


# Managing 3R In your Research

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› The Federal Council › FDHA › FSVO

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**Federal Food Safety and Veterinary Office**

Q Search ...

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Food and nutrition    Commodities    **Animals**    Import and Export    About the FSVO

FSVO > Animals > Animal experimentation

## Law

Animal Welfare Act from the 16<sup>th</sup> of Dec 2005

Animal Protection Ordinance (OPAn 2008)  
Ordinance on animal experimentation (2010)



Source: <https://www.fedlex.admin.ch/eli/cc/2008/414/en>

## Written in the law ?

Researchers in Switzerland are obliged to keep animal experiments to a minimum. Rather than animal experiments, alternative methods must be used where possible.

The **3R principles (replace, reduce, refine)** call for the development and use of **alternatives** to animal experiments, the **reduction** of animal experiments to the absolute minimum and **improvements** in experiments to ensure that animals are subjected to a **minimum of pain**.

# Why introducing 3R in my research ?

- Ethical question
- Impact on the **reliability** of the data obtained: stress and pain have effects on the behavior and physiology of the animal

## Increase in blood glucose concentrations in response to simple *in vivo* procedures

- Mice implanted with DSI sensors for measuring glycemia

Disturbance



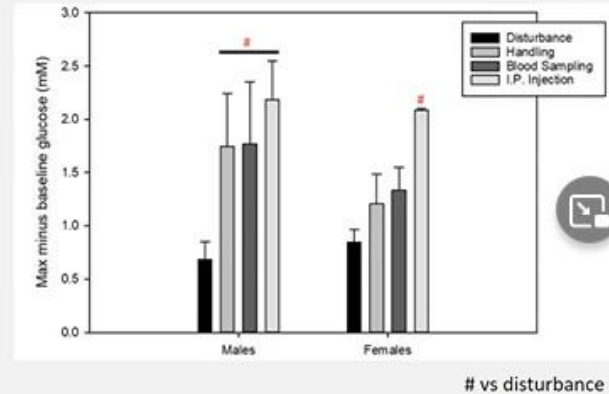
Handling



Blood sampling



Intraperitoneal injection



Source: <https://insidescientific.com/webinar/sex-drugs-and-protocol-how-researcher-choices-impact-experimental-outcomes-in-preclinical-diabetes-research/>

## How to Refine ?

**Refine** : how be ensured that animals are subjected to a **minimum of pain**.

1. Knowing animal welfare and how to increase it
2. Knowing suffering of the animal and how to decrease/avoid it
3. Evaluating accordingly the degree of severity of your experiment

# How to increase animal welfare/decrease animal suffering ?

- Be informed and trained (continuous education) - RESAL

**resal**  
Réseau des animaleries lémaniques

EN FR Recherche

RESAL - Réseau des animaleries lémaniques

Expérimentation animale Formation qualifiante Formation continue Accréditation de symposi... Offres d'emploi

Plus d'infos -

**Missions du ResAL**

Organisation des cours de formation qualifiante en expérimentation animale

- Cours de Module 1

Organisation des cours de formation continue en expérimentation animale

- Formation continue

Collaboration avec les autorités vétérinaires cantonales et fédérales

**Cours de formation continue à venir**

- Cours "Manipulation et contention souris" - 1er juillet 2024 - Matin
- Cours "Injections souris" - 1er juillet 2024 - Après-midi
- Cours "Prélèvements sanguins souris" - 2 juillet 2024 - Matin

**Conférences accréditées à venir**

- 3RCC Workshop "Animal experiments in research: Challenges and opportunities" - 8 avril 2024 - ETH (Zurich)
- Conférence Inotiv "GEMs - From Generation to colony management and husbandry" - 23 avril 2024 - En ligne (Zoom)
- Conférence - "Conscience de l'animal et perception de la douleur" - 13 mai 2024
- Symposium "Sex As a Biological Variable 2024" - 12-14 juin 2024 - Université de Berne

**La Newsletter du ResAL**

**Partenaires du ResAL**

- 3RCC
- SGV
- SCHAI
- swissuniversities
- SAFN
- LTK



## DRIVING 3RS ADVANCEMENT FOR BETTER ANIMAL WELFARE AND SCIENCE

The Swiss 3R Competence Centre (3RCC) promotes the 3Rs principle (replacement, reduction and refinement of animal experimentation) among the scientific community and the public. To facilitate the development and implementation of effective 3R methods and improve animal welfare it challenges research paradigms and creates innovative alternatives to animal use.







Pioneering Better Science



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PIONEERING BETTER SCIENCE

# Replacement, Reduction and Refinement

A UK-based scientific organisation dedicated to helping the research community worldwide to identify, develop and use 3Rs technologies and approaches.

[Our mission](#)



- Knowing your animal model

### Ex: mice

- Housed in group as much as possible (cage size)
- Improved detention conditions: minimizing transport, light and noise
- Enrichment in the cage
- Tunnel and/or cup handling



If working on  
Metabolism



Source: <https://www.unige.ch/recherche/expanim/>

# • Knowing pain/suffering in your animal model

**NC 3R<sup>s</sup>** National Centre for the Replacement, Refinement & Reduction of Animals in Research

## The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice. The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment. The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
<b>Orbital tightening</b> <ul style="list-style-type: none"> <li>Closing of the eyelid (narrowing of orbital area)</li> <li>A wrinkle may be visible around the eye</li> </ul>			
<b>Nose bulge</b> <ul style="list-style-type: none"> <li>Bulging on the bridge of the nose</li> <li>Vertical wrinkle on the side of the nose</li> </ul>			
<b>Cheek bulge</b> <ul style="list-style-type: none"> <li>Bulging of the cheeks</li> </ul>			
<b>Ear position</b> <ul style="list-style-type: none"> <li>Ears rotate outwards and/or backwards away from the head</li> <li>Ears may fold to form a 'curled' shape</li> <li>Space between the ears increases</li> </ul>			
<b>Whisker change</b> <ul style="list-style-type: none"> <li>Whiskers are either pulled back against the cheek, or pulled forward to stand on end</li> <li>Whiskers may clump together</li> <li>Whiskers lose their natural 'downward' curve</li> </ul>			

Read the original paper: <https://doi.org/10.1016/j.nxj.2015.05.001> (Mason, G., Brown, G., Cohen, G., Edwards, D., Gruber, S., et al. (2015) 'The Mouse Grimace Scale: A simple and robust method for measuring pain in mice', *Nature Reviews Neuroscience*, 18(10), 571-580. doi:10.1038/nrn3924. The NC3Rs provides a range of other resources on animal welfare, including <https://www.nc3rs.org.uk>

**NC 3R<sup>s</sup>** National Centre for the Replacement, Refinement & Reduction of Animals in Research

## The Rat Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rats. The specific facial action units shown below have been used to generate the Rat Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment. The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
<b>Orbital tightening</b> <ul style="list-style-type: none"> <li>Closing of the eyelid (narrowing of orbital area)</li> <li>A wrinkle may be visible around the eye</li> </ul>			
<b>Nose/cheek flattening</b> <ul style="list-style-type: none"> <li>Flattening and elongation of the bridge of the nose</li> <li>Flattening of the cheeks (generally, not the back)</li> </ul>			
<b>Ear changes</b> <ul style="list-style-type: none"> <li>Ears curl inwards and are angled forward to form a 'pruned' shape</li> <li>Space between the ears increases</li> </ul>			
<b>Whisker change</b> <ul style="list-style-type: none"> <li>Whiskers stiffen and angle along the face</li> <li>Whiskers may 'clump' together</li> <li>Whiskers lose their natural 'downward' curve</li> </ul>			

Read the original paper: <https://doi.org/10.1016/j.nxj.2015.05.001> (Mason, G., Brown, G., Cohen, G., Edwards, D., Gruber, S., et al. (2015) 'The Rat Grimace Scale: A simple and robust method for measuring pain in rats', *Nature Reviews Neuroscience*, 18(10), 571-580. doi:10.1038/nrn3924. The NC3Rs provides a range of other resources on animal welfare, including <https://www.nc3rs.org.uk>






- Knowing pain/suffering in your animal model

**NC 3R<sup>s</sup>** National Centre for the Replacement, Refinement & Reduction of Animals in Research

## The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a meaningful indicator of pain in mice. The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to pain-inducing agents and can be used as part of a clinical assessment.

Not present "0"

<b>Orbital tightening</b> <ul style="list-style-type: none"> <li>Closing of the eyelids (narrowing of orbital area)</li> <li>Asymmetry may be visible around the eye</li> </ul>	
<b>Nose badge</b> <ul style="list-style-type: none"> <li>Bulging on the bridge of the nose</li> <li>Vertical wrinkles on the side of the nose</li> </ul>	
<b>Cheek badge</b> <ul style="list-style-type: none"> <li>Bulging of the cheeks</li> </ul>	
<b>Ear position</b> <ul style="list-style-type: none"> <li>Ears rotate outward and/or backward away from the face</li> <li>Ears may fold to form a "pinned" shape</li> <li>Space between the ears increases</li> </ul>	
<b>Whisker change</b> <ul style="list-style-type: none"> <li>Whiskers are either pulled back against the cheek, or pulled forward to stand on end</li> <li>Whiskers may clump together</li> <li>Whiskers lose their natural "downward" curve</li> </ul>	

Read the original paper: <https://doi.org/10.1038/nrn3525>

The publication of using the Mouse Grimace Scale, research papers that undergo this methodology and to generate scales in other species, <http://www.3r.org.uk/information> to be used as part of a clinical assessment. The NC3R provides a range of information on animal welfare science.

Images kindly provided by Dr. Jeffrey Mogil, NC3R scientist.

**NC 3R<sup>s</sup>** National Centre for the Replacement, Refinement & Reduction of Animals in Research

**frontiers in Neuroscience**

**REVIEW**  
published: 08 April 2021  
doi: 10.3389/fnins.2021.632634

**Check for updates**

## Current Methods to Investigate Nociception and Pain in Zebrafish

Nils Ohnesorge<sup>1\*</sup>, Céline Heintz<sup>1</sup> and Lars Lewejohann<sup>1,2</sup>

<sup>1</sup> German Federal Institute for Risk Assessment (BfR), German Centre for the Protection of Laboratory Animals (B3fR), Berlin, Germany, <sup>2</sup> Institute of Animal Welfare, Animal Behavior and Laboratory Animal Science, Freie Universität Berlin, Berlin, Germany

• Whiskers stiffen and angle along the face  
• Whiskers may "clump" together  
• Whiskers lose their natural "downward" curve

Read the original paper: <https://doi.org/10.3389/fnins.2021.632634>

For guidance on using the Mouse Grimace Scale, research papers that undergo this methodology and to generate scales in other species, <http://www.3r.org.uk/information> to be used as part of a clinical assessment. The NC3R provides a range of information on animal welfare science.

Images kindly provided by Dr. Jeffrey Mogil, NC3R scientist.

- Evaluating accordingly the degree of severity of your experiment

According to article 24 of the Animal Experimentation Ordinance, the constraint suffered by animals caused by interventions or measures taken as part of animal experiments is divided into **four constraint categories**.

**Degree of severity 0:** No constraint/observational studies

**Degree of severity 1:** Slight constraint

**Degree of severity 2:** Moderate constraint

**Degree of severity 3:** Severe constraint



Source: <https://www.blv.admin.ch/blv/en/home/tiere/tierversuche.html>

Degree of severity (DS) also depends of the number/frequency of acts done on the same animal during its life

Addition of degree 1 experiments could be considered as degree 2



The DS will not influence how your FormA will be considered

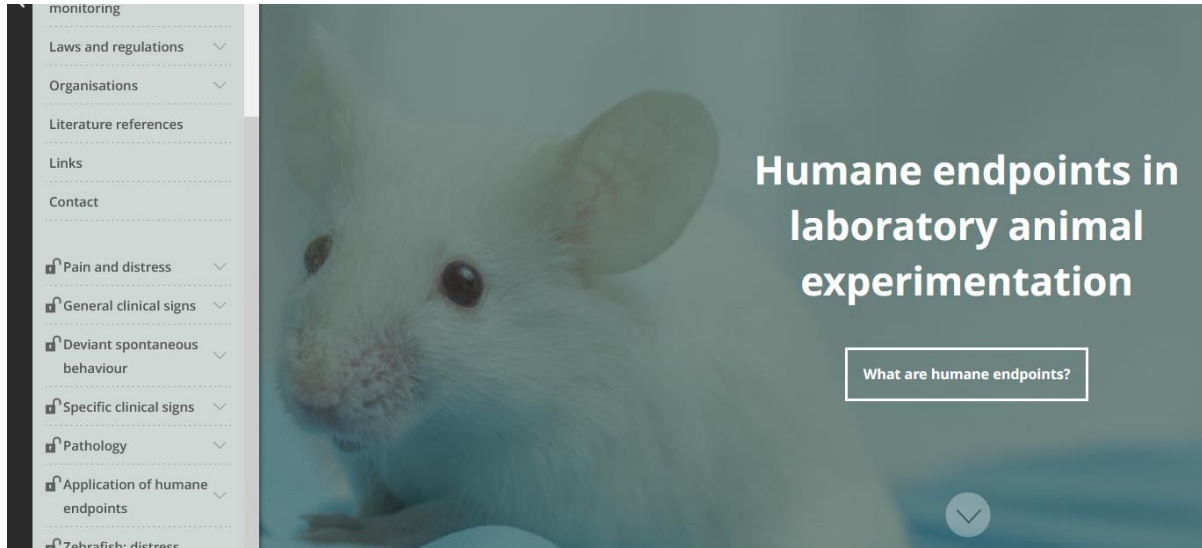
The most important thing : your DS should be evaluated correctly



## How to alleviate the DS ?

- Introduce rest period between two tests
- Equilibrate experiments between several groups
- Include a score sheet and define endpoints  
(could be [decisive for limiting the DS](#))

«Humane endpoints» : source of information on the detection of pain, distress and in laboratory animals. Tips, videos, information...



The image shows a screenshot of a website. On the left is a vertical navigation menu with the following items: monitoring, Laws and regulations, Organisations, Literature references, Links, Contact, Pain and distress, General clinical signs, Deviant spontaneous behaviour, Specific clinical signs, Pathology, Application of humane endpoints, and Zehrafisch: distress. On the right is a banner with a background image of a white mouse. The banner text reads: "Humane endpoints in laboratory animal experimentation" and "What are humane endpoints?".



Source: <https://www.humane-endpoints.info/en>



# How to Reduce ?

**Reduce** : how to decrease animal experiments to the absolute minimum

1. Statistics : Improve experimental design and statistical analysis  
specific courses/statistical support
2. Group experiments to reduce duplication of control groups
3. Longitudinal follow-up of the same animals (especially if using non-invasive  
technics)

#### 4. Discuss/Collaborate

- Colleagues
- Departments seminars
- Biobank/-80°C
- DEA / Core facilities...



# How the core facilities could help ?

- Technical help by trained people (Reduce/Refine)

## **2. Personal Abilities**

*The stress experienced by the animal during blood sampling does not only depend on the technique and the blood volume collected, but essentially the skill of the person who executes it. This is why it is essential that people taking blood samples are familiar with the animal species and the chosen technique. If not, they should learn and practice the chosen technique under the supervision of an experienced person. It's necessary take special care to handle the animals with care and calm.*

- Help in experiments plan (**Reduce/Refine**)
- Help in the management of Pain/Anesthesia (**Refine**)
- Help in animal handling and procedures (**Refine**)
- Help in the writing of SOPs/**Score sheets** in the respect of animal welfare

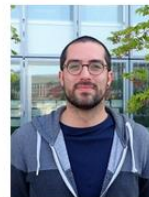
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Gain of time

# ChiRo (Surgeries in Rodent) Service from the PHYM department

- Technical help
- Technical development
- Protocol/technical advises/training
  - ✓ Animal welfare
  - ✓ Anesthesia/analgesia management
- SOP/score sheets/FormA redaction
- Continuous education

## Florian Visentin



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<https://www.unige.ch/medecine/phym/fr/le-departement/le-departement/personnel-commun>

# Anatomical and molecular preclinical imaging *in vivo* and *ex vivo*



Plateforme d'Imagerie  
Préclinique du Petit  
Animal (PIPPA)

## SMALL ANIMAL PRECLINICAL IMAGING PLATFORM (PIPPA)

Academic Director: **Prof. Éric Allémann**  
Operational Manager & contact: **Dr Didier Colin**  
[didier.colin@unige.ch](mailto:didier.colin@unige.ch); +41 79 553 44 58



[https://www.unige.ch/  
medecine/pippa/](https://www.unige.ch/medecine/pippa/)



## CENTER FOR BIOMEDICAL IMAGING (CIBM) PET HUG-UNIGE SECTION

Section Head: **Prof. Valentina Garibotto**  
Operational Manager & contact: **Olivia Bejuy**  
[olivia.bejuy@unige.ch](mailto:olivia.bejuy@unige.ch); +41 79 553 46 04



<https://cibm.ch>

Small animal imaging as a bridge between bench and bedside to increase translational power

- To validate *in vitro* results in preclinical models
- To translate preclinical results to clinical trials

Small animal imaging and 3Rs

- **Replace**
  - Partial replacement (fertilized egg models)
- **Reduce**
  - Longitudinal monitoring on the same animals
  - High translation power to clinical trials
  - Sharing imaging data between labs
- **Refine**
  - Non-invasive technics
  - Definition of novel endpoint criteria

# Metabolic phenotyping

## PHENOTYPING OF SMALL ANIMALS

Phenotyping of small animals through physiological  
and metabolic analyses

Clamp glucose / insulin



- Academic co-directors: Prof. Roberto Coppari
- Contact persons: Dr Franck Bontems
- Contact persons for clamp glucose / insulin: Dr Christelle Veyrat-Durebex

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[Franck.Bontems@unige.ch](mailto:Franck.Bontems@unige.ch)

<https://www.unige.ch/medecine/phenotypage/en>





Thank you