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Forum

Navigating complexity in modern toxicology: the role of omics in short-term in vivo studies

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Abstract

Toxicology is shifting toward predictive, mechanism-based approaches that support quicker, more human-relevant risk assessments and reduce reliance on animal testing. Central to this shift are short-term in vivo studies enriched with omics endpoints, which provide early molecular indicators of toxicity. These data enable the derivation of molecular points of departure and other biologically anchored metrics that can inform potency ranking, hazard identification, and risk assessment. This commentary summarizes insights from the 2025 Society of Toxicology session of the same name and highlights the importance of aligning technical advances with regulatory needs. Next-Generation Risk Assessment (NGRA) is a safety evaluation approach that incorporates emerging tools such as in vitro methods, computational models, and omics data to inform decision-making for human health and the environment, while aiming to reduce dependence on traditional animal testing. NGRA frameworks, while potentially generic in principle, must be tailored to the specific regulatory requirements and exposure contexts of different product sectors, including pharmaceuticals, industrial chemicals, agrochemicals, and cosmetics. Short-term mechanistic animal studies serve as a bridge between traditional long-term animal testing and new approach methodologies. From a technical standpoint, the generation, analysis, and interpretation of omics data have matured considerably, bringing regulatory acceptance within reach. Remaining challenges include standardizing bioinformatics pipelines, building confidence through validation against apical endpoints, and expanding training. Addressing these gaps through collaborative science and flexible regulatory frameworks will be key to realizing the full potential of omics-enabled hazard profiles to support NGRA.

Keywords: omics research; risk assessment; safety evaluation; mechanisms; hazard; assessment

Toxicology is undergoing a fundamental transformation, driven by the growing desire to improve mechanistic understanding of chemical effects while reducing animal use and accelerating safety evaluations (NAS 2017; Sewell et al. 2021; Schmeisser et al. 2023). Traditionally, toxicology has relied on subchronic and chronic in vivo studies (e.g. 90-day or 2-year repeat-dose rodent studies) to estimate no- or low-effect levels, identify target organ toxicity, and derive health-protective reference values. However, these approaches are resource-intensive, time-consuming, and increasingly misaligned with the current emphasis on human-relevant, high-throughput, and laboratory animal reduction and refinement methods.

Omics technologies offer powerful tools for understanding how biological systems respond to chemical exposures. These techniques, such as transcriptomics, metabolomics, proteomics, epigenomics, and even image-based phenomics (e.g. Cell Painting), enable the study of large sets of biological molecules to uncover molecular and cellular changes (Meier et al. 2025). Among these, transcriptomics is the most mature, but all omics approaches contribute to a deeper understanding of biological perturbations and help reveal mechanisms of toxicity.

When applied in the context of short-term in vivo studies (typically 5- to 28-day repeat-dose rodent studies), omics technologies can enhance the sensitivity and resolution of toxicity testing (Geter et al. 2014; Hester et al. 2015; Joseph 2017; Yuan and Nault 2025). These combined approaches enable early detection of mechanistic signals in response to chemical exposures, often before traditional apical outcomes (such as liver hyperplasia or adenomas) are observable (Calabrese et al. 2007; Kültz 2020; Johnson et al. 2022). Importantly, these mechanistic insights can be used to derive molecular points of departure (PODs) and support hazard identification, potency ranking, and risk assessment (Johnson et al. 2022). This paradigm presents a promising alternative to conventional long-term studies, providing a more efficient, mechanistically informed approach to chemical safety evaluation (EPA 2024b).

This commentary discusses recent efforts to operationalize these tools, with a focus on regulatory applicability and ongoing scientific challenges, as presented in the scientific session "Navigating Complexity: How Omics in Short-Term Animal Studies are Optimizing and Accelerating Chemical Risk Assessment" at the 2025 Society of Toxicology (SOT) Meeting.

Next-Generation Risk Assessment and Short-Term Mechanistic Animal Studies

Next-Generation Risk Assessment (NGRA) is an approach to chemical safety evaluation that integrates new technologies and methodologies, such as in vitro assays, computational modeling, and omics, to support more relevant decisions for human health or the environment and reduce reliance on traditional animal testing (Pallocca et al. 2022; Hristozov et al. 2024; Shao et al. 2024). It emphasizes mechanistic understanding, faster decision-making, and improved prediction of human health outcomes.

Short-term in vivo studies can be used within this framework to capture early biological responses to chemical exposures in a more cost-effective and mechanistically informative way. When paired with omics techniques, such as transcriptomics, metabolomics, and proteomics, these studies can generate rich molecular and cellular data to identify early biomarkers of toxicity (Corton et al. 2022), derive molecular PODs (Johnson et al. 2022), and inform risk assessments without the need for apical outcomes from long-term studies. Combining these short-term in vivo studies with omics-based endpoints can allow for the assessment of molecular perturbations and generate data that support hazard identification, potency ranking, and regulatory decision-making.

Importantly, the specific safety data requirements for chemical assessments vary considerably across sectors and are influenced by several factors, including the intended use, level of exposure, and whether the molecule is designed to have a targeted biological effect in humans (Fig. 1). For example, pharmaceuticals typically require comprehensive preclinical and clinical testing; pesticides must undergo extensive in vivo evaluation due to environmental and dietary exposures; industrial chemicals are assessed based on production volume and use scenarios; and cosmetics, in many regions, face strict prohibitions on animal testing, necessitating alternative methods. These differences pose a challenge to developing a universal NGRA framework. However, the adaptability of common approaches like the use of omics, applicable to both in vitro and in vivo models, makes it a powerful tool for cross-sector harmonization.

In this commentary, we highlight the value of short-term in vivo studies as a practical and scientifically robust bridge between developing in vitro approaches (e.g. new approach methodologies) and traditional long-term animal studies such as 90-day rodent toxicity tests (NAS 2023; van Ravenzwaay et al. 2024). We propose that integrating omics into these models can yield timely, mechanistic data to support protective, human-relevant decision-making, while reducing reliance on extended animal testing and building confidence in the robustness of the emerging new testing paradigm. Although hazard assessment strategies can be applied consistently across sectors such as pharmaceuticals, industrial chemicals, and agrochemicals, exposure considerations will vary by sector. These differences include factors such as use scenarios, population characteristics, and expected exposure duration and must be appropriately integrated into the risk assessment process.

Although growing evidence supports the use of short-term in vivo studies as alternatives to subchronic tests, further work is needed to evaluate their applicability in areas such as carcinogenicity and developmental and reproductive toxicity. From a regulatory decision standpoint, classification and labeling of the products should also be considered when making use of such data. It is also important to consider how these models perform across novel chemistries versus well-characterized analogs and how to implement them through an iterative, flexible process. Although many questions remain, we view short-term in vivo studies as a critical step in transitioning from long-term, pathology-focused animal studies toward mechanism-driven, fully in vitro-based hazard assessment.

Use of PODs from omics data: US EPA's approach and research

Short-term in vivo studies offer a powerful way to detect early biological changes that occur before observable toxicity, with gene expression data serving as sensitive indicators of biological perturbation. These molecular signatures can be modeled to derive transcriptomic points of departure (tPODs), typically based on the lower 95% confidence limit of the lowest median benchmark dose (BMD) showing consistent changes across pathways or biological processes (EPA 2024a). This approach is exemplified by the US EPA's Transcriptomic Assessment Product (ETAP) (EPA 2024b, 2024c), designed as a method to efficiently obtain data to inform decision-making for otherwise data-poor chemicals, particularly useful under the Toxic Substances Control Act, where many chemicals, including industrial chemicals like per- and polyfluoroalkyl substances (PFAS), lack repeat dose toxicity data suitable for human health assessment.

ETAP uses 5-day repeated oral dose rat studies with 8 or more dose groups to analyze gene expression of a series of potential target organs and derive transcriptomic reference values (TRVs) for health assessments, drastically reducing data turnaround of generating these values from years to months. Reference doses can also be updated as new data (e.g. from longer-term studies) become available (EPA 2024c). Notably, tPODs do not require mapping of adverse outcome pathways; instead, they rely on a concerted molecular change for BMD–response modeling, and no mechanistic inferences are reported.

The ETAP process has been demonstrated with perfluoro-3-methoxypropanoic acid (MOPA), a data-poor PFAS otherwise lacking in suitable studies for human health assessment. Targeted RNA-seq was applied to multiple tissues, and a tPOD was calculated based on the lower 95% confidence limit of the lowest median BMD for a gene ontology biological process set with at least 3 genes. The resulting TRV for MOPA was 0.09 μg/kg-day (EPA 2024a).

This concept is also being explored for developmental and reproductive toxicity and chemical co-exposures, where data gaps are even greater. For example, a pilot study on in utero exposure to dicyclohexyl phthalate found tPODs from fetal testis tissue that were within 2.5-fold of the lowest developmental and reproductive PODs (Waterbury et al. 2025). In another study, metabolomics and transcriptomic dose–response modeling was applied to a developmental PFAS and PFAS co-exposure scenario, allowing for comparisons between pup and maternal responses (Conley et al. 2022). Notably, the design of this study illustrates the potential of short-term, molecular-level approaches not only to inform target organ toxicity but also to address the complexities of developmental and reproductive toxicities. The study

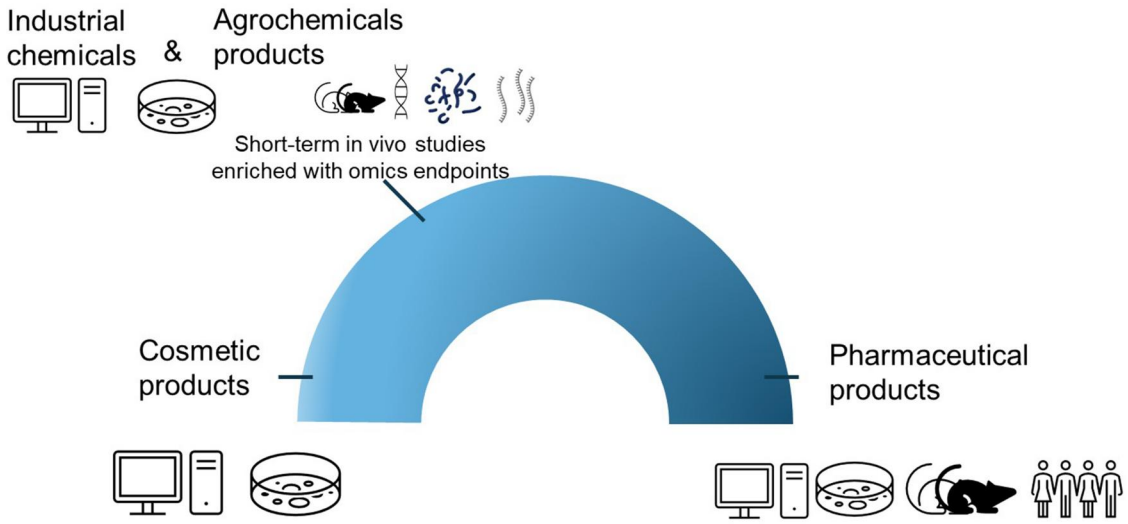

Fig. 1. Increasing data requirement across sectors for preclinical chemical safety assessment. Cosmetics typically do not allow animal studies and use new approach methodologies (NAMs) for decision-making, whereas agrochemicals and pharmaceuticals require multiple animal studies to be conducted, with industrial chemical requirements varying by jurisdiction and tonnage. Omics tools and short-term mechanistic studies can serve as a bridge from NAMs to longer animal studies.

identified metabolomic PODs and tPODs that were within 3- to 8-fold of the lowest concurrently measured apical data and enabled the ranking of individual chemicals and the chemical mixture by potency, which was consistent with life-stage sensitivity differences (Wehmas et al. in preparation). Taken together, these results suggest that short-term transcriptomic and metabolomic data may provide both conservative and practical alternatives for regulatory decision-making.

Toward regulatory application: standardization and confidence building

Although the scientific value of transcriptomics is well recognized, its regulatory application depends on consistent, reproducible bioinformatics pipelines for data processing, analysis, and interpretation (Johnson et al. 2022). A major challenge lies in the variability introduced by different workflows, such as normalization choices, filtering strategies, and BMD modeling methods, which can significantly influence resulting tPODs (O'Brien et al. 2025). To explore this, a multi-sector working group coordinated by the Health and Environmental Sciences Institute (HESI Global) has been evaluating how workflow differences affect gene expression outcomes, particularly tPODs.

A key factor influencing tPOD derivation is the statistical method used to filter responses, which serves to exclude non-responsive genes after preprocessing. Filters such as ANOVA and the Williams Trend test, commonly paired with *P*-value and fold-change thresholds, help reduce biological noise and computational burden. Although RNA-seq data inherently follow a negative binomial distribution, it is standard practice to normalize and transform the data (e.g. via variance stabilizing transformation or log-transformation) to better approximate normality prior to applying parametric tests like ANOVA. The Division of Translational Toxicology (formerly NTP) recommends a Williams Trend test with $P < 0.05$ and fold-change >1.5 (NTP 2018). Newer methods, such as the Curve Fit Pre-Filter and model averaging approaches implemented in BMDExpress v3.0, utilize ToxicR-based modeling to more selectively retain biologically plausible dose–response trends, though direct comparisons to older methods remain limited (O'Brien et al. 2025). Although stringent filters enhance model quality, overly aggressive filtering can exclude true signals and increase tPOD variability.

tPODs are also highly sensitive to the way gene-specific BMDs are derived. Traditional parametric models (e.g. Hill, linear, exponential) are used, often based on benchmark responses (BMRs) defined by SD thresholds. However, these thresholds may vary between experiments, prompting exploration of alternatives like relative deviation and effect size theory. Model selection is another key step; although many workflows choose a single best-fit model using criteria like the Akaike Information Criterion, this approach can underestimate the uncertainty by ignoring variability across alternative models. Model averaging, now available in BMDExpress, BBMD, Proast, and ToxicR, offers a more robust alternative by incorporating multiple fits. Nonparametric tools like ALOHA further expand options, particularly for complex dose–response shapes, though broader validation is needed (O'Brien et al. 2025).

Although tPODs offer great promise, differences in bioinformatics workflows, from filtering strategies to model selection, can meaningfully impact outcomes. Ensuring transparency and standardization across pipelines is critical for building confidence in transcriptomics-based risk assessment (O'Brien et al. 2025).

Expanding the toolbox: short-term mechanistic animal studies and multi-omics integration

In addition to transcriptomics, modern toxicology is increasingly turning to broader "omics" approaches, such as metabolomics, proteomics, epigenomics, and lipidomics. Researchers are also adopting integrated study designs that aim to extract the most information possible while minimizing animal use. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has proposed in vivo studies embedded within existing OECD guideline designs (e.g. TG 407: Repeated Dose 28-day Oral Toxicity Study in Rodents) and enhanced with additional endpoints (Ball et al. 2022; Botham et al. 2023).

A retrospective analysis of 113 chemical pairs with both 28- and 90-day toxicity studies revealed that the majority of adverse findings in 90-day studies were detectable at 28 days (ECETOC Smart Studies Task Force, manuscript in preparation; Escher et al. 2024; Meurer et al. 2024). However, certain endpoints, especially in hematology and liver, were missed in standard designs, suggesting that strategic additions (e.g. metabolomics, toxicokinetics, targeted transcriptomics) could help close these gaps.

ECETOC's task force has evaluated the technical readiness of adding such endpoints, noting that blood-based omics are particularly feasible because they are minimally invasive and can be repeated in the same animal. Tissue-based transcriptomics can also be integrated, although logistical challenges around tissue preservation, sample processing, and regulatory acceptability remain. These studies are designed not only to detect adverse effects but also to "de-risk" chemicals by confirming a lack of toxicity under defined conditions. Additionally, an OECD panel is currently developing best practices for tissue collection in omics studies, which is expected to enhance sample consistency and reproducibility, and align with interpretation frameworks such as the OECD's Omics Reporting Framework (OORF) (Harrill et al. 2021).

Interpretation frameworks and reporting standards

To make omics data actionable, consistent interpretation frameworks are essential. The OORF provides a structure for capturing metadata around study design, platform type, and analysis methods (Harrill et al. 2021). Although comprehensive, the OORF has not yet seen widespread adoption, likely due to its complexity and lack of regulatory mandate.

To support omics interpretation, an international, cross-sector group of experts developed the Regulatory Omics Data Analysis Framework (R-ODAF), which standardizes the selection of differentially expressed genes from RNA-seq and microarray data for toxicological applications. R-ODAF includes filtering steps to remove non-biologically relevant signals, such as low-expressed and non-stable genes, thereby focusing on the most consistent and biologically relevant gene expression changes (Verheijen et al. 2022).

One key insight from this work is that fold-change thresholds commonly used in transcriptomic sequencing-based studies (e.g. ±2-fold) may not be appropriate for decision making, as they tend to preferentially exclude highly expressed genes. In particular, the non-normally distributed read counts and limited coverage do not allow highly expressed genes to reach large fold changes, leading to their preferential removal. Instead, statistical consistency and biological coherence across pathways may be more reliable criteria for selecting gene sets.

Although this reporting framework is useful, there are challenges. First, other omics, like proteomics and epigenomics, are missing from the OORF. Similarly, modules for emerging transcriptomic technologies, such as single-cell and spatial transcriptomics, are not yet included. Although these elements can potentially be added, their absence highlights a broader issue: The rapid pace of technological advancement often outstrips the ability of standardized frameworks to fully capture the critical decisions made during data generation, analysis, and interpretation. Additionally, use of the OORF can be limited by factors such as lack of time and interest from researchers, absence of formal requirements, and the need for substantial technical expertise.

Despite these challenges, adopting the OORF offers important benefits. It promotes transparency and reproducibility, encourages consistency across studies, and helps ensure that key technical and biological considerations are clearly documented. This can improve data quality and facilitate meaningful comparisons across datasets, studies, and platforms.

Future outlook: integration and implementation

The integration of short-term studies and omics into chemical risk assessment has moved beyond theory. Programs like the US ETAP and international initiatives through the OECD show growing regulatory openness and progress toward standardized guidance for tPODs. Efforts led by organizations such as HESI and ECETOC are also helping to bridge the gap between innovation and implementation by generating usable data, defining workflows, and fostering collaborative dialogue.

Despite these advances, several key challenges remain:

- Harmonization of bioinformatics pipelines
- Validation of molecular PODs against traditional apical endpoints
- Global regulatory alignment on data acceptability
- Laboratory training to ensure high-quality omics generation
- Education of risk assessors and regulators on omics application

Additionally, the ultimate goal of human health toxicity testing is to protect humans, even when using animal models. Shorter-term rodent studies still have the same limitations as chronic rodent studies in that a rodent is not a human. A deeper discussion of this is beyond the scope of this paper, but includes species-specific differences in genes coding for key proteins, differences in sensitivity to compounds, and variability in metabolism (Van Norman 2019; Atkins et al. 2020). Additionally, leveraging existing clinical and epidemiological data for chemicals currently on the market, as well as those that have been withdrawn, can provide valuable insights into the interpretation of animal study relevance to human health and safety. This can help toxicologists understand the actual predictive power of animal data.

Although not a focus of the session, many of the concepts and tools discussed also apply to environmental health, particularly ecotoxicology. Fish and amphibians account for the highest vertebrate use in agrochemical registration (Lillicrap et al. 2016; Mitchell et al. 2023), and initiatives like EcoToxChip are demonstrating how omics can inform decision-making in this space (Basu et al. 2019).

Ultimately, realizing the full potential of these approaches will require sustained investment in collaborative research, transparent methods, and adaptive regulatory frameworks. Short-term in vivo studies with integrated omics endpoints offer a scientifically robust, mechanistically rich approach that unites traditional apical and molecular data within efficient workflows. Building regulatory and stakeholder confidence will depend on both scientific rigor and coordinated, cross-sector engagement to support adoption of innovative, ethically sound methods in chemical safety assessment.

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