The Challenges of Using Humane Endpoints in Fish Research

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The humanest possible treatment of experimental animals, far from being an obstacle, is actually a prerequisite for successful experiments’

Russell and Burch 1959

What is a humane endpoint?

- "the point at which an animal's pain and /or distress is terminated, minimised or reduced, by taking action such as killing the animal humanely, terminating a painful procedure or giving treatment to alleviate pain and/or distress"
- '**decision point**' at which **action is taken** to **alleviate animal suffering**
- Planned

When to intervene?

- The experimental aim is
  - Achieved
  - Cannot be achieved

- The suffering is
  - Disproportionate
  - Unacceptably high

Endpoints: Variable terminology

- Humane endpoints
- Scientific/experimental endpoints
- 'Objective-achieved' endpoints (Fry 1999)
  - outcome measures
- Surrogate (pre-lethal) endpoints
- **To go beyond what is required to achieve a scientific objective is 'avoidable' suffering and therefore 'inhumane**

The Challenges

- Attitudes
- Range of
  - species, sizes, stages
  - study types, models and pathologies
- Accessibility
- Defining the normal and abnormal: welfare indicators
- Regulatory acceptance

Attitudes

- Societal
- Scientific

- Considerations
  - Legal
    - Fish are protected under EC 86/609
  - Moral
    - Should assume & alleviate/ prevent suffering

Range -Types of fish used

- Zebrafish, salmon, brown & rainbow trout, sticklebacks, cod, fathead minnows, sheepshead minnows, tilapia, guppies, other cichlids (behavioural), turbot, seabass, pangassus catfish, goldfish, dab, sharks, plaice, haddock, halibut
- Round - flat, tropical – temperate, teleost – elasmobranch

  No best (adequate) welfare conditions defined
- Mature forms vs immature

Study types and models

- Development of medicines
  - Safety & efficacy - fish
  - Basic science – fish
  - Fast throughput screening - human
- Toxicology
  - Pollution
- Developmental Biology
- Understanding fish disease
- Understanding human disease
  - Genetics
- Behavioural

Variable Consequences

- Likely suffering related to:
  - Degree of compromise
  - Duration of compromise
  - Interventions
    - N.B. Specific handling issues for fish
- Additional potential suffering from
  - Continued use
  - Re-use

Low impact

- Usually low intervention studies, e.g.
  - Feed trials
  - Some genetics studies
  - Some Genetically Modified Organisms
- Few or no adverse effects likely
  - Scientific endpoint usually precedes welfare endpoint
- Adverse effects well characterised
  - early intervention possible

Moderate Impact

- Most types of study can fall into this category dependent on study design
  - Passage of pathogens
  - Some Genetically Modified Organisms
  - Vaccine safety tests
  - Disease models

- Adverse effects well characterised and intervention possible

Substantial Impact

- Significant, “long”-lasting clinical signs and/or mortalities
  - Ecotox
  - Disease models
  - Vaccine challenges
  - Virulence studies
- Impact on individual animals can be very high
- Important to reduce animal numbers
  - Intelligent Testing Strategy

Minimise suffering: Study design

- Correct design will minimise numbers
- Statistical advice
  - Numbers per group, replicates, intervention points
  - Size of effect required
- Avoid confounding factors
  - Intercurrent disease, poor quality stock

Accessibility

- Aquatic environment creates significant challenges:
  - Ability to visualise animals

Accessibility - view

- Size of enclosure/ access
- Numbers of animals held

Defining Normal and Abnormal

- Fish size
- Intrinsic mortality rate
- Identification of individuals
- Stocking density requirements

Welfare Indicators

- Positive or negative

- Positive = good welfare
- Negative = poor welfare

- Welfare indicators identified for (any) fish?

“Positive” welfare indicators

- Normal behaviours
  - Swimming /activity levels
  - Interactions with conspecifics
- Absence of disease
- Growing?
- Feeding?

Negative Welfare Indicators

- Not eating
- Lack of growth
- Poor swimming ability
- Obvious pathology (e.g. ulcers, exophthalmus, ascites, hemorrhage)
- Darkened/ mottled skin
- Pallor of gills
- Lethargy/ moribund
- Mortality (!)

Welfare Indicator limitations

- Better at identifying negative than neutral welfare indicators
- In some species/ stages - only able to identify huge deviations from adequate welfare
- Moribund or dead should not be considered as useful welfare indicators
- (it's a bit late)

What endpoint & why?

- Individual study
- Purpose
- Size of effect required
- Set endpoint to earliest possible time

What endpoint & why?

- Purpose
  - What is the hypothesis?
  - What needs to happen for you to disprove the null hypothesis?
  - What measures are you taking?
  - Is there a sequence of events?
  - What is the earliest you can use to achieve the scientific goal?

What endpoint & why?

- Size of effect required
  - Obtaining tissue – sea lice
  - “Enough” material
  - More fish – less consequence
- Difference expected between groups
  - Detectable difference vs clinically relevant difference
  - Protection

Use of pilot studies

- Identify clinical signs
- Predictive BUT NOT necessarily unique
- Consider signs in light of experimental protocol

Signs of Disease

Viral Haemorrhagic Septicaemia (VHS)

- Poor feeding
- Poor balance
- Erratic swimming behaviour
- Skin darkening
- Exophthalmia
- Pale gills

Bacterial Kidney Disease (BKD)

- Poor feeding
- Poor balance
- Erratic swimming behaviour
- Skin darkening
- Exophthalmia
- Ascites
- Pin-point haemorrhage

Event specific timing

- Identify time course
- Mortalities (FD) vs euthanased moribund records
  - Separate other (timed) sampling
- Target checks and sampling
- Increased frequency and duration at “high risk” times
  - Better samples (moribund more useful than post-mortem)
  - Improved welfare
  - Improved economics

Welfare assessments

- Qualitative
  - Subjective assessment
- Quantitative
  - Objective assessment of degree of disturbance
- Semi-quantitative
- Performed via “Cage side” assessments
- Use of distress/clinical ‘score sheets’ e.g. Morton and Griffiths 1985

Scoring systems

- Framework for humane endpoints and refinements
- Numerous examples
- Must adapt for maximum benefit
- Pilot studies are important
- Intervention points and actions clearly identified
- Must
  - Be easy to use, consistent, specific and sensitive
  - Specify intervention points

Bacterial Kidney Disease (BKD)

- Poor feeding
- Skin darkening
- Erratic swimming
- Exophthalmia
- Poor balance
- Pin-point haemorrhage
- Ascites

- Increase monitoring
- Vigilence
- Vigilence ++
- Early end point
- End point
- Absolute End point
- Absolute Endpoint

Plans / Score sheets

- Essential to modify with experience
- Review
  - suggested and actual clinical signs
  - “found dead” records
  - frequency and duration of checks
  - staff knowledge and training

Death as an Endpoint

- Avoid
  - Personal licensee requirement UK to kill animals undergoing severe suffering that cannot be relieved
  - OECD guideline humane endpoints
  - “mortality” ≠ death
- Frequent checks in acute phase
- Seek regulatory/ peer acceptance for clinical endpoints for your models
- Develop welfare assessments/ clinical scoring
- Embryonic / early lifestage lethality

Regulatory Acceptance

- Regulators require robust data to change requirements
  - Know what you are trying to prove (disprove)
  - Discuss with them what they need
  - Collect information to support change
- Rigidity of some fish regulatory studies
- Apply pressure for change!

Humane endpoints: Summary

- Scientifically valid, predictive and accurate
- (Regulatory acceptance)
- Developed on a study by study basis
- Use of pilot studies to define onset and time course of predicted adverse effects
- Requires a team approach
- Staff training and empowerment
- **Dynamic process (plan/implement/record/review)**

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- UK Licensees