Home Office

NON-TECHNICAL SUMMARY

Development and validation of New Approach Methodologies (NAMs) for Endocrine Disrupting Chemicals (EDCs)

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)
- (d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

endocrine disruption, xenoandrogens, thyroid disruptors, metabolic disruption, Adverse Outcome Pathways

Animal types	Life stages
Zebra fish (Danio rerio)	embryo, neonate, juvenile, adult
Medaka (Oryzias latipes)	embryo, neonate, juvenile, adult

Animal types	Life stages
Three-spined stickleback (Gasterosteus aculeatus)	embryo, neonate, juvenile, adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Endocrine Disrupting Chemicals (EDCs) interfere with the hormone systems of animals and current regulation requires a high number of vertebrate tests to assess their hazards. This project licence aims to develop and validate New Approach Methodologies (NAMs), a recognised international priority to modernise the hazard assessment of EDCs.

A retrospective assessment of these aims will be due by 6 September 2029

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The issue of endocrine disruption in the environment, known for almost three decades now, is still of very high concern from a policy perspective and generates an ever-increasing need for vertebrate testing. It is widely recognised that the number of chemicals that need to be assessed for hazard and

risk in the environment is too high for empirical testing, hence new, high throughput, reliable and preferably animal-free methods for assessing their hazard are urgently needed. In addition, the European Chemicals Agency (ECHA) recognised three areas of regulatory challenge in terms of chemical toxicity, namely neurotoxicity, immunotoxicity and endocrine disruption. For the latter, ECHA suggested that ''NAMs for both EATS (estrogen, androgen, thyroid and steroidogenic) and non-EATS modalities (e.g. the Retinoid system pathway) need to be improved and Adverse Outcome Pathways (AOPs) should be developed to facilitate the assessment and interpretation of observed endocrine activity and adverse effects (e.g. metabolic disorders)''. A prerequisite for NAM incorporation into regulatory decisions is that they should at least guarantee a similar protection level for humans and the environment that is in place currently. Under REACH (Registration, Evaluation and Authorisation of CHemicals) and CLP (Classification, Labelling and Packaging) regulations, it is only for hazard identification and classification of skin sensitisers that NAMs are sufficiently developed to substitute *in vivo* methods.

The proposed work is fully aligned with ECHA's recommendations (ECHA, 2023), aiming to characterise important endocrine pathways to develop and validate NAMs and AOPs that are useful to regulators. NAMs include a wide variety of methods, from frameworks where the knowledge is organised, such as AOPs, to non-animal (or non-protected life stage) testing and *in-silico* methods, including computational approaches. This licence aims to generate new endpoints and check the validity of existing proposed endpoints, for hazard assessment of EDCs, mainly in non-protected fish life stages (embryonic stages of fish, prior to first feeding). I**mportantly, this licence seeks to validate these early endpoints by demonstrating their presence is prognostic of adverse outcomes that are of regulatory concern.**

Ultimately, the outputs of the proposed work aim to replace or partially replace the need for using protected sentient vertebrates for regulatory testing of chemicals.

What outputs do you think you will see at the end of this project?

This project licence is seeking to obtain new or enhanced information on the adverse outcomes associated with EDCs, whilst minimising the future use of protected vertebrates to obtain such knowledge. The work will build confidence in a range of new, emerging or proposed endpoints of endocrine disruption in non-protected early life stages of fish (embryonic forms prior to first feeding). In addition to the (partial) replacement benefits for assessing harm from EDCs in the environment, the work may have refinement implications for human health. For example, one of the current methods used for such assessment, the Hershberger assay, requires the use of young rats that are surgically castrated before being tested.

Who or what will benefit from these outputs, and how?

The proposed work is of international relevance as management of chemicals in the environment is a global issue. We aim to better protect wildlife and halt the biodiversity loss experienced in aquatic environments, including marine, by acquiring new mechanistic knowledge on how chemicals affect the endocrine system. This new knowledge will not only allow better management of environmental chemicals benefiting society by protecting natural capital, but it will also help governments shape their policy on the risk management of EDCs. Importantly, the work aims to evaluate the validity of endpoints in non-protected fish life stages and characterise their relationship with apical endpoints that

are currently of regulatory relevance but require vertebrate testing. Hence, the benefits of this work are directly relevant to (partial) replacement of animal testing, a strong societal drive in the UK and internationally.

How will you look to maximise the outputs of this work?

The establishment is in a very good position to undertake relevant assessment work due to long term experience and a state of the art aquarium. The research team has representation at OECD Validation Management Group for the Environment (VMG-Eco), which oversees the development and validation of test guidelines for the assessment of chemical hazards. The team has been working in this area for over 25 years and has participated in the conception, development and/or validation of many OECD test Guidelines and guidance documents: the fish endocrine screen (TGs 229 and 230); the androgenised female stickleback screen (AFSS, GD148); the fish sexual development test (TG234); the fish embryo toxicity test (TG236); the two molluscan TGs on reproductive toxicity (TG242 and TG243); the most recent review of the fish acute toxicity test (TG203); the RADAR assay (Rapid Androgen Disruption Activity Reporter; TG251); the REACTIV assay (Rapid Estrogen ACTivity In Vivo assay, draft TG pending acceptance in April 2024).

In addition, we are contributing to PARC (Partnership for the Assessment of Risks from Chemicals), a 7-year partnership under Horizon Europe, which has total funding of €400 million, 50% from the European Union (in our case, UKRI) and 50% from Member States. PARC brings together chemical risk assessors and managers with scientists and stakeholders to accelerate method development and the production of necessary data and knowledge, responding to the needs of end-users.

The outputs of the proposed work will feed directly into test guidelines and guidance documents: AOPs with the endorsement of OECD (see AOP WIKI; https://aopwiki.org/) and IATAs (Integrated Approaches to Testing and Assessment). Unsuccessful approaches will directly inform TG development at OECD and PARC, whilst all data will be published in the peer review literature.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 2500
- Other fish: No answer provided
- Medaka (Oryzias latipes): 5000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are requesting to use three fish species (all common models for regulatory ecotoxicology) to underpin the aims of the project: new endpoints identified in non-protected life stages must be validated with adverse endpoints in protected stages as used for regulatory decisions. Although the

focus will be the development and validation of endpoints in embryonic, non-protected life stages, a few targeted experiments will follow these early endpoints into later, protected life stages to ensure they are not transient and linked with harm that is of regulatory concern, such as lack of reproduction, growth or development. The endocrine system is subject to homeostatic responses, hence early markers of disruption do not always translate into adverse outcomes, primarily because life has evolved multiple pathways to bring back homeostasis when the system is perturbed.

Typically, what will be done to an animal used in your project?

The fish will be exposed to the chemicals of interest in an environmentally relevant way, which includes waterborne and dietary exposures. The former is a very common route of exposure which we have implemented for over 20 years in our establishment. The latter is of great environmental importance especially for chemicals of low solubility in water but is used less frequently in regulatory testing. We have recently developed a robust system for dietary fish exposure, under a previous, short-term licence based on the same principles as those used by the fish bioaccumulation test (TG305).

Endocrine Disrupting Chemicals (EDCs) are not typically toxic, i.e. they do not induce visible signs of toxicity; rather they alter the signalling pathways of the endocrine system, affecting key processes for population sustainability, such as growth, development and reproduction. So, we do not expect routine animal suffering, as often anticipated in chemicals that present general toxicity (i.e. narcotics or irritants).

Exposures can be as short as few days or as long as a life cycle. In most cases we will follow the design of existing TGs for the assessment of EDCs as the aim is not only to seek new endpoints in fish embryos, but also to enhance existing test guidelines with additional endpoints. Most animals will be terminated before they reach sentient protected life stages, but some will need to be maintained for longer to link the early potentially diagnostic endpoints with a later adverse outcome of regulatory relevance.

In general, the only stressor we intend to apply is exposure to a single chemical or a binary mixture of chemicals. In some cases, however, when investigating metabolic disruption, we may need to additionally deprive fish of essential food micronutrients (e.g. vitamins) or alter the photoperiod and temperature cues of natural endocrine signalling. It should be emphasised that the endocrine system, which is the subject system of the proposed work, receives cues from the environment (light, temperature and food) and utilises them in a synchronised way to orchestrate development, growth and reproduction. It is therefore important to understand the interplay of these factors along chemical exposures as they may affect the outcome. Photoperiod and temperature changes will be applied well within the tolerance of each species (i.e. not outside the normal physiological range), whilst feed deprived from micronutrients will only take place in a few, selected cases and only when we have strong suspicions that adversity may be a combined effect of poor food quality and chemical endocrine disruption.

In addition, fish may be confined in a restricted water volume for a limited period to obtain non-invasive samples of hormone levels. Hormone levels are a key determinant of endocrine system functionality. Fish may be confined at specific life stages including juvenile, peripubertal, pubertal and sexually mature animals. The team has pioneered the development and validation of non-invasive techniques to obtain information on several sex steroids and cortisol as a stress marker under previous PPLs, and

have produced more than 50 relevant peer review publications. The fish may be returned in their tanks following confinement or euthanised. Our multiple observations in applying this method suggests that fish become accustomed to it very quickly and show little evidence of stress after confinement sessions. For example, male fish have displayed nest building activity within 15 minutes of confinement following habituation involving two past trials. Finally, behavioural assessments on feeding, swimming and reproduction may be conducted, involving potentially and principally transfer of fish into different tanks. These transfers are viewed as a mild procedure, similarly to common husbandry practices.

At the end of the exposure period protected animals will be terminated either using a Schedule 1 Method (S1M), or a humane non-schedule 1 method (non-S1M). The non-S1M method most likely to be used is immersion in liquid nitrogen, which is the only way we can obtain tissue gene expression data unaffected by an anaesthetic. All fish used in the proposed work are small teleosts, in which loss of consciousness (and death) are judged as instantaneous from freezing in liquid nitrogen. Infrequently, we may need to use another non-SM1 method, namely exsanguination (terminal bleeding) as this allows the collection of blood, which is required in some of the regulatory TGs. In this case, killing is under deep anaesthesia.

What are the expected impacts and/or adverse effects for the animals during your project?

We don't expect the fish to experience pain or any other discomfort except in cases where developmental exposures result in organ or systemic failures to perform basic functions e.g., inability to swim and feed normally, an expected outcome of swim bladder impairment following exposure to thyroid disruptors (AOP WIKI #155-159). The way most EDCs cause adversity is by altering important functions such as growth and reproduction without resulting in mortality, irritation or visible discomfort. Changes in behaviour are expected and are part of the series of endpoints investigated, hence they will be analysed both as humane intervention points (see below) and as supporting evidence of the affected pathway. It is expected however, that the study of androgenic xenobiotics, one of the main elements of the proposed work, will affect aggression levels that will need to be managed. Another element of expected adversity is weight loss, anticipated in investigations of the thyroid axis and of metabolic disruptions. On such occasions, the actual adverse effects will be managed by defined humane intervention points implemented by intensive monitoring which involves both direct visual checks and software analysis of videos from both in-tank underwater and external (glass aquaria) camera systems. Weight checks, a common husbandry procedure, albeit intrusive, will only be applied in cases where weight gain or loss are diagnostic endpoints for the chemical hazard assessment.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

For all fish species seeking authorisation in this licence we expect the following severities:

5% Non recovery - this percentage includes control and baseline (stock) animals killed via S1M or exsanguination (non-S1M)

25% Subthreshold - this percentage includes control and baseline (stock) animals killed via immersion in liquid nitrogen (non-S1M)

50% Mild - most of the exposed fish, and baseline (stock) samples subject to weight checks, skin swabs and/or confinement for non-invasive hormone measurement prior to humane killing.

15% Moderate - those animals that display clinical and/or behavioural signs that merit humane intervention.

5% Severe-those animals that are found moribund or dead in the first day check as the period of suffering may have been more than 10 hours (overnight).

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 6 September 2029

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We need to use protected animals to validate the early endpoints identified for non protected stages. This validation data will bridge the current gap between traditional regulatory endpoints and NAMs, ultimately waiving the need for further regulatory animal wastage. The use of animals is inevitable as they currently underpin all regulatory requirements, especially for EDCs. Collecting data to support NAM validity and confirming the relevance of early endpoints to the apical endpoints familiar to regulatory decision making, is the only viable way of generating confidence for the uptake of NAMs in regulation.

Which non-animal alternatives did you consider for use in this project?

We are working towards the development of non-animal alternatives via this work, as currently there are high animal data requirements for assessing hazards from EDCs. Specifically we will:

a) Generate a series of Adverse Outcome Pathways (AOPs), with full OECD endorsement, to guide future decisions in chemical read-across. AOPs are chemically blind, which means that they can capture any chemical with the same biological target, allowing chemical read-across which will waive

the need for further empirical testing. The chemicals used for this purpose under the proposed licence, will be listed as prototypic stressors in the OECD AOP wiki.

b) Generate a series of IATAs (Integrated Approaches to Testing and Assessment), another important framework that amongst other benefits helps to identify conservation of toxicity targets across species, allowing cross species extrapolations, and reducing duplication of testing in different countries.

c) Validate fish embryo endpoints, contributing directly to the development of NAMs that allow both the protection of the environment and reduction of the number of animals used. The exception here is the medaka, as under current UK HO guidance medaka embryos are protected as soon as they hatch. This means that the UK will not be able to demonstrate replacement via the application of at least two NAMs that have become part of the OECD TG programme (TG251, the RADAR assay) or expected to be as early as in April 2024 (the REACTIV assay); both require transgenic medaka embryos to be maintained for 24-72h after hatching.

Why were they not suitable?

These methods are under development and require validation to be suitable for regulatory decisions; we wish to contribute to this via the proposed work. NAMs need to demonstrate that they are fit for regulatory purposes rather than represent transient perturbations of a gene or metabolite. We strongly believe that most, if not all, of the non-animal or non-protected animal stages present great opportunities for (partial) replacement, and we strive to generate the validation data needed for their regulatory acceptance. In this perspective we are in an ideal position as institutionally we are directly connected to UK government needs for evidence and can prioritise work to meet requirements.

A retrospective assessment of replacement will be due by 6 September 2029

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We propose to use two highly used species for chemical hazard assessment (medaka and zebrafish) and an endemic UK species (the three-spined stickleback), which is also a commonly used fish in OECD TGs. The validation of markers for endocrine disruption in the stickleback will add significant

weight to the AOPs and provide the regulator with confidence that the markers of disruption are universal and not just relevant to laboratory fish strains.

The estimated numbers of fish are based on 1) an expected number of tests and 2) the number of fish per test. The number of tests is based upon the level of funding achieved over a 5-year period and our capacity to perform experiments over this time. Both tank and staff availability have been considered, including staff fatigue as monitoring will be intensive over long periods of experimentation.

The numbers of fish per test were calculated using requirements for existing relevant OECD TGs which already take into account the power needed for regulatory endpoints. These include the Fish Early Life Toxicity Test (FELT, TG210), the Fish Sexual Development Test (FSDT, TG234), the Fish Short Term Reproduction Assay (FSTRA, TG229) and the fish endocrine screen (TG230). Additional fish have been added as they will be sampled throughout the experimental duration, to provide means of tightening up AOPs (i.e. looking for relationships between molecular initiating events and key events). Most sampling will involve non-sentient embryonic stages, which are not counted; however, the higher medaka numbers reflect that under UK HO guidance all medaka post-hatch stages are protected, so account for additional animals in this species in the RADAR (TG251) and REACTIV (draft OECD TG) assays.

The point of protection for the three-spined stickleback has been calculated carefully for different temperatures during development over our long-term experience with this model. Embryonic development in fish is temperature-dependent, hence common use of degree-days. Protection for fish starts when capable of independent feeding, and for three-spined stickleback occurs later than 4dph (ca 11dpf) when raised at 17^{o}C. We have documented evidence (can provide videos to justify this suggestion) that exogenous feeding (ingestion of offered feed) doesn't take place prior to 5dph as we have repeatedly offered suitable prey (live *Artemia nauplii*) that was not ingested. Hence, the choice of 4dph (11dpf) at 17^{o}C as the point of becoming capable of independent feeding is conservative and protective.

For zebrafish, we will follow current HO guidance which states 5dpf.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Sampling of animals over the duration of the exposures has been calculated to account for biomarker or assay variability as well as natural variability so that no animal goes to waste because the power for analysis was too low. In general, we use 6 animals per time point for gene expression work. We also plan to use a novel technique that provides concomitant information on gene expression in all tissues - even in small specimens of fish embryos and early life stages. This novel platform operates on histological slides so all tissues are visible, both for histopathology and for gene network analysis associated with the pathology. The application of this novel technique alone dramatically reduces the number of fish used as all tissues can be simultaneously assessed in a single animal, adding statistical power to the gene network analysis.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In addition to the experimental design and the pioneering techniques we propose to use, we will further optimise the number of animals used for endocrine disruption method validation by:

Careful consideration of chemicals. As the work we plan to do involves the development of AOPs which are chemically blind (i.e. they need to stand up on their own as adverse outcomes, regardless of which chemical initiated the perturbation) the chemicals used in our experiments will either be listed as such under the AOP wiki or selected using a thorough review of the existing literature and examining the chemical structure in terms of ability to bind to proteins. This is because many of our targets are proteins (enzymes or receptors) that, although generally conserved across different animals, they do often present structural differences that hinder or promote interactions with chemicals.

Focus on endpoints for non-protected life stages of fish. All of our animals are bred in-house so that large numbers of eggs/embryos can be collected and used for screening selected chemicals including environmental EDCs before the main experimental procedures start.

In addition, pilot data and observations collected from stock fish will be used to better define sampling points which need to be concomitant with major physiological changes like hatching, initiation of feeding, metamorphosis and sexual maturation.

A retrospective assessment of reduction will be due by 6 September 2029

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We propose to use three different fish species to provide the necessary confidence for the resulting NAMs to be used at regulation. Two of the species are highly used for regulatory testing of EDCs (zebrafish and medaka), whilst the third (three-spined stickleback) is environmentally relevant and represents a reproductive strategy, which is shared with all fish that live in temperate waters. This contrasts with the core regulatory species that, although convenient model species, once they reach sexual maturity they spawn until they die which is not representative of the annual reproductive cycle of most fish species. This is of key importance for the development of AOPs as we expect differences in feedback loops between the brain and the gonads, stemming from the different needs inherent to the reproductive strategy.

We are extremely well positioned to undertake research with these species due to substantial past experience. However we plan to establish our first medaka colony (both wild type and transgenic animals that will be used for the RADAR and REACTIV assays) only after receiving formal training.

Why can’t you use animals that are less sentient?

In many ways, the work will focus on the lowest possible level of sentience as fish are considered lower vertebrates and within this category, embryonic or very early life stage animals are presumed to have even lower capacity to experience suffering. The whole research project aims to validate endpoints in non-sentient life stages of fish (embryonic forms, prior to the initiation of independent feeding). To do this, a number of fish need to be assessed at later, protected stages so the harmfull endpoints currently used in these tests are linked to the early markers.

We are very familiar with the physiology of the species we are proposing to use. The exception is the medaka, a species with which we have limited experience (under 3 years). For medaka, we plan to receive robust training before we initiate the in-house colony.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The procedures employed (dietary and/or aqueous exposures to chemicals with endocrine activity) are generally mild in nature and so are the anticipated harms. Toxicity of EDCs is subtle, not acute, and often linked with failure to reproduce, grow or behave in a natural manner rather than induction of pain or suffering. Nevertheless, the following steps are in place to ensure refinement:

a) Daily observations (a minimum of two but if signs of toxicity are evident this will increase to a minimum of four) and video analysis of fish appearance, behaviour and clinical signs via a comprehensive list as developed by the applicant’s team for TG203 (see OECD TG203, 2019, Annex).

b) Application of moribundity *in lieu* of mortality as an end-point of chronic toxicity. This is defined by the presence and severity of clinical signs. This can be considered as a humane intervention point (when suffering ends) to avoid confusion with endpoint (here meaning the response variable to the treatment that is measured and analysed).

c) Termination of treatments where mortality/moribundity exceeds 40% (acceptable mortality in the regulatory tests employed ranges between 25-30% as they are juvenile fish) of the initial population at any point during exposures.

d) Application of dietary exposure for chemicals of low solubility in water to avoid the use of high solvent levels; this option presents a refinement as high levels of solvents in waterborne exposure are associated with welfare issues.

e) Application of photoperiods and temperatures that are within natural seasonal variation for each fish species.

f) Provision of suitable environments, including enrichment (e.g. spawning substrates).

g) Active management of aggression levels by physical isolation of fish where observed. This is expected to be more intense in male fish as the levels of internal androgens are much higher than in female fish.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The applicant's team aspires to apply fully the 3Rs principles in their scientific use of animals. Some key literature that is relevant to the programme and is guiding our experimental approaches is listed below:

CCAC (Canadian Council on Animal Care), 1998: 24 p. [Online]. CCAC Guidelines On: Choosing an appropriate endpoint in experiments using animals for research, teaching and testing. http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/PDFs/APPOPEN.pdf

CEFIC, 2020. LRI-ECO51: Integrating the fish embryo test into the weight of evidence to inform acute fish toxicity. http://cefic-lri.org/request-for-proposals/lri-eco51-integrating-the-fish-embryo-test-into-the-weight-of-evidence-to-inform-acute-fish-toxicity/

Dennison, N., Ryder, K., 2009. The challenges of using humane endpoints in fish research. https://norecopa.no/media/6272/abstract-ryder-endpoints.pdf

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Goodwin, N., Westall, L., Karp, N.A., Hazlehurst, D., Kovacs, C., Keeble, R., Thompson, P., Collins, R., Bussell, J. 2016. Evaluating and Optimizing Fish Health and Welfare During Experimental Procedures. Zebrafish 13(Suppl 1): S-127–S-131, doi: 10.1089/zeb.2015.1165.

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NC3Rs online resources (e.g. https://www.nc3rs.org.uk/3rs-resources/breeding-and-colony-management?utm_campaign=January2024&utm_medium=email&utm_source=govdelivery).

OECD, 2002. Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Human Endpoints for Experimental Animals Used in Safety Evaluation, OECD Series on Testing and Assessment, No. 19, OECD Publishing, Paris.

OECD 2009. Test Guideline 230: A Short-Term Screening for Oestrogenic and Androgenic Activity, and Aromatase Inhibition (21-day Fish Assay), OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD, 2010. Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads, OECD Series on Testing and Assessment, No. 123, OECD Publishing, Paris.

OECD 2011. Test Guideline 234: Fish Sexual Development Test (FSDT), OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD 2012. Test Guideline 229: Fish Short Term Reproduction Assay (FSTRA), OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD 2013. Test Guideline 210: Fish, Early-life Stage Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD, 2013. Test Guideline 236: Fish Embryo Acute Toxicity (FET) Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

OECD, 2019. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, OECD Series on Testing and Assessment, OECD Publishing, Paris. OECD, 2014. Fish Toxicity Testing Framework, OECD Series on Testing and Assessment, No. 177, OECD Publishing, Paris.

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How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

This body of work aims to improve current test methods for regulating EDCs, and develop the next steps to meet future regulatory needs and minimise animal use. Engagement with the regulator and up to date good practice on animal welfare, chemical toxicity and regulatory needs will be maintained over the project through close links with: the NC3Rs; Defra (policy leads); Environment Agency (the UK regulator); the OECD VMG-Eco; the OECD Extended Advisory Group for Molecular Screening and Toxicogenomics (EAGMST); the Society for the Advancement of Adverse Outcome Pathways (SAAOP); SKIG, the advisory body of international experts that ensures the AOP wiki is functional and uses standardised information and nomenclature (ontology); SETAC Endocrine Disruptors and Animal alternatives special groups; the International Consortium to Advance Cross-Species Extrapolation in Regulation (ICACSER) which is providing shared resources/modern tools for the 21st century toxicology needs (https://www.setac.org/page/scixspecies). In this context a successful outcome would both refine the testing required and reduce the number of fish used by predicting chronic toxicity outcomes via early molecular initiating events.

A retrospective assessment of refinement will be due by 6 September 2029

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?