severity
& humane endpoints

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Learning Outcomes

2.8. Describe the concept of harms to animals including avoidable and unavoidable suffering, direct, contingent and cumulative suffering

2.9. Describe the severity classification system, and give examples of each category. Describe cumulative severity and the effect this may have on the severity classification.

5.5. Describe the severity classifications included in the Directive and give examples of each category; explain cumulative severity and the effect this may have on the severity classification.

2.10. Describe the regulations regarding re-use of animals.

5.4. Describe what a humane endpoint is. Identify criteria to be used to set humane endpoints. Define action to be taken when a humane endpoint is reached and consider possible options for refining methods to finish at an earlier endpoint.

5.6. Describe the circumstances when anaesthesia or analgesia may be necessary to minimise pain, suffering, distress or lasting harm

harms

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welfare

- general health
- happiness
- safety

harm

- damage
- injury

pain, suffering, distress and lasting harm

Directive 2010/63/EU

https://www.oxfordlearnersdictionaries.com/

harms > suffering

- frustration
- anxiety
- stress
- distress
- pain
- ill health

Recognition and Alleviation of Distress in LA, NRC 2008

harms > suffering

- frustration
- anxiety
- stress
- distress
- pain
- ill health

- avoidable
- unavoidable

- direct
- indirect

- contingent
- cumulative

Recognition and Alleviation of Distress in LA, NRC 2008

The Welfare of Animals in Research, Hubrecht UFAW 2014

cumulative effect

lifetime experiences

project

procedure(s)

iv | ip | surgery | MRI

induced disease

breeding husbandry transport

avoid

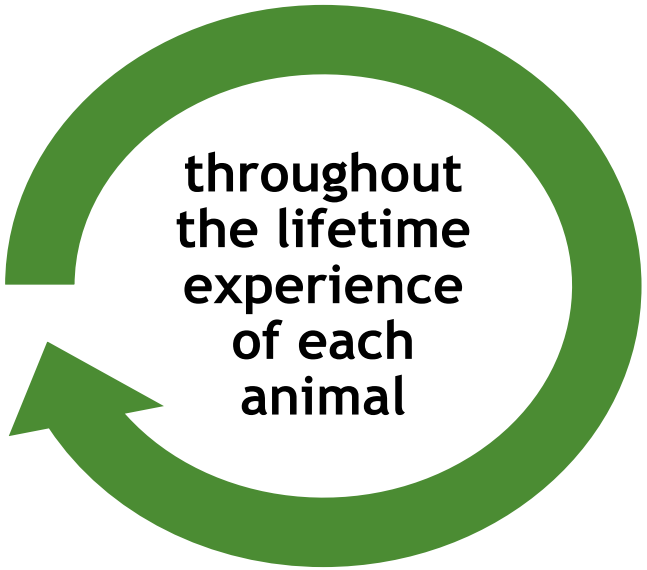
avoidable suffering

minimize

unavoidable suffering

plan for

unexpected suffering



Pictures are from https://awionline.org/

severity
of experimental procedures

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EN

Official Journal of the European Union

DIRECTIVES

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 22 September 2010

on the protection of animals used for scientific purposes

(Text with EEA relevance)

Directive 2010/63/EU

Article 16

Reuse

1. Member States shall ensure that an animal already used in one or more procedures, when a different animal on which no procedure has previously been carried out could also be used, may only be reused in a new procedure provided that the following conditions are met:

(a) the actual severity of 'moderate';

(b) it is demonstrated th and well-being has b

(c) the further procedur 'non-recovery'; and

(d) it is in accordance account the lifetime

2. In exceptional circu point (a) of paragraph 1 and after a veteri the animal, the competent authority may animal, provided the animal has not bee once in a procedure entailing severe pain, suffering.

Classificatio

1. Member States sl sified as 'non-recovery' by-case basis using the

2. Subject to the use Member States shall en it involves severe pain, long-lasting and canno

Article 54

Reporting

1. Member S years thereafter, this Directive a 39, 43 and 46

2. Member S on an annual ba in procedures, the procedures primates used i

ANNEX VIII

SEVERITY CLASSIFICATION OF PROCEDURES

The severity of a procedure shall be determined by the degree of pain, suffering, distress or lasting harm expected to be experienced by an individual animal during the course of the procedure.

Section I: Severity categories

Non-recovery:

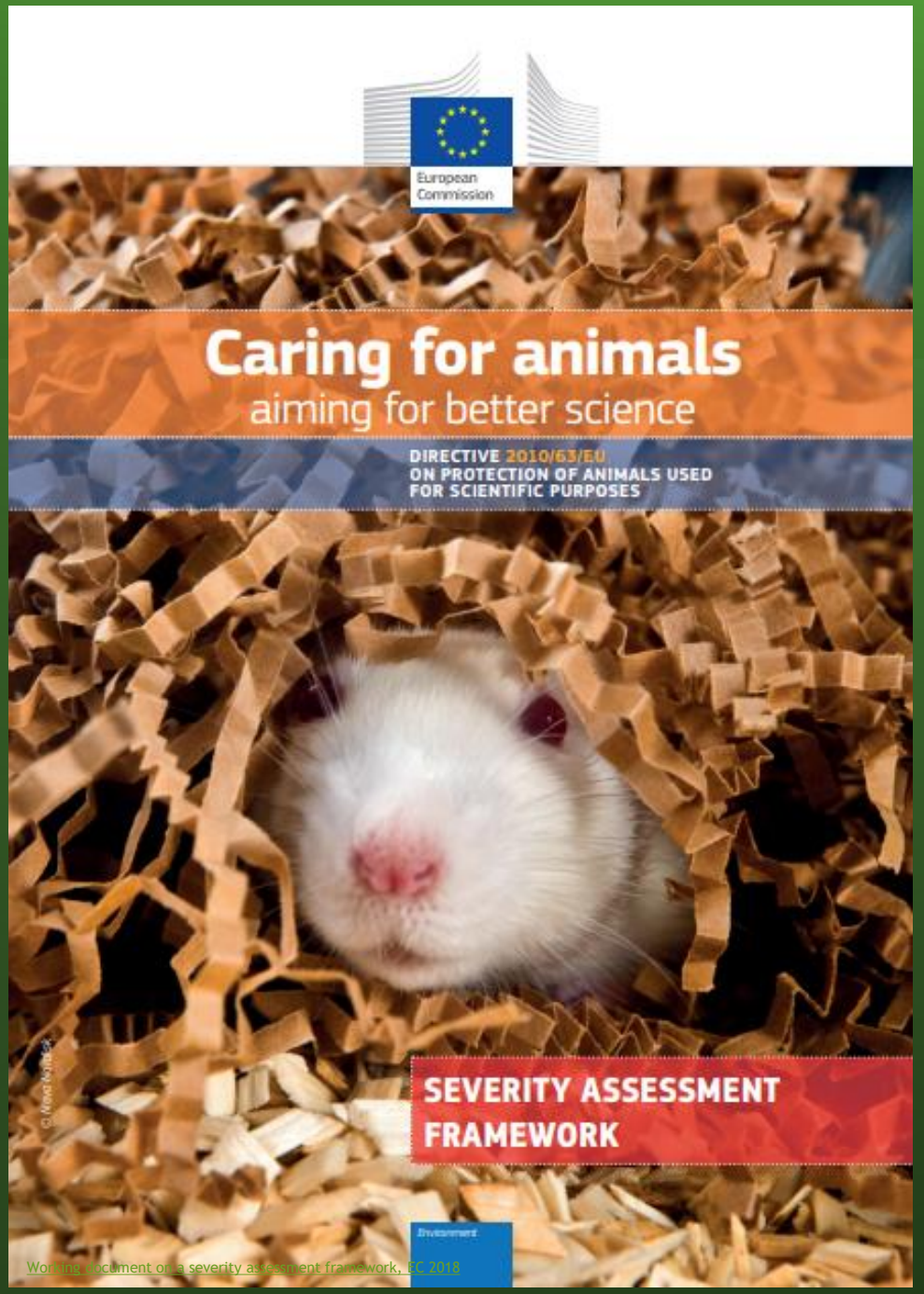
Procedures which are performed entirely under general anaesthesia from which the animal shall not recover consciousness shall be classified as 'non-recovery'.

Mild:

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

Moderate:

Procedures on animals as a result of which the animals are likely to experience short-term moderate pain, suffering or



Working document on a severity assessment framework, EC 2018

assignment criteria

procedure(s)

type

nature of pain, suffering, distress or lasting harm

prevention from natural behavior

duration, frequency, intervals

repeated

cumulative suffering

reuse

animals

animal traits (species, genotype, maturity, age, gender)

acclimatization to the procedure(s)

handler expertise

refinement methods

welfare monitoring

objective assessment of pain, suffering and distress

humane endpoints

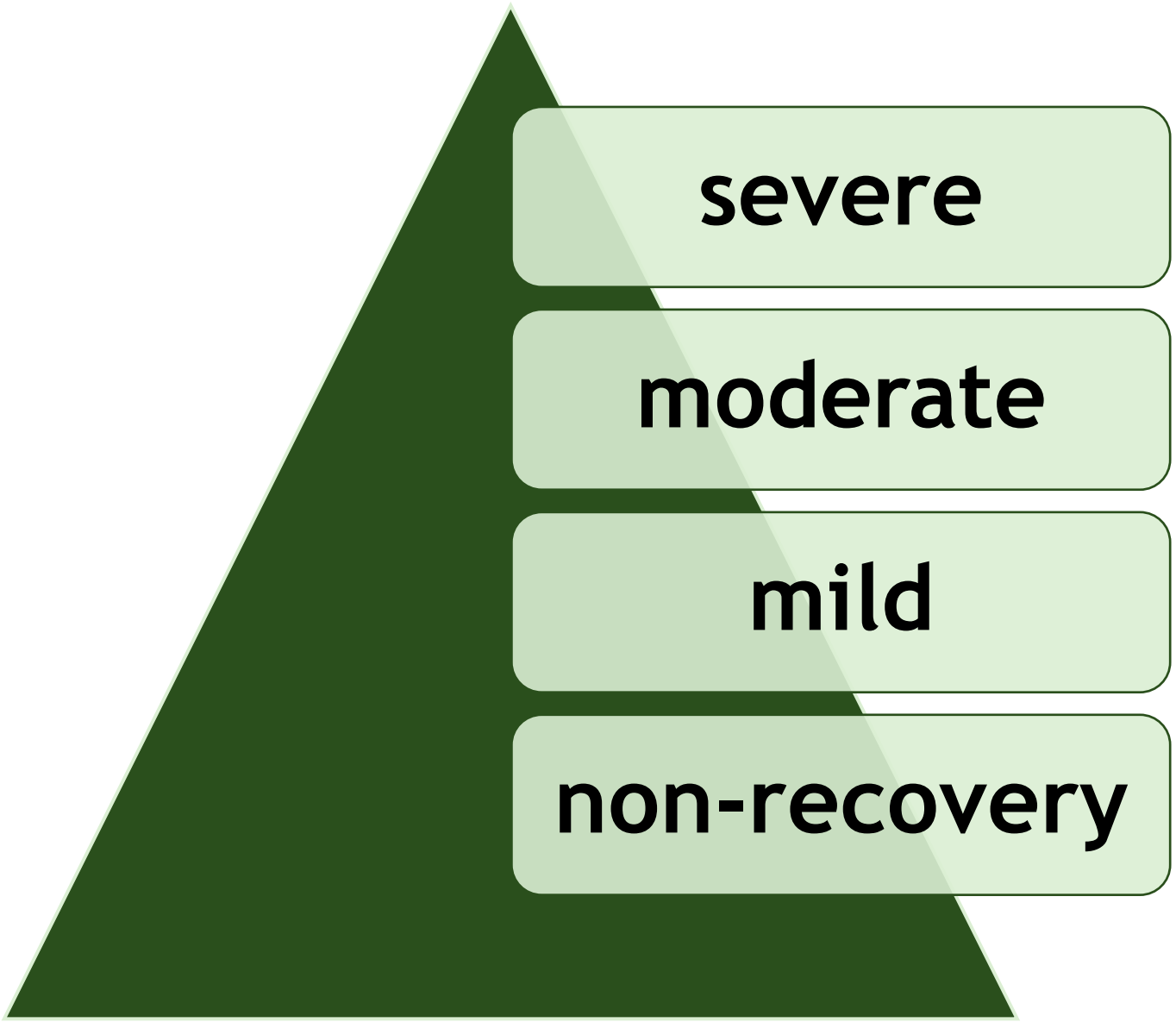
Directive 2010/63/EU Article 15, Annex VIII

Working document on a severity assessment framework, EC 2018

humane endpoints

refinement interventions to lower severity

severity categories



Directive 2010/63/EU Article 15, Annex VIII

severity categories

	non- recovery	mild	moderate	severe
pain suffering distress	under general anesthesia without recovery	short-term mild	short-term moderate long-lasting mild	short-term severe long-lasting moderate
impairment of the well-being/ general condition		not significant	moderate	severe

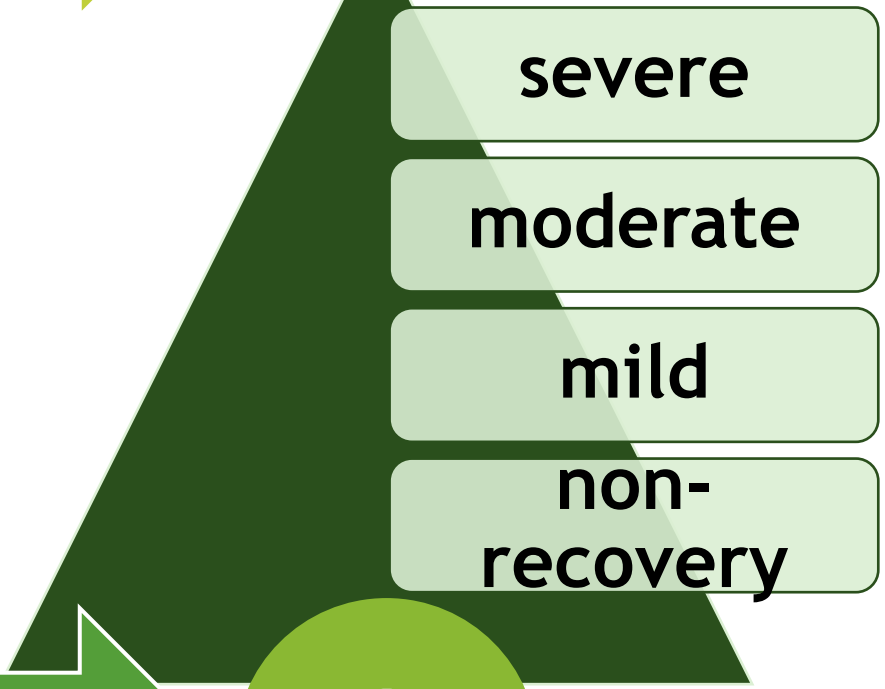
Directive 2010/63/EU Article 15, Annex VIII

thresholds

upper

severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated

not performed



not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice

lower

not licenced

Directive 2010/63/EU Article 15, Annex VIII

examples

mild	moderate	severe
administration of anesthesia	administration of substances with moderate effect, frequent administration	surgical interventions resulting in severe pain or harm
administration of substances (og, sc, im, ip, iv) with mild effect	blood collection (>10% of blood volume, frequent)	toxicity testing where death is the end-point, fatalities expected, severe pathophysiological states induced
blood collection (few samples, <10% of blood volume)	surgery under general anaesthesia and appropriate analgesia	testing of devices where failure causes severe effects
non-invasive imaging	acute dose-range finding studies, chronic toxicity/carcinogenicity tests, with non-lethal end-points	vaccine potency testing with severe effects
application of external telemetry devices	induction of tumours, or spontaneous tumours, with moderate effects	irradiation or chemotherapy with lethal dose without reconstitution of the immune system, or reconstitution with production of graft versus host disease
superficial procedures, ear and tail biopsies, non-surgical subcutaneous implantation of mini-pumps and transponders	irradiation or chemotherapy with a sublethal dose, or with reconstitution of the immune system, with mild or moderate and short-lived (< 5 days) effects	induction of tumours, or spontaneous tumours, with severe effects
induction of tumours, or spontaneous tumours, that cause no detectable clinical adverse effects	breeding of genetically altered animals with moderate phenotype	breeding animals with severe phenotype
noxious stimuli briefly associated with mild pain/suffering/distress, and which the animals can successfully avoid	prolonged (up to 5d) restraint in metabolic cages or withdrawal of food for 48 hours in adult rats	metabolic cages involving severe prolonged restriction
breeding of genetically altered animals, resulting in phenotype with mild effects	modified diets expected to cause moderate clinical abnormality	inescapable electric shock
feeding of modified diets expected to cause mild clinical abnormality	evoking escape and avoidance reactions unable to escape, expected to result in moderate distress	complete isolation for prolonged periods of social species
short-term (< 24h) restraint in metabolic cages, withdrawal of food for<24h in adult rats;		immobilisation stress to induce gastric ulcers or cardiac failure in rats
short-term deprivation of social partners, short-term solitary caging of social animals		forced swim or exercise tests with exhaustion as the end-point
open field testing		

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cumulative effect

lifetime experiences

project

procedure(s)

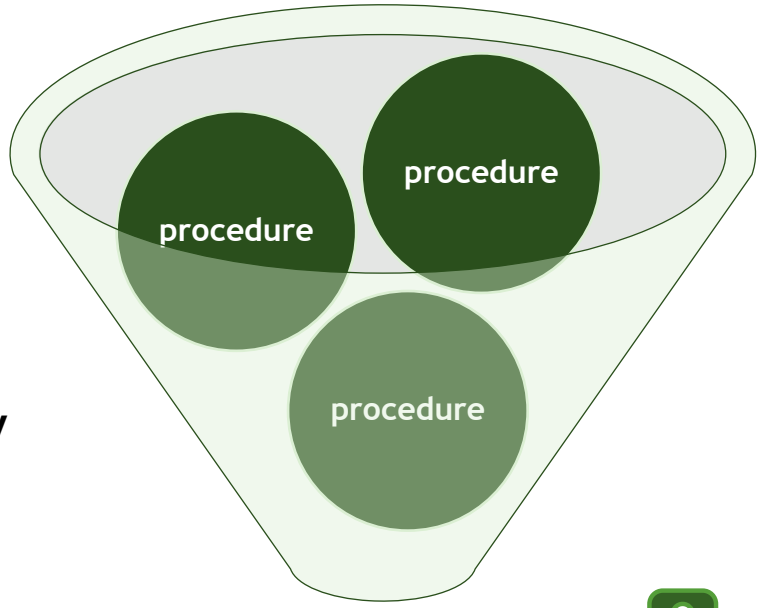
iv | ip | surgery | MRI

induced disease

breeding husbandry transport

prospective severity

- project's prospective severity
  - prediction of ONE category
  - worst case scenario
  - for all animals
  - in the application
- predictive severity
  - non technical summary
  - grouped expectation for each severity category



project

actual severity

- actual experience
- per animal
- annual report
- retrospective evaluation
- updated non technical summary

design → evaluation → authorization → implementation → completion retrospective assessment

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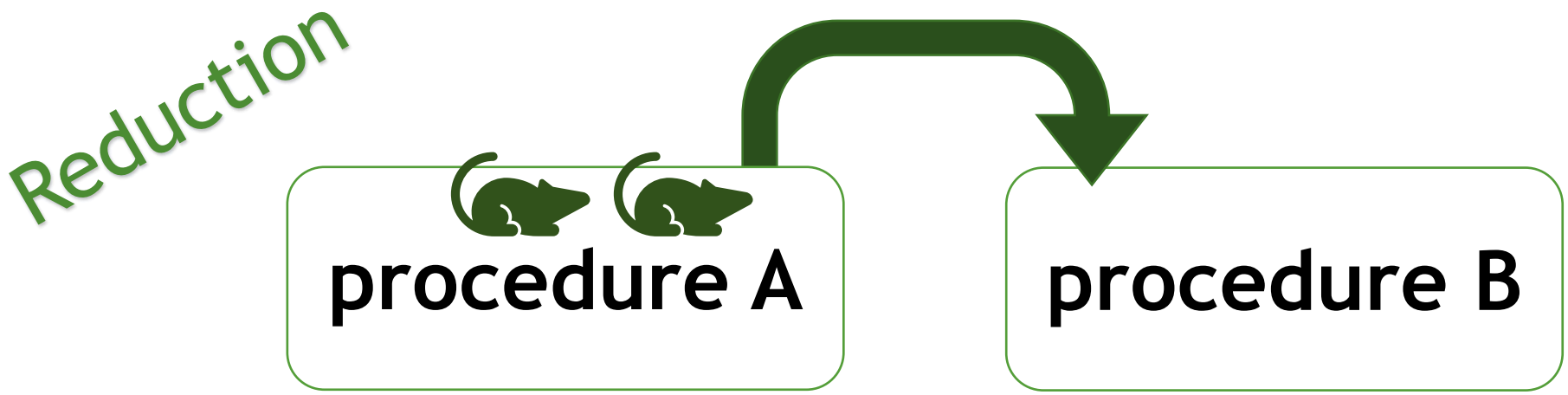
Working document on a severity assessment framework, EC 2018

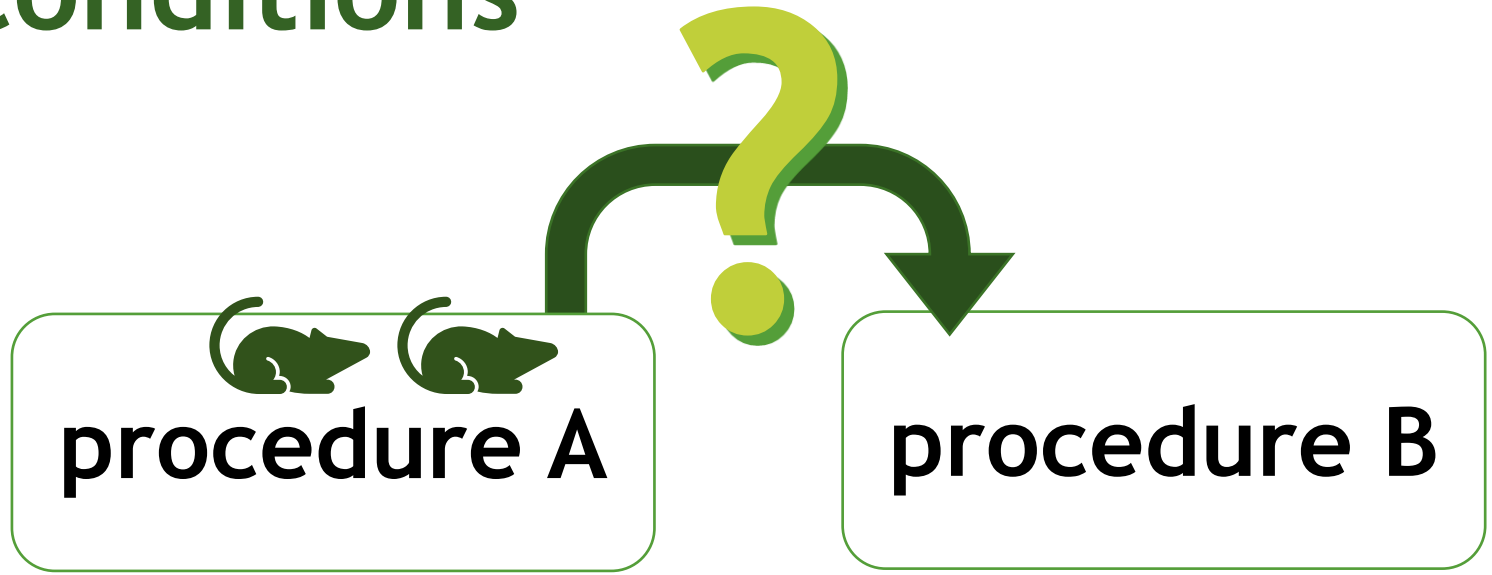
reuse



Directive 2010/63/EU Article 16

Working document on a severity assessment framework, EC 2018

reuse conditions



- actual severity mild/moderate
- fully restored health/well-being
- veterinary advice

- prospective severity mild/moderate/non-recovery
- scientific objective

Directive 2010/63/EU Article 16

Working document on a severity assessment framework, EC 2018

Refinement

humane intervention points

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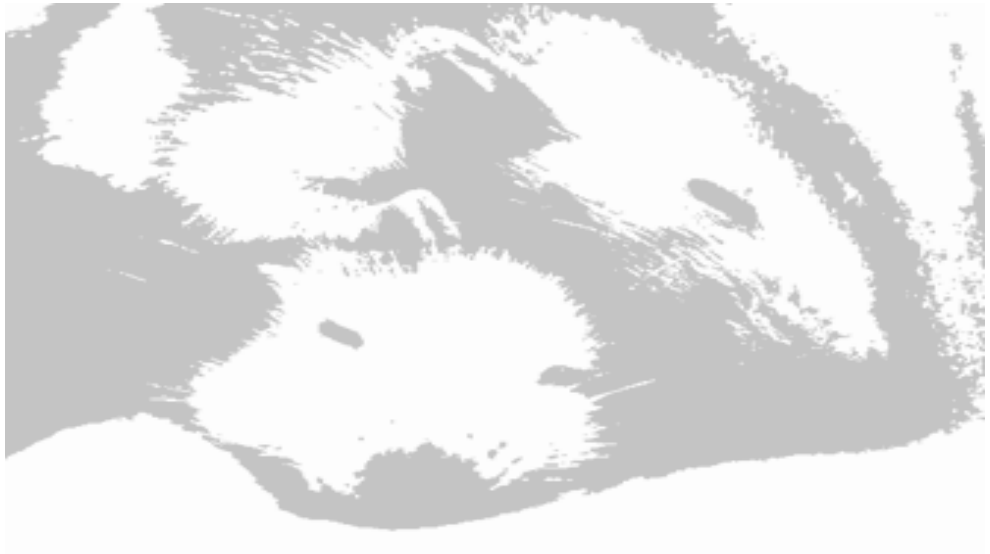
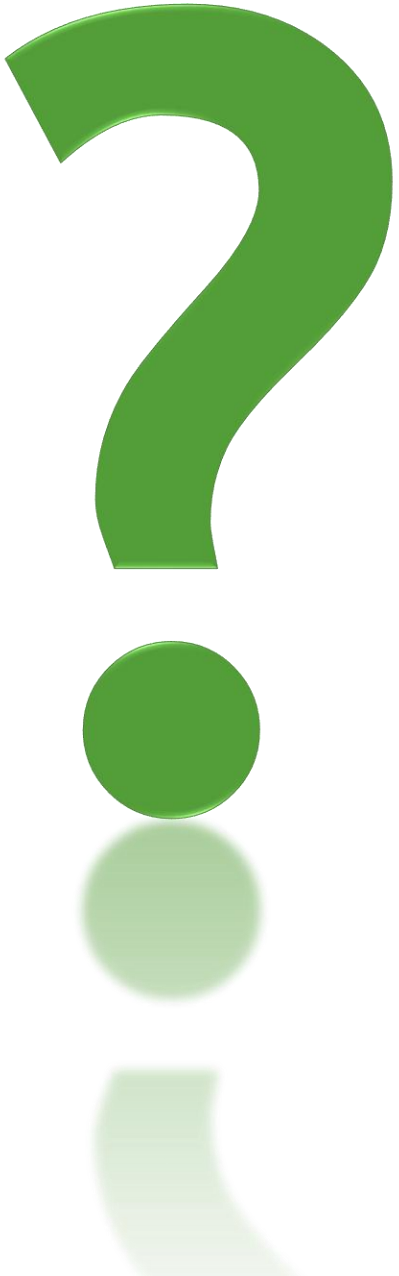
humane endpoints

a tool for refinement & reduction





humane endpoints

‘the earliest indicator(s) in an animal experiment of (potential) pain and/or distress that,

can be used to avoid or limit pain and/or distress

by taking actions such as humane killing or terminating or alleviating the pain and distress

within the context of moral justification and scientific endpoints to be met,’

(Hendriksen and Morton, 1999)

choice of methods

animals must not be used if alternatives exist
minimum number of animals must be used
animal models with the lowest capacity to experience pain, suffering, distress or lasting harm must be selected
the methods that cause the least pain, suffering, distress or lasting harm must be selected
methods must be refined to reduce the pain, suffering, distress or lasting harm they may inflict
when appropriate (e.g. surgical) procedures must be carried out under general or local anaesthesia
in case of expected or observed pain, an appropriate analgesic plan must exist
humane endpoints must be set
death as an endpoint must be avoided
early humane endpoints must be chosen
when the purpose of the procedure is achieved, animal suffering must be minimized
when a procedure ends or when further observations cannot be made, appropriate actions must be taken to relief the animals

Directive 2010/63/EU

indicators

- clinical signs (e.g. tumor formation)
- pathophysiological changes (e.g. hypothermia)
- behavioural changes (e.g. stereotypic behaviour)
- biochemical changes (e.g. ketonury)
- hormonal changes (e.g. prolactin)
- imaging changes (e.g. bioluminescence)

- preclinical changes (detectable before the onset of symptoms)

https://www.humane-endpoints.info/en/why-humane-endpoints

Directive 2010/63/EU

- termination of procedure
- removal from protocol
- modification of experimental design
- refinement
- anesthesia
- analgesia (local, general)
- husbandry
- other method(s) for alleviating pain/distress
- treatment
- euthanasia
- (earlier) sample collection?

Directive 2010/63/EU

https://www.humane-endpoints.info/en/why-humane-endpoints

actions

early

healthy	earlier indicators of disease onset	indicators of disease onset	earlier signs of disease	obvious signs of severe disease	dead
					
know what it looks like !?	e.g. MRI, CT, bioluminescence	e.g. blood glucose, tumor markers	e.g. weight loss, chromodacryrrhea	e.g. cachexia, moribund state	

start — experiment timeline — end?

increasing harms

Aurora Bronstad, 2019 - modified

Images from https://www.humane-endpoints.info/

Argyro Zacharioudaki DVM MLAS Dipl.ECLAM 2022

humane endpoints

intervention

- early study endpoints
- welfare monitoring & scoring
- other humane intervention points → actions

- quality of measurements/samples → collect?
- achievable goal vs suffering

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9583283/pdf/10.1177_00236772221090801.pdf

humane intervention points

refinement interventions to lower severity

the points where suffering is alleviated

Refinement

personnel must be proficient in the establishment and use of humane endpoints

harm benefit evaluation

information on the project's humane endpoints and severity shall be provided in the application for authorization to enable harm-benefit evaluation

the nature of the humane endpoints affects the severity classification of the procedure/project

early and painless humane endpoints shall be chosen and death as an endpoint must be avoided

harm benefit evaluation

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harm benefit analysis

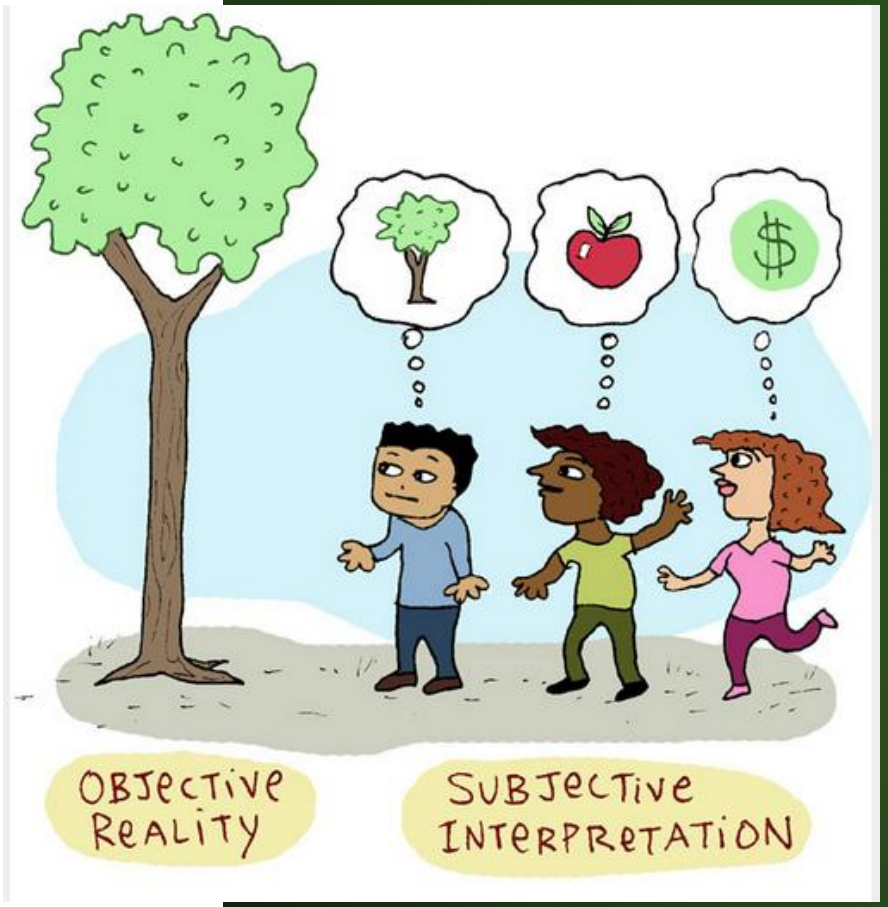
For each project a harm-benefit analysis is performed to assess whether the harm to the animals in terms of suffering, pain and distress is justified by the expected outcome, taking into account ethical considerations, and may ultimately benefit human beings, animals or the environment.

$$\text{Justification} = \frac{\text{Importance of objectives} \times \text{Probability of achievement}}{\text{Harms to animals}}$$

EC Guidance: Project Evaluation & Retrospective Assessment

harm benefit analysis

- culture
- environment
- economic situation
- acquired knowledge
- emerging unsolved scientific problems
- ethical values



harm benefit analysis

- sufficient information must be included in the application, to enable the evaluators to make a reasoned judgement

- harms to animals
- 3Rs

- benefits to society/nature
- likelihood to achieve benefits
- impact

EC Guidance: Project Evaluation & Retrospective Assessment

practice severity classification & humane intervention points selection

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