

*Addressing the needs for refinement in laboratory fish  
NC3Rs/Cefas, Weymouth, 26-28 April 2016*

## ***3Rs resources and guidelines for zebrafish***

[\*norecopa.no/zebrafish.pdf\*](http://norecopa.no/zebrafish.pdf)

Adrian Smith

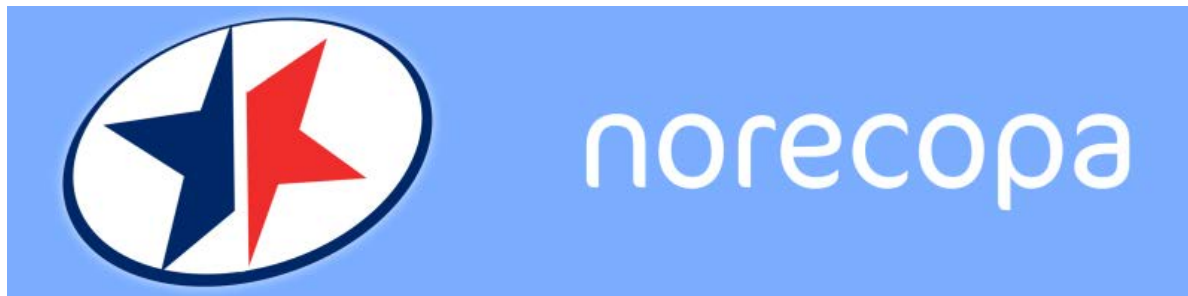
[\*adrian.smith@norecopa.no\*](mailto:adrian.smith@norecopa.no)

[\*www.norecopa.no\*](http://www.norecopa.no)



norecopa

National Consensus Platform for the  
Replacement, Reduction and Refinement of  
Animal Experiments



**a competence centre for the 3RS**

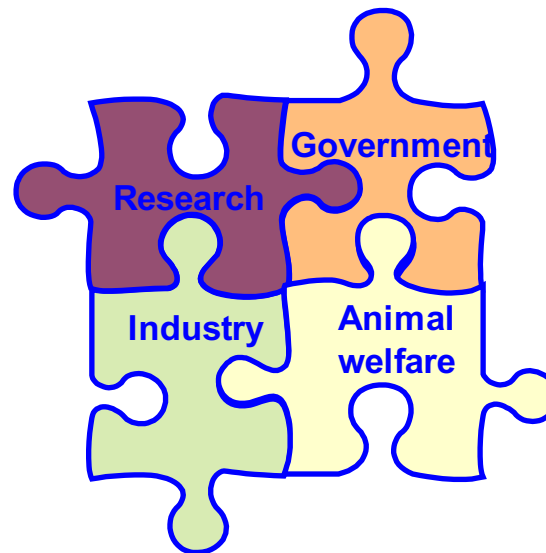
**Norecopa** is a member of **ecopa**

*European Consensus-Platform for Alternatives*

[www.ecopa.eu](http://www.ecopa.eu)



ecopa supports the establishment of National Consensus Platforms (NCPs) where all 4 stakeholders are equally represented:



## ***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*

## **An overview of existing guidelines for handling, bleeding, administration and identification techniques**

Penny Hawkins, Research Animals Department, RSPCA

[www.norecopa.no/norecopa/vedlegg/Fish-guidelines.pdf](http://www.norecopa.no/norecopa/vedlegg/Fish-guidelines.pdf)

## **Global update on guidelines for fish research**

Gilly Griffin, Canadian Council on Animal Care (CCAC)

[www.norecopa.no/fish2009](http://www.norecopa.no/fish2009)

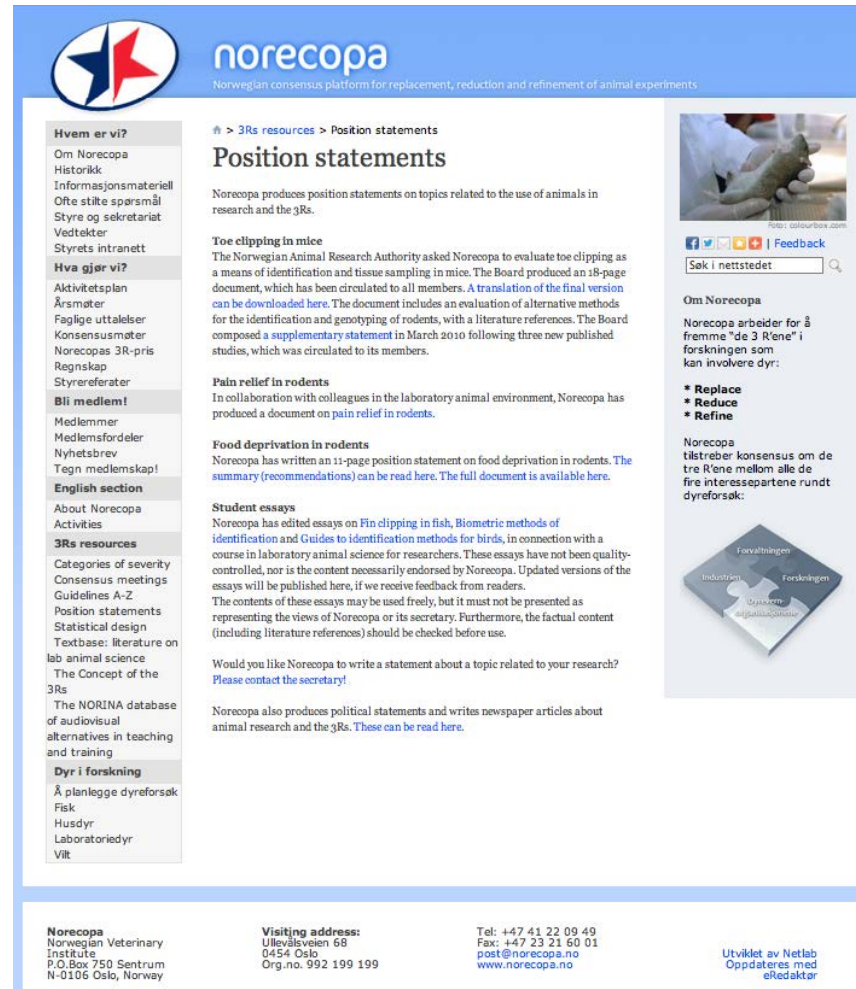
## **Guidelines for anaesthesia and analgesia of fish**

Gidona Goodman, University of Edinburgh

[www.norecopa.no/fish2009](http://www.norecopa.no/fish2009)

# Position Statements and Guidelines from Norecopa

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



The screenshot shows the Norecopa website with a blue header. The logo is a red and blue star. The text reads "norecopa Norwegian consensus platform for replacement, reduction and refinement of animal experiments".

**Hvem er vi?**  
Om Norecopa  
Historikk  
Informasjonsmaterieill  
Ofte stilte spørsmål  
Styre og sekretariat  
Vedtekter  
Styrets intranett

**Hva gjør vi?**  
Aktivitetsplan  
Årsmøter  
Faglige uttalelser  
Konsensumøter  
Norecopas 3R-pris  
Regnskap  
Styrereferater

**Bli medlem!**  
Medlemmer  
Medlemsfordeler  
Nyhetsbrev  
Tegn medlemskap!

**English section**  
About Norecopa  
Activities

**3Rs resources**  
Categories of severity  
Consensus meetings  
Guidelines A-Z  
Position statements  
Statistical design  
Textbase: literature on lab animal science  
The Concept of the 3Rs  
The NORINA database of audiovisual alternatives in teaching and training

**Dyr i forskning**  
Å planlegge dyreforsøk  
Fisk  
Husdyr  
Laboratoriedyr  
Vilt

**» > 3Rs resources > Position statements**  
**Position statements**  
Norecopa produces position statements on topics related to the use of animals in research and the 3Rs.

**Toe clipping in mice**  
The Norwegian Animal Research Authority asked Norecopa to evaluate toe clipping as a means of identification and tissue sampling in mice. The Board produced an 18-page document, which has been circulated to all members. A translation of the final version can be downloaded here. The document includes an evaluation of alternative methods for the identification and genotyping of rodents, with a literature references. The Board composed a supplementary statement in March 2010 following three new published studies, which was circulated to its members.

**Pain relief in rodents**  
In collaboration with colleagues in the laboratory animal environment, Norecopa has produced a document on pain relief in rodents.

**Food deprivation in rodents**  
Norecopa has written an 11-page position statement on food deprivation in rodents. The summary (recommendations) can be read here. The full document is available here.

**Student essays**  
Norecopa has edited essays on Fin clipping in fish, Biometric methods of identification and Guides to identification methods for birds, in connection with a course in laboratory animal science for researchers. These essays have not been quality-controlled, nor is the content necessarily endorsed by Norecopa. Updated versions of the essays will be published here, if we receive feedback from readers. The contents of these essays may be used freely, but it must not be presented as representing the views of Norecopa or its secretary. Furthermore, the factual content (including literature references) should be checked before use.

Would you like Norecopa to write a statement about a topic related to your research? Please contact the secretary!

Norecopa also produces political statements and writes newspaper articles about animal research and the 3Rs. These can be read here.

**Om Norecopa**  
Norecopa arbeider for å fremme "de 3 R'ene" i forskningen som kan involvere dyr:  
\* Replace  
\* Reduce  
\* Refine  
Norecopa tilstreber konsensus om de tre R'ene mellom alle de fire interessepartene rundt dyreforskning.

**Utviklet av Netlab**  
Oppdateres med eKodaker

**Norecopa**  
Norwegian Veterinary Institute  
P.O. Box 750 Sentrum  
N-0106 Oslo, Norway

**Visiting address:**  
Ullevålsveien 68  
0454 Oslo  
Org.no. 992 199 199

**Tel:** +47 41 22 09 49  
**Fax:** +47 23 21 60 01  
**post@norecopa.no**  
**www.norecopa.no**

**Compendium  
in  
Laboratory Animal  
Science  
for  
Fish Researchers**

edited by  
Trond Brattelid & Adrian J. Smith



**Norwegian School of Veterinary Science  
& Norecopia  
June 2011**



## TRAINING SCHOOL IN EXPERIMENTAL DESIGN & STATISTICAL ANALYSIS OF BIOMEDICAL EXPERIMENTS

FRAME Training School  
in Norway,

1 – 3 February 2016



Joining Information

<http://www.frame.org.uk/training-schools>

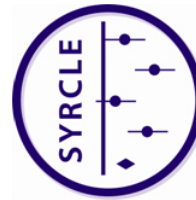


# *Systematic Reviews and Harm-Benefit Assessment*

*Voss, 27 – 28 May 2015*

*How to perform a systematic search of the scientific literature*

*Alice Tillema, Radboud University, Nijmegen*

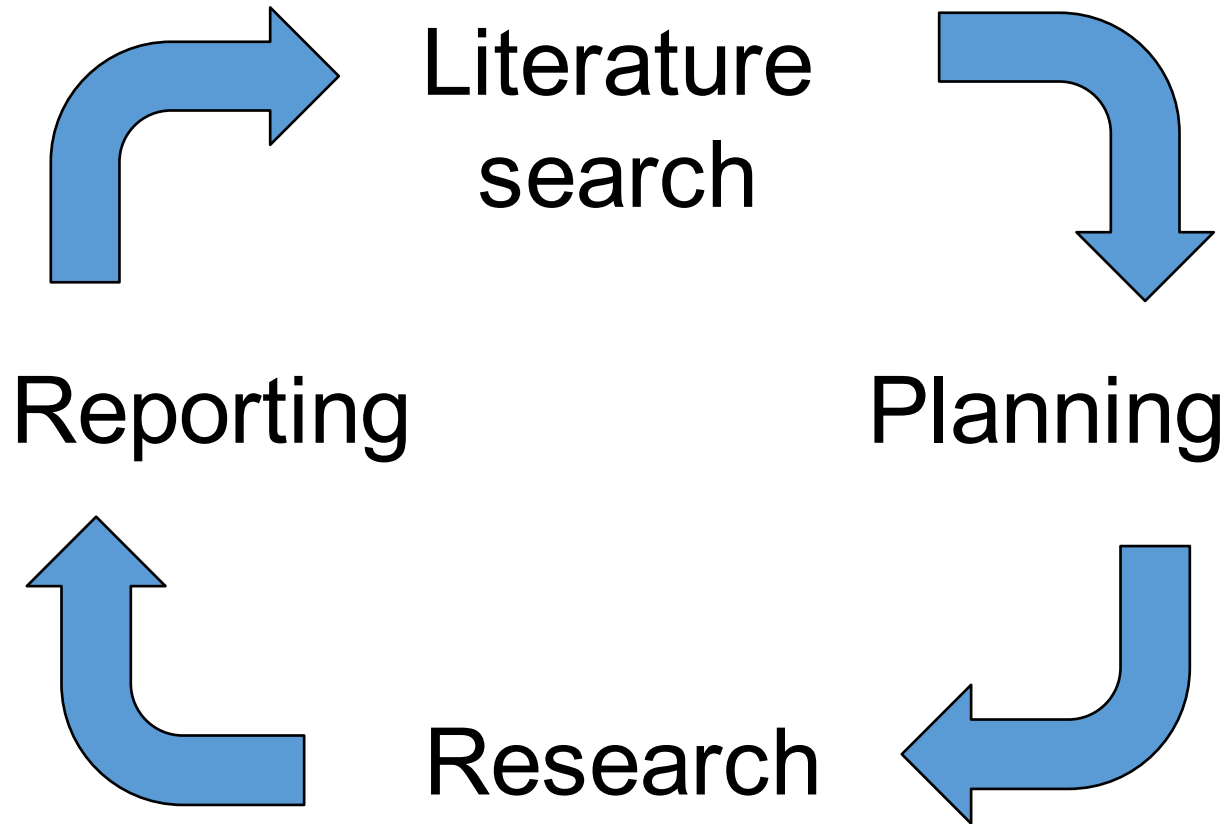


*My background (not necessarily like yours!)*

*A lab animal vet helping scientists implement the legislation while working with a wide range of species – many common denominators / minefields:*

- *Biology and physiology of the species*
- *Pharmacology of drugs in that species*
- *Housing and welfare requirements*
- *Procedures and choice of technique*
- *3R alternatives*
- *Literature searching*
- *Experimental design and statistical analysis*
- *Severity assessment and Humane endpoints*
- *Harm-Benefit Assessment*
- *Reporting (incl. dissemination of 3R knowledge gain)*

*Animal Science is all about quality assurance*



*History repeats itself when developing new models*

*GMO & GMM, nude mice, minipigs, isolators, IVC racks, cleaner fish, zebrafish*

*Two sets of “guidelines” emerge, that address very different questions:*

- Wow that was cool, how do you do it?*
- Hmm, should we really be doing this?*

*The first group are more **technical specifications** than **guidelines** in the 3R sense...*

*The second set tend to be running fast in an attempt to catch up with the first set.*

## **"Contingent suffering"**

indirect suffering

fear

boredom

transport stress



photo: colourbox.com

## **The lonely mouse**

Male mice housed singly develop symptoms which would be characterised as depression in humans.

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0111065>



## **Zebrafish?**

<http://www.tecniplast.it/usermedia/us/3-5LTankwithFish-small.jpg>

## **Abstract**

Emotional disturbances constitute a major health issue affecting a considerable portion of the population in western countries. In this context, animal models offer a relevant tool to address the underlying biological determinants and to screen novel therapeutic strategies. While rodents have traditionally constituted the species of choice, zebrafish are now becoming a viable alternative. As zebrafish gain momentum in biomedical sciences,

[online.liebertpub.com/doi/pdfplus/10.1089/zeb.2014.1041](http://online.liebertpub.com/doi/pdfplus/10.1089/zeb.2014.1041)

## The problem with animal-based research and development:

- Despite rigorous testing, failures still occur
- Many of these occur at a late stage - sometimes after the drug is on the market
- Many of these late failures lead to serious human disease or death
- Compounds fail in people with several diseases, which are impossible to model in an animal test system
- Can/should zebrafish be used as models in complex area such as behavioural research?



colourbox.com

# Leppefisk mot lakselus: Det store eksperimentet

KIM A. TALLAKSEN HALVORSEN STIPENDIAT VED UNIVERSITETET I AGDER  
OPPDATERT: 18.MAR. 2015 10:24 | PUBLISERT: 16.MAR. 2015 21:44



En grønngylt forsvarer redet sitt. Det er oppdrettsnæringen som selv har skapt luseproblemene, da er det urimelig at leppefisken og kysten vår betaler prisen, skriver artikkelforfatteren.

**Leppefisken skal redde oss fra lakselus. Men nå er den lille lusespiseren selv truet.**



*My claim:*

*We go to different sets of meetings and know relatively little about advances in other relevant areas.*



<https://kmonadollaraday.files.wordpress.com/2011/03/information-silos.jpg>



Jackson Laboratory @jacksonlab · 18h

The JAX mouse room has changed a lot since 1953! #ThrowbackThursday



A mouse room in 1953; note wooden mouse boxes.

*So what do we have for zebrafish...?*

*What qualifies as a guideline? Who decides? Every IACUC protocol?*

*Norecopa had that question in mind when we produced our database **3R Guide** ([3RGuide.info](http://3RGuide.info)).*



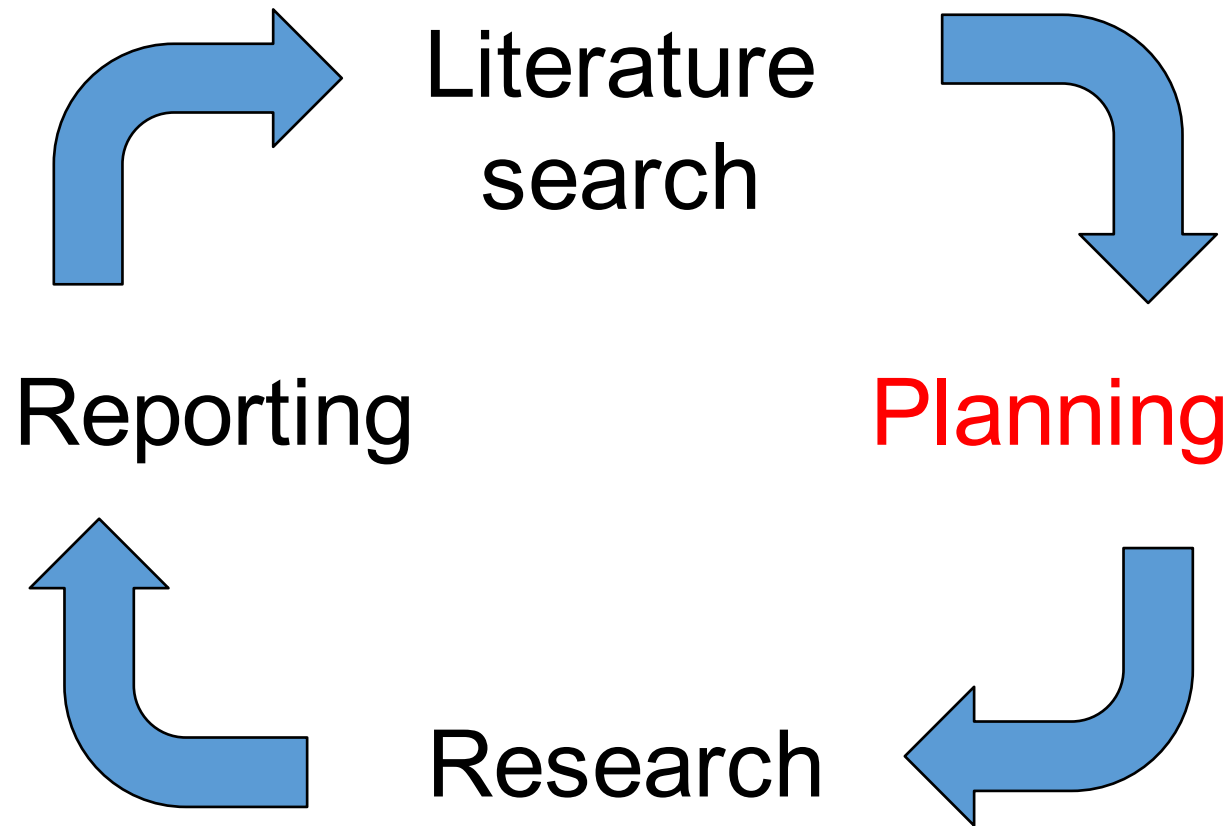
FONDAZIONE GUIDO BERNARDINI  
BETTER EDUCATION FOR BETTER SCIENCE

# Zebrafish: Husbandry, Care and Welfare

## 21st to 22nd of April 2016

### Thursday 21 April

Registration .....	09:00 - 09:45
Welcome and opening addresses.....	09:45 - 10:00
How to Run a Facility (Carole Wilson) .....	10:00 - 10:30
Coffee Break .....	10:30 - 11:00
<b>Current Practices – Practical Sessions and Group Discussion.....</b>	<b>11:00 - 12:00</b>
Housing (Heather Callaway)	Handling and Sexing (Jenna Hakkesteeg)
Feeding (Matt Wicks)	Environment Enrichment (Karen Dunford)
Recommendations and Guidelines (Carole Wilson).....	12:00 - 12:30



# ***What does Europe have to offer?***

## ***European Convention ETS123 for the Protection of Vertebrate Animals Used for Scientific or Other Purposes***

'The Convention is designed primarily to reduce both the number of experiments and the number of animals used for such purposes... Animals to be experimented on should be selected on the basis of clearly established quantitative criteria and must be well cared for and spared avoidable suffering whenever possible.'

### ***Appendix A: Guidelines for the accommodation and care of animals (revised, with species-specific guidelines from 2007).***

'Species-specific guidance on rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), tilapiine cichlids, zebra fish (*Danio rerio*), sea bass (*Dicentrarchus labrax*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic cod (*Gadus morhua*), turbot (*Scophthalmus maximus*), African catfish (*Clarias gariepinus*) is available in the background document elaborated by the Group of Experts.

*Further advice on the requirements of these and other species should be sought from expert specialists and care staff to ensure that any particular species needs are adequately addressed.'*

The FELASA library contains documents other than workinggroup reports, guidelines, recommendations or policy documents. These can be found under the respective tabs.

**Part B of Appendix A:  
Scientific background.  
No Part B for fish!**

>> ETS123 - Appendix A: guidelines for accommodation and care of animals (adopted version).  
Background information on the draft proposal for species-specific provisions presented by Groups of Experts for: amphibians, birds, cats, dogs, ferrets, non-human primates, reptiles, rodents and rabbits.

<http://www.felasa.eu/about-us/library/>

# EUROGUIDE

On the accommodation  
and care of animals used  
for experimental and  
other scientific purposes

BASED ON THE REVISED APPENDIX A  
OF THE EUROPEAN CONVENTION ETS 123

Published in conjunction with



The European Partnership  
for Alternative Approaches to Animal Testing

[http://www.felasa.eu/media/uploads/Euroguide\\_official\\_publication.pdf](http://www.felasa.eu/media/uploads/Euroguide_official_publication.pdf)

7 pages mention Zebra finches, nothing on Zebrafish...



## **EU Directive 2010/63 on the Protection of Animals Used for Scientific Purposes**

Detailed stocking densities for

- *Aquatic urodeles (salamanders, newts)*
- *Aquatic anurans (frogs, toads)*
- *Semi-aquatic anurans*
- *Semi-terrestrial anurans*
- *Arboreal anurans*
- *Aquatic chelonians (tortoises, turtles)*
- ...

### 10. Reptiles

Table 10.1.  
Aquatic chelonians

Body length (*) (cm)	Minimum water surface area (cm <sup>2</sup> )	Minimum water surface area for each additional animal in group holding (cm <sup>2</sup> )	Minimum water depth (cm)	Date referred to in Article 33(2)
up to 5	600	100	10	1 January 2017
Over 5 to 10	1 600	300	15	
Over 10 to 15	3 500	600	20	
Over 15 to 20	6 000	1 200	30	
Over 20 to 30	10 000	2 000	35	
Over 30	20 000	5 000	40	

(\*) Measured in a straight line from the front edge to the back edge of the shell.

*EU Directive 2010/63 on the  
Protection of Animals Used for  
Scientific Purposes*

**ETS 123 again!**

***Further advice on the requirements of  
these and other species should be  
sought from expert specialists and  
care staff to ensure that any  
particular species needs are  
adequately addressed.***

## “Fish” (all 30,000 spp.) !

### 11. Fish

#### 11.1. Water supply and quality

Adequate water supply of suitable quality shall be provided at all times. Water flow in re-circulatory systems or filtration within tanks shall be sufficient to ensure that water quality parameters are maintained within acceptable levels. Water supply shall be filtered or treated to remove substances harmful to fish, where necessary. Water-quality parameters shall at all times be within the acceptable range that sustains normal activity and physiology for a given species and stage of development. The water flow shall be appropriate to enable fish to swim correctly and to maintain normal behaviour. Fish shall be given an appropriate time for acclimatisation and adaptation to changes in water-quality conditions.

#### 11.2. Oxygen, nitrogen compounds, pH, and salinity

Oxygen concentration shall be appropriate to the species and to the context in which the fish are held. Where necessary, supplementary aeration of tank water shall be provided. The concentrations of nitrogen compounds shall be kept low.

The pH level shall be adapted to the species and kept as stable as possible. The salinity shall be adapted to the requirements of the fish species and to the life stage of the fish. Changes in salinity shall take place gradually.

#### 11.3. Temperature, lighting, noise

Temperature shall be maintained within the optimal range for the fish species concerned and kept as stable as possible. Changes in temperature shall take place gradually. Fish shall be maintained on an appropriate photo-period. Noise levels shall be kept to a minimum and, where possible, equipment causing noise or vibration, such as power generators or filtration systems, shall be separate from the fish-holding tanks.

#### 11.4. Stocking density and environmental complexity


The stocking density of fish shall be based on the total needs of the fish in respect of environmental conditions, health and welfare. Fish shall have sufficient water volume for normal swimming, taking account of their size, age, health and feeding method. Fish shall be provided with an appropriate environmental enrichment, such as hiding places or bottom substrate, unless behavioural traits suggest none is required.

#### 11.5. Feeding and handling

Fish shall be fed a diet suitable for the fish at an appropriate feeding rate and frequency. Particular attention shall be given to feeding of larval fish during any transition from live to artificial diets. Handling of fish shall be kept to a minimum.




About this site | Contact | Sitemap | Search | Legal notice | Cookies | English (en)



# ENVIRONMENT

European Commission > Environment > Chemicals > Animals used for scientific purposes

Home About us Policies Funding Legal compliance News & outreach



## 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 use of animals.

- Legislation and implementation
  - EU legislative framework
  - Implementation of Directive 2010/63/EU
    - Q&A and guidance documents
- The "Three Rs" and alternative approaches
  - Replacement, Reduction and Refinement – the "Three Rs"
  - Validation, acceptance and use
  - EU activities to advance alternatives
  - Member State activities to advance alternatives
  - Finding and distributing information on alternatives
  - Key resources
    - Search Tools
    - Databases
    - Portals and web-sites
    - Journals
    - Other resources and organisations

## Animals used for scientific purposes



Opinions of European Commission Expert Committees related to the use of animals in experiments



# Caring for animals

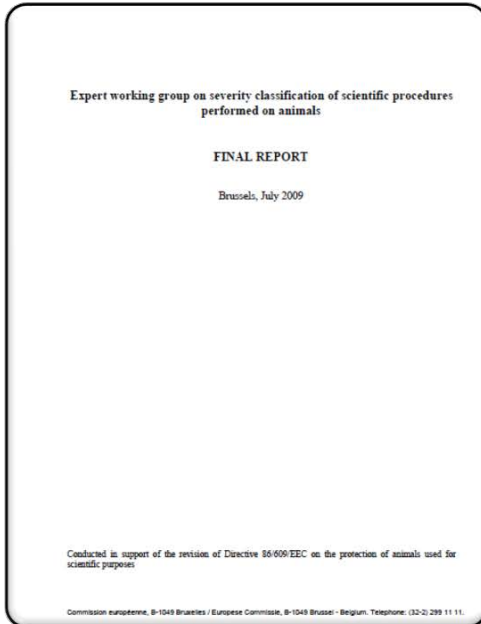
aiming for better science

DIRECTIVE 2010/63/EU  
ON PROTECTION OF ANIMALS USED  
FOR SCIENTIFIC PURPOSES



© Novo Nordisk

Environment



## ***Expert Working Group report on severity classification***

[http://ec.europa.eu/environment/chemicals/lab\\_animals/pdf/report\\_ewg.pdf](http://ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf)



**Guidance on the severity classification of procedures involving fish**

Report from a Working Group convened by Norecopa

Designed to be a supplement to the EU Working Group report on the same subject, which is most relevant for traditional lab animals

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](http://www.norecopa.no/categories)

**Code of Practice for the Housing and  
Care of Animals Bred, Supplied  
or Used for Scientific Purposes**

Refers to RSPCA Guidance

Presented to Parliament pursuant to Section 21 (5) of the  
Animals (Scientific Procedures) Act 1986

<https://www.gov.uk/government/publications/code-of-practice-for-the-housing-and-care-of-animals-bred-supplied-or-used-for-scientific-purposes>



**Guidance on the housing  
and care of  
Zebrafish**  
*Danio rerio*



**Barney Reed & Maggy Jennings**  
Research Animals Department, Science Group, RSPCA

**Guidance on the housing and  
care of the  
African clawed frog**  
*Xenopus laevis*



Barney T Reed

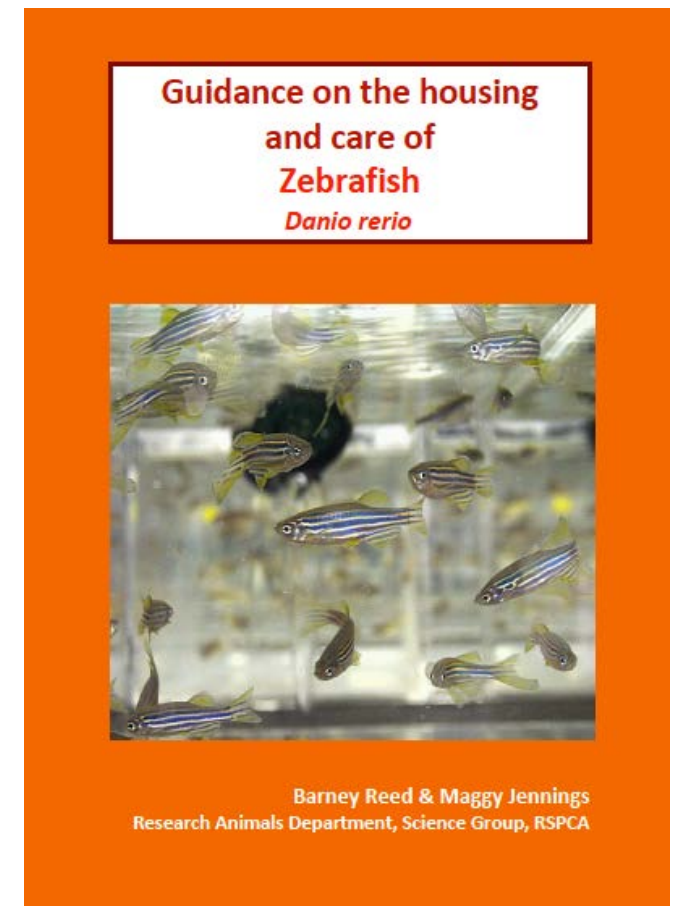
Research Animals Department - RSPCA



This report aims to improve the welfare of zebrafish by:

- facilitating understanding of zebrafish behaviour and thus a better appreciation of their requirements;
- highlighting current potential welfare and ethical concerns relating to the breeding, supply, housing and care of zebrafish;
- arriving, where possible, at consensus based on available evidence and sound scientific argument for appropriate environmental and care conditions for keeping zebrafish in the laboratory environment;
- providing recommendations for improving health, welfare and egg quality, for reducing the potential for stress and suffering, and for reducing the number of animals used;
- in areas where current knowledge is sparse or inconclusive, stimulating discussion and research to identify 'good practice'.

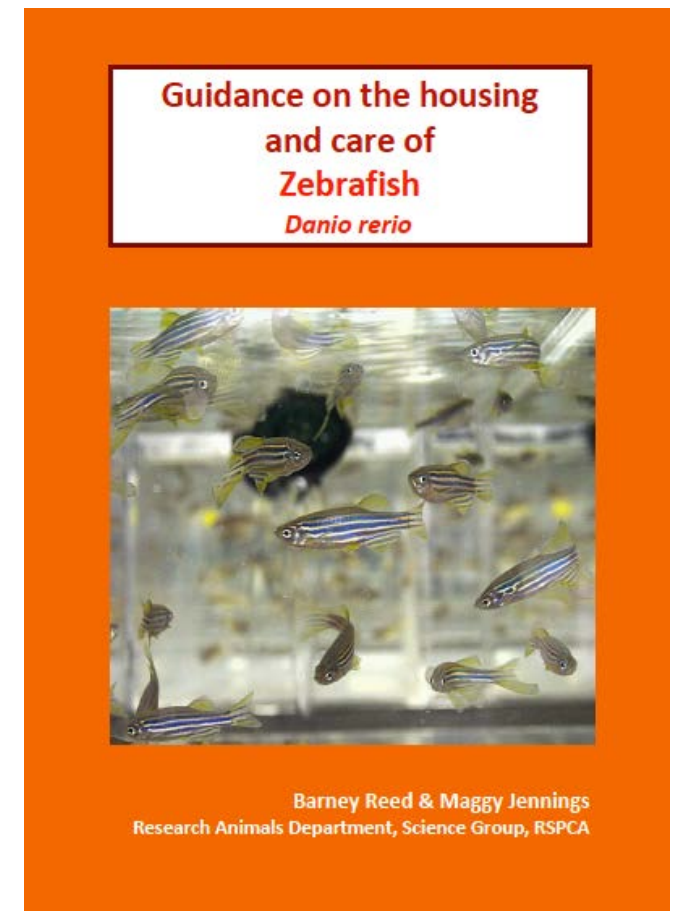
1. Introduction
2. Background information on zebrafish
  - Natural geographic range and habitat
  - Species characteristics
  - Use in research and teaching
3. Supply and transport
  - Source
  - Transport considerations
  - Quarantine
4. Housing and care
  - Lighting
  - Noise and other disturbances
  - Humidity
  - Water provision
  - Tank housing
  - Identification and marking techniques
  - Group housing
  - Catching and handling
  - Food type and feeding regime
  - Environmental enrichment
  - Assess of health and disease prevention

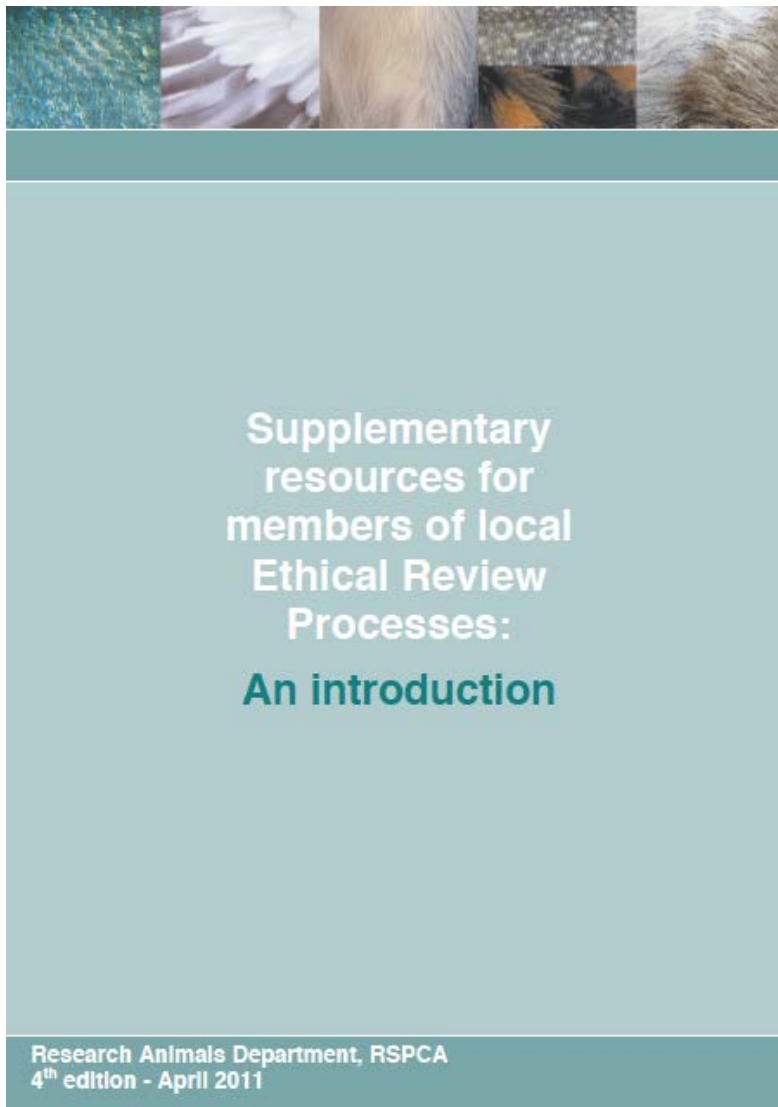


62 pages, including  
8 pages with  
literature references



5. Scientific procedures
  - Egg harvesting
  - Transgenesis
  - Mutagenesis
  - Genotyping
  - Cryopreservation
  - Blood collection
  - Injections
  - Analgesia and anaesthesia
  - Humane killing
6. Training of animal care staff and users
7. Concluding comments
8. References





African clawed frogs (*Xenopus laevis*)

Cattle

Dogs

Domestic fowl

Ducks and geese

Ferrets

Guinea pigs

Hamsters

Mice

Pigeons

Pigs

Quail

Rabbits

Rats

Sheep

Zebra finches

‘They are primarily intended for members of ethical review committees, though should also be useful for animal technologists, scientists and laboratory animal veterinarians’.

<http://science.rspca.org.uk/sciencegroup/researchanimals/reportsandresources/housingandcare>

# FELASA Guidelines under development

## Zebra fish: housing, husbandry, and health monitoring recommendations

This is a joint FELASA/ COST action BM0804 EuFishBioMed.

### Synopsis

The zebra fish (*Danio rerio*) has become a very popular and useful animal model in recent years. Zebra fish can replace mammalian models in a variety of scientific fields. Their mode of reproduction and the transparency of the embryos make them a unique tool for research. Many research institutions are adapting facilities and are educating personnel to be able to work with a species which in most cases is very different of the ones used traditionally. Information in the literature that can help professionals at research institutions to cope with this new challenge is spread and not easy to find. Some of the knowledge is in 'commercial' text about products and is sometimes not scientifically based. Of special interest are housing environment, husbandry practices, including health monitoring, veterinary care and experimental procedures.

<http://www.felasa.eu/working-groups/working-groups-present/zebrafish-housing-husbandry-and-health-monitoring-recommendations>

## Zebrafish 2015

28. June – 02. July / Oslo, Norway



<http://www.ccnorway.no/zebrafish>

*World Congresses on Animal Use in the Life Sciences and  
Alternatives*

*Important 3R-drivers and disseminators of information:*

[wc9prague.org](http://wc9prague.org) (2014)

*891 abstracts, 49 countries, 1000 participants  
(the next one is in August 2017 in Seattle)*

*Abstract book:*

<http://www.altex.ch/ALTEX-Proceedings/Current-Proceedings.97.html>





OPEN Options  
Access Available

**Editor-in-Chief:**  
Stephen C. Ekker, PhD

**Impact Factor:** 1.946  
\* 2014 Journal Citation Reports\* published by Thomson Reuters, 2015

**Frequency:** Bimonthly

**Archival Content:**  
11 Volumes • 46 Issues • 511 Articles

**ISSN:** 1545-8547    **Online ISSN:** 1557-8542

**Manuscript Submission:**  
Average time to first decision: 29 days

**Global Visibility and Reach:**  
More than 170 countries

**Indexed in:**  
MEDLINE, Current Contents, and all key indexing services

## Advancing Biomedical Research with Zebrafish and Other Aquarium Fish Models

2005 -

*Zebrafish* introduced the new section TechnoFish, which highlights these innovations for the general zebrafish community.

### TechnoFish features two types of articles:

- **TechnoFish Previews:** Important, generally useful technical advances or valuable transgenic lines
- **TechnoFish Methods:** Brief descriptions of new methods, reagents, or transgenic lines that will be of widespread use in the zebrafish community

### Zebrafish coverage includes:

- Comparative genomics and evolution
- Molecular/cellular mechanisms of cell growth
- Genetic analysis of embryogenesis and disease
- Toxicological and infectious disease models
- Models for neurological disorders and aging
- New methods, tools, and experimental approaches

## Toward an Integrated Zebrafish Health Management Program Supporting Cancer and Neuroscience Research

Sandra Martins,<sup>1</sup> Joana F. Monteiro,<sup>1</sup> Maria Vito,<sup>1</sup> David Weintraub,<sup>2</sup>  
Joana Almeida,<sup>1</sup> and Ana Catarina Certal<sup>1</sup>

### **Abstract**

Zebrafish is already one of the most used model organisms in biomedical sciences and other research fields. It is therefore becoming increasingly important to assure that zebrafish maintained in laboratory aquaculture conditions are raised and housed under rigorous standards that promote health and welfare to guarantee the required quality and reproducibility of research data. Specifying the programs each facility is adopting would be the first step to achieve this by allowing other facilities to compare, improve, and discuss their protocols and fish performance. We provide in this article a detailed description of an integrated facility health management program, with protocols and readouts, fully designed and aimed at maximizing fish health, welfare, and performance for research.

<http://online.liebertpub.com/doi/pdfplus/10.1089/zeb.2015.1198>





## Abstract

The presence of subclinical infection or clinical disease in laboratory zebrafish may have a significant impact on research results, animal health and welfare, and transfer of animals between institutions. As use of zebrafish as a model of disease increases, a harmonized method for monitoring and reporting the health status of animals will facilitate the transfer of animals, allow institutions to exclude diseases that may negatively impact their research programs, and improve animal health and welfare. All zebrafish facilities should implement a health monitoring program. In this study, we review important aspects of a health monitoring program, including choice of agents, samples for testing, available testing methodologies, housing and husbandry, cost, test subjects, and a harmonized method for reporting results. Facilities may use these recommendations to implement their own health monitoring program.

<http://online.liebertpub.com/doi/pdfplus/10.1089/zeb.2015.1210>



## Guidelines for health and welfare monitoring of fish used in research

R Johansen<sup>1</sup>, J R Needham<sup>1,2</sup>, D J Colquhoun<sup>3</sup>, T T Poppe<sup>4</sup> and A J Smith<sup>1</sup>

<sup>1</sup>Norwegian School of Veterinary Science, Laboratory Animal Unit, PO Box 8146 Dep., 0033 Oslo, Norway; <sup>2</sup>The Microbiology Laboratories, North Harrow, Middlesex HA2 7RE, UK; <sup>3</sup>Section of Fish Health, National Veterinary Institute, PO Box 8156 Dep., 0033 Oslo, Norway; <sup>4</sup>Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, PO Box 8146 Dep., 0033 Oslo, Norway

### Summary

The aim of this paper is to provide background material necessary for the development of international guidelines for the health and welfare monitoring of fish used in research. It provides an overview of present guidelines and discusses why more detailed and species-specific guidelines are needed. A major issue within fish research is to document the situation today and point out areas where improvements are needed.

**Keywords** Fish; health; welfare; monitoring; guidelines

Guidelines for monitoring and reporting the general health status and welfare of fish used in research are sparse compared with those available for mammalian laboratory animals. Despite the fact that there are more fish species than all other vertebrate species combined and that fish are studied in almost all biological disciplines [Powers 1989], most guidelines for fish encompass all species and all types of research [Casebolt *et al.* 1998]. There is a great need for more species-specific guidelines for health and welfare monitoring. In some cases, these guidelines may also have to be specific to the scientific topic where they are to be used.

The number of fish used in research is increasing, due both to the rapid expansion in the fish farming industry and an increased use of fish as model organisms in basic research and chemical testing [Kane *et al.* 1996]. The debate on whether to use fish or mice models started over 25 years ago [Dawe & Couch 1984]. Rodent models are now frequently being replaced by fish models

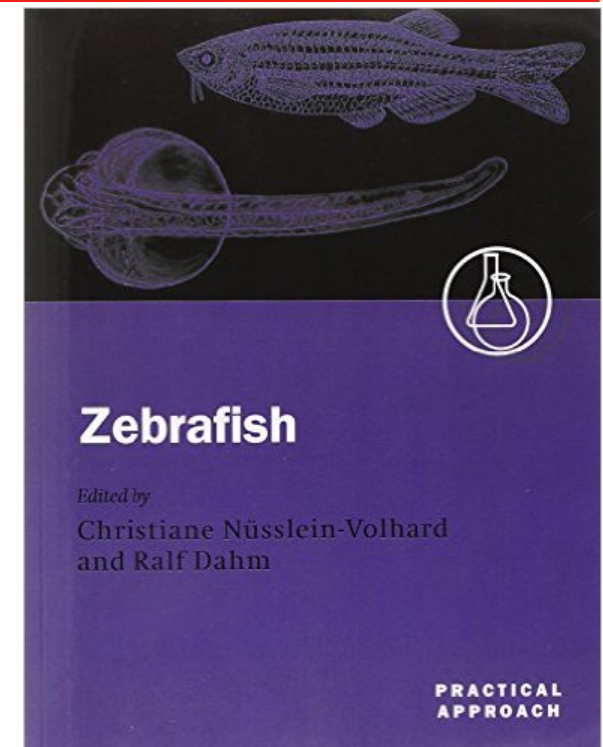
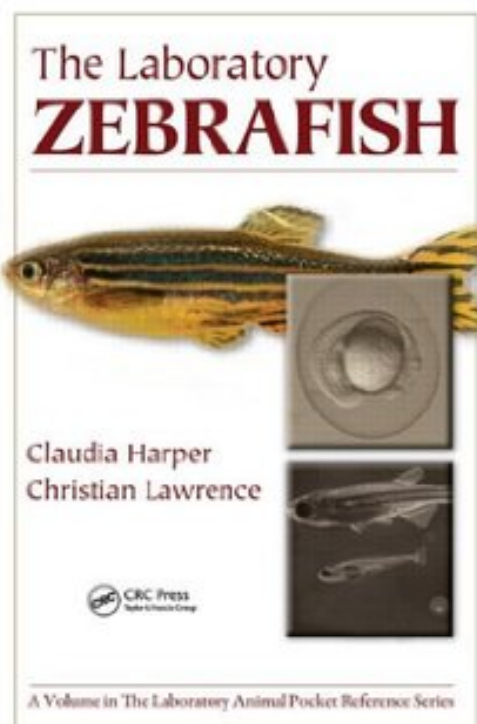
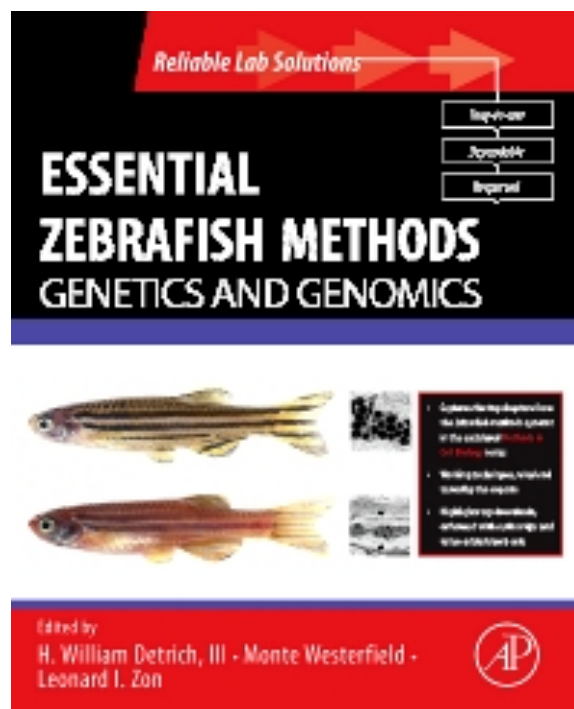
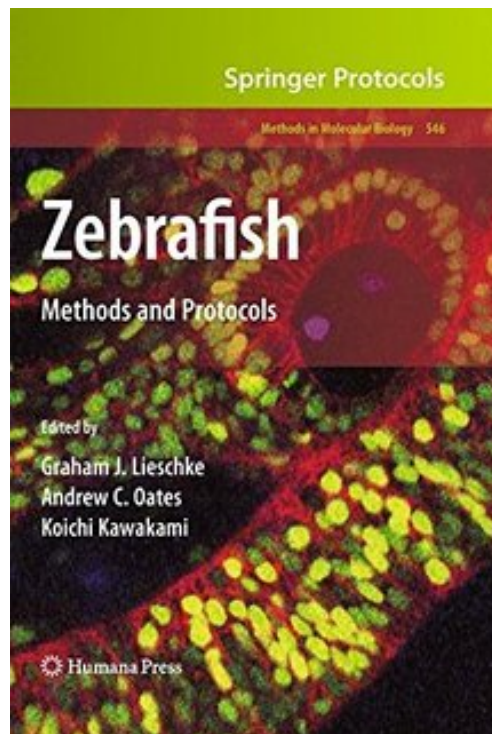
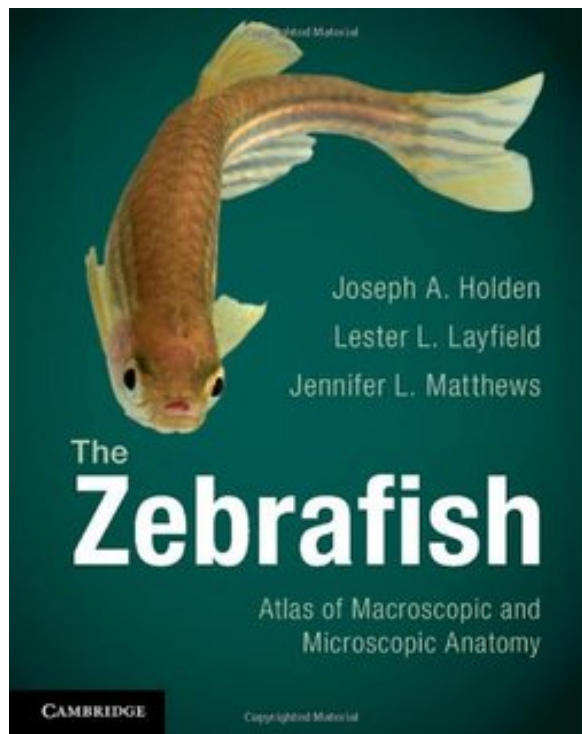
[May *et al.* 1987a, Powers 1989, DeTolla *et al.* 1995].

Guidelines and legislations are often more liberal towards the use of fish than mammals. This can be illustrated by the lack of focus on humane endpoints in fish models [Ryder 2005]. LD<sub>50</sub> testing is, for example, no longer allowed on mammals, but remains in use for fish [Braunbeck *et al.* 2004].

Even the reporting of numbers of fish used, and the type of research for which they are used, is confused by a lack of common international practice. Harmonization in this field is important to avoid the transfer of research from countries with high standards to those with lower ones. In Europe, all fish species and sizes are reported in the same statistical groups and the research disciplines reported are very general. Figure 1 shows, for example, an analysis of the use of live fish in Norway in 2004. This makes it difficult to monitor what fish are actually used for in research.

Reporting of the health and welfare of fish used in research is often sparse [Brattelid & Smith 2000], and may include general statements such as 'Healthy fish from a

Needs to be followed up by species-specific guidelines







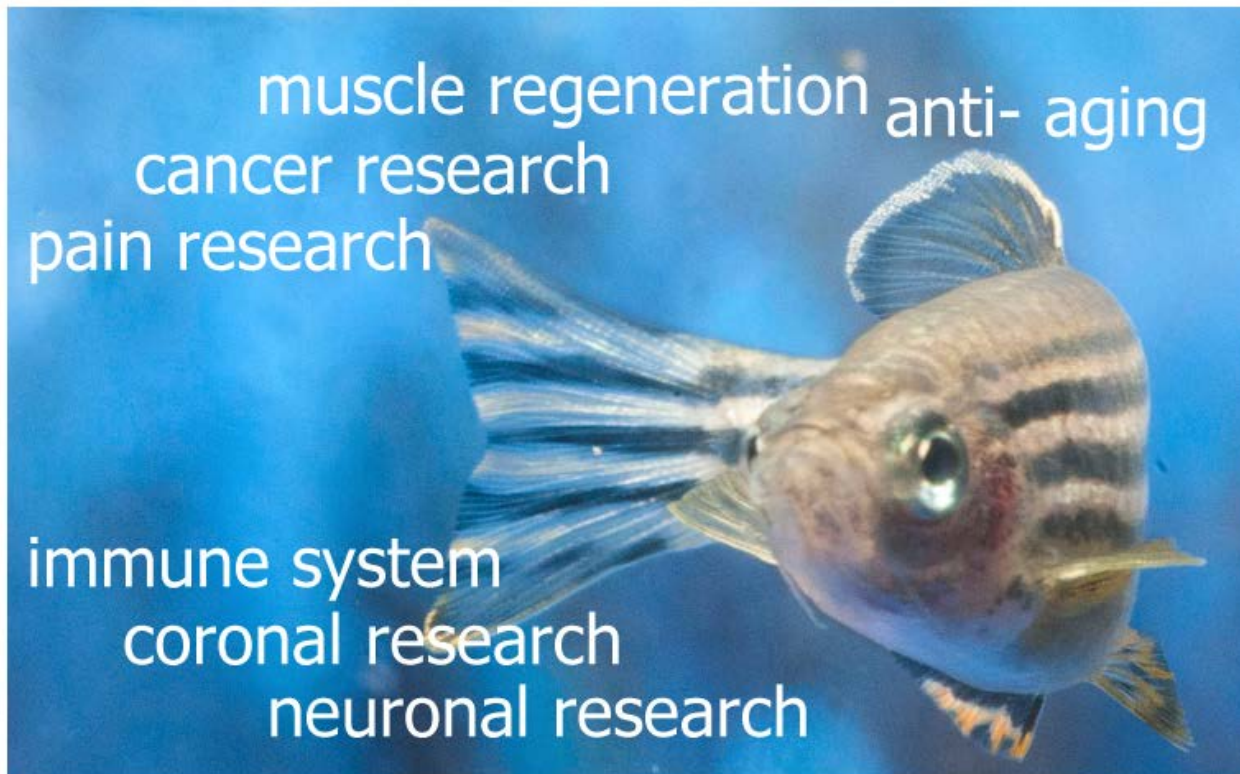
## The husbandry of zebrafish (*Danio rerio*): A review

Christian Lawrence  

### Abstract

The zebrafish (*Danio rerio*) has recently emerged as a pre-eminent vertebrate biomedical research model. The same favorable characteristics that have contributed to its popularity as a model of human disease and development; *i.e.* high fecundity, small size, rapid generation time, optical transparency during early embryogenesis, have also long endeared it to investigators in numerous other disciplines, including animal behavior, fish physiology, and aquatic toxicology. Despite this, the scientific rigour of zebrafish husbandry techniques is poorly developed. While there is a considerable body of literature on zebrafish that has both direct and indirect relevance to their husbandry, this information is from disparate sources, and little of it has been applied to developing standard protocols. This review is an attempt to integrate the available scientific information related to zebrafish biology and culture into an overview of the field that can be used to improve the efficiency with which this important model animal is used in research. The review also highlights those areas in which further studies are needed.

© 2007 Elsevier B.V. All rights reserved.



The zebrafish is a commonly used model organism in the biological sciences, similar to the mouse or rat. Zebrafish can grow to 4-5 cm and are easily handled in aquatic systems. Since their discovery for use in research in Prague in the 1980's, this fish has been established in many laboratories worldwide. The generation time of the zebrafish, from embryo to a breeding adult fish, is very short. Due to their transparent nature, developing Zebrafish embryos are very well suited for imaging purposes. There are very strict rules you have to follow if you want to work with laboratorial animals.

We will provide Information that describes all aspects of research with zebrafish and other fish models.

European Society for Fish Models in  
Biology and Medicine (EUFishBioMed)  
<http://www.eufishbiomed.kit.edu>



Home

Home Scientific community

Political Issues

Recommended Guidelines for Zebrafish

White Papers

Stress assessment in Zebrafish

EULoS

## Recommended Guidelines for Zebrafish

### EU Directive 2010\_63. Experiments with Zebrafish

The Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes was adopted on 22 September 2010. It is firmly based on the principle of the Three Rs, to replace, reduce and refine the use of animals used for scientific purposes. European scientist working with zebrafish have been consulted and involved in the development of common guidelines after what stage experiments with zebrafish should be considered animal experiments in regulatory terms:

[\[ ENGLISH \]](#)

[\[ SPANISH \]](#)

[\[ FRENCH \]](#)

[\[ GERMAN \]](#)

[\[ GREEK \]](#)

[\[ ISLANDIC \]](#)

### Recommended Guidelines for Zebrafish Husbandry Conditions

This guidelines are a joint FELASA (Federation of Laboratory Animal Science Associations) EuFishBioMed project and have been translated into seven languages:

[\[ ENGLISH \]](#)

[\[ FRENCH \]](#)

[\[ GERMAN \]](#)

[\[ ITALIAN \]](#)

[\[ GREEK \]](#)

[\[ DUTCH \]](#)

[\[ NORWEGIAN \]](#)

Please find below maintenance conditions for zebrafish (in German) written recently (Sept. 2014) by several German groups to support the ongoing discussions by German animal welfare associations to derive uniform recommendations for whole Germany.

[Husbandry conditions](#) (German)

<https://www.eufishbiomed.kit.edu/59.php>





Links to all on [www.eufishbiomed.kit.edu/58.php](http://www.eufishbiomed.kit.edu/58.php)



## British Association of Zebrafish Husbandry

BAZH is a volunteer non-profit making pan-institutional society that showcases the extent of zebrafish husbandry issues and research across the UK. Established in 2010 as an informal hub for all members of the zebrafish community, from researchers, vets, legislators, and animal technologists, to meet, discuss, and collate ideas, research and knowledge. BAZH holds biannual seminars on a vast range of topics, organises social events, and publishes newsletters, as well as hosting a range of media communication formats. If you want to get involved in any capacity, such as present a talk, contribute to newsletters, websites, etc please get in touch.

[www.bazh.org.uk](http://www.bazh.org.uk)

# Papers

Search:

## Papers

Abi-Ayad, A., Kestemont, P. 1994. Comparison of the nutritional status of goldfish (*Carassius auratus*) larvae fed with live, mixed or dry diet. *Aquaculture* 128: 163–176

Alabaster, J.S., Lloyd, R., 1980. *Water Quality Criteria for Freshwater Fish*. Butterworth. 297 pp.

Alsop, D., Matsumoto, J., Brown, S., Van Der Kraak, G. Retinoid requirements in the reproduction of zebrafish. 2007. *General and comparative endocrinology* 156:51 -62

American Veterinary Medical Association (AVMA) (2007) 'AVMA guidelines on euthanasia' [http://www.avma.org/issues/animal\\_welfare/euthanasia.pdf](http://www.avma.org/issues/animal_welfare/euthanasia.pdf).

Andrew, C. (1999) 'Freshwater fish' in *The IFAW Handbook on the Care and*





[www.zebrafish.org](http://www.zebrafish.org)

## **ZIRC Health Monitoring SOPs**

Sentinel Fish Program



[pdf](#)

Daily Monitoring of Fish Morbidity and Mortality SOP



[pdf](#)

Fixing Zebrafish for Histopathology



[pdf](#)

ZIRC PCR protocol for *P. neurophilia*



[pdf](#)

## D. Procedures

1. Fish exhibiting the behavioral and physical signs below should be removed. If you have time to remove the fish, go to step 3 or 4. If you do not have time to remove the fish immediately, place a red flag on the front of the tank and write the number of affected fish and observed clinical signs on the flag (i.e. "1 skinny").

Behavioral Abnormalities	Physical Abnormalities
Fish at surface or near water inlet Rapid breathing/opercular movements Sluggish movements/lethargy Flashing/rubbing on tank surfaces Circling, twirling, spinning Loss of equilibrium	Color change Weight loss Exophthalmia/pop-eyes Distended abdomen Skeletal deformity Mass/swelling Hemorrhage/redness Gas bubbles Protruding scales Fin erosion or lesion Skin ulceration or lesion



## The Zebrafish Book

[http://zfin.org/zf\\_info/zfbook/zfbk.html](http://zfin.org/zf_info/zfbook/zfbk.html)

## ZFIN Protocol Wiki

Created by Jonathan Knight, last modified on Mar 24, 2014

### Welcome to the Protocols Wiki

This is where zebrafish researchers can share experimental protocols and tips with the rest of the research community. Protocols are organized into sections corresponding to the chapters of [The Zebrafish Book, 5th edition \(4th edition on-line\)](#). Feel free to add new protocols to the appropriate section or add comments to any existing protocol.

<https://wiki.zfin.org/display/prot/ZFIN+Protocol+Wiki>

“ The Norwegian Zebrafish Platform had a National technology platform grant 2007- 2012 under the Functional Genomics Program, Research Council of Norway. Since 2013 zebrafish.no is part of NMBU Vetbio



### News [\(Archive...\)](#)

November 4th, 2015

## NMBU professors develop e-ZFbook, a new concept of e-learning

[Read more...](#)

November 3rd, 2015

## ZIG (Zebrafish Interest Group), Oslo

ZIG is a seminar forum for all interested in the zebrafish model. Contact: [Viola.Lobert@rr-research.no](mailto:Viola.Lobert@rr-research.no)

November 2nd, 2015

## The 9th European Zebrafish Meeting - EZM2015

Program, abstracts and "Zebrafish Blues" on <http://zebrafish2015.org>



### ZNN Forum

## ZNN

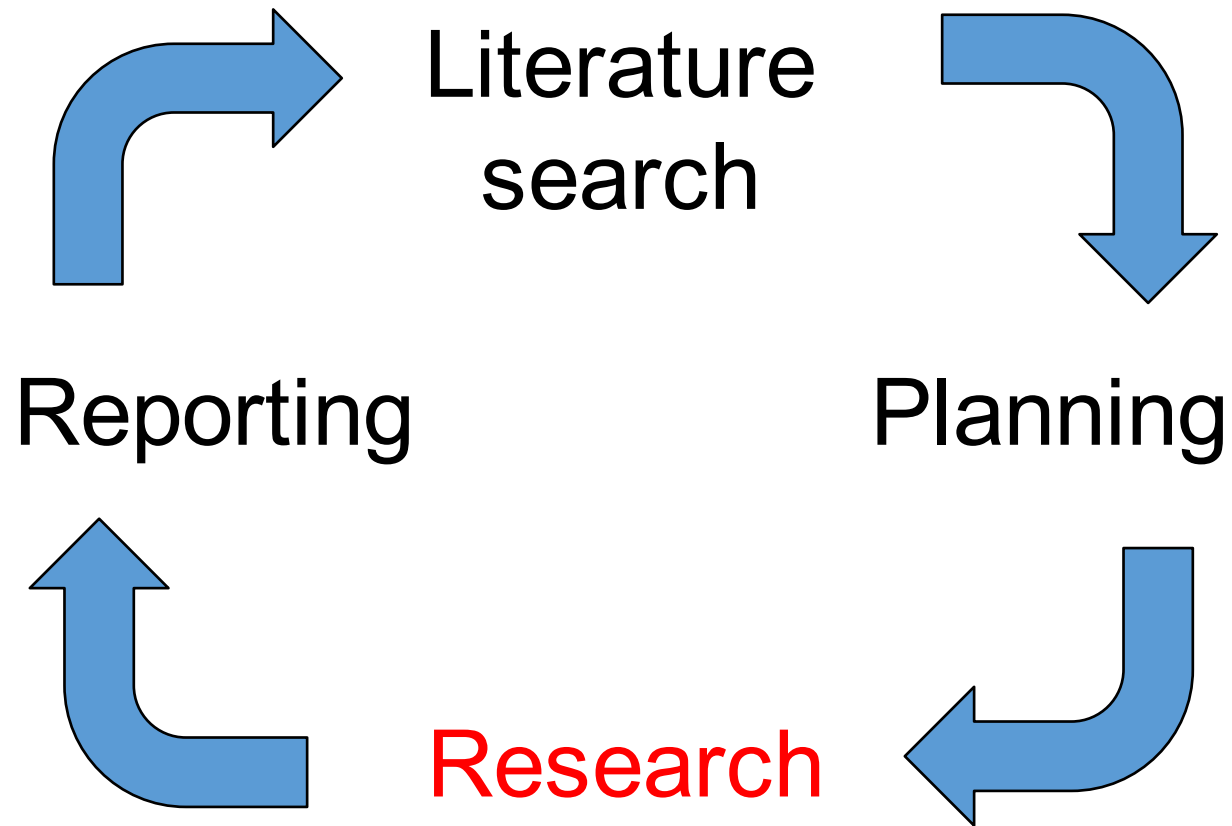
Zebrafish Network Norway



UNIVERSITY OF  
NORDLAND

### Links

# ZIG # Zebrafish Interest Group #





## Frequent challenges with research on non-traditional species

- Absence of standardised animals
- Health, housing and welfare monitoring
- Recognition of pain, suffering, distress



[https://speakingofresearch.com/2015/05/11/zebrafish\\_rising\\_star\\_animal\\_model](https://speakingofresearch.com/2015/05/11/zebrafish_rising_star_animal_model)



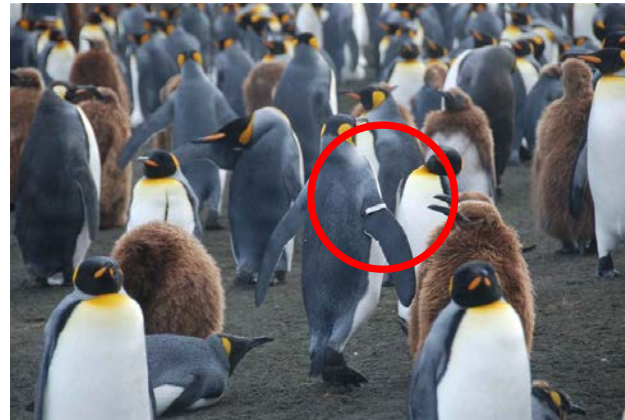
[http://ichef-1.bbci.co.uk/news/660/media/images/69786000/jpg/\\_69786238\\_69786233.jpg](http://ichef-1.bbci.co.uk/news/660/media/images/69786000/jpg/_69786238_69786233.jpg)

- “Normal” mortality
- Refinement of procedures
- Techniques for anaesthesia and humane killing

## *"Simple" techniques?*



Photo: T. Poppe, NMBU



[http://blogs.discovermagazine.com/notrocketscience/2011/01/12/flipper-bands-impair-penguin-survival-and-breeding-success/#.VLU6\\_8Y7\\_wo](http://blogs.discovermagazine.com/notrocketscience/2011/01/12/flipper-bands-impair-penguin-survival-and-breeding-success/#.VLU6_8Y7_wo)



Photo: NMBU



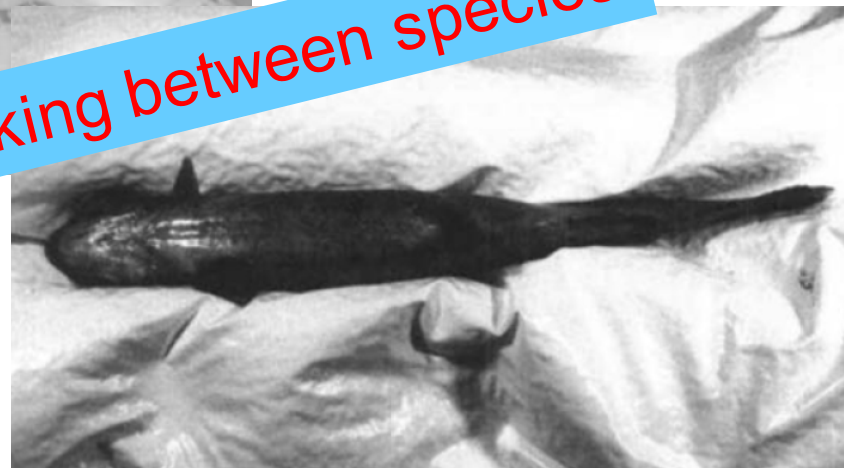
# Methods of positioning fish for surgery or other procedures out of water

**Trond Brattelid & Adrian J. Smith**

Laboratory Animal Unit, Norwegian School of Veterinary Science, PO Box 8146 Dep., N-0033 Oslo, Norway



**Lateral thinking between species**



Laboratory Animals, 2000, 34,  
430-433



The Norwegian Reference Centre for  
Laboratory Animal Science & Alternatives

Immobilisation and Intraperitoneal Injection in the Mouse



The injection is made by sticking first subcutaneously, then moving the needle slightly forward under the skin before finally penetrating the abdominal muscles. The injection should be made to one side of the midline. Rest the third, fourth and fifth fingers of the hand holding the syringe against your wrist, to avoid unwanted movement of the needle.



Restraint



Restraint



Restraint



Restraint



Restraint



Restraint



Injection



Injection

[Close this window to return to the main menu](#)

Slide show

Video

[film.oslovet.norecopa.no](http://film.oslovet.norecopa.no)

# Harm-Benefit Assessment

## No numerical 'formula'

- AND A SPECTRUM OF VIEWS



- The harm-benefit assessment involves value judgements, which depend on individual opinions on:
  1. The nature and likelihood of the benefit
  2. The value placed on animal life – may depend on species, numbers
  3. The nature and level of suffering
  4. Controversy around particular procedures
  5. The fate of the animals

***A useful additional (but largely unknown) tool...***

Carol M. Newton (1925-2014)



National Library of Medicine

## ***The three S's***

- *Good Science*
- *Good Sense\**
- *Good Sensibilities\**

*\*We can do this ourselves without scientific literature!*

Carol M Newton, quoted in Rowsell HC (1977): The Ethics of Biomedical Experimentation in The Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing pp. 267-281, National Academy of Sciences, Washington, D.C., ISBN 0-309-02603-2.

# Critical anthropomorphism

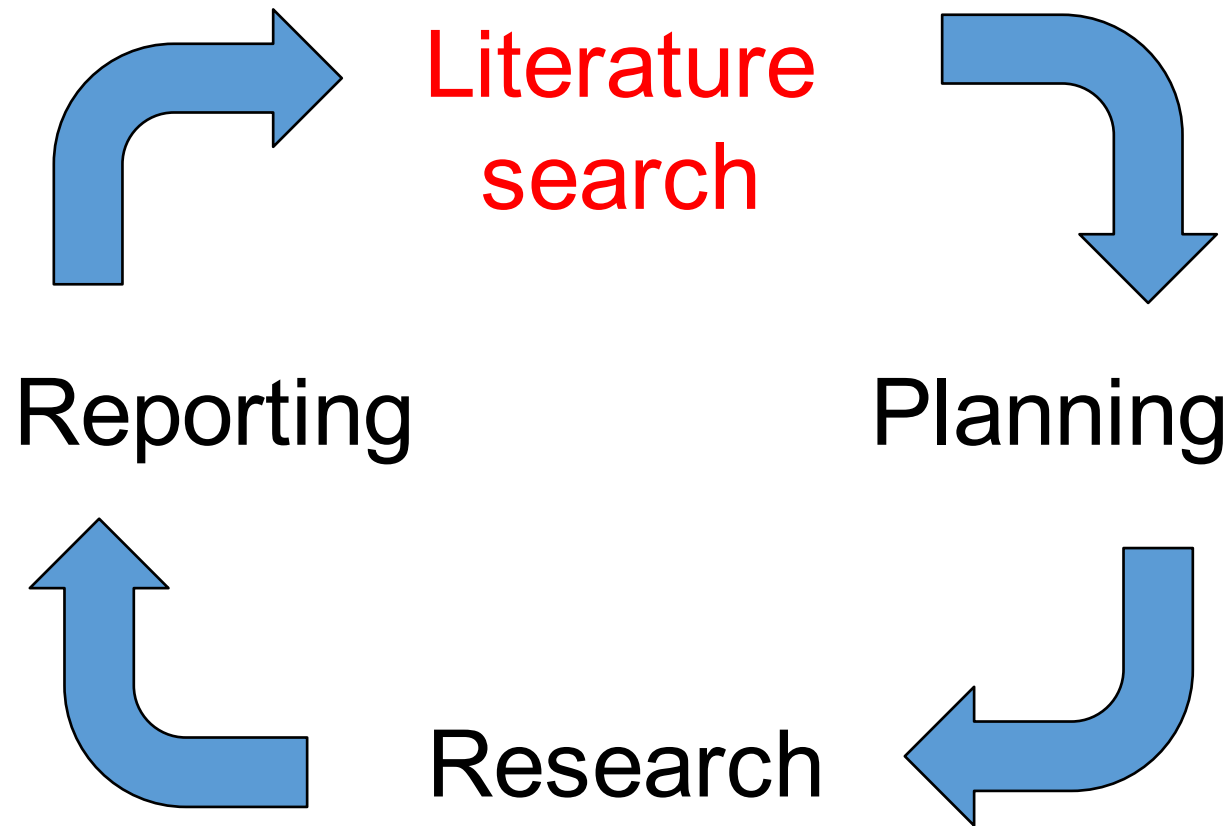


National Library of Medicine

= *empathy* + objective,  
knowledge-based  
consideration of what is  
likely to be significant to  
the animal

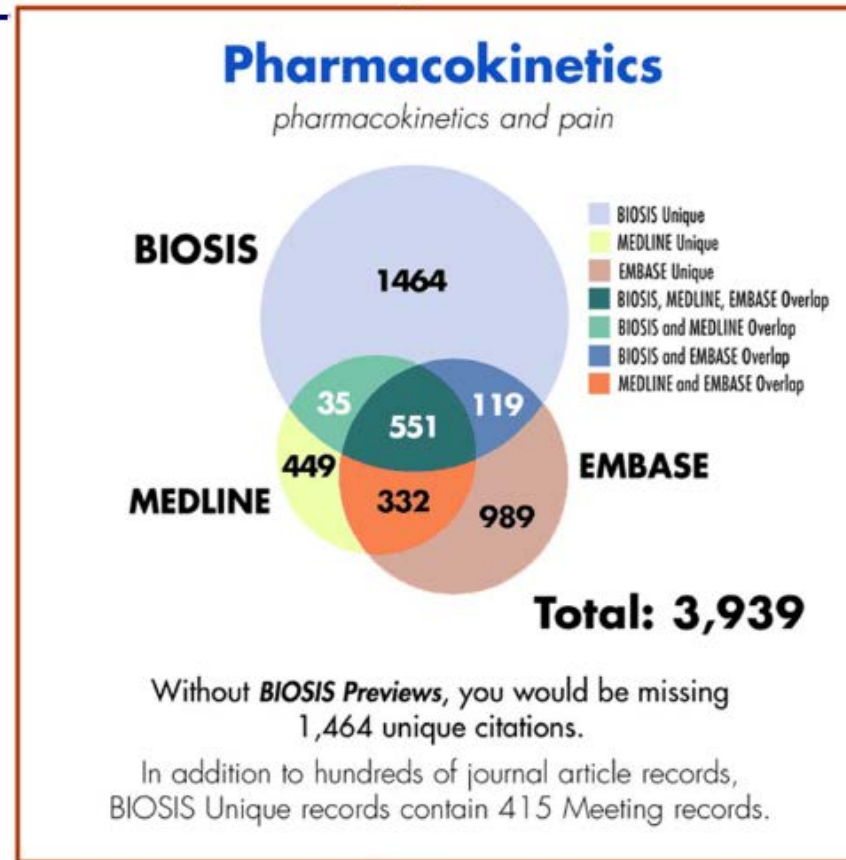
Penny Hawkins, Voss

<http://tjinfo.uib.no/Vedlegg?id=3753833e5de91342a40a2b95a5b4391a>



*What's the problem? We have Google...*

## Why Search Multiple Databases?





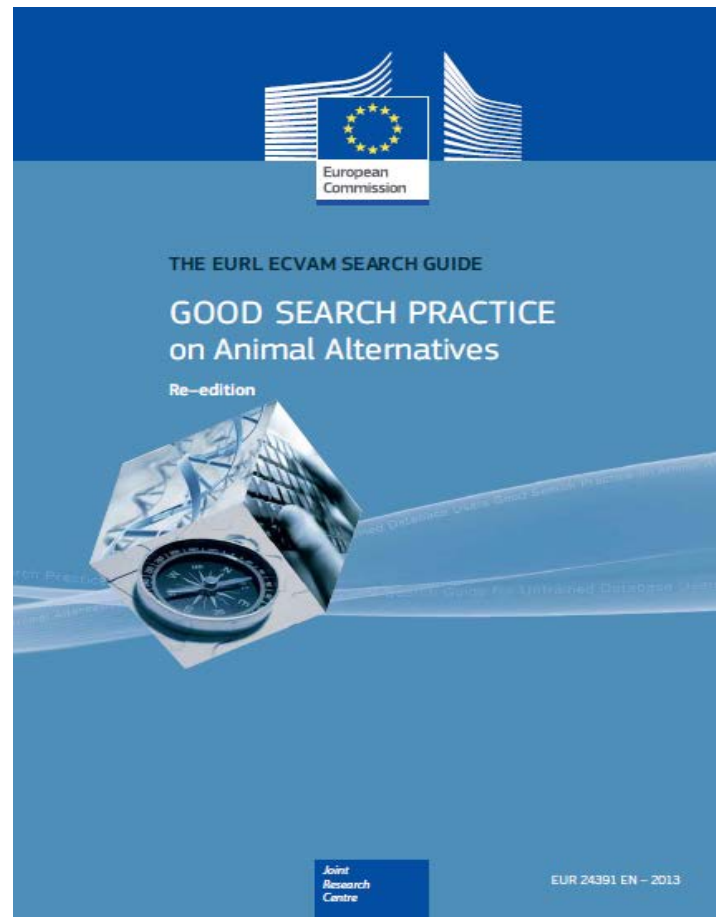
## ***Principles of setting up a search***

- Efficiency – minimise the number of irrelevant or poor-quality results
- Effectivity – maximise the number of high-quality results

# The EURL ECVAM Search Guide

Can be ordered free of charge from

[bookshop.europa.eu](http://bookshop.europa.eu)



# Advances within the 3Rs are often not highlighted in the literature by the scientists!



[http://www.theodora.com/rodent\\_laboratory/blood\\_collection.html](http://www.theodora.com/rodent_laboratory/blood_collection.html)



photo:NMBU

Saphenous vein puncture for  
blood sampling of the mouse, rat,  
hamster, gerbil, guinea-pig,  
ferret and mink



## Most-Cited Articles as of April 1, 2016- updated monthly

out of 153 | Indexed in Medline

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

### 1. Working Party Report:

W. Nicklas, P. Baneux, R. Bood, T. Decelle, A. A. Deeny, M. Fumanelli,  
and B. Illgen-Wilcke

**Recommendations for the health monitoring of rodent and rabbit  
colonies in breeding and experimental units**

Lab Anim January 1, 2002 36: 20-42, doi:10.1258/0023677021911740

» [Full Text \(PDF\)](#)

### 2. Articles:

C. Moolenbeek 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:10.1258/002367781780958577

» [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:10.1258/002367798780599866

» [Abstract](#) » [Full Text \(PDF\)](#)

Submit a Manuscript

Free Sample Copy

Email Alerts

RSS feed

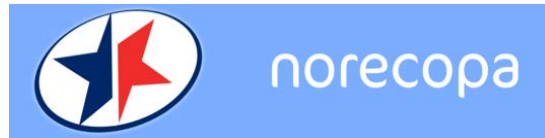
More about this journal



Laboratory Animals Ltd

Affiliated Societies

<http://lan.sagepub.com/reports/most-cited>



*AJ Smith & T Allen, 2005*

***The use of Databases, Information Centres and Guidelines  
when planning research that may involve animals***

*Animal Welfare, 14 (4): 347-359*

[norecopa.no/SmithAllen.pdf](http://norecopa.no/SmithAllen.pdf)

*This led to the construction of:*

**3R Guide**



**[www.3RGuide.info](http://www.3RGuide.info)**



[norecopa.no/3RGuide](http://norecopa.no/3RGuide)

## 3R Guide

[www.3RGuide.info](http://www.3RGuide.info)

*Databases, Guidelines, Regulations, Information Centres, Journals, E-mail lists*



*Audiovisual products*

**NORINA**

[norecopa.no/NORINA](http://norecopa.no/NORINA)

*Textbooks*

**TextBase**

[norecopa.no/TextBase](http://norecopa.no/TextBase)

**+ intelligent search engine**

www.norecopa.no

Guidelines bleeding mice

Search

Help  Enable synonyms and stemming Reset

Did you mean: guideline breeding mice(4)

Auto-complete function:

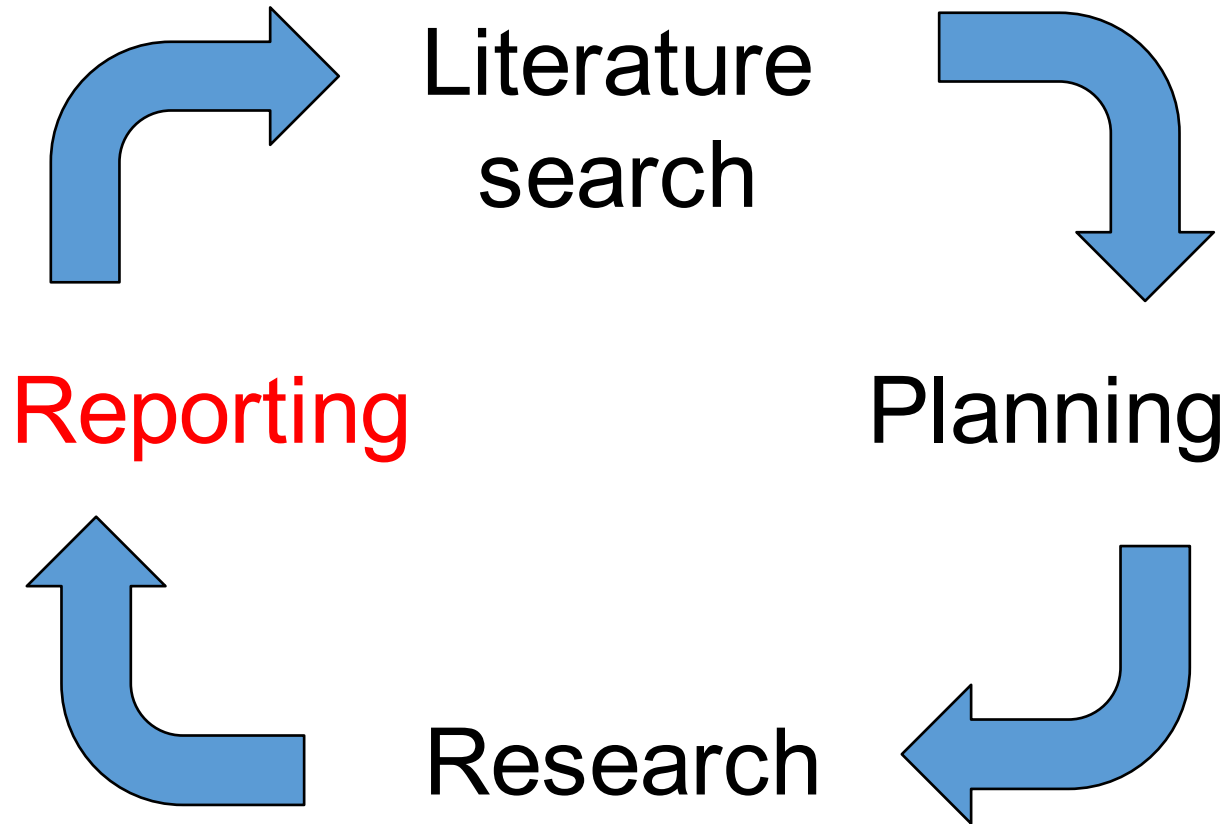
- blood
- blood
- blood collection
- blood from
- blood sampling

Category

- Agricultural animals (5)
- Anaesthesia (3)
- Anaesthesia and analgesia (4)
- Anatomy (175)
- Aquatic animals (4)
- Behaviour (4)
- Behavioural research (3)
- Biochemistry (7)
- Biology (4)
- Birds (5)
- Blood sampling (12)
- Cancer research (3)
- Design (7)
- Disease research (3)
- Dissection (7)
- Education and training (3)

Synonym list:

Bleeding, bloodsampling, blood sampling, venepuncture, blood collection, phlebotomy



## Missing mice: gaps in data plague animal research

Reports of hundreds of biomedical experiments lack essential information.

**Monya Baker**

05 January 2016

[Rights & Permissions](#)

Two studies have unveiled widespread flaws in the reporting of animal experiments — the latest in a series of papers to criticize [shoddy biomedical research](#).

Whereas reports of clinical trials in major medical journals routinely state how many patients die or drop out of analysis during the course of a study, animal studies generally fail to report this figure — or drop animals without saying why, according to a team led by Ulrich Dirnagl at the Charité Medical University in Berlin. That lapse could significantly bias results, the team reports in the journal *PLoS Biology*<sup>1</sup>.

In a second study in the same journal<sup>2</sup>, a team led by John Ioannidis, an epidemiologist at Stanford University in California who has repeatedly called for more reproducible and transparent research, criticizes the lack of data availability and detailed protocols in biomedical papers.

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%*

<i>Sex</i>	<i>28%</i>	<i>Room temperature</i>	<i>72%</i>
<i>Age</i>	<i>52%</i>	<i>Relative humidity</i>	<i>89%</i>
<i>Weight</i>	<i>71%</i>	<i>Photoperiod</i>	<i>72%</i>
<i>Source</i>	<i>53%</i>	<i>Number of animals/cage</i>	<i>73%</i>

*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.*

# Guidelines for reporting

We need

- General guidelines for reporting


e.g.


ARRIVE, ILAR, ICLAS, LASA/APC, GSPC


- Guidelines for reporting specific types of animal research or procedures


## Products


Your search for products of the type "Guidelines" containing the text "reporting" returned the following results (13 items, page 1 of 1):


 **Guidelines for reporting the results of experiments on fish.** A detailed account of experimental design, including an accurate description of the animals used, is an essential part of good research practice. This paper suggests guidelines for reporting fish experiments.

 **Guidelines for reporting the results of experiments using mammals.** A detailed account of experimental design, including an accurate description of the animals used, is an essential part of good research practice. This paper suggests guidelines for reporting experiments on mammals.

 **Recommendations for the conduct, reporting, editing and publication of scholarly work in medical journals.** The International Committee of Medical Journal Editors (ICMJE) developed these recommendations to review best practice and ethical standards in the conduct and reporting of research and other material published in medical journals, and to help authors, editors, and others involved in peer review and biomedical publishing create and distribute accurate, clear, unbiased medical journal articles.

 **The ARRIVE Guidelines.** The ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines have been developed by the NC3Rs to improve standards of reporting and ensure that the data from animal experiments can be fully evaluated and utilised. The guidelines are primarily aimed at scientists writing up their research for publication and for those who are involved in peer review.

 **Final report of a LASA/APC working group to examine the feasibility of reporting data on the severity of scientific procedures on animals.** This is the final report of a Working Group convened under the auspices of Laboratory Animal Science Association (LASA) and the Suffering and Severity Working Group of the Animal Procedures Committee (APC), to assess the feasibility of collecting and reporting data on the actual severity of adverse effects experienced by animals used in procedures regulated under the Animals (Scientific Procedures) Act 1986 [ASPA].

 **CCAC Guidelines on transgenic animals.** Topics covered include investigator and animal care committee responsibilities, proposals to create new or use existing strains, accounting, containment, reporting, responsibility of the CCAC and literature references.

 **Gold Standard Publication Checklist (GSPC).** Designed to help scientists to plan, design and perform animal experiments. Highlights the importance of reporting husbandry conditions and basic principles of the design of animal experiments by providing an overview of the literature on how and when interference with experimental results may occur.

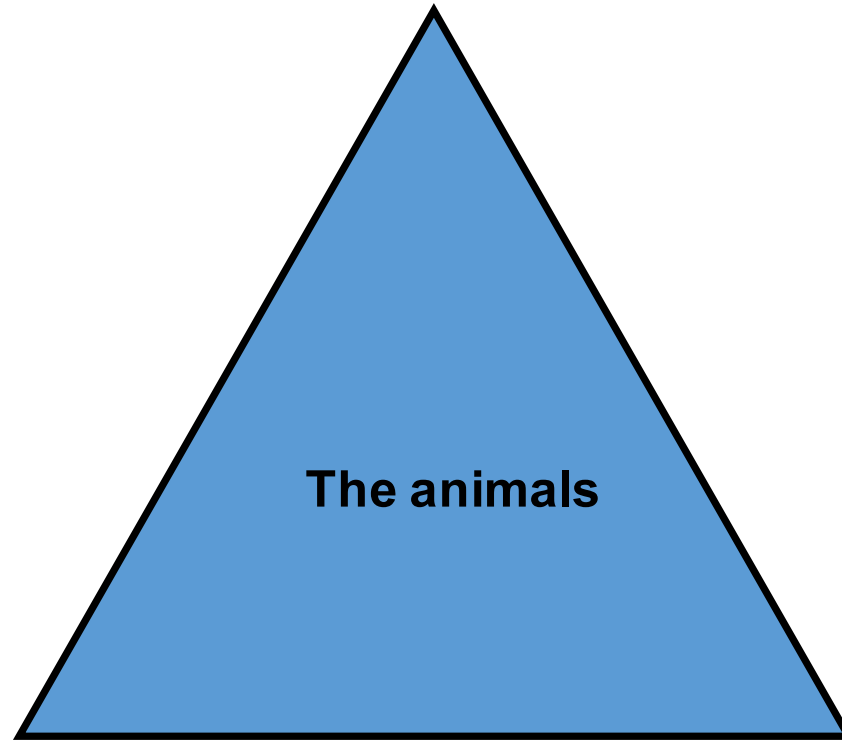
# The ARRIVE Guidelines

ITEM	RECOMMENDATION
Title	Provide as accurate and concise a description of the content of the article as possible.
Abstract	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.
<b>INTRODUCTION</b>	
Background	<p>3</p> <p>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.</p> <p>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.</p>
Objectives	4 Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.
<b>METHODS</b>	
Ethical statement	5 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.
Study design	<p>6</p> <p>For each experiment, give brief details of the study design including:</p> <p>a. The number of experimental and control groups.</p> <p>b. Any steps taken to minimise 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).</p> <p>c. The experimental unit (e.g. a single animal, group or cage of animals).</p> <p>A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.</p>
Experimental procedures	<p>7</p> <p>For each experiment and each experimental group, including controls, provide precise details of all procedures carried out.</p> <p>For example:</p> <p>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 supplier(s).</p> <p>b. When (e.g. time of day).</p> <p>c. Where (e.g. home cage, laboratory, water maze).</p> <p>d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).</p>
Experimental animals	<p>8</p> <p>a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).</p> <p>b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.</p>

Sample size	10	<p>a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group.</p> <p>b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.</p> <p>c. Indicate the number of independent replications of each experiment, if relevant.</p>
Allocating animals to experimental groups	11	<p>a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.</p> <p>b. Describe the order in which the animals in the different experimental groups were treated and assessed.</p>
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).
Statistical methods	13	<p>a. Provide details of the statistical methods used for each analysis.</p> <p>b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron).</p> <p>c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.</p>
<b>RESULTS</b>		
Baseline data	14	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).
Numbers analysed	15	<p>a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%<sup>2</sup>).</p> <p>b. If any animals or data were not included in the analysis, explain why.</p>
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).
Adverse events	17	<p>a. Give details of all important adverse events in each experimental group.</p> <p>b. Describe any modifications to the experimental protocols made to reduce adverse events.</p>
<b>DISCUSSION</b>		
Interpretation/scientific implications	18	<p>a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.</p> <p>b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results<sup>2</sup>.</p> <p>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.</p>
Generalisability/translation	19	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.
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.

***Animal research must be***

**Science-based**

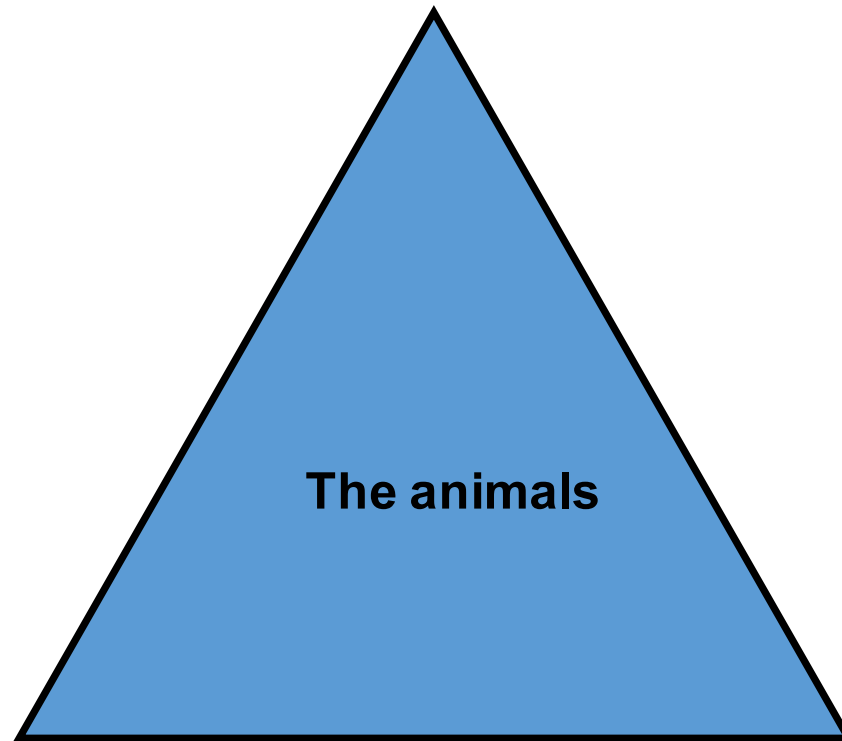


**The animals**

**Legal**

**Ethically acceptable**

**Those responsible for the animal facility**



**The scientists**

**The technicians**

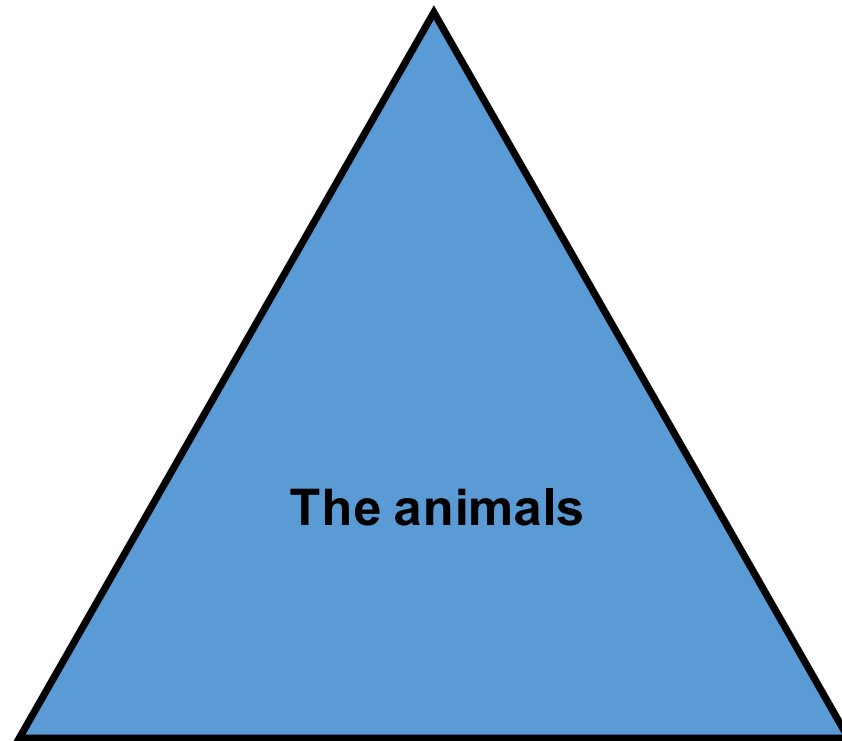
## **Those responsible for the animal facility**

A need for dissemination of best practice between

The AWERBs at the national level  
The National Committees at the EU level  
National 3R centres



**Those responsible for the animal facility**



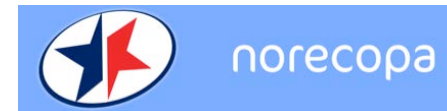
**The animals**

**The scientists**

**The technicians**

***Do you need more guidelines?***





### **Thanks to our main sponsors:**

- Dag S. Stiansen Foundation
- Laboratory Animals Ltd.
- Ministries of Agriculture and Fisheries
- Nordic Society Against Painful Experiments
- Norwegian Research Council
- Novo Nordisk
- Scottish Accreditation Board

### **Funding of the NORINA database:**

*Nordic Society Against Painful Experiments, Dag S. Stiansen Foundation, The Norwegian Research Council, the Norwegian School of Veterinary Science, Laboratory Animals Ltd., RSPCA, UFAW, AstraZeneca, Solvay Pharmaceuticals, the Swedish Fund for Research without Animal Experiments, Norwegian Federation for Animal Protection, Allkopi, The Humane Society of the United States, St. Andrew Animal Fund, Microsurgical Developments Foundation, AAALAC International, LASA, NEAVS, Amersham Health*

*Thank you for listening!*

