

Support of refinement by the NC3Rs: Experiences and recommendations

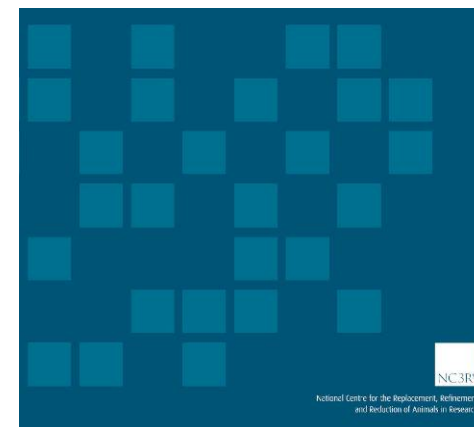
Mark Prescott, PhD

BfR-Forum, 13 December 2011

The NC3Rs

- Independent, scientific organisation
- Established by UK Government in 2004
- Use 3Rs as a framework to support science, innovation and animal welfare
- Work with academia, industry, research funders and regulators – broad remit
- Budget ~ £5.5 million per annum
- 18 staff based in London
- Activities divided between -
 1. Research funding
 2. Centre-led programmes

www.nc3rs.org.uk



House of Lords report 2002

“The development of scientifically valid non-animal systems of research and testing is important, not just to improve animal welfare, but to provide substantial benefits for human health”

“A Centre for the Three Rs should be set up...”



What are we creating?

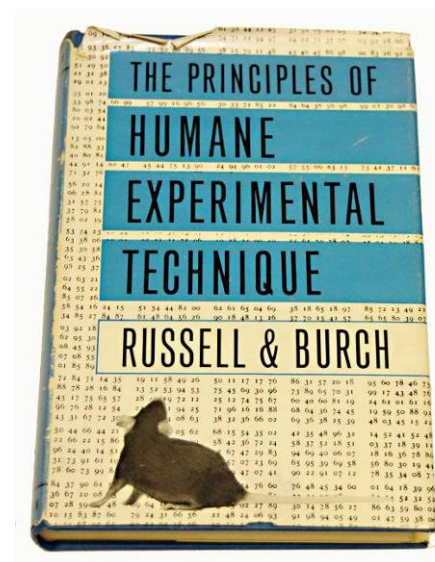
A scientific community where –

- The 3Rs are an integral part of mainstream life sciences
- There is greater willingness to challenge animal models in all sectors, and to implement the 3Rs
- There is increased investment in all 3Rs
- There is a new generation of researchers committed to the 3Rs from the early stages of their careers
- There is sustained and real progress



3Rs definitions

- **Replacement** – methods which avoid the use of animals (or ‘protected’ animals) in areas where they otherwise would have been used
- **Reduction** – methods which minimise the number of animals used (or maximise the information gained from a given number of animals)
- **Refinement** – improvements to scientific procedures and husbandry which minimise pain, suffering, distress or lasting harm and/or improve animal welfare



Importance of refinement

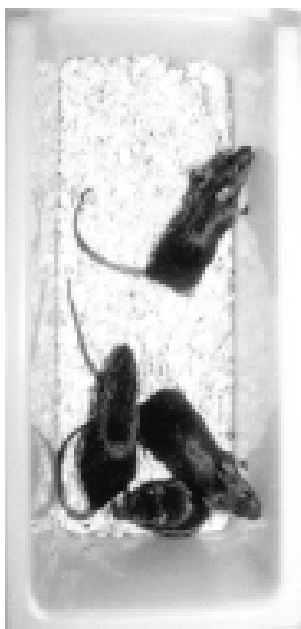
- Replacement is not yet possible in many areas
- Refinement has the greatest and widest potential impact
- Improving welfare can also improve quality of science



Enrichment and the brain

Huntington's disease mouse model

Hockly et al. 2002. Annals Neurol 51



A complex environment slows disease progression,
mimicking the human disease more accurately

A vertical decorative bar on the left side of the slide, composed of four stacked rectangular segments in varying shades of blue and grey.

Challenges

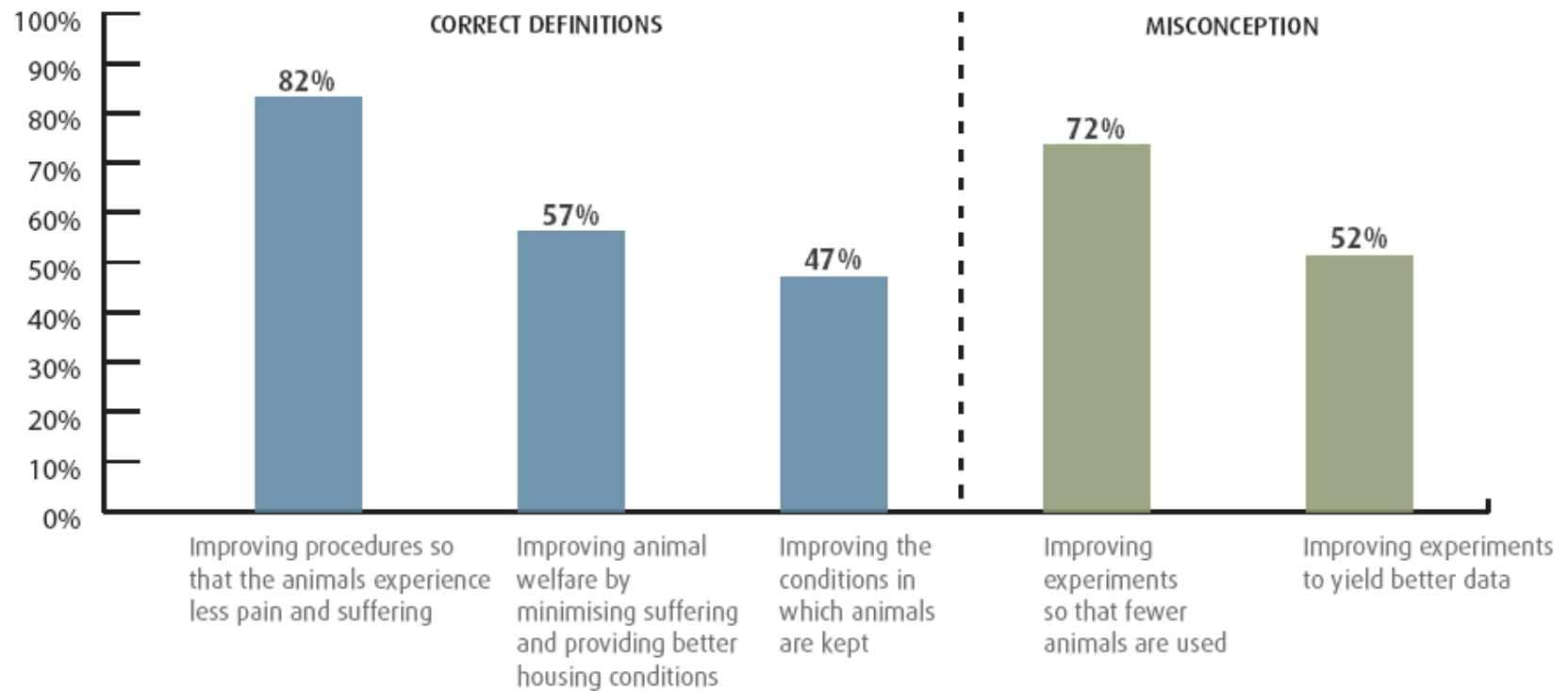
- Poor understanding of the term 'Refinement'
- Conflict with Reduction
- Lack of funding sources for, and credibility of, refinement research
- Need for new and improved refinement techniques, including tools for objective welfare assessment
- Poor awareness of existing refinement techniques
- Under-reporting of refinement information
- Lip service; lack of resources, institutional support and responsibility
- Regulatory conservatism

Which of the following definitions fits your understanding of REFINEMENT?

(Tick all that apply)



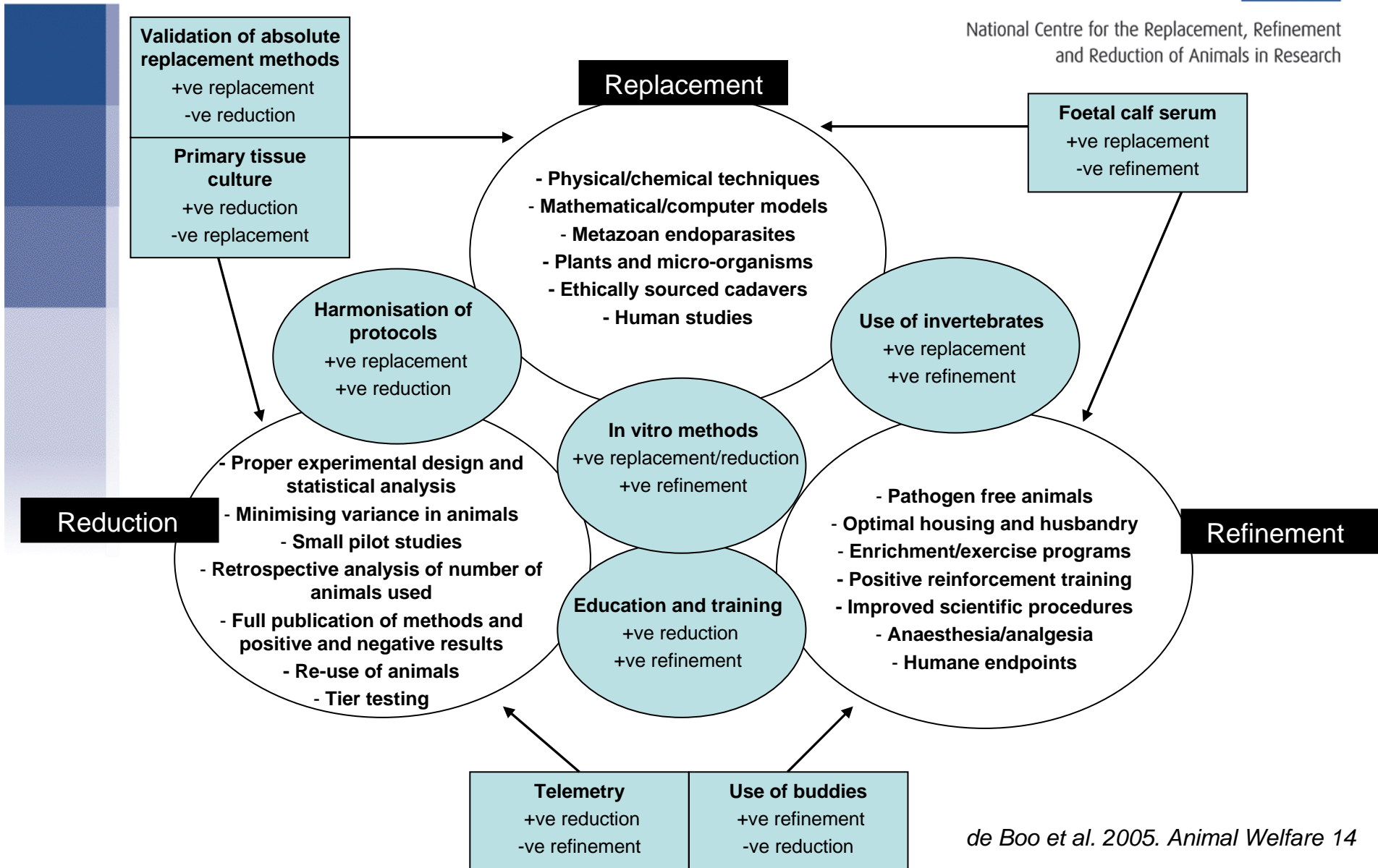
National Centre for the Replacement, Refinement and Reduction of Animals in Research



NC3Rs survey 2007, conducted by People, Science & Policy

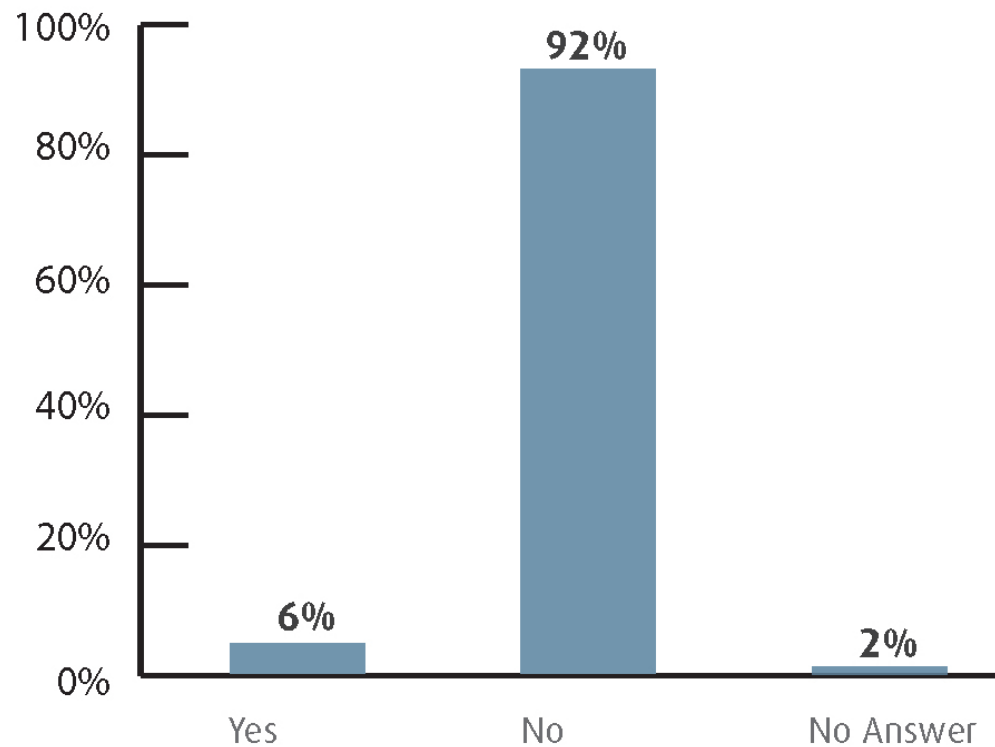
Base: All scientists (1,529)

Interplay between the 'R's



Have you ever applied for funding to undertake 3Rs research?

Base: All scientists (1,529)



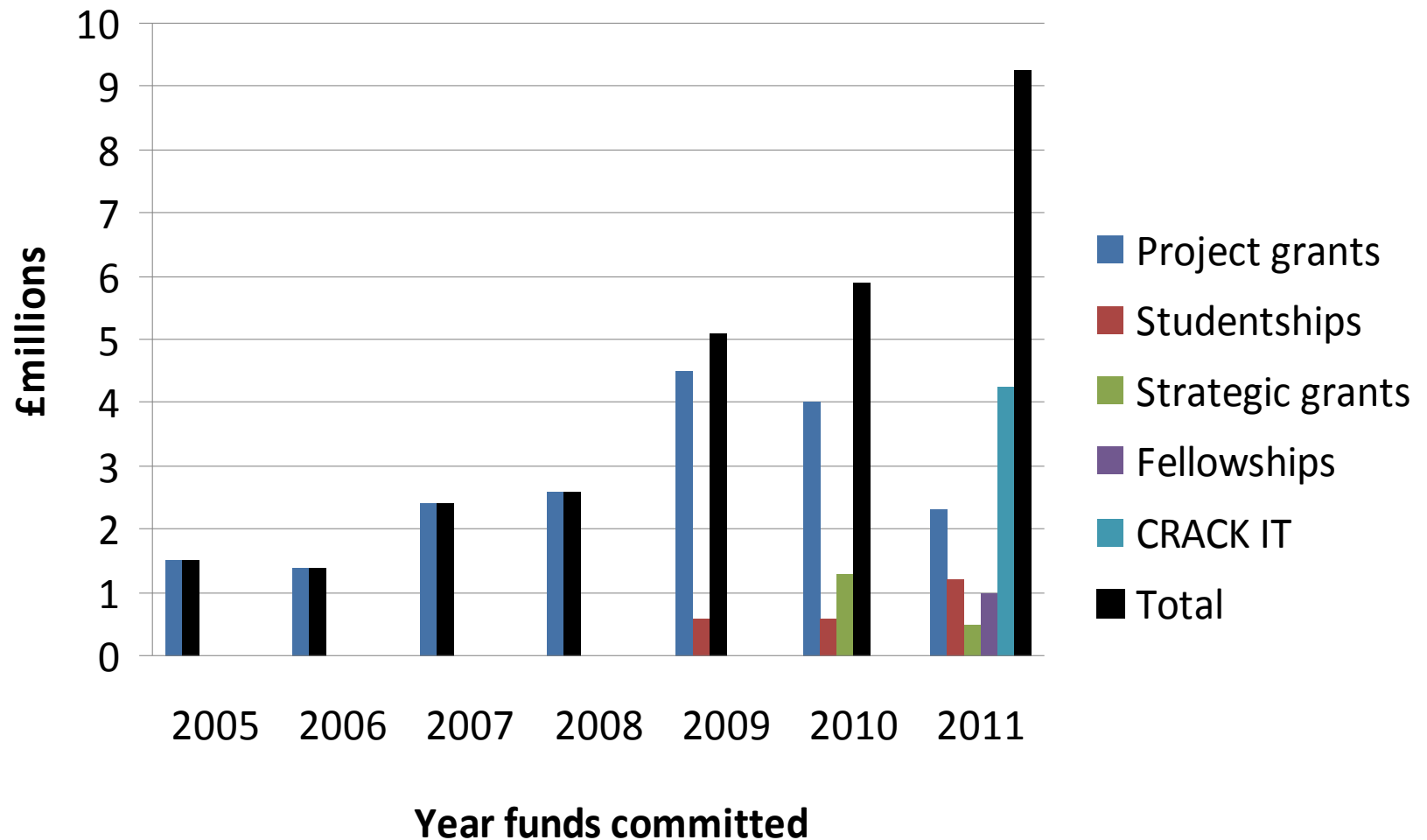
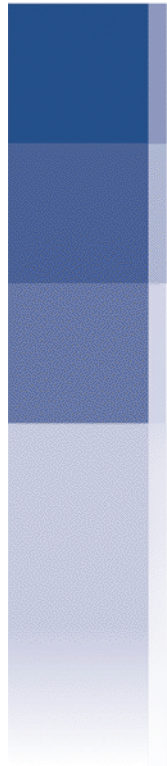
Research funding

- Motivate..... money talks!
- High quality..... robust peer review and assessment; same quality threshold as MRC; experienced panel members
- Capacity building..... opportunities for scientists at all stages of their careers
- Flexibility..... combination of responsive mode (best ideas, best scientists) with strategic priorities (influence research directions)
- Dissemination..... key to achieving larger impact



Funding schemes

– evolution and investment



Strategic calls and awards

Strategic calls (project grants)

2007 – [Refining procedures of substantial severity](#) – 3/11

– Replacement using tissue engineering (with BBSRC) – 4/11

2008 – [Refining rodent husbandry, care and procedures](#) – 2/10

– Fish and the 3Rs – 3/10

2009 – Replacement using invertebrate models (with BBSRC) – 2/13

2012 – [Animal welfare measures and assessment](#) (with BBSRC)

Strategic awards

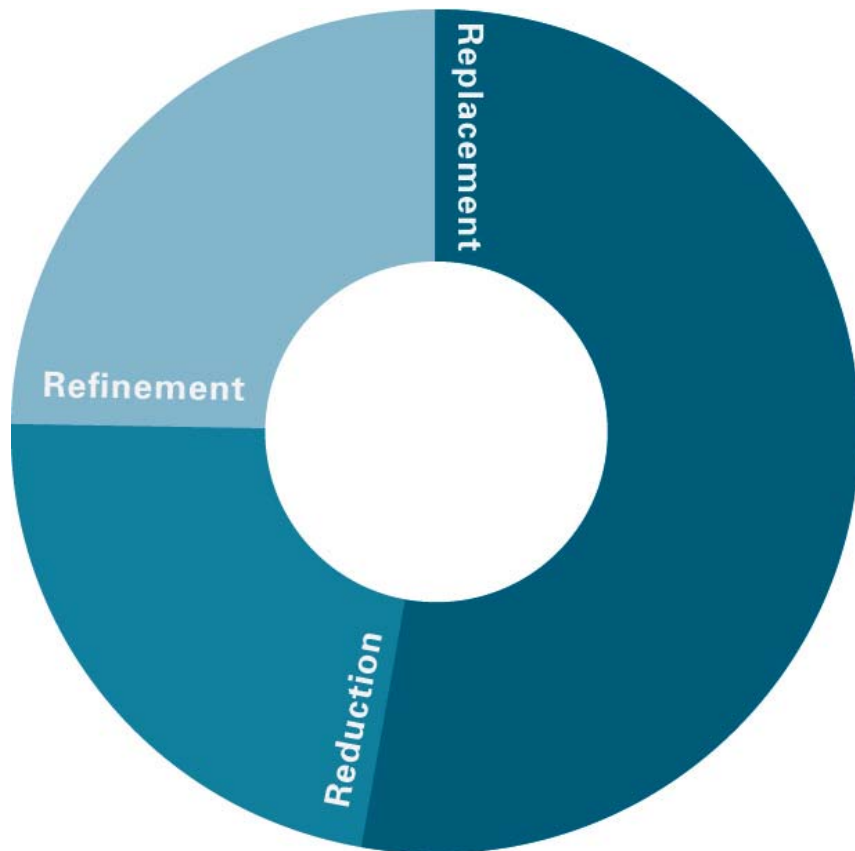
2010 – [Euthanasia of laboratory rodents](#) – 1

– 3Rs in asthma research – 2

2011 – Human carcinogenicity based assays



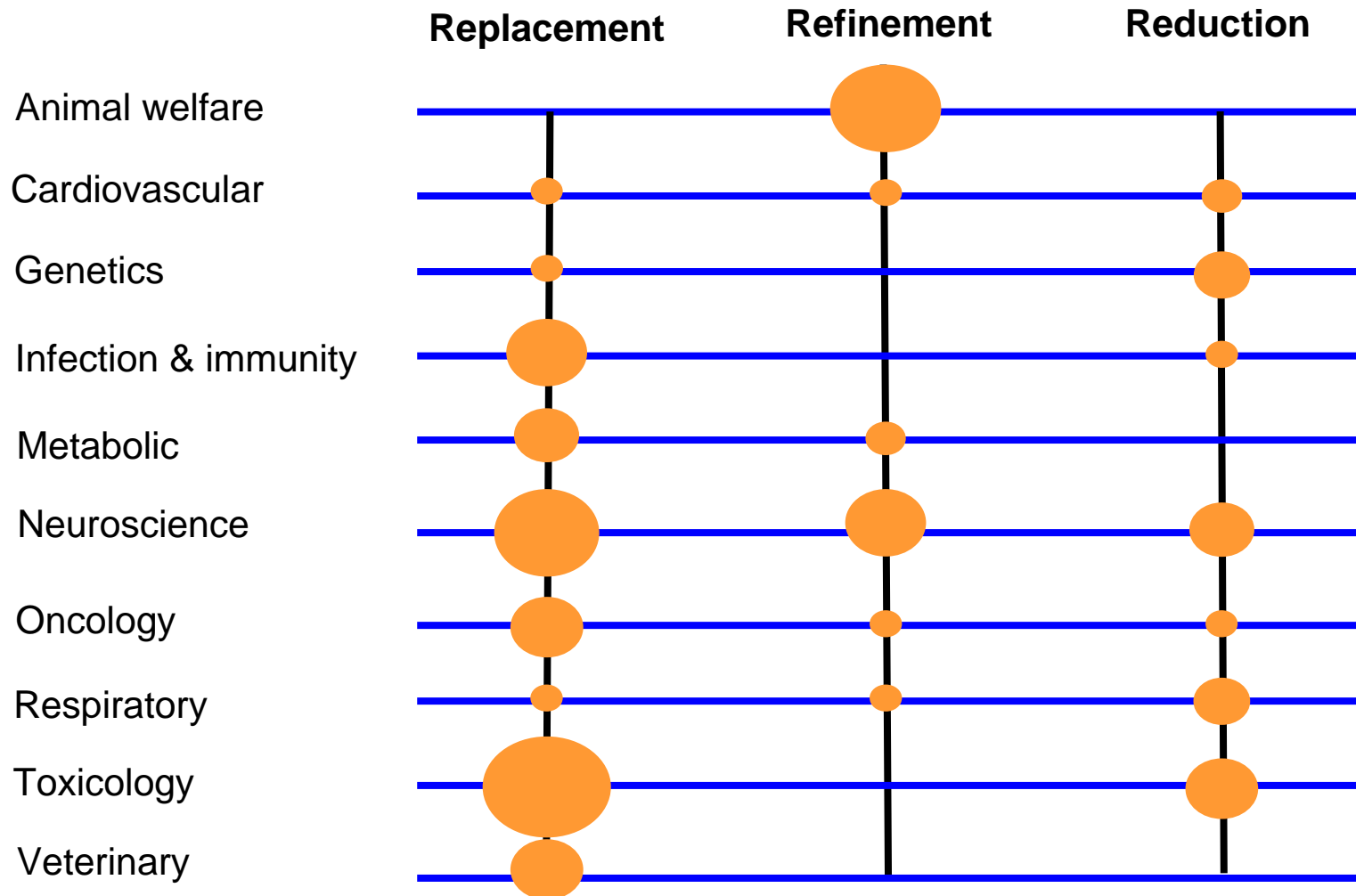
Analysis by 'R'



www.nc3rs.org.uk/researchportfolio



Analysis by discipline



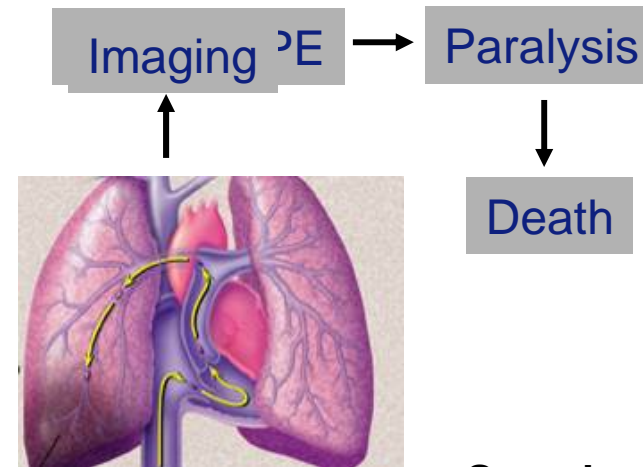
2006 grant: Dr Michael Emerson, Imperial College London



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

Refinement of a mouse model of pulmonary embolism

- Inflicts severe pain and suffering
- Novel method: drugs and gene function in PE
- Better scientific use
 - Models all levels of PE
 - Clinical PE information spectrum
- Major refinement: what is measured?

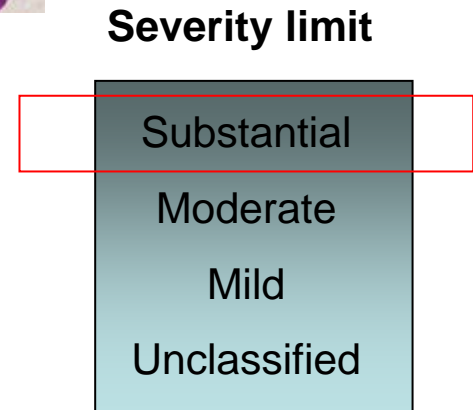
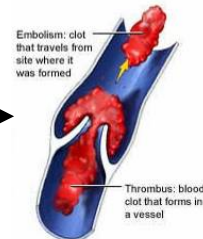


Lower concentrations

Clotting agent



Anaesthetise

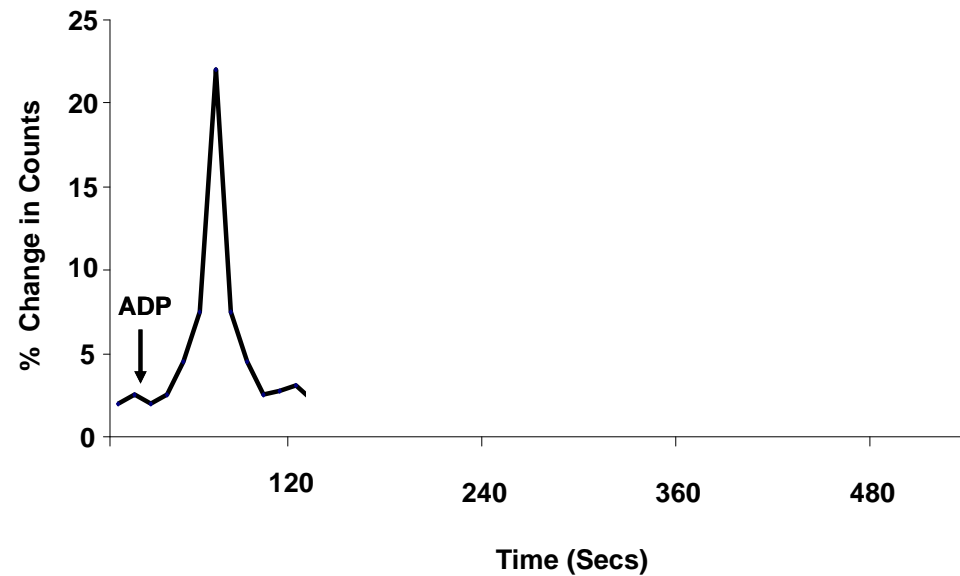


2006 grant: Dr Michael Emerson, Imperial College London



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

Refinement of a mouse model of pulmonary embolism



7 journal articles

Wellcome Trust and BHF grants

Adopted at 4 other UK establishments

Refinement and reduction:

Substantial severity procedure involving 200 mice

→ **unclassified procedure involving 30 mice**

→ **unclassified procedure involving 15 mice**

Centre-led programmes

- Challenge scientifically..... generates interest
- Collaborate with experts..... share data and expertise
- Publish in the peer reviewed literature..... kudos
- Websites, symposia..... inform, engage, maintain interest
- Influence funders and policy makers..... ensure change in practice



Examples of centre-led programmes



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



- Tissue engineering
- Improving models in asthma research
- Experimental reporting and design
- Primates in neuroscience research

- Pharmacokinetics in candidate selection
- Nausea and emesis research
- Primate use in abuse potential studies
- Peer review

- Primate use in mAb development
- Acute toxicity (pharmaceuticals)
- Acute toxicity (chemicals)
- Good practice in regulatory toxicology
- Novel approaches in chemical risk assessment

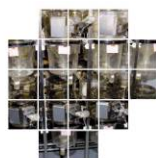
Safety Evaluation Working Group

NC3Rs

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

- Pharma industry, CROs and regulators
- Good practice in the conduct of regulatory toxicology studies

The cover features the logos of LASA (Laboratory Animals Science Association) and NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research). The title is 'Guidance on dose level selection for regulatory general toxicology studies for pharmaceuticals'. Below the title is a photograph of a scientist in a lab coat working with laboratory animals, including a dog and a monkey.



Urine Analysis in Regulatory Toxicology Studies: A Cross Company Comparison

Sally Robinson (AstraZeneca/LASA Animal Sciences Convenor), Sue Bolam (Sanofi Aventis), Andy Danks (Charles River), David Everett (Covance Laboratories), Rose Hill (Seqans), Engitte Mulker (Aptat), Sue Sparrow (GlaxoSmithKline), Derick Spencer-Engels (Huntingdon Life Sciences), Kathryn Chapman (NC3Rs)

- Urine analysis is traditionally seen as a non-invasive procedure however it has been shown to be stressful to animals
- Cross-company analysis of data has shown that there are variations in urine collection methods and frequency which may impact on animal welfare

What is the Safety Evaluation Working Group?

The UK Laboratory Animals Science Association (LASA) and the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) established a Safety Evaluation Working Group comprising toxicologists from the UK's major pharmaceutical companies and contract research organisations. Regulatory toxicology studies required for pharmaceutical development are well defined in terms of purpose, study design and types of data collected. The objective of the group is to share best practice and identify areas for improving animal welfare whilst ensuring that the scientific objectives and regulatory requirements of these studies are still met.

Regulatory requirement for urine analysis

Urine is routinely collected on regulatory toxicology studies as its analysis is considered a core regulatory parameter. One of the scientific reasons for this is that it may be used to determine early markers of acute and chronic nephrotoxicity and/or early changes in kidney function that can then be monitored non-invasively in clinical trials in humans.

Table 1: Rat and dog urine collection details illustrating differences between companies.

Parameter	Company 1	Company 2	Company 3	Company 4	Company 5	Company 6	Company 7	Company 8	Company 9
Species	Rat	Dog	Rat	Dog	Rat	Dog	Rat	Dog	Rat
Method	Metabolic cage	Metabolic cage	Metabolic cage	Metabolic cage	Metabolic cage	Metabolic cage	Metabolic cage	Metabolic cage	Metabolic cage
Frequency	Once	Once	Once	Once	Once	Once	Once	Once	Once
Volume	10-15 ml	10-15 ml	10-15 ml	10-15 ml	10-15 ml	10-15 ml	10-15 ml	10-15 ml	10-15 ml
Analysis	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea

Why investigate urine analysis?

Urine analysis is traditionally seen as a non-invasive procedure. However, isolation in a metabolism cage in a barren environment, separated from cage mates without food and sometimes water has been shown to be stressful for animals (for a review see reference 1). For larger species, the use of catheterisation also has associated welfare issues. There are examples showing the added value of urine analysis in rare individual cases. However, the quality of urine samples is highly variable and this may limit its value on a routine basis. Investigating the differences in practices between companies could have an impact on animal welfare by establishing whether a standard refined method of collection could be applied across companies.

Data collection

Data was collected from each company on i) methods for urine collection, ii) frequency of urine collection and iii) parameters analysed. The data was collected for the mouse, rat, dog and non-human primate.

Table 2: Frequency of dog and primate urine collection illustrating differences between companies.

Study Type	Species	Frequency	Method	Parameters
Non-urgent study	Primate	Once	Metabolic cage	Urea
	Dog	Once	Metabolic cage	Urea
Urgent study	Primate	Once	Metabolic cage	Urea
	Dog	Once	Metabolic cage	Urea
Urgent study	Primate	Once	Metabolic cage	Urea
	Dog	Once	Metabolic cage	Urea
Urgent study	Primate	Once	Metabolic cage	Urea
	Dog	Once	Metabolic cage	Urea

Results

There were differences in urine analysis methods between companies. These included:

- length of time in metabolism cage
- food/water deprivation
- catheter use (for larger animals)

 Data were collected for rat, dog, mouse and primate and the data for rat and dog are illustrated in table 1. Urine is rarely collected from mice as their size means it is difficult to obtain a sample of adequate volume. When it is collected the samples are usually pooled from a group of animals. There were differences in frequency of collection between companies for rat, dog and primate (see table 2). The main differences were:

- not all companies collect urine on a dose range finding study
- on longer term studies some companies collect interim urine samples whereas others do not
- there were differences in parameters analysed (see table 3).

Table 3: Parameters measured in rat urine illustrating differences between companies.

Parameter	Company
Urea	A B C D E F
Urea	A B C D E F
Specific gravity (density)	A B C D E F
Urea	A B C D E F
Glucose	A B C D E F
Bilirubin	A B C D E F
Albumin	A B C D E F
Haemat	A B C D E F
Colour/Appearance/Clarity	A B C D E F
Colour/Transmittance	A B C D E F
Conductivity (mS/cm)	B
Cellulose	C
Microbiology	D
Endocrine substances	D

Please take this opportunity to share your thoughts and experiences with the authors of the poster.

Summary

The cross-company collection of data has shown that there are variations in i) the time animals spend in a metabolism cage, ii) the use of catheters, iii) whether animals are food and/or water deprived and iv) the frequency of collection of urine samples and v) parameters analysed.

Discussion and Further Work

Further work on the most suitable method and the frequency of urine collection, from a scientific and welfare perspective is ongoing within the group. This includes work with clinical pathologists to:

- clarify the scientific rationale behind urine analysis to balance scientific objectives with welfare implications for the animal
- investigate opportunities for refining methods of urine collection and minimising the number of collections to benefit animal welfare

 The group will be investigating questions such as:

- is a urine sample necessary from every study?
- can we improve sample quality whilst optimising animal welfare?
- what volume of urine is needed to achieve the scientific objective of the study?

References
1. EF Kuiper, NE Everts, PH Scofield. Experimental animal urine collection. *Journal of Laboratory Animals* 33: 333-351 (2004)





Welcome :: Home :: Mouse :: Tail vein

- ☐ Home
- ☐ General principles
- ☐ Vascular catheters
- ☐ Mouse
 - ☐ Decision tree
 - ☐ Blood vessel cannulation
 - ☐ Tail vein
 - ☐ Tail snip
 - ☐ Saphenous vein
 - ☐ Retro-orbital
 - ☐ Abdominal/thoracic blood vessel
 - ☐ Cardiac puncture
 - ☐ Schedule 1 stunning followed by decapitation
 - ☐ Decapitation
- ☐ Rat
- ☐ Hamster
- ☐ Guinea pig
- ☐ Rabbit
- ☐ Ferret
- ☐ Dog
- ☐ Pig
- ☐ Marmoset

Mouse : Tail vein (non-surgical)

Tail vein sampling is suitable for all strains but is more difficult in black or pigmented mice. It is quick and simple to perform for competent individuals. This technique may require the animals to be warmed in order to dilate the blood vessel prior to taking the sample. This may be stressful and can cause dehydration due to salivation, in addition to increasing metabolic rate, which may affect the experimental data. As a result, other routes such as [saphenous vein](#) sampling should be used where possible and, in particular, where there is a need to take multiple samples. View a video of the mouse tail vein sampling technique below.



The lateral tail vein is usually used and 50 μ l to 0.2 ml of blood can be obtained per sample depending on the size of the animal and specific requirements. The tail may need to be washed with diluted Hibiscrub (1%) in order to see the blood vessel.

To avoid bruising and damage to the tail, normally no more than two blood samples should be taken per session and in any one 24-hour period. Where it is necessary and justifiable to take more, the use of temporary or [surgical cannulation](#) methods should be considered. The number of attempts to take a blood sample should be minimised (no more than three needle sticks in any one attempt) and sufficient time should be given for the tail to recover between blood sampling sessions. Alternate sides of the tail should be used and successive needle punctures moved towards the tail base.

If it is necessary to warm the animal, a warming cabinet should be used (39°C for 10 to 15 minutes). Mice should be carefully monitored, including checking for signs of dehydration. The time the mouse is in the warming cabinet should be recorded and the cabinet should be calibrated regularly to avoid [hyperthermia](#); digital displays should not be relied upon. It is important to ensure the temperature in the cabinet is uniform and that there are no 'hot spots'. Male mice may need to be warmed singly to avoid fighting.

The lateral tail vein is usually accessed approximately one-third along the length of the tail from the tail tip, moving towards the base of the tail for multiple samples. Aseptic technique should be used. A [local anaesthetic cream](#) (e.g. EMLA cream) can be applied to the site 30 minutes prior to blood sampling. Animals need to be [restrained](#) which can cause stress and therefore the duration of restraint should be minimised. Where a restraint tube is used, it should be appropriate for the size of the mouse in order to avoid damage to the tail, testes and limbs. All forms of restraining equipment should be frequently washed to prevent pheromonally-induced stress or cross-infection.



Search

Resources

References

A good practice guide to the administration of substances and removal of blood, including routes and volumes.

☐ [Open Link](#)

Ness RD (1999), Clinical pathology and sample collection of exotic small animals. *The Veterinary Clinics of North America: Exotic Animal Practice*. 2 (3), pp 591-620

Lucas RL, Lentz KD, Hale AS (2004), Collection and preparation of blood products. *Clinical Techniques in Small Animal Practice*. 19(2), pp 55-62

Tuli JS, Smith JA, Morton DB (1995), Corticosterone, adrenal and spleen weight in mice after tail bleeding, and its effect on nearby animals. *Laboratory Animals*. 29(1), pp 90-95

Nemzek JA, Bolgos GL, Williams BA, Remick DG (2001), Differences in normal values for murine white blood cell counts and other hematological parameters based on sampling site. *Inflammation Research*. 50(10), pp 523-527

Schnell MA, Hardy C, Hawley M, Probert KJ, Wilson JM (2002), Effect of blood collection technique in mice on clinical pathology parameters. *Human Gene Therapy*. 13(1), pp 155-161

Methods of blood collection in the mouse.

☐ [View PDF](#)

Removal of blood from laboratory animals and birds.

☐ [View PDF \(131KB\)](#)

Procedures With Care

ADMINISTRATION OF SUBSTANCES

SEARCH

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General Introduction

This web site provides information to assist research workers develop their skills in the administration of substances to laboratory animals. Initially we have provided material dealing with rats and mice, since they are the animals most widely used in research. Further material will be added to expand the range of techniques and species.

This site focuses particularly on the manual skills needed to carry out the different procedures humanely and efficiently, and on the need to handle animals carefully to reduce any distress caused by the procedure.



Administering substances to animals, for whatever reason, can have a significant impact on their welfare. If carried out incorrectly, not only can animal welfare be compromised, but the scientific goals of study can be affected. If the administration is for a therapeutic purpose, then incorrect administration can lead to a failure of the treatment.



The selection of a particular route of administration must balance a number of factors – for example the volume and physicochemical properties of the substance, the required speed of onset, and other factors such as the degree of tissue irritation that could be caused. These topics are discussed in more detail in a number of different [guidelines](#).

[View Full Introduction](#)

WELCOME TO THE PROCEDURES WITH CARE WEBSITE

This website shows recommended techniques for the administration of substances to common species of laboratory animals.

The material was developed with the support of the Institute of Animal Technology and NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research).



TUTORIALS



[Subcutaneous Injection in the Rat](#)



[Intravenous Injection in the Rat](#)



[Intramuscular Injection in the Rat](#)



[Intraperitoneal Injection in the Rat](#)



[Subcutaneous Injection in the Mouse](#)



[Intravenous Injection in the Mouse](#)



[Intraperitoneal Injection in the Mouse](#)

VIEW BY SPECIES

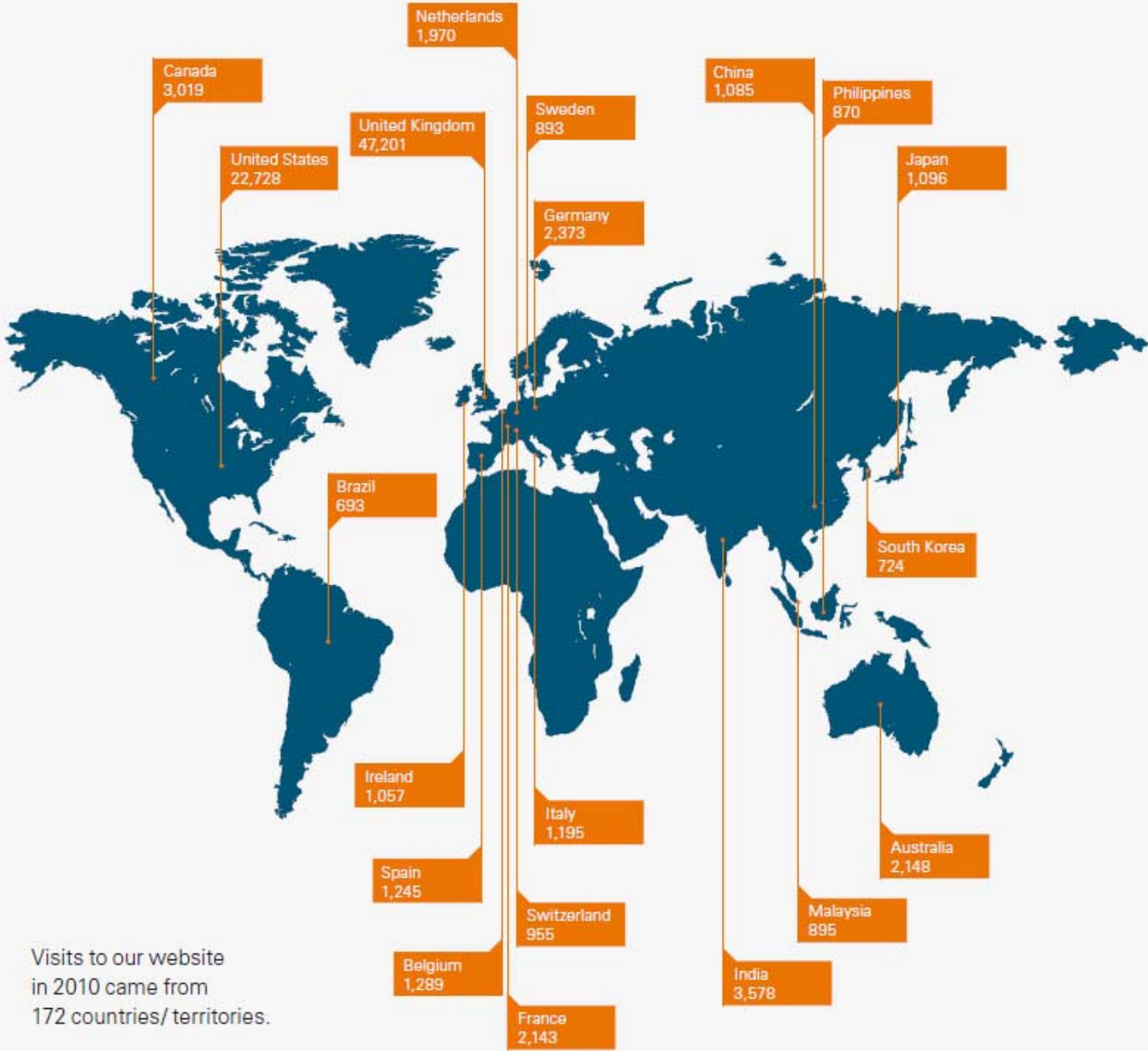
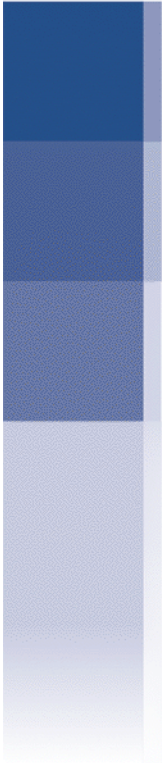
- [Mouse](#)
- [Rat](#)
- [View All](#)

VIEW BY TECHNIQUE

- [Intramuscular](#)
- [Intraperitoneal](#)
- [Intravenous](#)
- [Subcutaneous](#)
- [View All](#)

EXTERNAL LINKS

- [Institute of Animal Technology](#)
Advancing and promoting excellence in the care and welfare of animals in research.
- [NC3Rs National Centre for the Replacement, Refinement and Reduction of Animals in Research.](#)



Visits to our website
in 2010 came from
172 countries/ territories.

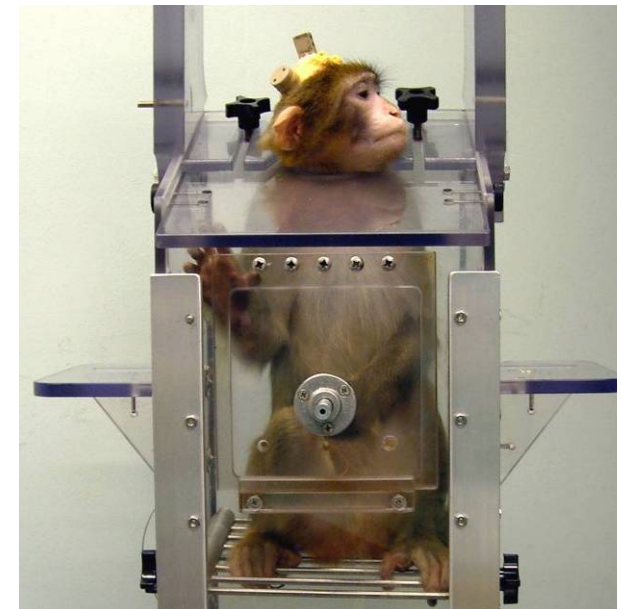
Top 20 shown

Refining food and fluid control

- Controversial area of concern; wide variation in practice; few data in the literature
- Convened a working group for data sharing, analysis and expert opinion
- Published peer-reviewed report
 - Reviews and summarises current practice
 - Identifies the animal welfare issues
 - Identifies refinements
 - Makes recommendations on best practice
 - Highlights data and research gaps
- Promoted at scientific conferences and institutes in the UK and overseas
- Further support, e.g. NC3Rs studentship

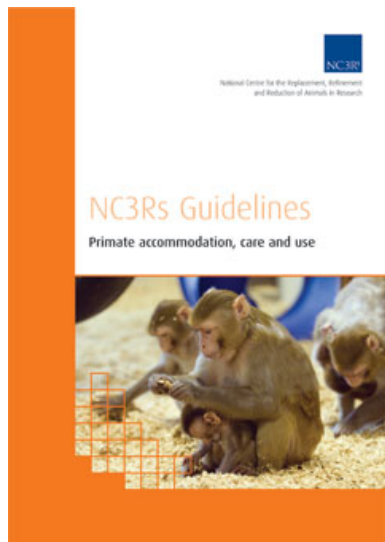
*Prescott et al. 2010.
J Neurosci Methods 193*

*Prescott et al. 2011.
J Neurosci Methods (in press)*



Peer review

- All MRC, BBSRC and Wellcome Trust grant, studentship and fellowship applications involving non-human primates, cats, dogs and equines
 - Identify and address any animal welfare concerns
 - Ensure 3Rs opportunities are exploited
 - Monitor the implementation of guidelines produced with the funders to support best practice
- Enhances science, animal welfare and reputation



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



Thank you

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