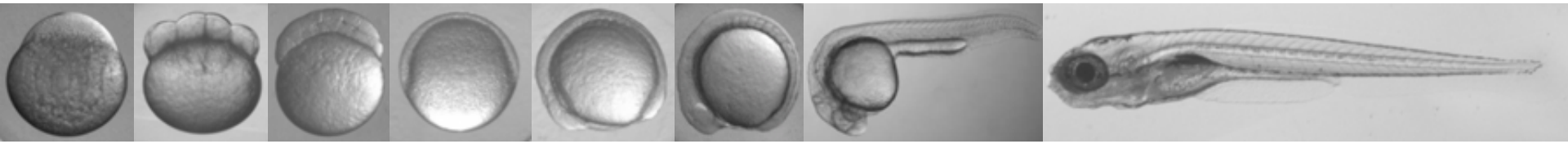
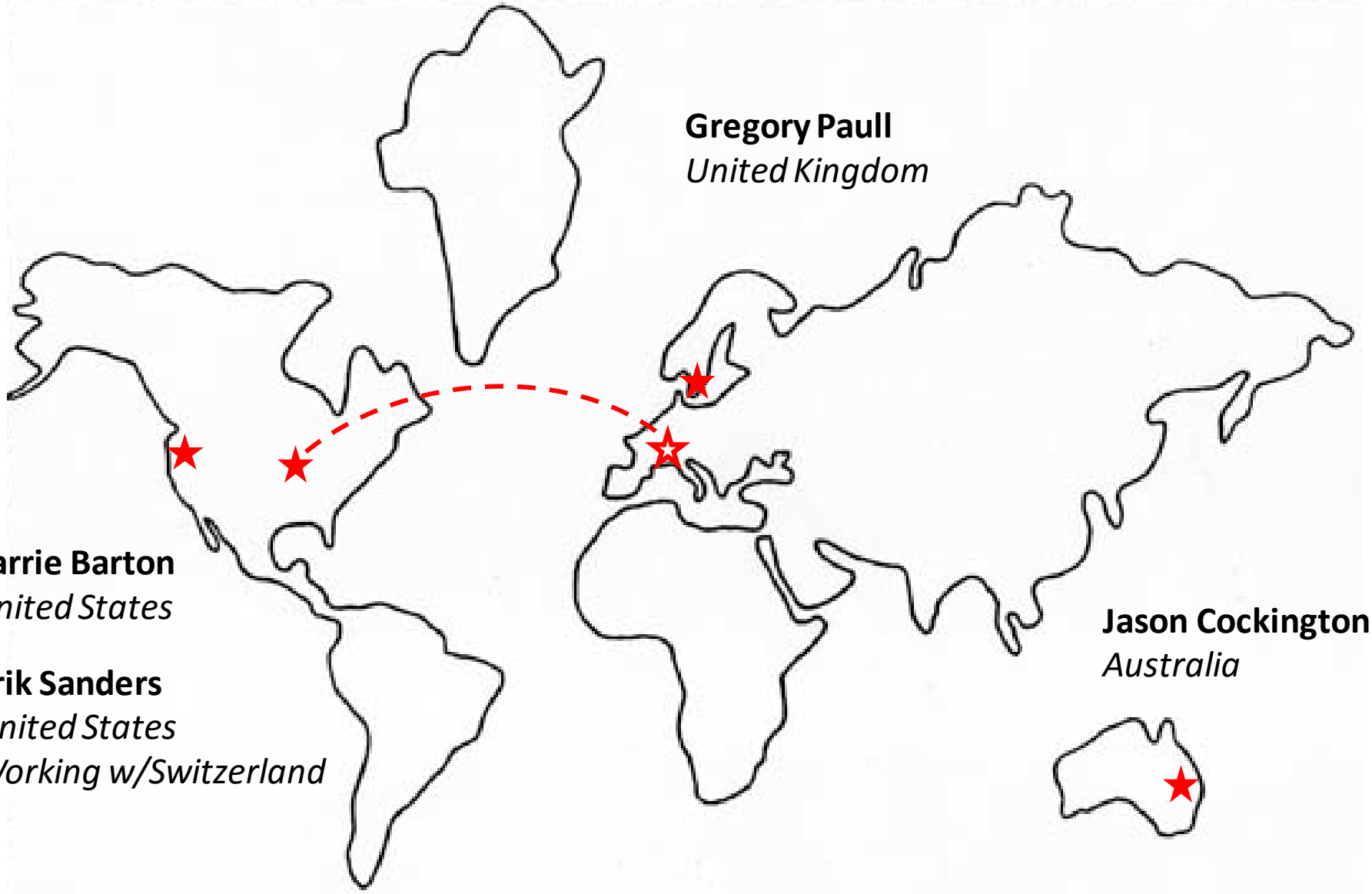
Ethics and the Research Zebrafish

A Multi-Continent Review



5th Annual International Zebrafish Husbandry Course
Buguggiate, Italy 2016

Perspectives



Objectives

Hierarchy of Oversight

Overview of the systems responsible for ensuring ethical use of zebrafish by region

New and Changing Standards

Keeping up with changing ethical standards - *Real world examples of adaptation*

Implementation

Advice for incorporating a proactive approach to ethics management into a program

Perspectives

Carrie Barton
United States

Hierarchy of Oversight – Federal Level

U.S. Dept. of Health and Human Services (DHHS)

- Agency for Healthcare Research and Quality (AHRQ)*
- Agency for Toxic Substances and Disease Registry (ATSDR)*
- Centers for Disease Control and Prevention (CDC)*
- Food and Drug Administration (FDA)*
- Health Resources and Services Admin. (HRSA)*
- Indian Health Service (IHS)*
- National Institutes of Health (NIH)*
- Substance Abuse and Mental Health Services Admin. (SAMHSA)*

PHS Policy on Humane Care and Use of Animals (1971)

U.S. Government Principles for the Care of Vertebrate Animals Used in Testing, Research and Training

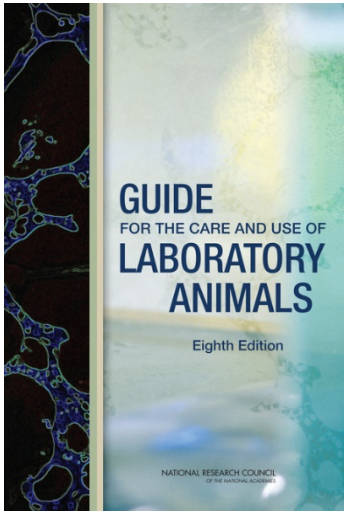
Health Research Extension Act (1985)

-Establish guidelines
-Animal welfare assurance mechanism (OLAW)
-Internal Animal Care and Use Committee (IACUC)
-Reporting of noncompliance and deficiency correction

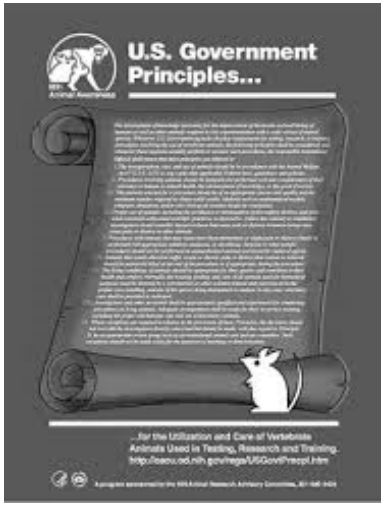
OLAW – Mission Statement and Guidance

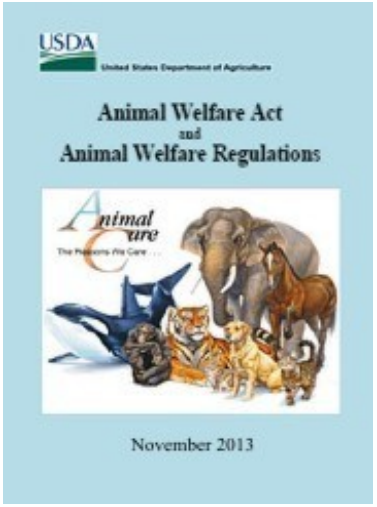
Mission Statement:

The Office of Laboratory Animal Welfare (OLAW) provides guidance and interpretation of the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, supports educational programs, and monitors compliance with the Policy by Assured institutions and PHS funding components to ensure the humane care and use of animals in PHS-supported research, testing, and training, thereby contributing to the quality of PHS-supported activities



AVMA Guidelines for the Euthanasia of Animals





Institutional Oversight

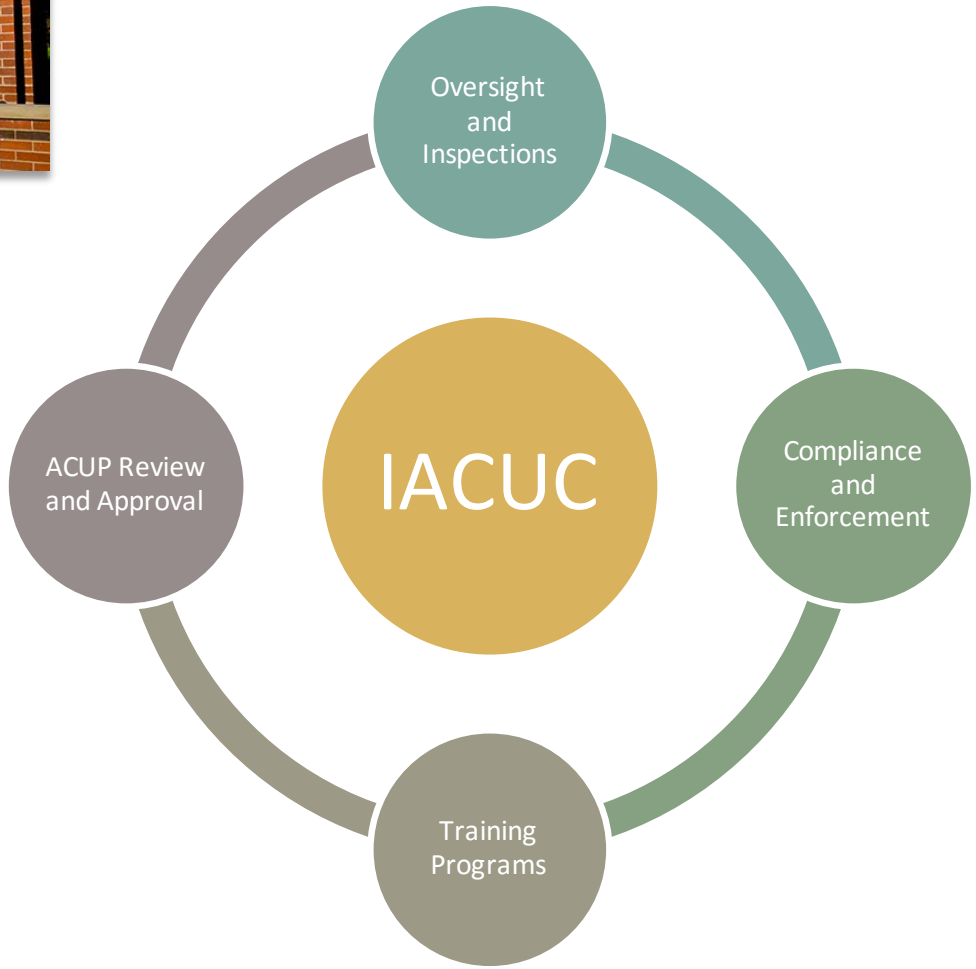
Office of Research

↓

Office of Research Integrity

↓

Internal Animal Care and Use Committee (IACUC)



Independent Oversight

The **Association for Assessment and Accreditation of Laboratory Animal Care** International

AAALAC International is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs.

Voluntary

Institution is open to inspections

Detailed program description

Annual reporting

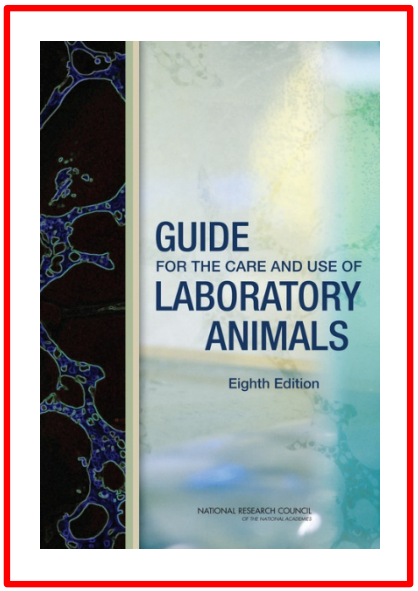
AAALAC status must be renewed every three years

New and Changing Standards

The "Guide"

8th Edition specifically mentions aquatic species (2011)

- Pages dedicated to "aquatic species" = ~10
- Contains few mandates/directives regarding care



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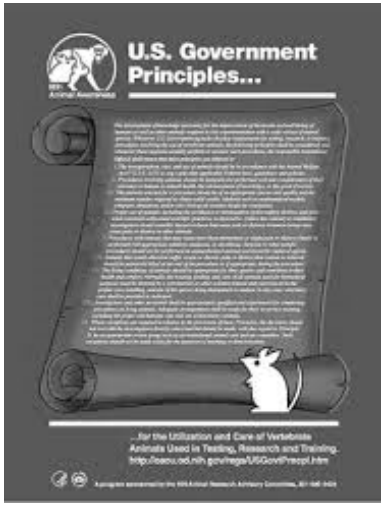
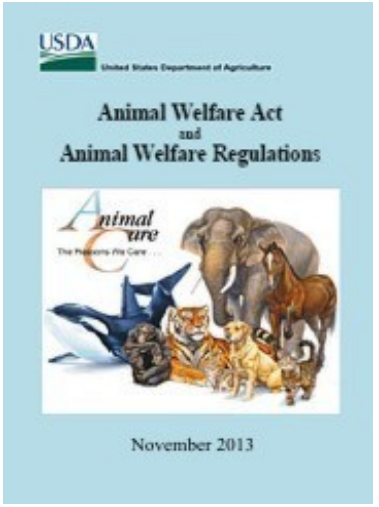
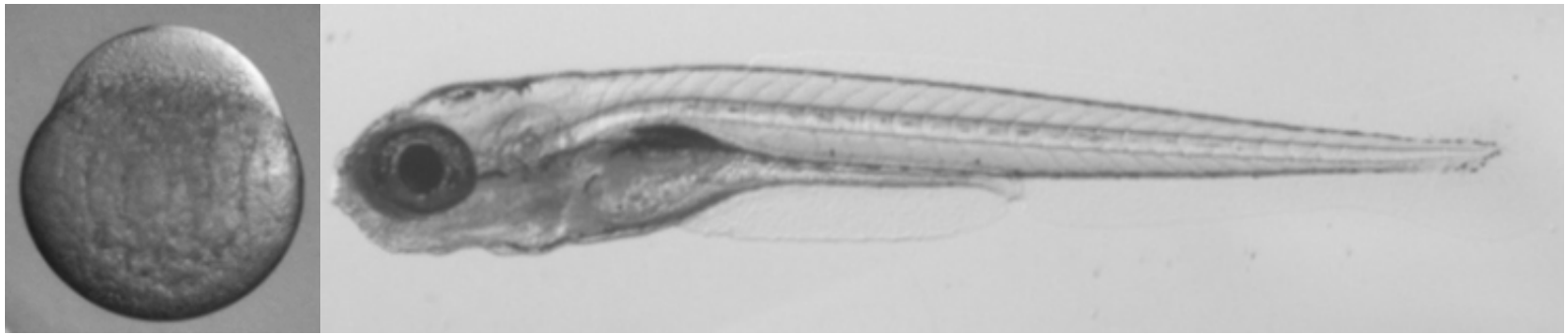




Examples of Major Change

At what age is a fish covered by regulation?

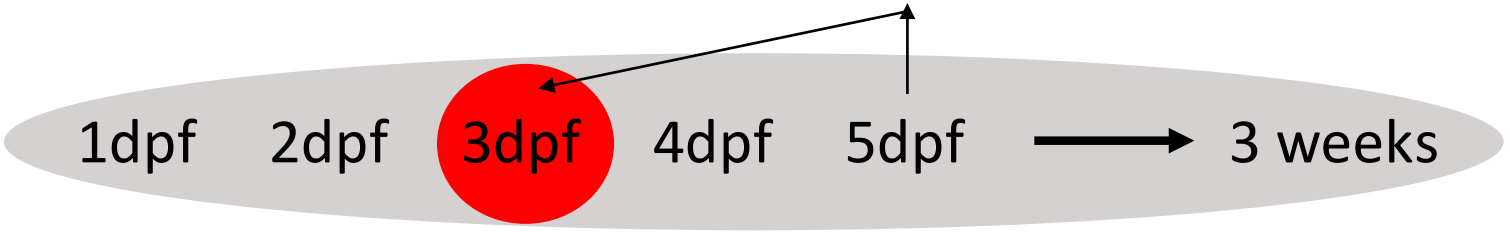
Specific system development? | Free feeding? | Free swimming? | Husbandry?



Time point varied wildly by institution

OSU Oregon State University

All users at OSU had ACUPs
Using the 5dpf time point



Does the PHS Policy apply to larval forms of amphibians and fish? Yes, larval forms of fish and amphibians have vertebrae and are covered by the PHS Policy. As noted in FAQ A4, the PHS Policy applies to the offspring of egg-laying vertebrates only after hatching. Zebrafish larvae, for example, typically hatch 3 days post-fertilization.

Implementation of Internal Policy Change

-Identified all current users effected by the change

-Invited all users to an informative webinar regarding new policy

-Q & A Sessions

-Implemented a multistage implementation schedule – biggest to smallest users

-Set a realistic time line for users to comply to new standards, and re-train staff

-Followed up with all users to ensure complete understanding of the new standard

Pre-emptive Management of Ethics

Program Description

AAALAC Template
http://aaalac.org/programdesc/index.cfm

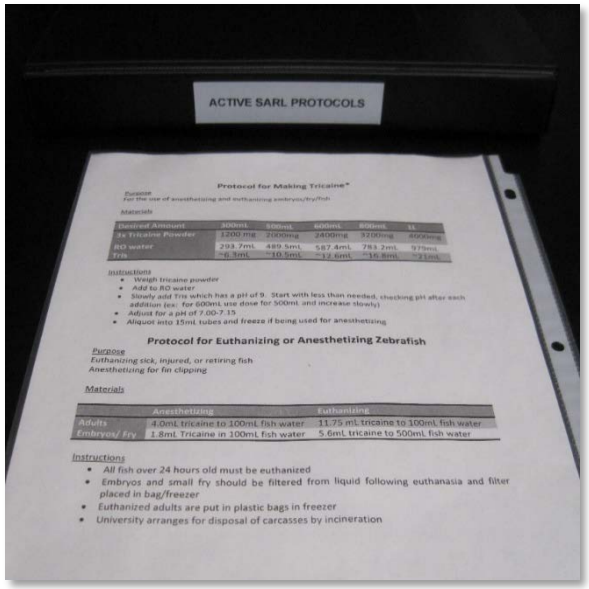
Standard Operating Procedures (SOP)

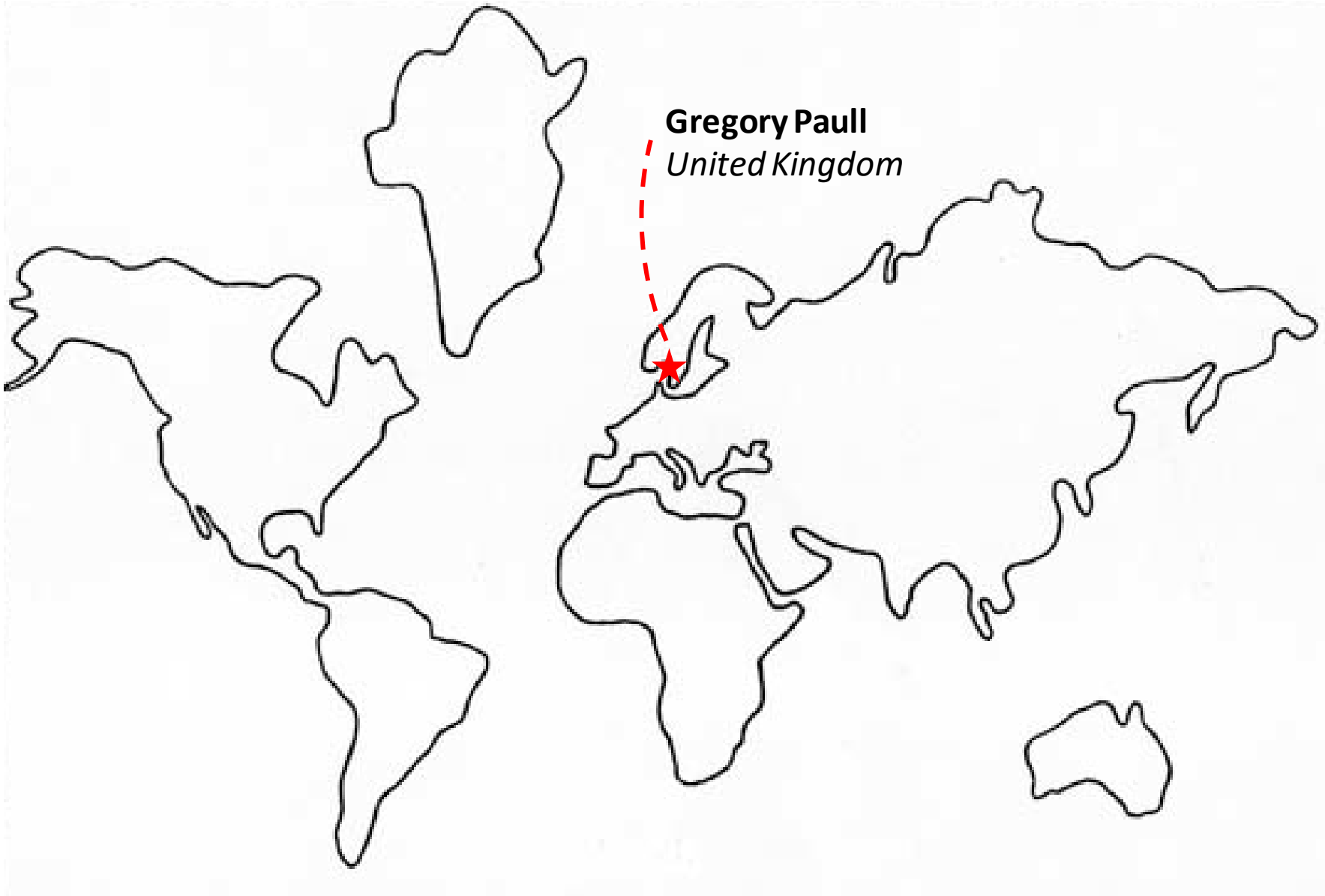
Animal Care and Use Program
Program Mgmt.
Program Oversight

Veterinary Care
Animal Procurement and Transport
Preventative Medicine
Clinical Care and Mgmt.
Surgery
Pain and Distress
Anesthesia and Analgesia
Euthanasia

Physical Plant
Facilities Overview
Centralized Animal Facilities
Satellite Animal Housing Facilities
Emergency Power and Life Support Systems
Other Facilities

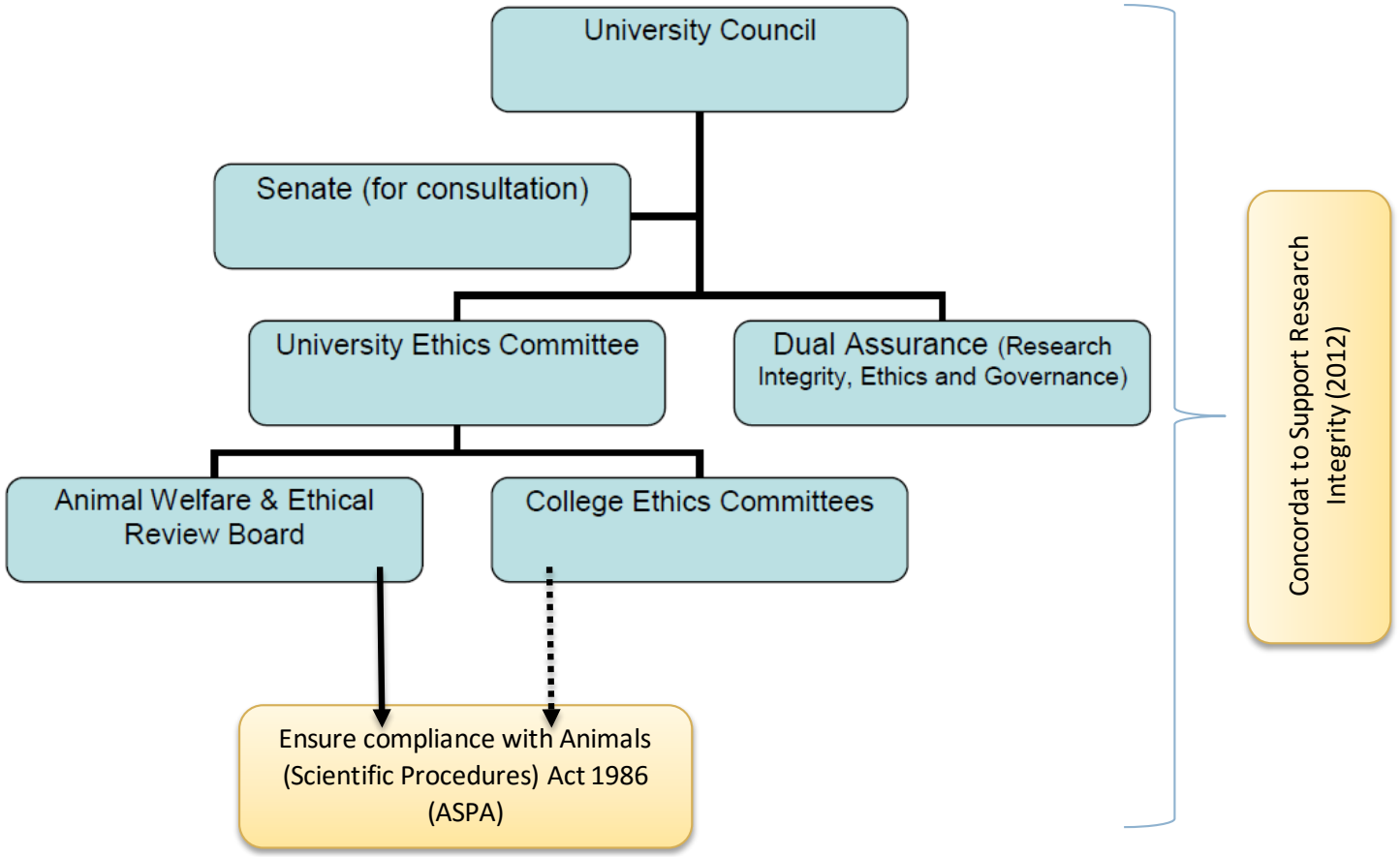
Animal Environment, Housing and Mgmt.
Animal Environment
Animal Housing
Animal Facility Mgmt.




Gregory Paull
United Kingdom

Research Ethics: Hierarchy of Enforcement UK

- University approval - College or discipline specific review processes



- Consideration of ethics encompasses teaching, research misconduct and the more obvious aspects of research with animals and human participants

Research Ethics: Framework

- The Concordat to Support Research Integrity (2012)

This concordat seeks to provide a comprehensive national framework for good research conduct and its governance. As signatories to and supporters of the concordat to support research integrity, we are committed to:

- maintaining the highest standards of rigour and integrity in all aspects of research
- ensuring that research is conducted according to appropriate ethical, legal and professional frameworks, obligations and standards
- supporting a research environment that is underpinned by a culture of integrity and based on good governance, best practice and support for the development of researchers
- using transparent, robust and fair processes to deal with allegations of research misconduct should they arise
- working together to strengthen the integrity of research and to reviewing progress regularly and openly

University of Exeter Code of Good practice in the Conduct of Research

Signatories

RESEARCH COUNCILS UK

wellcome trust

Scottish Funding Council
Promoting further and higher education

NHS
National Institute for Health Research

Department for Employment and Learning
www.delni.gov.uk

HIGHER EDUCATION FUNDING COUNCIL FOR ENGLAND hefce

Universities UK

Cyngor Cyllido Addysg Uwch Cymru
Higher Education Funding Council for Wales
hefcw

Research Ethics: Integrity

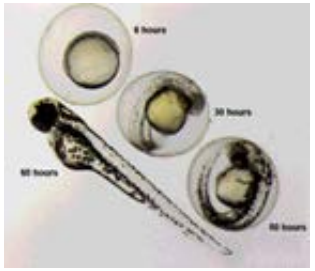
- Why is this relevant to me?

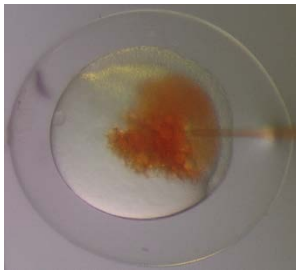
❖ High quality research

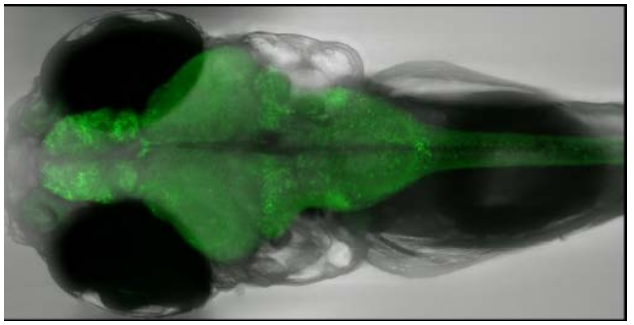
❖ Public trust

❖ Core values and moral responsibility

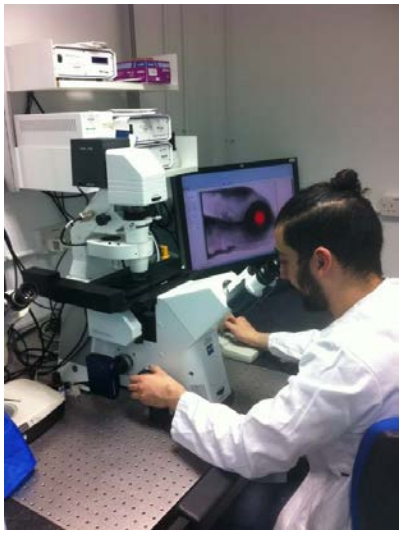
❖ Integral part of all stages of research – not just at the start







❖ Compliance requirements!



When does a project require ethical approval?

All research involving:

human participants, their data or tissue

animals

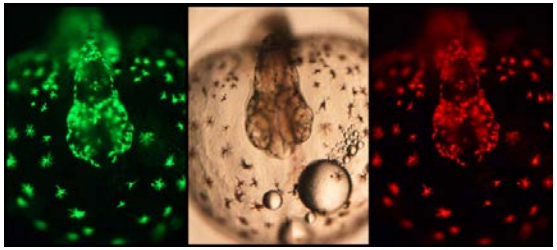
UK: Animals (Scientific Procedures) Act 1986

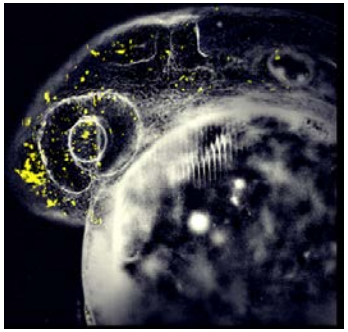
Regulates procedures carried out on protected animals for scientific research and testing that may cause pain, suffering, distress or lasting harm

- Definition of protected animal:

Any living vertebrate, other than man, and any living cephalopod.
A protected animal is living until its circulation stops permanently or its brain is destroyed

Fish become protected when they are capable of independent feeding.





'Grey Areas'

Repeated use of animals in unregulated procedures, behavior, confinement

Cumulative/additive effects?

Animals (Scientific Procedures) Act 1986

- Promotes '3Rs' - replacement, reduction, refinement

3Rs policy – animal research is carried out where no practical alternative exists and under controls which keep suffering to a minimum. ASPA is committed to ensuring that any licence they grant under the act must rigorously and demonstrably apply the 3Rs principles.

- Three-tier licensing system administered through Home Office:

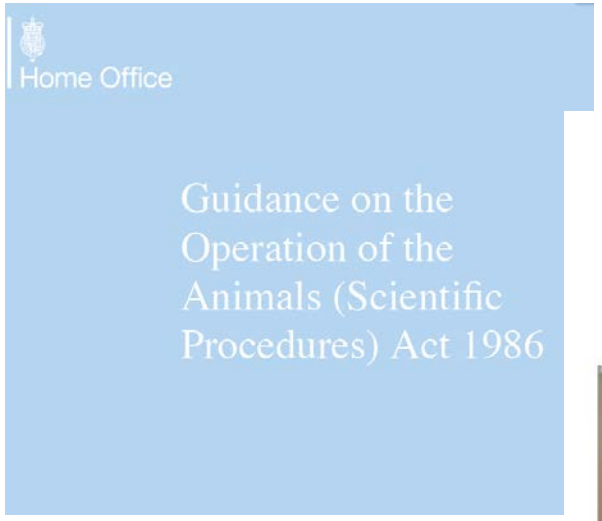
  Establishment Licence

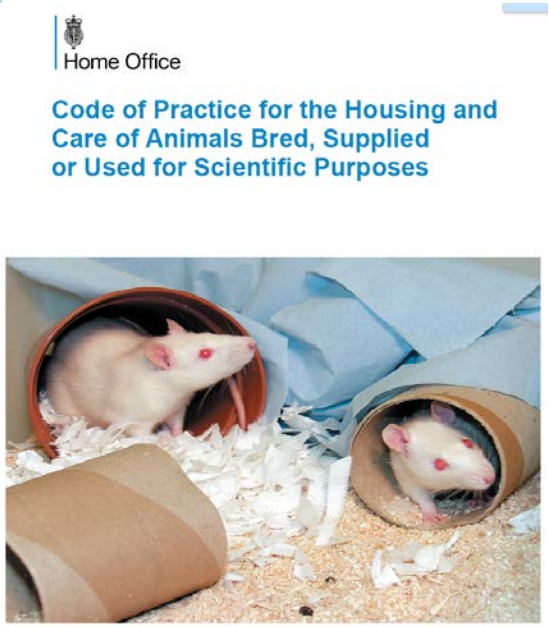
  Project Licence

  Personal Licence

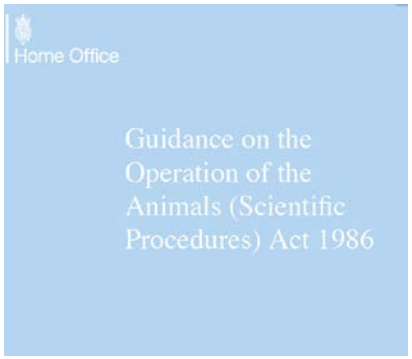
- Home Office Inspectorate

  Compliance





Animals (Scientific Procedures) Act 1986 – regulated by the HO



Background to the Act

The 3Rs and choice of methods

Establishment licences

Personal licences

Project licences

Humane killing of protected animals

Care and accommodation of protected animals

Named Persons

Training

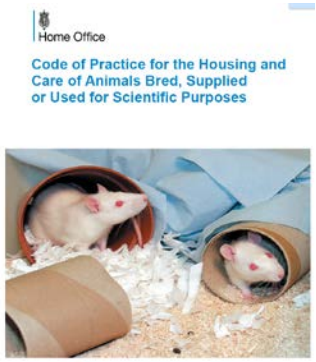
Animal Welfare and Ethical Review Group

About Home Office Inspectors

Non-Compliance – condition 18!

The Animals in Science Committee

Other advisers to the Secretary of State



Background

- Why? To whom? Where applies?
- Comes into Force?

The Physical facilities

Environmental Conditions

Care of Animals

- Health, housing, enrichment, wild
- Feeding, watering, handling

Mice

Birds

Fish etc.

Animals (Scientific Procedures) Act 1986 – Locally enforced by:

Named Persons:

- **Establishment Licence Holder (ELH)**
- **Named Veterinary Surgeon (NVS)**
- **Named Animal Care and Welfare Officer (NACWO)**
- **Named Information Officer (NIO)**
- **Named Training and Competency Officer (NTCO)**
- **Home Office Liaison Officer (HOLO) – optional for the institute**

AWERB Members:

- **Member of academic staff that doesn't carry out animal research**
- **Member(s) of academic staff that does carry out animal research**
- **Lay member**
- **AWERB Secretary**
- **HO Inspector has the right to attend**

AWERBs role: review applications for Project Licences

PPL BACKGROUND – expertise, project duration, place of work, background, expected benefits

PROGRAMME OF WORK – purpose, aims, should be unambiguous, realistic and achievable!

THE 3Rs – Replacement, Reduction, Refinement

SPECIAL SPECIES – domestic, wild, endangered, origins

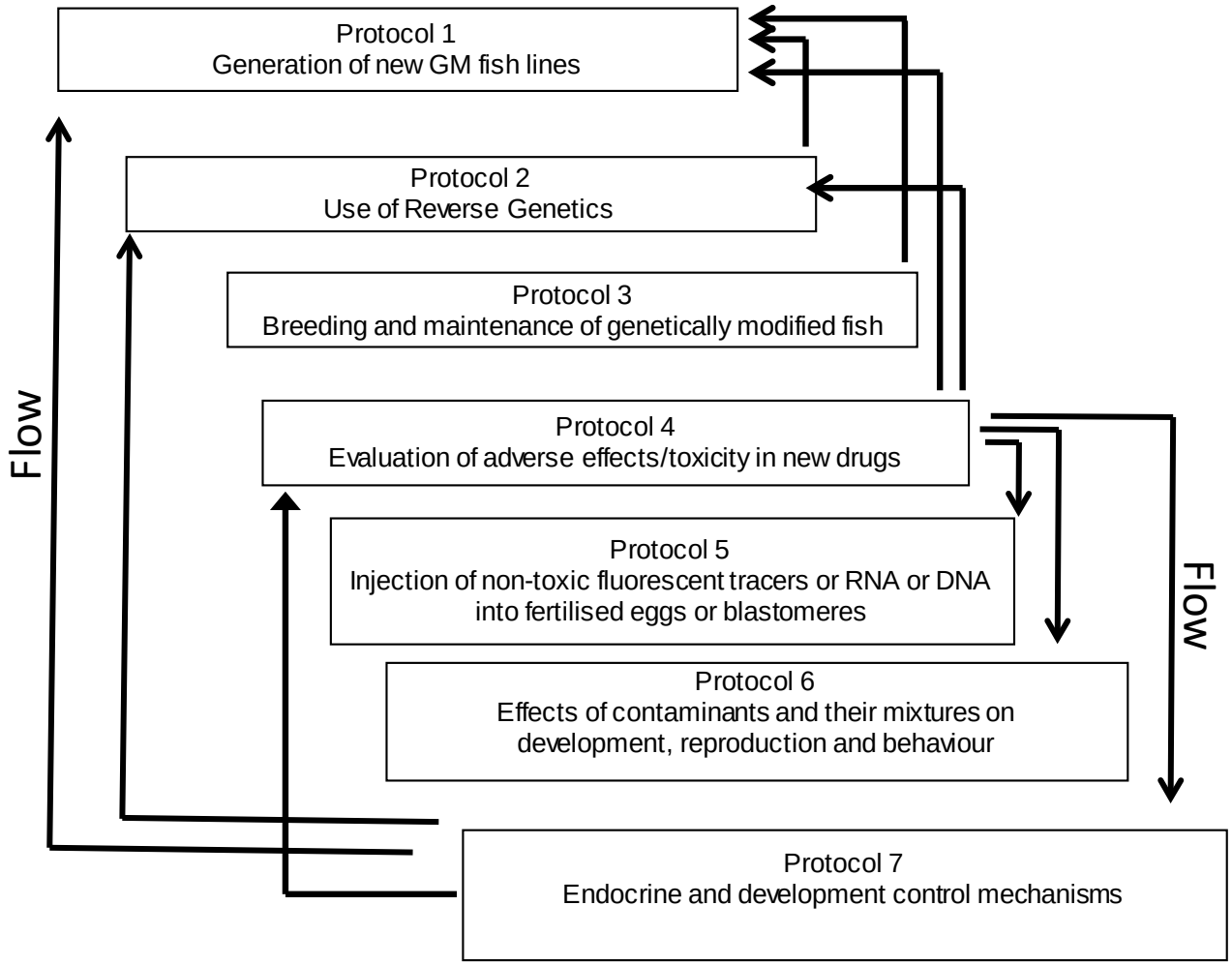
USE OF NEUROMUSCULAR BLOCKING AGENTS

TRANSFER OF ANIMALS – Export/Import, licence, welfare

PROTOCOLS – Experience (flow) of the animal in the facility.

NON-TECHNICAL SUMMARY

Animals (Scientific Procedures) Act 1986 – Example of Project Licence:



Protocol No.	Estimated numbers over the duration of the project	Severity limit
1	Adults (500), juveniles (2000) and embryos (2000) per year	moderate
2	Adults (400), juveniles (600) per year	Mild
3	Adults (100), juveniles (100) per year	moderate
4	2000 per year	severe
5	500 per week	Mild
6	500 per year	Mild
7	2000 per year	Moderate

Animals (Scientific Procedures) Act 1986 – Enforced by:

- Expected Adverse effects

❖ Likely incidence

❖ How recognised

❖ Refinement control measures

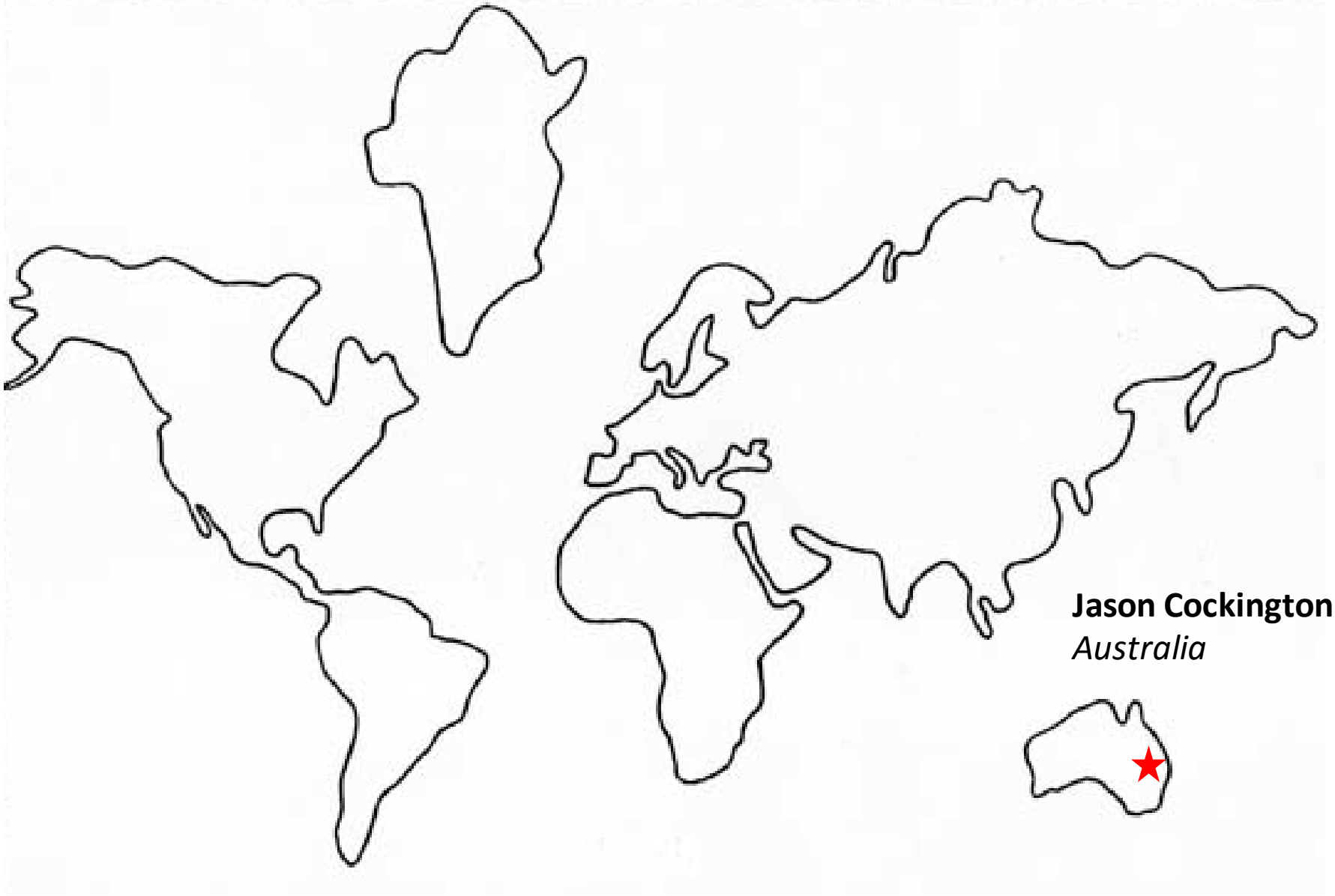
❖ Humane end points and limits of severity

Humane end-points and limits of severity

The following humane endpoints and limits of severity will be applied from the point of protection (5 dpf) onwards:

- Overall mortality should be no more than 2% greater than that expected through normal husbandry practices otherwise that batch of larvae will be humanely killed.
- The majority of animals should be responsive to seeker touch or a Petri dish tap otherwise that batch of larvae will be humanely killed.
- <20% should exhibit abnormal posture (e.g. loss of dorsoventral balance) otherwise that batch of larvae will be humanely killed.
- Any animals exhibiting unusual morphology (e.g. abnormal spinal curvature) will be humanely killed. Estimated occurrence <1%.
- Any animals showing severe oedema (which is easily visible with the naked eye and inhibits the normal movement of the animal) or body malformation (e.g. shortened body or facial-cranial defects) will be humanely killed. Estimated occurrence <1%.
- If abnormal locomotor activity is seen in >20% of animals, then that batch of animals will be humanely killed.
- Any spontaneous seizure-behaviour (e.g. rapid circular swimming, head twitching) observed in any animals then these animals will be humanely killed.

Perspectives



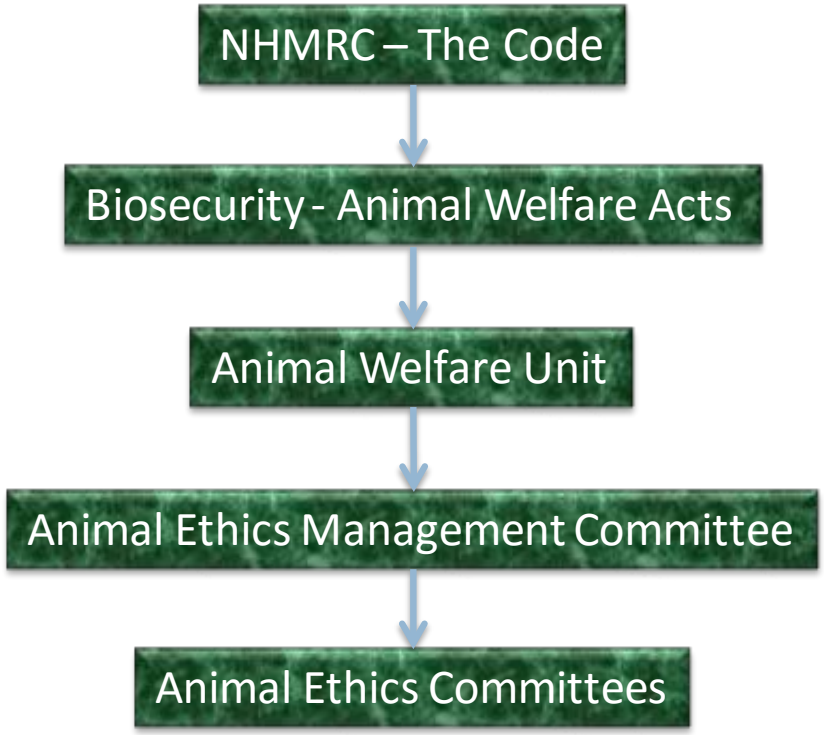
Australia : Hierarchy of Enforcement

Federal Government

State Government

Institution

Independent authority



Research Ethics: Framework – The Code

- Australian code for the care and use of animals for scientific purposes 8th ed. (2013)

- Details roles and responsibilities
- Compliance is requisite for government grant funding

Legislation: The Animal Care and Protection Act (2001)

- Does The Code apply to fish?

- An animal for the purpose of scientific use means:
  - all vertebrates excluding human beings (e.g. mammals, birds, fish, amphibians, reptiles)
  - cephalopods (e.g. octopus, squid, cuttlefish and nautilus)
  - crustaceans (e.g. lobster, crab and crayfish) (except in QLD)
- The term “animal” also includes vertebrates, cephalopods and crustaceans in the early stages of their development
- Fish eggs excluded

Animal Ethics Management Committee

Defines the University's interpretation of The Act

- When is a fish a fish?

Varies sate to state

- VIC = 7dpf
- NSW = 5dpf
- QLD = Exogenous Feeding Onset
- NZ = 30dpf

Not a Fish?

- Experiments require an ANRFA
  (Application Not Requiring Full Application)

Animal Ethics Committees

Ensures the institution remains compliant with *The Code*

- Reviews applications for research, breeding & teaching
- Reviews project performance and approves project continuation
- Monitors animal welfare by inspection of holding facilities
- Approves animal use SOPs
- Reports to the regulators as required

Animal Ethics Committees

Categories of membership

A. Registered Veterinarian

B. Research scientist with substantial recent animal experience

C. Animal Welfarist (e.g. RSPCA)

D. Lay person

Advisory guests

- OIC (Facility Manager)
- AWO (Animal Welfare Officer)
- Institutional Veterinarian

Animal Welfare Enforcement

Monitoring by the regulator Department of Agriculture and Fisheries (DAF)

Annual inspections of laboratories & animal holding facilities by AEC

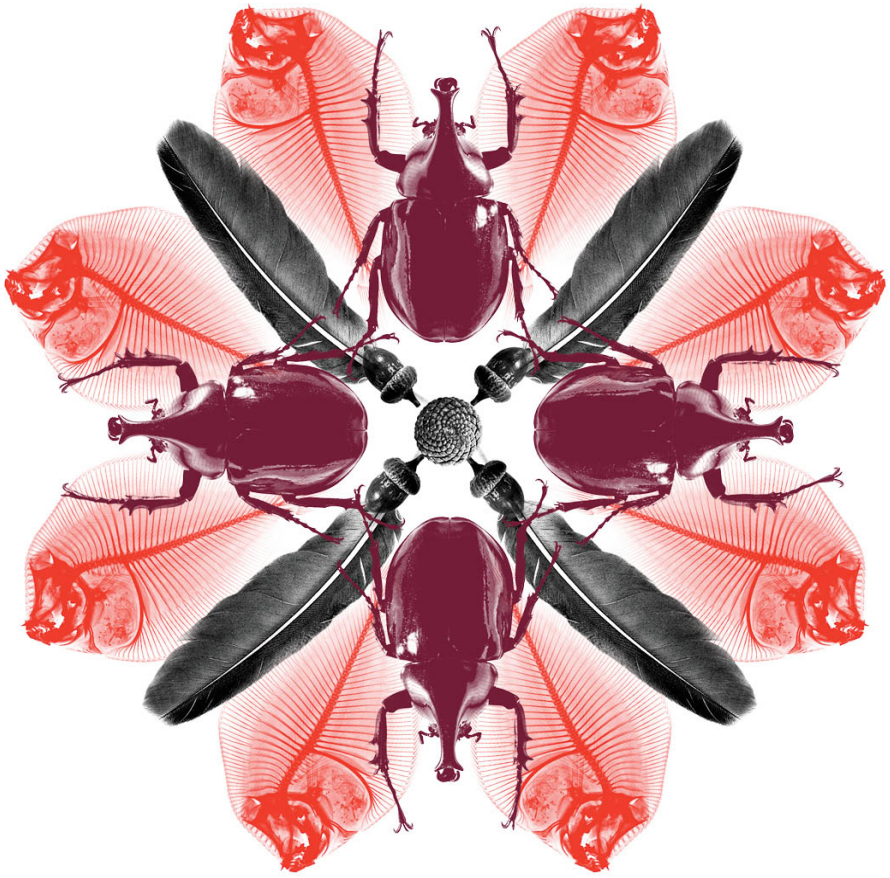
Mandatory Annual Report

Conclusion Report

Animal Ethics Vs Biosecurity

Biosecurity much greater common concern in Australia

- Quarantine
- Gene Technology Regulation



Department of Agriculture and Fisheries : Biosecurity

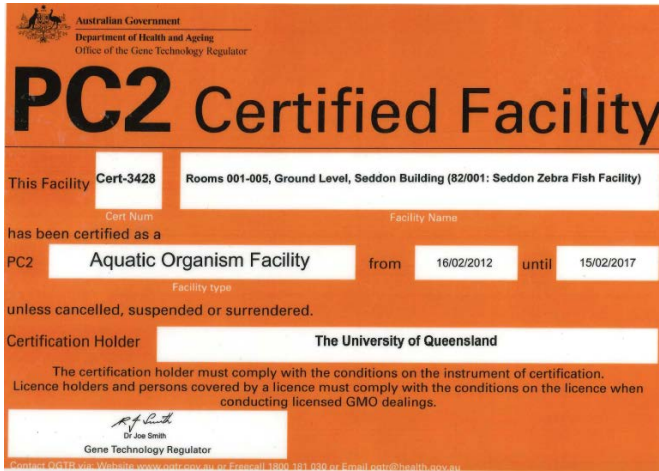
Quarantine Approved Arrangement Class 7.7 – Live Laboratory Fish

- Life-time quarantine
  - Pre-release holding period = >7 days
  - Release inspection by DAF Biosecurity
  - Only offspring permitted for release – must be bleached
- Shipping Lines Internationally
  - Live fish must be declared = No FedEx Shipments!
  - IATA Packing Instruction 959 if GMOs
  - Accurate animal numbers
  - Government Vet signed Health Certificates

Department of Health :
Office of the Gene Technology Regulator

Notifiable Low Risk Dealing (NLRD) required to hold GMO zebrafish

- Assessed by Institutional Biosafety Committee (IBC)
- Handled by trained personnel only
- Held in Phisical Containment Level 2 – Aquatic Facilities
- Storage, transport & disposal prescribed under regulation
  - Label must declare GMO
  - All GMOs must be accounted for
  - Exact location known when stored



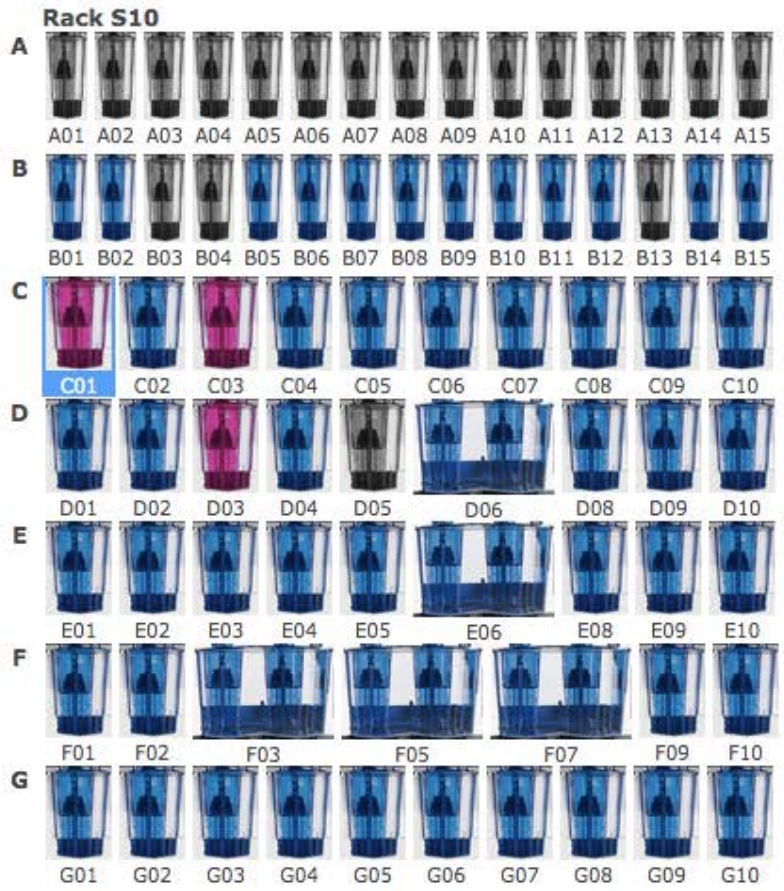
Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

PC2 Certified Facility

This Facility Cert-3428 (Cert Num) Rooms 001-005, Ground Level, Seddon Building (82/001: Seddon Zebra Fish Facility) (Facility Name)
has been certified as a
PC2 Aquatic Organism Facility (Facility type) from 16/02/2012 until 15/02/2017
unless cancelled, suspended or surrendered.
Certification Holder The University of Queensland
The certification holder must comply with the conditions on the instrument of certification.
Licence holders and persons covered by a licence must comply with the conditions on the licence when conducting licensed GMO dealings.
Dr Joe Smith
Gene Technology Regulator

OGTR : Storage of GMOs example

Zebrafish	
Tank:	109574
Clutch:	206134
Line/Label [Edit]:	nfliGFP;lyvedsRed
Line/Label Fullname:	nfliGFP;lyvedsRed
Background:	
Group:	Hogan
Owner:	Kaska Koltowska
Genotype:	
DOB:	03-07-15
Father Tank:	
Mother Tank:	
Transgenic:	✓
IBC/OGTR Approval:	IBC/176E/IMB/2009
Ethics Approval:	IMB/181/13/BREED
Import:	
Location [Split]:	S10C01
Export:	
Count:	46
Males:	0
Females:	0
Notes:	from outx with lyve, selected GFP DsRed positive 13/06/16 Death : 1 dead 14/06/16 Outbox : 1 Body Score. Score:0 27/07/16 Death : 1 dead 10/09/16 Death : 1 dead
Merged Into:	

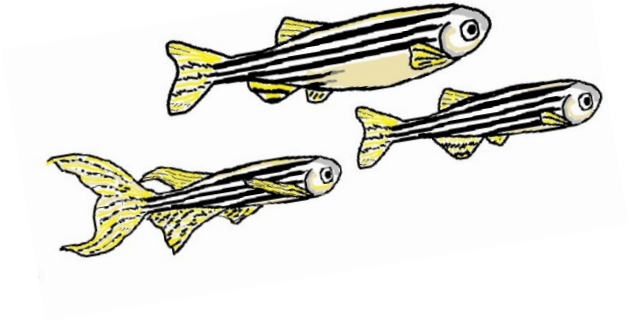


Fishy Scenario : Gone Fishing

Fish from a research project are found very unwell. What do I do if the CI is not contactable?

- Section 2.4.1: Researchers take personal responsibility for all matters that relate to the wellbeing of animals that they use, including housing, husbandry and care.

- Section 2.5.6: If an emergency welfare intervention is considered necessary for the animal allocated to a project, animal carers must take reasonable steps to first contact the responsible investigator. However, welfare of the animal must be the priority at all times and may necessitate immediate intervention



Animals (Scientific Procedures) Act 1986 – Enforced by:

- Importantly AWERB is not just a rubber stamp for projects!

❖ Project Licence Review should be a maximum 40% of AWERB activity

❖ AWERB designed as a review process for all animal work

❖ Notably a forum for refinements and new techniques to be brought to the attention of researchers focusing on 3Rs

❖ Cost/Harm Benefits!

AWERB meets every 2-3 months

NVS inspects monthly

Named persons meet every 2 months

HO inspections (unannounced) twice a year