
PAIN

SUFFERING

DISTRESS

LASTING HARM

# Implementation of the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) Guidelines for Zebrafish Research.

BATESON CENTRE ZEBRAFISH FACILITY
THE UNIVERSITY OF SHEFFIELD
UK

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# Aquarium Manager

- Postdoctoral Zebrafish Research Associate, Philip Ingham Laboratory 2000-2008
- Aquarium Manager 2008-present day
- Named Animal Care and Welfare Officer 2008-present day

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# Implementation of the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) Guidelines for Zebrafish Research.

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

https://www.nc3rs.org.uk/

- commissioned a survey reviewing reporting on animal experimentation in publications (271).

All publically funded biomedical studies in UK and US

Only 60% stated number and characteristics

Only 70% used statistical methods

Most (88%) did not use randomisation
(86%) did not use blinding

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# Webinar Contents

## Introductions and background

- Overview of the Bateson Centre Zebrafish Facility
- The iCARE role
- The 3Rs in brief

## The Official Information

- ARRIVE guidelines
- 2016 “Surge in support/Failure to implement”

## Managing ARRIVE

- How to manage the ARRIVE guidelines in your facility
- Individual study plans
- Experimental challenges
- Useful websites and links

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# Zebrafish Facility


TECNIPLAST


AQUANEERING



FAIRFIELD (GLASS)

TECNIPLAST

Home to > 4000 tanks ranging from 1 litre – 15 litres

5 holding rooms
- -3 main rooms
- -1 quarantine
- -1 behaviour analysis room

Team of 8 (6.7 FTE) technicians

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## Level of research

> 770 lines
390 transgenics
140 mutants
240 both
6 wild types

2014 70 new lines
2015 100 new lines
2016 14 new lines to date

>20 Project licences

Approx. 25 groups

>80 Personal licence holders

>160 registered users

Important point is 50 % users are not formally trained.

Dangerous situation for husbandry and compliance.

Research management is essential.

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# iCARE Institutional Care and Animal welfare REsponsible (person)

- High standards of husbandry and care
- Culture of care
- Communication and diplomacy skills
- Good judgement – balance scientific and welfare needs
- Understanding of scientific procedures
- Input to the Animal Welfare Body (AWB)
- Work closely with the Designated Veterinarian (DV)
- Unbiased research input
- Implement good record keeping

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## The 3Rs are internationally established principles.

| Replacement | Reduction | Refinement |
| --- | --- | --- |
| Methods which avoid or replace the use of animals | Methods which minimise the number of animals used per experiment | Methods which minimise suffering and improve animal welfare |

Guiding principles underpinning the humane use of animals in scientific research.

Good science and good animal welfare go hand in hand

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# 3Rs for Zebrafish - Historical point of view

"The zebrafish is a lower neuro-physiological vertebrate, which can replace mammalian models."

"To date mainly unprotected larval or embryonic forms have been used"

"There is less ethical concern for keeping fish in laboratories, their mental and physical concerns may be deemed less then those of a rodent for example."

3Rs have concentrated on welfare and husbandry and not experimental design.

"Remember they may shoal but they are all individuals!"

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# 3Rs for Zebrafish - Current point of view

Zebrafish are no longer just a replacement for mammals, they need 3R considerations of their own.

Significant progress has been made using zebrafish as a model of disease.

Protected animals are increasingly important in research.

Recommendations for advanced husbandry techniques are emerging.

Refinements are increasingly being identified and implemented, such as

- anaesthesia
- analgesics
- health status
- environmental enrichment
- acclimatisation techniques
- behavioural analysis

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National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

# The ARRIVE guidelines:

## *Animal Research: Reporting of In Vivo Experiments*

Pioneering Better Science

# ARRIVE Guidelines

- What are the ARRIVE guidelines?
- Why were the ARRIVE guidelines developed?
- Why do we need to improve the reporting of animal research?
- Who supports the ARRIVE guidelines?
- How can you use the ARRIVE guidelines?
- Why should you use the ARRIVE guidelines?
- What resources are available?
- Conclusions

NC 3Rs

# What are the ARRIVE guidelines?

The ARRIVE guidelines were developed as part of an NC3Rs initiative to improve the reporting of biomedical research using animals.

The ARRIVE guidelines consist of a checklist of 20 items, containing key information necessary to describe a study comprehensively and transparently.

The ARRIVE guidelines can be used to ensure reproducibility of animal research and avoid unnecessary animal use.


www.nc3rs.org.uk/ARRIVE

# Why were the ARRIVE guidelines developed?

- The ARRIVE guidelines were proposed following an extensive review on the reporting of animals in research (Kilkenny et al., 2009). This was the largest survey of the quality of reporting of publically funded animal research in the UK and US.

OPEN ACCESS Freely available online PLoS one

**Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals**

Carol Kilkenny[1]*, Nick Parsons[2], Ed Kadyszewski[3], Michael F. W. Festing[4], Innes C. Cuthill[5], Derek Fry[6], Jane Hutton[7], Douglas G. Altman[8]

- The survey identified key areas for improvement:

| Experimental design | Statistical analysis | Reporting of studies |
|---|---|---|
| Most papers did not report randomisation (88%) or blinding (86%) to reduce bias in animal selection and outcome measurements. | Only 70% of publications fully described statistical methods and presented the result with a measure of variability. | Only 59% included three important pieces of information: hypothesis, number of animals and characteristics of animals. |

- The ARRIVE guidelines were created in response to this survey to improve the reporting of animal research.

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ARRIVE

# Why do we need to improve the reporting of animal research?

- Improved reporting is needed to maximise information published and minimise unnecessary animal studies leading to improved translation of pre-clinical research.

- Failures in reporting of animal research have been demonstrated in a variety of research fields.

**Cancer**

Hess, KR. Statistical design considerations in animal studies published recently in Cancer Research. *Cancer Research* (2011) 71:625.

**Stroke**

Macleod, MR et al., Systematic review and metaanalysis of the efficacy of FK506 in experimental stoke. *Journal of Cerebral Blood Flow & Metabolism* (2005) 1-9.

**Pain**

Rice, ASC et al., Animal models and the prediction of efficacy in clinical trials of analgesic drugs: A critical appraisal and call for uniform reporting standards. *Pain* (2008) 139(2):243-7.

**Multiple sclerosis**

Vesterinen, HM et al., Improving the translational hit of experimental treatments in multiple sclerosis. *Multiple Sclerosis* (2010) 16(9): 1044-55.

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# Who supports the ARRIVE guidelines?

The ARRIVE guidelines are endorsed by journals, funders and learned societies.

## Journals





Over 400 journals have incorporated the ARRIVE guidelines in their Instructions to Authors

## Funders





The major funding bodies of biomedical research in the UK support the ARRIVE guidelines.

## Universities








Universities endorse the ARRIVE guidelines by encouraging staff and students to use the guidelines.

## Learned Societies






A growing number of learned societies endorse the ARRIVE guidelines and share the guidelines with their members.

NC 3Rs

# How can you use the ARRIVE guidelines?

The guidelines can be used when reporting research. In brief, the ARRIVE guidelines include the following:

## Title

1. Accurate & concise description

## Abstract

2. Background, objectives, methods, key findings and conclusions

## Introduction

3. Background
4. Objectives

## Methods

5. Ethical statement
6. Study design (blinding/randomisation)
7. Experimental procedures (How? When? Where? Why?)
8. Experimental animals (species, sex, weight)
9. Housing and husbandry
10. Sample size
11. Allocation experimental groups
12. Experimental outcomes
13. Statistical methods

## Results

14. Baseline Data
15. Numbers Analysed
16. Outcomes & estimation
17. Adverse events

## Discussion

18. Interpretation & implications
19. Generalisability and translation
20. Funding

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For a full description, see the 20-point check list at www.nc3rs.org.uk/ARRIVE

ARRIVE

# Why should you use the ARRIVE guidelines?

The ARRIVE guidelines can help the reporting of your research to be:

- Reproducible
- Transparent
- Accurate
- Comprehensive
- Concise
- Logically ordered
- Well written

The ARRIVE guidelines can be used when:

- Writing a manuscript
- Preparing a PhD thesis
- Designing experiments

The ARRIVE guidelines can help promote the 3Rs by ensuring maximal output from animal experiments and reduce the need for excessive animal use.

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ARRIVE

# What resources are available?

The following resources are available :

## Checklist

A checklist that can be used when writing manuscripts to record each item of the ARRIVE guidelines.

## Examples

Examples for each point of the ARRIVE guidelines demonstrating how they can be used in practice to report animal research across a variety of research fields.

## Presentation

A copy of this presentation and accompanying speaker notes can be used as reference.

Resources can be downloaded by visiting www.nc3rs.org.uk/ARRIVE

## Pocket Guidelines

A handy pocket sized reference guide of the ARRIVE guidelines, available upon request from the NC3Rs


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ARRIVE

# Conclusions & Further Information

The ARRIVE guidelines are designed to improve reproducibility and reporting standards.

To download the ARRIVE guidelines and for further information, please visit:

## www.nc3rs.org.uk/ARRIVE

ARRIVE

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*, *8*(6), e1000412. doi:10.1371/journal.pbio.1000412

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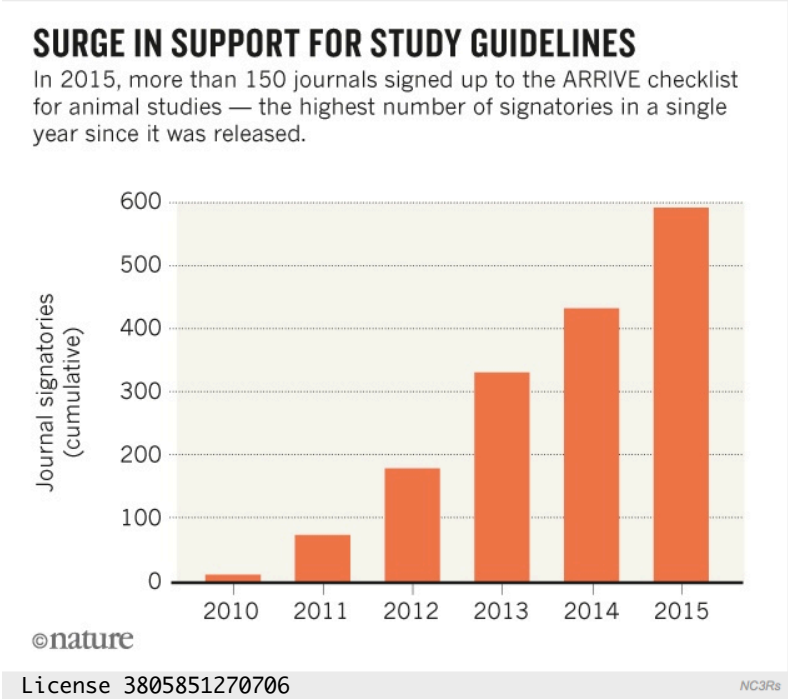
# 2016 “Surge in support/Failure to implement”

## Endorsement unfortunately does not mean enforcement




More than 600 research journals voluntarily enforce guidelines to improve the reporting of animal experiments.

Scientists still repeatedly pointed out that many published papers on animal studies suffer from poor study design and sloppy reporting – leaving the research at a substantial risk of bias.


“Voluntary guidelines are very important, but journals need to take tangible steps to implement them with standards flexible enough to work for a broad range of studies,” says *Nature*’s Editor-in-Chief

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# How to manage the ARRIVE guidelines in your facility

## iCARE roles in research management (welfare accepted)

1. Good judgement – balance scientific and welfare needs
2. Understanding of scientific procedures
3. Unbiased research input
4. Implement good record keeping

## Implementation

1. Help with experimental design
   - Project Licence
   - Personal Licence
   - Individual discussion
   - Individual Study Plan
2. Institutional committees (cross facility)
   - NACWO meetings
   - 3Rs committee
   - Ethical Review Process
3. Senior Statistical Consultant: Experimental design expert

# Individual Study Plans

Experimental design/ARRIVE guidelines

Works closely with manager/NACWO on severity limits

Critical thinking is developed

Controls are discussed and conducted alongside experiment

Husbandry comparison is discussed

Husbandry and welfare are considered

Informed decision making is explained

Appropriate end point is planned

# INDIVIDUAL STUDY PLAN (ISP)

| | |
|---|---|
| ISP reference (e.g. JMB 1) This must be written on the cage card | |
| PPL number | |
| Name of PPL holder | |
| Protocol number (or 19b number) and severity | |
| PIL/Researcher name(s) | |

| Animal holding room | Start date of experiment | End date |
|---|---|---|
| | | |

**CONTACT DETAILS** (of all PILs working under this ISP and the PPL holder, plus anyone else who may be contacted in an emergency, e.g. to collect tissues):

| Name | Email address | Phone number | Emergency/out-of-hours phone number |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

Indicate a preferred method of contact in an emergency using a #

**ANIMALS:**

| Species/strain | Number of animals per group | Number of groups |
|---|---|---|
| | | |

**HUSBANDRY:**

If animals need altered husbandry or extra requirements from the animal care staff, provide details:

**AIM OF THE EXPERIMENT:** Describe briefly, in a few sentences only

**EXPERIMENT DETAILS:**

Describe (using lay terms) what will happen to **these** animals during **this** experiment, using bullet points as much as possible. Include: time-frame (e.g. on day 1 ….), names of drugs/substances, volumes, routes, surgical summary and control animals (do NOT cut and paste from the PPL)

**ADVERSE EFFECTS and END-POINTS** (for **these** animals):

| | |
|---|---|
| Expected adverse | |

**UNEXPECTED DEATHS:**

| |
|---|
| If an animal reaches its end-point or has to be culled on welfare grounds and the researcher is unavailable, describe how you would like the cadaver to be stored (e.g. fridge, freezer) and any tissues to be taken: |
| |

**HEALTH AND SAFETY:**

I have filled out a COSHH risk assessment for this work (delete as applicable) ..... YES ..... NO →

**SIGNATURES:**

Contact details
Licencing details
Experimental detail
Husbandry considerations

Experiment location and dates
Aim of the experiment
Adverse effects and end-points
Health and safety

# Problems getting experimental information for previous publications

- Availability of publications
- Referencing previous journals
- Lack of experimental details
- Methods refer to confidential/national programmes of work
- Refinements are often hidden within publications
- Methodology papers are more scarce

Difficult to implement comments such as

"All efforts were made to minimise animal suffering and the number of animals used"

"Fish were anaesthetised using Tricaine (Sigma)"

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# Problems optimising experimental information without published methodology

Acclimitisation is extremely important before many experiments
- drug screens
- drug dosing
- behavioural testing
- cortisol measuring

Chemicals maybe absorbed by plastic so glass is required

Some chemicals may need to be metabolised e.g. tamoxifen

Immersion of older fish in drugs is usually problematic

Some chemicals may need to be injected or fed to the fis

Zebrafish are potentially too small for repeated injections

# Concluding remarks

The iCARE role implies that facility management should assist in the implementation of the ARRIVE guidelines

Individual study plans can be designed by researchers to help collect data in line with ARRIVE and promote good experimental design

The ARRIVE guidelines promote the 3Rs (replacement, refinement, reduction)

The ARRIVE guidelines are widely endorsed but rarely enforced

# Useful websites

National Centre for the Replacement Refinement & Reduction of Animals in Research

https://www.nc3rs.org.uk/

http://www.understandinganimalresearch.org.uk/

http://science.rspca.org.uk/sciencegroup/home

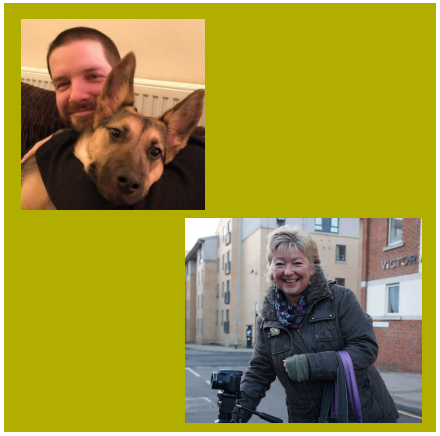
Home Office

https://www.gov.uk/guidance/research-and-testing-using-animals

European Commission

http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

# Many thanks from Sheffield

Understanding ANIMAL RESEARCH

RSPCA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Home Office