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

Support of refinement by the NC3Rs: Experiences and recommendations

Mark Prescott, PhD

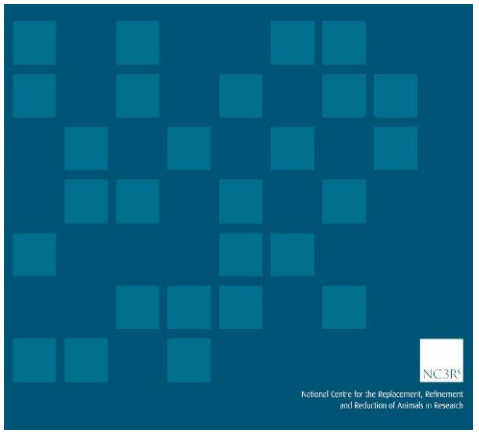
BfR-Forum, 13 December 2011

The NC3Rs

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

- 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

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

"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?

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

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

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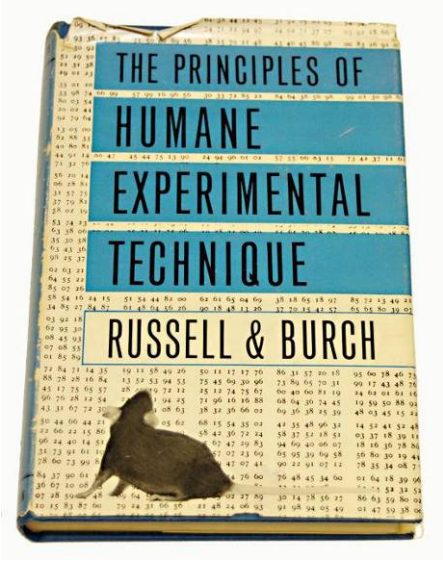
- **Replacement** – methods which avoid the use of animals (or 'protected' animals) in areas where they otherwise would have been used

- **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



Buchanan-Smith et al. 2005. Animal Welfare 14

Importance of refinement

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- 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

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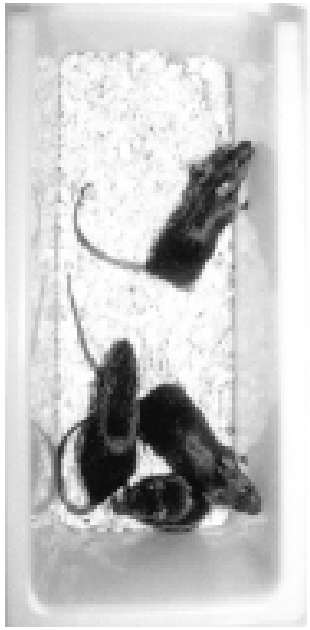
Hockly et al. 2002. Annals Neurol 51



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

Challenges

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- 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)

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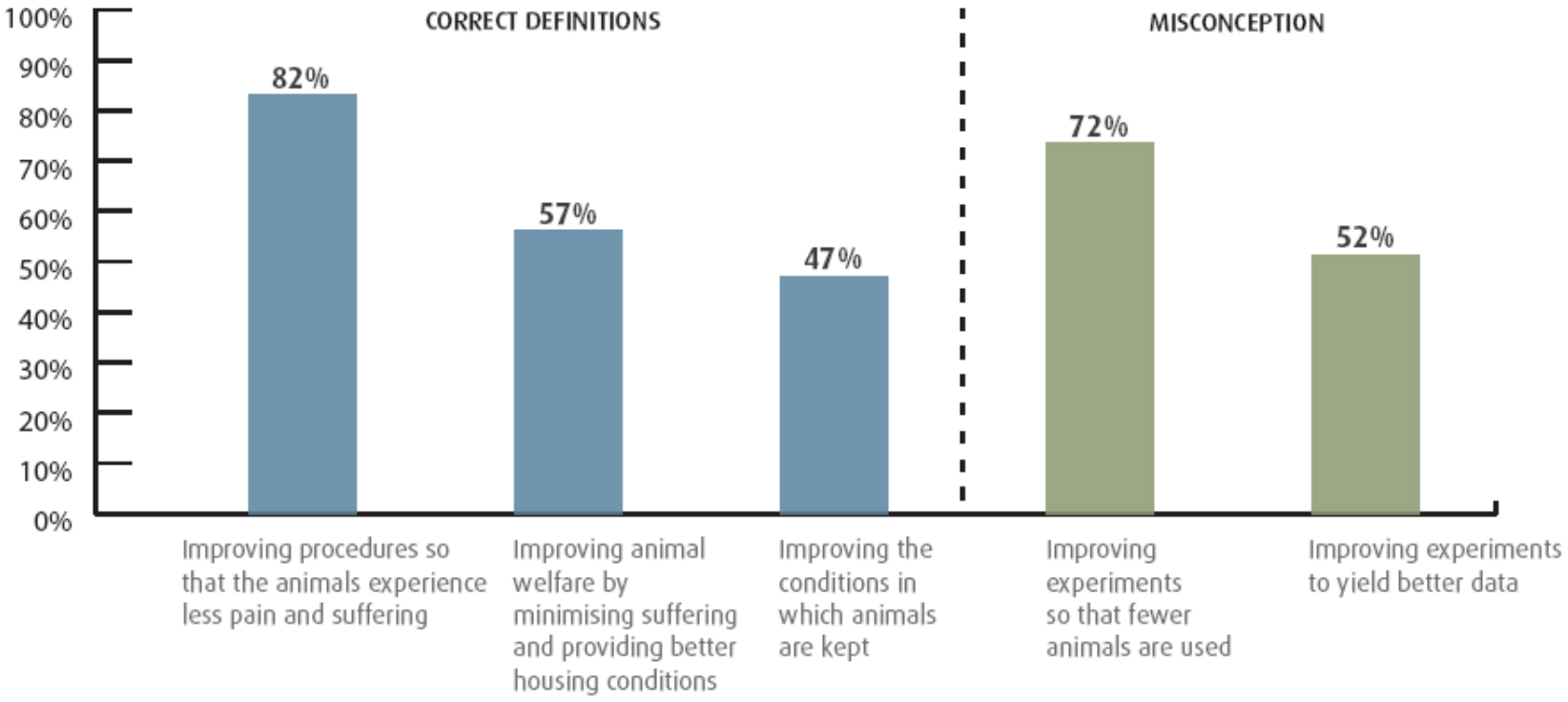

NC3Rs survey 2007, conducted by People, Science & Policy

Base: All scientists (1,529)

Interplay between the ‘R’s

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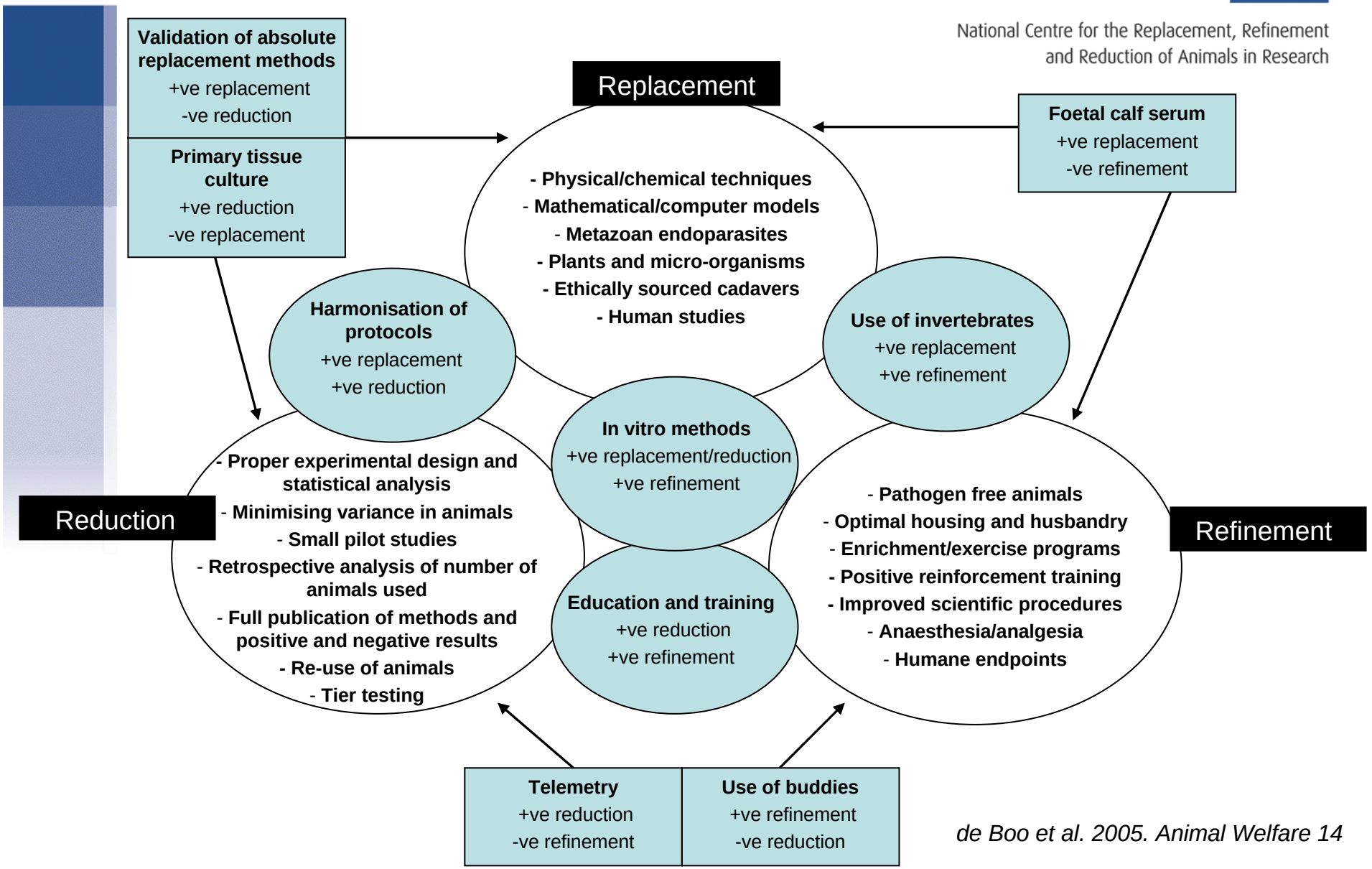
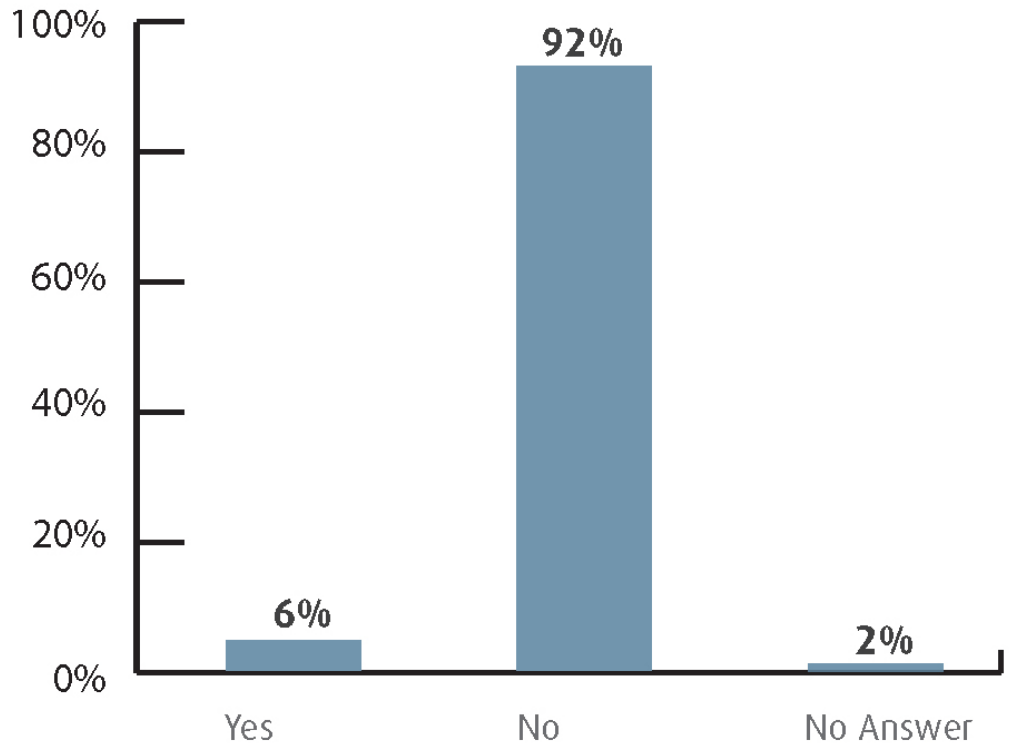


de Boo et al. 2005. Animal Welfare 14

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

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

Base: All scientists (1,529)



NC3Rs survey 2007, conducted by People, Science & Policy

Research funding

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- 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

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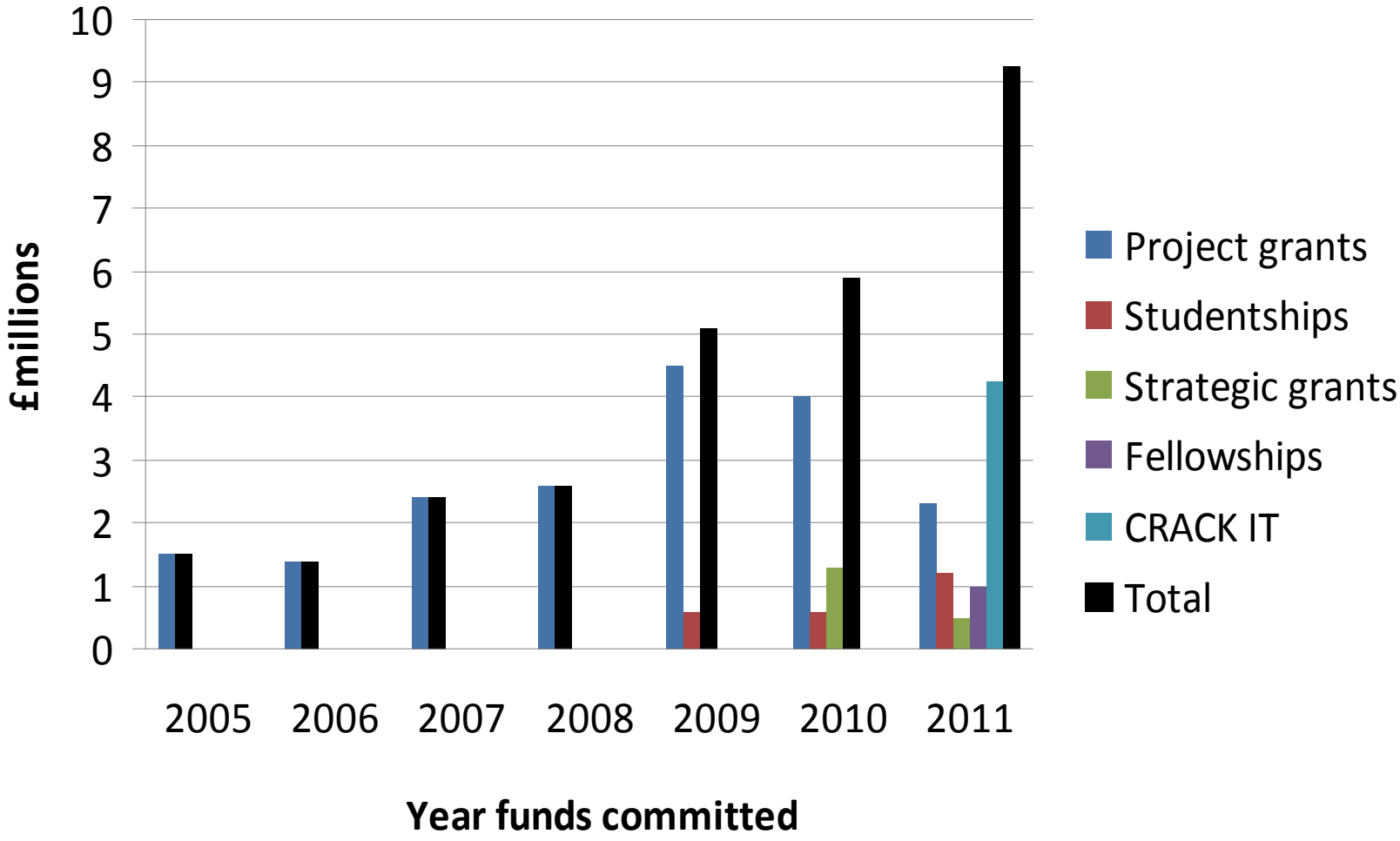


Strategic calls and awards

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

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)

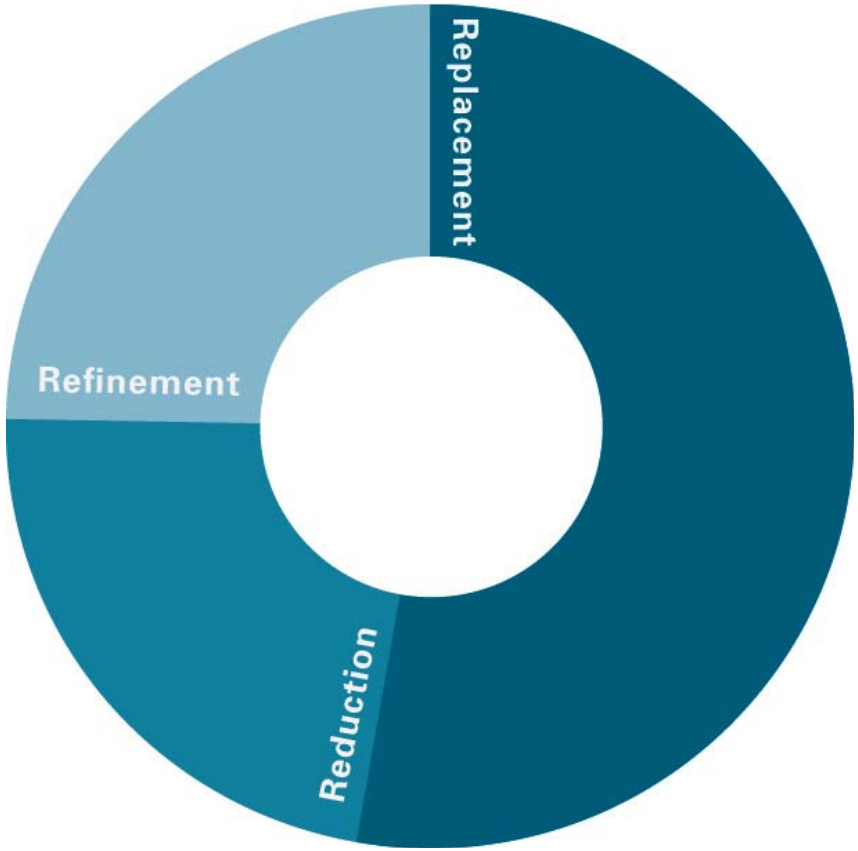
Strategic awards

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

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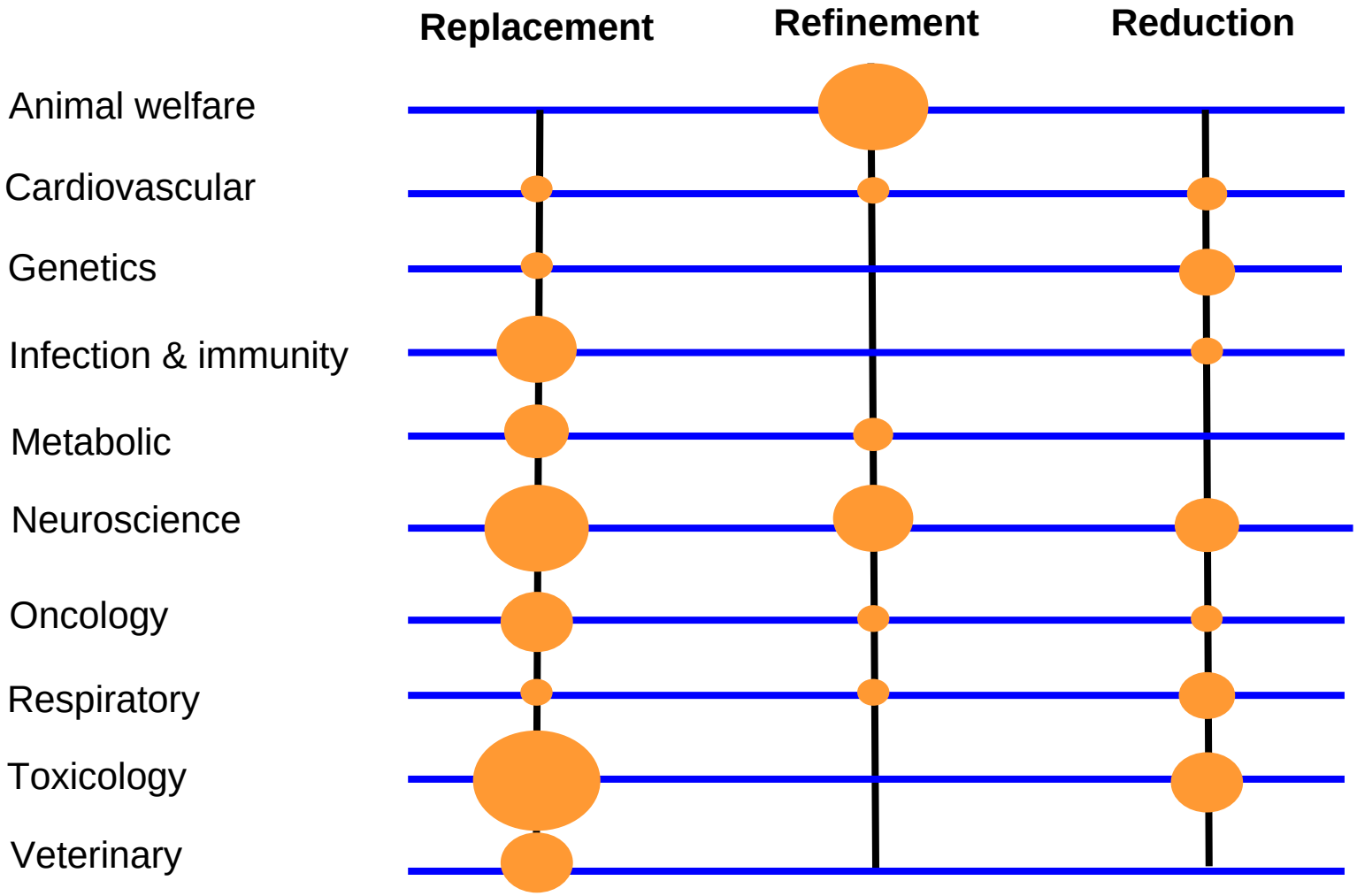

2006 grant: Dr Michael Emerson, Imperial College London

Refinement of a mouse model of pulmonary embolism

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- Novel method: drugs and gene function in PE
- Better scientifically
  - Models several, fatal embolism
  - Clinical PE has a broad spectrum
- Major refinement

~~Inflicts severe pain and suffering~~

~~Limited scientific use~~

~~Models one, fatal embolism~~

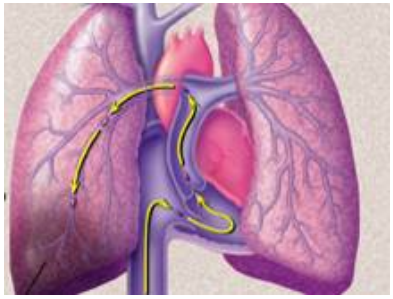
~~More information~~

~~Major refinement~~

~~Major refinement~~

~~What is measured?~~

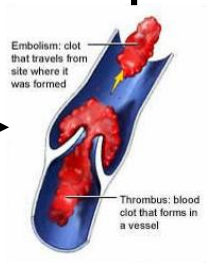
Imaging PE → Paralysis → Death



Lower concentrations

Clotting agent

Anaesthetise



Severity limit

- Substantial
- Moderate
- Mild
- Unclassified

2006 grant: Dr Michael Emerson, Imperial College London

Refinement of a mouse model of pulmonary embolism

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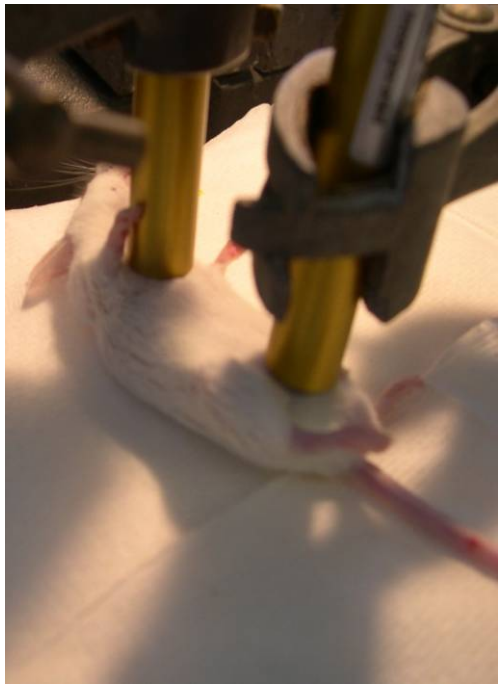

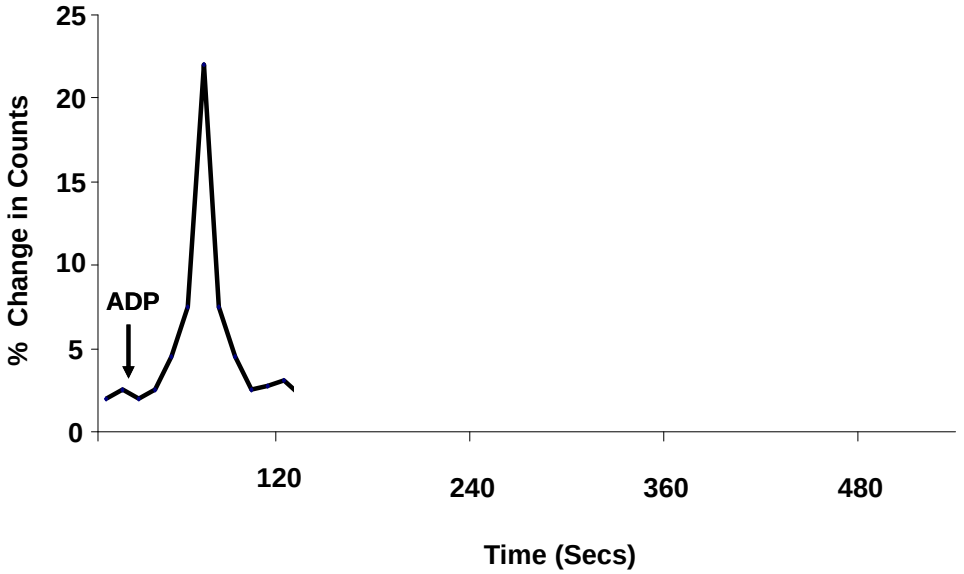

7 journal articles

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

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- 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

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Basic research / **Applied research**

Disease modelling / **Pharmaceutical/Chemical Development** / **Safety/Toxicity Testing**

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

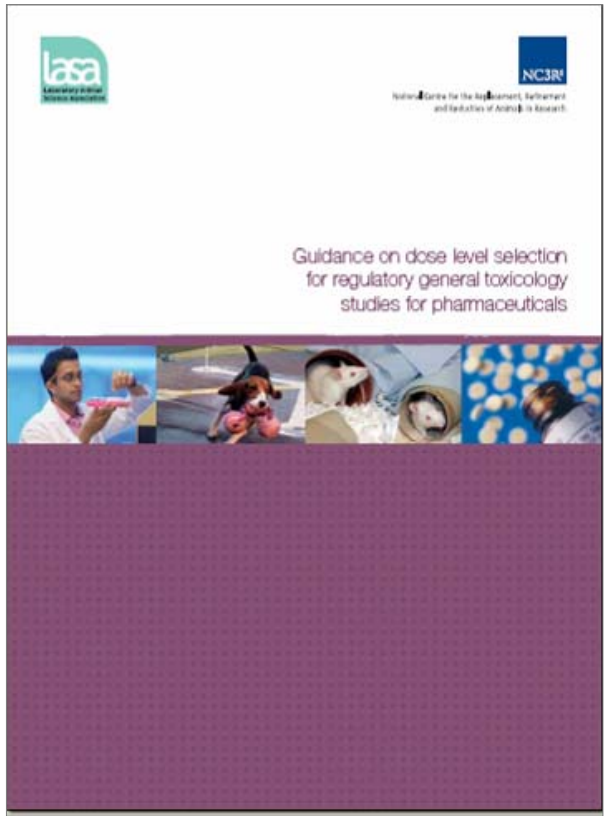
- Pharmacokinetics in candidate selection
- Nausea and emesis research
- Primate use in abuse potential studies
- Peer review

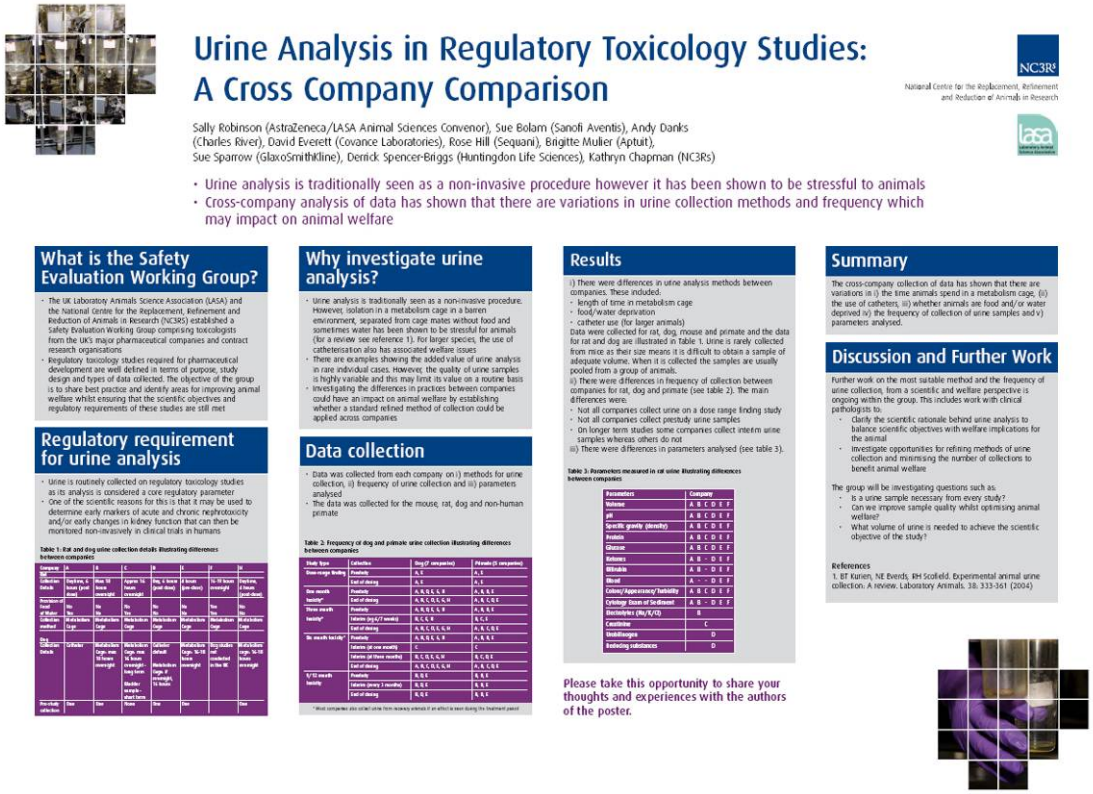
- Primate use in mAb development
- Acute toxicity (pharmaceuticals)
- Acute toxicity (chemicals)
- Good practice in regulatory toxicology
- Novel approaches in chemical risk assessment

Safety Evaluation Working Group

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





- 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 ul 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^{o}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 trail, testes and limbs. All forms of restraining equipment should be frequently washed to prevent pheromonally-induced stress or cross-infection.

Search

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, Propert 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

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.


Netherlands
1,970
Canada
3,019
China
1,085
Philippines
870
Sweden
893
United Kingdom
47,201
United States
22,728
Japan
1,096
Germany
2,373
Brazil
693
South Korea
724
Ireland
1,057
Italy
1,195
Australia
2,148
Spain
1,245
Malaysia
895
Switzerland
955
Belgium
1,289
India
3,578
France
2,143
Visits to our website
in 2010 came from
172 countries/ territories.
Top 20 shown

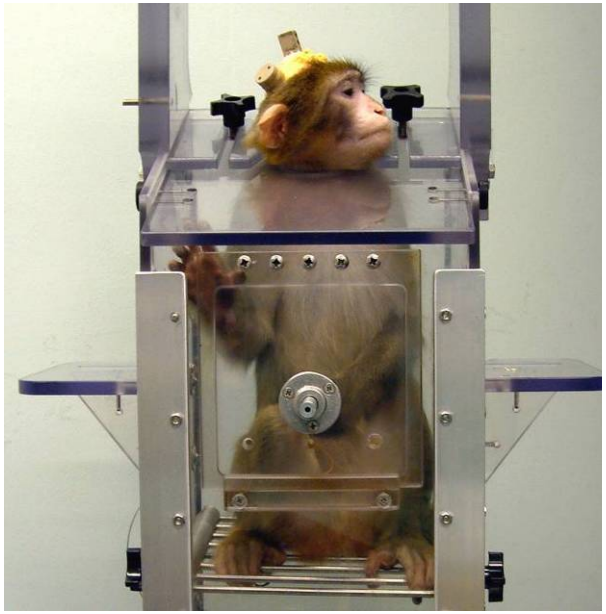
Refining food and fluid control

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

- 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

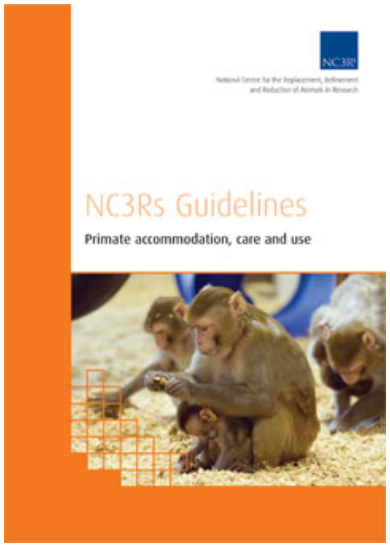
Prescott et al. 2010.
J Neurosci Methods 193

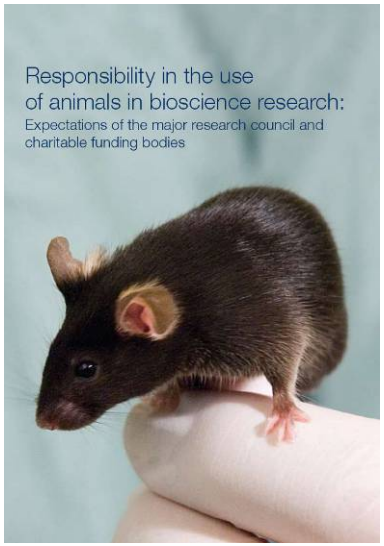
Prescott et al. 2011.
J Neurosci Methods (in press)



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





NC3Rs
National Centre for the Replacement, Refinement and Reduction of Animals in Research

MRC Medical Research Council

BBSRC bioscience for the future

wellcome trust

The Royal Society Celebrating 350 years

amrc Association of Medical Research Charities

Natural Environment Research Council

defra Department for Environment Food and Rural Affairs

Thank you

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

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