Practical guidance for applying for animal experiments in FOTS

All applicants are expected to read and consult this guidance document when writing FOTS-applications.

Submitted FOTS-applications that lack much of the essential information described in this guide, will be returned to the applicant without any further comment than to consult the guide!

Content of this guidance document

Background and scope

The legal requirements for applications can be found in the Norwegian Regulation on animal experimentation which is concurrent with the EU Directive for animal research (2010/63/EU).

The competent authority for animal research in Norway is the **Norwegian Food Safety Authority (Mattilsynet)**

Applicants can find guidance information on applications at Mattilsynet, but the information is only in Norwegian:

- Hvordan søker du om godkjenning av forsøk? | Mattilsynet
- Hvilke forsøk må du søke om? | Mattilsynet
- Hvilke forsøk må du ikke søke om? | Mattilsynet
- Gebyr for godkjenninger av forsøk på dyr | Mattilsynet
- Tips ved etablering og bruk av scoreskjema i et dyreforsøk | Mattilsynet

This guidance document has been compiled to advice applicants on how to apply for experiment in the Norwegian electronic system for animal research – FOTS. It is based on the current legislative demands, the requirements and recommendations published by Mattilsynet, as well as the «Routine for animal experiments at Helsefak» (hereby called "the Routine") decided by the Faculty Director at Helsefak 7.7.2015 and last updated 23.04.20.

User account, password, and login to FOTS

Contact the Department Manager at the animal facility AKM (PMSK) by email to obatin username/password to FOTS.

The following information is needed from the applicant as well as from all planned co-workers in the experiment in order to be registered in FOTS:

1. Full name, E-mail adress and Phone
2. Documentation of required **theoretical training** according to the legal requirments (EU Commission Education and Training Framework
3. Documentaiton of **practical skills** as required by legislation (EU Commission Education and Training Framework)
4. Documentation of **CPD (continous updating of competence)** as required by the legislation (EU Commission Education and Training Framework)

FOTS can be accessed here: https://asp.gitek.no/fdu/pmws.dll/Login .

An English version of FOTS can be chosen before log-in by clicking the English flag .

Brief description on the rules of procedure for FOTS applications

Local assessment of FOTS applications prior to submision to Mattilsynet:

Mattilsynet requires that all applications are quality controlled locally by the the named animal care and welfare officer (= Person med særskilt kontrollansvar, **PMSK**) at the institution before applications are submitted to Mattilsynet.

When the responsible applicant submit a FOTS-application, the application first goes to the PMSK at AKM (the head of AKM) for quality control.

Upon submission of a FOTS-application by the applicant, the application first goes to local review at PMSK. The FOTS-application ***is NOT send automatically to the authority (Mattilsynet)*.**

The application is reviewed by PMSK, and is then either forwarded to Mattilsynet *or* reset to "DRAFT" in FOTS and send back to the applicant for revision if it has short-coming and is regarded as incomplete, c.f the Routine pt. 5B.

The processing time for the local assessment by PMSK depend on the number of applications in the pipeline and can vary between 1-3 weeks.

Only applications that fulfil the legal and formal requirements will be forwarded to Mattilsynet for processing.

Public summary and transparency

Certain fields in the FOTS-application are marked with a globe. Fields marked with a globe will be collected into a public summary which will be published at **Mattilsynet's web page** and in the **EU Commission ALURES database for non-technical summaries.**

Each field marked with a globe in the FOTS-application, has a text limit of 2,500 characters.

NOTE: Fields marked with a globe must not contain personal information or information that is to be except for public access.

When an application is submitted to Mattilsynet via FOTS it is automatically registered in the Norwegian official database for public the public sector, Einnsyn

In the Einnsyn register, the FOTS ID, title and name of insitution is provided, see example here: eInnsyn - Case: 2021/75510 - Norwegian Food Safety Authority

NOTE: **Anyone have the right to search for and order access to the documents published on eInnsyn. Unless the FOTS application and/or related documents in the case contain confidential information, it will be released in full at any stage of the application process after it has been submitted to Mattilsynet.**

NOTE: Applications that have the status of "DRAFT" or is submitted to "LOCAL REVIEW" in FOTS is not available for anyone else than the applicant, the co-workers listed in the application, and PMSK.

In our experience animal welfare organizations regularly ask for access to FOTS applications (in any stage of the process after submission to Mattilsynet). In addition, we also experience that journalists may ask for access.

NOTE: It is possible to withheld confidential information, please see page 7 for more information

Application assesment by Mattilsynet

The processing time in Mattilsynet can be up to 40 working days (+ 15 days for especially complicated applications). Mattilsynet can decide to use external experts to review applications. Mattilsynet can also contact the applicant for supplementary information.

The decision letter from Mattilsynet is communicated to the applicant by email, with copy to PMSK.

NOTE: *Mattilsynet may set conditions in their decision, so it is important to read the decison letter thoroughly.*

NOTE: A copy of the decision letter is send to UiTs offical post (postmottak@uit.no) and archived in E-phorte and forwarded to the applicant´s institute/department

Mattilsynet charges fees for handling of applications. The current fees (as of 2022) are as follows:

Category*	***Type of application***	***Fee (NOK)***	***Comment***
B	Application for change in a current FOTS application	1660,00	If the application only regards extension of the approval date, fee is *not* charged.
C	Application for pilot study	4165,00	
D	New FOTS appliation	6665,00	Mattilsynet can decide that this fee can be reduced to category C for *"thoroughly prepared and non-complex applications"*

** The categories are defined in the "Regulation on fees for defined services of Mattilsynet"*

The applicant can appeal to Mattilsynet if the applicant believe the fee has been set unreasonablly high. The applicant will have to appeal within 3 weeks following Mattilsynet decision.
NOTE: PMSK will not be involved/contribute to such appeals as this is regarded as being a matter between the applicant and the Mattilsynet.

Application for changes in a current FOTS application is charged with fee (unless the only change is to extend the project approval period). *Notification of changes* is free of charge. Please see the chapter on **Changes in approved protocols in FOTS** in this guidance document for more information.
NOTE: *Only projects that have a valid approval can be extended* (e.g. if approval date is expired you will have to send in a new complete application in order to continue the experiment)

THE APPLICATION FORM IN FOTS – STEP BY STEP

The table on the following pages, follows the heading titles and format found in the online FOTS-form and provides guidance and advices based on the legal requirements as well as some tips and advice for typical "pit-falls".

NOTE: The field marked in RED and with a globe is part of the public summary that will be collected into the EU Public summary. These fields must not contain any confidential informaiton nor information that can identify persons or institutions.

NOTE: tabeles or tabulated text that are copied/made in the online FOTS-form becomes unreadable nonsense in the pdf-file that is generated of the application form when the application is submitted. Tables may be very useful, but **tables and figures have to be *uploaded as attachments* in FOTS** and not included in the text field in the electronic application form.

General information

Working title:
Provide a short and descriptive title for the project

International ID:
ID in the EU's Public summary database ALURES. The number is generated automatically, not to be filled in by the user.

Establishment where the experiment/project will be conducted:

Only topical if you are registered as user in FOTS of more than one animal facility in Norway. Select the animal facility at which the experiment will be conducted if you are registered as a user at several animal facilities in Norway.

Application category

Two possibilities, either "Pilot experiment" or "New Experiment"

Keywords:

Enter keywords that are clear, specific, and informative. The purpose of the keywords is for those who want to search the EU Alures database of public summaries.
You can either choose keywords from the available list or create new keywords. You can provide up to 5 keywords. Animal species nor objective of the study should not be listed as keywords (as these are generated into the public summary from other fields of the FOTS-application).

Other
Field experiment, including localization and scientific justification: Experiments outside approved facilities. Not relevant at AKM.

Multiple generic projects cf. § 6, part 5, refers to special projects performed within industria/commercial toxicology/pharmacology. Not relevant at AKM.

Previous experience with comparable procedures (YES/NO)

It is relevant and recommended that the applicant includes the experience of the applicant and all co-workers when answering this question

Experiments/procedures funded by

Choose the most suiable option in the drop-down-menu are:
"Annen finansieringskilde" = Other finance source
"Den norske kreftforening" = The Norwegian cancer association
"EU/EØS midler" = EU/EEA (European Economic Area) funding
"Forskningsrådet" = The Norwegian Research Council
"På oppdrag fra offentlig etat" = Commisioned by a public agency
"På oppdrag fra privat bedrift" = Commisioned by a private company

NOTE: All applications are expected to hold the same quality irrespective of funding source. Applications supported by large and important funding institutions (like EU and/or Forskningsrådet) obviously is an indicator of relevance and high scientific quality, but it is important to realize that funding from these sources does not consitute an ethical approval to perform animal experiments. Consequently, **all applications are expected to contain the same level of detail and be complete, irrespective of funding source.**

Planned start and end dates
The applicant is advised to apply for sufficient project time. Projects tend to take more time than planned, the approved project time is without any ethical consequence and all applications will be invoiced by Mattilsynet. Applications can be approved for up to 4 years and it is advicable to take advantage of the full availbe project approval period.

NOTE: Start date should must not be prior to application submission, as it will imply that the project was started prior to submission. Take into consideration both local control of application (1-3 weeks) and Mattilsynet´s handling time (up to 40 working days) when setting the start date.

Invoice information (reference number and invoice adress)

Mattilsynet charges fees for handling of applications (see page 4) and the applicant needs to provide the correct invoice address in FOTS.

NOTE: UiT (and many other public institutions, like e.g., UNN) have electronic and centralized invoicing systems requiring that correct invoice address and invoice reference is provided.

1. **For users at UiT:**

If the fee should be paid by funds that the applicant has on UiT, the following information should be provided:

a) Invoice reference

Provide the "Delprosjektnummer" (9 digits) and "Koststed" (8 digits)

If in doubt which Delprosjektnr/Kossted to use, please contact the administration at your institute/department.

b) Invoice adress:

UiT, Fakturamottak DFØ, PB 4710 Torgarden
7468 Trondheim

NOTE: UiT has recently (2022) changed invoice adress (it is now in Trondheim, not Tromsø). The field to provide invoice adress in FOTS has limited space (max. no. of characters) so please use the adress provided above

2. **Invoice reference and address for external users:**
   Provide *correct invoice address and valid reference number* (contact your institution for advice).
   Most public institutions have centralized invoice adress.
   For UNN the invoice adress is:
   UNN HF C/O, Fakturamottak, PB 3232
   7439 Trondheim

   Make sure to provide invoice reference to!

Public access (confidential information)

Product sensitive details are neither required nor relevant in an ethical application. Generic information on the type and class of test substance and its expected effect on the animals is most often sufficient. The sections of the application that is to exempt from public access must be specified – the entire application cannot be exempt.

Do the application contain confidential information. YES/NO.

If yes, please refer to relevant act(s) and regulation(s)

The relevant Acts are § 13 in the Norwegian Public Adminstration Act and/or the § 13 in the Norwegian Freedom of Information Act (contact PMSK if you need advice)

Common/legally accepted reasons for withholding information include patent process, protection of IP rights, competitive research, economoical/business relations

If yes, which information do you want to keep from public access?

Product sensitive details are very seldom required in an ethical application. Generic information on the type and class of test substance are most often sufficient as long. Mattilsynet will rarely need sensitive information in their review of and decision on an ethical application. Information related to the type/class of test substance will usually be sufficient and equally relevant substitute for sensitive information.

The **sections of the application that is to be exempt from access must be specified** – the entire application cannot be exempt. Mattilsynet will not consider your wish for denied public access until a public access demand is being raised. Mattilsynet will confer with the applicant before taking a decision regarded public access to the information.

NOTE: All fileds marked with a globe must **NOT** contain confidential information

NOTE: If the application contains confidential information, Mattilsynet will not decide on the matter of confidentiality until *until they recieve a request for access from the public*. If Mattilsynet receives such a request, the applicant will normally be contacted by Mattilsynet and consulted. In order for Mattilsynet to withold information they have to doument that there are legal grounds (e.g. authorization according to relevant §§ in the Public Administration Act and the Freedom of Informaiton Act). If Mattilsynet decides that the applicant has valid legal grounds and the information is witheld, the one requesting access can still apeal Mattilsynet´s decision. Then the applicant will be consulted again.

NOTE : If the applicant indicate that NO information is confidential, Mattilsynet will release the full application if they recieve a request for acess of information from the public. Then the applicant will *not* be consulted in advance, but notified retrospectively.
In our experience is it mostly animal welfare organisations and journalists that request access to information.

Applicant and participants (co-workers)

Institution:

Name	Access	Role/education	Course in animal research:
Applicant	NA		
Participant 1	Read or write		
Participant 2	Read or write		
Etc.	Read or write		

All participants that are involved in **planning or conducting the animal experiment** must be registered as participants in the application.
All participants needs to fullfill the legal requirments for theoretical and practial training suitable for their role in the experiment.

NOTE: If a planned co-worker is not previously registered in FOTS, the responsible applicant must send name, telephone number, mail address, course and training documentation (pdf copy of course certificates and documentation of the required practical training) to PMSK.

NOTE: If the lab animal course is a non-Norwegian course and not accredited by FELASA, please include an official course description and other relevant details (course length (days/hours), lab animal species involved, practical training) with the course certificate. All documents must be in Norwegian or English and only officially translated document will be accepted!

NOTE: There is also a requirement for a course module on Norwegian legislation for persons who have taken courses abroad. Contact PMSK for further information.

NOTE: Personnel that only do ex vivo work (handle animal cadavers, organs or other samples after termination) and/or personnel that only assist in data capture without handling live animals are *not* considered participants and are hence not relevant to include in an ethical application. The same goes for the permanent staff at AKM (the animal caretakers).

NOTE: Information on how to add a new co-worker to an already approved FOTS-application can be found in the section on Changes in approved FOTS applications in this guidance document.

Background and purpose

Purpose:

Select the relevant purpose in the drop-down menu. A similar purpose and structure, is used in annual reporting.

The experiment's objectives

Give a short presentation of the background and purpose addressing scientific unknowns, and/or scientific or clinical needs.

Clarify the current state of knowledge on which your project intends to build. Explain the way in which the project will help to advance knowledge through filling a knowledge/information gap.

As a general rule one og more **clearly defined hypothesis** need to be described. However, it is acceptable and normal that early phase projects often are descriptive and/or explorative by nature, with few or no defined hypotheses. All other projects, and particularly projects describing continuation of ongoing research project, are expected to describe one or more hypotheses that warrant the activity and provide context to the study design and practical performance.

NOTE: Keep in mind that this text field constitute a part of the public summary and therefore should not be too technical and not contain confidential information.

NOTE: For complex/larger projects, it is advisable **to attach a more through description of background and objectives**. Where relevant, **literature references** are important to include such an attachment.

NOTE: Attachement cannot replace requested information in the FOTS-form. Therefore all fields in FOTS needs to be filled out properly. Attachements should be used to supplement and provide additional informaiton that is not suitable to include in the online form.

What are the potential benefits likely to derive from this project?

'Potential benefits' are to which extent humans, animals, plants, or the environment may potentially benefit if the project meets its objectives. It relates to the value that may be placed directly on the outcomes of the programme of work, both in the short term and taking account of possible longer-term impact.

Describe scientific and societal utility, directly and indirectly: Who will benefit? In what way, and what will it mean for society? Both immediate/short-term and long-term benefits should preferably be adressed.

It can be useful to address some/or all of the following points in your application to provide detailed information **about the benefits of your project**:

- Wherever possible, "increased knowledge" as the primary benefit should be linked to a more tangible strategic goal, even though any wider benefits may be much further in the future and less predictable.
- Explain why the benefits go beyond "it would be nice to know".
- Scale of improvement (for humans, animals or the environment) and burden to society of the problem (both in basic and applied research).
- Basic research driven by hypotheses needs to confirm that the hypothesis is scientifically sound and realistic
- In some areas of basic research expanding knowledge can be a suitable objective in its own right – but *should always be linked to dissemination* of results, whether positive or negative (having regard to intellectual property), and potential longer-term benefits.

Common problems:
Failing to adequately adress benefits (lacking wider context; unsubstantiated/unrealistic claims of potential benefits; benefits not linked to the objectives set out in the application).

When assessing a FOTS application Mattilsynet will perform a **Harm-benefit analysis,** in which the potential benefits of a research project are weighed against the harms likely to be caused to the animala.
A judgement is made as to **whether the likely harms are justified by the likely benefits.** *This judgement is fundamental with regard to granting or rejection of the application. It is the applicant that must provide enough information about benefits and harms, in order for a HB-analysis to be performed!*

Research animals (characterisation, severity, anesthesia/analgesia, etc.)

Animal species and strain/line, age, and weight

NOTE: Each species AND each strain must be **registered seperately** .

NOTE: Use correct scientific names for strains/substrains/lines. For commercially available strains it is advisable to include stock number and/or web-link to the specific strain

Specification of age, weight and duration of the experiment need to be in accordance with each other (e.g. if it is stated that 5 weeks old male Wistar rats will be used, it cannot be stated that the start weight is 300g). Growth- and weight curves for commercial available rodents can be found at the web page of the suppliers: Charles River; Taconic; Envigo; The Jackson Laboratory
NOTE: if you plan to use a new strain, please contact PMSK in advance so that availability and microbiolgical health status of the animals can be checked at an early stage of the planning phase. Animals coming from non-commercial sources (e.g. other universities) are not accepted to the SPOF-barrier for rodents at AKM. Please contact head of AKM for further advice.

Number of animals:
The number of animals stated here **must conform with what is stated under "Calculation of numbers of animals".** *If the numbers in these two sections of the application diverge, the application will automatically be returned to the applicant for revison.*

Number of reused animals (cf. § 17)/Reuse not applicable:
NOTE : the regulation is strict on reuse, requiring special grounds for allowing reuse. This is to reduce the harms to individual animals. **Reuse means using animals in a new FOTS id that have previously been used in another experiment (another FOTS id).** Multiple planned procedures in the same experiment (same FOTS id) does not represent "reuse".

Experience with this species: No/Yes.
It is relevant and recommended that the applicant includes the experience of the applicant and all participants when answering this question (provided that the experienced participants actually participate in performing the study).

Duration for the most affected individual animal (d, h, min).
It is the maximum duration of the experiment for the most affected individual animals that is to be stated. Assumptions, averages, or the time for doing the surgical intervention alone will be erroneous and misleading. Information about the duration for the individual animal is vital for review of the application and for follow-up when the experiment is being carried out.

The duration must be based on the needs of the scientific endpoints, not what entails the greatest possible flexibility. Acclimatization, where no procedures occur, does not count as part of the duration of trials.

What are the expected severities and the numbers of animals in each severity category?

The number of animals assigned to the different severity categories must conform what is described under "procedures" and "adverse effects" in the Methods part of the application!

The severity categories are defined as follows (according to increasing severity):

I. Non-recovery: Experiments which are performed entirely under the same general anesthesia from which the animal shall not recover consciousness.

NOTE: This type of experiment is often also referred to as "acute experiment" or "terminal". This category **only applies** for animals that are anesthetized and the procedures are performed and the animal killed *while still being in the same anesthesia*. If any regulated procedures (like for instance an injection) is given to the animal prior to anesthesia, it is not a non-recovery experiment. Similarly, if the animal is allowed to wake up from the anesthesia before it is euthanized it is not a non-recovery experiment.

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

III. 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 as well as procedures that are likely to cause moderate impairment of the well-being or general condition of the animals.

IV. 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 as well as procedures, that are likely to cause severe impairment of the wellbeing or general condition of the animals

NOTE: experiments that result in severe pain, suffering or distress, which is likely to be long-lasting and cannot be ameliorated, is prohibited (§ 13).

NOTE: Consult **the examples provided** in Part III in the Appendix B of the Norwegian Regulation (equivalent to Part III in Annex VIII in the EU directive).

For further guidance and examples of severity classifications, the applicant should consult the EUCommissions Guidance Document on Severity Classification

Animals with a deviant phenotype: Do the animals have any congenital or hereditary disease/illness or other abnormalities related to their phenotype that may impair their welfare (e.g. diabetes, autoimmune disease, increased tumor incidence, dental defects)?

All genetically altered animal strains with known hereditary disposition to a harmful phenotype, including animals with genetic defects that do not arise from genetic modification, are to be considered.

"Harmful or deviant phenotype": is to be understood as an animal who is likely to experience, as a consequence of the genetic alteration pain, distress, suffering or lasting harm equivalent to, or higher than that caused by the introduction of a needle in accordance with good veterinary practice (c.f. Norwegian regulation and Directive 2010/63/EU).

NOTE: "genetically altered animals" include genetically modified (transgenic, knock-out and other forms of genetic alteration) and naturally occurring or induced mutant animals).

NOTE: Note that breeding and use of GM lines is subject to application until the applicant has completed an Animal Welfare Assessment as required by the European Commission, and documented the absence of a harmful phenotype.

NOTE: Induced models, like for instance high-fat feeding to cause obesity/metabolic syndrom shoud not be described here. Induced models should be described under "methods".

Provide a description of the phenotype. If the clinical effects of the altered phenotype depend on the age of the animal, describe the typical progression of the alterations, and the age at which the animals will be used and/or terminated must be defined.

If you claim that a genetically altered animal line does not have a harmful phenotype *this claim needs to be documented*

Describe which precautions, efforts and/or treatments the animals will be given in order to safeguard their wellbeing and welfare and when this will be relevant:

Actions can range from altering of the diet (e.g. autoclaved water/feed to immunocompromised animals), altering the husbandry routines (e.g. aseptic housing or increased frequency of cage changes for diabetic animals due to increased urine production), aseptic handling (immuno-compromised animals) to more frequent monitoring routines, to termination.

If the clinical effect of the deviant phenotype is dependent upon the age of the animal, the age at which the animals will be used and/or terminated must be defined.

Sedation, analgesia and anesthesia:

Appropriate use of anesthesia and analgesia is very important information during application review. Information on this topic is quite often incomplete and the applicant is advised to contact PMSK for advice if in doubt.

Anesthesia and analgesia has to be described in terms of:
- active ingredient
- concentration (mg/ml)
- dose (mg/kg or similar)
- administration route
- adminsitration frequency
- duration of administration. T

NOTE: The frequency and duration of post operative analgesia treatment is very important information, frequently lacking.

NOTE: Pre-emptive analgesia is a general requirement for all anaesthetic protocols. Isoflurane or other anaesthetics that lack analgetic properties are not allowed to use as single agents for painful procedures.

NOTE: Several variants of common anesthesia mixtures are known in the literature (e.g. at least 3 different mixtures for rodents containing Zoletil are known). When using mixtures of anesthetic agents, the dosing volume (ml/kg) and the mixing recipe and/or the concentration of active ingredients (mg/ml) must be described.

Double-check that the anesthesia and analgesia protocol concurs with the description in the Methods section.

Use of neuromuscular blockers:
The Norwegian regulation states the following (§14): “Animals must not be administered drugs that abolish the expression of pain unless a suitable anesthesia or analgesia is provided. Scientific documentation of the need and proposed procedure, including details of anesthesia, analgesia and monitoring routines, must be submitted”.

Analgesia not applicable/Rationale for why analgesia cannot be used

You should ***only*** tick of this alternative if the procedure will cause pain, but the use of analgesia is incompatible with the scientific purpose of the study. The omission of analgesia in general or classes of analgesics must be described in the application (cf Norwegian regulation § 14 and Appendix B, 3f).

What will happen to the animals kept alive at the end of the procedure?

Only the first option, re-use, may be relevant at AKM. It is reminded that “re-use” means using animal in another FOTS application. (Performing multiple procedures on animals included in the same FOTS-application does not represent re-use)

(The number of animals to be “returned” or “relocated” is not applicable for animals used at AKM).

Calculating number of animals

Explain the choice of species and the related life stages

Provide the scientific justification and relevance for the chosen species, strain, genotype, sex and age class.

Give the rationale and justifications for the numbers of animals that will be used.

The justification and **prospective calculations** on the required number of animals is a common shortcoming in applications.

The Regulation (§ 6.2) specifically requires that a **pilot study** is carried out if there are uncertainties about the number of animals needed or the effect on the animals of new methods are unkown.

The Regulation (§§ 9, 11) also specifies the number of animals used in experiments should be *reduced to a minimum without compromising the objectives of the project and the experiment should be designed in order to use as few animals as possible*.

Delayed processing of the ethical application is likely if animal numbers are not justified by valid prospective calculations. If (pilot-) studies with the same or similar animal models have been previously performed or are available in the literature, the results from these should be used for prospective calculation of required number of animals. Refer to previous FOTS ids from pilot studies and/or refer to literature of previous similar experiments when relevant.

NOTE: The number of animals must be in accordance with the numbers stated in the section «Research Animals».

NOTE: Any replication of the experiment and/or experimental groups needs to be justified.

In some experiments (e.g. surgical interventions, post mortem processing of organs/tissues etc.), a certain degree of technical failure or data loss is expected when the operated animal or organ does not comply with defined inclusion criteria. A certain number of extra animals, in addition to the calculated number, is then reasonable to include to compensate for excluded animals. However, the extra animals needs to be justified and reasonable and included in the total number of animals. Do not describe the need for 100 animals and apply for 110 (under «Reseach Animals) without explaining why 10 extra animals are needed.

Calculating the number of animals in breeding projects (i.e. FOTS application needed to breed animals with a non-documented or harmful phenotype) can obviously be difficult. The input that should be included to calculate the estimated number of animals is fertility rate, genetics and how it affects the number of offspring with the desired genotype, and the number of offspring required for experiments.

Describe all experimental groups and group sizes. A table describing the groups and group sizes may be added as an attachment to this application.

NOTE: tables copied directly into the text fields in FOTS and table-like organization of data directly in the text fields becomes chaotic nonsense in the pdf print-out that is generated when the application is submitted. Tables are often very illustrative, but include tables as Attachments.

For complex project with many different animal groups and/or many different procedures/interventions/tests a timeline or flow chart should be included as attachment in order to get an overview of «what happens when».

Describe the statistical method used to determine the number of animals. If statistics can not been applied, describe why.

It is not sufficient to write “Power analysis” or “Resource equation” and not provide any further information. If Power analysis, Resource equation or similar prospective method of calculation have been applied, the input parameters and justification for the chosen values of these parameters should be included, e.g. like size of the effect of biological parameter of interest, variation, desired power, significance level, etc.

Alternatives/3Rs

Replacement

Why is it not possible to achieve the aim of this experiment without the use of animals? What alternatives have been considered and why were they rejected?

The requirements for documenting evaluation of possible alternatives to animal experiments are stringent in the legislation. Stating "not relevant" or "in vitro studies are the only ones that will answer the research question" or "cannot be peformed in humans" will *not* be accepted.

It is relevant to include description of in vitro studies that have been conducted prior to or in parallell with the animal experiments, but then an explantation should be provided why the use of animals is still needed for the research in question (e.g. which questions/investigations cannot be achived using in vitro methods).

To fulfill the legal requirements the alternatives must be presented *as objectively and in depth as possible with a presentation of pros and cons of available alternatives versus the applied use of research animals*. Only presenting your own views and conclusions on the relevance of alternatives will not be sufficient.

Conclude with the up- and downsides of the available alternatives versus the applied use of research animals.

Reduction*:*

When the use of animals is unavoidable: What has been done to minimize the number of animals and still achieve valid scientific results?

The most important measure will be a logic and transparent prospective calculation of animals. Critical review of how and when control animals are used could also be relevant.

Performing an experiment step-by-step and analysing data before including further animals/new cohorts may also be an effective way of reducing animal numbers.

Refinement

What measures have been planned to optimise the wellbeing and welfare of the animals? (Keywords: analgesia, anaesthesia, endpoints, environmental enrichment, surgical techniques, sampling techniques etc.)

Experience from prior (pilot) experiments or literature must be used to reduce and refine future protocols with the goal to perform the planned experiment as carefully as possible with regard to animal welfare.

NOTE: if the methods that will be used are new and the effects on the animal is unknown, the regulation demand that a pilot study is carried out.

Some key words regarding refinement include:

- Setting early humane endpoints
- Appropriate use of analgesia
- Appropriate acclimatisation and adaptation (habituation) of the animals to handling and other procedures when relevant
- Considering the animal´s natural needs (use of environmental enrichment, limit social isolation of gregarious species etc)
- Providing support therapy (heat, fluid, recovery gels, etc.) following surgery

Please provide reasons for the planned fate of the animals after the procedure

In most studies using laboratory animals, the animals will be euthanized as samples/tissues are needed for further ex vivo analysis.

Methods description

When writing ethical applications and performing animal experiments, one simple rule applies: ***Write what you do - and do what you write*.** The description in the application must enable Mattilsynet to assess: the planned technical design and methods used in the experiment, how this will affect the animals and the research results, and how the study will meet the described study objectives. Describe all the planned procedure **in a chronological order** so it is possible to evaluate the cumulative effect on the animals and to understand "what happens when". The technical methods should also be described clear in order to facilitate evaluation of compliance when the experiment is actually being carried out.

NOTE: Refer to attachments (literature, figures, tables, flow-charts) *when relevant* to supplement the text, but attachment should not be a substitute for submitting a complete text in the methods section of the online application form.

Preparation of the animals before the actual laboratory experiment

Describe any purchase, transport, quarantine/acclimation, housing, environmental enrichment, feeding regime.

Answer all the points listed, included cage type, number of animals in cage/bin, type of environmental enrichment etc.

If the animals will be handled frequently during the experiment, a period of gradual adaptation to handling should be included. Similarly, use of equipment that requires an activity that is not voluntary and/or normal (e.g. running on a treadmill) will require gradual training and adaptation of the animals prior to study start.

NOTE: If the housing conditions will be different than standard for AKM it has to be described clearly in the application. This includes single housing of social species (like rodents and pigs). Justification as well as indication of necessary time should be provided in the application if single housing is regarded necessary. Similarly, other deviation from standard housing conditions (light, temperature, altered feeding regime etc.) needs to be thoroughly described.

NOTE: cage groups should not be reorganized after acclimatization and the number of animals per cage should therefore be considered carefully when setting up the study plan.

Which procedures will be applied to each animal during the experiment *(e.g. restraint, handling, tagging, internal transport, anesthesia, surgery, administering of test substance, and more)? Describe the procedures as regards method, duration, and frequency. For blood samples the volume and method must be described. For injections location, method administering, and volume to be administered have to be described. Describe incarceration/immobilisation necessary to perform the procedure.*

- All procedures, also those not involving surgery or invasive techniques should be described.
- ID marking must be described, and also geno-/phenotyping if relevant. If ID-identification of the animal is regarded unnecessary this should be stated (in order for it not to appear as an omission)
- Write the text chronologically so that it is clear when the different interventions and procedures are taking place. If multiple interventions are performed, attach and refer to a timeline or flow-chart to supplement what is written in the text so it is possible to evaluate "what happens when" with each individual animal. It has to be clearly described the number of times and when an intervention/procedure is being performed on the animal. The expected (cumulative) effects on the animals have to taken into account and described.

NOTE: if multiple interventions are performed you need to consider the need for recovery period in between different interventions (e.g. anaestheisa may cause intermediate weightloss; blood can not be sampled in too large volumes too often; etc).

- The regulation has a general requirement for anesthesia when performing procedures on research animals. However, anesthesia can be omitted *if anesthesia is considered more stressful than the procedure itself* or if anesthesia is considered incompatible with the study purpose. Literature clearly document that physical restraint is very stressful for rodents. Only routine procedures, that can be completed rapidly by skilled technique and require short periods of physical restraint, can be performed under physical restraint only. Routine SC/IP injection and standard blood sampling are examples of such procedures. When physical restraint or anesthesia will be used for sampling, injection and/or other procedures, the number and duration of (repeated) procedures, anesthesia and physical restraints has to be described.

- Even if anesthesia/analgesia has been described in the table in the "Animal section" of the application, it is recommended to include a complementary description of the methodology and timing of the anesthesia and analgesia protocol in the methods section as well. Analgesia, observation time after conclusion of the surgical intervention and supportive therapy (heat, fluids, other) should also be described. Ensure that the drugs and dose levels of anesthesia and analgesia in the Methods section and Animal sections are identical. Anesthesia and analgesia are central elements in the cost-benefit analysis and one of the topics that most often give rise to questions and requests for change.

- Provide a detailed and supplementary technical description of all interventions:

    - Surgery (incl. preparation, the surgical interventions, equipment used; wound closing, etc.)

    - Administration of substances (oral, injections etc): provide VOLUME, DOSE and ADMINSTRATION METHOD

    - For guidance on the max. recommended volumes for injection/oral administration to common laboratory animals see i.e. this article: http://onlinelibrary.wiley.com/doi/10.1002/jat.727/epdf

    - Add all test substances, drugs and chemicals that the animals will be exposed to. Dose levels are always relevant, but remember to also include information on dosing VOLUME.

NOTE: If the test substances might cause adverse or toxic effect (intended or unintended) the expected clinical effects of the animals have to be clearly described. If you state that the substances will *not* have any effects on the animal, references needs to be provided – the burden of proof is on the applicant! A pilot study will have to be performed if the effect on the animal is unknown.

    - The VOLUME to be administrated is very often lacking. This is essential information in order to evaluate if the volumes are adapted to the animal species and animal size/age. Please use and refer to published guidelines regarding max. volumes (e.g. http://onlinelibrary.wiley.com/doi/10.1002/jat.727/epdf)

    Also, provide details on dose level (volume and quantity of active substance) per dosing, number of dosages, administration method and speed of injection for iv. (ml/min or ml/kg/h).

    - BLOOD SAMPLING: Include volume per sample, number of samples, sample frequency and sampling technique. This is essential information in order to evaluate whether the planned blood sampling regime is adapted to the animal species and animal size/weight/physiological status. Follow published guidelines. UKs 3R-center has a very useful webpage providing guidance for blood sampling of common laboratory animals.

NOTE: terminal blood sampling from the heart from rodents requires surgical anaesthesia (use of CO_2 is not allowed)

NOTE: the retro-orbital blood sampling technique for rodents is not a method that is recommended and will usually not be allowed in Norway as routine method

NOTE: attachments may supplement and expand the information supplied, but should not be a substitute for submitting a complementary text in application form.

Make a description of the experiment's design, and pinpoint any experimental endpoints. *You are encouraged to include an illustration displaying what will be done to the animals or groups of animals at what time (timeline).*

Describe clearly the different groups; the scientific endpoints/outcome measures, and how animals are allocated to groups (randomization or other procedure); describe which analyses that are performed and if blinding is being used.

For larger/complex project it is advisable to attach more information in tables/flowchart

What are the expected impact/adverse effects on the animals. *Examples: pain, weight loss, inactivity/reduced mobility, stress, abnormal behaviour, and the duration of those effects?*

NOTE: in the pdf that is generated of the application the title of this point is "Reason for indicated accumulated severity for the animals that are most affected"

You should address the potential adverse effects (= harms) and the description and assessment should *be systematic, realistic, and based on scientific and clinical knowledge*. If there are uncertainty which harms that will be caused, the legislation require that a pilot study is performed.

Describe which effects that may occur, when they might occur, and for how long (duration).

If you state that "no adverse effect" is expected, it needs to be validated/documented – the proof of the burden lies on the applicant!

If different animal groups will experience different harms, be clear and systematic when describing the various groups.

The assessment of adverse effects must conform with what is listed under "Procedures".

NOTE: Assessment of potential adverse effects includes *more* than just evaluating potential pain, it also includes stress, aversion, any limitations in expressing natural behavior (including modifications on the housing, husbandry and care standards), etc.

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If multiple procedures are performed, consideration must be given to the cumulative strain/burdens (and the need for recovery periods to reduce such burdens).

Follow-up and supervision *before, during and after procedures.*

Take into account all the adverse effects that can be expected during the whole experiment ("critical periods"). This provides the foundation for the requirements for welfare assessement, follow-up and supervision of the animals during the experiment (as well as for defining the humane endpoints).

For terminal experiments and for experiments involving surgery: assessing the health status of the animal *prior to* anesthesia as well as continuous monitoring of anesthetic depth and the animal´s physiological condition during the surgical procedure are essential components of follow-up and supervision.

The animals should minimally be observed once daily, but for some experiments more frequent observation of the animal than 1/day is needed either during special critical periods or throught the whole experiment.

If no adverse effect are expected (again: the burden of proof is on you the applicant!), it will be sufficient to state that the animal will be under "standard daily observation by the staff at AKM". In all other circumstances a detailed plan for follow-up and supervision of the animals have to be provided with identification of critical periods, welfare assessment scheme and frequency of observation.

A score sheet should be used when relevant. The score sheet should be species- and procedure specific with relevant, measurable clinicial parameters. It should include examples for the different scores and which

measures that will be implemented following a specific score. **An example Score sheet for rodents is attached at the end of this guidance document.**

NOTE: You must adapt the score sheet with relevant parameters and assessment criteria suitable for your particular experiment. For example, if surgery is peformed assessment of post-operative pain and wound healing is essential. If weight loss is expected, weighing the animal as well as body condition assessment is essential, etc.

NOTE: Score sheets should not be included when you do not expected markedly reduced condition or marked body weight loss (i.e. not for non-recovery and not for mild severity experiments)

Method of euthanasia *If animals are to be euthanised:Which method of euthanasia will be used (cf. regulations § 16, part 2 and appendix C)?*

Appendix C in the Norwegian Regulation (equal to Annex IV in the EU directive) contains **a table listing allowed euthanasia methods** for various species that you need to consult.

NOTE: for several of the methods listed in App. C **there are restrictions and conditions for its use**.

When using anesthetic overdose: Provide active ingredient , dose and administration method. When using pentobarbital i.p. to rodents it is recommended to sedate the animals first with gas anaesthesia.

NOTE: The dose for pentobarbital for euthanasia should be at least 4 x anaesthetic dose (e.g. > 200 mg/kg for rodents and pigs)

NOTE: Some of the methods described in App C, including killing of rodents and rabbits by concussive/ percussive blow to the head and killing of rabbits by electrical stunning, will NOT BE ALLOWED routine methods at AKM, Helsefak.

Deviating method of euthanasia *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).*

NOTE: **You should only fill out this field, if you are using a method that is NOT listed as approved method in the regulation!**

Any method of killing that deviates from those described in App.C must be explicitly described and scientific arguments provided for why the methods in App. C cannot be used.
It has to be scientfically documented either that the methods is as equally effective on welfare grounds as listed methods or that the listed methods cannot be used due to conflict with the research objective.
You need strong arguments to receive approval for using a non-listed method!

Criteria for humane endpoints., *i.e. setting of clear, predictable and irreversible criteria that allow early termination of the experiments before the animals experience significant harm whilst still meeting the experimental objectives. An adapted score form may be attached to the application.*

The purpose of humane endpoints is to prevent uncessary animal suffering, e.g. suffering that is not necessary to achive the objective of the study.

Humane endpoints (HEPs), are predefined criteria that determine when the procedure/experiment should be ended for the individual animal. The humane endpoints provided will heavily influence the severity classification of the experiment.

Subjective and little specific HEPs, which give no guidance on interpretation and actions, are often seen in applications. "The animals will be killed if they suffer unnecessarily" is an examples of a meaningless HEP that will not be accepted.

HEPs should be precise and specific and based on measurable changes in the animal (biochemical,

behavioural, clinical). HEPs should be set as early as possible in relation to the scientific objectives of the experiment. In many (most) experiments, it is unnecessary that the animal become critically ill/moribund as the objective often is to look at the earlier stages of the disease development rather than severe or advanced stages.

NOTE: §11 in the Regulation states the following: Death as an endpoint should be avoided as far as possible. If death is unavoidable as endpoint the experiment should be designed so that:

a) as few as possible animals dies
b) the duration and intensity of the suffering is reduced as much as possible, and
c) a painless death is ensured as far as possible

In this contexts "death as endpoint" means that animals are allowed to deterioate to a point where they are moribund/dying without interventions. Usually there is no need in most experiment to push it to this extreme end. Usually the experiment can be ended before the animal become terminal ill.

As a general rule the max. allowed weight loss should not exceed 10 % unless there is scientific reasons for allowing a higher weight loss. In some animal models a transient higher weight loss is expected. In such cases this must be clearly described (incl. when weight loss occur, expected % of loss and when regain of weight is expected)

The HEPs should be adjusted to the specific experiment, e.g. with clear, relevant clinically measurable parameters. **Score sheets** should be used when relevant. The score sheet should be **cumulative** and it should be clearly indicated at which specific scores measures and actions will be taken.

NOTE: humane endpoints are also relevant for terminal experiments (inability to maintain the animal in stable surgical anesthesia or critical, unintended change in physiology for example due to bleeding) as well as for breeding projects (deviations from defined/approved phenotype, max. number of litter pr animal etc).

It is reminded that the **predefined humane endpoints are binding** and not subject of discussion when the experiment is ongoing!

Which actions will be taken if animals reach the humane endpoint *(examples: treatment of symptoms, reduced exposure, or euthanasia)*

Therapy by analgesia, support-diet (for instance recovery-gel for rodents or i.v. fluid for pigs), antibiotics or killing are typical actions.

If actions other than killing are planned, more frequent monitoring must be part of the plan. Monitoring in itself is not an action.

The described measures must be linked to the human endpoints, ie. different endpoints may have different actions (other and earlier measures than euthanasia). Such measures can be, for example, nutritional gel, soaked feed in the bottom of the cage, fluid treatment, etc.

Attachments to the FOTS application

NOTE: Attachement should be supplement to the online application form when needed. **Attachment should not be a substitute for submitting a complete text in the online application form.**

All text field in the online form should be completely filled out, and a statements like "See attachment for details" and no further text in the text field of the FOTS form will be returned to the applicant for revision.

Examples of attachments:

A. Score sheet (when relevant)
B. Detailed project description for more complex/larger experiments
C. Table with experimental groups and treatments
D. Timeline or flow chart illustrating the various phases, interventions and duration of the experiment
E. Relevant scientific publications (as pdfs) and/or reference list

Changes in approved FOTS applications

It is not allowed to deviate from approved protocols without applying or notifying the Food Safety Authority (Mattilsynet)!

Depending on the nature of the change you will either have to "**Apply for change**"; or you will have to send a "**Notification of change**" (see the table below for examples); or if the changes are too substantial/deviating too much from the original FOTS-application – you will have to submit a completly new FOTS-application.

The functions for applying/notifying changes in a particular FOTS-application, can be found in the left hand side menu under the specific FOTSid.

PROLONGATION of the approval period for the experiment always require APPLICATION for change as it is only Mattilsynet that has authority to grant project approval.
NOTE: only applications that still have a valid approval can be prolonged, meaning that you must apply in due time in advance before the approval date expire if a prolongation is needed. If the approval date has expired you will have to send in a new complete FOTS application

Changes that are considered to have no negative impact on animal welfare can be notified by sending "NOTIFICATION of change" (see table ahead for examples).

For both application and notification of changes the applicant must describe the following: 1) Purpose of the change, 2) description of the required changes, and 3) update project summary if relevant, and 4) For notification of changes the applicant also need to explain why the changes are considered to have no negative impact on animal welfare.

NOTE: When an application/notification of change is submitted in FOTS, it first goes to PMSK for control. The PMSK will consider whether the change fall under the cateory of application/notification and whether it contains sufficient information.

Guidance on typical changes in approved FOTS protocols

Change	**Category**	**Comment**
Extension of project end date (aproval period)	Application	Extending the project end date has no negative effect on animal welfare and could be notified. However, changing the end date of an approved project requires an authority that only Mattilsynet has. NOTE Only applications that still have a valid approval can be extended.
Expand animal numbers	Application	Expanding the animal numbers will have an overall negative effect on animal welfare.
Altered composition of animal lines and genotypes within same species. No change in total animal numbers within species in question	Notification	If the desired change is a relative change between different animal lines, with or without altered phenotype, and the previously described severity classification is not altered.
Altered composition of animal lines and genotypes within same species. No change in total animal numbers within species in question	Application	If the desired change will increase the number of animals in a higher versus a lower severity category compared to the original application.
Altered anesthesia/analgesia	Most often notification	Notification requires that the qualitative effects of the altered anesthesia and analgesia remains as previously described.
Including new participant	Notification	Requires that the participant can document the required competence and education. See also point below

NOTE: Notificaiton of change as well as extension of project approval period is free of charge. Application of changes (except for extension of approval period) are charged with a fee.

ATTACHMENT 1 EXAMPLE SCORE SHEET

EXAMPLE SCORE SHEET for assessment of health, welfare and humane endpoints adapted to rodents

NOTE: the score sheet needs to be adapted to the specific experiment with relevant observation parameters, monitoring frequencies, examples and measures for various scores, defined scores and max. cumulative score and defined humane endpoints in relation to the scientific objective of the study.

Score 0 =	**Normal**
Score 1 =	**Slightly affected**
Score 2 =	**Moderately affected**
Score 5 =	**Significantly affected**

Note: value set for each score and cumulaltive score will vary with type of experiment.

Example cumulative score: The animal will be euthanized with an overall score of 10. The animal will be euthanized if the weight loss is >10 % independent of other scores.

Examples Score 1:
- Weight loss < 5 %
- Slightly reduced grooming
- Sign of mild pain which is alleviated by providing analgesia

Measures effected at score 1:
- Additional analgesia if sign of pain
- Additional environmental enrichment
- Supplementary/support diet

Examples score 2:
- Weight loss 5-9 %
- Sign of moderate pain that is alleviated by providing analgesia
- Mild/localized piloerection
- Reduced activity level or tendency hiding when, but not inactive
- Reduced grooming, mild discharge from eyes (mild chromodacryorrhea)

Measures effected at score 2:
- Additional analgesia if sign of pain
- Support diet (wet feed or Diet Recovery Gel)
- Increase monitoring frequency to 3-4 times/day and euthanizing if no improvement within 48 hours

Example Score 5:
- Piloerection on larger part/whole body
- Nasal/eye discharge («red tears»/chromodacryorrhea)
- Inactivity or abnormal motor activity
- Hunched body position
- Sign of pain which is not alleviated despite analgesia

See example score sheet next page

Animal ID:	Cage nr/id:					
FOTS id	Baseline body weight:					
Date intervention(s):	Description of intervention(s):					
Observation date						
Time:						
Undisturbed observation (without handling)						
Activity level/mobility						
Behaviour						
Body posture:						
Fur:						
Grooming:						
Response when handled						
Indication of pain						
General clinical signs						
Food intake						
Drinking						
Stool (colour, consistency, amount)						
Body weight (g)						
% of baseline						
Body condition (musculature, fat)						
Respiration						
Nose/eyes						
Specific clincial signs						
ADD ALL OTHER RELEVANT PARAMETERS/SIGNS BASED ON THE THE CHARACTERISTICS OF THE EXPERIMENT FORSØKSRELEVANTE PARAMETRE						
Signature						