

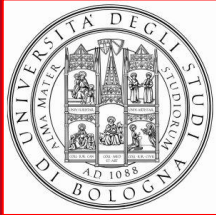


ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Ozzano dell'Emilia 05 Febbraio 2020

Principi di eutanasia, informazioni e descrizioni dei diversi metodi disponibili specifici per specie (teoria)

Dott. Ludovico Scenna Medico Veterinario
specialista in Scienza e Medicina degli Animali da Laboratorio
Libero professionista



D. Lgs. 26/2014

Art. 13 comma 3

3. Nelle procedure di cui al comma 2, va evitata la morte come punto finale, preferendo punti finali più precoci e umanitari. Qualora la morte come punto finale è inevitabile, la procedura soddisfa le seguenti condizioni:

a) comportare la morte del minor numero possibile di animali;

b) ridurre al minimo la durata e l'intensità della sofferenza dell'animale, garantendo per quanto possibile una morte senza dolore.

Art. 22 comma 3

c) le condizioni fisiche in cui gli animali allevati, tenuti o utilizzati sono soggette a controlli giornalieri;



Training, self-examination

http://cbctraining.ncl.ac.uk/eM-EU5/story_html5.html

Please allow 80 minutes to fully complete this standalone tutorial.

RECOGNITION & PREVENTION OF PAIN, SUFFERING & DISTRESS IN LABORATORY ANIMALS EU MODULE 5

NC
3R^s

National Centre
for the Replacement,
Refinement & Reduction
of Animals in Research



Newcastle
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Training, self-examination
<https://www.humane-endpoints.info/en>

A screenshot of the Humane Endpoints website homepage. The page features a dark green background with a white mouse. The main heading reads 'Humane endpoints in laboratory animal experimentation'. A call-to-action button says 'What are humane endpoints?'. The left sidebar contains a navigation menu with items like 'Home', 'About this website', 'Humane Endpoints', 'Normal behaviour and physiology', 'Observation and monitoring', 'Laws and regulations', 'Organisations', 'Literature references', 'Links', and 'Contact'. The footer includes copyright information: 'Copyright © 2016 3Rs-Centre Utrecht Life Sciences | All rights reserved. | Terms and Conditions'.



Clinical Score Systems

Humane endpoints and use of score sheets



Timo Nevalainen
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Finland

Body Condition Score - BCS

THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

IACUC / LARC STANDARD PROCEDURES

Title: Body Condition Scoring (BCS) of Mice

Description of procedure:

Scoring the body condition of rodents is a non-invasive method for assessing health and establishing endpoints for adults where body weight is not a viable monitoring tool, such as with tumor models, ascites production and pregnancy, or young growing animals. Body condition scores (BCS) range from 1 (emaciation) to 5 (obesity). An anticipated BCS of 2 (under conditioned) or lower requires justification in the protocol. Scores are determined by frequent visual and hands-on examination of each animal. The hands-on evaluation is done by gently holding the mouse by the base of the tail and passing a finger over the sacroiliac bones. Match the findings to the descriptions and diagrams provided to determine a score.

Body Condition Score Chart

			<p>BC 1 Mouse is emaciated</p> <ul style="list-style-type: none"> Skeletal structure extremely prominent; little or no flesh cover Vertebrae distinctly segmented
			<p>BC 2 Mouse is under conditioned</p> <ul style="list-style-type: none"> Segmentation of vertebral column evident Dorsal pelvic bones are readily palpable
			<p>BC 3 Mouse is well-conditioned</p> <ul style="list-style-type: none"> Vertebrae and dorsal pelvis not prominent; palpable with slight pressure
			<p>BC 4 Mouse is over conditioned</p> <ul style="list-style-type: none"> Spine is a continuous column Vertebrae palpable only with firm pressure
			<p>BC 5 Mouse is obese</p> <ul style="list-style-type: none"> Mouse is smooth and bulky Bone structure disappears under flesh and subcutaneous fat
			<p>BC 3, 4, 5</p>

Note: A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2-, 2-...)

Updated 7/2013



re Systems


https://www.bu.edu/researchsupport/compliance/animal-care/working-with-animals/procedures/body-condition-scoring-for-mice-iacu...

Body Condition Scores

[Collapse all](#)


Body Condition 1: Mouse is emaciated

- Skeletal structure extremely prominent, little or no flesh cover.
- Vertebrae distinctly segmented



Body Condition 2: Mouse is underweight

- Segmentation of vertebral column evident.
- Dorsal pelvic bones are readily palpable.

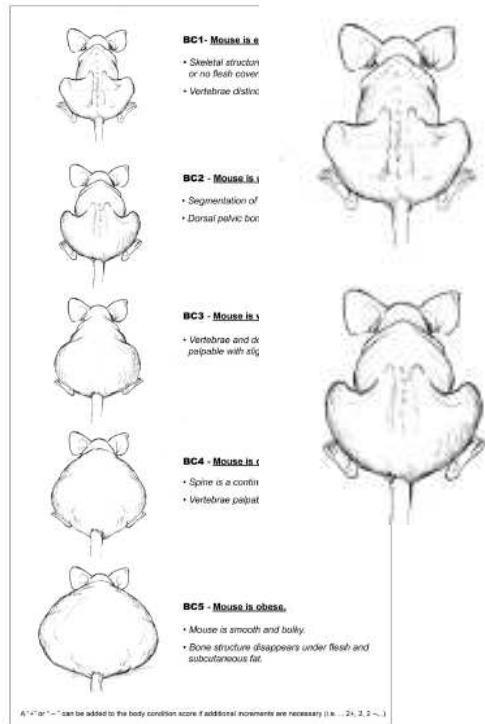


Body Condition 3: Mouse is well-conditioned



Burkholder et al.

Page 16

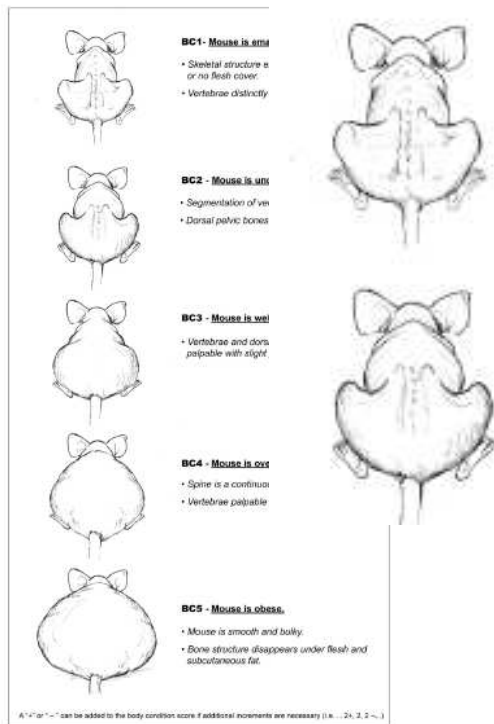


Body condition scoring is a quick and easy methodology that is useful in assessing animal health. It is particularly helpful when body weight might not reflect body condition (e.g. presence of tumors, ascites, organomegaly, pregnancy). Simply run your finger over the sacral area and score the animal according to the chart.

Figure 2. Body Condition Scoring (BCS) is a quick, easy and reliable method for assessing mouse health. It utilizes a scoring system of 1 to 5 with 3 being the optimal condition, 1 being emaciated and 5 being obese.



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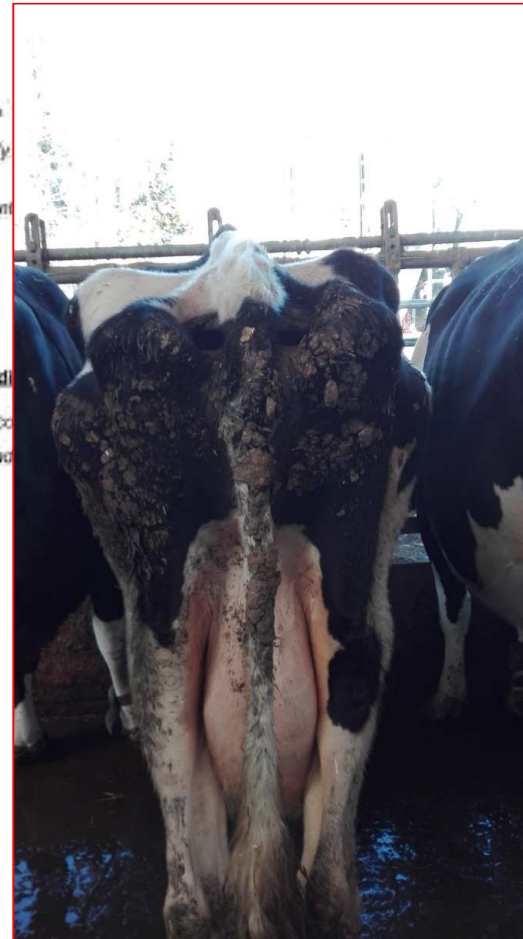


BC1 - Mouse is emaciated.

- Skeletal structure extremely or no flesh cover.
- Vertebrae distinctly segment

BC2 - Mouse is undercondi...

- Segmentation of vertebral co
- Dorsal pelvic bones are read



Nuova BCS Cowdftion app

La nuova BCS Cowdftion App sviluppata da Bayer migliora e facilita la determinazione dei corretti valori di BCS per angole visive.

Valutazione del Body Condition Score (BCS)

BCS 1 - molto magra

BCS 2 - magra

BCS 3 - stato ottimale

BCS 4 - grassa

BCS 5 - molto grassa

molto grassa

Punti dell'esame ispettivo per il BCS

Figure 2. Body Condition Scoring (BCS) is a quick, easy and reliable method for assessing mouse health. It utilizes a scoring system of 1 to 5 with 3 being the optimal condition, 1 being emaciated and 5 being obese.



Clinical Score Systems

Clinical Score System – Roberta Aiello - IzsVe

Humane endpoints and use of score sheets



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Manifestazione clinica	scoring system
Nessuna	0
Arruffamento del pelo	1
Arruffamento del pelo, diminuzione di attività fisica	2
Arruffamento del pelo, diminuzione di attività fisica, perdita di peso tra 10 e 15%	3
Arruffamento del pelo, diminuzione di attività fisica, difficoltà respiratoria, perdita di peso tra 15 e 20%	4
Arruffamento del pelo, diminuzione di attività fisica, difficoltà respiratoria, perdita di peso di più del 20%, cifosi	5



Clinical Score Systems

Humane endpoints and use of score sheets

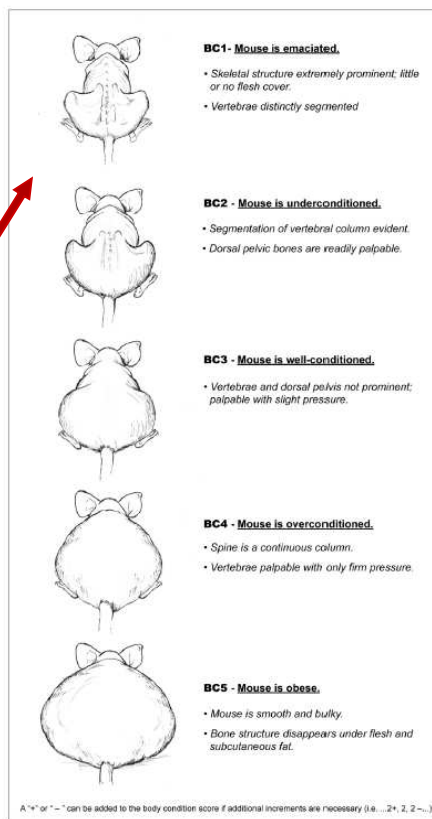


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Clinical Score System – OPBA – UNITN

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Author Manuscript
Curr Protoc Mouse Biol. Author manuscript, available in PMC 2013 June 01.
Published in final edited form as:
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Health Evaluation of Experimental Laboratory Mice
Tanya Burkholder, Charmaine Foltz¹, Eleanor Karlsson, C. Garry Linton, and Joanne M Smith



BCS

Manifestazione clinica	Clinical score
Pelo normale, mucose normali, normale attività fisica, normale consumo di cibo ed acqua	0
Arruffamento del pelo (piloerezione, lesioni, disidratazione)	1
Arruffamento del pelo, < attività fisica (grooming, esplorazione...)	2
Arruffamento del pelo, < attività fisica (letargia), *perdita di peso < 10 %	3
Arruffamento del pelo, letargia, *perdita di peso < 10% , difficoltà respiratoria (dispnea, tachipnea)	4
Arruffamento del pelo, letargia, *perdita di peso tra 10 e 20% , dispnea/tachipnea, lordosi/cifosi	5
Arruffamento del pelo, letargia, dispnea/tachipnea, lordosi/cifosi, *perdita di peso > 20%	6

Body condition scoring is a quick and easy methodology that is useful in assessing animal health. It is particularly helpful when body weight might not reflect body condition (e.g. presence of tumors, ascites, organomegaly, pregnancy). Simply run your finger over the sacral area and score the animal according to the chart.



Clinical Score Systems

Humane endpoints and use of score sheets



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Per tutte le specie tutelate dal Decreto → Es. Zebrafish

Manifestazione	Punteggio
Nuoto in gruppo nella colonna d'acqua; respirazione normale; normale forma corporea; scaglie normali	0
Respirazione accelerata; pesci vicino alla superficie/ingresso dell'acqua; forma e scaglie normali; crescita lenta	1
Magrezza; lieve scoliosi; ritenzione uova; obesità; scaglie normali	2
Tumori; pesci emaciati con scoliosi; occhi sporgenti; scaglie sollevate; lesioni/ulcere; pesci adagiati sul fondo; nuoto disorientato	3

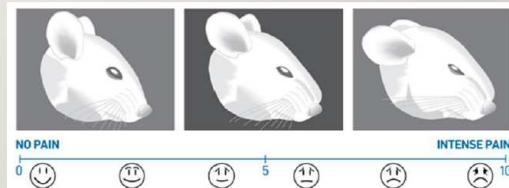


Grimace scale

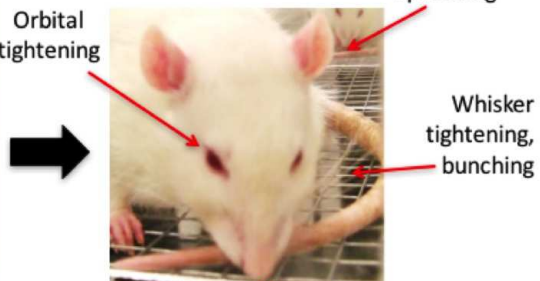
<https://iacuc.ucsf.edu/sites/g/files/tkssra751/f/wysiwyg/STD%20PROCEDURE%20-%20Misc%20Rodent%20Procedures%20-%20BCS%20Rats.pdf>

MOUSE GRIMACE SCALE

- **History:** Facial expressions are widely used to assess pain in human infants → Langford et al. developed a similar grimace scale for mice in 2010
- **Purpose:** To become a useful measurement tool in pain research and in clinical assessment of mice



No Pain



Pain

B. ACTION UNITS:

1. Orbital Tightening

Mouse must display a narrowing of the orbital area, a tightly closed eyelid, or an eye squeeze. An eye squeeze is defined as the orbital muscles around the eyes being contracted. A wrinkle may be visible around the eye. As a guideline, any eye closure that reduces the eye size by more than half should be coded as a "2". *Note that sleeping mice display closed eyes, and this may be mistaken for a tightly closed eyelid. Photographs of sleeping mice should therefore not be taken and/or coded.



0 1 2

3. Cheek Bulge

The cheek muscle is contracted and extended relative to the baseline condition; it will appear to be convex from its neutral position. Note: The cheek is considered to be the area directly below the eye and extending to the beginning of the whiskers on the nose (in humans, the infraorbital triangle). The distance from eye to whisker pad may appear shortened relative to baseline.



0 1 2

2. Nose Bulge

Mouse must display a bulge on top of the nose. The skin and muscles around the nose will be contracted creating a rounded extension of skin visible on the bridge of the nose. A nose bulge may also be coded if a coder sees vertical wrinkles extending down the side of the nose from the bridge. In frontal headshots, a bulge may be seen as a widening of the nose area (i.e., V-shape connecting eyes to nose appears broader). *Note that a nose bulge may also appear when mice are actively exploring (i.e., sniffing). Ideally, these photographs should not be taken and/or coded, as they may inflate baseline MGS scores.



0 1 2

4. Ear Position

Ears may be pulled back from their baseline position, or may be seen as laid flat against the head. In a typical baseline position ears are roughly perpendicular to the head and are directed forward. In pain, the ears tend to rotate outwards and/or back, away from the face. As a result, the space between the ears may appear wider relative to baseline. *Note that mice engaged in exploration or grooming may also pull ears back, but distance between ears tends to narrow rather than widen. In any case, these may cause confusion, and it is advised that photographs of mice actively exploring or grooming not be taken and/or coded.



0 1 2

5. Whisker Change

Whiskers must have moved from the baseline position. They could either be pulled back to lay flat against the cheek or pulled forward as if to be "standing on end". Whiskers may also clump together compared to baseline whiskers, which tend to be fairly evenly spaced.



0 1 2



Clinical Score Systems

Grimace Scale

Humane endpoints and use of score sheets



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BRIEF COMMUNICATIONS

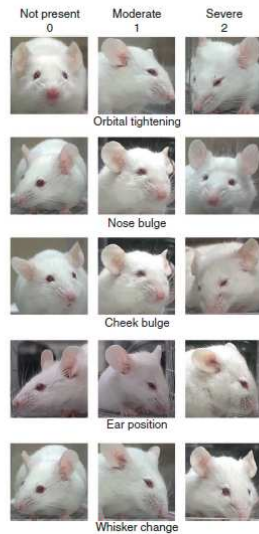


Figure 1 | In the MGS, intensity of each feature is coded on a three-point scale. For each of the five features, images of mice exhibiting behavior corresponding to the three values are shown.

improvement in accuracy, such that over 97% of pain versus no-pain calls were correct (Supplementary Fig. 1a).

We also assessed the scale on 14 commonly used preclinical pain assays (Online Methods), comparing average action unit scores assigned to 'pain' photos to those assigned to 'no-pain' photos and then comparing the resulting difference scores to zero by one-sample *t*-tests. The MGS revealed significant changes from baseline in

a 0.87

MOUSE GRIMACE SCALE (MGS): THE MANUAL

B. ACTION UNITS:

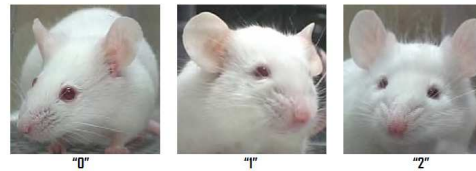
1. Orbital Tightening

Mouse must display a narrowing of the orbital area, a tightly closed eyelid, or an eye squeeze. An eye squeeze is defined as the orbital muscles around the eyes being contracted. A wrinkle may be visible around the eye. As a guideline, any eye closure that reduces the eye size by more than half should be coded as a "2". *Note that sleeping mice display closed eyes, and this may be mistaken for a tightly closed eyelid. Photographs of sleeping mice should therefore not be taken and/or coded.



2. Nose Bulge

Mouse must display a bulge on top of the nose. The skin and muscles around the nose will be contracted creating a rounded extension of skin visible on the bridge of the nose. A nose bulge may also be coded if a coder sees vertical wrinkles extending down the side of the nose from the bridge. In frontal headshots, a bulge may be seen as a widening of the nose area (i.e., V-shape connecting eyes to nose appears broader). *Note that a nose bulge may also appear when mice are actively exploring (i.e., sniffing). Ideally, these photographs should not be taken and/or coded, as they may inflate baseline MGS scores.

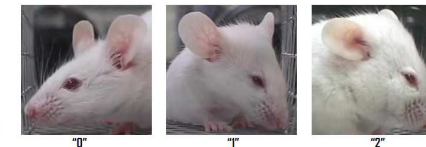


baseline condition; it will appear to be convex and to be the area directly below the eye and extending to the beginning of the whiskers on the nose (in humans, the infraorbital triangle). The distance from eye to whisker pad may appear shortened relative to baseline.



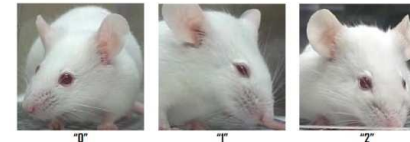
4. Ear Position

Ears may be pulled back from their baseline position, or may be seen as laid flat against the head. In a typical baseline position ears are roughly perpendicular to the head and are directed forward. In pain, the ears tend to rotate outwards and/or back, away from the face. As a result, the space between the ears may appear wider relative to baseline.*Note that mice engaged in exploration or grooming may also pull ears back, but distance between ears tends to narrow rather than widen. In any case, these may cause confusion, and it is advised that photographs of mice actively exploring or grooming not be taken and/or coded.



5. Whisker Change

Whiskers must have moved from the baseline position. They could either be pulled back to lay flat against the cheek or pulled forward as if to be "standing on end". Whiskers may also clump together compared to baseline whiskers, which tend to be fairly evenly spaced.





Clinical Score Systems Grimace Scale

Humane endpoints and use of score sheets



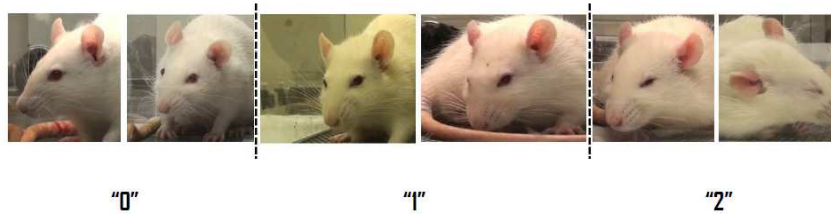
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Universities of Kuopio and Helsinki
Finland

RAT GRIMACE SCALE (RGS) THE MANUAL

B. ACTION UNITS:

1. Orbital Tightening

Rats in pain display a narrowing of the orbital area, a tightly closed eyelid, or an eye squeeze. squeeze is defined as the orbital muscles around the eyes being contracted. The nictitating membrane may be visible around the eye and becomes more pronounced as the pain intensifies. As a guideline, any eye closure that reduces the eye size by more than half should be coded as a "2". *Note that sleeping rats display closed eyes, but of a relaxed nature, whereas a rat in pain may display a closed eye with tight orbital muscles. Photographs of sleeping rats should not be taken and/or coded.



"0"

"1"

"2"

2. Nose/Cheek Flattening

Rats in pain display a *lack* of bulge on top of the nose (i.e., a flattening of the nose). In the "no pain" condition a clear bulge is present at the bridge of the nose. The whisker pads are also rounded and slightly puffed out, leaving a clear crease between the pads and the cheek. When in pain, the bridge of the nose flattens and elongates, causing the whisker pads to flatten. At this time the crease between the pads and the cheek is no longer present. In frontal headshots, the nose may appear narrower and longer.



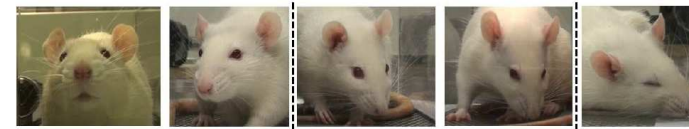
"0"

"1"

"2"

3. Ear Changes (Position, Orientation, Shape)

The ears of rats in pain may be curled and pointed more than in the baseline position. In the baseline position ears are roughly perpendicular to the head, face forward, and are angled slightly backward. Importantly, the ears also have a rounded shape. In pain, the ears tend to fold, curl inwards and are angled forward. This curling of the ears tends to result in a "pointed" shape of the ears. In pronounced pain states, the ears are angled outward and are held close to 45° away from both the perpendicular axis and the nose. As a result, the space between the ears may appear wider relative to baseline.



"0"

"1"

"2"



Clinical Score Systems

Grimace Scale

Humane endpoints and use of score sheets



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Rabbit Grimace Scale (RbtGS) Manual

This manual describes the five action units that comprise Rabbit Grimace Scale (RbtGS) and how these action units should be scored. These action units have been shown to increase in intensity in response to post-procedural pain (Keating et al. 2012).

The five action units in the Rabbit Grimace Scale are:

- Orbital tightening
- Cheek flattening
- Nostril shape
- Whisker change & position
- Ear shape & position

Orbital Tightening

0	1	2
Not Present	Moderately Present	Obviously Present
<ul style="list-style-type: none"> • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye 		

Cheek Flattening

0	1	2
Not Present	Moderately Present	Obviously Present
<ul style="list-style-type: none"> • Flattening of the cheeks. When 'obviously present', cheeks have a sunken look. • The face become 		

Ear Shape & Position

0	1	2
Not Present	Moderately Present	Obviously Present
<ul style="list-style-type: none"> • Ears become more tightly folded / curled (more cylindrical) in shape • Ears rotate from facing towards the source of sound to facing towards the hindquarters • Ears may be held closer to the back or sides of the body 		

Nostril shape

0	1	2
Not Present	Moderately Present	Obviously Present
<ul style="list-style-type: none"> • Nostrils (nares) are drawn vertically forming a 'V' rather than 'U' shape • Nose tip is moved down towards the chin 		

Whisker Change & Position

0	1	2
Not Present	Moderately Present	Obviously Present
<ul style="list-style-type: none"> • Whiskers are pushed away from the face to 'stand on end' • Whiskers stiffen and lose their natural, downward curve • Whiskers increasingly point in the same direction. When 'obviously present', whiskers move downwards 		



Clinical Score Systems Grimace Scale

Humane endpoints and use of score sheets



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Estimating Sheep Pain Level Using Facial Action Unit Detection

Yiting Lu, Marwa Mahmoud and Peter Robinson
Computer Laboratory, University of Cambridge, Cambridge, UK

Abstract—Assessing pain levels in animals is a crucial, but time-consuming process in maintaining their welfare. Facial expressions in sheep are an efficient and reliable indicator of pain levels. In this paper, we have extended techniques for recognising human facial expressions to encompass facial action units in sheep, which can then facilitate automatic estimation of pain levels. Our multi-level approach starts with detection of sheep faces, localisation of facial landmarks, normalisation and then extraction of facial features. These are described using Histogram of Oriented Gradients, and then classified using Support Vector Machines. Our experiments show an overall accuracy of 67% on sheep Action Units classification. We argue that with more data, our approach on automated pain level assessment can be generalised to other animals.

I. INTRODUCTION

Pain level assessment is critical to the welfare of sheep.

Finally, we argue that - with their pain scales calibrated - the proposed automatic pain level estimation approach can be generalised to other animals, such as mice [12] [5], rabbits [14] and horses [13].

We start by reviewing the related work in Section 2. A description of our dataset is discussed in Section 3. Our methodology is described in section 4 followed by the experimental evaluation in Section 5. Finally, conclusions and future work are presented in Section 6.

II. RELATED WORK

Analysing facial expressions of animals was first introduced by Langford *et al.* [4] to facilitate detection of pain level in mice. This approach has been advanced and



Fig. 2. Sheep facial AU taxonomy with brief description & image. The taxonomy is based on the SAFES [1].

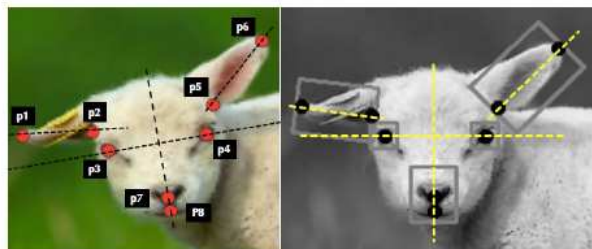
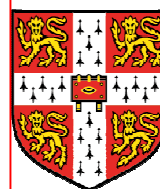


Fig. 3. Left: Localised facial landmarks (Note: the eight facial landmarks are labelled from p1 to p8) Right: Normalised sheep face marked with feature bounding boxes



UNIVERSITY OF CAMBRIDGE




 Commissione europea

Cura per gli animali

Verso una scienza migliore

DIRETTIVA 2010/63/EU DEL PARLAMENTO EUROPEO E DEL CONSIGLIO, DEL 22 SETTEMBRE 2010, SULLA PROTEZIONE DEGLI ANIMALI UTILIZZATI A FINI SCIENTIFICI



QUADRO DI VALUTAZIONE DELLA GRAVITÀ DELLE PROCEDURE

Ambiente

Come adattare un Clinical Score Systems alle proprie esigenze

Humane endpoints and use of score sheets



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Come adattare un Clinical Score Systems alle proprie esigenze

Humane endpoints and use of score sheets



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Finland



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Curr Protoc Mouse Biol. Author manuscript; available in PM

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3399545/>

Parole chiave:

- Tanya Burkholder
- Mice Health Evaluation

Published in final edited form as:

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Burkholder et al.

Health Evaluation of Experimental Laboratory Mice

Tanya Burkholder, Charmaine Foltz¹, Eleanor Karlsson, C Garry Linton, and Joanne M Smith

Abstract

Good science and good animal care go hand in hand. A sick or distressed animal does not produce the reliable results that a healthy and unstressed animal produces. This unit describes the essential of assessing mouse health, colony health surveillance, common conditions, and determination of appropriate endpoints. Understanding the health and well-being of the mice used in research enables the investigator to optimize research results and animal care.

Keywords

Mouse health evaluation

Table 1

Assessing Pain and Distress in Mice

PAIN AND DISTRESS ASSESSMENT	EXAMPLES
1) no indication of pain and distress	Normal; well groomed; alert; active; good condition; asleep or calm; normal appetite; BCS=3,4 or 5
2) mild or anticipated pain and distress	Not well groomed; awkward gait; slightly hunched; looks at wound or pulls away when area touched; mildly agitated; BCS=2
3) moderate pain and distress	Rough hair coat; dirty incision; squinted eyes; moves slowly; walks hunched and/or slowly; depressed or moderately agitated; slight dehydration; pruritic; restless; uncomfortable; not eating or drinking; BCS=2-
*4) severe pain and distress	Very rough hair coat; eyes sunken (severe dehydration); slow to move or non-responsive when coaxed; hunched; large abdominal mass; dyspnea; self mutilating; violent reaction to stimuli or when approached; BCS=1



Humane Endpoint

- ***The humanest possible treatment of experimental animals, far from being an obstacle, is actually a prerequisite for successful animal experiments.***

Russel & Burch 1959

- ***Animals found dead in the course of experiments not only represent missed opportunity for implementing alternative and more humane endpoints but possible loss of valuable data as well. Such deaths mark unnecessary suffering and bad science.***

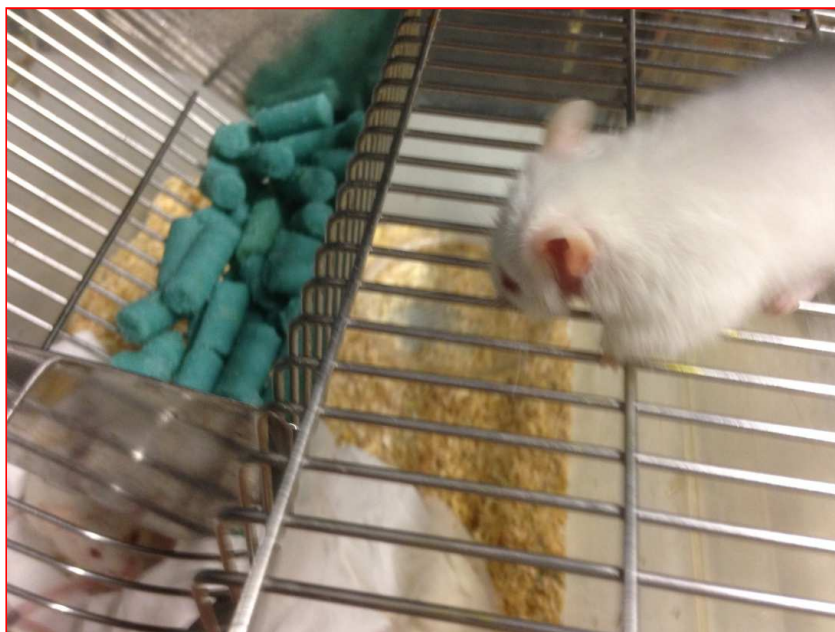
Morton DB et al 1998.

- ***Use of humane endpoints in animal experiments describes the 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. Some examples of possible criteria include body temperature, body weight, precise clinical signs and blood markers. Suffering may be alleviated by, for example, modifying the experimental design, or treatment or euthanasia of the animals. Thus, for experiments that previously might have used the number of animals dying versus the number surviving as their scientific output, the scientific output becomes the number of animals surviving versus the numbers humanely killed after exhibiting certain clinical signs that reliably suggest that death was inevitable***

NC3RS LINK Humane Endpoint 2012

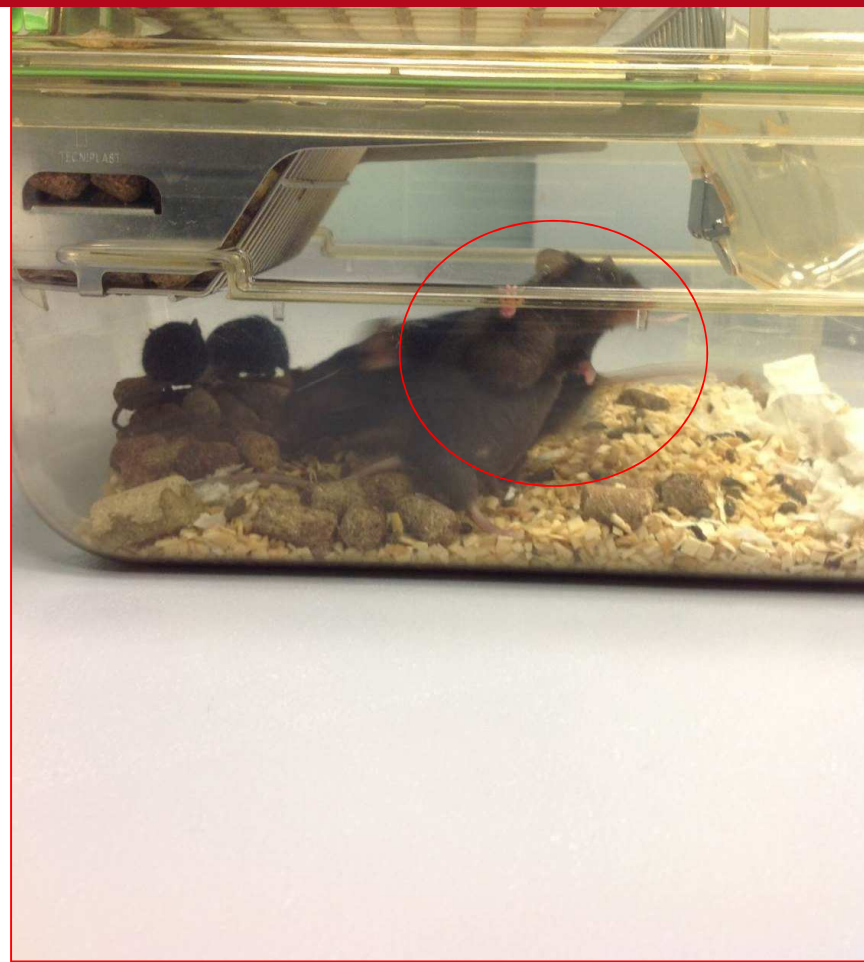
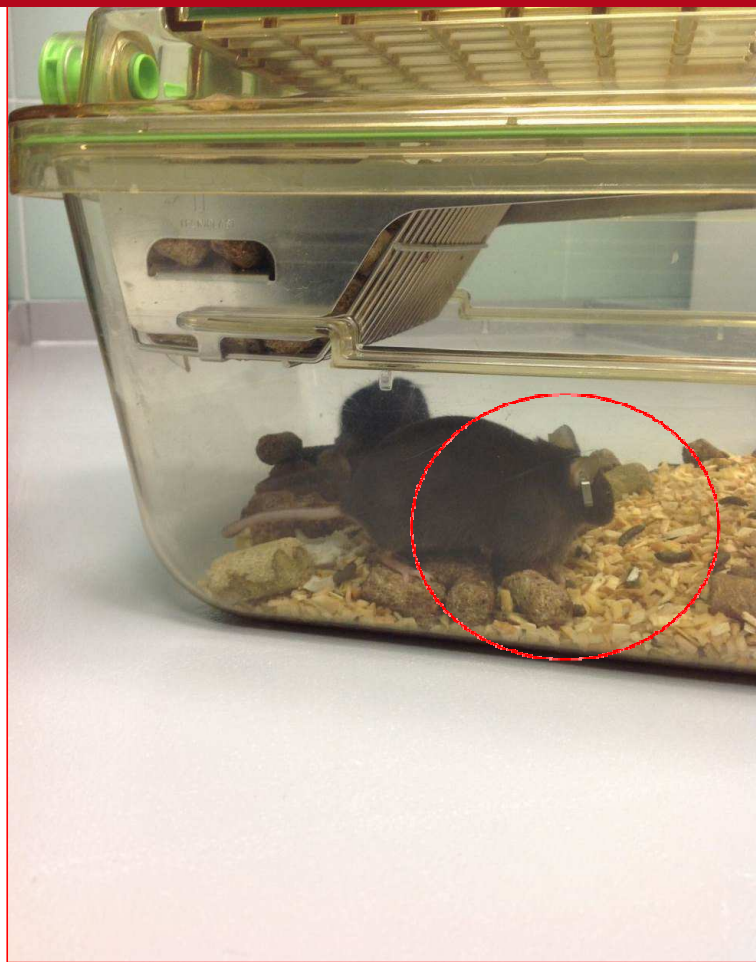


Humane Endpoint





Humane Endpoint





Humane Endpoint



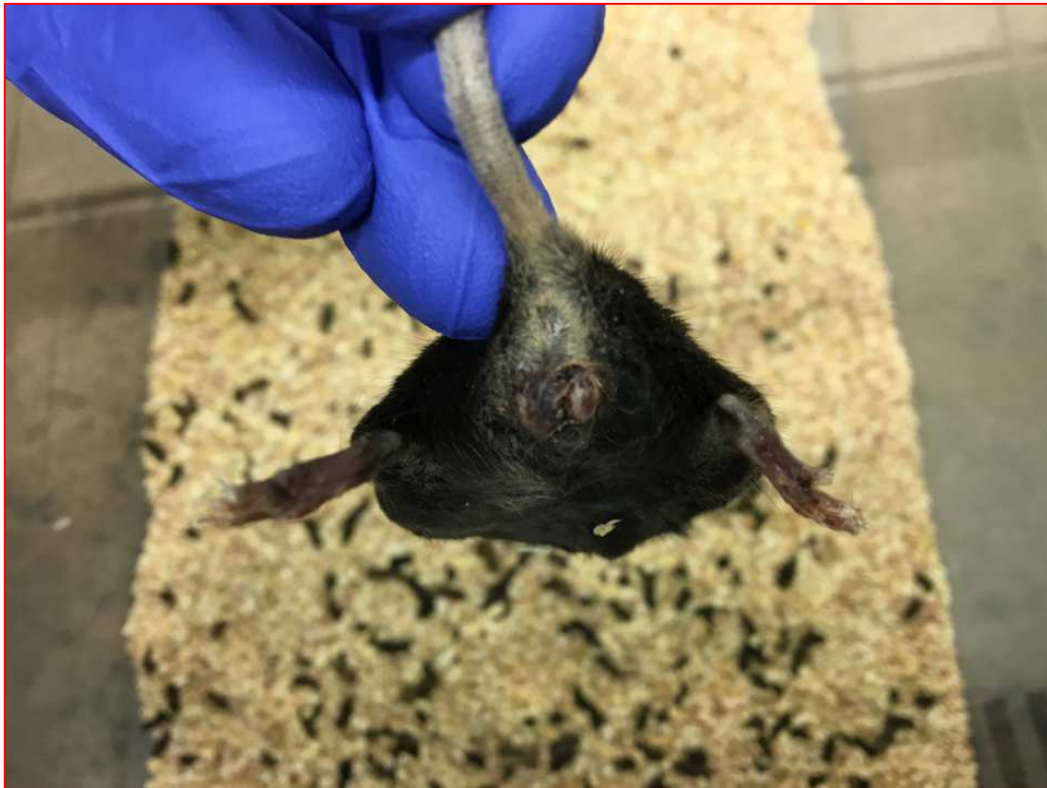


Humane Endpoint





Humane Endpoint





Humane Endpoint



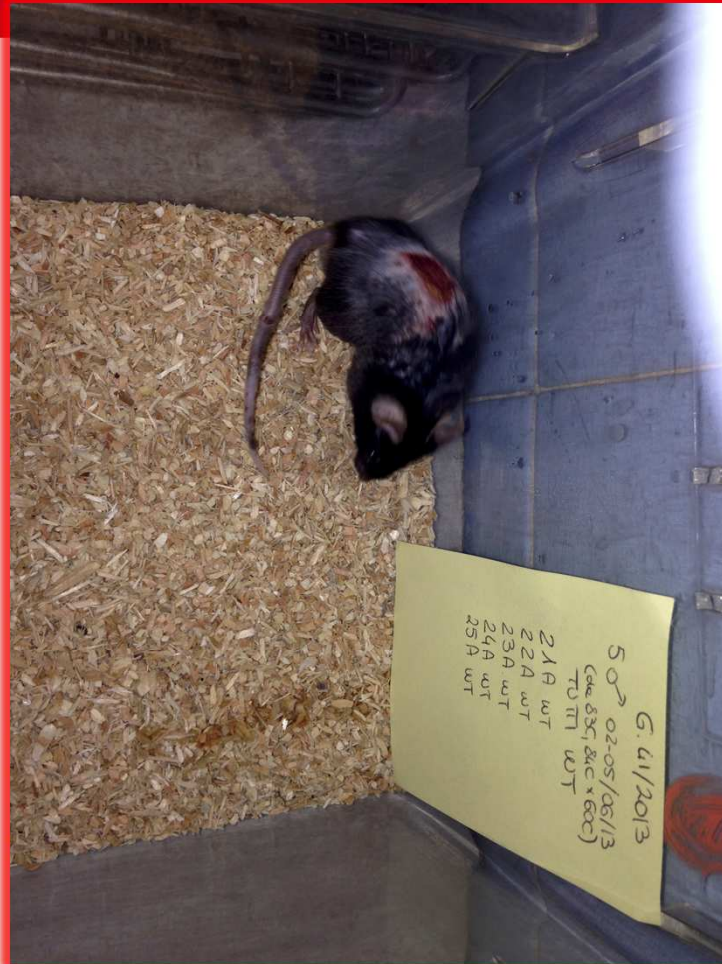


Humane Endpoint

Humane endpoints and use of score sheets



Timo Nevalainen
Universities of Kuopio and Helsinki
Finland



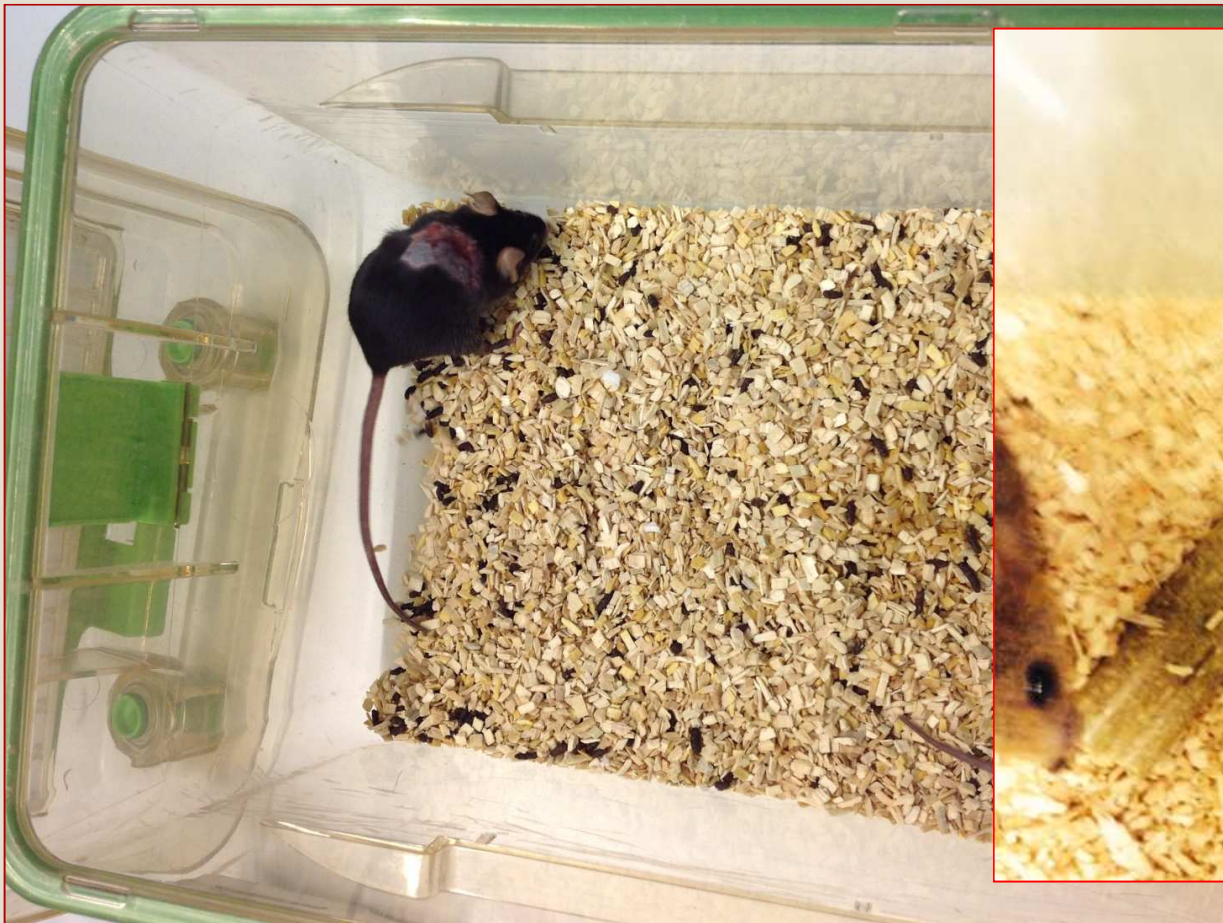


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Humane Endpoint

Vaginal prolapse
+
Ulcerative dermatitis



Rectal prolapse



Comparative Medicine
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Vol 64, No 3
June 2014
Pages 171-178

Original Research
Isolation of *Helicobacter* spp. from Mice with Rectal Prolapses

Cassandra L Miller, Sureshkumar Muthupalani, Zeli Shen, and James G Fox*

Fredrickson et al.

Page 2

environment. Recently, however, we observed disease and death from ulcerative colitis in mice with overt MAIDS.

A naturally occurring disease of mice, transmissible murine colonic hyperplasia (TMCH), is caused by *Citrobacter rodentium*, formerly known as *Citrobacter freundii* biotype 4280.^{2,15,25} Features of TMCH include rectal prolapse, diarrhea, and thickened, dilated colon, as well as a histologic picture of epithelial cell hyperplasia and crypt elongation. The severity of lesions varies with mouse strain and age, C57BL/6 being relatively resistant, and most infections resolve spontaneously.^{15,19} This report describes the colonic lesions seen in MAIDS mice and their etiologic association with infection by *C. rodentium*.

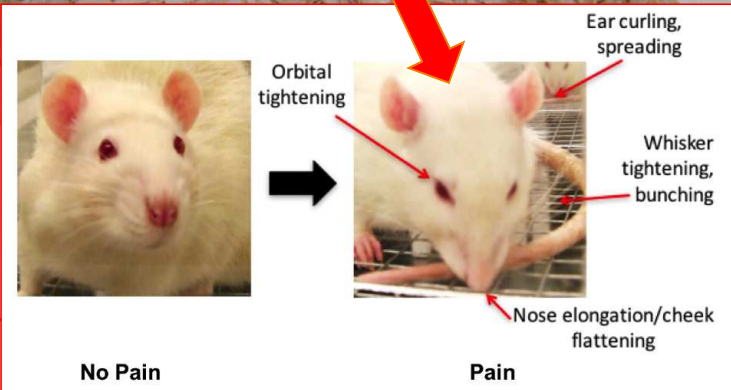
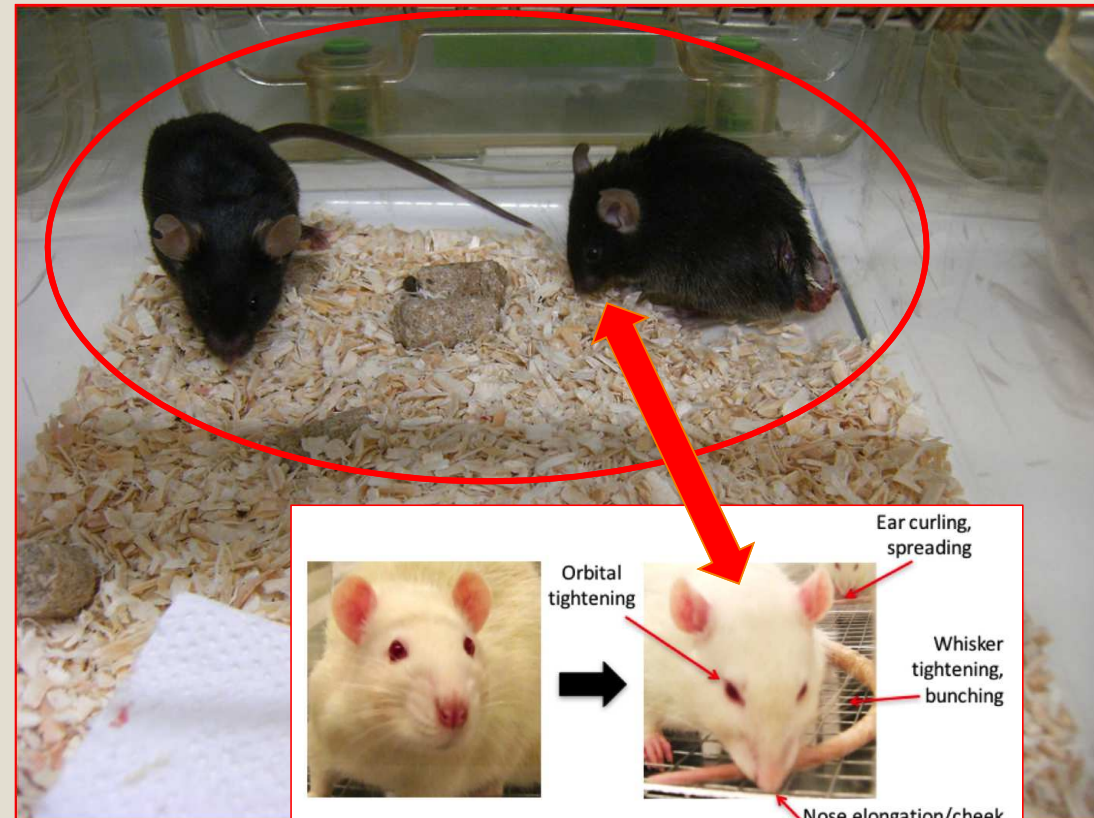


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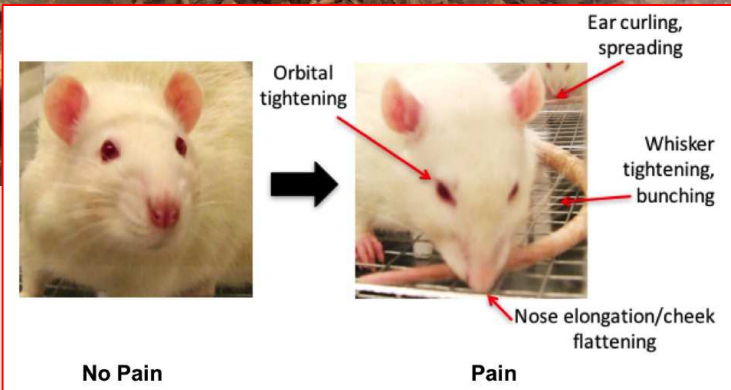


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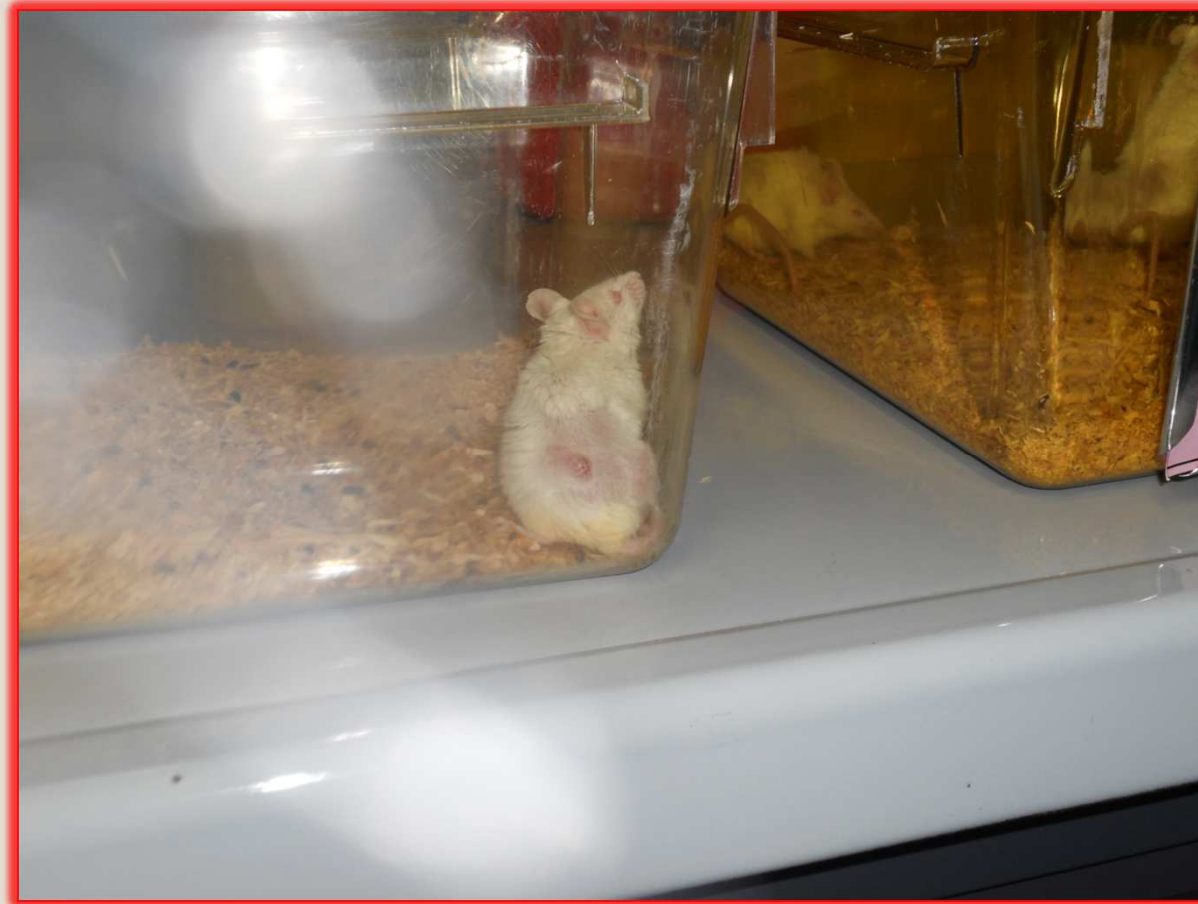


Humane Endpoint





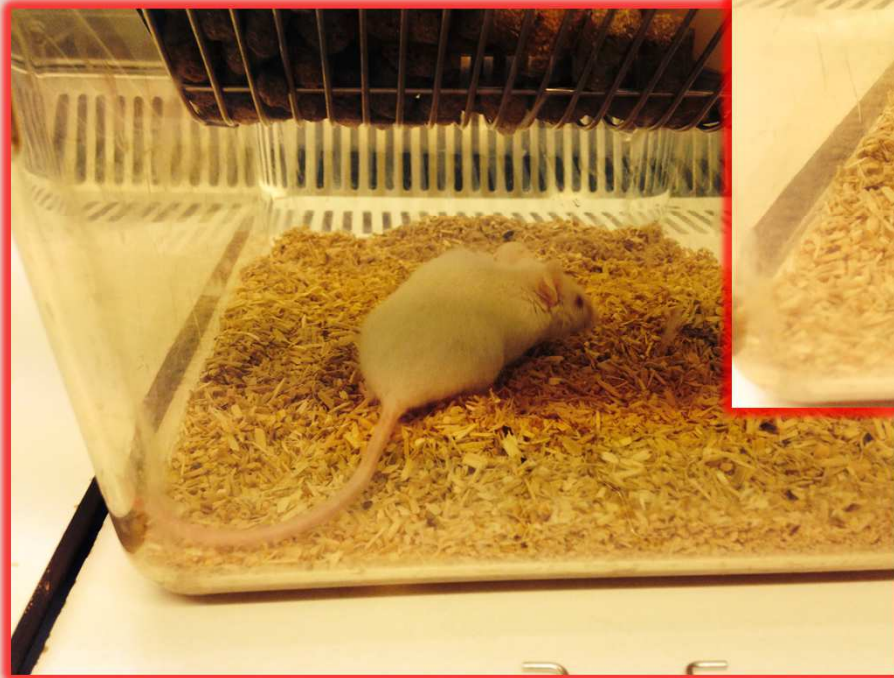
Humane Endpoint

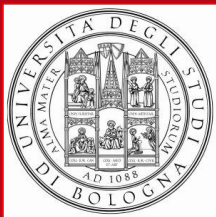




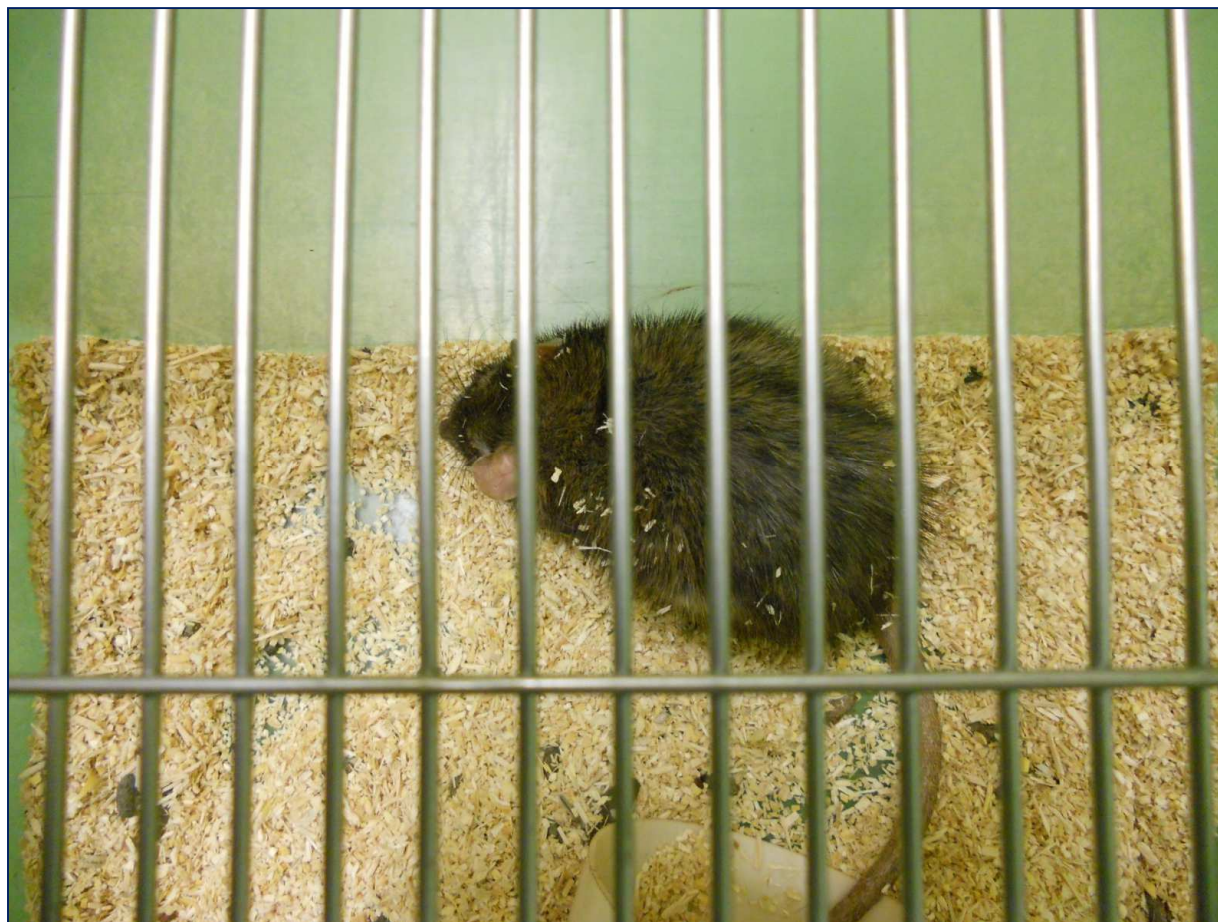
Humane Endpoint

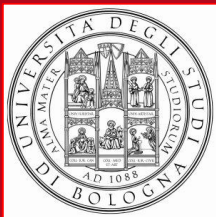
IACUC Handbook Table 17.1 pag 314





Humane Endpoint





Humane Endpoint





Training, self-examination

https://www.google.it/search?q=rat+humane+endpoint+laboratory&client=firefox-b-ab&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiJobXfsefTAhVMJcAKHQ4C4gQ_AUICigB&biw=1920&bih=923#imgdii=T_wdaJm4KtHg_M:&imgcr=iAuL6_hXsr1ICM:

rat 1



rat 2



rat 3





Humane Endpoint



Check????

Laboratory Animal Care

BOSTON UNIVERSITY
Animal Health Monitoring

The Boston University logo, consisting of the words 'BOSTON UNIVERSITY' in white text on a red rectangular background.



Humane Endpoint



Check:

1. Eyes
2. Ears
3. Tails
4. Hunched posture?

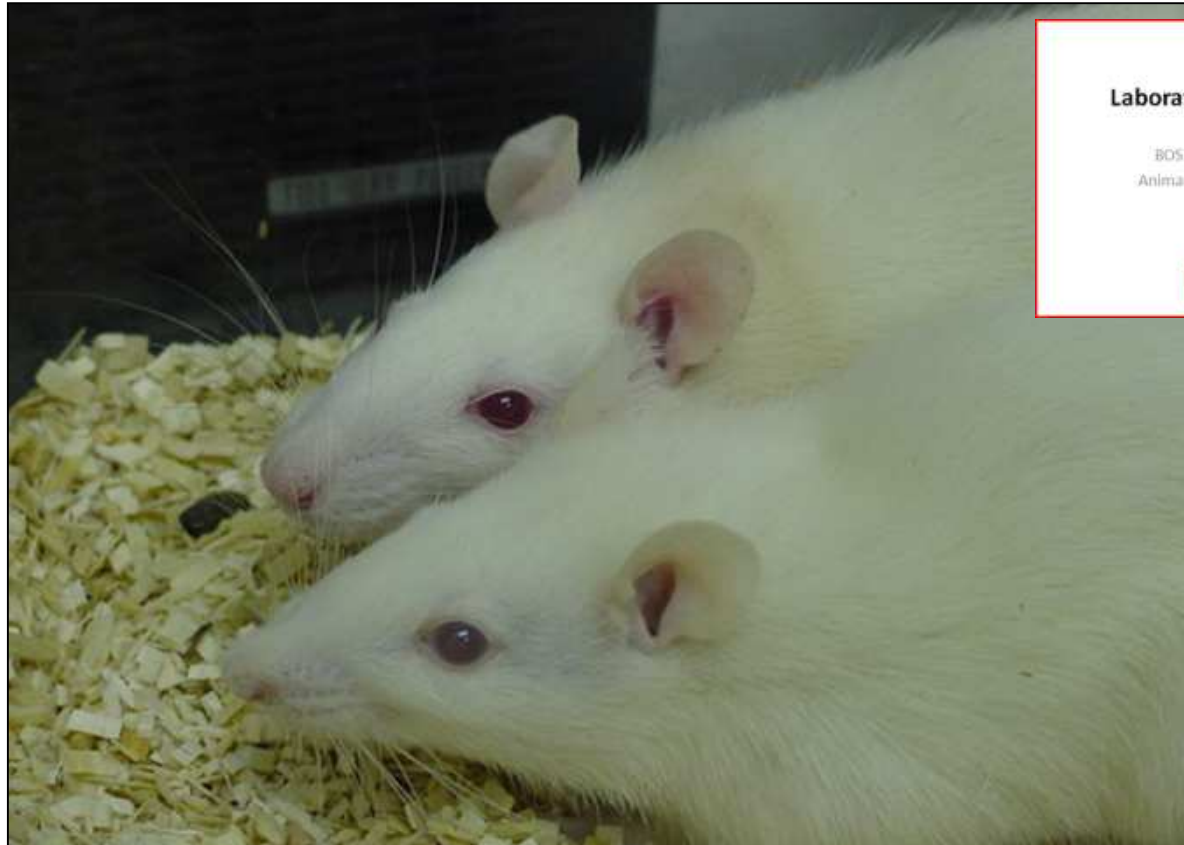
Laboratory Animal Care

BOSTON UNIVERSITY
Animal Health Monitoring





Humane Endpoint



Laboratory Animal Care

BOSTON UNIVERSITY
Animal Health Monitoring





Humane Endpoint

Your help in caring for all the animals is greatly appreciated!

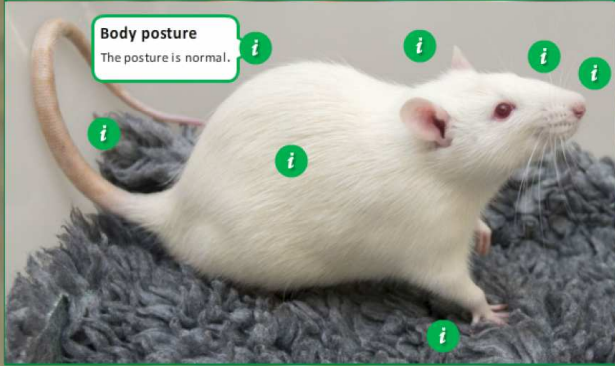
Glossary

RECOGNITION & PREVENTION OF PAIN, SUFFERING & DISTRESS IN LABORATORY ANIMALS

We can carry out similar assessments on other species. This rat shows **positive** signs of good health and welfare. Hover over the icons for more information.

NOTES

Body posture
The posture is normal.

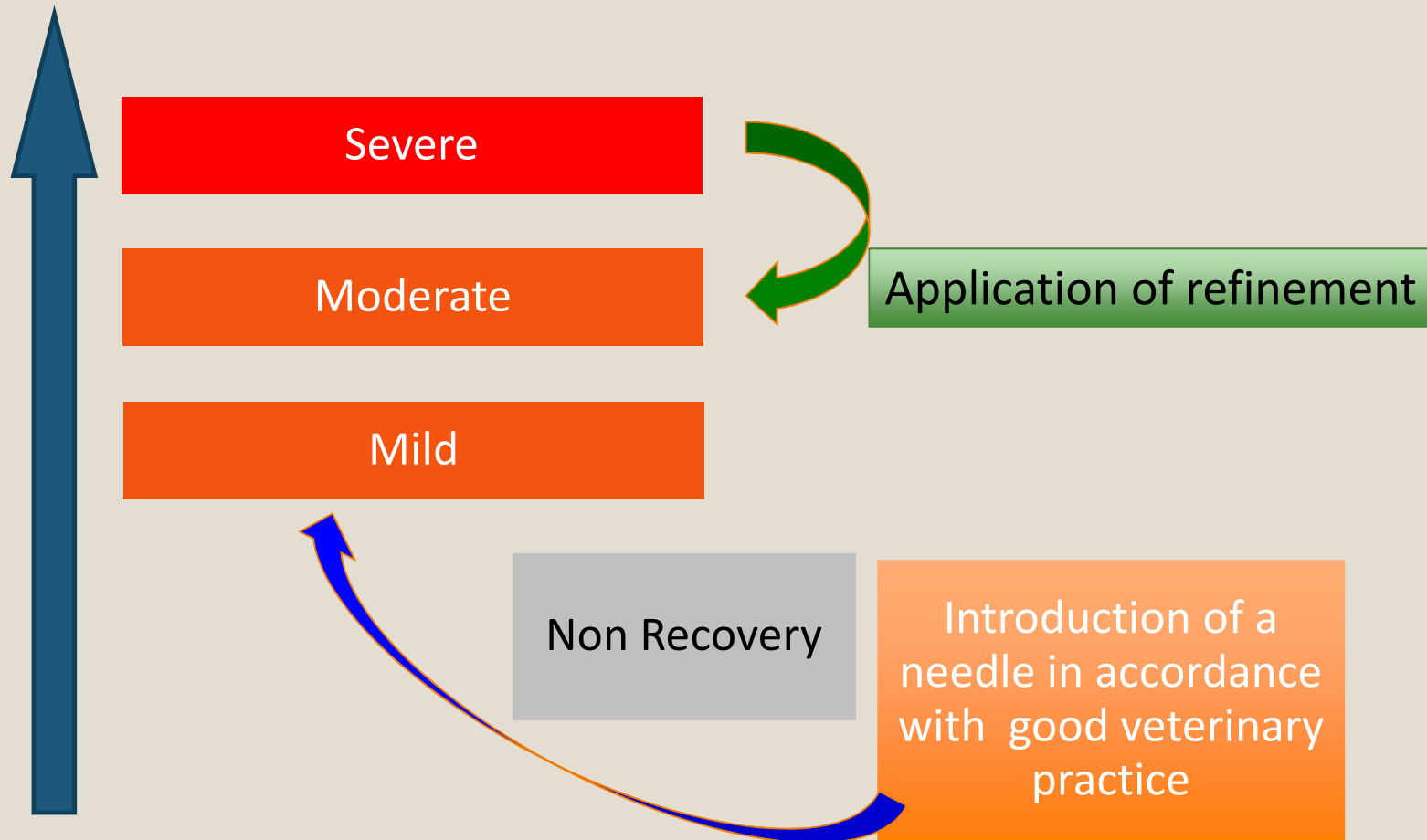


▶▶

NC3r

<https://www.nc3rs.org.uk/news/fresh-approach-training-welfare-assessment-laboratory-animals>

Gravità delle procedure





Eutanasia/Soppressione

SERIE GENERALE

*Spediz. abb. post. - art. 1, comma 1
Legge 27-02-2004, n. 46 - Filiale di Roma*

Anno 155° - Numero 61

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Roma - Venerdì, 14 marzo 2014

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Soppressione Allegato IV

14-3-2014

GAZZETTA UFFICIALE DELLA REPUBBLICA ITALIANA

Serie generale - n. 61

ALLEGATO IV

ALLEGATO IV

Metodi di soppressione degli animali

1. Nel processo di soppressione degli animali sono utilizzati i metodi elencati nella tabella seguente.

Possono essere utilizzati metodi diversi da quelli elencati nella tabella:

- a) su animali non coscienti, a condizione che l'animale non riprenda conoscenza prima della morte;
 - b) su animali impiegati nella ricerca nel settore agricolo, qualora la finalità del progetto preveda che gli animali siano tenuti in condizioni analoghe a quelle degli animali negli allevamenti commerciali; tali animali possono essere soppressi conformemente alle disposizioni di cui all'allegato I del regolamento (CE) n. 1099/2009 del Consiglio, del 24 settembre 2009, relativo alla protezione degli animali durante l'abbattimento⁶.
2. La soppressione degli animali è completata mediante uno dei seguenti metodi:
 - a) conferma dell'arresto permanente della circolazione;
 - b) distruzione del cervello;
 - c) dislocazione del collo;
 - d) dissanguamento; o
 - e) conferma dell'insorgenza del *rigor mortis*.



Soppressione Allegato IV tabella 3

14-3-2014

GAZZETTA UFFICIALE DELLA REPUBBLICA ITALIANA

Serie generale - n. 61

3. Tabella

Animali - osservazioni/metodi	Pesci	Anfibi	Rettili	Uccelli	Roditori	Conigli	Cani, gatti, furetti	Grandi mammiferi	Primati non umani
Overdose di anestetico	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Proiettile captivo			(2)						
Biossido di carbonio					(3)				
Dislocazione cervicale				(4)	(5)	(6)			
Colpo da percussione alla testa				(7)	(8)	(9)	(10)		
Decapitazione				(11)	(12)				
Elettrocuzione	(13)	(13)		(13)		(13)	(13)	(13)	
Gas inerti (Ar, N ₂)								(14)	
Colpo a proiettile libero con fucili, pistole e munizioni adeguate			(15)				(16)	(15)	



Soppressione Allegato IV tabella 3

14-3-2014

GAZZETTA UFFICIALE DELLA REPUBBLICA ITALIANA

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Requisiti

- 1) Da utilizzarsi, se del caso, previa sedazione.
- 2) Da utilizzarsi solo per i grandi rettili.
- 3) Da utilizzarsi solo in quantità sufficiente. Da non utilizzare per roditori allo stato fetale e neonatale.
- 4) Da utilizzarsi solo per i volatili di peso inferiore a 1 kg. I volatili di peso superiore a 250 g vengono sedati.
- 5) Da utilizzarsi solo per i roditori di peso inferiore a 1 kg. I roditori di peso superiore a 150 g vengono sedati.
- 6) Da utilizzarsi solo per i conigli di peso inferiore a 1 kg. I conigli di peso superiore a 150 g vengono sedati.
- 7) Da utilizzarsi solo per i volatili di peso inferiore a 5 kg.
- 8) Da utilizzarsi solo per i roditori di peso inferiore a 1 kg.
- 9) Da utilizzarsi solo per i conigli di peso inferiore a 5 kg.
- 10) Da utilizzarsi solo sui neonati.
- 11) Da utilizzarsi solo per i volatili di peso inferiore a 250 g.
- 12) Da utilizzarsi solo se altri metodi non sono praticabili.
- 13) Necessita di attrezzature specifiche.
- 14) Da utilizzarsi solo sui suini.
- 15) Da utilizzarsi solo in ambiente naturale da tiratori esperti.
- 16) Da utilizzarsi solo in ambiente naturale da tiratori esperti quando altri metodi non sono praticabili.

Euthanasia

Typically, initial CO₂ delivery to the micro-isolator is accomplished by opening the CO₂ cylinder valve so that animal(s) are slowly exposed to increasing levels of CO₂ (e.g., displacing approximately 10-30% of the chamber volume per minute)

<https://www.usf.edu/research-innovation/comparative-medicine/documents/training/euthanasia.pdf>

Confirmation of Euthanasia

- **Performance of Euthanasia**
- All animal euthanasia must be performed by appropriately trained personnel approved on the Animal Protocol. All euthanasia procedures must be continuously monitored by the person(s) performing the procedure, until confirmation of euthanasia is complete.
- <https://animal.research.uiowa.edu/iacuc-guidelines-euthanasia>

Soppressione/Eutanasia Tabella 3 Allegato IV D. Lgs. 26/2014



GENERALE
Anno 155° - Numero 61

UFFICIALE
REPUBBLICA ITALIANA

Mercoledì, 14 marzo 2014

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AMMINISTRAZIONI

REPUBBLICA ITALIANA Serie generale - n. 61

Roditori	Conigli	Cani, gatti, furetteri	Grandi mammiferi	Primates non umani
(1)	(1)	(1)	(1)	(1)
(3)				
(5)	(6)			
(8)	(9)	(10)		
(12)				
	(13)	(13)	(13)	
			(14)	
		(16)	(15)	

Soppressione/Eutanasia Tabella 3 Allegato IV D. Lgs. 26/2014

Standard method of euthanasia:

Overdose with MS-222 (200-300 mg/l) by prolonged immersion. Fish should be left in the solution at least 10 minutes following cessation of opercular movement.

Death should be assured by decapitation

Journal of the American Association for Laboratory Animal Science
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Vol 54, No. 1
January 2015
Pages 76-79

Effectiveness of Recommended Euthanasia Methods in Larval Zebrafish (*Danio rerio*)

Jennifer L. Strykowski^{1,2} and Joseph M. Schech²*

The standard solution for euthanizing zebrafish contains 300 mg/L MS222 buffered to a pH of 7.0.5. It is recommended that adult fish remain in a solution of this concentration for at least 10 min after the cessation of opercular movement. However, our facility noted that the hearts of larval zebrafish continued to beat even after 30 min or more in MS222.



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



Ozzano dell'Emilia 05 Febbraio 2020

Your help in caring for all the animals is greatly appreciated!

Dott. Ludovico Scenna Medico Veterinario
specialista in Scienza e Medicina degli Animali da Laboratorio
Libero professionista