New Approach Methodologies (NAMs) for the Endocrine Activity Toolbox: Environmental Assessment of Fish and Amphibians

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INTRODUCTION

Testing requirements for chemicals acting via an endocrine mechanism vary globally and vary in coverage. They include testing on all substances, exposure-based requirements, and/or additional testing based on an initial trigger. Evaluation of endocrine activity in humans and wildlife involves specific in vivo and in vitro assays used to evaluate relevant pathways. Multiple in vivo test guidelines have been validated for mammals, amphibians, or fish by the OECD, focusing on oestrogen, androgen, thyroid, and steroidogenesis (EATS) pathways. However, these toxicity tests often require the use of a large number of laboratory animals, which are cost inefficient and contradict the 3R principles for animal welfare considerations and increasing mandates to move towards an 'animal free' testing paradigm worldwide. New approach methodologies (NAMs) hold promise to move away from animal testing for endocrine activity. While there are limitations with NAMs, they hold great promise to identify molecular changes that can be used to predict individual or population effects and to reduce the numbers of animals used in ecotoxicology testing.

METHODOLOGY

In a collaborative effort led by the Health and Environmental Sciences Institute (HESI) and the UK's NC3Rs, experts from government, academia, and industry met in early 2020 to discuss the current challenges of endocrine toxicity testing for fish and amphibians. In continuing the cross-sector initiative, this presentation outlines the current state of the science to evaluate chemical endocrine disruption potential in amphibians and fish using NAMs for EATS pathways. We also discuss the challenges of using NAMs for risk or hazard assessment and what is needed to reduce the uncertainties with using these tests. Movement towards development and utilisation of NAMs will lead to a replacement, reduction, and refinement of animal testing needed plus increase the robustness, fitness-for-purpose, and environmental relevance of regulatory assessments to identify chemicals acting via endocrine mechanisms.

RESULTS

Cross sector experts have summarised current NAM methods available for EDC assessment including in silico, cell-free and cell-based methods, and embryo models. This collaborative effort focuses on endocrine activity evaluation for fish and amphibians, but learnings can be used for other organisms and potentially non-endocrine pathways. Some common limitations or issues for NAMs include lack of metabolic competence or ability to capture ADME properties and feedback loops that in vivo tests can measure. As with in vivo endocrine pathway assays, non-monotonic dose response can make interpretation difficult. Mixtures are difficult to test in all systems, including in vitro and embryos. The currently available in silico tools cannot be applied to evaluate mixtures, and therefore individual components need to be evaluated separately. Species coverage is also somewhat limited. Despite some of the shortcomings related to NAMs and NAM development described above, these tools have promise for integration into AOP frameworks within a WoE approach for hazard and risk assessment.

DISCUSSION

New approach methodologies can be utilised to both reduce the number of animals needed to assess a chemical's endocrine potential but also to simultaneously improve evaluation of endocrine activity. While NAMs are currently used in some regulatory assessments, advances need to be made to be able to move away from in vivo testing to a fit-for-purpose test battery of NAMs. Next steps include performing case studies on compounds that have been evaluated with NAMs and in vivo methods in a regulatory context and comparing the outcomes.