RSPCA ANIMALS IN SCIENCE

Working together to end severe suffering

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norecopa.no/RSPCA/FOSS

BARNEY REED

RSPCA 200

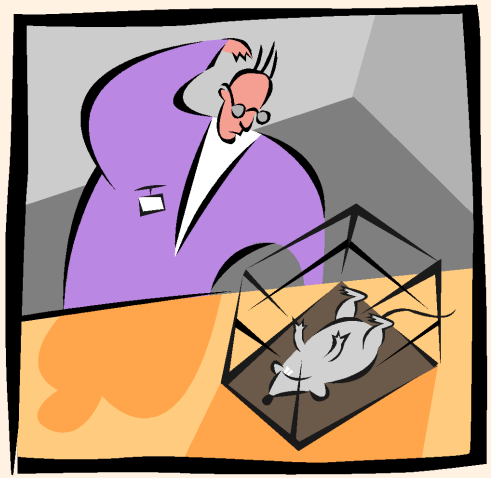
1824-2024

Within the European Union and the UK, '**severe**' procedures are those where animals used in science are likely to experience either:

- **severe pain, suffering** or **distress**
- **long-lasting moderate** pain, suffering or distress,
- **severe impairment** to their wellbeing or general condition

e.g. Annex VIII of EU Directive 2010/63

Causes of severe suffering

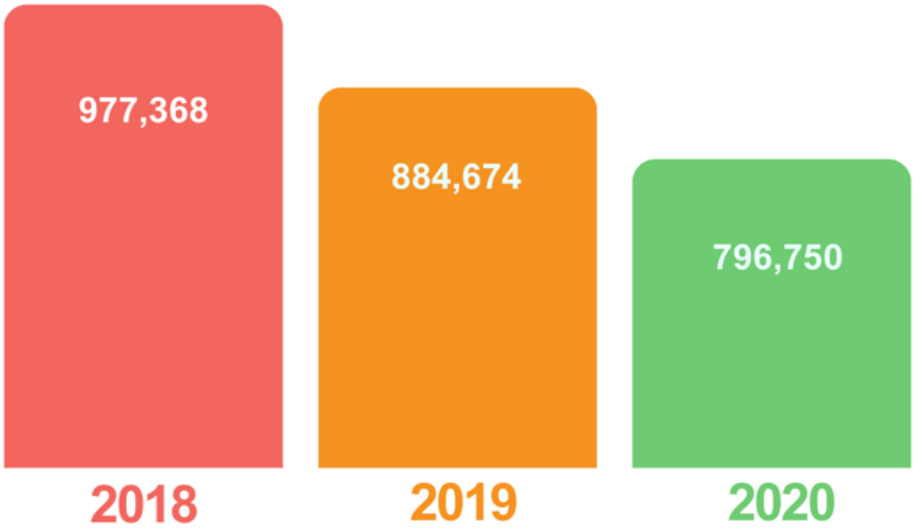


THREE MAIN REASONS

- Animals may be used in studies of **diseases or conditions** that by their nature can cause severe suffering
- A **combination** or series of less severe factors can combine to lead to an increase in overall suffering
- Where animals **die unexpectedly**, or where the **death** of an animal is used an **'endpoint'** of the study

SEVERE PROCEDURES IN THE EU AND NORWAY

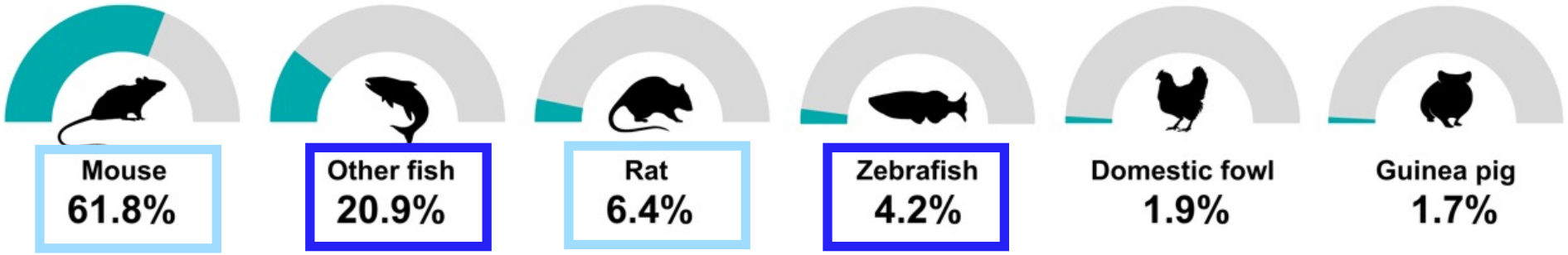
ADJUSTED TO OMIT UK FIGURES



	2018	2019	2020
Non-recovery	6% (521,765)	6% (494,368)	**4% (330,392)**
Mild [up to and including]	48% (4,311,312)	50% (4,380,747)	**49% (3,921,024)**
Moderate	35% (3,169,559)	34% (2,955,923)	**37% (3,006,764)**
Severe	11% (976,445)	10% (884,186)	**10% (796,750)**
Total	**100% (8,979,081)**	**100% (8,715,224)**	**100% (8,054,930)**

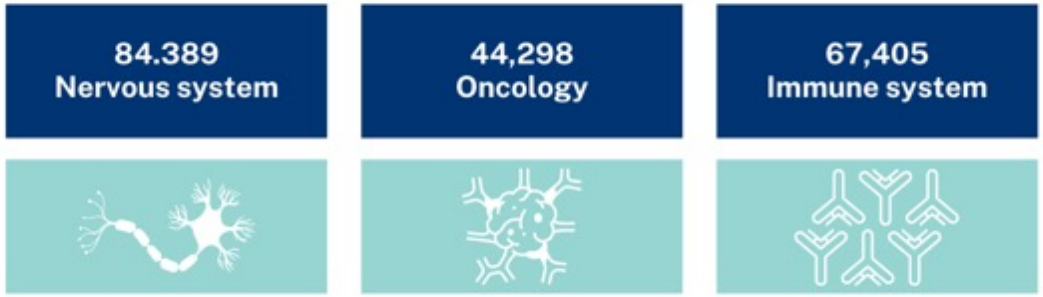
https://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

796,750 animals experienced 'severe' suffering in the EU & Norway in 2020



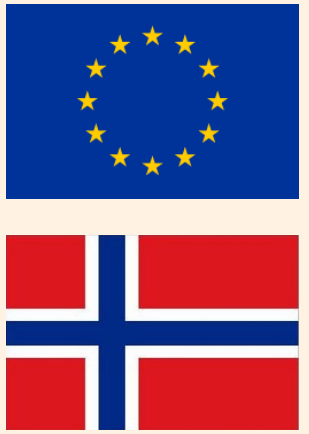
LAVA istockphoto.com/PaulBull

Most 'severe' basic research



Most 'severe' translational research

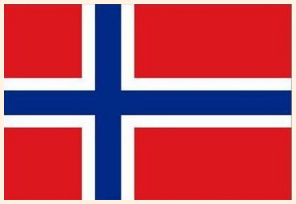
Most 'severe' regulatory use



Data for EU27 and Norway for 2000

Main species involved in ‘severe’ procedures

76,728
total procedures



Atlantic salmon

In 2020, 51,700 Atlantic salmon were used in severe procedures.

Number	Research area
29,925	Animal diseases and disorders
10,053	Quality control
5,963	Immune system

Rainbow trout

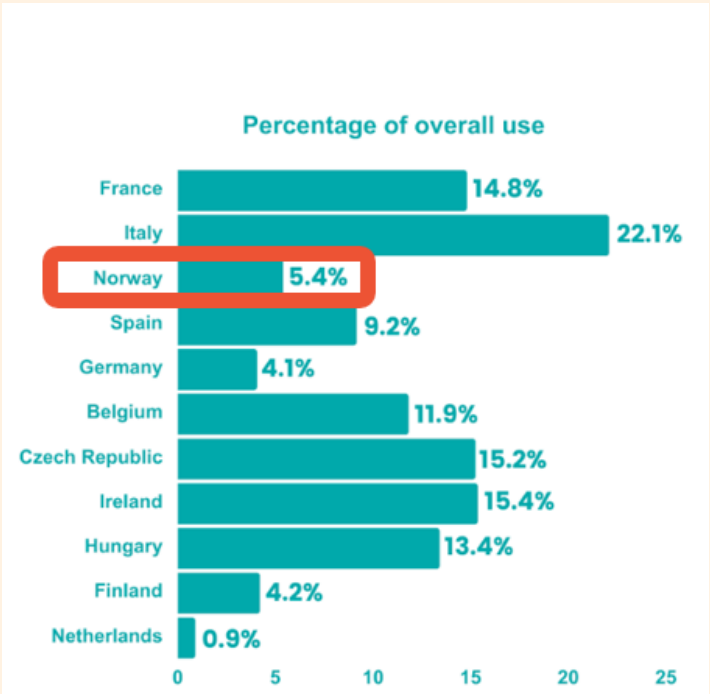
In 2020, 6,877 rainbow trout were used in severe procedures.

Number	Research area
5,011	Animal diseases and disorders
1,370	Quality control
496	Immune system

Zebrafish

In 2020, 14,875 zebrafish were used in severe procedures.

Number	Research area
12,827	Nervous system
2000	Human infectious disorders
48	Gastrointestinal system including liver

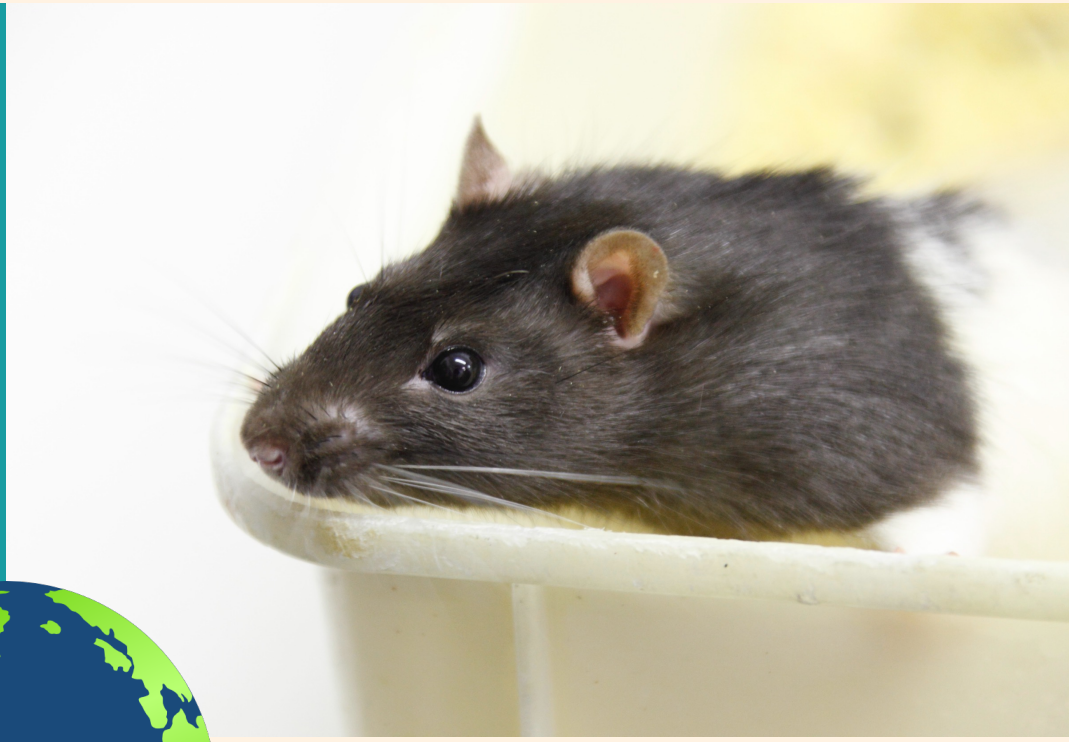


https://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

10M *

animals across the world experience severe suffering each year

*estimate



All laboratory animal suffering is a concern, but reducing and avoiding 'severe' suffering should be a top priority

- ✓ **Ethical** and animal welfare benefits
- ✓ **Legal** requirements to minimise suffering
- ✓ **Societal** concerns about harms to animals
- ✓ **Scientific** benefits - better welfare means better science

Everyone has a role to play

- Scientists
- Animal technologists
- Persons with responsibilities under Articles 24 and 25 (attending veterinarians, staff responsible for ensuring information access, training and competency etc)
- Animal Welfare Bodies
- Competent authorities
- National Committees
- National 3Rs centres
- NGOs

FOCUS ON SEVERE SUFFERING

Our initiative

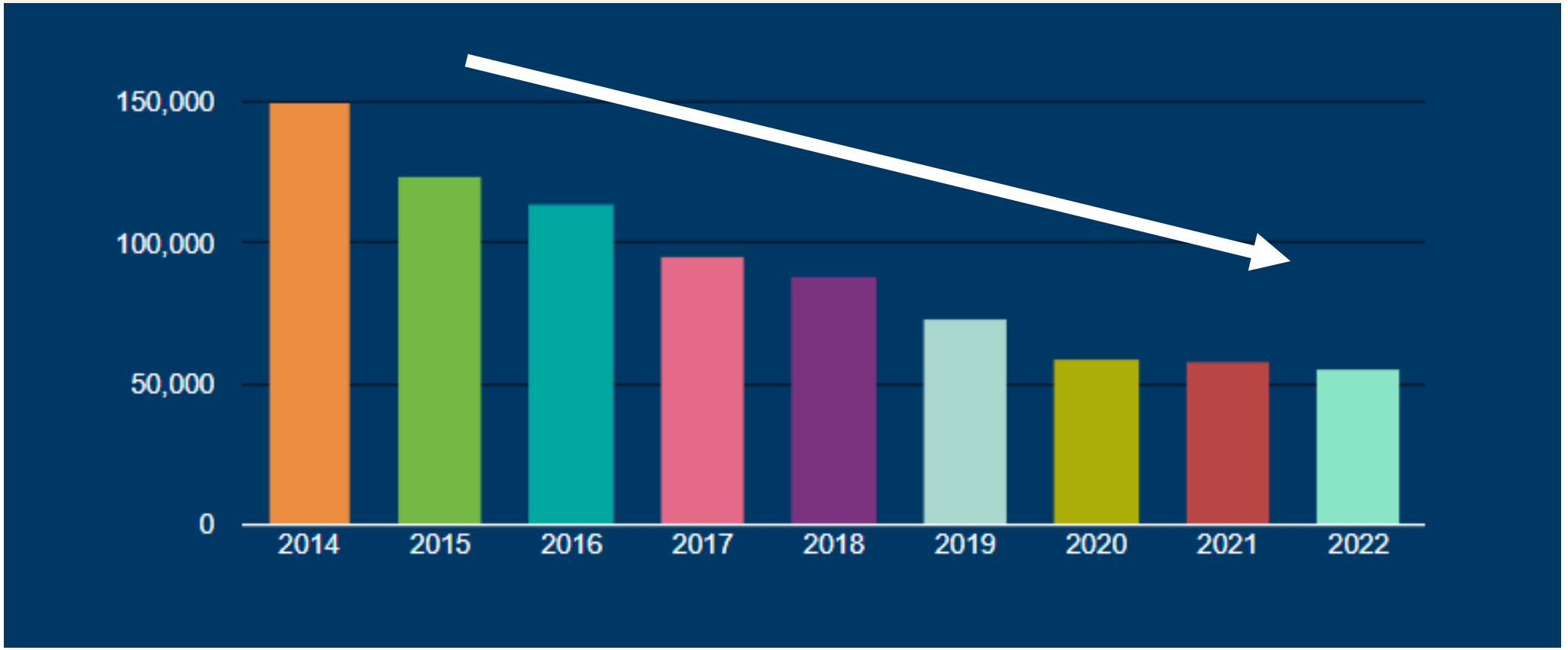
Since 2012, the RSPCA has been **working collaboratively** with the scientific community in the UK, EU and internationally, to initiate and promote a range of activities aimed at identifying and promoting **practical steps** which will help people to **reduce** or, ideally, **avoid** 'severe' suffering.

FOCUS ON SEVERE SUFFERING

Key objectives

- Refine models to bring them to a **lower severity** where possible
  - applies to other levels of suffering too

- Ensure there has been **robust discussion** and a rationale that **justifies** the need for 'severe' limits, where they still exist

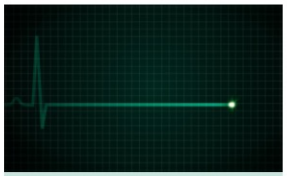


61% reduction in experimental procedures causing severe suffering in the **UK** since 2014

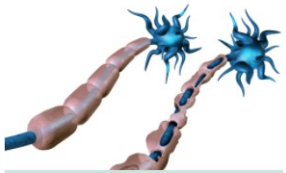
focusonseveresuffering.co.uk

Website

focusonseveresuffering.co.uk

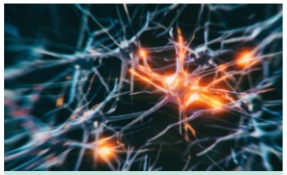


Avoiding mortality
Hawkins et al. (2019)
Avoiding mortality in animal research and testing.
ISBN: 978-0-901098-17-7A

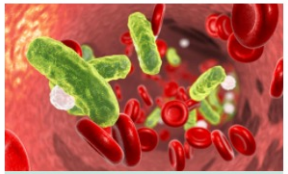


Experimental Autoimmune Encephalomyelitis (EAE)
Wolfensohn et al. (2013)
Reducing suffering in experimental autoimmune encephalomyelitis (EAE).
Journal of Pharmacological & Toxicological Methods 67, 169-176

Rheumatoid arthritis
Hawkins et al. (2015)
Applying refinement to the use of mice and rats in rheumatoid arthritis research.
Inflammopharmacology 23, 131-150



Seizures, convulsions and epilepsy
Wolfensohn et al. (2013)
Reducing suffering in animal models and procedures involving seizures, convulsions and epilepsy.
Journal of Pharmacological & Toxicological Methods 67, 9-15



Sepsis
Lilley et al. (2015)
Refinement of animal models of sepsis and septic shock.
Shock 43, 304-316

Spinal cord injury
Lilley et al. (2020)
Refining rodent models of spinal cord injury.
Experimental Neurology 328, 113273

PERCEIVED OR ACTUAL REGULATORY REQUIREMENTS

The OECD recognises that '*with increasing knowledge and experience, investigators in animal research will be able to identify more specific, early humane endpoints in the form of clinical signs for impending death or severe pain and distress. This would permit international harmonisation of these humane endpoints*'. **Researchers and establishments should challenge regulatory bodies to accept evidence that death can be predicted and to accept data from tests in which humane endpoints have been defined and implemented.**

PREDICTING ANIMAL DEATHS

There is always scope to better predict mortality, and to challenge any assumptions that a proportion of deaths is 'inevitable' or that endpoints cannot be refined. Perceptions about the ability to predict death often change; for example, telemetered body temperature using microchips has improved the ability to define humane endpoints and avoid severe suffering in a number of fields. It is good practice to keep up with the literature and to identify any new approaches that may be suitable for trialling at the facility.

ACTIONS FOR THE AWERB (OR EQUIVALENT BODY)

The AWERB, AWB, IACUC or AEC should ask for explanations of humane endpoints, including how they are defined, refined and implemented. They can also ask to see, and discuss, animal 'fate' data, including a breakdown of animals humanely killed as part of the experiment, found dead, killed because they are close to a humane endpoint, or because they are not needed (surplus). This will allow the institution to monitor wastage, identify where endpoints may need to be revised and see where additional welfare monitoring should be applied.

For further information about humane endpoints, see www.humane-endpoints.info and www.nc3rs.org.uk/humane-endpoints.

SCAN ME

RSPCA ANIMALS IN SCIENCE

lasa Laboratory Animal Science Association

LAVA Laboratory Animals Veterinary Association

iat

EXAMPLES OF POTENTIALLY 'SEVERE' PROCEDURES

Batch potency testing of vaccines (where control animals experience 'severe' disease symptoms) **and other biologics** e.g. botulinum toxin, for regulatory purposes

Studies involving infectious disease models, including the development of vaccines or other treatments, where animals may experience 'severe' disease symptoms

Various tests involved in regulatory toxicology, including ecotoxicology, especially where animals may become moribund or die

Monoclonal antibody production using the mouse ascites method – NB this method has not been used in the UK since 2012 but is still used elsewhere in the world

Some cancer models – involving large tumours, resection, bone metastasis, brain tumours, pancreatic tumours

Some heart disease models – myocardial infarction induction; monocrotaline (MCT)-induced pulmonary arterial hypertension; transverse aortic constriction/banding

Multi-organ failure models

Demyelination of the central nervous system (CNS)

Models of motor neurone disease (MND)

Spinal cord injury models

Neuroscience studies using non-human primates, involving the cumulative effects of numerous surgeries, regular and long periods of restraint, and/or fluid or food control

Tamoxifen as an inducer of gene function

Irradiation with reconstitution of bone marrow

Cerebral malaria in rodents

Pancreatitis models

focusonseveresuffering.co.uk/severe-procedures

Expert Working Groups

- Seizures, convulsions and epilepsy
- Experimental autoimmune encephalomyelitis (EAE)
- Rheumatoid arthritis
- Sepsis
- Spinal cord injury
- Bone marrow ablation and reconstitution*

*Being completed 2024

- Avoiding mortality

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Events

- Brussels, Belgium - 2016
- Berlin, Germany - 2017
- Stevenage, UK - 2019
- Athens, Greece - 2019
- Manchester, UK - 2022
- Stockholm, Sweden - 2022
- Leiden, Netherlands - 2023
- Weybridge, UK - 2023

100s of participants: regulators, scientists, veterinarians, animal technologists and care staff, members of Animal Welfare Bodies and National Committees etc.

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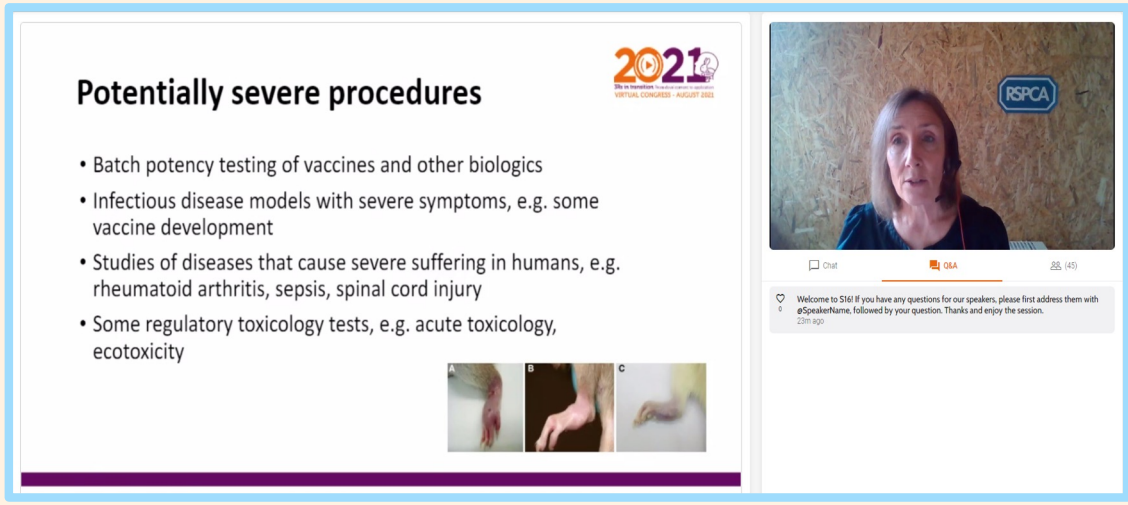
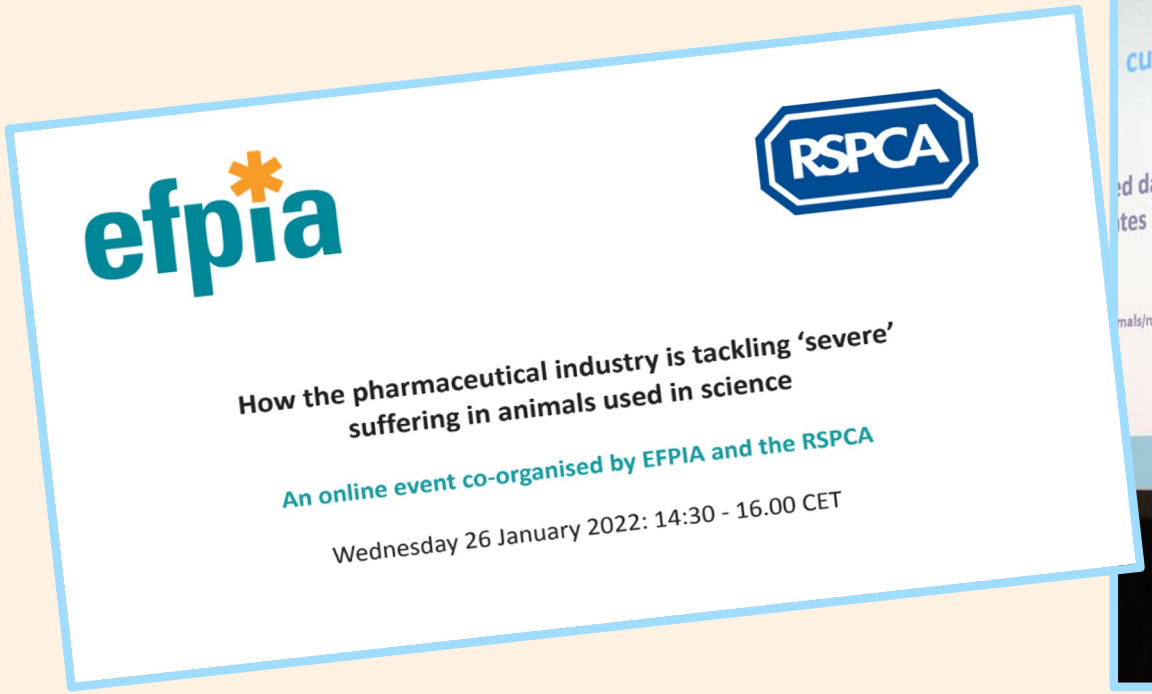
Nuno Henrique Franco @Nuno_H_Franco · Dec 10, 2021
So happy to have Penny Hawkins @RSPCA_LabAnimal delivering a talk at the SPCAL Scientific Day on the challenging goal of ending severe suffering, in #animalresearch.
PENNY HAWKINS
ANIMALS IN SCIENCE DEPARTMENT
@RSPCA_LabAnimal


Potentially severe procedures
• Batch potency testing of vaccines and other biologics
• Infectious disease models with severe symptoms, e.g. some vaccine development
• Studies of diseases that cause severe suffering in humans, e.g. rheumatoid arthritis, sepsis, spinal cord injury
• Some regulatory toxicology tests, e.g. acute toxicology, ecotoxicity
RSPCA
Chat
Q&A
Welcome to S16! If you have any questions for our speakers, please first address them with @SpeakerName, followed by your question. Thanks and enjoy the session.


efpia
RSPCA
How the pharmaceutical industry is tackling 'severe' suffering in animals used in science
An online event co-organised by EFPIA and the RSPCA
Wednesday 26 January 2022: 14:30 - 16.00 CET


current situation
felasa
clasa
14th felasa congress 2019
Prague, Czech Republic

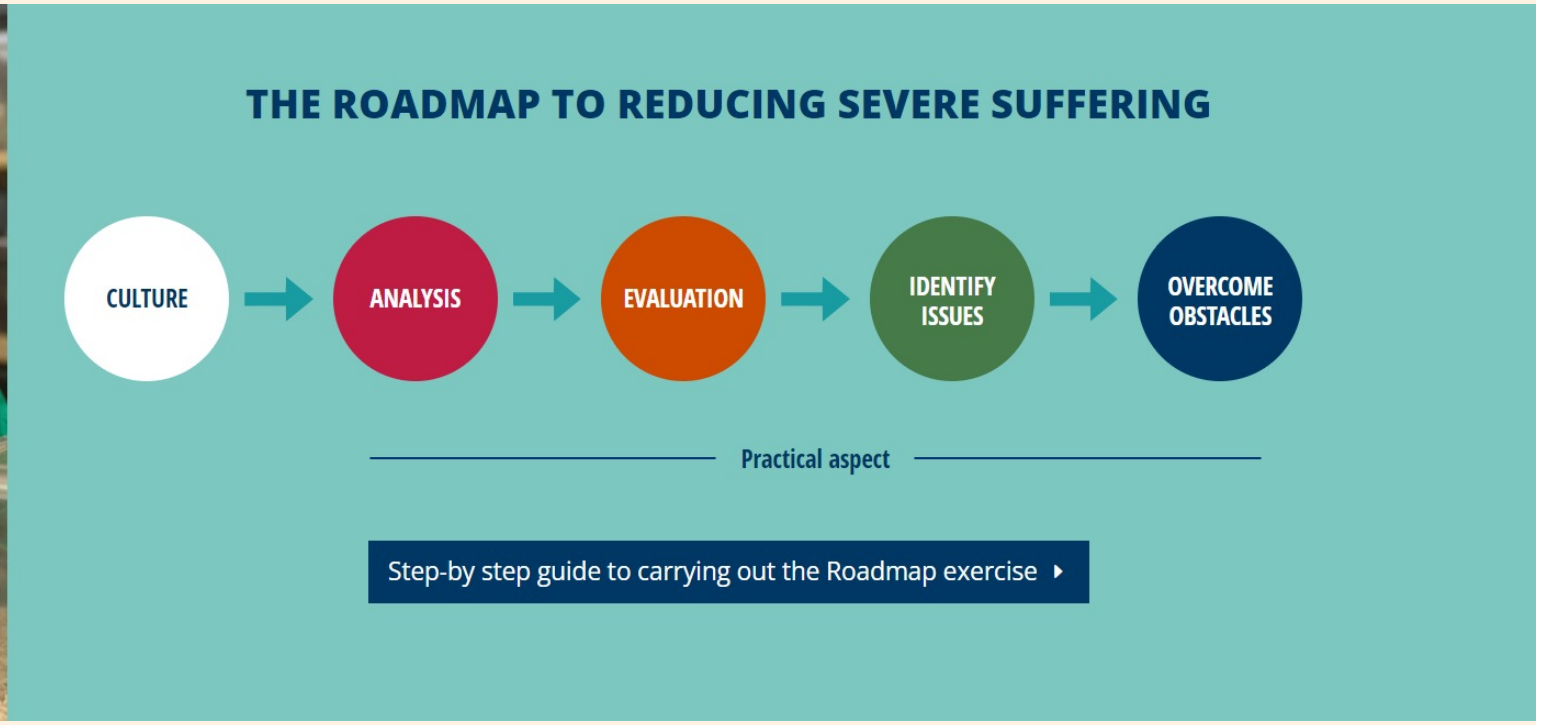
Establishments should adopt a commitment to address severe suffering

- Agreement as a **priority area** for attention and action
- Institutional **strategy** and responsibilities
- Setting of **clear objectives**

Consider as part of the 'Culture of Care'

FOCUS ON SEVERE SUFFERING

The Roadmap



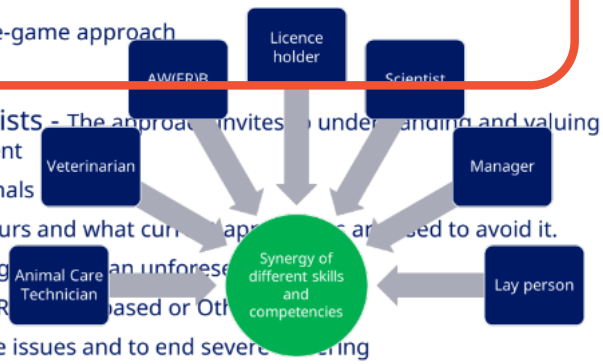
focusonseveresuffering.co.uk/roadmap

istockphoto.com/fotografixx

Novo Nordisk®

Why the roadmap works

- The RSPCA approach facilitates a **cooperative response** from licence holders and scientists, because:
  - Objective, data driven, systematic and no blame-game approach
- Dialogue with licence holders and scientists - The approach invites to understanding and valuing the roles of different people within an establishment
  - Data check: Is the scoring as 'severe' for all animals
  - Evaluation: Looking at why severe suffering occurs and what current practices are used to avoid it.
  - Is the harm prospective or does severe suffering an unforese
  - Define obstacles: Are the obstacles, - Scientific, R based or Oth
  - Overcome obstacles: Set out a plan to overcome issues and to end severe ering
  - Action plan
  - Evaluate



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Applying the roadmap at Novo Nordisk

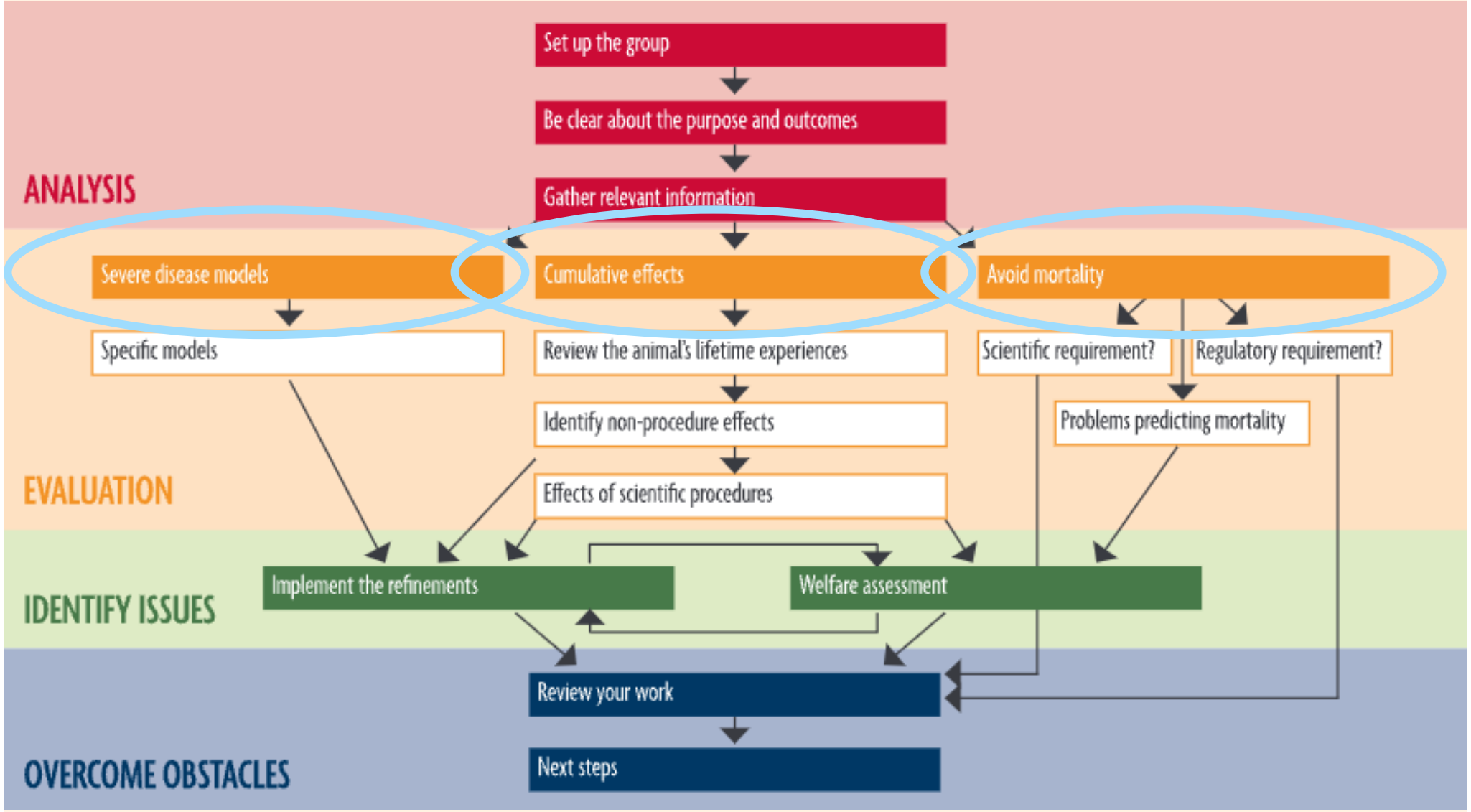
Prospectively

- Identify as many sources of harm as possible
  - Related to the (disease) model
  - Related to procedures
  - Related to housing, husbandry and care
- Agree on Humane Endpoints
  - General
  - Model specific
- Agree on procedures for welfare assessment

Retrospectively

- Assess actual severity
  - Identify when and why severe harm was experienced by the animal
  - Identify if avoidable harm unintentionally occurred
  - Evaluate the effectiveness of the implemented Humane Endpoints
  - Evaluate the effectiveness of how animal welfare was assessed
- Agree on revisions
- Agree on how learnings are captured and communicated to all relevant people

From presentation by Thomas Bertelsen (tsbt@novonordisk.com) at 'Refining severe disease models and procedures' international meeting - August 2022


ANALYSIS
Set up the group
Be clear about the purpose and outcomes
Gather relevant information
Severe disease models
Cumulative effects
Avoid mortality
Specific models
Review the animal's lifetime experiences
Scientific requirement?
Regulatory requirement?
Identify non-procedure effects
Problems predicting mortality
EVALUATION
Effects of scientific procedures
IDENTIFY ISSUES
Implement the refinements
Welfare assessment
Review your work
OVERCOME OBSTACLES
Next steps

1. Refining specific models and procedures

What does this study involve doing to the animals?	What will the animals experience? How much suffering might it cause? What might make it worse?	How will suffering be reduced to a minimum?	
	Adverse effects and indicators of these	Methodology and interventions	Humane endpoints
Administration of rheumatoid arthritis inducer	Capture and restraint – distress. Aggression, vocalisation, unwilling to be caught. Administration i.d. or s.c. – pain. Flinching, vocalisation, aggression. Pain or ulceration around injection site. Attention to site, reduction in nest quality, body weight/food intake reduction,	Competent, empathetic capture (e.g. not by tail) and handling, habituate to handling and restraint. Use gaseous anaesthesia for i.d.; inject into rump, not tail base (if tail base is painful, restraint by the tail will hurt). Minimise volumes and doses, use multiple sites if large volumes. Ensure injectate formulated to minimise adverse effects Inject into rump (less risk of ulceration); never inject into the foot; if attention paid to site apply topical anaesthesia and review	Humane endpoints with respect to administration of inducer in general: - Ulceration that is painful, shows no signs of healing or becomes infected. - If an ulcer reaches >5 mm, the vet or senior animal technologist should be informed and consulted about treatment. Animal should be humanely killed if no signs of healing within 3 days.

In practice - a case study

Mouse models of rheumatoid arthritis

A pharmaceutical company introduced the G6PI, CIA and CAIA mouse models of rheumatoid arthritis, which have the potential to cause severe suffering. This prompted a re-evaluation of the company's welfare scoring sheets and husbandry refinement protocols, with the aim of reducing suffering. The scientists and animal technologists worked together to tailor and refine monitoring systems, husbandry and procedures.

Mice used in G6PI and CAIA studies were very carefully monitored by scientists and animal technologists, to identify indicators of adverse effects and collate data on weight loss and disease scores. The observations were specific to each model, although standardised terminology was created to describe indicators. As a result, the following refinements were adopted:

- the humane endpoint for weight loss was reduced from 25% to 20%, and another endpoint added of a 15% weight loss that persisted for 5 days
- the tailored indicators (such as soft stools for CAIA) enabled study length to be reduced; e.g. the CIA studies were reduced from 30 days to 20
- disease scores were revised to include a range of indicators, as opposed to paw volume only, capturing severity more effectively and enabling endpoints to be further refined
- additional refuges are provided for DBA/1 male mice, eliminating aggression
- non-tangling nesting material is provided
- when mobility is restricted, longer sipper nozzles are fitted and food given in dishes on the cage floor
- the Mouse Grimace Scale is used to help assess acute pain

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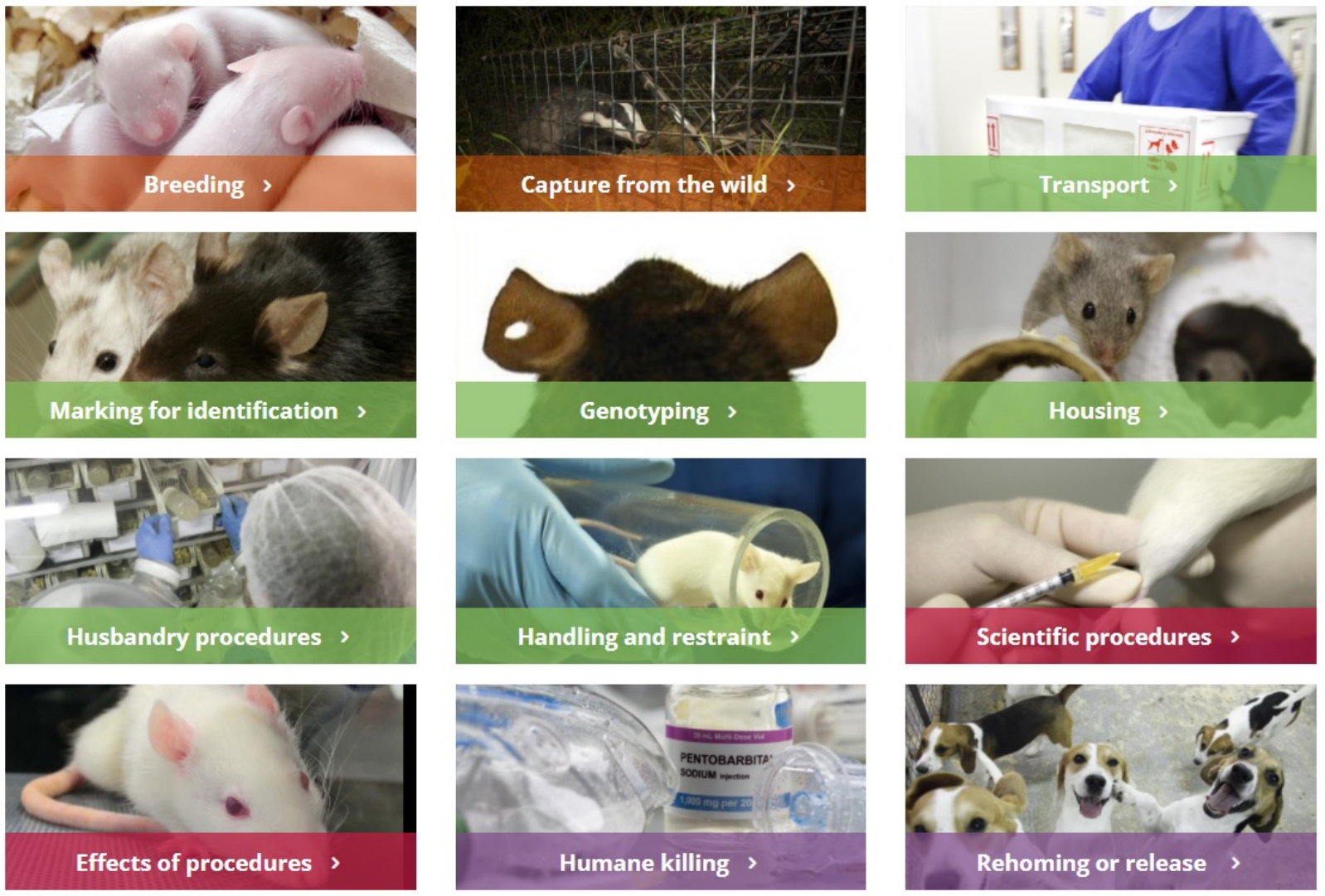
2. Thinking about the lifetime experience of animals

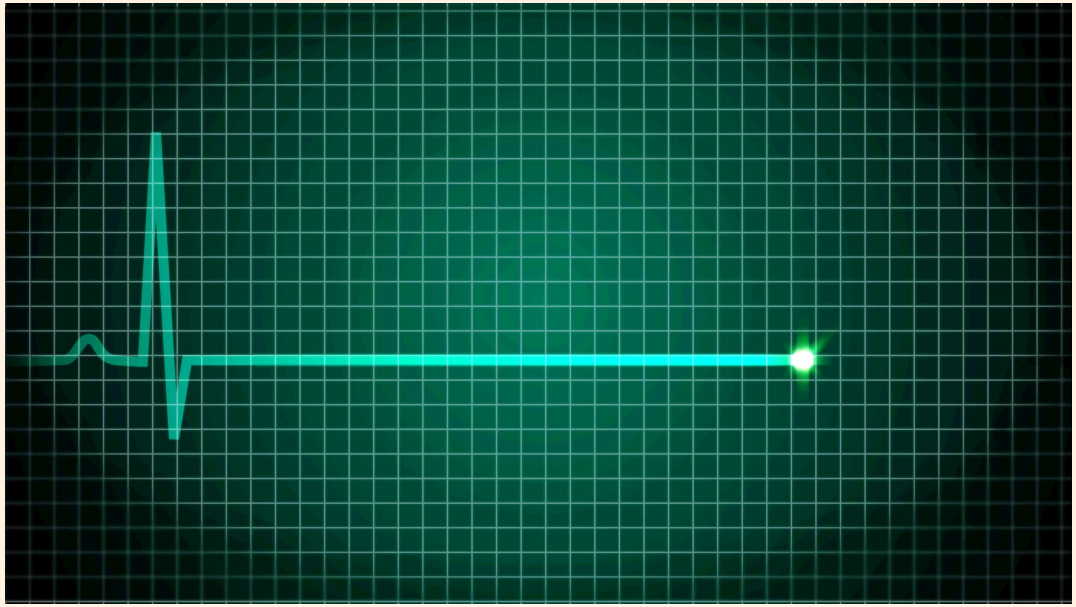


focusonseveresuffering.co.uk/lifetime-experiences

Factor	Experience of the animal	Welfare issues	Ways of mitigating these
Sourcing	Mice are bred in house. Supply and demand are carefully matched and animals provided with litter, nest boxes and nesting material. Cages are cleaned weekly.	Distress due to separation of dam and pups at weaning.	Ensure removal from dam is appropriately timed and keep litters together wherever possible. Review frequency of cage change (e.g. fortnightly?) to ensure cage is sufficiently clean but with minimal disturbance.
Transport	Once, between rooms within the same building before procedures begin.	Stress and anxiety due to movement.	Move in home cages, minimise distance, think about timing, ensure sufficient time to recover before any other interventions or procedures.
Marking for identification	Animals are identified using microchips, which involves capture and restraint for insertion.	Distress due to restraint, short term pain of chip insertion.	Trial less aversive capture techniques (see below). Research pros and cons of sedating or anaesthetising mice. Ensure adequate checks in case of longer term discomfort.

3. Avoiding mortality

- Is mortality difficult to predict in the strain or model?
- Is there a scientific requirement for death as an endpoint?
- Is there a regulatory requirement for mortality?



istockphoto.com/sweetandsour

Improving ability to predict death

Review records and refine welfare assessment protocols

- What signs looking for?
- How often looking?
  (frequency of monitoring)
- When looking?
  (e.g. after specific interventions; day vs night)
- How looking?
  (e.g. use of latest technology)

istockphoto.com/fotografixx

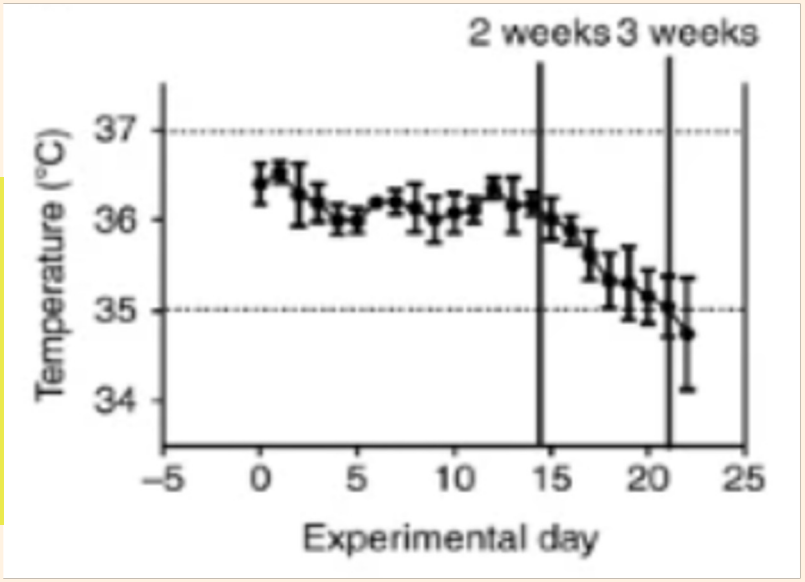
Improving ability to predict death

Frequently occurring indicators

- Body temperature
- Body weight
- Difficulty in rising or moving

"all mice that had a mean decrease in body temperature of 0.7 °C or greater had lymph nodes heavier than 0.5 g (100% sensitivity)"

Hunter et al 2014



doi.org/10.1038/bjc.2013.818

Humane endpoints in regulatory toxicology

***'with increasing knowledge and experience, investigators … will be able to identify more specific, early humane endpoints in the form of clinical signs for impending death or severe pain and distress**. This would permit international harmonisation of these humane endpoints'*

OECD

The Organisation for Economic Cooperation and Development (OECD) is the regulatory body for chemical testing. Many tests, such as OECD tests 203 and 210, describe death as an endpoint when determining LC50 or LD50, but also recommend that labs familiarise themselves with sub-lethal clinical signs to avoid mortality. According to the guidelines, if fish are showing signs of considerable suffering (very severe and death can be reliably predicted) and considered moribund, animals should be anaesthetised and euthanised. However, the lack of clear guidance on what constitutes a sub-lethal clinical sign, as well as standardised recording methods, leads to variations in data recording within and between companies, which may not meet regulatory standards. Consequently, while humane endpoints are acknowledged, they are not consistently applied due to this lack of guidance.

RSPCA

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Humane Endpoints in Regulatory Toxicology in Fishes

FOCUS ON SEVERE SUFFERING

Surrey, 2023

Summary Report

In November 2023, the RSPCA organised an in-person meeting at the Animal and Plant Health Agency in Weybridge, focusing on humane endpoints in regulatory toxicology studies using fishes. The aim was to identify and share practical refinements to reduce and avoid 'severe' suffering. The discussion focused on the need to standardise practices, ensuring that refinements can be widely adopted. This report will summarise the presentations and discussions, and suggest action points for both immediate and future steps.

Chloe Stevens from the RSPCA opened the meeting by explaining that the topic was chosen on the basis of a report commissioned by the RSPCA and conducted by Alyson Leyshon, of LeyshonBanks Consulting. The comprehensive report investigated severe suffering within regulatory testing and highlighted the importance of standardising good practices for staff training and the development of humane endpoints.

The first session of the day was devoted to **case studies - identifying indicators in acute toxicology**.

Nic Bury, from the University of Southampton, introduced the challenges posed by novel chemicals to wildlife and the environment. There are an estimated 350,000 chemicals on the global market with little understanding of their impact at a cellular level. The ethical issues with using fish in toxicity tests, plus the enormous number of compounds to be tested, means that an alternative approach is required to risk-assess these chemicals. Nic is working on an innovative approach to identify how chemicals interact with stress receptor proteins using **bioinformatic tools** to predict differences in chemical docking between proteins from different fish species, and with predictions confirmed using functional assays. Nic hopes to expand to include more proteins and chemicals, thus circumventing the need to perform tests with fish.

In the next presentation, Karen Thorpe from Fera provided valuable insights into animal welfare challenges associated with **OECD tests 203 and 210**[1], and presented ways of

Recommendation	Application	Challenges
Define and implement a standardised approach to identify sub-lethal clinical signs and apply humane endpoints.	Develop frameworks for monitoring that include reference guidelines, checklists, and record sheets. Include information on normal behaviour, species-specific norms, and indicators of clinical signs. [E] Develop and adopt a standardised language for describing fish behaviours and clinical signs. This will help to achieve consistent interpretation between individuals and organisations. [E] Promote collaboration and information/process sharing around applying humane endpoints between organisations. Create a platform for sharing best practices, research findings, and experiences. [E] [S] [M] Treat a standardisation framework as a living document that evolves over time. Regularly update guidelines to align with the latest advances in fish behaviour and welfare science. [B] [E] [S] Involve regulatory bodies in developing and endorsing standardised practices. Ensure that regulatory standards align with the agreed framework. [E] [R] [B] Establish feedback mechanisms to assess the effectiveness of the standardised approach. Encourage internal audits, reviews, and continuous improvement based on feedback from the scientific community. [R] [S]	A standardised approach is likely to be difficult to develop for a large number of fish species with different characteristics. Individual observer variations in interpreting fish behaviour may hinder standardised assessments. Identifying objective indicators will be a priority. Divergent practices between industries (e.g. company-specific or sector-specific) may impede universal adoption. It may be difficult to achieve widespread acceptance of the standards. Access could be limited to advanced technologies for effective detection of clinical signs. Globally, there are differences in both national regulations and cultural perspectives on fish welfare. These measures will all require resources, leadership, development and management, which may be difficult to access.
Establish standardised approaches to staff training on identifying clinical signs and education in fish behaviour and welfare.	Develop standardised training programmes for personnel involved in fish monitoring. These programmes should focus on species-specific behaviours and indicators of welfare and adverse effects. [T] [S] [E] Establish Continuing Professional Development (CPD)-friendly courses with flexible formats, making education more accessible. [T] [S] Encourage participation in meetings, workshops, and knowledge exchange forums. [M] Emphasise the importance of a Culture of Care within training frameworks. Ensure that training goes beyond technical skills to instil care, empathy, and compassion among staff. [T] [M] [R]	Resources and leadership will be required to establish training standards. Establishments may resist external input into education and training. Staff might resist adopting new methodologies or altering established routines. Variations in staff experience levels may lead to disparities in assessments, which will need to be monitored and addressed if necessary. Access could be limited to advanced technologies for effective training methods. There might be increased time, personnel, and financial requirements for continuing training programs.
Promote collaboration and information-sharing networks within the scientific community to facilitate the generation and dissemination of resources.	Engage stakeholders, including researchers, technicians, and regulatory bodies in discussions on standardisation. Encourage feedback, collaboration, and the sharing of experiences. [M] [S] Establish platforms for collaborative efforts among organisations. [M] [S] Create information-sharing networks. [S]	Some CROs are resistant to revealing what they perceive to be ‘company sensitive information’. These will need to agree that information shared to help train staff, and implement humane endpoints, is not commercially sensitive. Identifying which organisation or group will oversee a collaborative effort could be problematic.

To summarise the conclusions from this meeting, people who are responsible for the care and welfare of fish in regulatory toxicology tests, including implementing humane endpoints, need:

- Adequate resources, including training materials, to aid standardisation of fish monitoring and implementing humane endpoints.
- Access to comprehensive training modules for assessing fish welfare and clinical signs, covering a variety of species and experimental conditions.
- Standardised language for fish behaviours and clinical signs to avoid individual variability.
- Continuing Professional Development in recognising clinical signs in fish toxicology.
- Opportunities to actively participate in meetings, workshops, and webinars to stay updated and enhance expertise.
- Access to a network for sharing information and best practices, facilitating collaboration and mutual support within the research community.
- Technologies to complement their expertise, such as video monitoring hardware and software to enhance clinical sign identification.

Examples of questions to consider

- Why is severe suffering needed? Is there a robust scientific justification?
- Could the protocol be run with a moderate severity limit?
- Is the ‘model’ translatable? How significant are the proposed benefits of the work?
- Is there a regulatory requirement for the experimental design and ‘endpoint’? Can this be challenged?
- Are welfare assessment and monitoring protocols optimised?
- What more could be done to mitigate impacts on animals?

Harm-benefit analysis

"*Where severe suffering is unavoidable, there should be an exceptionally high level of benefit and likelihood of achievement…"*

UK Animals in Science Committee

https://assets.publishing.service.gov.uk/media/5a81edade5274a2e8ab5695b/Review_of_harm_benefit_analysis_in_use_of_animals_18Jan18.pdf

Upcoming

- Next **events**:
  5 November 2024: Newcastle, **UK** (pain)
  20-21 November 2024: Paris, **France**
- Next **Expert Working Group**: Studies involving **respiratory distress**
- Series of **workshops** on **using the Roadmap**: various institutions - 'in person' or online
- Creating an **interactive version** of the '**Roadmap**'
- Development of a 'cumulative severity' **tool**

For more information

Visit our website

focusonseveresuffering.co.uk

Email us

animalsinscience@rspca.org.uk

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