Humane Endpoints in Regulatory Toxicology in Fishes

Surrey, 2023

FOCUS ON SEVERE SUFFERING

Summary Report

In November 2023, the RSPCA organised an in-person meeting at the Animal and Plant Health Agency in Weybridge, focusing on humane endpoints in regulatory toxicology studies using fishes. The aim was to identify and share practical refinements to reduce and avoid 'severe' suffering. The discussion focused on the need to standardise practices, ensuring that refinements can be widely adopted. This report will summarise the presentations and discussions, and suggest action points for both immediate and future steps.

Chloe Stevens from the RSPCA opened the meeting by explaining that the topic was chosen on the basis of a report commissioned by the RSPCA and conducted by Alyson Leyshon, of LeyshonBanks Consulting. The comprehensive report investigated severe suffering within regulatory testing and highlighted the importance of standardising good practices for staff training and the development of humane endpoints.

The first session of the day was devoted to **case studies - identifying indicators in acute toxicology**.

Nic Bury, from the University of Southampton, introduced the challenges posed by novel chemicals to wildlife and the environment. There are an estimated 350,000 chemicals on the global market with little understanding of their impact at a cellular level. The ethical issues with using fish in toxicity tests, plus the enormous number of compounds to be tested, means that an alternative approach is required to risk-assess these chemicals. Nic is working on an innovative approach to identify how chemicals interact with stress receptor proteins using **bioinformatic tools** to predict differences in chemical docking between proteins from different fish species, and with predictions confirmed using functional assays. Nic hopes to expand to include more proteins and chemicals, thus circumventing the need to perform tests with fish.

In the next presentation, Karen Thorpe from Fera provided valuable insights into animal welfare challenges associated with **OECD tests 203 and 210**[1], and presented ways of addressing these. She advocated for using the 'threshold approach' for testing fish toxicity. In contrast to full toxicity testing involving five concentrations, this approach employs a single concentration test which significantly reduces animal use and suffering whilst generating data acceptable to regulatory bodies. The threshold approach uses a 'limit test', testing fish toxicity at a concentration determined by reliable data from algae and acute invertebrate toxicity data. Karen also highlighted the significance of 'intervention' endpoints in reducing severe suffering in fishes. Interventions are based on clinical signs and may include increased observation frequency, or a humane endpoint being applied. In order for this approach to be effective, the observer has to be well-trained in observing fish behaviour, and be aware that premature killing can affect the validity of the study which may result in more fish undergoing testing.

[1] The OECD guidelines are a collection of the most relevant internationally agreed testing methods used by governments, industry and independent laboratories. OECD test 203 describes 'Fish, Acute Toxicity Test', OECD test 210 describes 'Fish, Early-life Stage Toxicity Test'.

Chris Ramsden from AgroChemex Environmental presented a different perspective on minimising harm in regulatory testing, particularly focusing on the **OECD 203** guideline. He explained how the intention behind the test is to model a worst-case scenario for chemical exposure on the one hand, but on the other hand the protocol also optimises fish survival by eliminating all other variables, including predators, and water flow. This is not a natural situation, so the test is not necessarily a valid predictor of what would happen in the environment. Chris explained that a 2019 revision of test guidelines resulted in the incorporation of the threshold approach, and limit tests, along with more comprehensive observation assessments. However, the requirement within the test guideline for mortality as the endpoint still unfortunately remains. Chris suggested that prior confirmation of the acceptability of the threshold approach or the LC50 moribund study (which allows for euthanasia prior to death to reduce suffering in terminally ill fish) is essential, although if there is any doubt in the acceptability of this design to the regulatory authority the study should default to using mortality as the endpoint to avoid the risk of the study being rejected. He outlined criteria for a high quality study, involving the sourcing of fish, optimal staff expertise, robust analytical support and all other aspects required to fulfil regulatory standards. Chris concluded by explaining that until the requirement for this study type is amended and unless the guideline itself is updated to reduce suffering, a well-executed study that meets validity criteria, ought not to be rejected or repeated by regulatory authorities, which therefore prevents further fish from experiencing severe suffering.

Following this, Ioanna Katsiadaki from Cefas described the challenges and opportunities in potentially **deleting OECD 203 and 210 testing guidelines**. Focusing 3Rs efforts on these tests was highlighted as a priority due to the significant numbers of animals used in them. Ioanna cited a DEFRA-sponsored workshop on refining test 203 in 2020 that identified two refinement opportunities: (1) the application of clinical signs that predict mortality and (2) shortening the test duration. However, there are challenges involved in implementing these refinements, which are largely due to a lack of consensus at the OECD level. Ioanna discussed the emergence of non-animal testing methods (NAMs) and their significance in addressing the sheer volume of chemicals requiring assessment, as well as reducing the ethical and financial concerns of traditional testing methods. While it is unlikely that OECD tests 203 and 210 will be ‘deleted’ in the near future, the use of alternatives and application of humane endpoints by CROs will significantly reduce the suffering experienced by large numbers of fishes.

In the final presentation of this session, Claire Morgan from Cefas discussed the importance of **checking laboratory fishes**, their housing systems, and environmental conditions to monitor their welfare. Claire highlighted the need for meticulous record-keeping, including observed clinical signs, to track issues, inform staff, assess severity, and provide data on treatments. There should be a commitment to high standards of checks on fishes, with responsible, empathetic and well-trained personnel. The presentation concluded by mentioning ongoing efforts aimed at providing foundational guidance for checking laboratory fishes and the necessity of regular staff reassessment, to ensure competence and adherence to established standards.

The next session focused on training. Robin Labesse from the Institute of Animal Technology (IAT) highlighted the Institute's role in promoting animal welfare through **higher education**

for animal technologists. The introduction of new Continuing Professional Development (CPD)-friendly courses was emphasised, featuring a more flexible format with smaller units, making education more accessible and tailored to individual needs (see iateducation.co.uk).

Following this, Felicity Hood and Elaine Wardrop from Charles River presented a talk on delivering training, focusing on the challenges of training in the industry and emphasising the need to develop and maintain a **Culture of Care**. They discussed strategies for making training relevant, informative, and legally compliant. Demonstrating that individuals are making a positive impact helps to recognise the importance of staff well-being within the Culture of Care. The speakers addressed the importance of teaching care, empathy, and compassion, and utilising various media for effective training. Finally, Rebecca Cohen from Fera shared her experience working as an animal technician specialising in fishes. She emphasised **the importance of taking time** during training on husbandry and welfare, detailing her extensive shadowing of senior staff during health checks and feeding. Rebecca's experience highlighted the effectiveness of a practical, hands-on training approach, because understanding animal behaviours requires substantial time investment.

The group then discussed **how training for technicians could be improved**. During the discussion on training staff how to monitor fishes and identify sub-lethal indicators, several **key issues** were raised:

- The diverse range of fish species poses a challenge, with each having unique characteristics that complicate the standardisation of monitoring practices.
- The sheer number of individual fish used in studies also poses a practical challenge.
- There is a notable lack of training materials for personnel involved in monitoring fishes.
- Some sub-lethal indicators may not be observable as they involve subtle physiological changes. For example, waterborne indicators like cortisol were acknowledged as useful under controlled conditions, but there are practical limitations including the time to get results. Indicators that can be used rapidly at the tankside are required.
- It can be difficult to monitor individuals effectively, due to the high density, difficulty tracking fast-moving fishes in 3D environments, and the difficulty distinguishing between individuals. This can make tracking symptoms and determining the likelihood of recovery difficult.
- Staffing levels can be an issue, particularly for unsocial hours.

Participants agreed that better standardisation of good practices for monitoring fishes could be achieved by improving training for animal care staff and researchers in fish welfare. Emphasising the importance of continuous learning, the group suggested regular updates to training programmes to align with advancements in fish welfare science. In-house "practical refresher" sessions, revised annually, could facilitate ongoing professional development. Additionally, the group encouraged technicians to actively participate in meetings and workshops, fostering a collaborative environment for knowledge exchange.

A small-group discussion on **standardising how sub-lethal clinical signs are identified within acute regulatory toxicology** identified some essential **action points**, which are summarised and discussed in Table 1. The discussion underscored the need for

collaboration, faster adaptation by regulators, and a cultural shift in considering the welfare of fishes in toxicology studies. As a meeting outcome, we plan to use the Table to help enable further initiatives towards standardisation.

Table 1: Working towards a framework for standardisation in applying humane endpoints in fish toxicology. This table lists action points for regulators of animal use, e.g. the UK Animals in Science Regulation Unit [R]; regulatory bodies such as the OECD [B]; the scientific community [S]; animal unit management [M]; training organisations [T]; external bodies, such as dedicated groups formed to achieve the task [E]. These would usually include members with a range of expertise and perspectives, e.g. scientists, animal technologists, veterinarians, regulators and participants from NGOs.

Recommendation	Application	Challenges
Define and implement a standardised approach to identify sub-lethal clinical signs and apply humane endpoints.	Develop frameworks for monitoring that include reference guidelines, checklists, and record sheets. Include information on normal behaviour, species-specific norms, and indicators of clinical signs. [E] Develop and adopt a standardised language for describing fish behaviours and clinical signs. This will help to achieve consistent interpretation between individuals and organisations. [E] Promote collaboration and information/process sharing around applying humane endpoints between organisations. Create a platform for sharing best practices, research findings, and experiences. [E] [S] [M] Treat a standardisation framework as a living document that evolves over time. Regularly update guidelines to align with the latest advances in fish behaviour and welfare science. [B] [E] [S] Involve regulatory bodies in developing and endorsing standardised practices. Ensure that regulatory standards align with the agreed framework. [E] [R] [B] Establish feedback mechanisms to assess the effectiveness of the standardised approach. Encourage internal audits, reviews, and continuous improvement based on feedback from the scientific community. [R] [S]	A standardised approach is likely to be difficult to develop for a large number of fish species with different characteristics. Individual observer variations in interpreting fish behaviour may hinder standardised assessments. Identifying objective indicators will be a priority. Divergent practices between industries (e.g. company-specific or sector-specific) may impede universal adoption. It may be difficult to achieve widespread acceptance of the standards. Access could be limited to advanced technologies for effective detection of clinical signs. Globally, there are differences in both national regulations and cultural perspectives on fish welfare. These measures will all require resources, leadership, development and management, which may be difficult to access.
Establish standardised approaches to staff training on identifying clinical signs and education in fish behaviour and welfare.	Develop standardised training programmes for personnel involved in fish monitoring. These programmes should focus on species-specific behaviours and indicators of welfare and adverse effects. [T] [S] [E] Establish Continuing Professional Development (CPD)-friendly courses with flexible formats, making education more accessible. [T] [S] Encourage participation in meetings, workshops, and knowledge exchange forums. [M] Emphasise the importance of a Culture of Care within training frameworks. Ensure that training goes beyond technical skills to instil care, empathy, and compassion among staff. [T] [M] [R]	Resources and leadership will be required to establish training standards. Establishments may resist external input into education and training. Staff might resist adopting new methodologies or altering established routines. Variations in staff experience levels may lead to disparities in assessments, which will need to be monitored and addressed if necessary. Access could be limited to advanced technologies for effective training methods. There might be increased time, personnel, and financial requirements for continuing training programs.
Promote collaboration and information-sharing networks within the scientific community to facilitate the generation and dissemination of resources.	Engage stakeholders, including researchers, technicians, and regulatory bodies in discussions on standardisation. Encourage feedback, collaboration, and the sharing of experiences. [M] [S] Establish platforms for collaborative efforts among organisations. [M] [S] Create information-sharing networks. [S]	Some CROs are resistant to revealing what they perceive to be ‘company sensitive information’. These will need to agree that information shared to help train staff, and implement humane endpoints, is not commercially sensitive. Identifying which organisation or group will oversee a collaborative effort could be problematic.

	Support initiatives by organisations such as HOLTIF, RSPCA, and NC3Rs. [S] [M] Work towards the developing a global guidance system for fish welfare in toxicology studies. Include high-level and specific indicators, minimum standards at the start of a test, and a clear definition of clinical signs. [R] [B]	Regulatory bodies can be slow to change guidelines; this may require significant negotiation and persuasion.

The final session of the day involved a discussion of **the use of technology in monitoring fishes**. A 2016 NC3Rs/CEFAS meeting on 'addressing the needs for refinement in laboratory fish' discussed the potential for using video-monitoring of fishes, including not only recording and reviewing fish behaviour, but also using specialist software to recognise behavioural indicators of adverse effects. Some obstacles preventing greater use of these technologies were highlighted as technical limitations, financial costs and lack of awareness, and a pre-meeting survey suggested that the same issues were relevant in 2023. While the majority of participants currently do not employ any technology for monitoring, there was unanimous agreement on its potential utility. Remote monitoring, specifically, was highlighted for its ability to identify humane endpoints without altering fish behaviour. However, there is still a lack of advanced technology for data analysis and continued reliance on human observation. The Fish Behaviour Index (FBI) tool, recognised for its value, employs a camera and tracking software to assess fish welfare. Practical challenges, such as potential camera obstructions and electrical issues in water-intensive facility rooms, were also noted.

To summarise the conclusions from this meeting, people who are responsible for the care and welfare of fish in regulatory toxicology tests, including implementing humane endpoints, need:

- Adequate resources, including training materials, to aid standardisation of fish monitoring and implementing humane endpoints.
- Access to comprehensive training modules for assessing fish welfare and clinical signs, covering a variety of species and experimental conditions.
- Standardised language for fish behaviours and clinical signs to avoid individual variability.
- Continuing Professional Development in recognising clinical signs in fish toxicology.
- Opportunities to actively participate in meetings, workshops, and webinars to stay updated and enhance expertise.
- Access to a network for sharing information and best practices, facilitating collaboration and mutual support within the research community.
- Technologies to complement their expertise, such as video monitoring hardware and software to enhance clinical sign identification.

We invite you to review the above list at your establishment and assess the current availability or potential implementation of the mentioned measures. Some may be readily achievable, while others might require investment in order to achieve good practice. Further considerations will require actions by professional bodies and trainers. We welcome any comments or feedback you may have, which can be shared with us at animalsinscience@rspca.org.uk.