FILLING INSTRUCTIVE

In red appear the specific filling instructions that do not appear in the protocol

SECTION 1. ADMINISTRATIVE BACKGROUND

Indicate protocol code:	XXXXXXXX *Here indicate the 9-digit CODE assigned on the platform* *E.g: 200512007*	**Indicate sending date of 1st version:**	XX/XX/XXXX *Write date*

Version, mark with X:	**1:**		**2:**		**3:**		**4:**		**5:**		**Another version:**

Project title:	… Write Title.
Indicate Funding Source(s) and assigned number: (E.g. Fondecyt Regular 11170303)	…Indicate the funding of the project. E.g: FONDECYT Regular 2022, number xxxxxx, FONDECYT postdoctorate number xxxxxxx, Ring project xxxxxx, departmental funds, etc. *Remember, the CEC-CAA evaluates awarded projects, unless there is an exceptional case that must be consulted with the CEC-CAA coordination. If you indicate "Own funds" you must specify and justify.*
Indicate if this research is: research unit / undergraduate thesis / doctorate /magister / teaching / etc.:	… If it contemplates several items or it is not yet defined, mention it.
Other participating institutions (example: INACH, industry, other universities):	…

RESEARCH TEAM					
TYPE OF MEMBER	**Name:**	**Role: PI, Researcher, Co-investigator, Associate Researcher, Postdoctorate, doctoral thesis student, lab manager, laboratory technician, etc.) and Academic Category if applicable (Instructor, Associate Professor, etc.):**	**Institution:**	**E-mail:**	**Training in Research Ethics with Animals** ✧ Indicate ***Yes or **No**
Academic Responsible UC	*Name and surname*	*E.g:* *Coinvestigator, Associate Professor*	*E.g:* Pontificia Universidad Católica de Chile	*xxxx@uc.cl*	*E.g:* *YES*
Principal investigator		*E.g:* *Postdoc, Adjunct Instructor*			*E.g:* *NO*

Team member					
…Add more rows if required					

All research must have an Academic Responsible UC

The academic responsible UC is the Tutor, Sponsor who supervises and accompanies the Researcher Responsible.

Researcher Responsible: Is the person intellectually responsible for the research (undergraduate thesis holder, doctorate, postdoctoral project) and the one who performs most of the experiments or procedures included in the project.
There are times when the Academic Responsible and Researcher Responsible are the same. E.g. UC academic, leader of their own laboratory who is Principal Investigator at Fondecyt of Initiation.

✧ **Training in Animal Research Ethics and/or welfare and management of experimental animals.**
***Attach certification. **The following link will take you to the training program in ethics and handling of research animals: Cursos AALAS Learning Library. Once the mandatory courses are completed, a certificate will be automatically generated that you must attach.**

IN THE EVENT OF AN EMERGENCY WITH THE ANIMALS DURING NON-WORKING HOURS, NOTIFY:	
Name:	**Phone number:**
Name:	**Phone number:**

… Add more rows if required

Identify the person(s) who will be responsible for going to the vivarium during weekends, holidays, in case of emergency such as: natural disasters (e.g. earthquake), fires, lanimal escape, damage to the operator, etc.

SECTION 2. PURPOSES AND JUSTIFICATION OF THE RESEARCH

2.1. SCIENTIFIC FOUNDATION. Indicate the main relevance(s) of the Project.

Approximately 300 words. Mention what the project will be about and the animal model to be used (delete this sentence when completing).

… The scientific foundation must present the research, what it is required for, what is the question it will answer and/or new information it will provide, as well as what the project will consist of in general. Justify its relevance. E.g. It must clearly demonstrate that the study is relevant to: human or animal health, the advancement of scientific knowledge in general or society. Don't forget to mention the use of animals.

2.2. FOUNDATION FOR THE ORDINARY CITIZEN. This section may be public knowledge.

Approximately 300 words. Use language that is easy for common citizens to understand, mention what the project will be about and the animal model to be used (delete this sentence when completing).

… The scientific foundation must present the research, what it is required for, what is the question it will answer and/or new information it will provide, as well as what the project will consist of in general. Justify its relevance. E.g.

You must clearly demonstrate that the study is relevant to: human or animal health, the advancement of scientific knowledge in general or society. Don't forget to mention the use of animals.

2.3. HYPOTHESIS	…	
2.4. GENERAL OBJECTIVE	…	
2.5. SPECIFIC OBJECTIVES	**Specific objective**	**Is it done at the UC? YES/NO***
	1.	
	2.	
	3.	
	… Add more rows if required	

***NOTE: If your project is financed by a collaborative research program (Millennium Science Initiative, Research Ring, FONDAP, BASAL or some other that has collaboration), It is important that you specify the objectives that will be executed in this institution and which in the collaborating institution.**

SECTION 3. EXPERIMENTAL DESIGN

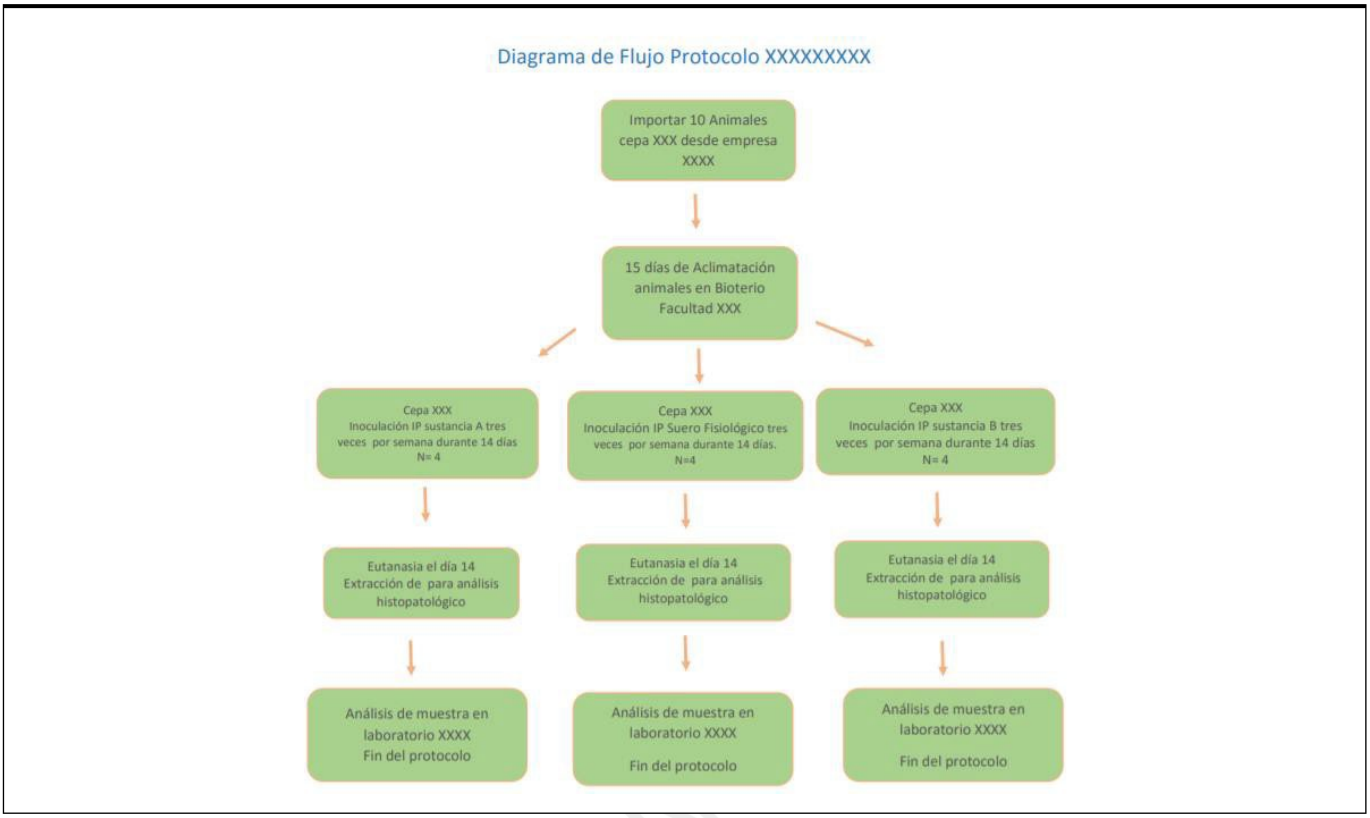
3.1. FLOW CHART. Make a(some) flow chart(s), including the experimental groups, controls, treatments, timing of the treatments, number of animals, parameters/variables to be analyzed, etc.
You can use this link to generate your chart: https://eda.nc3rs.org.uk/

Remember that your chart must contain the number of animals to be used, experimental groups and temporality. You can make more than one chart if you deem it convenient. E.g: one for the design that includes the groups and another for the temporary scheme (delete this instruction when completing).

…

In this section you must include a chart that describes the procedures of the experimental design. The chart must contain the n of animals by objective and temporality.

Chart Example



3.1. DESCRIPTION OF THE EXPERIMENTAL DESIGN. Describe the experimental groups (controls and treatments) that are compared as part of the objectives. Indicate the n (n=number) of each group (identify experimental unit and replicates if applicable) and the total n per objective. Mention the variables that will be quantified and that will be subject to subsequent statistical analysis. Mention the measures taken to avoid bias in the results: will you use random selection of animals or cages to assign treatments? What part of the results will you analyze blindly? Consult ARRIVE Guides

Describe in narrative form the experimental procedures of the project and their temporality (delete this sentence when completing).

… The description must be brief, with a desirable length of one page. Do not include here the details of biochemical analyses, nor of procedures. There are sections for this later (section 5).

3.3. JUSTIFICATION OF THE NUMBER OF ANIMALS. Justify the number of animals (n) to be used, include the calculation of the sample size and justify if there is an exception. Consider if you will have a percentage of animal loss and justify. Use INSTRUCTIVE.

Justify the number of animals to be used, remember to include the calculation of the sample size if applicable (delete this sentence when completing).

… Justification of the Number of Animals

A guiding principle for the ethical use of animals in research and teaching is that no animal life is wasted: the number of animals used in each project must be the minimum necessary to obtain valid and meaningful results.

All animals must be accounted for and justified, including experimental animals, donors, live offspring of pregnant animals, and animals that are generated in colonies but cannot be used for research. Animal losses due to morbidity, mortality, or other difficulties expected with experimental procedures must be carefully described to justify the need for additional animals.

Research must be designed to produce a significant result with the minimum number of animals, so the method for determining this number must be clearly specified. In most cases, statistical techniques and/or calculation of statistical power (Power Analysis) are adequate to maximize the analysis of the data generated by each animal. However, it must be recognized that the basis for an adequate justification of the number of animals depends largely on the nature of the study. Previous experience and familiarity with the model may also be taken into account, but must be carefully documented in the protocol.

A consultation with a statistician or the use of a statistical program during the design stage may be helpful. One of the sites that can support the calculation of statistical power (Power Analysis) is the following http://statpages.info/

Below, 5 types of studies are detailed, along with guidelines to justify the number of animals for each type. These guidelines provide support, but a study may not fit precisely into one of the 5 categories.

Teaching protocols: The number of animals is determined by the index of the number of students, which must be explained in the description of the justification. Consider minimizing the number of animals without sacrificing the quality of direct experience for students.

Tissue collection for in vitro studies (including antibody generation): The number of animals must be determined by the total amount of tissue required and the yield provided by one animal. Explain and justify in detail how the required number was calculated.

Pilot studies that do not require statistical analysis (use of animals to determine success or failure of an objective, such as the generation of transgenic animals): The number of animals must be justified according to the estimate of success of the experimental procedure, which must be detailed in the description.

Pilot studies: The number of animals is determined by the experience and personal judgment of the researcher and they are typically small. The data collected in the pilot studies are generally used to determine the sample size of future experiments.

Studies that require inferential statistical analysis: These studies make inferences about a whole, based on a sample. The number of animals must be determined by a statistical power analysis. The justification must include the values of alpha, beta, sigma and the size effect used in the power analysis to determine the sample size. Alternatively, the minimum number of animals can be determined and justified by references to comparable studies in which desirable sizes demonstrated statistical significance.

NOTE: The number of animals cannot be justified by the number of experiments that the laboratory staff can carry out in a given time (a week, a month, etc.)

This text has been adapted from University of Californa Office of Research. Last update December 2021

SECTION 4. USE OF ANIMALS

Type	Animal Animal	Farm animal	Wild animal*	Animal companion	Other(s) (detail)
Mark with an x					

**If you do not know which species you will sample, indicate which are the most common species that you could find and which of them are protected.*

4.1. DETAIL OF ANIMALS TO BE USED PER OBJECTIVE. Indicate the number of animals to be used according to species, strain, weight, sex and stage of development. Check that it is consistent with the flow chart.

Objective	Species / Strain	Age / State of development	Weight	Sex	Number to be used	Conservation status of the species	SAG authorization/ Sernapesca/other
1	*E.g: Mus musculus/C57BL/6*	*E.g: 8 weeks, E7, adult*	*E.g: 20 gr*	*Male* *Female*		*Indicate: endangered/vulnerable/rare/N/A* *If it does not correspond to a protected species, indicate Not Applicable (NA). Generally this question applies to wild animals.*	*Indicate: Yes/No/ In process/N/A* *Generally this question applies to wild animals.*
2							
3							
				***TOTAL number TO USE =**		*Total sum, separate by species.*	

… Add more rows if required
**If you are changing the n in an amendment, add space in this row and write what the original and amended numbers were by marking the new text with color.*

4.2. JUSTIFICATION OF THE USE OF ANIMALS versus alternative models:

Justify why you need to use animals, reflect on replacement of the 3 Rs (delete this sentence when completing)

… Justify why currently existing alternative methods (cell lines, computational modeling, meta-analysis of literature) fail to answer the research question, making the use of animals necessary.

The following are databases where you can search whether or not there is an alternative to the use of animals in the framework of your research
http://cidportal.jrc.ec.europa.eu/ftp/jrc-opendata/EURL-ECVAM/datasets/DBALM/LATEST/online/dbalm.html
https://www.nlm.nih.gov/enviro/altbib.html
https://www.nal.usda.gov/awic/alternatives-literature-searching

4.3. JUSTIFICATION OF SPECIES(s) to be used:

Justify why you need to use the particular species (delete this sentence when completing)

… Justify based on the answers that the use of this particular species will give you. Also, explain the differences with other animal models that may give similar responses.

The cost of the animal must not be considered as a primary justification for using a particulahour species or model.

4.4. ORIGIN AND HOUSING OF ANIMALS:			
a) Origin of animals:	…*Indicate where the animals come from. E.g: The Jackson Laboratory, CIBEM Vivarium, donation from another institution (specify).*		
b) Indicate if you will transport animals within the institution, from one institution to another, from land (field) to an institution. Describe from and to where, as well as the means and conditions of transport:	… *In this box you must enter the information regarding how you will transport the animals either within the institution, from one institution to another, from the field study to our institution or vice versa. Indicate the characteristics of the means of transport, the time this process takes, the number of animals to be transported, also indicate whether they will be transported in cages, boxes or plastic containers (the medium must be suitable for the species) if they have ventilation, etc.* ***You can access the transport instructive for rodents generated by the CEC****. If required, consult the following link:* ***Instructivo traslado de roedores de experimentación Welfare of Animal During transport***		
c) Housing place of the animals during the development of the protocol:	… *Indicate the place(s) where you will house the animals during the execution of your project*		
d) Name and email of the person in charge of the housing place of the animals:	…		
e) Do you have a letter or email authorizing the use of animals from the person in charge of the enclosure? **Mark with an x** **All researchers must contact the person in charge of the enclosure for feasibility advice.**		**YES**	*Don't forget to attach it*
		In process	*Remember to attach it for the next round of reviews*
		NO	*You must start this process as soon as possible, as it is a requirement for the approval of the protocol*
		N/A	*If it does not apply or you cannot obtain it, justify:* …
d) In vivariums, the use of environmental enrichment is mandatory. In case of not using or deciding to use different elements, describe and justify. If not applicable, write N/A.	… *In rodent vivariums, environmental enrichment is used for all animals, for example, tissue paper, cardboard cones, etc. If you will indicate the use of different elements or you will not use them, you must provide a justification.*		
e) Animal density (number of animals per cage, pen, enclosure, also indicate their dimensions: floor area/animal and height).	… *Links of interest:* ***Densidades apropiadas para ratas y ratones*** ***Directiva Europea 2010*** ***Guide For The Care and Use of Laboratory Animals (ed. 2011)*** *This must only be completed by those who carry out research outside of the UC vivariums.*		

f) Site of procedures and physical location of the site of procedures:	… *Indicate the place where you will perform the procedures with animals. E.g: CIM surgery room, CIBEM procedure room.* *If it is outside the vivarium, indicate the name and contact of the person in charge: E.g. "Neurophysiology Laboratory", 3rd floor, Building XX. Person in charge: John Doe, Lab manager, jdoe@uc.cl*

g) Identification method(s) of the animal, mark with an X:	**Marker**	**Ear notch**	**Other (describe)**

4.5. USE or GENERATION OF GENETICALLY MODIFIED ANIMALS. Mark with an X. If not applicable, check N/A.					
	Use		**Generation**		**N/A**
a) Describe the genotype and phenotype of the genetically modified animals to be used, and the timing of the appearance of signs/symptoms:					
… *Indicate the genotype and phenotype of the genetically modified animal. E.g., Mouse Genotype B6.129P2-Il10tm1Cgn/J. Phenotype: does not produce the cytokine IL-10 and has spontaneous colitis, which usually manifests from 6 weeks of age. Cite reference if available.*					
b) Indicate the type of cross (refer to the parental genotype) used to generate the animals:					
… E.g. Homozygous x Heterozygous, X % of genotype X (the desired one) is expected in the offspring.					

SECTION 5. PROCEDURES TO BE CARRIED OUT WITH THE

5.1. AUTHORIZED PERSONNEL
Complete the following List of Persons Authorized for the Handling of Animals. If you include personnel with animal handling experience, attach certifications. If you plan to recruit personnel, but have not done so yet, identify them as NN and indicate what training they should have. Remember that any new inclusion of personnel must be informed to the committee by means of an amendment before the person begins their work with animals.

LIST OF PERSONS AUTHORIZED FOR THE HANDLING OF ANIMALS

Name:				
Function and techniques to perform in this protocol:	*Indicate the functions related to animal handling to be performed in this protocol: E.g. surgical procedures, monitoring of animals, administration of compounds. Also indicate the specific techniques to be performed for this protocol, e.g: stereotaxic surgery, intraperitoneal injections, etc.*			
Animal handling experience. Mark with X:		**YES**		**NO**

If you indicated "YES", mention who trained them and the years of experience in the functions and techniques to be performed in this protocol:	*Describe the years of experience and the techniques they use: surgical, sexing, IP injections, EV. Supervision of animals, etc; as well as the person(s) responsible for their training.*
If you indicated "NO, indicate who will train them in the functions and techniques to be performed in this protocol:	*Indicate who will be in charge of training, the time that will be invested and the techniques in which they will be trained. If you still can't specify, explain. Remember that the veterinary doctors in charge of the vivariums can perform these trainings.*

Copy and complete this table for each member of the research team associated with this protocol who will handle animals.

5.2. NON-SURGICAL PROCEDURES. Detail NON-SURGICAL procedures, including those performed under anesthesia. Examples: administration of substances, behavioral tests, different types of diet, restraint or immobilization methods, temperature conditions, survival studies, bronchoalveolar lavage, etc.

… You must describe in detail the non-surgical procedures to be performed. In case of using a standard procedure (SOP) previously approved by the CEC-CAA, mention the approval number and attach it as an annex. Remember that it is necessary for the committee that each protocol be self-explanatory: instead of just naming a previous protocol in which you used the same procedure, copy the corresponding description here.
Detail the housing conditions of the animals during and between the non-surgical procedures to be performed. For example, if you are going to carry out the water maze test, indicate where you will keep the animals during this test, under what conditions of temperature and humidity, if you will use support measures, etc.

5.2. a) BLOOD DRAW. In case of blood draw, complete the following table (review INSTRUCTIVE):

Species	Route	Volume to extract each time	Frequency	Needle gauge	Person Responsible

… Add more rows if required

Check:
https://www.nc3rs.org.uk/3rs-resources/blood-sampling
Diehl et al, J. Appl. Toxicol. 21, 15–23 (2001)

Blood sample collection.
The volume of blood to be extracted is determined both by the experimental requirements and by the limit that ensures that the animal will not be harmed. As a general rule, the smallest possible volume should be extracted; for this procedure to be classified as mild in severity, no more than 10% of the blood volume should be extracted at once and less than 15% of the blood volume may be extracted in a period of 30 days.
The animals have approximately 70 ml of blood per Kg of weight, this varies according to the species (see table 1 A and B), and the extraction of smaller volumes can generate harmful effects if the animal is compromised in some way. Extraction of larger volumes may have minor effects if a replacement of the extracted volume is performed.
The maximum volume of blood to be extracted, as well as its frequency, must be stipulated in the experimental protocol.

REFERENTIAL TABLES THAT CAN SERVE AS A GUIDE:

Table 1A

Species	*Reference Weight*	*Blood volume (ml/kg)*	*Total blood volume in adult (ml)*	*Safe volume to extract at once (ml)**	*Exsanguination volume (ml)*
Mouse	*18-40 g*	*58.5*	*M 1.5-2.4* *H 1.0-2.4*	*0.1-0.2*	*M 0.8-1.4* *H 0.6-1.4*
Rat	*250-500 g*	*54-70*	*M 29-33* *H 16-19*	*M 2.9-3.3* *H 1.6-1.9*	*M 13-15* *H 7.5-9*
Guinea pig	*700 g-1.2 kg*	*69-75*	*M 59-84* *H 48-63*	*M 6-8* *H 5-6*	*M 29-42* *H 24-31*

** A single blood draw of 10% of the blood volume, average 7ml/kg.*

Table 1B

Species	*Blood volume (ml/kg)*	*Total blood volume in adult (ml)*	*Safe volume to extract at once (ml)**
Large White pig	*56-69*	*13.200-15.000*	*1320-1500*
Yucatan Pig	*56-69*	*4.200-4.800*	*420-480*
Sheep	*58-64*	*4060-4480*	*400-450*
Goat	*57-90*	*3990-6300*	*400-630*
Cattle	*60*	*27.000-36.000***	*2700-3600*

** A single blood draw of 10% of the blood volume*
*** Adult weight assumed to be 450-600 kg.*

***Table** 1 (A) Blood draw volumes for laboratory animals. (B) Blood draw volumes in older animals. All the information was extracted from the "Handbook of Laboratory Animal Management and Welfare. Sarah Wolfensohn and Maggie Lloyd. Fourth Edition (2013)."*

IF YOU ARE GOING TO PERFORM REPEATED EXTRACTIONS, CHECK: Diehl et al, J. Appl. Toxicol. 21, 15–23 (2001)

5.2. b) ADMINISTRATION OF COMPOUNDS, EXCEPT anesthetics, analgesics and related, which will be requested in SECTION 6 of Animal Welfare. That is, in this table include for example: diet, alcohol, microorganisms, viruses, drugs, etc**.**									
Identify the Compound	…		…		…		…		…	
Finished pharmaceutical product, mark with an X:		**YES**		**YES**		**YES**		**YES**		**YES**
		NO		**NO**		**NO**		**NO**		**NO**
		N/A		**N/A**		**N/A**		**N/A**		**N/A**
Pharmaceutical presentation:	*Tablet, Solution,*									

	lyophilisate, ointment, vaccine, other (explain if you choose another)				
Indicate lethal dose 50 (cite reference or link in pubchem.ncbi.nlm.nih.gov. If you cannot get it, explain:					
Indicate how the compound is prepared:	*No preparation required, prepared according to factory instructions, own preparation (describe)*				
Dose:	*Give the dose in mg per kg of the compound to be administered.*				
Route:	*Indicate the route of administration of the compound: Oral, IV, IP, SC, ID, etc.*				
Volume:	*Volume of the product to be administered per time.*				
Administration Frequency:	*E.g. once a week, once a day, once throughout the protocol, etc.*				
Treatment duration:	*E.g. a month, a week, a day, etc.*				

Compound administration manager:	*Name of the person responsible for administering the compound.* *Make sure this person has the respective training*				

Indicate the source (bibliography or other) on which you relied for the choice of the compounds indicated above, or justify the choice of method:

…In this section you must indicate the bibliography consulted for the choice of the compounds to be used, if the choice was not based on the bibliography, detail where it comes from and the reason for your choice.

REFERENTIAL TABLES THAT CAN SERVE AS A GUIDE:

Administration of volumes for laboratory animals

Species	*Reference Weight*	*Intravenous or Intra-arterial (ml)*	*Intra-peritoneal (ml/site)*	*Intra-muscular (ml/site)*	*Subcutaneous (ml/site)*	*Oral (ml)*	*Intradermal (ul/site)*
Mouse	*20 g*	*0.2*	*1-2*	*0.05*	*0.5 **	*0.4*	*100*
Rat	*250 g*	*1*	*2-4*	*0.1*	*1-2 **	*5*	*100*
Guinea pig	*500 g*	*1*	*5-7*	*0.1*	*1-2 **	*5*	*100*

Note: The values have been calculated according to the average size of the species. Adjust if necessary for the specific weight of each individual.

** Maximum of 4 sites.*

Needle gauge recommended for work with laboratory animals (G)

Species	*Intraperitoneal*	*Intramuscular*	*Intravenous*	*Subcutaneous*
Mouse	*27*	*29-30*	*27-28*	*25*
Rat	*23-25*	*25*	*25-27*	*25*
Guinea pig	*21-25*	*25*	*25-27*	*23-25*
Sheep	*19-21*	*21*	*19-21*	*19-21*

Species	*Site*	*Gauge*	*Length*
Rat	*Tail vein*	*24-25*	*12-19 mm*
Pig	*Ear vein*	*21-23*	*25-40 mm*
Sheep, goats, cattle	*Jugular vein*	*19-21*	*40 mm*

Information extracted from "Handbook of Laboratory Animal Management and Welfare. Sarah Wolfensohn and Maggie Lloyd. Fourth Edition, 2013."

5.3. SURGICAL PROCEDURES. Write here the details of the surgical procedures to be performed:				
… We suggest visiting https://module.researchanimaltraining.com/nc3rs/21-3/#/ *Describe the surgical procedure(s) you will perform on the animals from the start to the end of the study. Indicate how you will control the depth of anesthesia before starting the procedure. Do not forget to include the intraoperative monitoring, mentioning which parameter(s) you will monitor. Indicate who will be responsible for performing each procedure. Remember that it is necessary for the committee that each protocol be self-explanatory: instead of just naming a previous protocol in which you used the same procedure, copy the corresponding description here.* *In case of using a standard procedure (SOP) previously approved by the CEC-CAA, mention the approval number and attach it as an annex.*				
a) Indicate intraoperative support measures. Mark with an X.		**Serum**		**Ophthalmic ointment**
		Heat (indicate how you will provide it): …		**Other (specify): …**
b) Asepsis methods during surgery:	*Describe the method of asepsis to be used before and during surgery, examples: Sterile gloves, sterile field cloths and surgical instruments, 70% alcohol, chlorhexidine solution, etc.*			
c) Number of animals to be operated/processed per day and estimated duration of the surgery/intervention:	*Indicate how many animals you will undergo surgery per day. You can consider standardization of surgical techniques, prior to the development of the experiment, in order to verify if the surgical technique(s) and postoperative care are adequate. If you decide to do this, you must indicate this within section 3.1. and consider the number of animals needed.*			
d) Describe post-mortem procedures. Remember that asepsis is just as important.	*Briefly explain post-mortem "surgical" procedures, if applicable, including aseptic measures.*			
e) Conditions of the place where the surgical procedure will be performed.	*Indicate the conditions of the place where you will perform the surgeries. Temperature control, if the surface where it will be carried out is washable, disinfectable. Mention what equipment and instruments you have to carry out the procedure efficiently and aseptically.*			
f) If the surgical procedure(s) include(s) animal survival, define the duration and care of the immediate and mediate postoperative period. Indicate the frequency of care. Identify the person responsible.	*After the surgical procedure, a series of postoperative care must be provided to ensure the survival and well-being of the animal. In this section you must detail what these cares will be, frequency, duration and identify the person in charge of them.*			
g) Indicate if the animals to be used have previously undergone or will undergo any invasive or surgical procedure more than once. If yes, indicate time interval between surgeries and/or invasive procedures.	*If the animals to be used in your study have already undergone a procedure, and you are going to reuse them, you must indicate which procedures they were previously subjected to.* *The reuse of animals will be allowed only if the severities of previous procedures were mild to moderate, the current procedure is mild to moderate or without recovery and veterinary advice is available.*			

5.4. PROBABLE STARTING DATE of procedures with animals (if possible include Gantt Chart).

… *In this section you must indicate the probable starting date of the experiments with animals. The vivarium must have this information to have the space to keep the animals of the different projects.*
The Gantt Chart will allow the committee to organize itself to monitor the project.

SECTION 6. ANIMAL WELFARE

6.1. IMPACT ON ANIMAL WELFARE. Indicate what impact on animal welfare you expect with your protocol, considering the procedures, species, physiological state and/or phenotypes of the animals, and what actions you will take**.**

… *It is imperative to avoid all unnecessary pain and suffering in each animal that participates in a protocol. Any manipulation that causes pain or affliction of the animal(s), must be justified in a solid and detailed manner. Specify actions such as analgesia and/or increased supervision, or other specific measures required by your study model. Also consider adequate hydration and the use of serum, at the appropriate temperature, as well as nutritional supplements in animals that may require it. Describe interventions to alleviate painful, stressful procedures, e.g. use of analgesics, anti-inflammatories, also consider taking into account and evaluating the effectiveness of these interventions during the execution of the procedures. Faced with surgeries, having personnel specialized in the surgical techniques to be performed represents a form of refinement, which will result in less animal suffering, avoiding the risk of deterioration of the animal and the consequent loss of data.*

For example, if your project contemplates infection with a microorganism, consider what its effects would be on the animal, and what measures you will take to minimize suffering. If the above has not been described, then you should consider ***conducting a pilot****.*

Examples of Morbidity Criteria

Morbidity criteria and action to be taken	
PARAMETER TO BE ASSESSED	***ACTION TO TAKE***
Coarse hair	*Increase supervision. Consult the Veterinary Doctor (VD).*
Separation from the group	*Increase supervision. Consult the Veterinary Doctor (VD).*
Weight loss	*Increase supervision. Consult the VD.*
Eye discharge	*Consult the VD.*
Lethargy	*Increase supervision. Consult the VD*
Arched back	*Consult VD. Consider analgesia. Increase supervision.*
Jerky movements or twitching	*Consult VD. Consider analgesia. Increase supervision.*
Loss of appetite	*Consult VD. Provide feeding, deliver palatable food. Increase supervision.*
Ataxia	*Consult VD. Increase supervision.*
Tremor	*Consult VD. Increase supervision*
Ulceration (tumor, surgical wound, injection site)	*Consult VD. Increase supervision*
Infection (tumor, surgical wound, injection site)	*Consult VD. Increase supervision*
Diarrhea	*Consult VD. Moisturize Increase supervision.*
Loss of balance and jerky movements.	*Consult VD. Consider analgesia. Increase supervision.*

Change in respiratory rate (slow, shallow, fast, labored)	*Consult VD. Increase supervision.*

Extracted from the protocol used by the IACUC of the Roswell Park Comprehensive Cancer Center, version year 2010.

To reduce animal suffering during the procedures, we suggest you follow the next hyperlink.
http://www.procedureswithcare.org.uk/

6.2. SUPERVISION. Indicate frequency and period of supervision of animals if required. Remember this information must also be established in the animal supervision guidelines.

…*We suggest visiting*: http://cbctraining.ncl.ac.uk/eM-EU5/story_html5.html

Attach the guideline(s) for animal superision: this must be SPECIFIC (applicable to the experimental condition of each proposed animal model) and, as far as possible, ARRANGED according to the order in which the animal is checked (the first thing that is reviewed must go first in the guideline). Review Examples of Supervision Guidelines

*The **supervision guideline** allows quantifying, through the measurement of welfare indicators, the effect of the treatment applied to the animals, as well as early detection of signs of abnormality that may or may not be the product of the experimental manipulation. Its use makes it possible to define humane endpoints when an animal deteriorates, allowing it to be removed from the experiment in time and retrieve information that could be lost if the animal dies.*
There are numerous supervision guidelines, these must be chosen or generated, according to the procedures to be carried out during your protocol. This is how, a protocol in which surgeries will be performed, must include among its indicators the evaluation of the state of the wound. On the other hand, a protocol where only behavioral tests are carried out, without surgical intervention, will not require the measurement of this indicator but of others related to the procedure performed on the animal.

Do you attach the animal supervision guideline(s) or does it not apply? Mark with an X Remember this guideline must be SPECIFIC, that is, applicable to the experimental condition of each proposed animal model. Review Examples of Supervision Guidelines		**YES**
		NO
		N/A

6.3. ANESTHESIA AND ANALGESIA. Indicate the compounds that you will use to induce anesthesia, analgesia and other palliative care, that is, include anti-inflammatories, tranquilizers and sedatives**.**

Information in:

https://www.flairelearning.com/eModule/21-2/#/
http://cbctraining.ncl.ac.uk/eM-EU20/story_html5.html
https://module.researchanimaltraining.com/nc3rs/21-5/#/lessons/WNkSJdjJU4LWEP-ABE2R0ZcsxdMsuJcN

Anesthetics	Dose	Route	Volume	Frequency	Treatment duration	Procedure in which it will be used
E.g. Isoflurane	*4-5% Induction*	*Inhala-tion*		*1 time*	*1-3 minutes*	*Osmotic pump implant surgery*
E.g. Isoflurane	*1-2 % maintena nce*	*Inhala-tion*		*1 time*	*30 minutes*	*Osmotic pump implant surgery*

Analgesics and/or anti-inflammatories	Dose	Route	Volume	Frequency	Treatment duration	Procedure in which it will be used
E.g. Ketoprofen	*5 mg/kg*	*SC*	*0.5 ml*	*1 time*	*30 minutes*	*Pre surgical*
E.g. Tramadol	*20 mg/kg*	*Oral*	*1 drop*	*every 12 hours*	*3 days*	*Post surgical*

Sedatives and/or Tranquilizers	Dose	Route	Volume	Frequency	Treatment duration	Procedure in which it will be used

… Add more rows if required

SECTION 7. EUTHANASIA

7.1. EUTHANASIA CRITERIA AS A HUMANITARIAN END POINT. Describe the criteria(s) for stopping work with animals, according to what is said in the supervision guideline or if there are any extra criteria to be evaluated. In the latter case, remember to add it as a note to the supervision guideline.

… Describe at what time and under what specific criteria (indicators that allow you to perceive animal suffering) it will be decided to interrupt the experiment for each animal, and what actions will be taken to avoid death as an end point.
*In this section you must indicate the **humane end point**, this refers to the sign or signs that indicate progressive deterioration of an animal during an experiment and that are close to the "point of no return".*
The humane endpoint does not necessarily mean the humane sacrifice of the animal, however, in the case of rodents, deterioration is so fast when the signs already appear that the most frequent procedure is to proceed with euthanasia (in other species, there are more possibilities of intervention). For this reason, it is important to adequately set the potential humane end points, thus preventing the rodent from dying in suffering and carrying out a timely euthanasia, which also allows the rescue of the animal's information (e.g. tissue samples).

7.2. EUTHANASIA METHOD(S)			
PROTOCOL STAGE	**Indicate method, dose and route of administration**	**Species and stage of development**	**Person responsible for the procedure**
Euthanasia because of End of Protocol	Method:…… Dose:…… Administration route:…… *E.g. Anesthesia overdose, CO2, cervical dislocation prior anesthesia, decapitation prior anesthesia, exsanguination under deep anesthesia, overdose of anesthesia Ketamine/Xylazine, etc. Indicate the dose to be administered and the route to be used for it.*	Species and stage of development: …….	Person Responsible:…….
Euthanasia as Humane End point	Method:…… Dose:…… Administration route:……	Species and stage of development: …….	Person Responsible:…….

… Add more rows if required

Indicate the method of euthanasia to be used **both for the humane end point and for the end of the protocol**. You can enter the link: AVMA Euthanasia 2020. (American Veterinary Medical Association Guidelines for the Euthanasia of Animals: 2020 Edition) and consult the methods accepted per species.

SECTION 8. SEVERITY LEVEL

Complete the following table with **each of the procedures you will perform on animals, according to their level of severity**. To classify as Mild, Moderate and/or Severe, follow the INSTRUCTIVE. For moderate or severe procedures, palliative measures must be adopted, such as the use of analgesics and the establishment of end point criteria.

PROCEDURES	MILD	MODERATE	SEVERE	NO RECOVERY
NON-SURGICAL	*E.g. Write here the non-surgical procedures that qualify as minor. (Use the table below to estimate the severity of the procedure)*			
SURGICAL				*E.g. Write down the surgical procedures to be performed that are without survival of the animal.*

LEVEL OF SEVERITY OF THE EXPERIMENTAL PROCEDURES

NON-RECOVERY
Procedures that are performed entirely under general anesthesia from which the animal will not regain consciousness must be classified as <<non-recovery>>.
MILD (Procedures as a result of which the animals are likely to experience mild and short-term pain, suffering or distress, as well as procedures without significant alteration of the welfare or general condition of the animals).
Administration of anesthesia, except for the sole purpose of euthanasia.
Pharmacokinetic study where a single dose is administered and a limited number of blood samples are collected (totaling < 10% of the circulating volume) and the substance is not expected to cause any discernible adverse effects.
Administration of substances through subcutaneous, intramuscular, intraperitoneal routes, by gastric tube and intravenously through superficial blood vessels, where the substance has only a minor effect on the animal, and the volumes are within limits appropriate for the size and species of the animal.
Application of external telemetry devices that cause only minor debilitation to the animal or minor interference with normal activity and behavior.
Superficial procedures, for example: ear and tail biopsy, non-surgical subcutaneous implantation of mini-pumps and transponders.
Short-term confinement (< 24 hours) in metabolic cages.
Non-invasive imaging techniques in animals (for example: MRI), with appropriate sedation or anesthesia.
Studies involving short-term (<24 hours) deprivation of social partners, short-term solitary caging of adult rats or mice.
Feeding with modified diets, which do not cover all the nutritional needs of all animals, and are expected to cause a mild clinical abnormality in the study period.
Induction of tumors, or spontaneous tumors, that do not cause perceptible clinical deleterious effects (e.g., small, subcutaneous, non-invasive nodules).
Breeding of genetically modified animals that is expected to result in a phenotype with mild effects.
Models that expose the animal to noxious stimuli that are briefly associated with mild pain, suffering, or distress, and that the animal can avoid.
A combination of or an accumulation of the following example procedures may result in a minor classification: *.-Body composition assessment through non-invasive measurements and minimal restraint.* *.-ECG monitoring using non-invasive techniques with minimal or no restriction of habituated animals.* *.-Application of external telemetry devices that are not expected to cause any impairment to socially adapted animals and that do not interfere with normal activity and behavior.* *.-Breeding of genetically modified animals that are not expected to have any clinically detectable adverse phenotypes.* *.-Addition of inert markers to the diet to follow the passage of the food bolus.* *.-Withdrawal of food for a period of less than 24 hours, in adult rats.* *.-Open field trials.*

MODERATE *(Procedures as a result of which animals are likely to experience short-term or mild but long-term moderate pain, suffering or distress, as well as procedures that could cause a moderate alteration in the welfare or general condition of the animals).*
Frequent application of substances producing moderate clinical effects, and/or blood sampling (>10% of circulating volume), in a conscious animal within 14 days without volume replacement.
Withdrawal of food for 48 hours in adult rats.
Use of metabolic cages or other implements that imply a moderate restriction of movements.
Surgeries under general anesthesia and analgesia and appropriate palliative measures to control post-surgical pain or suffering, or post-surgical alteration of the general condition. Examples include: Thoracotomy, craniotomy, laparotomy or orchiectomy, lymphodenectomy, thyroidectomy, orthopedic surgery with
effective stabilization and wound care, organ transplantation with effective treatment of rejection, surgical implantation of catheters or biomedical devices (e.g., telemetry transmitters, mini-irradiation
or chemotherapy at a sub-lethal dose, or at an otherwise lethal dose, but with reconstitution of the immune system. Harmful effects would be expected to be mild to moderate and short-lived (< 5 days).
Tumor induction models, or spontaneous tumors, expected to cause moderate pain or distress using analgesia, or to lead to moderate interference with normal behavior.
Acute toxicity dose range determination studies, chronic toxicity/carcinogenicity tests, with non-lethal end points.
Production of genetically modified animals through surgical procedures.
Breeding of genetically modified animals, which are expected to give rise to a phenotype with moderate effects.
Studies with modified diets that do not cover the nutritional needs of all animals, and that are expected to cause a moderate clinical abnormality in the study period.
Elicit escape reactions in which the animal is unable to escape or avoid the stimulus, and which are expected to result in moderate distress.
SEVERE *(Procedures as a result of which the animals are likely to experience severe or moderate but long-lasting pain, suffering or distress, as well as procedures that could cause a serious alteration in the welfare or general condition of the animals).*
Use of metabolic cages that imply a severe restriction of movement for a long period (> 48 hours).
Irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or reconstruction with the production of graft-versus-host disease.
Potency trial of a vaccine characterized by persistent alteration of the animal's condition, progressive disease causing death, associated with long-lasting moderate pain, distress or suffering.
Trials of devices where failure could cause severe pain or distress, or death of the animal (e.g: cardiac resuscitation devices).
Surgical and other interventions, under general anesthesia, expected to result in moderate, severe or persistent post-operative pain, suffering or distress, or a severe and persistent alteration in the general condition of the animal. For example: producing unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multi-organ failure.
Toxicity tests where death is the endpoint or deaths are anticipated and severe pathophysiological states are caused, (e.g: single dose acute toxicity test, LD50).

Breeding of animals with genetic disorders that are expected to experience severe and persistent alteration of their general condition, e.g: Huntington's disease, muscular dystrophy, models of chronic relapsing neuritis.
Organ transplant where organ rejection is likely to cause distress or severe alteration of the general condition of the animal (e.g: xenotransplantation).
Complete isolation for prolonged periods of time of gregarious species, e.g: mice, rats, guinea pigs, dogs, cattle, sheep, pigs.
Inescapable electric shock (e.g: to produce learned helplessness).
Immobilization stress to induce gastric ulcers or heart failure in rats.
Models with tumor induction, or with spontaneous tumors, expected to cause progressive fatal disease, associated with long-lasting moderate pain, distress or suffering. (e.g: tumors causing cachexia)
Forced swimming or exercise tests with exhaustion as the end point.

Extracted and adapted from Annex IX "Severity Classification of Procedures" Royal Decree 53/2013, of February 1, which establishes the basic applicable standards for the protection of animals used in experimentation and other scientific purposes, including teaching.