18 Settembre 2018

UNIVERSITÀ
DEGLI STUDI
DI FERRARA
- EX LABORE FRUCTUS -

Riconoscimento del dolore, distress, sofferenza ed eutanasia ai sensi del D. Lgs. 26/2014

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;

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Training, self-examination
http://cbctraining.ncl.ac.uk/eM-EU5/story_html5.html

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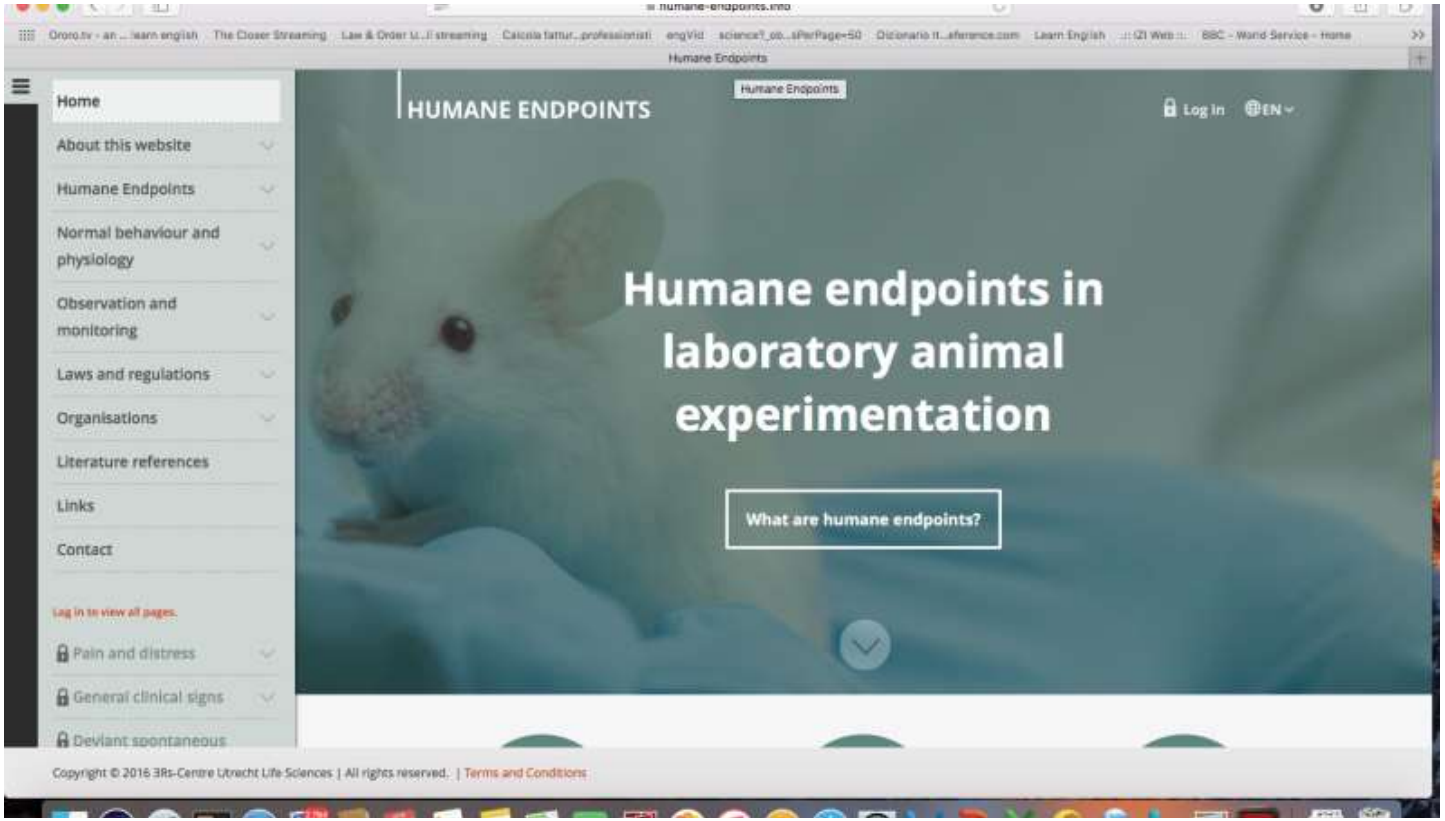
Training, self-examination
https://www.humane-endpoints.info/en



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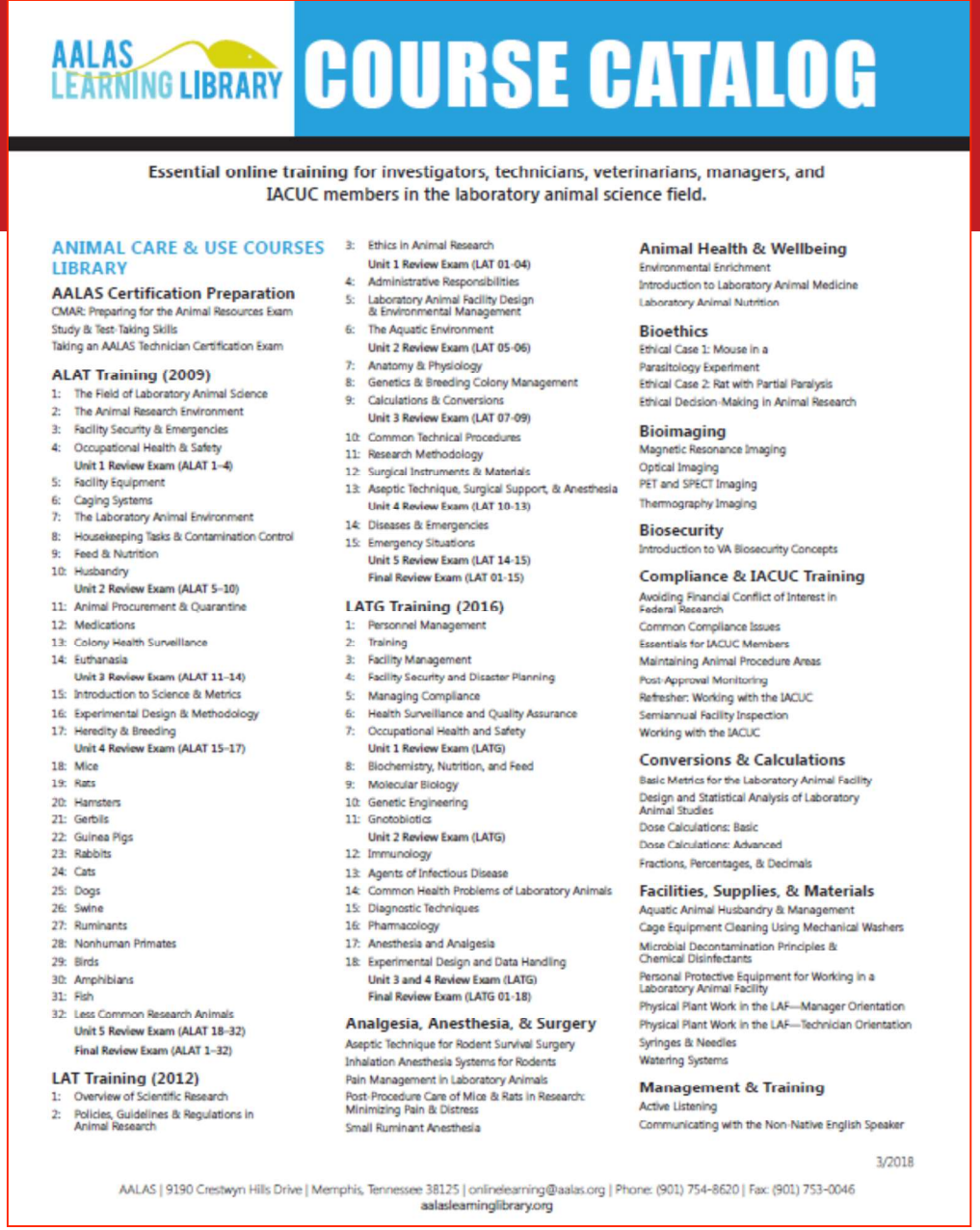

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Unit 3 and 4 Review Exam (LATG)
Final Review Exam (LATG 01-18)

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Aseptic Technique for Rodent Survival Surgery
Inhalation Anesthesia Systems for Rodents
Pain Management in Laboratory Animals
Post-Procedure Care of Mice & Rats in Research: Minimizing Pain & Distress
Small Ruminant Anesthesia

Animal Health & Wellbeing

Environmental Enrichment
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Laboratory Animal Nutrition

Bioethics

Ethical Case 1: Mouse in a Parasitology Experiment
Ethical Case 2: Rat with Partial Paralysis
Ethical Decision-Making in Animal Research

Bioimaging

Magnetic Resonance Imaging
Optical Imaging
PET and SPECT Imaging
Thermography Imaging

Biosecurity

Introduction to VA Biosecurity Concepts

Compliance & IACUC Training

Avoiding Financial Conflict of Interest in Federal Research
Common Compliance Issues
Essentials for IACUC Members
Maintaining Animal Procedure Areas
Post-Approval Monitoring
Refresher: Working with the IACUC
Semiannual Facility Inspection
Working with the IACUC

Conversions & Calculations

Basic Metrics for the Laboratory Animal Facility
Design and Statistical Analysis of Laboratory Animal Studies
Dose Calculations: Basic
Dose Calculations: Advanced
Fractions, Percentages, & Decimals

Facilities, Supplies, & Materials

Aquatic Animal Husbandry & Management
Cage Equipment Cleaning Using Mechanical Washers
Microbial Decontamination Principles & Chemical Disinfectants
Personal Protective Equipment for Working in a Laboratory Animal Facility
Physical Plant Work in the LAF—Manager Orientation
Physical Plant Work in the LAF—Technician Orientation
Syringes & Needles
Watering Systems

Management & Training

Active Listening
Communicating with the Non-Native English Speaker

3/2018

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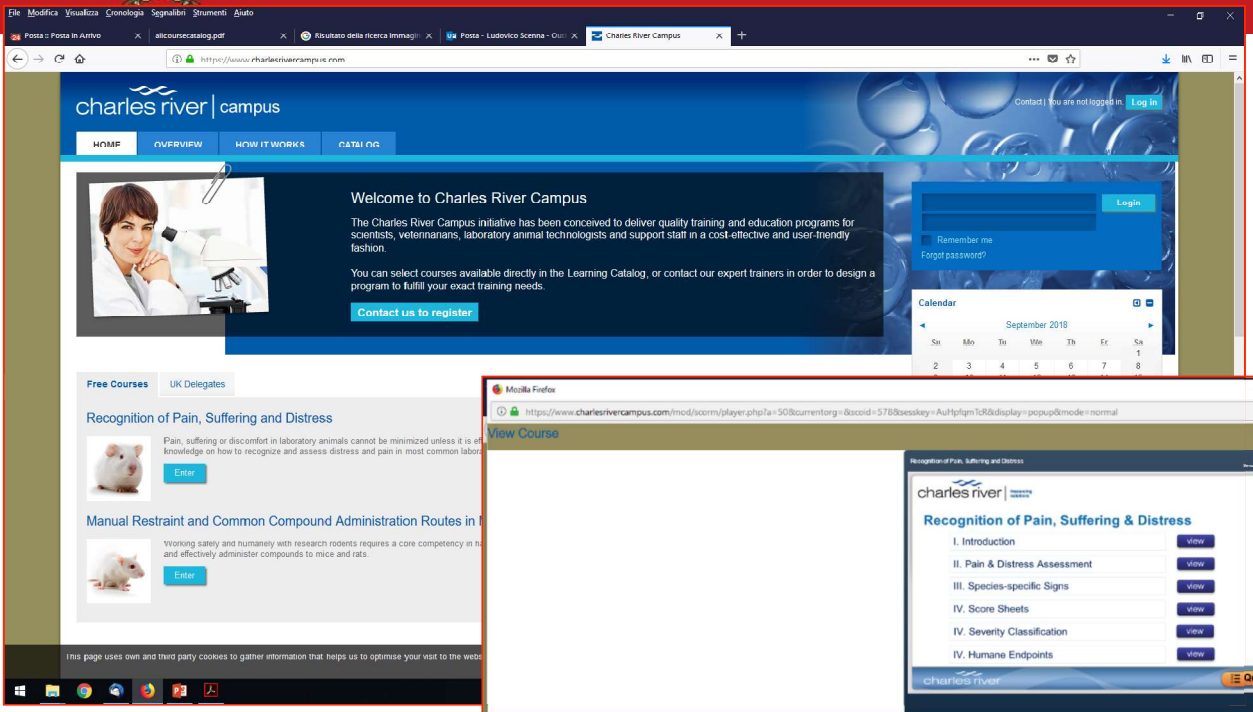

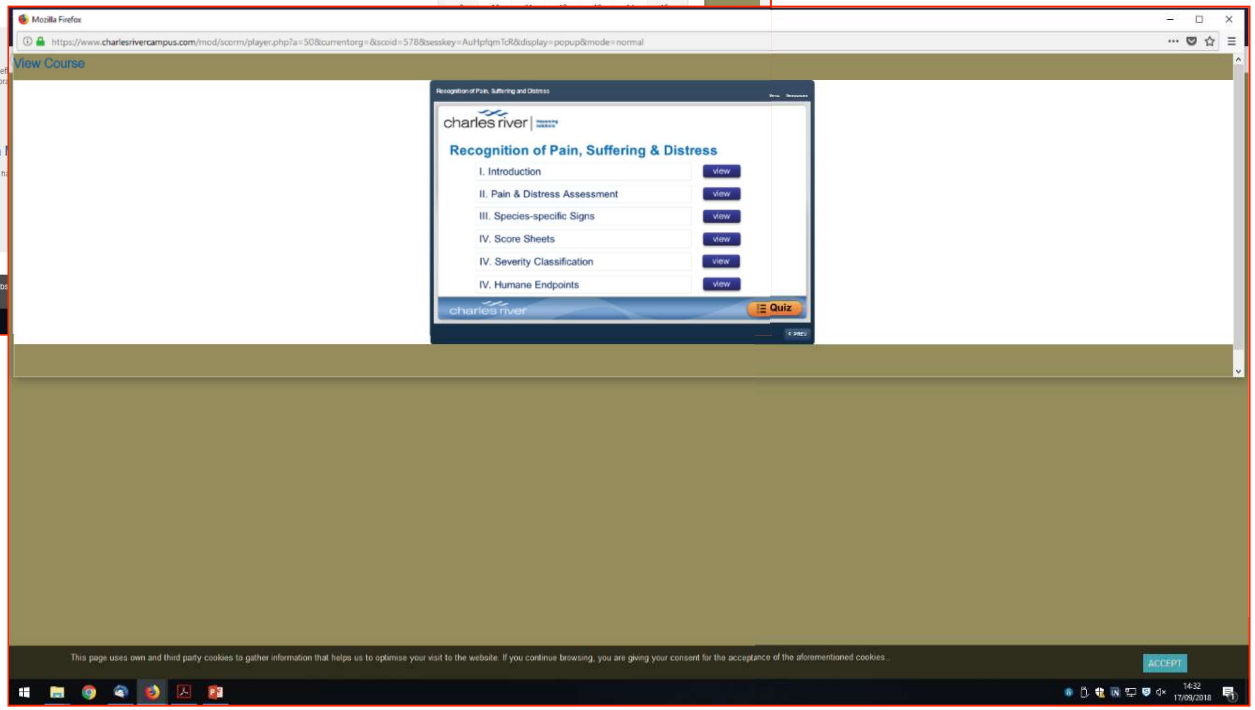

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Clinical Score Systems

Body Condition Score - BCS

Humane endpoints and use of score sheets

Timo Nevalainen
Universities of Kuopio and Helsinki
Finland

THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

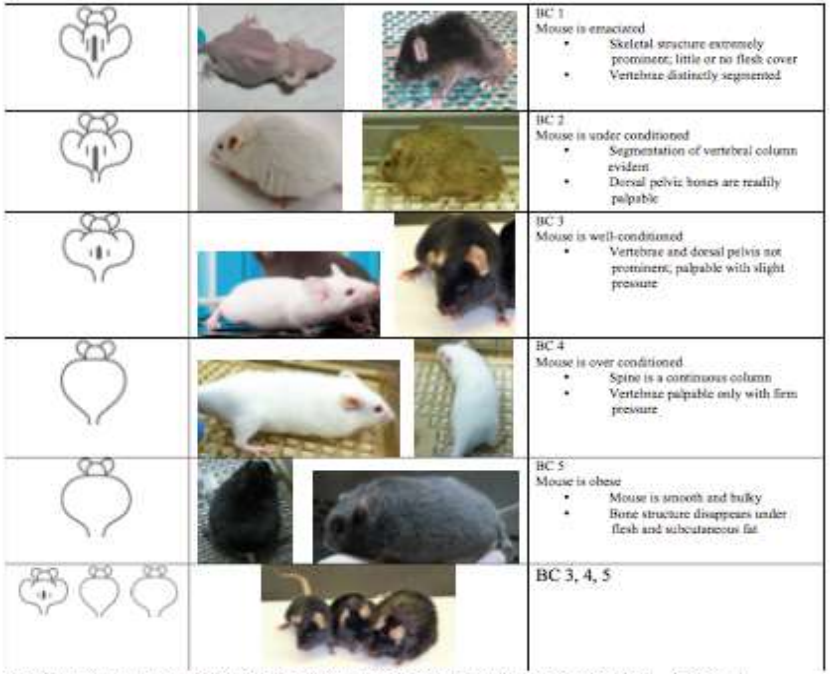
IACUC / LARC STANDARD PROCEDURES

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



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

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Clinical Score Systems

Clinical Score System – Roberta Aiello - IzsVe

Humane endpoints and use of score sheets

Timo Nevalainen
Universities of Kuopio and Helsinki
Finland

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

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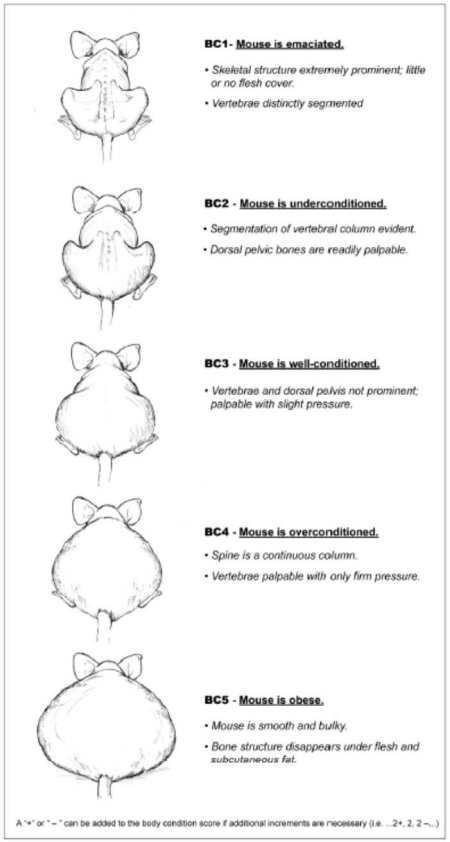
Clinical Score Systems

Clinical Score System – OPBA – UNITN



Published in final edited form as:
Curr Protoc Mouse Biol. 2012 June ; 2: 145–165. doi:10.1002/9780470942390.mo110217.

Health Evaluation of Experimental Laboratory Mice

Tanya Burkholder, Charmaine Foltz[1], Eleanor Karlsson, C Garry Linton, and Joanne M Smith

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

Clinical Score Systems

Per tutte le specie tutelate dal Decreto → Es. Zebrafish

Manifestazione	Punteggio
Nuoto in gruppo nella colonna d'acqua; respirazione normale; normale forma corporea; scaglie nomali	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

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Clinical Score Systems
Grimace Scale

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 0.8 ***

MOUSE GRIMACE SCALE (MGS): THE MANUAL

he baseline condition; it will appear to be convex
ed 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.

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

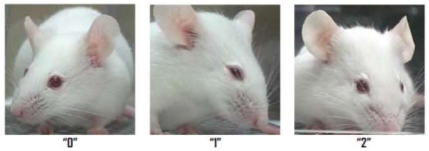
Mouse must display a bulge on top of the nose. The skin and muscles around the nose will be contracted creating a rounded extension of skin visible on the bridge of the nose. A nose bulge may also be coded if a coder sees vertical wrinkles extending down the side of the nose from the bridge. In frontal 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.

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.



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Clinical Score Systems
Grimace Scale

RAT GRIMACE SCALE (RGS)
THE MANUAL

B. ACTION UNITS:

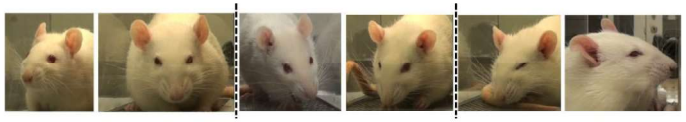
1. Orbital Tightening

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

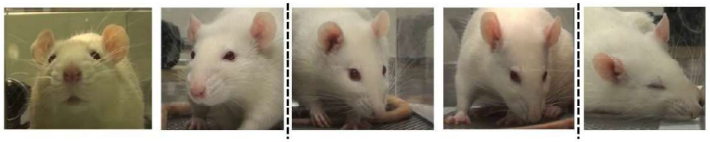
Rats in pain display a *lack* of bulge on top of the nose (i.e., a flattening of the nose). In the "no pain" condition a clear bulge is present at the bridge of the nose. The whisker pads are also rounded and slightly puffed out, leaving a clear crease between the pads and the cheek. When in pain, the 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"

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Clinical Score Systems
Grimace Scale

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

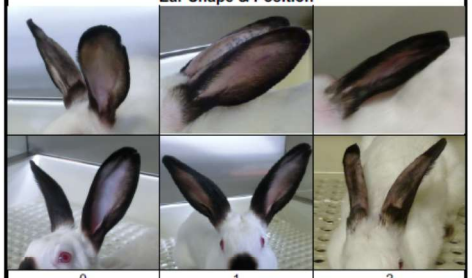
- Closing of the eyelid (narrowing of orbital area)
- A wrinkle may be visible around the eye

Cheek Flattening

0	1	2
Not Present	Moderately Present	Obviously Present

- Flattening of the cheeks. When 'obviously present', cheeks have a sunken look.
- The face become

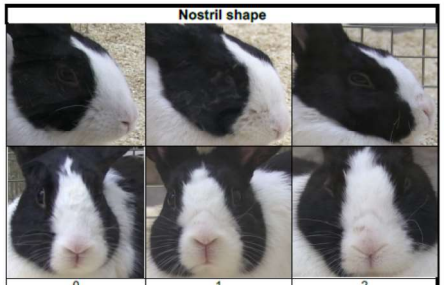
Ear Shape & Position



0	1	2
Not Present	Moderately Present	Obviously Present

- 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

- 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

- 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

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Clinical Score Systems
Grimace Scale

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

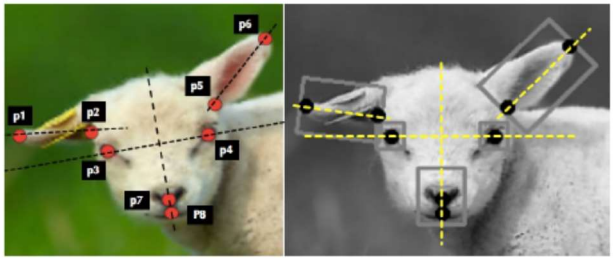

Fig. 3. Left: Localised facial landmarks (Note: the eight facial landmarks are labelled from p1 to p8) Right: Normalised sheep face marked with feature bounding boxes

Fig. 2. Sheep facial AU taxonomy with their description & example. The taxonomy is based on the SPFES [1]

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How to make a Clinical Score Systems

Indice

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Punto 24 dell'Allegato VI del D. Lgs. 26/2014

Valutazione preventiva iniziale, considerazione di perfezionamenti specifici e punti finali umanitari

L | M | G | NR

Quali interventi devono essere effettuati sugli animali utilizzati in questo studio?	Che cosa proveranno gli animali? Quanta sofferenza potrebbero procedure? Che cosa potrebbe aggravare la sofferenza?	In che modo verrà ridotta al minimo la sofferenza?	
	Effetti avversi	**Metodologia e interventi (refinement)**	**Punto finale**
Allevamento C57BL/6J Pak6$^{-/-}$ e Pak6$^{-/-}$/Pak7$^{-/-}$	• Fenotipo "lieve"	• In omozigosi → no distress per handling dovuto a genotipizzazione • Monitoraggio quotidiano del peso e dello stato di idratazione come in tutto l'allevamento delle colonie. • Monitoraggio quotidiano anche nei festivi	• Clinical score system = 1
Allevamento C57BL/6J Aldh1a1$^{-/-}$ /Aldh2$^{-/-}$	• Fenotipo "lieve"	• In omozigosi → no distress per handling dovuto a genotipizzazione • Monitoraggio quotidiano del peso e dello stato di idratazione come in tutto l'allevamento delle colonie. • Monitoraggio quotidiano anche nei festivi	• Clinical score system = 1
Genotipizzazione col taglio della coda	• Stress per l'handling. • Infiammazione nella parte più distale della coda • Perdita della falange distale nei primi sette giorni di vita • Infiammazione regione distale della mano	• Monitoraggio quotidiano anche nei festivi del peso e dello stato di idratazione come in tutto l'allevamento delle colonie attraverso standardizzatp clinical score system e Body Condition Score (BCS)	• Necrosi in direzione craniale in caso di genotipizzazione attraverso la parte distale della coda.
Iniezione intracranica AAV in pups	• Distress operatorio	• Controllo visivo dell'animale che riprende le normali funzionalità vitali compreso il colore • Si è cercato il miglior piano anestesiologico possibile per l'età dell'animale • Nessun effetto tossico è stato descritto né su animali e né su neuroni dopo l'inoculo del virus AAV	• Colore diverso dai pari età dovuto ad una troppo lunga fase di raffreddamento •

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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

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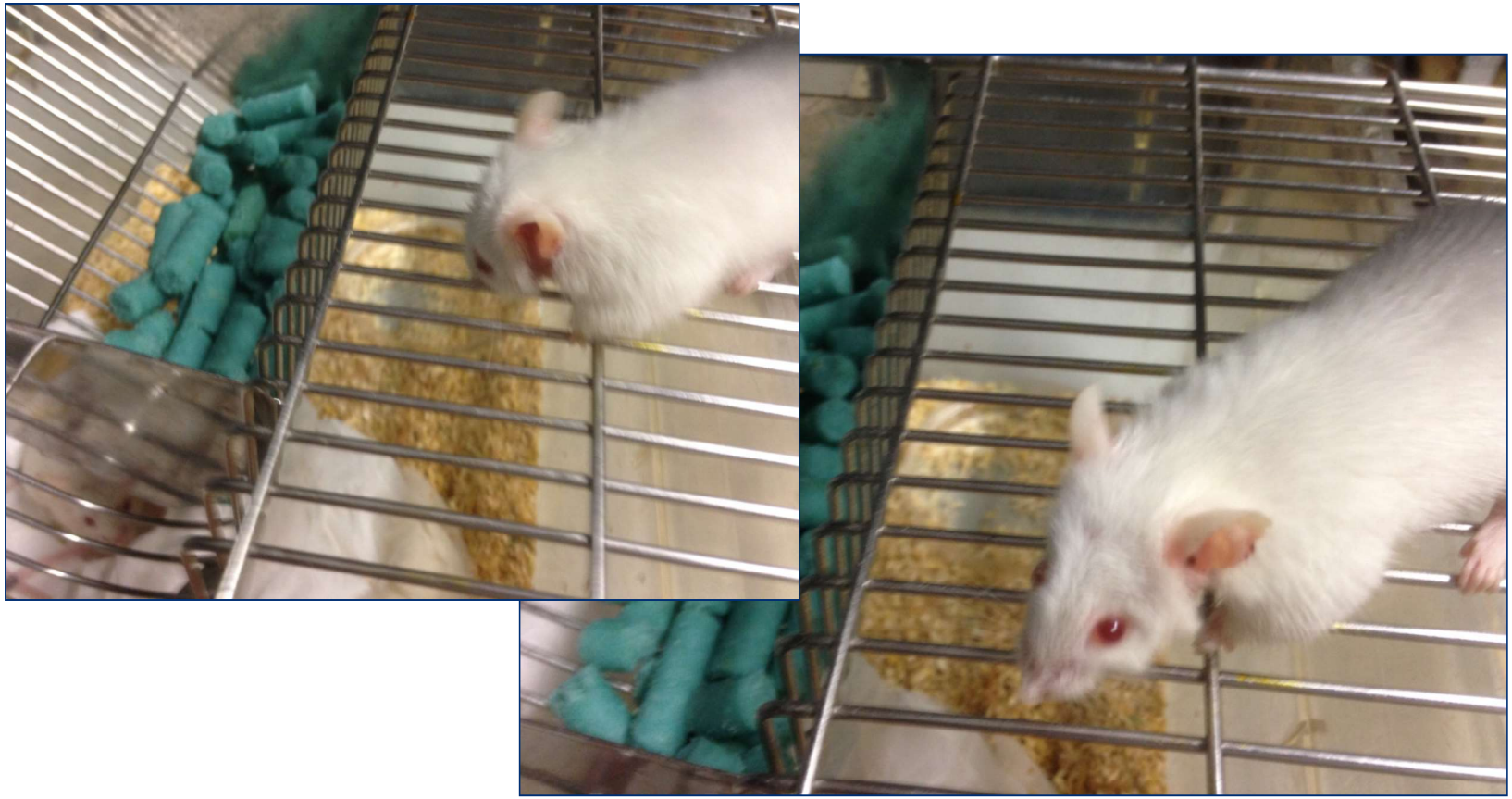
Humane endpoint



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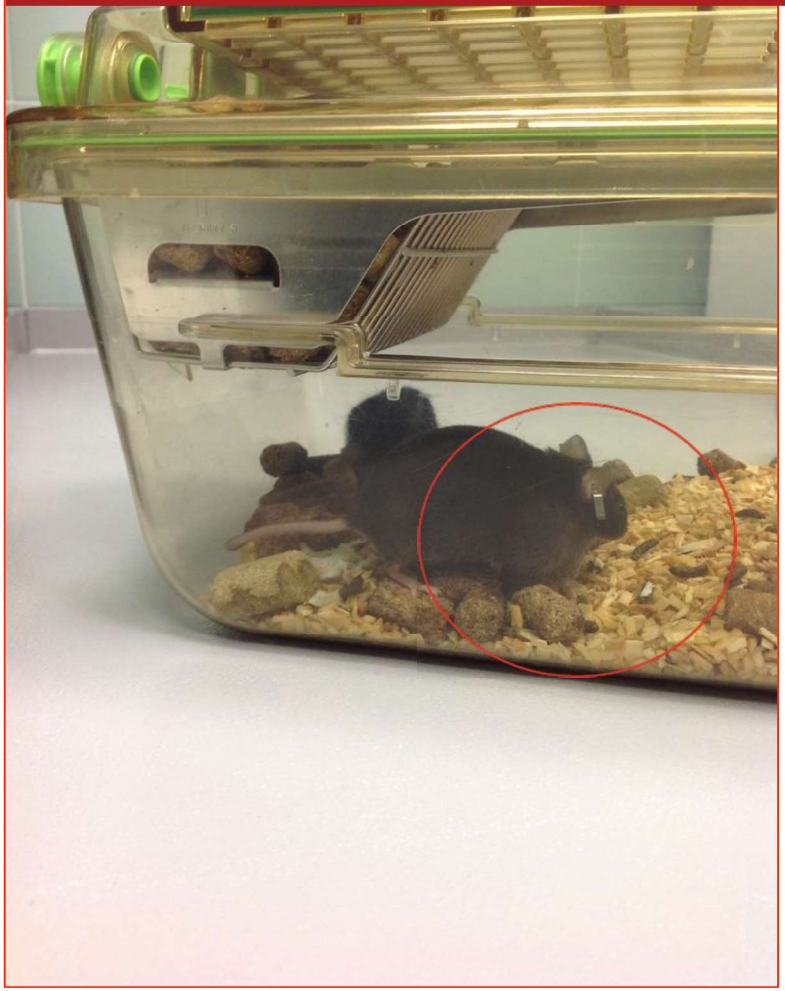
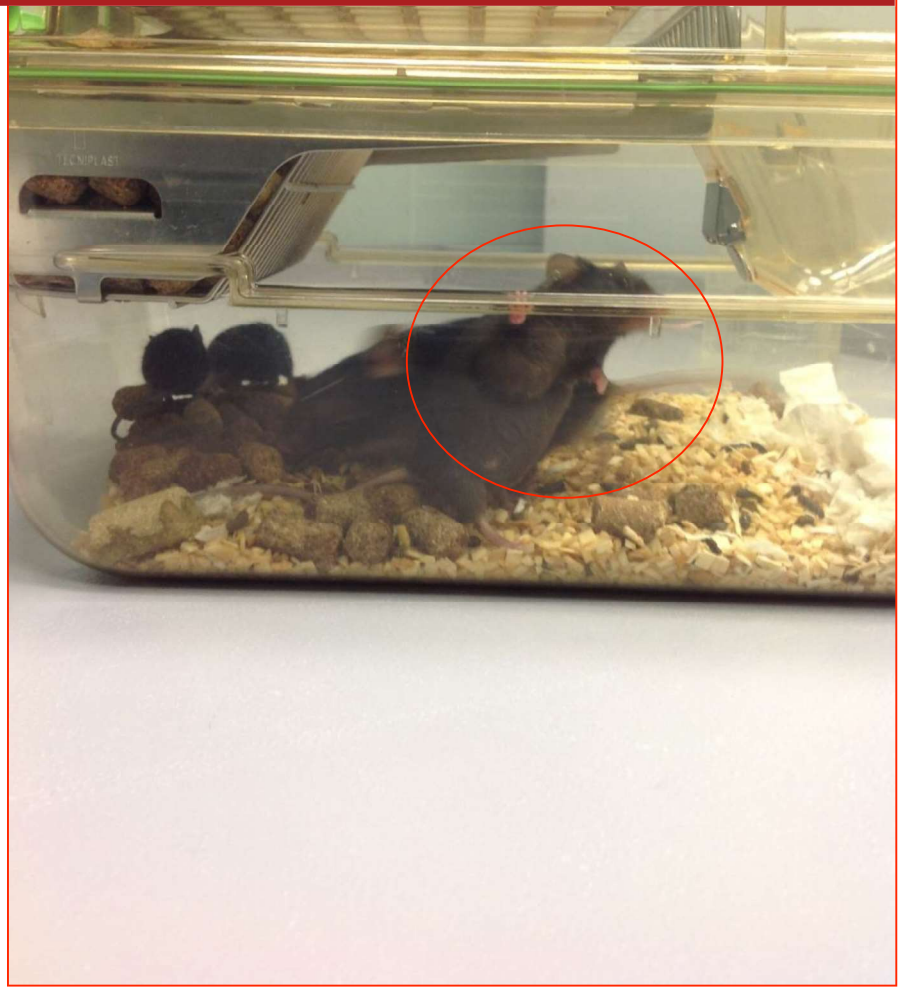
Humane endpoint





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Humane endpoint

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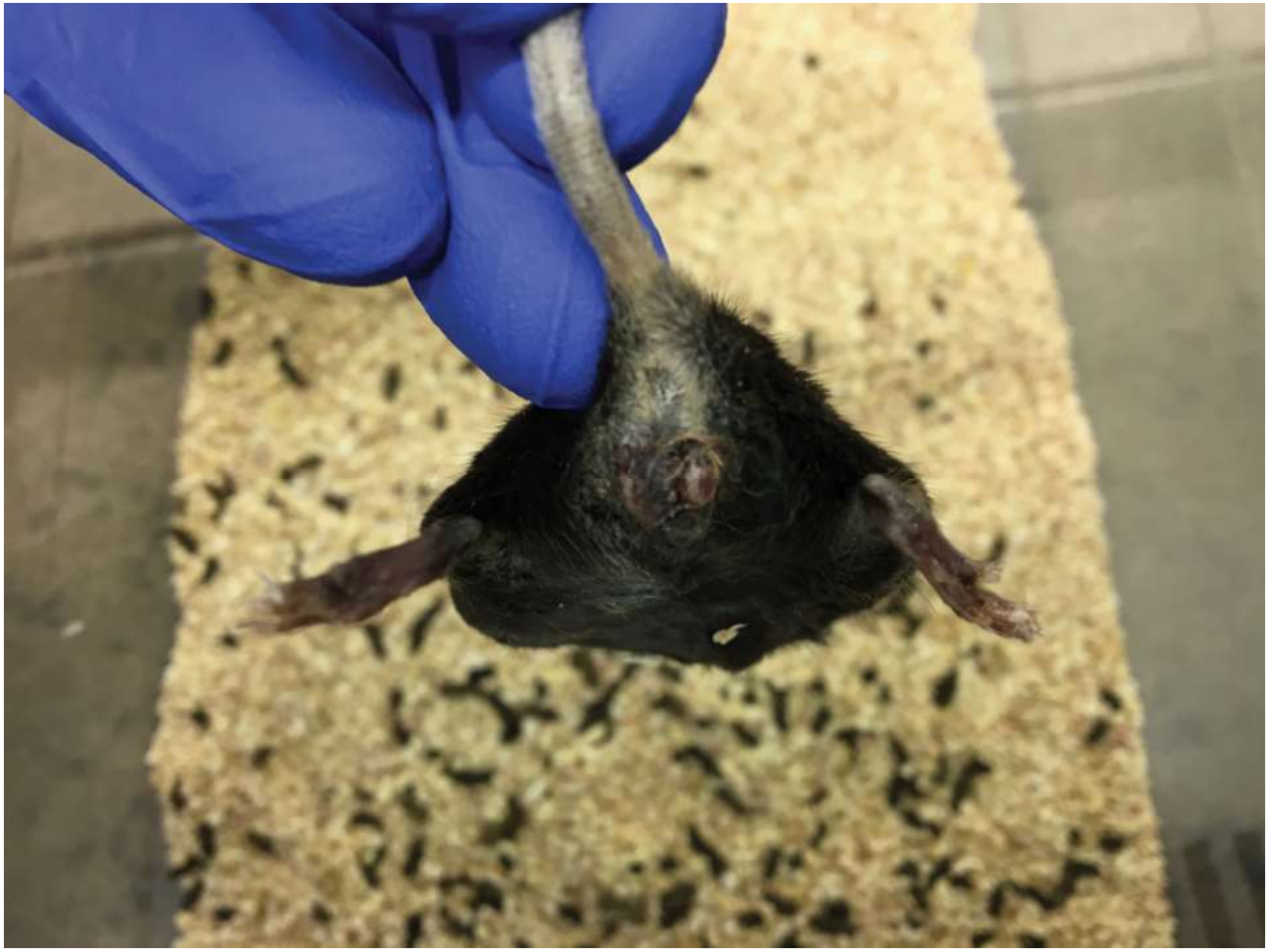

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Humane endpoint

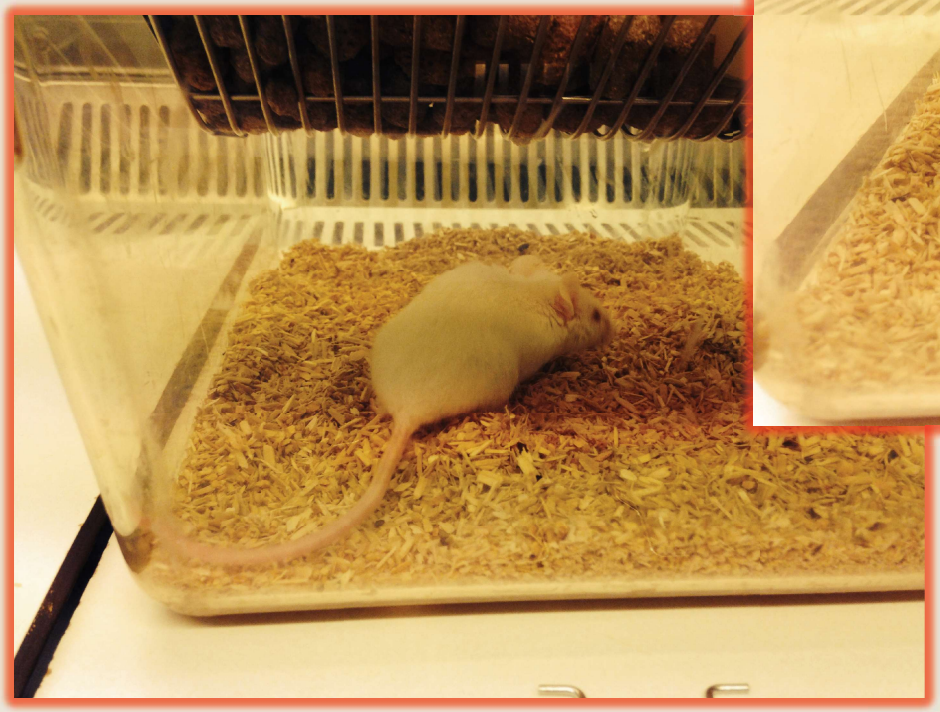
Humane Endpoint

Humane Endpoint

Humane Endpoint

Humane Endpoint

IACUC Handbook Table 17.1 pag 314



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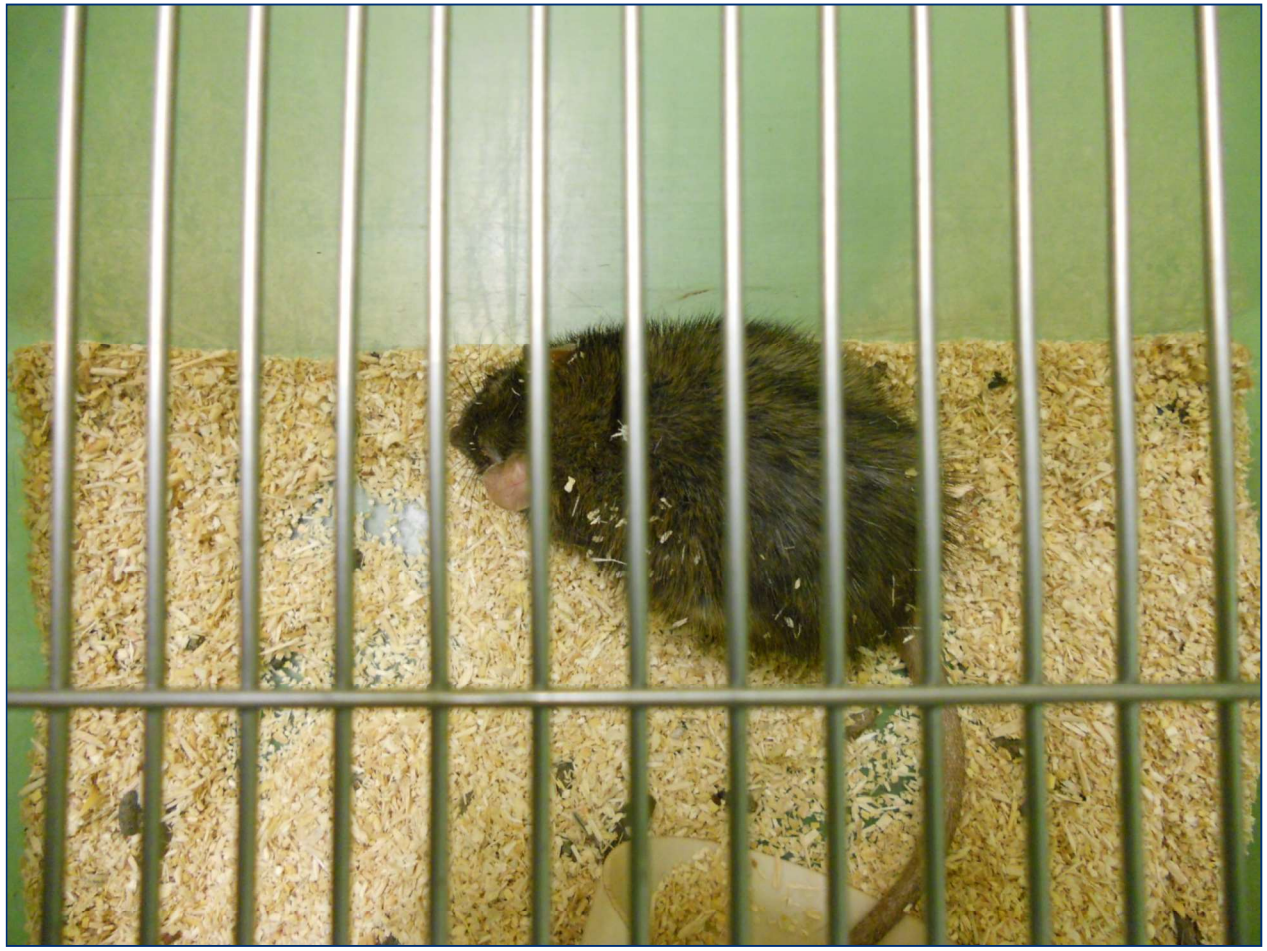

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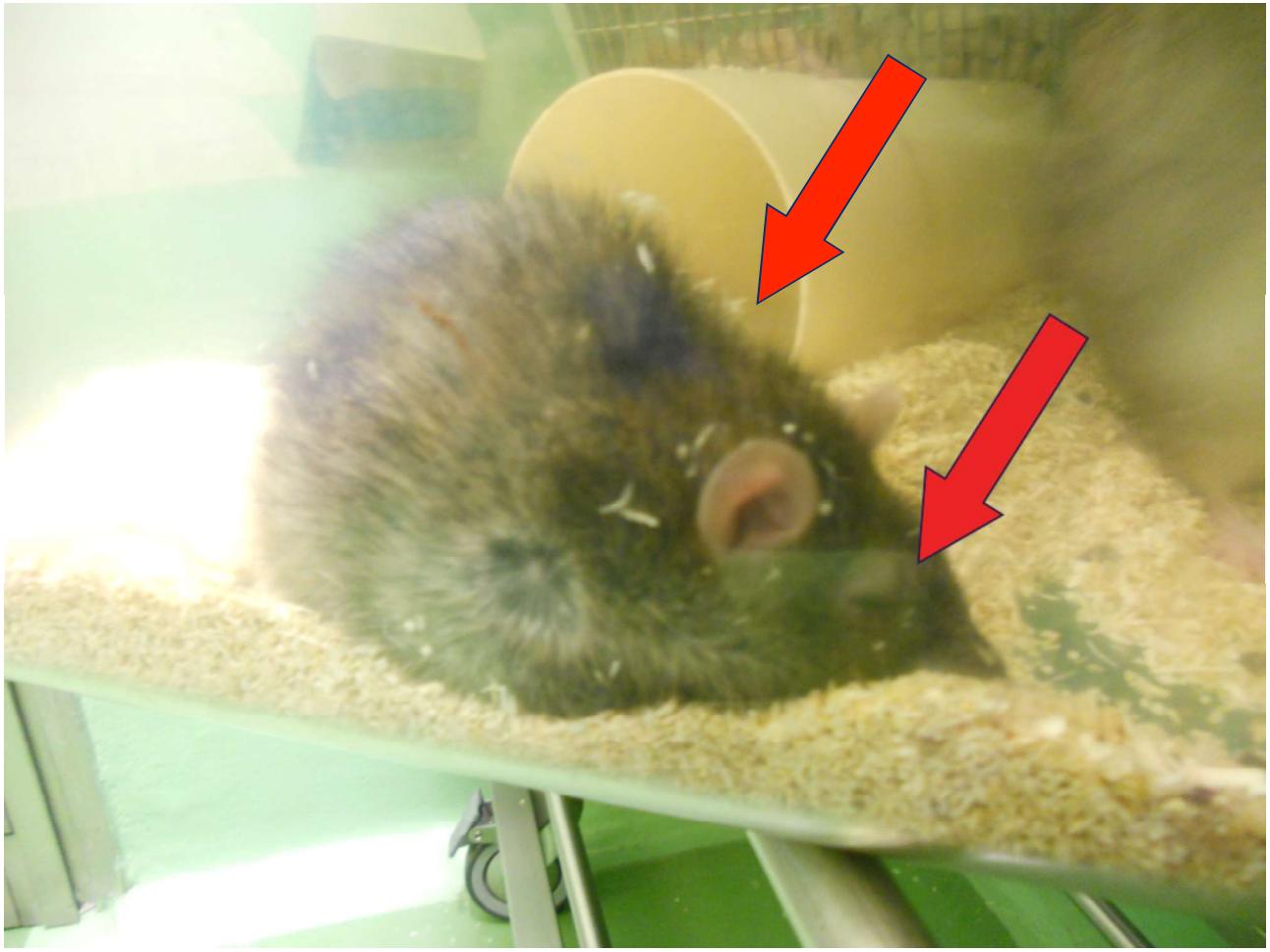
Humane endpoint



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Training, self-examination

https://www.google.it/search?q=rat+humane+endpoint+laboratory&client=firefox-b-ab&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiJobXfsefTAhVMJcAKHQI4C4gQ_AUICigB&biw=1920&bih=923#imgdii=T_wdaJm4KtHg_M:&imgrc=iAuL6_hXsr1ICM:

rat 1

rat 2

rat 3

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Humane endpoint

Check????

Laboratory Animal Care

BOSTON UNIVERISTY
Animal Health Monitoring

BOSTON UNIVERSITY

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Humane endpoint

Check:
1. **Eyes**
2. **Ears**
3. **Tails**
4. **Hunched posture?**

Laboratory Animal Care

BOSTON UNIVERSITY
Animal Health Monitoring

BOSTON UNIVERSITY

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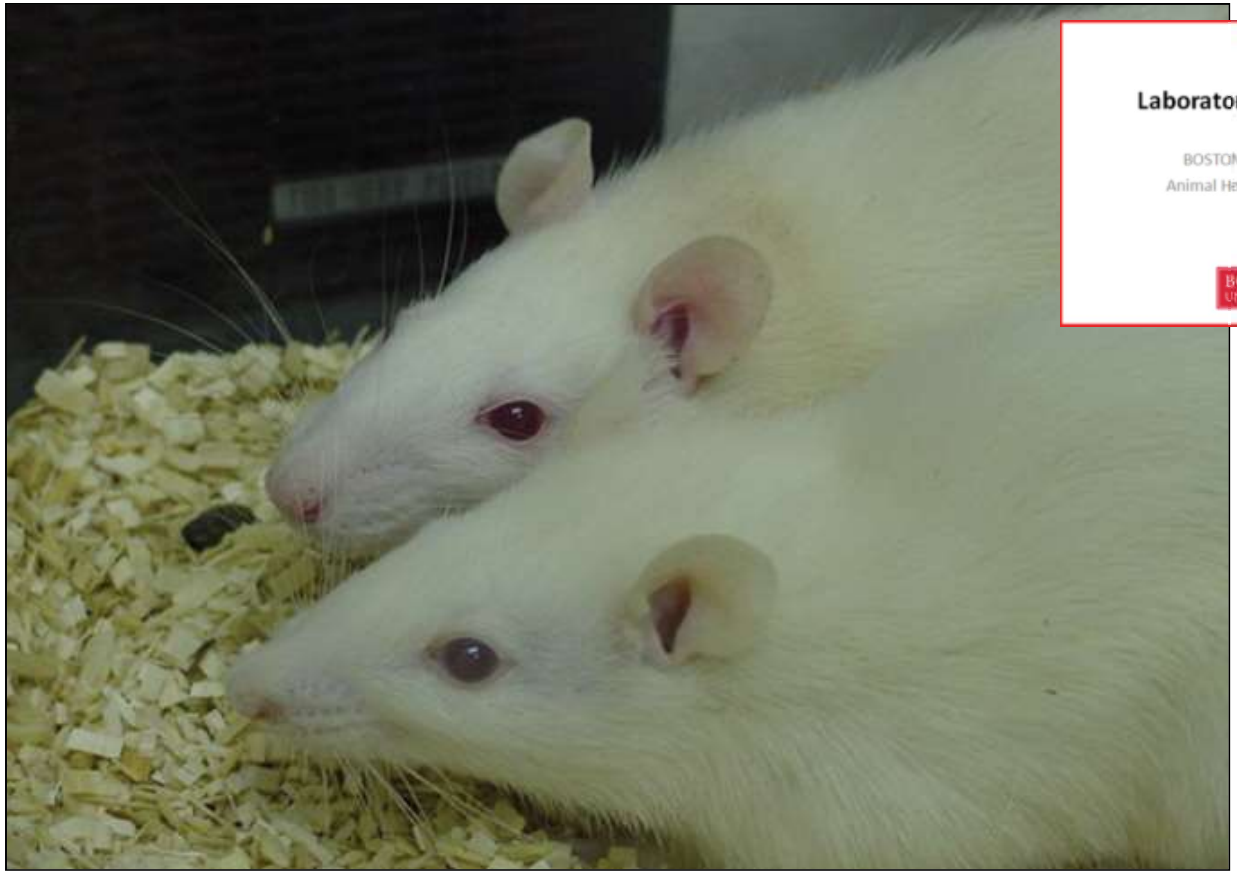
Humane endpoint



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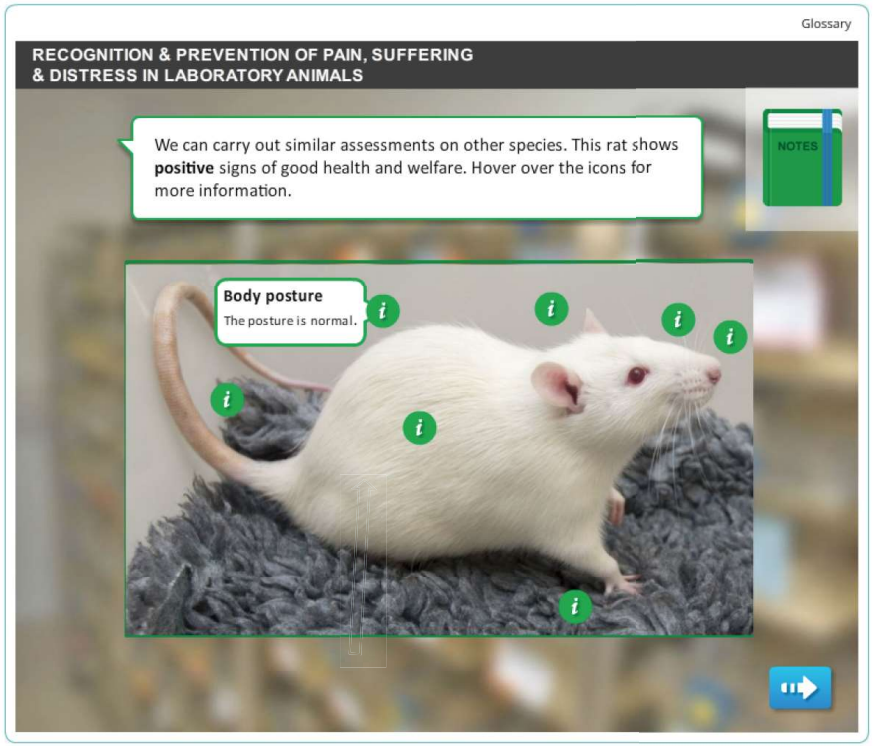
Humane endpoint

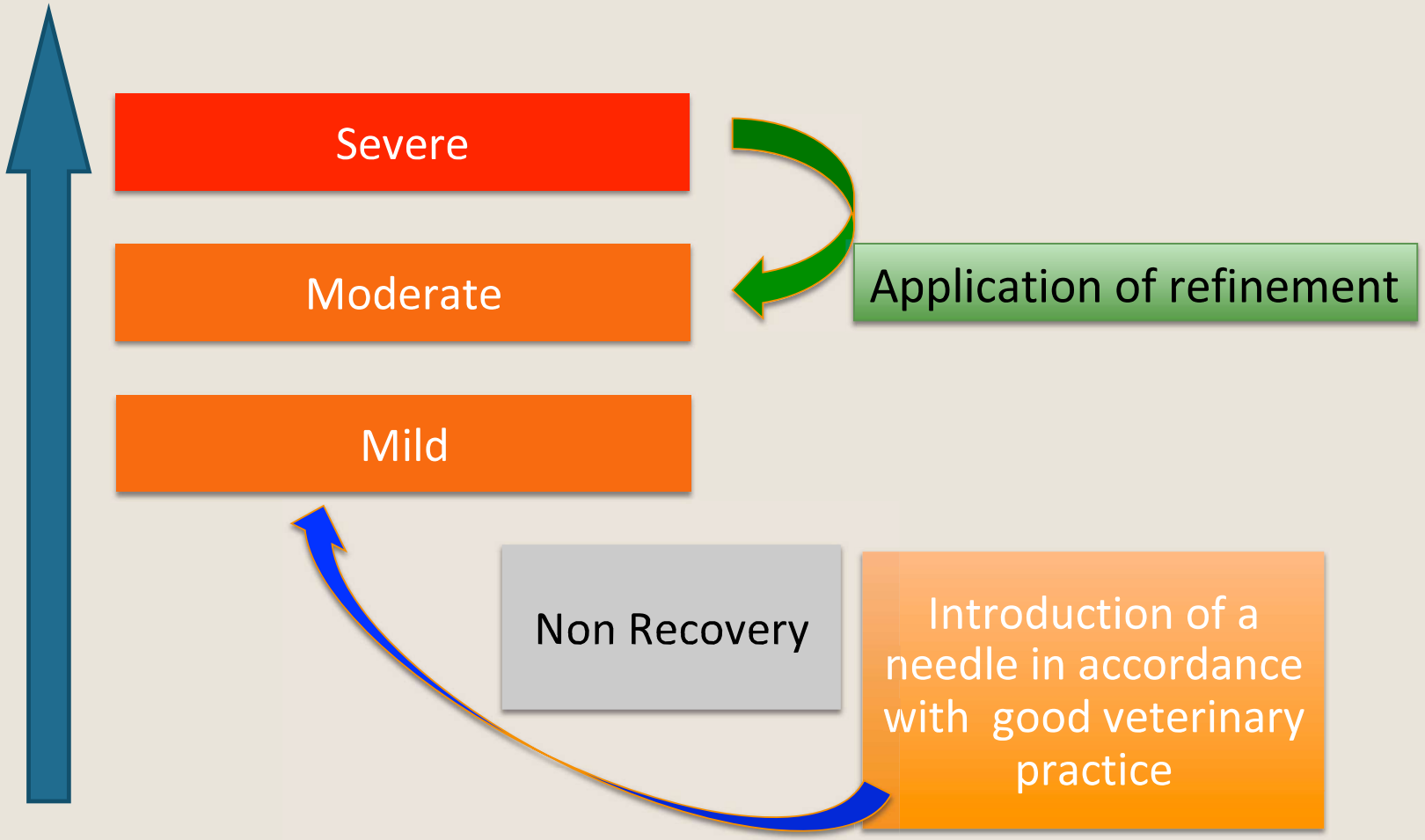
Your help in caring for all the animals is greatly appreciated!



NC3r

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

Gravità delle procedure



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Eutanasia

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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AVVISO ALLE AMMINISTRAZIONI

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'abbattimento[6].

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*.

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Eutanasia:
3. Tabella: Allegato IV del D. Lgs. 26/2017

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	X	X	(2)	X	X		X		X
Biossido di carbonio	X	X	X		(3)	X	X	X	X
Dislocazione cervicale	X	X	X	(4)	(5)	(6)	X	X	X
Colpo da percussione alla testa				(7)	(8)	(9)	(10)	X	X
Decapitazione	X	X	X	(11)	(12)	X	X	X	X
Elettrocuzione	(13)	(13)	X	(13)	X	(13)	(13)	(13)	X
Gas inerti (Ar, N_2)	X	X	X			X	X	(14)	X
Colpo a proiettile libero con fucili, pistole e munizioni adeguate	X	X	(15)	X	X	X	(16)	(15)	X

Eutanasia Tabella Allegato IV

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.

Università degli Studi di Ferrara

Organismo Preposto al Benessere Animale

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

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Riconoscimento del dolore, distress, sofferenza ed eutanasia ai sensi del D. Lgs. 26/2014