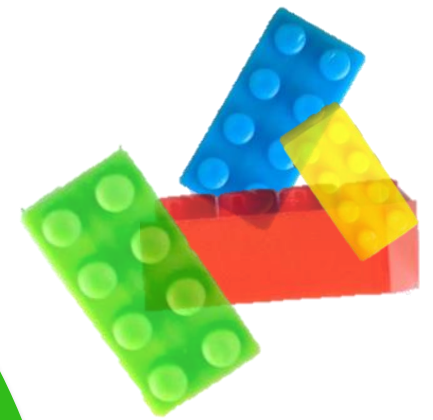


LO 2.4, 2.6, 2.10, 2.11, 2.13, 2.14, 11.10-11.14, 11.17, 11.18

3Rs

**Argyro
Zacharioudaki**

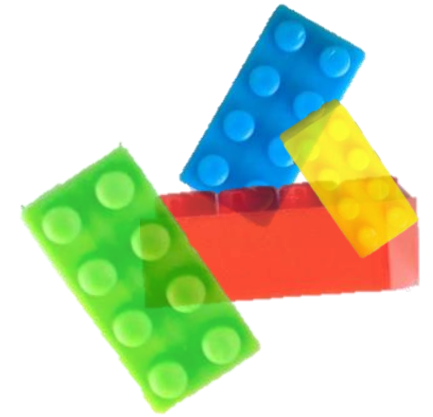
DVM MLAS Dipl.ECLAM
BIOEMTECH CRO Laboratories



what are the
3Rs?

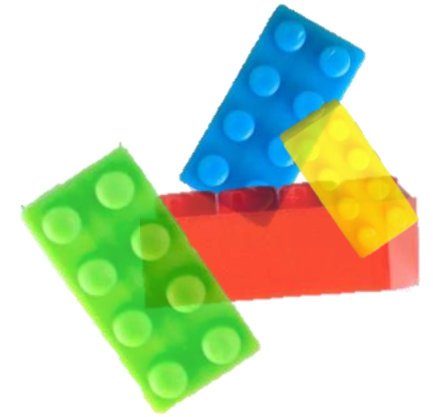


...they considered:



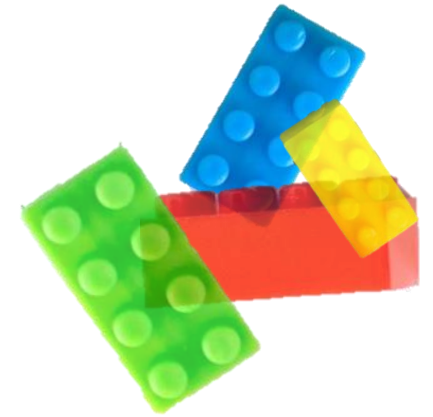
- “The experimental biologist almost always requires animals in a **stable and known** physiological state; he commonly requires a number of animals in as nearly as possible the same physiological state.”
- “The psychosomatics of experimental animals are perhaps the most important single subject for the development of **humane and efficient** technique in animal experiment.”

...they considered:



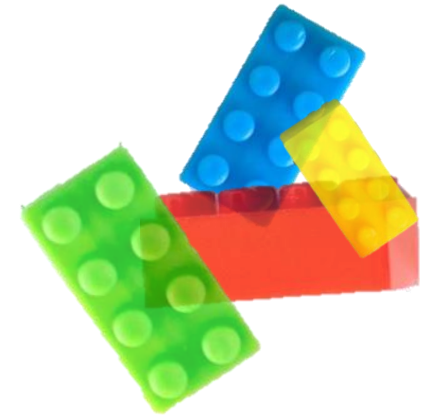
- quality of research results
- scientific evidence of effect of pain and distress to animals
- ethical concerns for humane care and use of animals

...and they raised the question:



- is there an irreconcilable conflict between the claims of science and medicine and those of humanity in our treatment of animals?

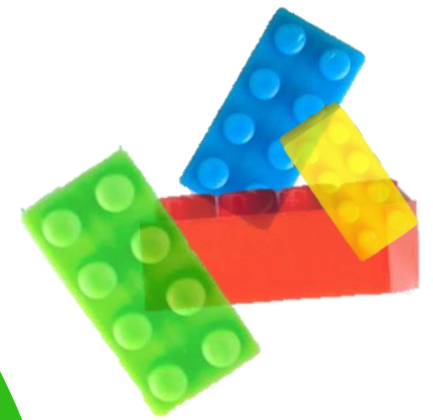
... and they propose the solution:



- ✓ Replacement
- ✓ Reduction
- ✓ Refinement

... exaggerate the positive
eliminate the negative
latch on to the alternative
always replace reduce refine...

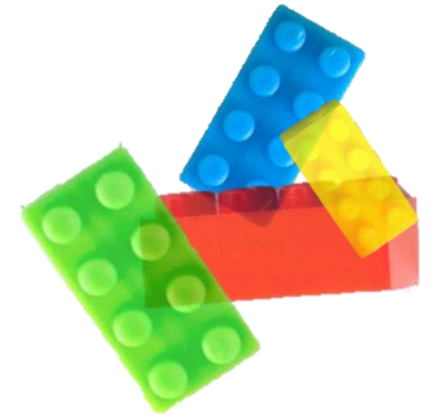




is it obligatory
to follow the
3Rs?

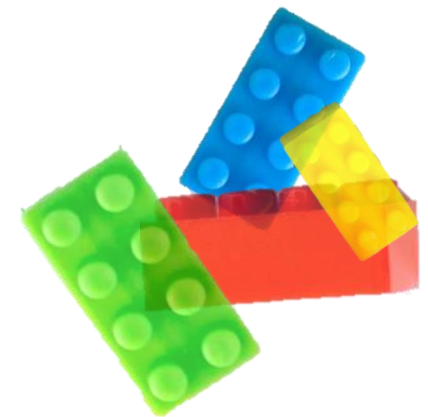


...today



- 3Rs are part of the **legislation** on the care and use of laboratory animals in most of the world!
- “**principle of replacement, reduction and refinement**”

Directive 2010/63/EU

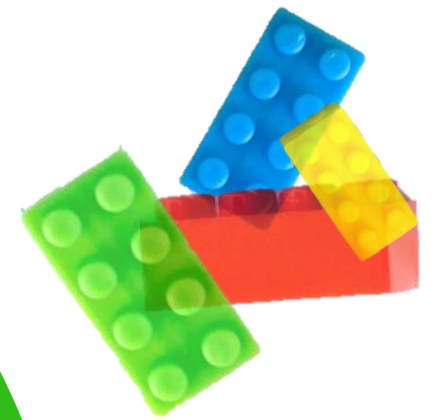


[Directive 2010/63/EU](#)

Article 4

Principle of replacement, reduction and refinement

1. Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure.
2. Member States shall ensure that the number of animals used in projects is reduced to a minimum without compromising the objectives of the project.
3. Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals.
4. This Article shall, in the choice of methods, be implemented in accordance with Article 13.



why follow the
3Rs?

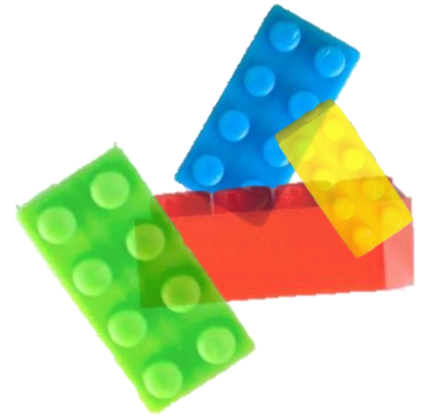




Good Animal Care and Good Science Go Hand in Hand

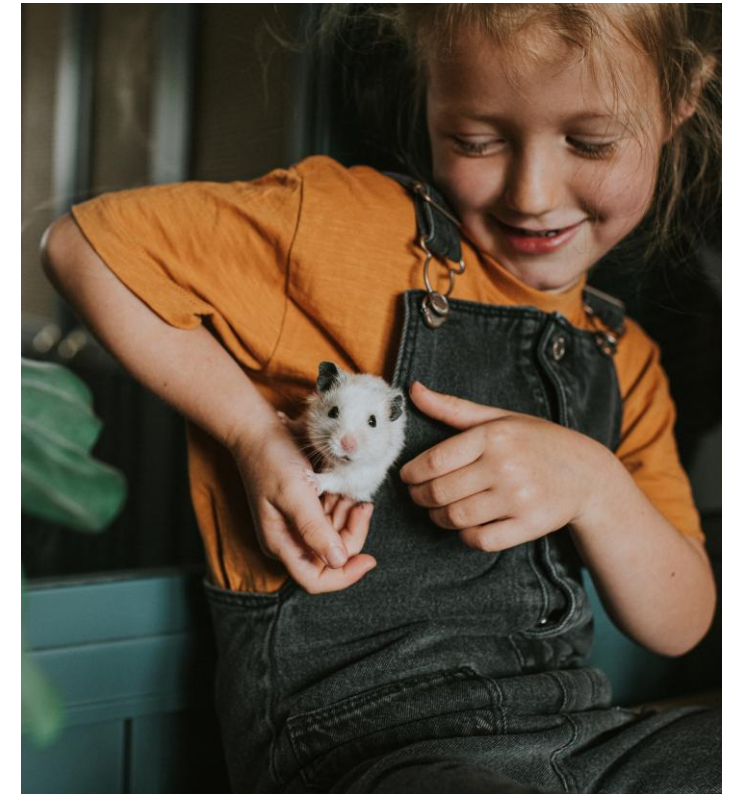
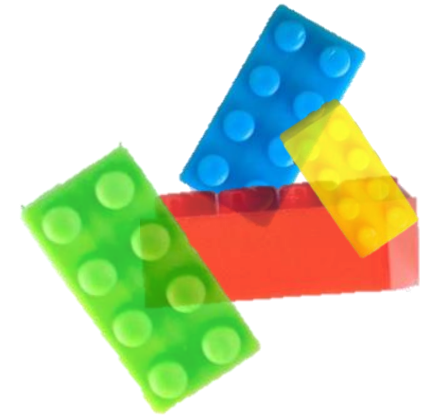


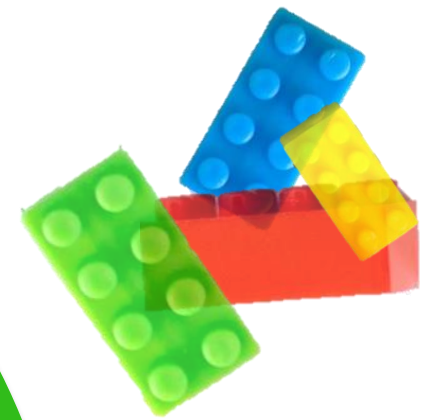
NIH Animal Research Advisory Committee
301-496-5424
SecOACU@od.nih.gov



...why?

- legal obligation
- ethical concerns
 - personal
 - public
 - publishing
- scientific evidence
 - better welfare = better research

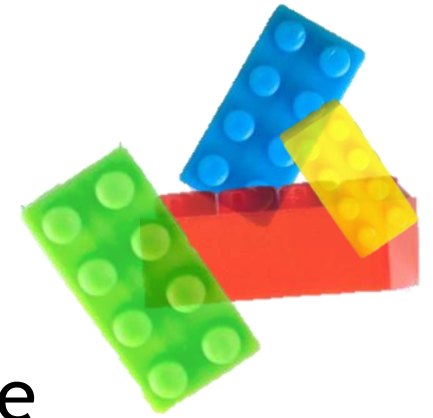




how to...
REPLACE?



Replace: Search for alternatives



- search for valid alternative(s) to replace animal use
 - present search in your application
 - use it!!



guide searching for alternatives

norecopa.no / [Alternatives](#) / [Alternatives to animal research and testing](#)

ALTEX

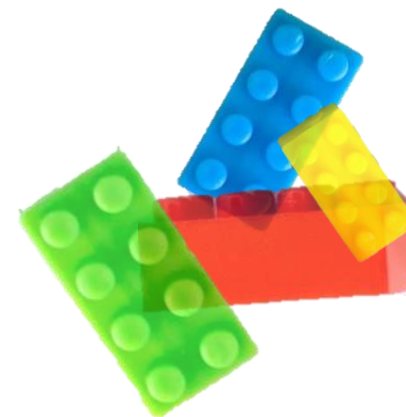
ALTERNATIVES TO ANIMAL EXPERIMENTATION

EU Reference Laboratory for alternatives to animal testing

Welcome to the European Union Reference Laboratory for alternatives to animal testing - EURL ECVAM!



Replacement examples

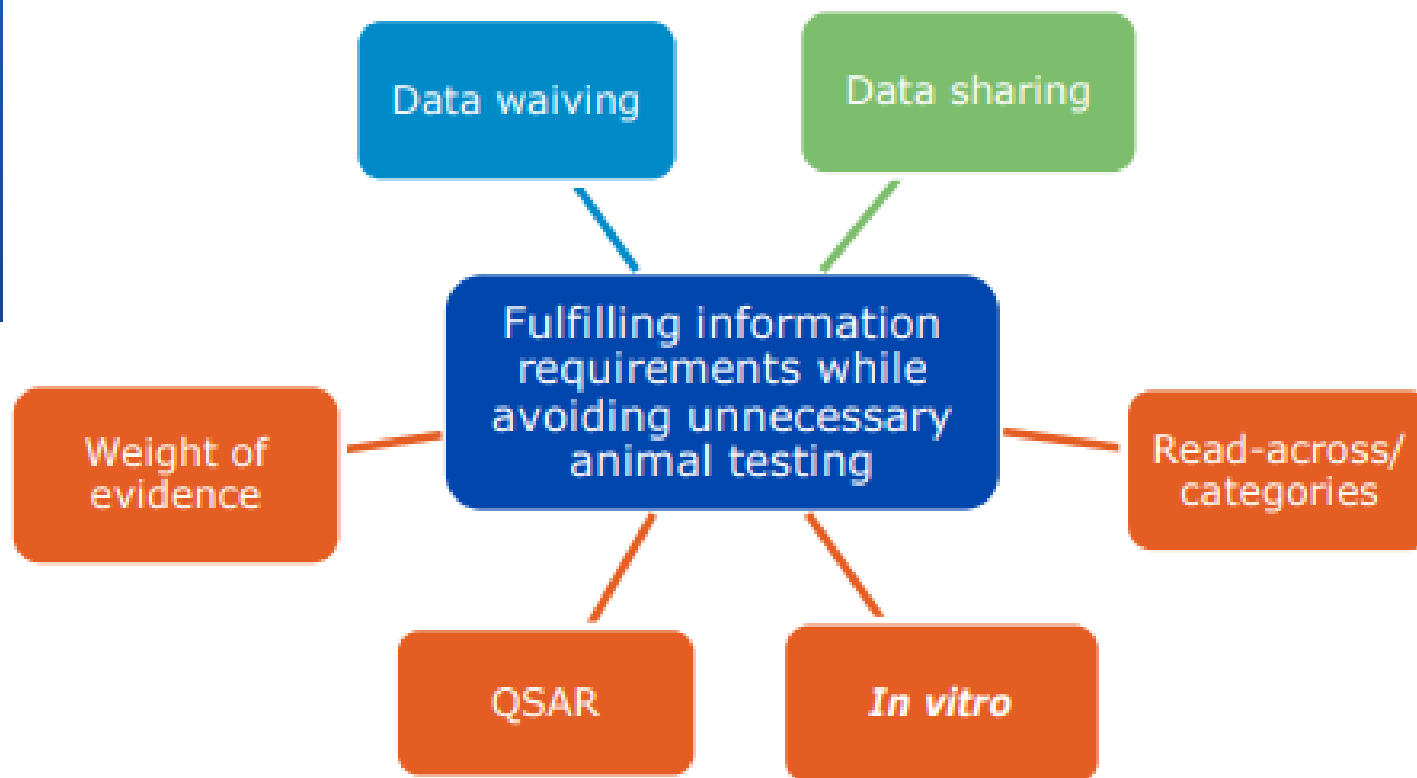


- chemical safety

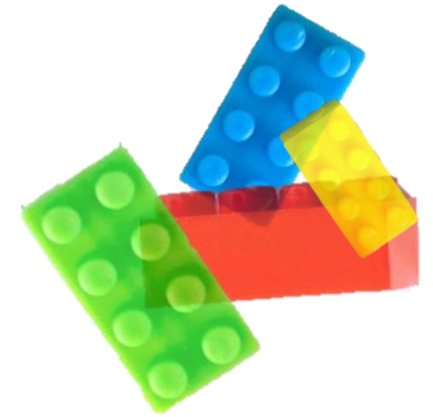


Practical guide

How to use alternatives to animal testing to fulfil your information requirements for REACH registration



Replacement examples



- validated test methods

Eye irritation/Serious eye damage

Eye irritation is the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.

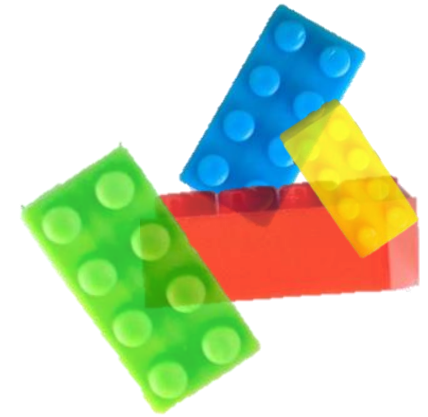
Read more [here](#).

- The Cytosensor Microphysiometer (CM) toxicity test
- The Fluorescein Leakage (FL) test
- Bovine Corneal Opacity and Permeability (BCOP) test
- Isolated Chicken Eye (ICE) test
- EpiOcular™ Eye Irritation Test (EIT)
- SkinEthic™ HCE Eye Irritation Test (EIT)
- Ocular irritation assay
- Low Volume Eye Test (LVET)
- In vivo rabbit eye test template for pre-existing data

<https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/validated-test-methods>



Replacement examples



- cosmetics testing

<https://www.euronews.com/living/2019/03/03/cosmetics-what-are-the-alternatives-to-animal-testing>

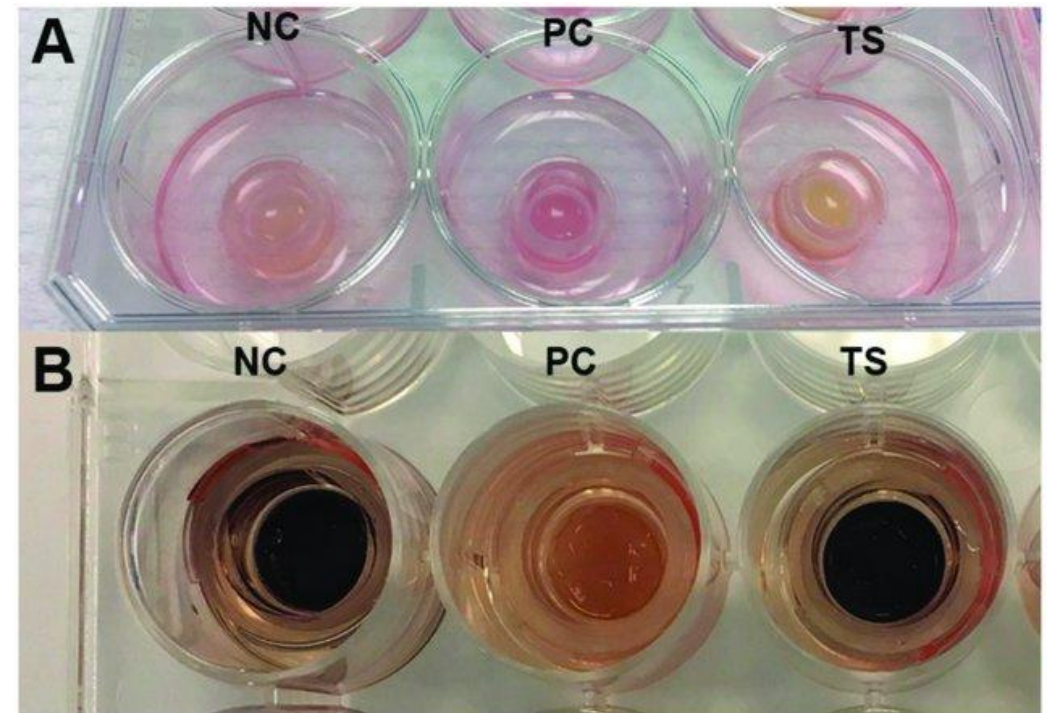
<https://www.unilever.com/planet-and-society/responsible-business/alternatives-to-animal-testing/>

<https://www.eara.eu/a-history-of-the-eu-testing-ban>

The view of 3D reconstructed human skin inserts during in vitro skin irritation evaluation before (A) and after (B) cell viability test after 18 h of incubation.
Čižauskaitė et al. 2018

Selected non-animal screening and alternative methods used in Henkel laboratories:

- *Indicator assays for determining cytotoxic effects*
- *Organotypical skin models for studying irritation of the skin*
- *Hen's egg test for mucous membrane compatibility (Hen's Egg Test on the Chorionallantoic Membrane, HET-CAM Test)*
- *Hen's egg test for determining mutagenic properties (Hen's Egg Test for Micronucleus Formation, HET-MN Test)*
- *Testing of skin absorption (OECD 428)*
- *Photohemolysis test for determining phototoxic potential*
- *Dendritic cells for determining sensitizing potential*
- *In-silico methods: quantitative structure-activity relationships using chemical informatics systems*



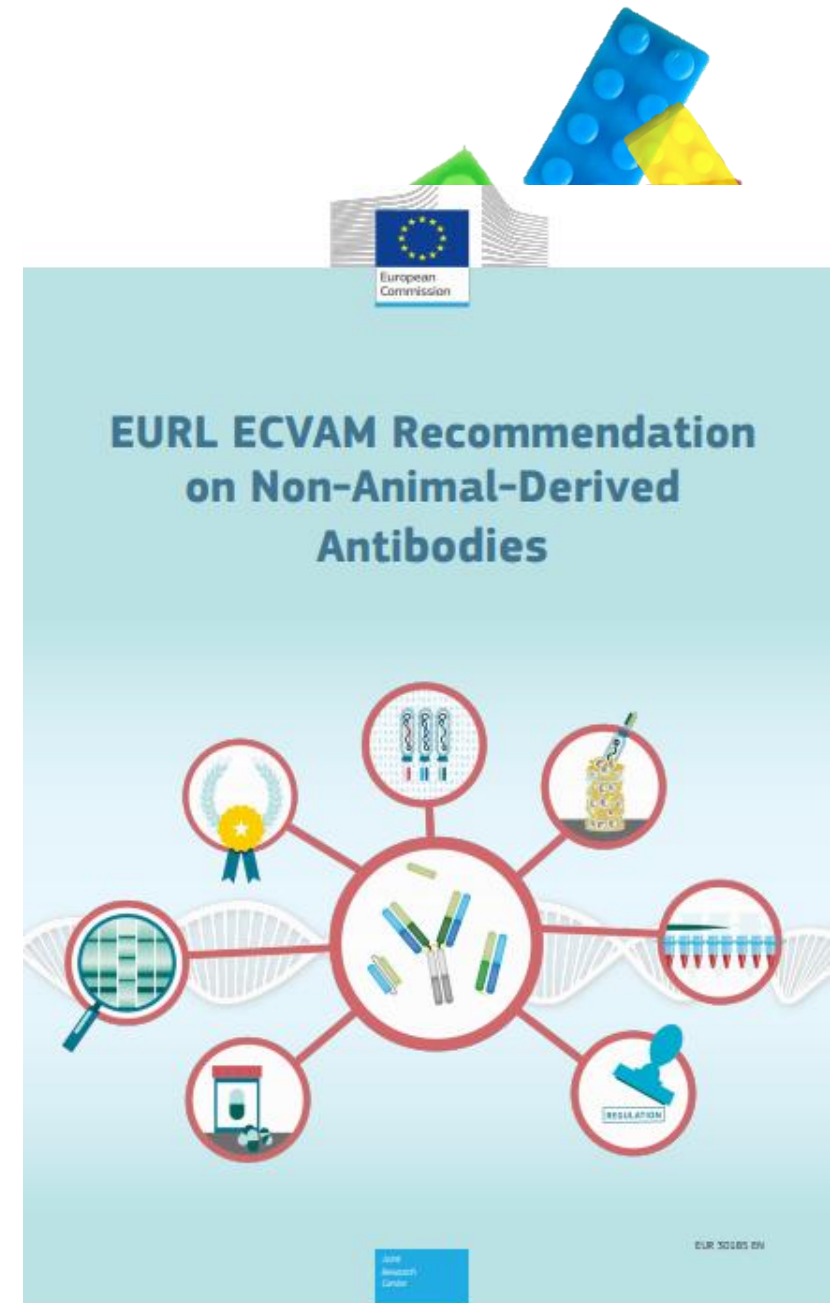
Replacement examples

- antibody production

<https://publications.jrc.ec.europa.eu/repository/handle/JRC120199>

<https://www.eara.eu/post/feature-antibodies>

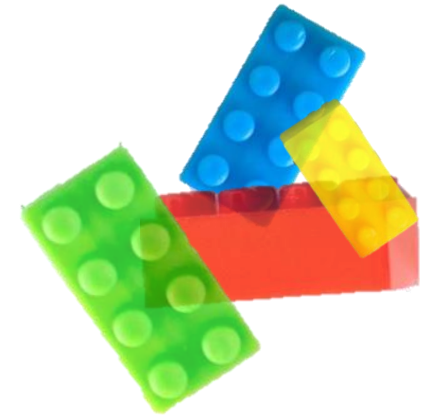
The Nobel Prize in Chemistry 2018 was divided, one half awarded to Frances H. Arnold "for the directed evolution of enzymes", the other half jointly to George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies."



Replacement examples

- education and training

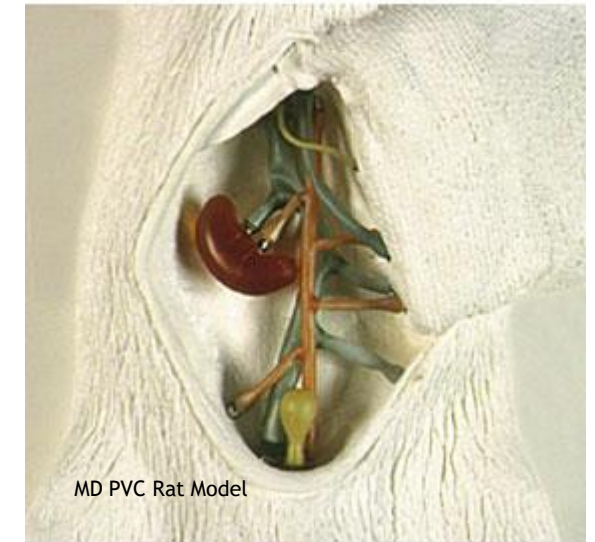
- <https://www.braintreesci.com/products.asp?dept=109>



Laparoscopy simulators

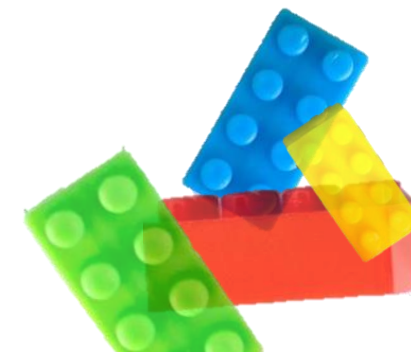


Mimicky® Mouse Simulator



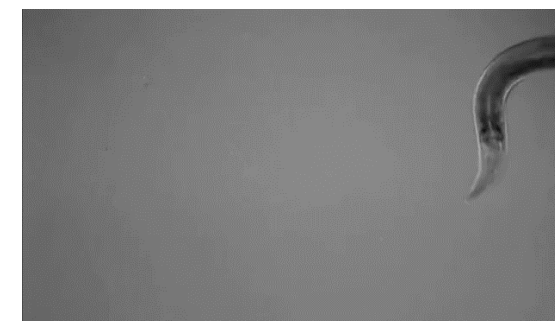
MD PVC Rat Model

Replacement



- “lowest” species
- ✓ select species with the lowest capacity to experience pain, suffering, distress or lasting harm
- species not covered by Directive 2010/63/EU
 - single-cell organisms
 - *Drosophila melanogaster*
 - *Caenorhabditis elegans*
 - etc
- species covered by Directive 2010/63/EU
 - live non-human vertebrate animals, including
 - independently feeding larval forms; and
 - fetal forms of mammals as from the last third of their normal development;
 - live cephalopods

Using Model Animals to Assess and Understand Developmental Toxicity



An Updated Comparison of Common Model Organisms

	 Cell Cultures	 <i>C. elegans</i>	 <i>Drosophila</i>	 Zebrafish	 Mouse
Connectome	★☆☆☆☆	★★★★★	★★★★☆	★★★★☆	★★★★☆
Availability of off-the-shelf models	★★★★☆	★★★★☆	★★★★☆	★★★★☆	★★★★☆
Ease of genetic manipulation	★★★☆☆	★★★★★	★★★★★	★★★★☆	★★★★☆
Amendable to drug screening	★★★☆☆	★★★★☆	★★★★☆	★★★★☆	★★★★☆
Safety and ethics considerations	★★★★☆	★★★★☆	★★★★☆	★★★★☆	★★★☆☆
Strains can be frozen and revived	✓	✓	✗	✗	✗



how to...
REDUCE?



Reduction

- experimental design
 - replace
 - hypothesis, outcomes, groups, power analysis, randomization, blinding...
 - no “waste”
 - minimum number of animals
 - use statistics to select appropriate number of animals per group

PREPARE



The PREPARE Guidelines Checklist

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith^a, R. Eddie Clutton^b, Elliot Lilley^c, Kristine E. Aa. Hansen^d & Trond Bratteli^{d*}

^aNorecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; ^bRoyal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; ^cResearch Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.; ^dSection of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; ^{d*}Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

ARRIVE guidelines

The ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments) are a checklist of recommendations to improve the reporting of research involving animals – maximising the quality and reliability of published research papers to better scrutinise, evaluate and reproduce it.



Experimental
Design
Assistant



Michael Festing

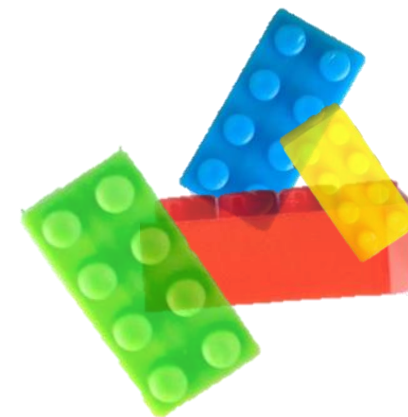
G*Power

Statistical Power Analyses for Mac and Windows

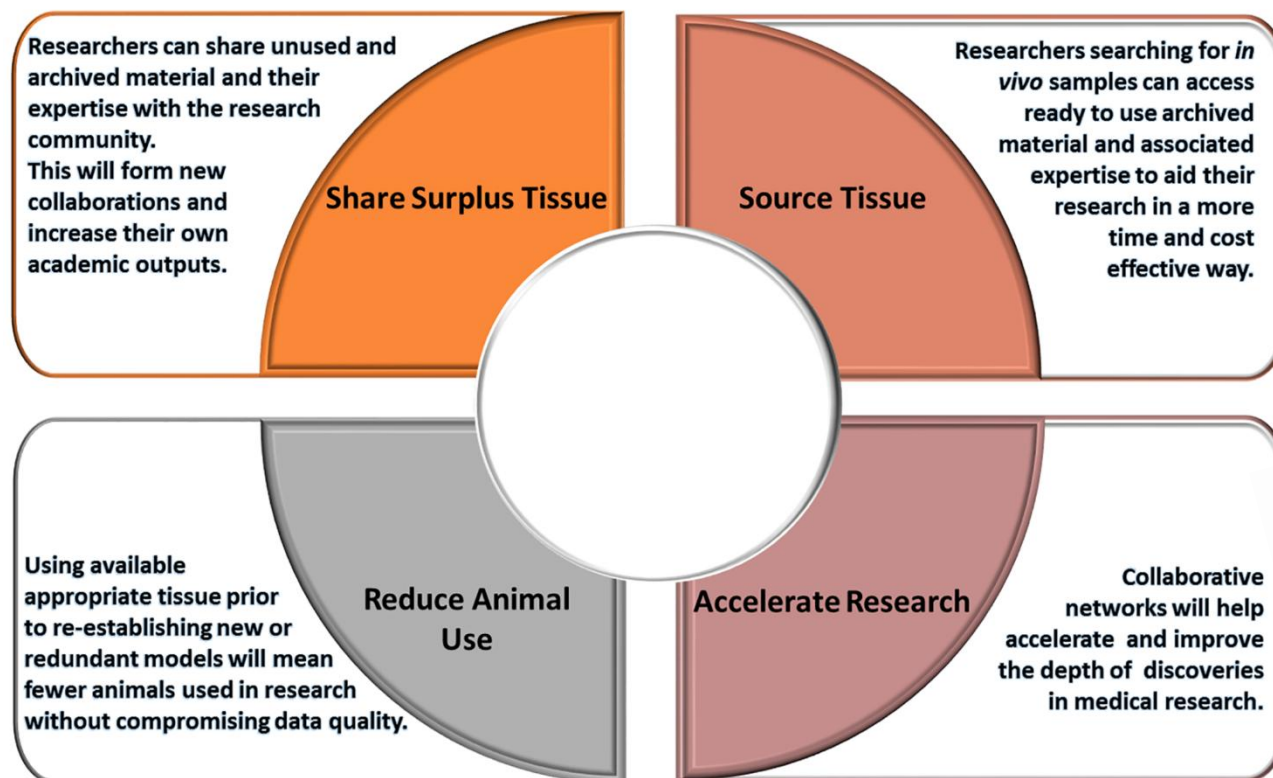
G*Power is a tool to compute statistical power analyses for many different t tests, F tests, χ^2 tests, z tests and some exact tests. G*Power can also be used to compute effect sizes and to display graphically the results of power analyses.

Reduction

- share



THE SEARCH FRAMEWORK

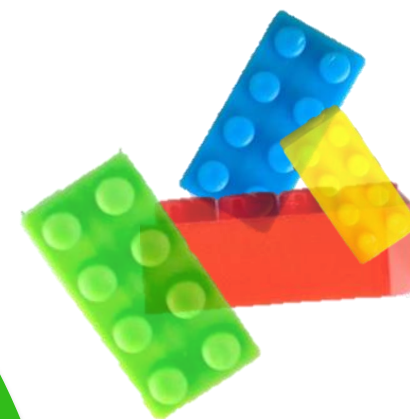


The Sharing Experimental Animal Resources, Coordinating Holdings (SEARCH) Framework: Encouraging Reduction, Replacement, and Refinement in Animal Research

Bethny Morrissey, Karen Blyth, Phil Carter, Claude Chelala, Louise Jones, Ingunn Holen, Valerie Speirs 

Published: January 12, 2017 • <https://doi.org/10.1371/journal.pbio.2000719>

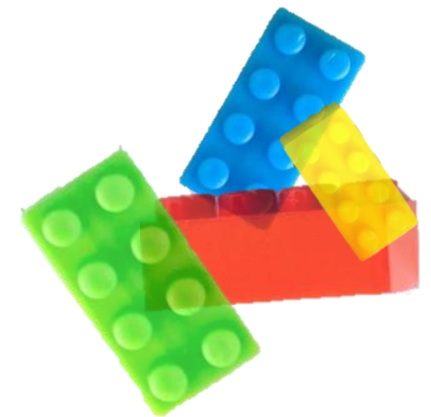
Article 18
Sharing organs and tissues
Member States shall facilitate, where appropriate, the establishment of programmes for the sharing of organs and tissues of animals killed.



is Rehoming
allowed?



Rehoming



Directive 2010/63/EU

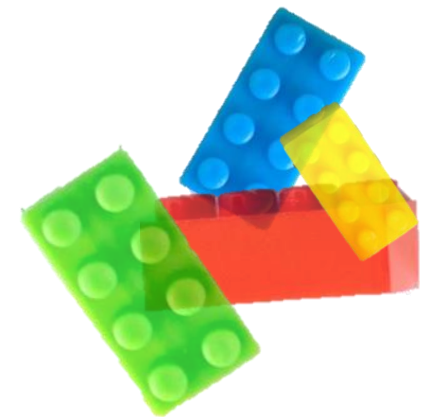
Article 19

Setting free of animals and rehoming

Member States may allow animals used or intended to be used in procedures to be rehomed, or returned to a suitable habitat or husbandry system appropriate to the species, provided that the following conditions are met:

- (a) the state of health of the animal allows it;
- (b) there is no danger to public health, animal health or the environment; and
- (c) appropriate measures have been taken to safeguard the well-being of the animal.

Rehoming



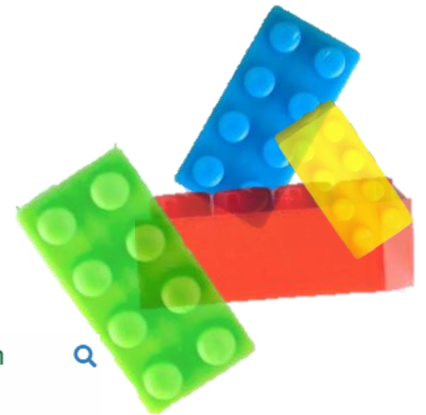
Directive 2010/63/EU

Article 29

Scheme for rehoming or setting free of animals

Where Member States allow rehoming, the breeders, suppliers and users from which animals are intended to be rehomed shall have a rehoming scheme in place that ensures socialisation of the animals that are rehomed. In the case of wild animals, where appropriate, a programme of rehabilitation shall be in place before they are returned to their habitat.

Rehoming



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[Reports](#)

Rehoming of animals used for scientific and educational purposes

Publications

Recommendations

[FELASA recommendations for the rehoming of animals used for scientific and educational purposes.](#) Ecuer E, Boxall J, Louwerse AL, Mikkelsen LF, Moons CP, Roth M, Spiri AM. Lab Anim. 2023. 57(5):572-582. doi: 10.1177/00236772231158863.

[Supplementary Material](#)

[Species specific requirements for rehoming](#)

Rehoming



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Rehoming of animals used for scientific and educational purposes

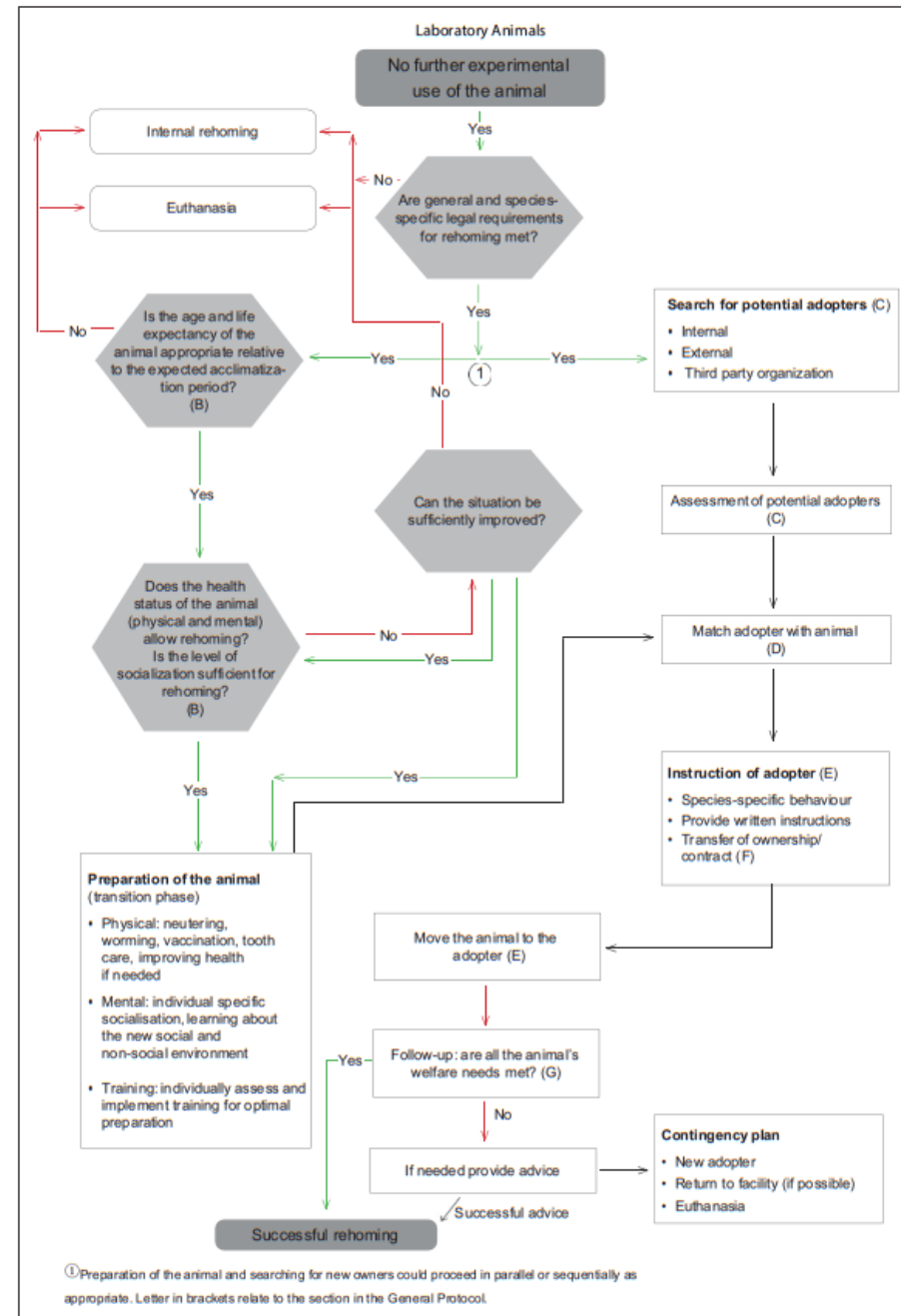
Publications

Recommendations

[FELASA recommendations for the rehoming of animals used for scientific and educational purposes](#), Ecuer E, Boxall J, Louwerse AL, Mikkelsen LF, Moons CP, Roth M, Spiri AM. Lab Anim. 2023. 57(5):572-582. doi: 10.1177/00236772231158863.

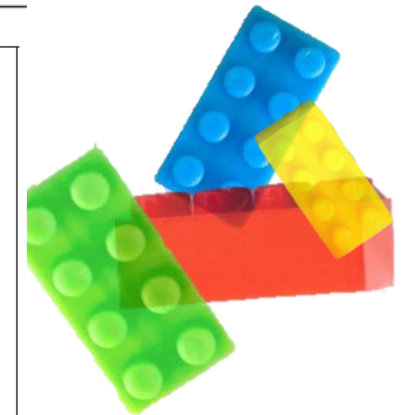
[Supplementary Material](#)

[Species specific requirements for rehoming](#)



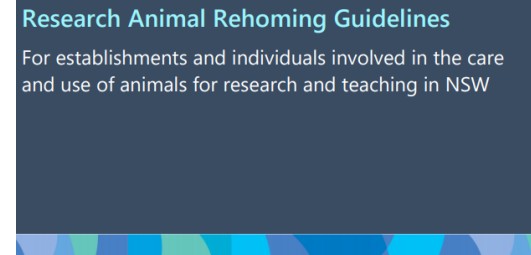
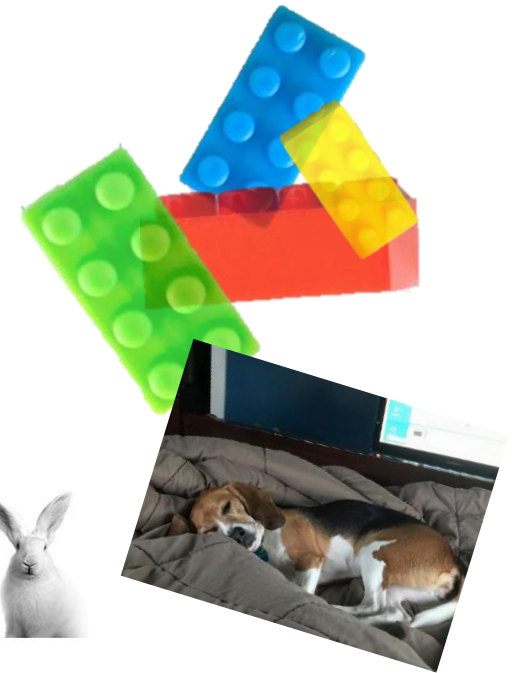
Argy

Figure 1. Flowchart of the rehoming process.



Rehoming

- appropriate scheme
 - ✓ ensure success
 - animal status
 - suitable home
 - ✓ ensure animal well-being
 - ✓ public safety
- veterinary evaluation & supervision
- & AWB advice
 - animal health status
 - socialization
 - avoid unnecessary distress
 - accompanying health records



www.dpi.nsw.gov.au

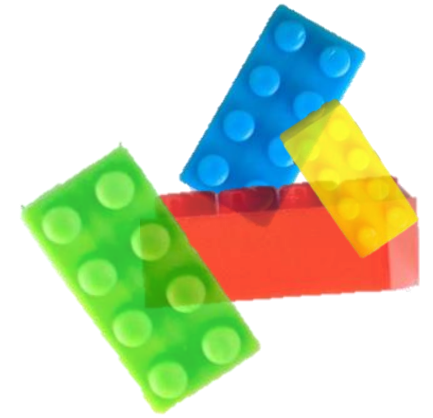
A semi-structured questionnaire survey of laboratory animal rehoming practice across 41 UK animal research facilities

Tess Skidmore , Emma Roe

Published: June 19, 2020 • <https://doi.org/10.1371/journal.pone.0234922>

Rehoming

- for reduction!

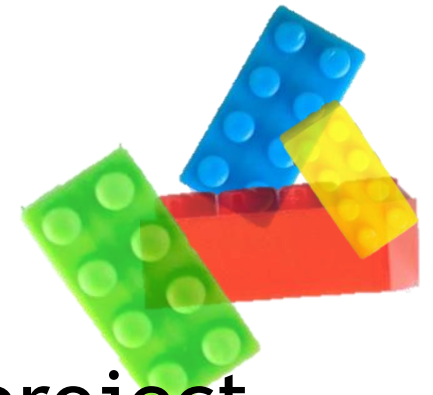




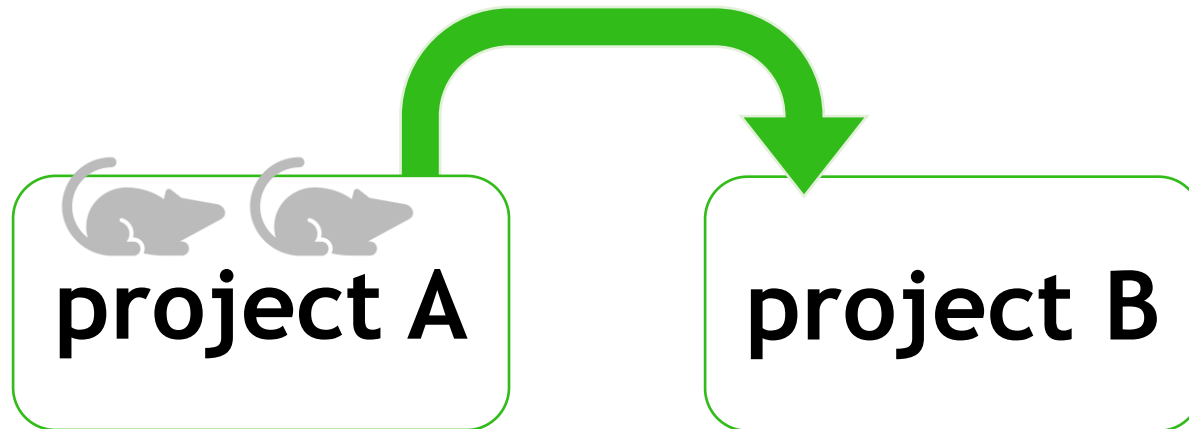
is Reuse
allowed?



Reuse?



- use an animal from one project on another project
- when a different animal on which no procedure has previously been carried out could also be used



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 the previous procedure is 'moderate';

(b) it is demonstrated that the animal's health and well-being have not been affected;

(c) the further use of the animal is justified, taking into account the lifetime of the animal;

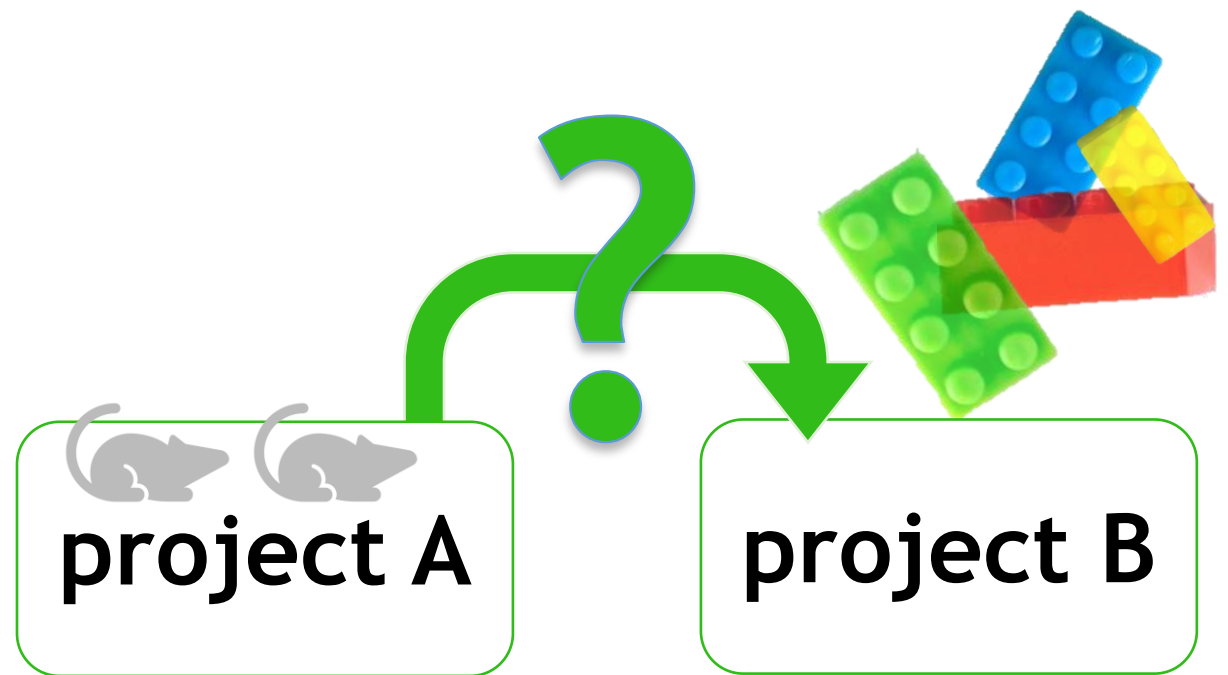
(d) it is in accordance with the Directive, taking into account the lifetime of the animal.

2. In exceptional circumstances, by way of derogation from point (a) of paragraph 1 and after a veterinary examination of the animal, the competent authority may allow reuse of an animal, provided the animal has not been used more than once in a procedure entailing severe pain, distress or equivalent suffering.

National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes
Working document on specific articles in Directive 2010/63/EU
Brussels, 6-7 October 2011

Reuse conditions

- to not detract from the scientific objective
- to not result in poor animal welfare
- considered on a case-by-case basis balancing the benefit of re-use against adverse effects on welfare, taking into account the lifetime experience of the individual animal

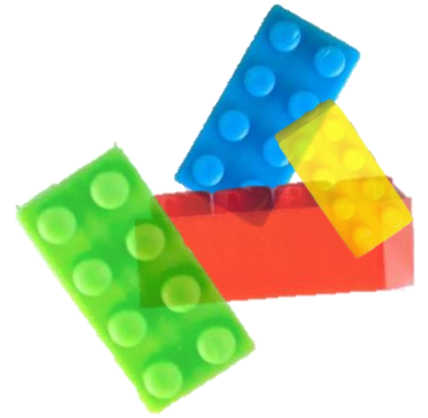


- actual severity of 1st project must be mild/moderate
 - exemption may be allowed after veterinary examination and only after 1 severe procedure
- animal's health/well-being must be fully restored

- prospective severity of 2nd project is mild/moderate/non-recovery
- in accordance with veterinary advice

Re-use

- for reduction!





how to...
REFINE?



Refine

study your animal model

select refined model/design
pilot?

identify harms

identify refined methods for procedures to reduce harms

cause the least pain, suffering, distress or lasting harm;

and are most likely to provide satisfactory results.

use anesthesia & analgesia when needed

get trained!

select early humane endpoints

avoid death as endpoint

acceptable euthanasia methods

assess severity

minimize

higher threshold

monitor welfare

plan & organize carefully

continuously re-evaluate

retrospective evaluation

publish the details!

Article 13

Choice of methods

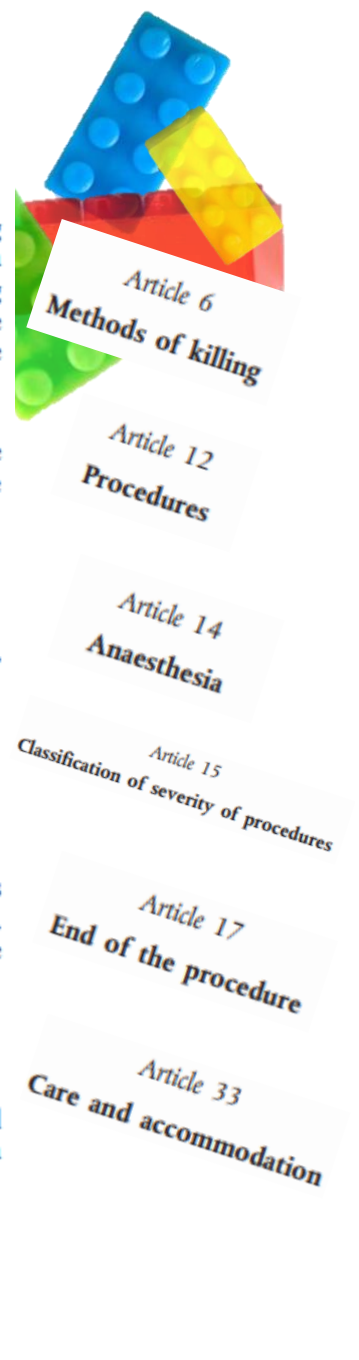
1. Without prejudice to national legislation prohibiting certain types of methods, Member States shall ensure that a procedure is not carried out if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognised under the legislation of the Union.

2. In choosing between procedures, those which to the greatest extent meet the following requirements shall be selected:

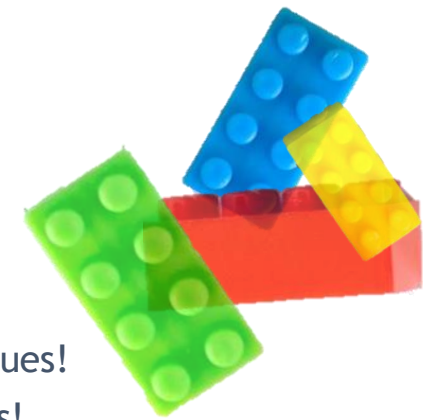
- (a) use the minimum number of animals;
- (b) involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm;
- (c) cause the least pain, suffering, distress or lasting harm; and are most likely to provide satisfactory results.

3. Death as the end-point of a procedure shall be avoided as far as possible and replaced by early and humane end-points. Where death as the end-point is unavoidable, the procedure shall be designed so as to:

- (a) result in the deaths of as few animals as possible; and
- (b) reduce the duration and intensity of suffering to the animal to the minimum possible and, as far as possible, ensure a painless death.

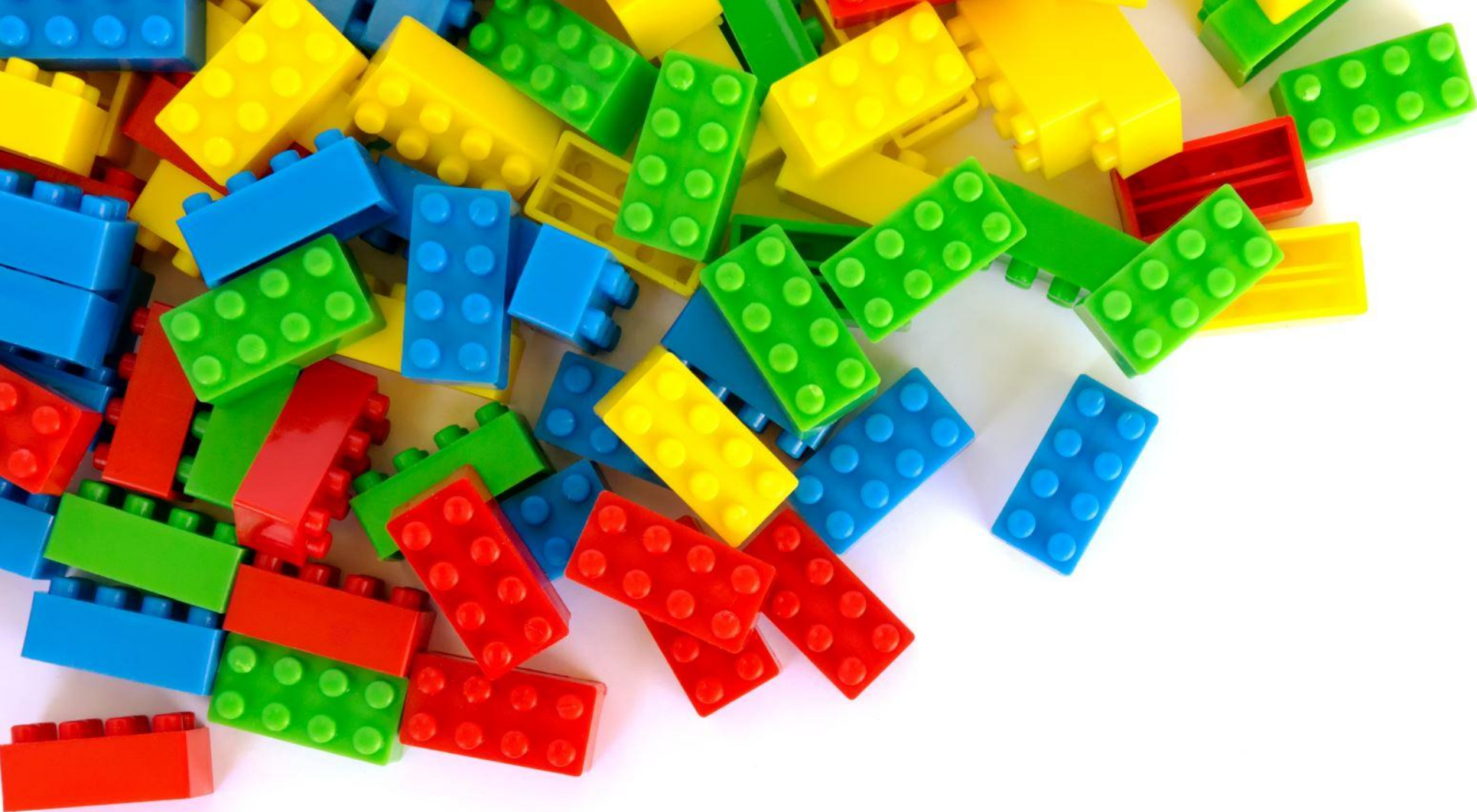


Literature Search



- google it!
- Google Scholar
- PubMed
- Laboratory Animals
- LabAnimal
- AALAS Journals
- Books
- Consult colleagues!
- Contact experts!



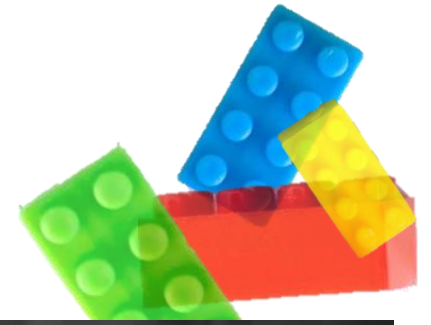


How to implement 3Rs?

by thinking out of the box

Argyro Zacharioudaki DVM MLAS Dipl.ECLAM

3Ss



- Good Science
 - experimental design and reporting
 - reproducibility
- Good Sense
 - “the Right animal is used for the Right Reason” (the Three Rs of Harry Rowsell)
 - Culture of Challenge (Louhimies, 2015) - Look for the acceptable, rather than choosing the accepted.
- Good Sensibilities
 - Culture of Care
 - Humane and responsible use
 - Respect

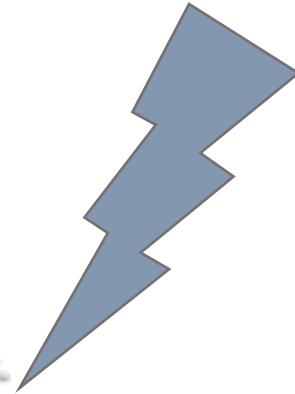
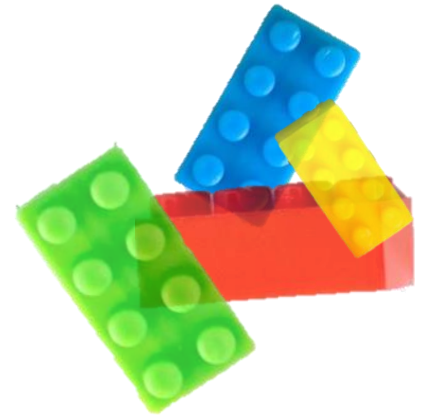


<https://norecopa.no/media/7963/3ss-presentation.pdf>

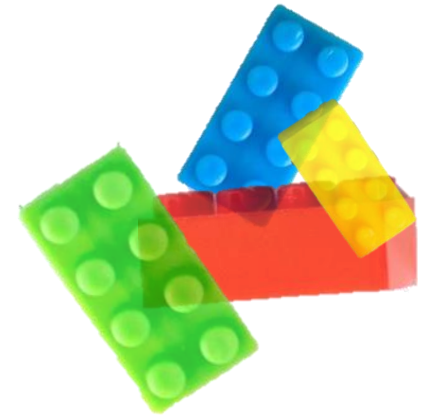
Carol M. Newton (1925-2014)

Refinement examples

- housing and care



Refinement examples



- handling

NC 3R^s National Centre for the Replacement Refinement & Reduction of Animals in Research

[Who we are](#) ▾ [Our portfolio](#) [Our funding schemes](#)

[Home](#) > [3Rs resource library](#)

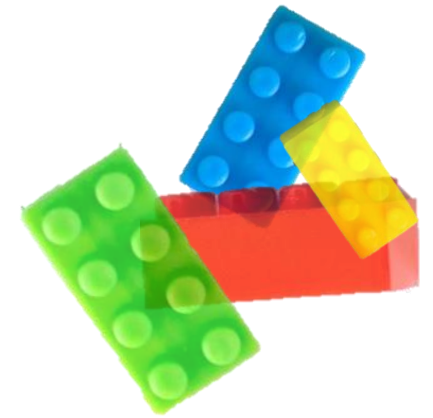
HANDLING

Mouse handling

Research has shown that picking up mice by the tail induces aversion and high anxiety levels, as assessed by a range of measures, which can be minimised by instead using a tunnel or cupped hands.



Refinement examples



- procedures

- <https://www.ri.se/en/what-we-do/expertises/3r-refinement>



- <https://nc3rs.org.uk/3rs-resource-library/handling-and-training-mice-and-rats-low-stress-procedures>



Refinement examples

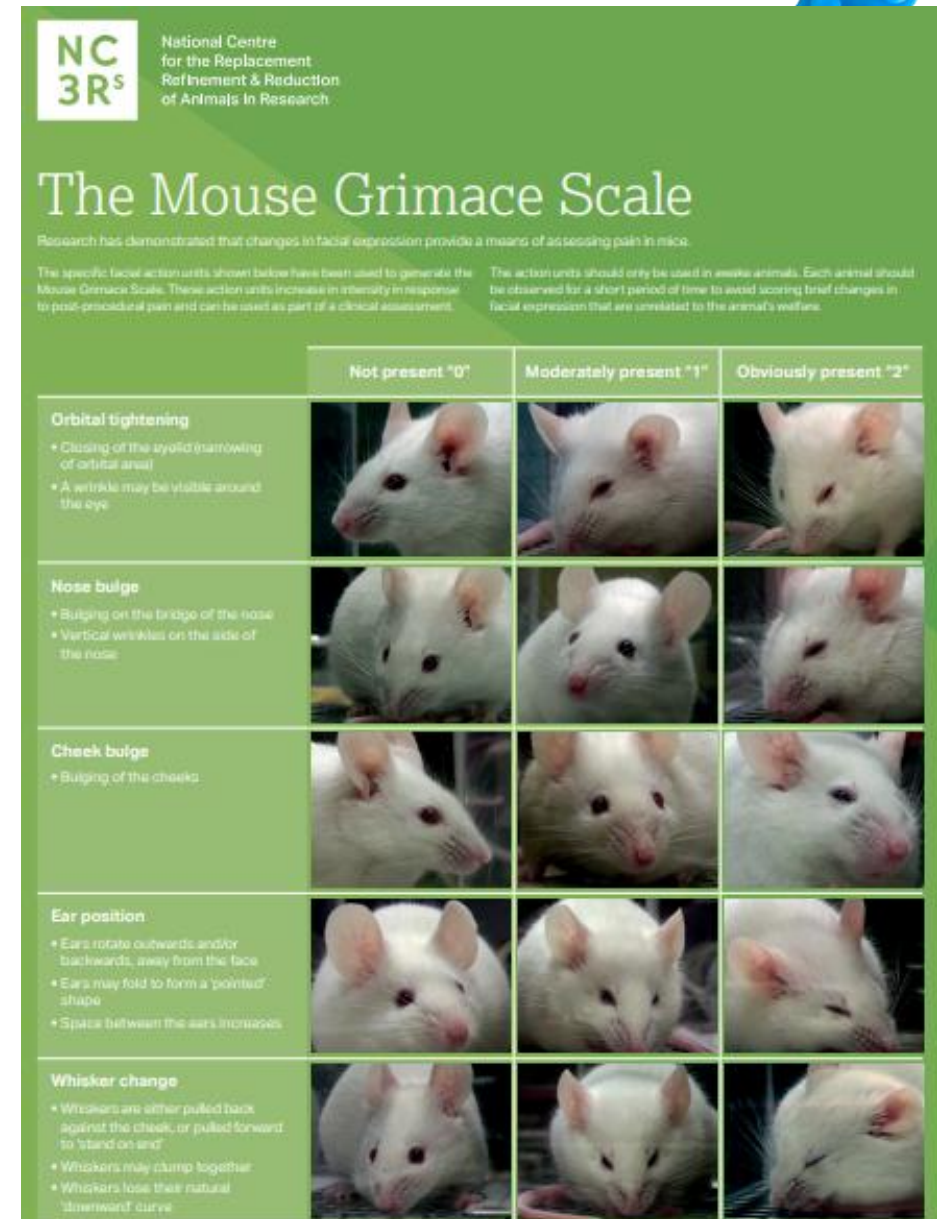
- assess and relieve pain
- support the use of analgesia
- use early humane endpoints

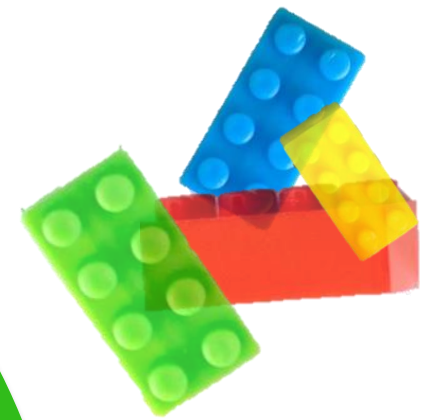
Published: 22 March 2017

Side effects of pain and analgesia in animal experimentation

Paulin Jirkof 

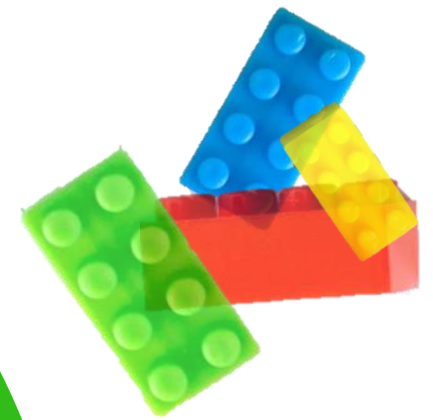
Lab Animal **46**, 123–128(2017) | [Cite this article](#)





Reduction vs Refinement?





3Rs in testing
of a new drug?



Resources

- must read!

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)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 114 thereof,

Having regard to the proposal from the European Commission,

Having regard to the opinion of the European Economic and Social Committee ⁽¹⁾,

After consulting the Committee of the Regions,

Acting in accordance with the ordinary legislative procedure ⁽²⁾,

Whereas:

- (1) On 24 November 1986 the Council adopted Directive 86/609/EEC ⁽³⁾ in order to eliminate disparities between laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Since the adoption of that Directive, further disparities between Member States have emerged. Certain Member States have adopted national implementing measures that ensure a high level of protection of animals used for scientific purposes, while others only apply the minimum requirements laid down in Directive 86/609/EEC. These disparities are liable to constitute barriers to trade in products and substances the development of which involves experiments on animals. Accordingly, this Directive should provide for more detailed rules in order to reduce such disparities by approximating the rules applicable in that area and to ensure a proper functioning of the internal market.
- (2) Animal welfare is a value of the Union that is enshrined in Article 13 of the Treaty on the Functioning of the European Union (TFEU).
- (3) On 23 March 1998 the Council adopted Decision 1999/575/EC concerning the conclusion by the Community of the European Convention for the protection of vertebrate animals used for experimental

and other scientific purposes ⁽⁴⁾. By becoming party to that Convention, the Community acknowledged the importance of the protection and welfare of animals used for scientific purposes at international level.

- (4) The European Parliament in its resolution of 5 December 2002 on Directive 86/609/EEC called for the Commission to come forward with a proposal for a revision of that Directive with more stringent and transparent measures in the area of animal experimentation.
- (5) On 15 June 2006, the Fourth Multilateral Consultation of Parties to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes adopted a revised Appendix A to that Convention, which set out guidelines for the accommodation and care of experimental animals. Commission Recommendation 2007/526/EC of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes ⁽⁵⁾ incorporated those guidelines.
- (6) New scientific knowledge is available in respect of factors influencing animal welfare as well as the capacity of animals to sense and express pain, suffering, distress and lasting harm. It is therefore necessary to improve the welfare of animals used in scientific procedures by raising the minimum standards for their protection in line with the latest scientific developments.
- (7) Attitudes towards animals also depend on national perceptions, and there is a demand in certain Member States to maintain more extensive animal-welfare rules than those agreed upon at the level of the Union. In the interests of the animals, and provided it does not affect the functioning of the internal market, it is appropriate to allow the Member States certain flexibility to maintain national rules aimed at more extensive protection of animals in so far as they are compatible with the TFEU.

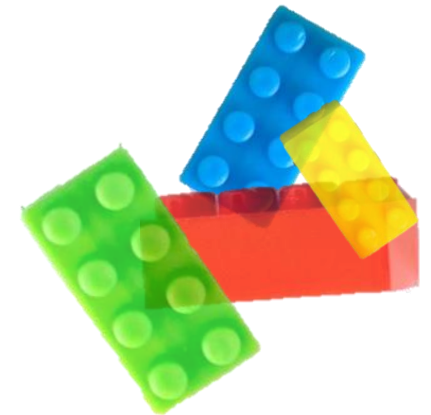
⁽¹⁾ OJ C 277, 17.11.2009, p. 51.

⁽²⁾ Position of the European Parliament of 5 May 2009 (OJ C 212 E, 5.8.2010, p. 170), position of the Council of 13 September 2010 (not yet published in the Official Journal) and position of the European Parliament of 8 September 2010 (not yet published in the Official Journal).

⁽³⁾ OJ L 358, 18.12.1986, p. 1.

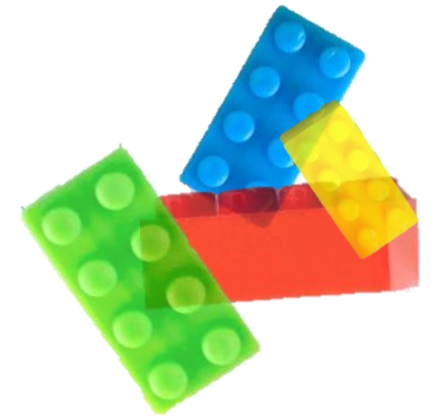
⁽⁴⁾ OJ L 222, 24.8.1999, p. 29.

⁽⁵⁾ OJ L 197, 30.7.2007, p. 1.



Directive 2010/63/EU

Resources



[Energy, Climate change, Environment](#)

[Environment](#)

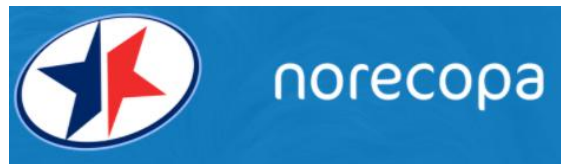
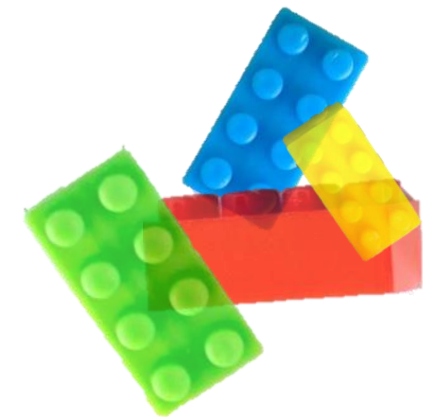
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Animals in science

EU actions for the protection of animals used for scientific purposes

Resources

Εθνική και Ευρωπαϊκή Νομοθεσία
για την προστασία των ζώων που χρησιμοποιούνται για επιστημονικούς σκοπούς



Procedures With Care



EEBEZE



Προεδρικό Διάταγμα 56/2013

Οδηγία 63/2010/ΕΕ

Directive 2010/63/EU

Ιστοσελίδες Εθνικών & Ευρωπαϊκών Φορέων

ΥΠΟΥΡΓΕΙΟ
ΑΓΡΟΤΙΚΗΣ
ΑΝΑΠΤΥΞΗΣ

ΕΘΝΙΚΗ ΕΠΙΤΡΟΠΗ
ΓΙΑ ΤΗΝ ΕΥΖΩΙΑ
ΖΩΩΝ
ΕΡΓΑΣΤΗΡΙΟΥ

ΕΥΡΩΠΑΪΚΗ
ΕΠΙΤΡΟΠΗ

FELASA

LO 2.4, 2.6, 2.10, 2.11, 2.13, 2.14, 11.10-11.14, 11.17, 11.18

?3Rs

**Argyro
Zacharioudaki**

DVM MLAS Dipl.ECLAM
BIOEMTECH CRO Laboratories

Thank you!