EPAA Environmental Safety Assessment project

Moving towards alternatives to animal testing for ESA

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-with support from Jose Tarazona (ISCIII)

3RS Integrating 3 Worlds
Human, Animal and Environmental Health
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Background: EPAA Partners Forum on ESA

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Use of alternatives to animal testing for Environmental Safety Assessment (ESA): Report from the 2023 EPAA partners' forum

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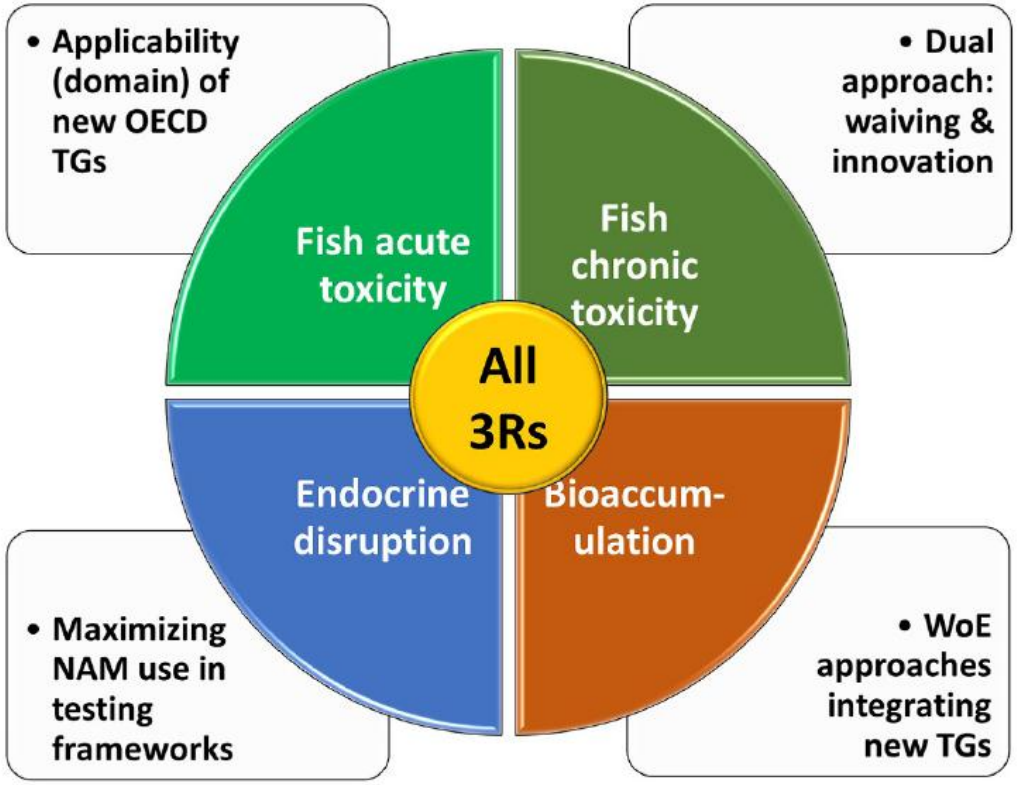


Fig. 1. Main priority areas and proposed actions, to be complemented with a long-term initiative for developing a new ESA paradigm.

EPAA Environmental Safety Assessment (ESA) project-SETUP

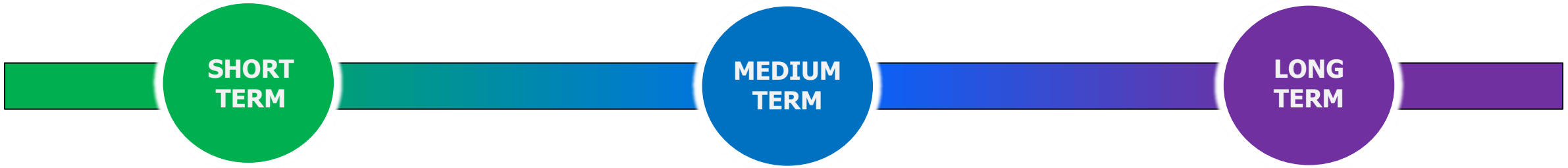
- **Project Team:** 40 members from EPAA (EC, EU Agencies, Companies, Associations) and external organizations

- **Objective:** To facilitate the integration of non-animal alternatives in ESA, contributing to the EC roadmap, and paving the way towards a more relevant paradigm for environmental assessments.

- **Working method:** Identification of main areas. For each area, WG has elaborated a short document discussing current State of the science, obstacles for regulatory uptake, and recommendations for the EC Roadmap

Short-term	Short-Medium term	Medium-Long term
Fish acute toxicity	Chronic fish testing	Envisioning a new ESA paradigm
Bioaccumulation	Endocrine disruption	
	Birds and mammals in ESA	
	Validation of new methods	

3 baskets approach – short / medium / long-term actions



- ✓ Existing (combination) of methods that could be implemented in legislation already today.

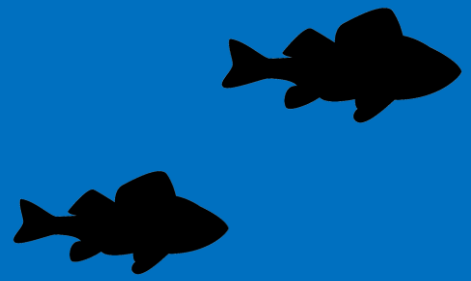
- ✓ Existing (combination of) methods or methods in development.
- ✓ Further development needed or expansion of domain of applicability or validation.

- ✓ New (combination) of methods that allow to reach protection goal without use of animals.
- ➢ Paradigm shift.
- ➢ Likely revision of regulatory assessment.

Acute Toxicity- Aquatic / Fish

- Understand sensitivity at different trophic levels for specific chemical classes to waive fish acute testing
- Use QSARs (domain of applicability)
- OECD TG 249: Fish cell line acute toxicity test (domain of applicability)
- OECD TG 236: Fish embryo acute toxicity (FET) test (domain of applicability)

- Additional applicability domain mapping & expansion



- Advance mechanistic NAMs for MoA understanding
- Move to completely animal-free (such as foetal bovine serum (FBS) and embryos)

Bioaccumulation

- OECD TG 319 A/B (in vitro intrinsic clearance rainbow trout hepatocytes) with IVIVE (domain of applicability)
- OECD TG 321 Hybit (domain of applicab.)

- Phase out requiring new OECD TG 305 (in vivo Bioaccumulation in fish)
- Promote the use of OECD IATA on bioaccumulation (includes multiple Lines of Evidence).
- Advance in vitro methods and IVIVE for mammals

- Transition to cell-line based assays and Physiologically based toxicokinetic (PBK) modelling
- Develop NAMs for avian species (e.g. HESI avian in vitro work, NC3Rs CRACK-IT "Wings of Change")

Chronic Toxicity – Aquatic / Fish

Growth, Developmental, Reproductive, Behavioral, Survival, Mechanistic

- ❑ Develop case studies of how different NAMs can be used together (as integrated approaches)
- ❑ **Develop and evaluate an in vivo reference dataset:** Effect and endpoint sensitivity, assays inherent variability
- ❑ **Support in vitro to in vivo extrapolation (IVIVE) development:** Predictive endpoints, sensitive cell lines, mechanistic info, effects correlation
- ❑ **Further investigate and develop predictive capacity of NAMS:** Develop new & expand current *in vitro* NAMs + *in silico* NAMs

- ❑ Advance mechanistic NAMs for MoA understanding
- ❑ Move to completely animal-free (such as foetal bovine serum (FBS) and embryos)

Endocrine Disruption

- ❑ Implement Eleutheroembryos tests in legislation

- ❑ Implement Eleutheroembryos tests in legislation (further case studies needed for extending chemical space)
- ❑ Improvement the existing OECD conceptual framework - consider it as toolbox instead of a tiered approach only

- ❑ Development of guidance on the use of AOP framework to map non-animal methods
- ❑ Gather data on cross-species extrapolation – are molecular key events conserved?
- ❑ Identify endocrine pathways other than EATS, and develop non-animal methods and AOPs for those, so that it is possible to distinguish endocrine from non-endocrine (systemic) pathways

- ❑ Define mechanistic based panel of in silico and in vitro assays that allows for a hypothesis driven safety assessment
- ❑ Develop quantitative Adverse Outcome Pathways (qAOPs) enabling higher tier assessments.

Birds and Mammals Toxicology

Designed by Freepik

- Leverage concepts from Human for human safety assessments, such as virtual control groups (VCGs), *in vitro-in vivo* extrapolation (IVIVE), dynamic energy budget-toxicokinetic-toxicodynamic (DEB-TKTD) model development and qAOP progress is recommended
- Build back ecological relevance to risk assessment, beyond replacement of *in vivo* models.
- Further investment into ecological research and cross-species extrapolation, is required to further inform assessments and to monitor effectiveness
- Identify relevant effects from in vivo studies which drive regulatory decisions and define research program re animal-free approaches to report on them

Long term Paradigm shift

- ❖ One-to-one replacements will not be possible for most endpoints

- ❖ Hypothesis-driven environmental Next Generation Risk Assessment based on animal-free methods is needed that allows for
  - A system for classification and labelling based on animal-free testing approaches only
  - Information on hazard characterisation/derivation of thresholds/risks

- **Recommendations**:
  - ❑ Describe in the Roadmap elements needed for NGRA
  - ❑ Developing a **generic bioactivity battery** covering the most **relevant environmental pathways for hazard identification** of chemicals with unknown modes of action.
  - ❑ **Developing tiered strategies** for the use of NAMs-based methods for hazard identification and characterisation.

What next?

https://www.propsychhealth.com/2021/03/14/picture-of-the-future/

→ Support Roadmap implementation

→ Define next focus activities based on roadmap needs, including case-studies development.

→ A lookout to global needs and synergies

→ Further thoughts are welcome…

Thank you!

With special thanks to the EPAA Project Team 40+ members