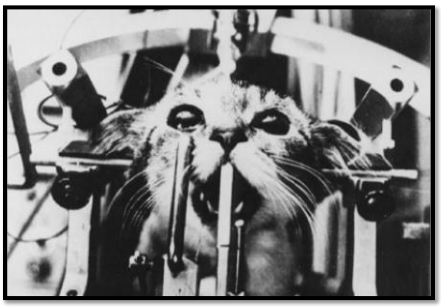
Russell & Burch - The principal of humane experimental techniques 1959

Replacement	Substitution for conscious living higher animals of insentient animals, or methods not involving animals (in vitro methods)
Reduction	Reduction in the number of animals used to obtain information of a given amount and precision
Refinement	Decrease in the incidence or severity of inhumane procedures

3R must be implemented in planning & performing experiments using animals

Ethics – how do we justify difficult decisions

Different opinions



Spontaneous attitudes to animal experiments

- Animal experiments can be acceptable in the following context and conditions
- If there are no other options (No replacements for animals)
- If the animals do not suffer (refinement)

http://www.vr.se/inenglish/fromus/news/newsarchive/news2008/news2008/publicopinioninswedenontheuseofanimalsinresearch.5.1d4cbbbb11a00d342b0800010843.html

Research animal ethics

- Ethics
  - How do we justify animal experiments

Use of animals is justified because of the greater goodness for the majority (utilitarian, consequence ethics)

  - Ethical guidelines

Reduction of harm to all sentient being is a moral issue. The 3Rs represent a practical method for harm reduction and provide a framework for morally acceptable use of animals

Ethical toolbox - A practical strategy for decision making

*Article 4 - Principle of **replacement**, reduction and refinement*

1. Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure.

2. Member States shall ensure that the number of animals used in projects is **reduced** to a minimum without compromising the objectives of the project.

3. Member States shall ensure **refinement** of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals.

The new regulation is based on
EU Directive 2010/63
Protection of animals used for scientific purposes.

3R: A practical strategy for decision making

- Key questions to ask
- Are we sure there is not an alternative to achieve the information we need??
  - How can we document it?
- Are we sure that we use the least number of animals to obtain information of a given amount and precision
  - How can we document it?
- Are we sure that we have taken all possible steps to minimize/eliminate any harm to the animals and optimized their well being?
  - How can we document it?

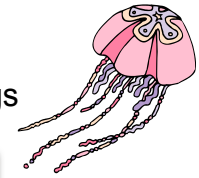
REPLACEMENT
rEDUCTION
REFINEMENT

Examples

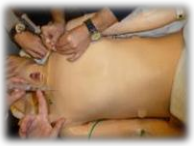
REPLACEMENT

Replacement

- In vitro models
- Computer modeling
- Replace sentient beings with less sentient beings



- Training
  - Models



- Routine testing
  - Chemical test for shell-fish toxins
  - Pregnancy test (50 years ago we used animals!)

Replacement

- Replacements methods are easier to automate for large scale testing and optimizing of productivity
- Replacements methods are therefore cheaper and commonly preferred wherever alternative methods exists!

rEDUCTION

Reduction

- Reduce the number of animals
- Design your experiments so you get as much information of each animal as possible
  - Without unreasonable burden on the individual animal
- Do your calculations carefully
  - Using too few animals might cause inconclusive studies
  - Inconclusive studies is unnecessary use of animals
- Avoid any variation (noise) that force you to increase number of animals in groups
  - Standardization of environment, animals (genetics, microbiology) and procedures
- Avoid bias

REDUCTION: CONSULT A STATISTICIAN!!

Reduction in the number of animals used to obtain information of a given amount and precision

However you still have do decide what are the important or relevant biological effects to measure!

Biostatistics vs. Lab Research

http://www.youtube.com/watch?v=PbODigCZqL8

REFINEMENT

Refinement

- Reduce or eliminate harm or damage to sentient beings
- Decrease
  - Animal use
  - Reduce impact of harmful procedures
- Increase animal welfare
  - Optimize well being

- Refinement is a complex concept. The following slides pinpoint some targets that commonly have potential for refinement.

Refinement strategies - Injections

- Use subcutaneous route whenever possible
- Use solutions with physiological properties
  - Salinity, pH, temperature
- Use pyrogenfree solutions intended and produced for injection in live animals or humans
  - Avoid injection solutions mixed on the lab bench
  - If necessary, use a pharmacy equipped with necessary competence and facilities to prepare solutions for injections

Refinement strategies for Anaesthesia

- Minimize handling and restrain unless the animal is especially trained for this
- Minimize induction and recovery time
- Use an approach that ease adjustment of anaesthesia depth
- Monitor and support vital physiological functions
  - (Temperature, breathing and O_2-uptake, Circulation)
- Keep monitoring the animal until it is completely recovered and waked up after anaesthesia
- Allow animal to recover and take up normal activities between repeated anaesthesia

Refinement strategies for Surgery

- Consider Non-Survival surgery
- Use pre-emptive analgesia
- Use post-surgical analgesia
- Use aseptic techniques
- Use the least invasive techniques
- Use least traumatic needles and sutures
- Use skilled experienced personnel
- Avoid bleedings

Refinement strategies for Surgery Minimal incisions and trauma will:

- Reduce
  - Recovery time
- Trauma (surgery)
  - Increase cytokines (IL-6, C protein)
  - Catecholamine, cortisol, blood glucose
  - O_2 consumption, pulmonary and kidney workload
  - Responses might last 10-14 days after surgery

Refinement strategies for Surgery Minimal incisions and trauma

- Preserve immune function
  - Preserve T-cell mitogen response
  - Monocyte responses
  - Intestinal transit recovers faster
  - Metabolic activities less disturbed
  - Reduce need for Antibiotics and analgesics post op
  - Reduce post op mortality and morbidity
  - Reduce number of animal

Refinement of surgical techniques

- Refinement of surgery also cause reduction
- Reduce
  - Animal suffering
  - Animal number
  - Data inaccuracy
  - Repetition of experiment
- Always aim for “best practices” / “gold standards” for surgical techniques

Refinement (+ Reduction) by Embryo Freezing

- Often mice are kept on shelf without using them
  - saves costs
  - saves animals
- Loss of mice / lines due to
  - genetic drift
  - infections
  - catastrophes
- Exchange of lines between labs
  - no need to transport live mice
  - protection against cross contaminations

HOW TO FIND INFORMATION ON 3RS

Information available - norecopa

http://www.norecopa.no

Information available

http://www.nc3rs.org.uk/

http://threers.ccac.ca/en/alternatives/

Information available

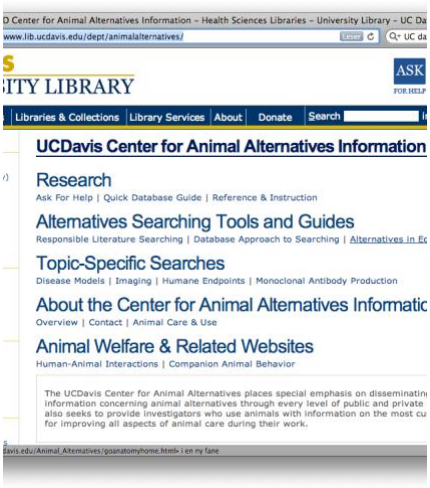
http://altweb.jhsph.edu

ALTWEB

http://www.frame.org.uk

FRAME

Information available



http://www.lib.ucdavis.edu/dept/animalalternatives/

Norina database

Journals

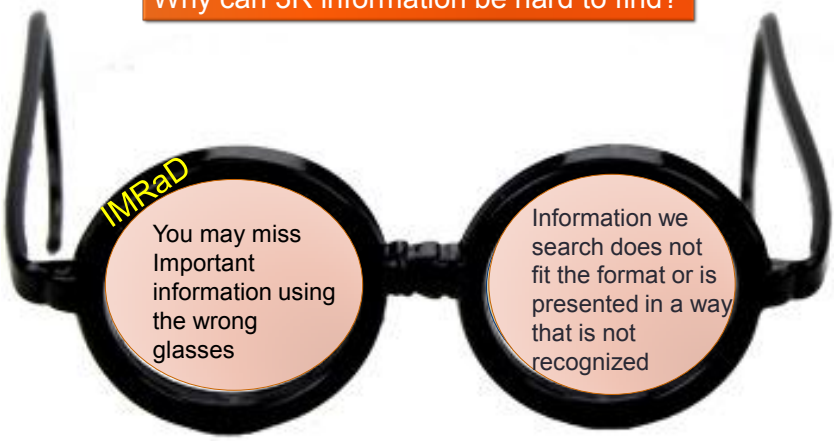
- ATLA
  - Alternatives to Laboratory Animals
- Animal Welfare
  - (UFAW)
- ILAR Journal
  - Institute for Laboratory Animal Research - http://dels.nas.edu/ilar
- Laboratory Animals
  - The Royal Society of Medicine Press
- Comparative Medicine
  - American Association for Laboratory Animal Science (AALAS)

norecopa 3R guide

http://oslovet.norecopa.no/3R/fag.aspx?fag=83

There are much, much more!!

Why can 3R information be hard to find?



Don't limit your search to PubMed and IMRaD structured papers!!!

IMRAD is an acronym for Introduction, Methods, Results And Discussion

3R summary

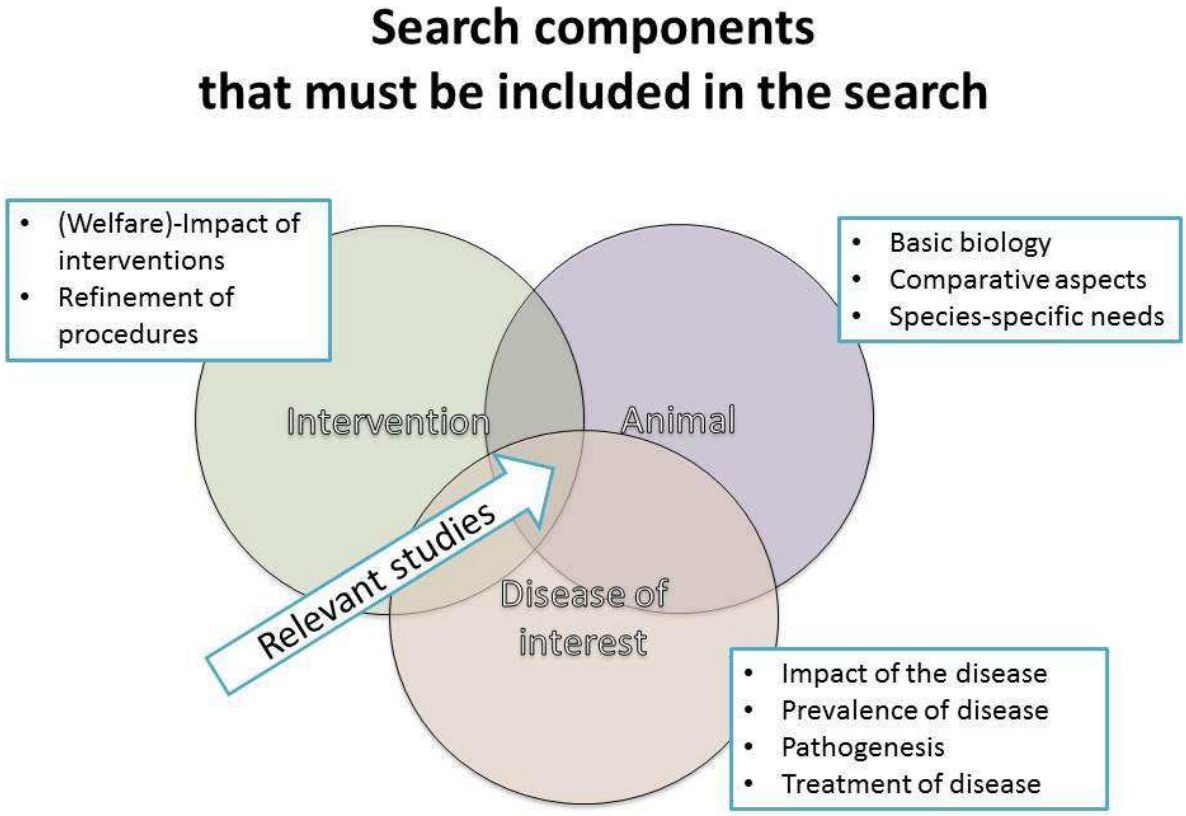
- Replacement
  - Use alternatives to animals whenever possible
- Reduction
  - Reduce number of animals to minimum
- Refinement
  - Reduce or eliminate harm or damage to sensitive beings
  - Optimize well being
  - Refinement is a complex concept. The aim of this presentation was to pinpoint some targets that commonly have potential for refinement.
- Much information is available

Conflicts between the Rs

- Is it better to use few animals that suffer more, or is it better to use more animals that each suffer less?
- How will you justify this?
- Discuss this in the break!!

Literature studies, choice of animal model and design of experiments

Below you find some presentations and teaching tools that are helpful in planning and evaluation of animal experiments.



Search key words

When you are planning an experiment it is common to do a literature search.

You must include more than 1 search component in your search. For biomedical research it is advised to include search for:

- **Intervention**: With focus on Welfare)-Impact of interventions and refinement of procedures
- **Animal**: With focus on basic biology, comparative aspects human vs animal and species-specific needs
- **Disease of interest**: With focus on impact of the disease, prevalence of disease, pathogenesis and treatment of disease

This can give useful information when you plan experiments both with regard to using the best model and to animal welfare.

Systematic reviews

Systematic review is a structured, thorough and transparent way of doing a literature-search. Several relevant sources and/or databases are included in the swerach that is based on relevant keywords, including commonly used synonyms («MeSH terms (http://www.nlm.nih.gov/mesh/)»). The word "mouse (http://www.ncbi.nlm.nih.gov/mesh/?term=mice+%5BMesH%5D)" can have several synonyms that you have to include. Similarly you find some for "rat (http://www.ncbi.nlm.nih.gov/mesh/?term=rat+%5BMesH%5D)" here.

Reasons to perform systematic review: Lessons learned from a study of omega -3 fatty acid supplementation in animal models for Alzheimer's disease. (Radboud%20university%20medical%20center%20in%20the%20Netherland%20has%20built%20a%20resource%20center%20for%20those%20that%20are%20interested%20in%20learning%20more%20about%20systematic%20reviews%20of%20animal%20experiments.%20You%20find%20more%20on%20this%20link.)

Radboud university medical center in the Netherland has built a resource center for those that are interested in learning more about systematic reviews of animal experiments. You find more on this link. (https://www.radboudumc.nl/Research/Organisationofresearch/Departments/cdl/SYRCLE/Pages/default.aspx)

The have also published video presentations from 1st International Symposium on Systematic Reviews in Laboratory Animal Science (http://www.umcn.nl/Research/Departments/cdl/SYRCLE/Pages/3RRCsymposium.aspx)

What have we learned from Cochrane Collaboration?

- Critical review of animal experiments.
- Translational value for humans
- Choice of statistical methods and how it influence results.

Paper: Systematic reviews and meta-analyses of preclinical studies: publication bias in laboratory animal experiments (http://lan.sagepub.com/content/45/4/225.full)

Meta analysis

Conclusions from several relevant studies are analyzed in a meta-analysis (http://en.wikipedia.org/wiki/Meta-analysis). This way evidence from several studies are used to confirm or reject theories for example between a drug and its effect on the body, disease-mechanisms et cetera that you don't easily get from a single study.

Meta analyses are useful in planning animal experiments for example to evaluate if a model is really suitable to predict an outcome or relevant effects. If 50% of studies show an effect and the other 50% show an opposite effect, which studies shall we trust, or can we trust any of them? Based on this analysis you can make a more qualified evaluation whether or not it is reasonable to set up new animal studies or if it's better to use other approaches to achieve more knowledge about a phenomenon.

Meta analyses are also useful and necessary in translational research from preclinical studies in animals to clinical studies in humans. Are conclusions from the animals studies so clear and reliable that they support continuous studies in patients, or do the result diverge in different directions?

Design of animal experiments

Michael Festing has developed a teaching (http://www.3rs-reduction.co.uk/) tool for planning of animal experiments including

- Proper design of animal experiments
- What factors influence the number of animals that are needed in a study

- Statistical methods
- How to save animal, time and resources by better planning of animal experiments

(recommended web browsers explorer, safari)

There is also a lot of control questions and self tests included

Michael Festing is a toxicologist and has been working with problems related to design of animal experiments for several years and he is the author of the book "Design of Animal Experiments (http://www.uk.sagepub.com/books/Book242188?siteId=sage-uk&prodTypes=any&q=9781853155130&pageTitle=productsSearch)" (SAGE)

Read more about Michael Festing her (http://en.wikipedia.org/wiki/Michael_Festing)e.

Calculating number of animals

Even when you base your group sizes in similar studies like:" We know from previous experiments that this study requires a minimum of n animals to obtain statistical power."

What are the relevant treatment effects, significance level and/or power in this study? Do you have an estimate of variation?

These numbers should be mentioned even if it's based on previous experience from similar studies.

Challenges in statistics and design of experiments

Below you find some cartoon videos from YouTube illustrating some common misunderstandings and challenges with regards to design of animal experiments and evaluating the result.

- Biostatistics vs. Lab Research (http://www.youtube.com/watch?v=PbODigCZqL8)
- Power of the test, p-values, publication bias and statistical evidence (http://www.youtube.com/watch?v=kMYxd6QeAss)
- What the p-value? (http://www.youtube.com/watch?v=ax0tDcFkPic)