SAFE: innovative Safety Assessment of Fish adverse Effects

Overall aim

1. The aim of this Challenge is to develop a suite of innovative, scalable bioassays for key adverse outcome pathways (AOPs) to replace *in vivo* fish studies in chemical safety screening and regulatory environmental risk assessment.

Duration

2. This is a two-Phase Challenge with funding for up to three years. Phase 1: six months, Phase 2: up to three years.

Budget

3. Phase 1: up to £100k, Phase 2: up to £700k.

Sponsors

4. Challenge Sponsors define the Challenges in collaboration with the NC3Rs to set out the business case and 3Rs benefits, with a view to using the product developed. Sponsors are required to provide in-kind contributions to help solve the Challenge.

5. The Challenge is sponsored by the following companies: AstraZeneca, Bayer AG, and Unilever.

Partners

6. Challenge Partners collaborate with the NC3Rs to provide additional resources to successful applicants to help deliver the Challenge.

7. The Challenge Partners are the Department for Environment, Food and Rural Affairs (DEFRA), the Health and Safety Executive and the Environment Agency.

Co-funders

8. DEFRA and the Environment Agency.

Background

9. Environmental Risk Assessment (ERA) evaluates the likelihood that the environment may be impacted as a result of exposure to one or more chemical stressors by addressing the relationship between the exposure of a specific environmental compartment to a chemical (e.g. emissions resulting from consumer use or from industrial processes) and the inherent hazard of that chemical (e.g. potential for it to cause harm to relevant species). Traditionally, the ERA framework relies on *in vivo* testing across three trophic levels: unicellular algae, invertebrates (most commonly *Daphnia*) and vertebrates (fish). Manufacturers use the concepts that underpin ERA when designing new chemicals, aiming to reduce potential environmental impact, but rigorous safety assessment is still required for regulatory approval.

10. *In vivo* fish testing is frequently required for regulatory purposes, but global research efforts into the development and application of New Approach Methodologies (NAMs), broadly defined as any non-animal technology, methodology, approach, or combination thereof, aim to provide improved information on chemical hazard and risk assessment and reduce the reliance on *in vivo* studies (1,2). For example, the RTgill-W1 cell line assay (3) has been accepted by regulators under OECD (OECD TG 249) ISO (ISO 21115:2019) to predict fish acute toxicity. NAMs using chemical read-across (4) and biological cross-species extrapolation (5) are also increasingly accepted by regulators to replace *in vivo* studies. These approaches, however, are limited by their reliance on the identification of an analogue chemical or species for which similar characteristics can be demonstrated and the presence of associated hazard data. These methods can therefore only be used for pathways that are known to be highly conserved across species and lead to a common adverse outcome.

11. There is a need for the development of innovative NAMs-based testing strategies that can holistically assess the risk and impacts of chemicals on fish and replace their use in safety assessment. These approaches will require:

    - Understanding of the specific targets and pathways of toxicological concern that are identified as critical for their growth, reproduction and survival.
    - Identification of biomarkers of exposure able to act as early reporters of those adverse effects.

12. The AOPs framework collates existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organisation relevant to risk assessment. The use of AOPs can improve toxicity testing by identifying species and endpoint selection, enhancing cross-chemical extrapolation, and supporting the prediction of mixture effects. AOPs can also facilitate the use of molecular or biochemical endpoints (biomarkers) to predict chemical impacts on individuals and populations (6). Using the AOP approach in combination with the development of novel bioassays to interrogate biomarkers could fill data gaps in cases where finding a suitable, data-rich chemical or biological analogue may be not feasible and expand the use of NAMs in ERA.

The Challenge

13. This Challenge aims to develop a suite of scalable bioassays to permit reliable chemical screening and risk assessment for relevant fish adverse outcomes that can be standardised and, in the future, accepted within global regulatory frameworks. Information generated by these assays coupled with the capability to understand fish internal kinetics (TK) dynamics (TD) of compounds (7), reverse dosimetry calculations, and the use of quantitative in vitro to in vivo extrapolation (qIVIVE) models will permit optimised environmental protection without the need for further animal testing.

14. Examples of relevant pathways that are in scope are shown in Tables 1 and 2 (pathways in scope are not limited to this list). Development of this technology will represent a major step forward in the use of NAM-based protection of the environment, while maximising the potential to design safer chemicals early in the development pipeline.

Table 1: Examples of fish-specific adverse outcomes. Population level adverse outcome: survival.

Individual level adverse outcomes	Mode of action
Swim bladder inflation	Thyroid signalling
Osmoregulation	Oestrogen signalling
Lateral line impairment	Oestrogen signalling
Olfactory rosette	Oestrogen signalling
Fin regeneration	Oestrogen signalling
Oocyte maturation	Progesterone

Table 2: Examples of fish-specific adverse outcomes. Population level adverse outcome: reproduction.

Individual level adverse outcomes	Mode of action
Vitellogenesis	Oestrogen signalling
Oestrogen signalling	11 keto-testosterone enzymes
Poor quality sperm	Inhibited synthesis of androgens
Decreased E2 levels/decreased synthesis of VTG	Aromatase
Oocyte maturation	Progesterone

3Rs Benefits

15. Between 2015 to 2017, approximately 1.3 million procedures were performed on fish in the EU (190,000 in the United Kingdom), of which a significant number were to support regulatory safety requirements (8). As an example, the most frequently used bioassay for chronic fish toxicity — the fish early life stage test (OECD 210) — requires a minimum of 480 fish individuals from a recommended species, for example zebrafish, excluding breeding stock and animals used for dose range-finding. The early life stage test assesses lethal and sub-lethal effects, including mortality, abnormal behaviour and morphology, and the majority of these tests are conducted at moderate severity (e.g. likely to cause short-term moderate pain, suffering or distress for the animals used). Dose range finding studies are conducted at the highest severity and there is a significant risk of mortality, with animals likely to experience severe pain, suffering and distress.

16. There are very few validated *in vitro* assays that have been accepted within regulatory frameworks, particularly those addressing potential long-term sub-lethal effects. Identification, development and validation of new bioassays able to detect potential impairment due to chemical exposure in fish will represent a leap forward in the ability to perform refined ERA without the need to rely on vertebrate testing.

17. Completion of this Challenge will deliver a reliable, widely available NAM-based platform to address chemical safety in fish. In the short-term, this will result in a reduction in the number of *in vivo* fish studies carried out for safety purposes at candidate early screening stages and ERA. In the longer term, with increasing acceptance of the validity and reliability of NAMs, there will be an opportunity to fully replace *in vivo* animal studies for regulatory purposes in ERA.

Key deliverables

18. The key deliverables are:

19. The identification and characterisation of relevant fish-specific adverse outcomes that are aligned with relevant environmental protection goals (i.e. individual fitness reduction and/or population effects). Pathways may be grouped if they lead to common adverse outcomes. The selected fish-specific adverse outcomes should:

    - Be critical for growth, reproduction and survival.
    - Identify biomarkers of exposure able to act as early reporters of those adverse effects.
    - Have robust justification and characterisation and maximise coverage across the mechanisms through which a fish population can be impaired.

20. The biomarkers identified should be able to act as early reporters of the critical, fish-specific adverse outcomes and robust bioassays are needed to interrogate them. Approaches should focus on

innovative assays to address selected adverse outcomes rather than on the individual pathways selected.

- Biomarkers should be evidence-based, with associated mechanistic information and read-outs that are amenable to the generation of Points of Departure [1] to assess toxicity responses.
- Assays should be robust, scalable, reproducible and anchored on mechanistic information clearly linked to the adverse outcome.
- Uncertainty associated with the predictive performance of the identified biomarkers/bioassays must be characterised by assessing an adequate number of compounds (i.e. four positive chemicals and two negative controls) with relevant modes-of-action to demonstrate the suitability of the read-outs and the ability to generate predictive statistical models with the data generated.
- Models must perform to a level comparable with historic in vivo data.
- Bioassays must be amenable for transfer to industry standard platforms and future validation for potential regulatory acceptance.
- Plans for commercialisation and initiation of discussions with regulators are expected during the project period.

Scope

21. Proposals must **not** require the generation of primary cells or the culling of fish. Fish-based *in vitro* assays must use immortalised cell lines. Alternative model organisms for *in vitro* assays such as single cell organisms, or *in silico* approaches are within scope and welcomed.

Phase 1 deliverables

22. The deliverables for Phase 1 are:

- Delivery of a desk-based knowledge gap analysis, to include identification and evidence of fish-specific relevant adverse outcomes critical to fish growth, reproduction and survival which are not predicted by the currently available *in vitro* assays based on other vertebrate cell lines.
- Identification of suitable fish-specific relevant AOPs to be used for developing robust bioassays.
- Detailed plans to develop the proposed bioassays in Phase 2 including:

[1] Point of departure (POD) is the point on a toxicological dose-response curve established from experimental data, *in silico* models or observational data, that corresponds to an estimated low effect level or no effect level.

- Detailed rationale for the approach taken to developing the bioassays.
- Demonstration of the required technical experience in the team to successfully develop the bioassays.
- A detailed work plan for Phase 2 including technical approaches to address false discovery and/or negatives and an appropriate standard test set of chemicals.
- Robust plans for the commercialisation and dissemination of the technology as part of Phase 2.

Phase 2 Deliverables

23. Phase 2 includes essential and desirable deliverables. **Essential deliverables** are:

- Development of a suite of bioassays for the AOPs selected in Phase 1. Bioassays should:
  - Be developed and evaluated using relevant positive and negative controls.
  - Enable the prediction of adverse outcomes that are anchored in mechanistic knowledge and cover a broad chemical space including, but not limited to, pharmaceuticals, plant protection products and home and personal care products.
  - Permit the characterisation of the uncertainties including, but not limited to, false discovery and false negatives.
  - Be amenable to scale-up for high throughput use.
  - Be robust and reproducible.
  - Teams should initiate commercialisation and dissemination of the assays and associated evidence, including publications where appropriate.

24. The **desirable** deliverable is:

- Clear plans to begin exploring regulatory acceptance of the proposed approach (e.g. early engagement with OECD National co-ordinators).

Sponsor in-kind contributions

25. The Sponsors will provide:

- Early input and insight to the prioritisation of adverse outcomes.
- A focus on end-user application to support ERA of novel chemicals for use in a global regulatory context and access to extensive ERA expertise for a wide range of chemicals, including home and personal care compounds, pharmaceuticals and plant protection products.
- Expertise and guidance in mechanistic-based approaches to unravel toxicity effects.

- Access to:
  - Extensive chemical and toxicological information owned by the Sponsors.
  - Relevant findings from ongoing research programmes focusing on NAMs-based approaches to mechanistic-based risk assessment of human relevant toxicity.
  - A wide network of in-house expertise (including chemistry, toxicology, physiology, molecular and cell biology, computational science, statistics, exposure and effect modelling, risk assessment, etc.) to provide scientific advice, analysis and critique.
  - In-house assessment of the approaches developed through this Challenge, as appropriate (i.e. assessment of performance in terms of sensitivity and specificity) to facilitate industry uptake, including the opportunity to test and deploy the developed product(s) across different industry sectors, including home and personal care, pharmaceuticals and plant protection products to aid product commercialisation.

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