3 September 2025

Public comment prepared for the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) meeting, September 11 and 12, 2025.

Dear Dr. Marty and members of the committee,

We represent the international PrecisionTox consortium, a collaborative network composed of 15 partners spanning eight countries, including two institutions within the United States. Our mission is to better protect human and environmental health by developing and applying New Approach Methodologies (NAMs) for chemical safety testing, with the goal of establishing mechanistic causality between chemical exposure and toxicity. This is accomplished by defining biomolecular toxicity pathways through the integration of genomics, metabolomics, and evolutionary theory. PrecisionTox uses human cell lines, a suite of well-established biomedical model organisms, fruit flies, water fleas, roundworms, and frog and zebrafish embryos, and artificial intelligence to uncover molecular toxicity pathways shared across the animal kingdom. These models have proven to be invaluable in understanding human disease and are now for assessing environmental impacts on human health. The result is a new regulatory paradigm, phylotoxicology, which provides greater certainty in predicting chemical hazards and risks without using traditional animal models. Importantly, the *in vivo* models employed by PrecisionTox are 3R-compliant and, under European Union Directive 2010/63/EU, are not classified or protected as animals in Europe. Ultimately, these NAMs are being utilized to draft new laws and policies at both national and international levels.

We are submitting this comment because we are concerned that the current NIH definition of NAMs, which is also being adopted by other US federal agencies, excludes these important alternative *in vivo* models. PrecisionTox *in vivo* models have proven themselves to be invaluable contributors to our understanding of human biology and systemic toxicity. This is most easily exemplified by the multiple Nobel Prizes in Medicine awarded for research using these systems.

NIH defines NAMs as:

> "New Approach Methodologies (NAMs) - NAMs are laboratory (*in vitro* and *in chemico*) or computer-based (*in silico*) research approaches intended to more accurately model human biology more accurately, and when used alone, or in concert with others, enable improved disease models, including toxicology as well as complex human-relevant models, and as a result, complement animal models in biomedical research."

The NIH is establishing the core of its NAMs program with recent calls to fund NAMs developers, a central NAMs data hub, and NAMs validation centers that explicitly exclude alternative *in vivo* models. This exclusion is both ironic and disturbing in that the NIH and NTP have been international leaders in the utilization of these *in vivo* models for toxicological research for over 25 years. It is the complete antithesis to the desire for NAMs to improve our understanding of mechanism-based toxicity. In the context of chemical safety assessment, *in vivo* NAMs contribute to our knowledge of mechanism-based toxicity.

To enhance confidence in the extrapolation of mechanistic effects observed in NAMs to adverse effects on individuals or populations for regulatory purposes, investigators often uses organisms and life stages that are not considered as protected animals, such as whole-organism invertebrates (e.g. *Daphnia*, *C. elegans*, *Drosophila*) and early life stages of aquatic vertebrates (e.g. zebrafish and frog embryos). **Put simply, as tests with mammalian models are phased out, these non-protected, biomedically relevant alternative model species become even more crucial to chemical safety testing and complement efforts to embrace human *in vitro* models.**

There are many reasons to adopt biomedically relevant alternative model species. We emphasize two that we consider most pertinent to this committee.

1) The European Commission recognizes the importance of these biomedically relevant, non-traditional model species, which are not protected as animals in Europe, as it works towards meeting its goals of using animal testing only as a last resort under European directive No 1907/2006.

   Member of the European Parliament (MEP) Jutta Paulus (Greens/EFA) recently petitioned the European Commission to address the following three questions to clarify its stance on the importance and use of these alternative *in vivo* models as it works towards eliminating animal testing in chemical safety assessments. MEP Jutta Paulus asked the following:

   (i) To what extent will the Commission implement NAMs for chemical safety assessment in order to simplify REACH and fulfil its stated goal of employing animal testing only as a last resort?
   (ii) Does the Commission agree that the use of organisms and life stages that are not considered as protected animals under Directive 2010/63/EU is crucial for the medium-term replacement of animal testing?
   (iii) Does the Commission intend to change the scope of protected animals under Directive 2010/63/EU to also include non-vertebrate animals beyond cephalopods, and early life stages of all vertebrates other than mammals?

   The Commission's response (EC document # E-002468/2025) states that they consider these alternative models crucial for their aim to eliminate animal testing.

   > *"The Commission plans to present a proposal to revise the Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) by the fourth quarter of 2025. In that context, it is exploring options for a better uptake of new approach methodologies (NAMs) for chemical safety assessment.* ***The Commission agrees that the use of organisms and life stages that are not considered as protected animals under Directive 2010/63/EU2 is important for the medium-term replacement of testing with animals considered as protected.***
   >
   > *Currently, the Commission is not considering amending the scope of the Directive beyond species and life stages listed in its Article 1(3). It plans however to publish by early 2026 a Roadmap to phase-out animal testing for chemical safety assessments, with attention also to testing with non-vertebrate animals and with vertebrates other than mammals in their early life stages."*

   It is generally accepted that *in vitro* models cannot replicate in *in vivo* systems: a monolayer of primary hepatocytes is not a liver. By extension, more complex *in vitro* systems; organoids,

spheroids, and 3D bioprinting; are artificial constructs that can provide valuable information, but are still not intact organs. Additionally, they do not replicate whole organisms and their applicably to hazard and risk assessment is highly problematic. ***We request that federal agencies in the United States, including the NIH, also recognize the importance of these non-protected, biomedically-relevant model species when moving towards eliminating mammalian testing***. These alternative *in vivo* models, which have proven central to understanding human biology, enable the integration of responses in whole animals, complementing efforts that focus on human *in vitro* models, while also reducing ethically challenging mammalian tests. Clear guidance from this committee would be a valuable step in this direction.

2) The NIH has traditionally embraced alternative model species, an approach that has proven incredibly successful. Historically, studies in *Drosophila melanogaster, C. elegans*, and zebrafish embryos have proven essential in dissecting molecular mechanisms that underlie human diseases, including cancer, diabetes, neurodegeneration, and immune disorders. The power of these systems is evident by the outsized number of human gene families whose names are simply derived from fly or worm gene names (*e.g.,* Notch, Smad, EGLN). Moreover, ten Nobel Prizes have been awarded to research based on studies in *Drosophila* and *C. elegans,* with the most recent prize awarded in 2024 to Ruvkun and Ambros for their discovery of microRNAs in the worm. The unparalleled genetic toolkits available for studies in *C. elegans, Drosophila,* and zebrafish, together with the emerging model system *Daphnia*, ideally position these organisms to transform our approach to chemical safety testing. It should be noted that this idea of using these invertebrate models is not new. In their 2008 editorial in *Science* magazine, NIH Director Francis Collins, Assistant Administrator for the EPA George Gray, and Associate Director, U.S. National Toxicology Program John Bucher highlighted a need to use these exact same organisms as the starting point for transforming environmental health protection (**Fig 1**, DOI: 10.1126/science.1154619).

We appreciate SACATM's attention to our concerns and hope that biomedically-relevant, non-protected animals will be included in future NIH definitions of New Approach Methodologies.

Respectfully yours,

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On behalf of the PrecisionTox Consortium