Where can we find 3R literature?

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www.norecopa.no
• What is 3R literature?
• Why is it hard to find?
• What can we do about?
• Examples of 3R sources
• Tools for searching the literature
Reporting

Literature search

Planning

Research
3R literature embraces all 3R alternatives

1) Replacement alternatives

3D models
Audiovisual aids
Computer simulations
Mannekins, simulators, robots
QSAR (Quantitative Analysis of Structure/Activity Relationships)
Cell and tissue cultures, organoids, organ perfusion
    High Throughput Screening (HTS), organs-on-a-chip
Biochemical & immunological methods (RIA, ELISA)
Hybrid DNA technique, GMM
Trials on “lower” organisms
Acute experiments (terminal anaesthesia)
Trials on dead animals
Trials on humans (microdosing and medical imaging)
Synthesis of new evidence from experiments that have already been performed
High Throughput Screening (HTS)

Cell culture and compound management

Robotic platform with high-throughput liquid handler for sample dilution and treatment.
Optical plate reader and incubator

Data management system

Automated imaging microscope for high-content screening

Lung-on-a-chip
Wyss Institute, Harvard
Next generation Multi-Organ-Chip

Integrating natural science and technology: Fish and fish robots

Prof. Maarja Kruusma

photo: Norecopa

centre for biorobotics
www.biorobotics.ttu.ee
Rats from IKEA
Fidelity: overall proportionate difference (e.g. HiFi)

Discrimination: the extent to which the model reproduces one particular property in which we are interested

https://www.wardsci.com/store/catalog/product.jsp?catalog_number=813015#

http://www.interniche.org/ko/node/5134
The potential for 3R alternatives cannot be evaluated until the objective of the study is known. This applies to all use of animals in research, testing, education and training.

Possible objectives in education & training:

- Teaching and practising:
  - laboratory skills
  - general animal handling skills
  - preparation-specific animal skills
- imparting good ethical thinking
- new knowledge and reinforcing existing
- data handling skills
- experimental design skills
- communication skills (oral, written)
- groupwork
- staff-student interaction
AJ Smith & K Smith, 2004

Guidelines for humane education: Alternatives to the use of animals in teaching and training

Proceedings of the 4th World Congress on Alternatives and Animal Use in the Life Sciences, New Orleans, August 2002.

http://www.atla.org.uk/wp-content/plugins/s2member-files/32_S1a_3_Plenary_specialcontribution.pdf (log-in required, pages 16-26)
Where do I find information about alternatives for use in Education and Training?

Databases
- NORINA (oslovet.norecopa.no/NORINA)
- InterNICHE (interniche.org/en/alternatives)

Loan system
- InterNICHE (interniche.org/en/loansystem)
Do we need an alternative?

3) Reduction alternatives

A good statistician is the lab animal’s best friend.

Combined with methods to reduce background “noise”.

http://norecopa.no/norecopa/vedlegg/Berdoy-handout.pdf
Sources of background “noise”:

• Age, sex, weight  
• Stress, subclinical disease  
• Room temperature, animal cage  
• Environmental “enrichment”  
• Temporal differences between treatments  
• Climatic factors  
• Position of cage in the room  
• Experimenter  
• Animal Technician (weekend workers)  
• and many more
3) Refinement alternatives

"Simple" techniques?

Are they feasible? e.g. i.m. injections
"Simple" identification methods?
Do they affect the animal?

#.VLU6_8Y7_wo

Photo: T. Poppe, NMBU

Photo: colourbox.com
Refinement to avoid *contingent suffering* (not just direct suffering caused by the procedure)
e.g. fear, boredom, discomfort
which may caused by
e.g. transport, housing, husbandry, social hierarchy

Single-housed male mice show symptoms of what in humans would be characterised as depression

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0111065
An useful additional (but largely unknown) tool...
Carol M. Newton (1925-2014)

The three S’s

• Good Science
• Good Sense*
• Good Sensibilities*

*We can do this ourselves without scientific literature!

Creating a culture of care

Friday 22 August 2014

Dr Marilyn Brown, Corporate Vice President of Global Animal Welfare at the contract research organisation Charles River, has many years of experience managing experimental facilities and animal care programmes.

https://www.nc3rs.org.uk/news/creating-culture-care

Establishing a Culture of Care, Conscience, and Responsibility: Addressing the Improvement of Scientific Discovery and Animal Welfare Through Science-based Performance Standards

H. J. Klein and K. A. Bayne
Address correspondence and reprint requests to Dr. Klein, Merck Research Laboratories, WP42–211, West Point, PA 19486, or email Hilton_klein@merck.com.

http://ilarjournal.oxfordjournals.org/content/48/1/3.full
Why is 3R literature hard to find?

• Bibliographic databases are often not used adequately (poor overlapping between the databases)
• Too few scientists are aware of the specialist 3R-databases
• Scientists rarely use ”3R” words when they write titles/abstracts/keywords for their papers
• Databases rarely flag 3R-papers with explicit thesaurus terms 😞
• We have no single ”Journal of Alternatives”
Reporting has historically been poor:

Jane Smith et al. (1997): 149 papers in 8 journals from 1990-1991:

Parameters not mentioned:

Number of animals: 30%

How the animals were killed: 45%

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>28%</td>
</tr>
<tr>
<td>Age</td>
<td>52%</td>
</tr>
<tr>
<td>Weight</td>
<td>71%</td>
</tr>
<tr>
<td>Source</td>
<td>53%</td>
</tr>
<tr>
<td>Room temperature</td>
<td>72%</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>89%</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>72%</td>
</tr>
<tr>
<td>Number of animals/cage</td>
<td>73%</td>
</tr>
</tbody>
</table>
Often detailed descriptions of chemicals, equipment and treatments, but very little about the animals, choice of sample size, randomisation etc:

‘white mice were used’

Many of these omissions make it harder to advance the 3Rs, e.g.

• methods of drug administration and blood sampling
• details of anaesthesia and analgesia
• humane endpoints
Kilkenny C et al. (2009)

271 papers, mostly in 2003-2005

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0007824

Many studies did not

• describe the animals adequately
• describe how the sample size was chosen
• describe how the animals were allocated to the treatment groups, and whether the observations were performed blind.
Even the titles, keywords and abstracts are often not very informative and lack 3R terms.

The development of Response Surface Pathway Design to reduce animal numbers in toxicity studies.
3R methods are often not highlighted in the scientific literature. SCID-Hu mice immunized with a pneumococcal vaccine produce specific human antibodies and show increased resistance to infection.
Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea-pig, ferret and mink

Visibility! Not necessarily in a high-impact journal.
Most-Cited Articles as of May 1, 2015 -- updated monthly

Rankings based on citations to online articles from HighWire-hosted articles.

1. Working Party Report:
   Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units
   » Full Text (PDF)

2. Articles:
   C. Moolenbok and E. J. Ruitenber
   The 'Swiss roll': a simple technique for histological studies of the rodent intestine
   Lab Anim January 1, 1981 15: 57-59,
   doi:10.1258/0023677811780958577
   » Abstract  » Full Text (PDF)

3. Papers:
   Annelise Hem, Adrian J. Smith, and Per Solberg
   Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guineapig, ferret and mink
   Lab Anim October 1, 1998 32: 364-368,
   doi:10.1258/002367798780509866
   » Abstract  » Full Text (PDF)
Part of the problem:

Reporting (the Materials & Methods section) should ideally be so detailed that it is possible to reproduce the study in another lab.

But this information takes space.
Although space is limited, we waste space...

‘drinking’ water

‘farm’ pigs

‘under approved conditions’ (who approved them?)

‘housed under standard conditions’

‘given analgesia’
Many journals now offer supplementary online space (generally unlimited) where more information about the methods and results can be posted.

And most people have access to a website where this could be posted...
Publication of negative results

• Bias automatically occurs if only positive results are reported!
• Negative results may be just as important for the scientific community, even if they are less newsworthy
• Many medical journals require registration of trials before they start, to prevent the under-reporting of negative results (http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html)

There are a number of journals now that report negative results, e.g.

J of Negative Results (http://www.jnr-eeb.org/index.php/jnr)
J of Negative Results in Biomedicine (http://www.jnrbm.com)
J of Pharmaceutical Negative Results (http://www.pnrjournal.com)
The All Results Journals (http://www.arjournals.com/ojs)
Identify and ensure the quality of (at least) the critical points in the experiment:
critical for animal welfare and scientific value
Good reporting and increased implementation of the 3Rs is dependent upon:

*Quality assurance and a culture of care at all levels of the animal facility.*

- SOPs describing good techniques, carried out by *competent* operators
- A Checklist (“contract”) between researcher and the facility
- The AAALAC Program Description template or something similar as an overall quality assurance checklist for the facility
- A *Master Plan* as a weekly checklist for the whole facility
Template for a Program Description from AAALAC International

- Animal care and use policies and responsibilities
- Animal environment, housing and management
- Veterinary medical care
- Physical plant

https://www.aaalac.org/programdesc/index.cfm
OUTLINE

DESCRIPTION OF INSTITUTIONAL ANIMAL CARE AND USE PROGRAM

I. Introduction
   A. Name of Program Unit
   B. Overview and Purpose
   C. Description of the Organization (Attach organizational chart plus any support comments needed)
   D. Key Institutional Representatives
   E. Accreditation History
   F. Nature of Research, Testing, and Teaching Programs
   G. Research Funding Source(s)
   H. Summary of Facilities
   I. Other Units not Included in This Description
   J. Contract Facilities
   K. Other Relevant Background

II. Description
   A. Institutional Policies and Responsibilities
      1. Monitoring the Care and Use of Animals
         a. Institutional Animal Care and Use Committee(s) (IACUC)
            1) Who appoints Committee/Who is Institutional Official
            2) Composition/Frequency of Meetings/Responsibilities of the Committee

10/97
A simple but effective Master Plan
Guidelines
e.g.
• ARRIVE, ILAR, ICLAS, LASA/APC
• GSPC
• Guidelines for specific types of animal research
# The ARRIVE Guidelines

**Introduction**

1. **Background**
   - a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.
   - b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.

2. **Objectives**
   - Clearly describe the primary and any secondary objectives of the study or specific hypotheses being tested.

**Methods**

3. **Ethical Statement**
   - Indicate the nature of the ethical review permissions, relevant licences (e.g., Animal (Scientific Procedures) Act 1986), and national or institutional guidelines for the care and use of animals that cover the research.

4. **Study Design**
   - For each experiment, give brief details of the study design including:
     - a. The number of experimental and control groups.
     - b. Any steps taken to minimize the effects of subjective bias when allocating animals to treatment (e.g., randomisation procedure) and when assessing results (e.g., if done, describe who was blinded and when).
     - c. The experimental unit (e.g., a single animal, group of animals, cage of animals).
   - A timeline diagram or flow chart can be useful to illustrate how complex study designs were carried out.

5. **Experimental Procedures**
   - For each experiment and each experimental group, including controls, provide precise details of all procedures carried out.
   - a. How (e.g., drug formulation and dose, site and route of administration, anaesthesia and analgesia used (including monitoring), surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including suppliers.
   - b. When (e.g., time of day).
   - c. Where (e.g., home cage, laboratory, water maze).
   - d. Why (e.g., rationale for choice of specific anaesthetic, route of administration, drug dose used).

6. **Experimental Animals**
   - a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g., mouse or rat, age, weight), and weight (e.g., mean or median weight plus weight range).
   - b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g., knockout or transgenic), genotype, health immune status, drug or test naïve, previous procedures, etc.

**Results**

7. **Baseline Data**
   - For each experimental group, report relevant characteristics and health status of animals (e.g., weight, microbiological status, and drug or test naïve) prior to treatment or testing. This information can often be tabulated.

8. **Numbers Analyzed**
   - a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g., 15/20, not 50%).
   - b. If any animals or data were not included in the analysis, explain why.

9. **Outcomes and estimation**
   - Report the results for each analysis carried out, with a measure of precision (e.g., standard error or confidence interval).

10. **Adverse events**
    - a. Give details of all important adverse events in each experimental group.
    - b. Describe any modifications to the experimental protocols made to reduce adverse events.

**Discussion**

11. **Interpretation**
    - a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.
    - b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the implications associated with the results.
    - c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.

12. **Generalisability/translation**
    - Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.

13. **Funding**
    - List all funding sources (including grant number) and the role of the funder(s) in the study.

[https://www.nc3rs.org.uk/arrive-guidelines](https://www.nc3rs.org.uk/arrive-guidelines)
The ARRIVE guidelines

Animal Research: Reporting of In Vivo Experiments

ARRIVE (动物研究: 体内实验报告) 是由国家3R中心创设，旨在通过提高动物研究设计、分析和报告的质量，使报告的信息量最大并要求不必要的研究减至最低程度。该指南于2010年6月在PLOS Biology网络杂志发表，并得到多家科研杂志、主要的资助机构和学术团体的赞同。

ARRIVE（动物研究: 体内实验报告）指南的宗旨：

- 提高动物研究报告的质量。
- 指导作者在稿件中提供必要的信息，但它并不是硬性规定。
- 报告的灵活性使其广泛适用于各种研究领域和实验方案。
- 促进具有可重复性、透明性、精确性、全面性、简明性、逻辑性的高质量论文。
- 促进科研成果在科学界更广泛的交流。

ARRIVE（动物研究: 体内实验报告）指南适用于：

- 统一性、可接受性，或鼓励作者条理清晰。有些条款并不适用于所有的研究，有些数据只适用于某些研究设计和执行的指南。
- 提供实验设计和执行的指南。但是研究中有些条款如随机化、临床和使用对照组等对于设计试验时减少偏倚和提高研究的稳健性是有帮助的。

ARRIVE指南适用于哪些科研领域?

- 最适用于比较研究，即两个或多个实验动物组进行比较，其中一组或多组常设为对照组。也适用于比较药物不同剂量的研究。或者用单一动物作为其自身对照（未受内对照）。
- 大多数建议也适用于不会对照组的研究。
- 适用于涉及实验动物科学研究的任何领域。

如何使用ARRIVE指南?

该指南提供了一个对研究设计有改进的框架，适用于研究设计的准备和实施。

参考文献


致谢

国家3R中心 (NC3Rs) 对所有参与ARRIVE指南的创建中提供宝贵经验和建议的各位专家致以最衷心的感谢。特别感谢NC3Rs报告指南工作组所有成员。我们同时要感谢NC3Rs的研究者、医学研究委员会、生物技术和生物科学研究委员会、威康信托、帕金森氏病协会、英国心脏基金会、其基金会持有人和基金委员会成员对指南所提的反馈意见。

感谢张晖编审 (《环境与健康简报》)、颜万勇博士 (艾尔森研发中心) 和吴东华博士 (伦敦大学玛丽女王学院) 帮助审校中文译稿。

Further Information

www.nc3rs.org.uk/ARRIVE
enquiries@nc3rs.org.uk
@NC3Rs
So we would find more 3R literature if there was greater transparency...

Improved publication standards
Open Access to primary data and negative results
Clear implementation of the 3Rs
Editorial action
  • More structured M&M sections in papers
  • Information on the ethical review process and justification
  • Experimental design and appropriate analysis
  • Compliance with guidelines
    • ARRIVE, GSPC, ILAR, ICLAS, LASA/APC
    • local guidelines, AAALAC template
    http://oslovet.norecopa.no/3R/produkter.aspx?search=reporting
  • Compliance with the Basel Declaration
basel-declaration.org

- emphasises the 3Rs
- encourages transparency and collaboration to avoid repetition of animal studies
- implement and monitor the highest training standards
- invites animal welfare organisations to open discussion
- promotes balanced dialogue
We would also find more 3R literature if there was greater use of 3R descriptors...

Using PubMed to access data in MEDLINE:

MESH (Medical Subject Headings) thesaurus

Other databases have their own thesauri. A thesaurus can be useful to build up a list of suitable keywords, even if you use another database.
Examples of 3R sources

• National 3R centres
• 3R congress proceedings
• Guidelines papers
• Journals
• Discussion groups
• Training schools
National 3Rs Centres

www.nc3rs.org.uk
Three Rs Search Guide

If you plan to use animals for scientific purposes, you must complete an animal use protocol and submit it to an animal care committee for approval prior to commencement of the study. The animal use protocol outlines how the Three Rs will be implemented in the proposed animal-based procedures. To find the most up-to-date information on the Three Rs, investigators typically conduct a structured information search. To assist investigators with this search, the CCAC has produced the Three Rs Search Guide.

The Three Rs Search Guide provides detailed instructions on how to conduct a Three Rs information search in the Step-by-Step Three Rs Search Strategy.
Animal welfare organisations

www.rspca.org.uk/sciencegroup/researchanimals

Reducing suffering - Rabbit welfare

Reducing suffering: introduction
For as long as animals are used in research and testing, every step must be taken to reduce suffering and improve welfare...

More

Refinement
The research animals department promotes initiatives that will lead to improvements in laboratory animal housing and care and reductions in suffering caused by procedures...

More

Rodent welfare
Working to improve the welfare of laboratory rodents is extremely important because the vast majority of animals used in research and testing are mice and rats...

More

Rabbit welfare
The lives of laboratory rabbits can be greatly improved by providing housing and care that caters for their physical and behavioural needs...

More

Thousands of rabbits are used in research and testing throughout the European Union every year, mostly in pharmaceutical research and development. The lives of laboratory rabbits can be greatly improved by providing housing and care that caters for their physical and behavioural needs.

UFAW/RSPCA Rabbit Behaviour and Welfare Group
During 2008, the UFAW/RSPCA Rabbit Behaviour and Welfare Group published a report providing practical guidance on refining laboratory rabbit husbandry.
A resource book for lay members of ethical review and similar bodies worldwide

3rd edition
January 2015

Maggy Jennings and Jane A. Smith
Centres giving information on alternatives

UCCAA
University of California Center for Animal Alternatives
www.lib.ucdavis.edu/dept/animalalternatives

awic.nal.usda.gov
Future collaboration: retrieval of specific, flagged 3R-records from a Unified Repository

accessing references from many of the large databases such as MEDLINE
The world congresses on the 3Rs are important 3R-drivers and disseminators of information: 
wc9prague.org
891 abstracts, 49 countries, 1000 participants
(the next one is in September 2017 in Seattle)

1996: 2nd World Congress on Alternatives and Animal Use in the Life Sciences, Utrecht

1997: Altweb (Alternatives to animals on the web)
http://altweb.jhsph.edu
International consensus meetings

Harmonisation of the Care and Use of:
  Fish (2005)
  Wildlife (2008)
  Fish (2009)
  Agricultural animals (2012)

http://norecopa.no/consensus-meetings

All presentations and consensus statements are on the internet: a lasting resource
Guidelines as a portal to more information

R Johansen, JR Needham, DJ Colquhoun, TT Poppe & AJ Smith

Guidelines for health and welfare monitoring of fish used in research

Laboratory Animals, 2006, 40: 323-340
http://www.lal.org.uk/pdffiles/GuidelinesFish.pdf

For a global view of guidelines, see 3R Guide:

Expert Working Group report on severity classification

Methods of positioning fish for surgery or other procedures out of water

Trond Brattelid & Adrian J. Smith
Laboratory Animal Unit, Norwegian School of Veterinary Science, PO Box 8146 Dep., N-0033 Oslo, Norway
We need more guidelines for specific research areas

Guidance on the severity classification of procedures involving fish

P Hawkins, N Dennison, G Goodman, S Hetherington, S Llywelyn-Jones, K Ryder and AJ Smith

Laboratory Animals, 45: 219-224, 2011

www.norecopa.no/categories
Position Statements and Guidelines

- Food deprivation
- Toe clipping
- Pain relief
- Fin clipping of fish
- Biometric methods of identification
- Methods for identification of birds
Animals used for scientific purposes

Retrieval and provision of information on the "Three Rs" and alternatives

Accessing accurate, relevant and up-to-date information on the Three Rs is a challenge for all those using animals.

Legislation and implementation

EU legislative framework
Implementation of Directive 2010/63/EU
Q&A and guidance documents

Opinions of European Commission Expert Committees related to the use of animals in experiments
European Directive, Article 47: 3R-alternative approaches

1. The Commission and Member States shall contribute to the development and validation of 3R-alternatives, and encourage research in this field

2. Member States shall assist the Commission in identifying laboratories for validation studies

3. The Commission shall set the priorities for these studies and allocate tasks

4. Member States shall promote alternatives and disseminate information on them

5. Member States shall nominate a single point of contact to provide advice on the regulatory relevance and suitability of alternatives proposed for validation (PARERE: Preliminary Assessment of Regulatory Relevance)
Journals

ATLA (Alternatives to Laboratory Animals)
Animal Welfare (UFAW)
ILAR Journal
Laboratory Animals
Comparative Medicine

See www.3RGuide.info for more
It doesn’t have to be the latest issue or most recent report...

Humane Endpoints for Animals Used in Biomedical Research and Testing

* Contents
* Front Matter

http://ilarjournal.oxfordjournals.org
Email discussion groups

e.g.  CompMed + archive
     LAREF
     VOLE
     Local competent persons...

See www.3RGuide.info for more
FRAME Training Schools

Portugal, 30 March-1 April 2015
Norway, February 2016

www.frame.org.uk/training-schools

NC3Rs website

http://nc3rs.org.uk/experimental-design
Guidelines for the Design and Statistical Analysis of Experiments Using Laboratory Animals
http://ilarjournal.oxfordjournals.org/content/43/4/244.full

NC3Rs Experimental Design Assistant (EDA)
http://nc3rs.org.uk/experimental-design-assistant-eda

TextBase

Your search for Textbase publications containing the text "design" in the title returned the following results (13 items, page 1 of 1):

1. The Design of Animal Experiments: Reducing the Use of Animals in Research Through Better Experimental Design. By Festing, Michael F.W., Ovendom, Philip, Dau, Rose, Games, Dorja, Marie Cortina & Berotoy, Manuel (2002). This handbook is aimed at all research scientists who use laboratory animals, with the aim of helping them to design their own experiments more effectively and/or to improve their ability to communicate with professional statisticians when designing more complex experiments.

http://www.uk.sagepub.com/books/Book242188?sitId=sage-uk&prodTypes=any&q=9781853155130

oslovet.norecopa.no/textbase