Wings of Change: Advancing avian toxicity assessments with new approach methodologies (NAMs)

Overall aim

1. The aim of this Challenge is to develop new approach methodologies (NAMs) to assess acute and chronic avian toxicity for chemical screening and environmental risk assessment.

Duration

2. This is a two-phase Challenge with funding for up to three years.

3. Phase 1: nine months; Phase 2: up to three years (subject to successful completion of Phase 1).

Budget

4. Phase 1: up to £200k[1]; Phase 2: up to £1.5M.

Sponsors

5. 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.

6. The Sponsors for this Challenge are BASF, Bayer Crop Science, Corteva and Syngenta.

Partners

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

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[1] £100k is available for Phase 1 projects covering one work package; £200k is available for projects covering two work packages. The total budget for all Phase 1 awards is £500k. Please refer to the Guide for Participants for more detail.

8. The Challenge Partners are the American Chemistry Council and the Health and Environmental Sciences Institute.

Background

9. Environmental risk assessment evaluates the likelihood that organisms in the environment will be affected due to exposure to chemicals. For pesticides, the potential for exposure to a chemical to cause toxicity in avian species must be assessed to meet regulatory requirements. The studies are carried out *in vivo* to determine the effects of acute and chronic exposure to the test chemical. The testing is conducted for the registration of pesticides in adherence to the Organisation for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (US EPA) Office of Chemical Safety and Pollution (OCSPP) guidelines. Modified versions of the studies described in the guidelines are also conducted by companies for internal screening to prioritise candidates in development. *In vivo* avian studies are increasingly required for substances covered by the Toxic Substances Control Act that has been implemented following reforms that mandate US EPA review of the safety of the thousands of chemicals produced or imported into the USA.

10. The guidelines require the use of large numbers of birds and there are ongoing efforts to reduce the number of *in vivo* studies required. The US EPA published guidance in 2020 for waiving the sub-chronic avian dietary study [1, 2] in cases where it would not provide additional value to the risk assessments, such as where sufficient toxicity data is already available from other studies (e.g. for the acute oral toxicity study) [3, 4]. This study is also no longer required in the European Union (EU) for the registration of new pesticide active ingredients [5, 6]. A collaboration between the US EPA and the animal protection group the People for the Ethical Treatment of Animals is evaluating whether a protective risk assessment can be calculated using chronic testing data from a single bird species instead of two, as is currently required in the USA and Canada. These programmes have the potential to reduce animal use in environmental risk assessments, nevertheless a significant amount of avian *in vivo* testing will continue to be required.

11. The increasing global interest in the development and use of NAMs, defined as full and partial replacement approaches for assessing chemical or drug toxicity, provide opportunities to move away from *in vivo* studies. For example, avian NAMs that use chicken and quail embryos for the assessment of endocrine disruption have recently been proposed for consideration within the OECD framework. Other NAMs in development incorporate *in silico* and *in vitro* approaches and include:

    - Quantitative Structure Activity Relationship (QSAR) models for the assessment of acute toxicity [7, 8].
    - A dynamic energy budget – toxicokinetic-toxicodynamic (DEB-TKTD) model supported by bobwhite quail embryo injection data to predict key reproductive endpoints for the assessment of chronic toxicity [9].

- EcoToxChips [10] that use quantitative PCR technology to assess the effects of chemical exposure on the transcription of around 380 toxicologically-relevant genes. EcoToxChips are available for two avian species – the Japanese quail and the double-crested cormorant, although neither of these species are regularly used for regulatory testing.

- Immortalised cell lines are available for some avian species, including chicken, the Japanese quail and the double-crested cormorant [11, 12], and have been used for toxicity testing of chemicals such as flame retardants, but not pesticides [13].

12. All NAMs currently in development require further development and validation for use in a regulatory context and this is likely to be a lengthy process before they are able to replace the use of animals. In the meantime, there is also the potential for NAMs to be used in Integrated Approaches to Testing and Assessment (IATA) [14]. IATAs are frameworks in which multiple lines of evidence including data from *in vitro* assays and *in silico* models are combined to guide custom testing strategies that use as few animals as possible.

The Challenge

13. The aim of this Challenge is to develop a suite of NAMs for acute and chronic toxicity that can be:

    - Used to screen candidate chemicals in early development.

    - Integrated into an IATA to enable regulatory testing with fewer animals in the short-term and in the longer term, lay the foundations for moving away from *in vivo* avian studies for environmental risk assessment purposes.

14. The Challenge will require the integration of knowledge and experience within the *in silico*, *in vitro*, mechanistic toxicology and adverse outcome pathway (AOP) areas, alongside existing data and strategies.

3Rs benefits

15. The avian acute oral [15, 16] and reproduction [17, 18] toxicity tests are required for the regulatory assessment of acute and chronic avian toxicity respectively.

16. Acute toxicity assessments typically use either an upland game bird or a waterfowl (usually the bobwhite quail) and a passerine (usually the canary or zebra finch) with the number of birds used depending on the guideline. In OCSPP 850.2100, ten birds per dose with five doses plus controls (60 birds in total) or a limit test of a single dose plus control (20 birds in total) are used [16]. OECD test guideline 223 is an “up-and-down test”, with results from the previous stage used to determine the next phase of testing. It typically uses between ten and 24 birds but can use up to 49 animals [15]. Effects include mortality and there are significant animal welfare concerns because of the potential suffering involved. The test substance is administered either by capsule or gavage and is

followed by a 14-day observation period which can be extended if required.

17. Chronic toxicity assessments require the dietary exposure of test chemicals to young adult animals for approximately 20 weeks. Depending on the countries in which a pesticide is registered, testing in one or two species is required, in most cases an upland game bird and a waterfowl (usually the bobwhite quail and mallard duck). These studies use around 128 adult animals (16 pairs (male and female) per dose level, three dose levels plus controls) and 2,560 chicks (at an average of 40 chicks per pair) per study. Effects most often seen on reproductive endpoints, include the numbers of eggs laid, egg viability, embryo survival, hatching success, hatchling survival, and eggshell thickness.

18. Delivery of this Challenge will enable scientists (and regulators) to:

    - Replace the use of animals for internal decision making by providing predictive approaches that can be used for early screening to rank or prioritise candidate chemicals for further development and prevent unsafe chemicals progressing into regulatory animal studies.

    - Reduce the number of animals used for regulatory risk assessment by integrating NAMs into an IATA.

19. Learnings from this Challenge could also have the potential to further the development of NAMs for other taxonomically relevant species such as reptiles. New knowledge on avian toxicokinetics and/or metabolism could also be applied to predict the metabolism of chemicals, where currently *in vivo* studies are required for the purposes of human safety assessment for the consumption of poultry products from hens exposed to pesticides.

Key deliverables

20. Avian toxicity assessment is complex, and in the case of the avian reproduction study (to assess chronic toxicity), is a lengthy study with multiple endpoints. Furthering development of NAMs to provide comparable toxicity information will first require in-depth understanding of what can be derived from available data.

21. The Challenge has two Phases. Phase 1 is primarily desk-based, focusing on the analysis of avian toxicity data to inform the NAMs that will be developed in Phase 2. Phase 2 focuses on the development of these NAMs and their integration into an IATA for avian toxicity testing in a regulatory context.

Approaches that are in scope:

- *In silico* – QSARs, exposure and effects modelling (e.g. dynamic energy budget models and general unified threshold models for survival).

- *In vitro* – cell lines and sub-cellular fractions are preferred, and these should be from species used in regulatory tests (bobwhite quail and mallard duck for chronic toxicity; bobwhite quail and canary or zebra finch for acute toxicity), but other species would be accepted if they can be shown to be predictive. Primary cells may be considered if there are no cell line alternatives.

- Embryo (*in ovo*) – from species used in regulatory tests. Embryo studies should not involve protected life stages. Avian protected life stages are defined under UK Home Office guidance and the EU Directive 2010/63/EU.

Phase 1 deliverables

22. For Phase 1 projects, applicants can apply to deliver either the **acute work package**, **the chronic work package or both**. Applicants can apply for up to £100k to deliver one work package or up to £200k to deliver both. It is expected that Phase 2 addresses both acute and chronic toxicity deliverables. Applicants focusing on a single work package during Phase 1 are expected to include the required expertise for Phase 2 to address both aspects – either by collaborating with another Phase 1 team or securing additional external partners.

23. Phase 1 requires the collection and analysis of existing avian toxicity data. There are publicly available databases such as the US EPA ECOTOX database and the Pesticide Properties DataBase that applicants can access with data for acute (e.g. LD50) and chronic (No Observed Effect Level (NOEL)/Lowest Observed Effect Level (LOEL)) study assessment endpoints. However, these databases may not provide the detailed datasets that are needed, including dose-response data and effects on individual endpoints (e.g. number of eggs laid and chick weight). It is expected that applicants will identify additional databases or sources of data for use within the project and consult with the Sponsors on this.

Chronic work package

24. The chronic work package deliverables for Phase 1 are:

    - Analysis of currently available chronic toxicity data in line with the OECD/OCSPP avian reproduction guidelines to identify which endpoints are frequently the most sensitive and use these to define the NOEL/LOEL/Points of Departure). The endpoints should then be prioritised for further development.

        - The analysis should include information on the uncertainty and biological variability of each endpoint.

        - Effects may be observed in the adult birds, the eggshell, as well as the embryo and hatched chick.

    - Proposal of putative AOPs for prioritised endpoints or groups of closely-related endpoints that could aid in the development of NAMs.

- A plan for the NAMs that will be developed during Phase 2.
- Plans for the integration of Phase 1 work packages to deliver the objectives for Phase 2.

Acute work package

25. The acute work package Phase 1 deliverables are:

- Collection and analysis of acute toxicity data in line with the OECD/OCSPP guidelines.
- Evaluation of existing *in silico* (e.g. QSAR) approaches to assess acute toxicity, including a critical evaluation of the limitations and needs.
- Development of a proposal for a NAMs strategy to predict acute toxicity (LD50). This should include consideration of both *in silico* (e.g. QSAR) and *in vitro* approaches.
- Plans for the development of NAMs for Phase 2.
- Plans for the integration of Phase 1 work packages to deliver the objectives for Phase 2.

26. The Phase 2 deliverables are:

- Development of the NAMs identified in Phase 1 for the assessment of acute and chronic avian toxicity.
    - Approaches should have acceptable sensitivity and specificity as agreed with the Sponsors and informed by historical *in vivo* data derived from OECD/OCSPP guidelines.
    - Assays must be amenable for transfer to industry standard platforms and future validation for potential regulatory acceptance. The transferability of the methods developed should be discussed in Phase 1 with the Sponsors based on the proposed assay formats.
- Integrate the acute and chronic NAMs into an IATA for avian testing in a regulatory context. The strategy should take into account that one-to-one replacements for *in vivo* animal studies in a regulatory context are not likely to provide sufficient confidence for stakeholders to make decisions. The IATA framework should:
    - Present an alternative approach to acute and chronic avian toxicity assessment by integrating additional lines of evidence. For example, data from other species, chemical class, mode-of-action specific information and exposure.
    - Deliver a clear avian testing approach that reduces the amount of *in vivo* testing required by providing a roadmap for how to incorporate NAMs into risk assessment and

regulatory decision making. The IATA can include bioassays, toxicokinetics, *in silico* predictions and effects modelling approaches.

- A clear plan for exploring regulatory acceptance of the approach including early engagement with regulators and industry.
- Journal publications outlining the NAMs and IATA framework and any comparisons to historical data to build the evidence base to move towards future regulatory acceptance.

27. It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The application must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

In-kind contributions

28. The Challenge will be supported through the provision of in-kind support from the Sponsors. The in-kind support offered includes:
    - Extensive expertise in avian toxicity testing and risk assessment for regulatory purposes for a wide range of chemicals.
    - Expertise and guidance in mechanistic-based approaches to unravel toxicity effects.
    - Access to:
        - Extensive chemical and toxicological information owned by the Sponsors.
        - A wide network of in-house expertise (including chemistry, toxicology, physiology, molecular and cell biology, computational science, statistics, exposure and effect modelling, risk assessment) 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.

Partner contributions

29. In Phase 2, the Health and Environmental Sciences Institute will provide access to a network of potential North American-based collaborators offering their resources (e.g. avian *in vitro* models) to the Challenge winner. There is the potential for financial support to facilitate this access through the Health and Environmental Sciences Institute and the American Chemistry Council, subject to approval. Further information will be provided during Phase 1.

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