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Review

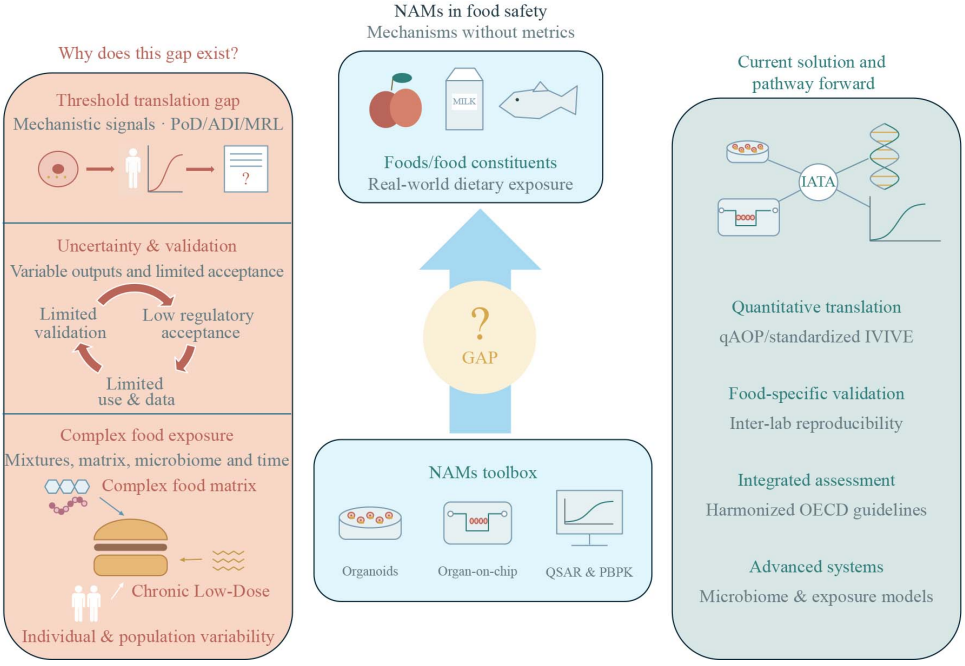
# NAMs in Food Safety: Mechanisms Without Metrics

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## GRAPHICAL ABSTRACT




## HIGHLIGHTS

- A gap exists between NAM outputs and quantitative regulatory endpoints.
- Thresholds, uncertainty, and complex exposure limit NAM regulatory adoption.
- Integrating NAMs with existing approaches enables more effective risk assessment.

**ABSTRACT** New Approach Methodologies (NAMs) have the potential to transform toxicology, yet their impact on food safety regulation remains limited. This review summarizes their current applications in mechanistic screening, toxicokinetic modeling, exposure assessment, and integrated risk evaluation, and examines regulatory perspectives on their use. It argues that the limited uptake of NAMs reflects a structural mismatch between mechanistic or semi-quantitative outputs and the stable, reproducible, and legally defensible quantitative evidence required for food-safety decisions. This mismatch is conceptualized through three interconnected challenges: the threshold-translation gap, the uncertainty paradox associated with emerging methodologies, and the complexity of chronic, mixture-based, and matrix-dependent food exposures. Accordingly, NAMs are currently better positioned as complementary tools that strengthen existing risk-assessment frameworks rather than as complete replacements for conventional toxicology.

**KEYWORDS** New Approach Methodologies; Food safety regulation; Risk assessment; Quantitative thresholds; Evidence translation

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## 1 Introduction

In recent years, New Approach Methodologies, commonly referred to as NAMs, have advanced rapidly in pharmaceutical research and development. Liver-on-a-chip systems are increasingly applied to predict drug-induced hepatotoxicity [1], lung-on-a-chip models replicate respiratory motion for the assessment of inhalation safety, and intestine-on-a-chip platforms mimic peristaltic activity to investigate host–microbe interactions. By recapitulating key organ functions in human-relevant systems, NAMs offer improved mechanistic insight and, when integrated with kinetic modeling, may enable more credible extrapolation to humans than traditional animal models. However, comparatively less attention has been given to a broader systemic question: why has the increasing availability of NAM technologies not yet produced a coherent and self-sustaining framework for food-safety regulation?

In food-safety research, technological progress has not translated into a comparable level of regulatory influence. This discrepancy cannot be explained solely by the maturity of individual platforms. It also reflects a structural misalignment between the evidence currently generated by NAMs and the evidence required for food-safety decision-making. Most NAMs primarily generate mechanistic, molecular, or cellular bioactivity data, which are often qualitative or semi-quantitative. Food regulation, by contrast, depends on population-protective quantitative outputs, including points of departure, health-based guidance values, and enforceable residue or contaminant limits. Such values must remain sufficiently robust, reproducible, comparable, and legally defensible across chronic low-dose exposure, heterogeneous dietary patterns, chemical mixtures, and population-wide variability. Consequently, the central question is not simply whether NAMs are technically feasible or biologically informative, but whether they are fit for clearly defined regulatory purposes and can contribute to a cumulative and internally consistent evidentiary framework. This structural misalignment between outputs of NAMs and regulatory requirements in food safety is illustrated in Fig. 1.

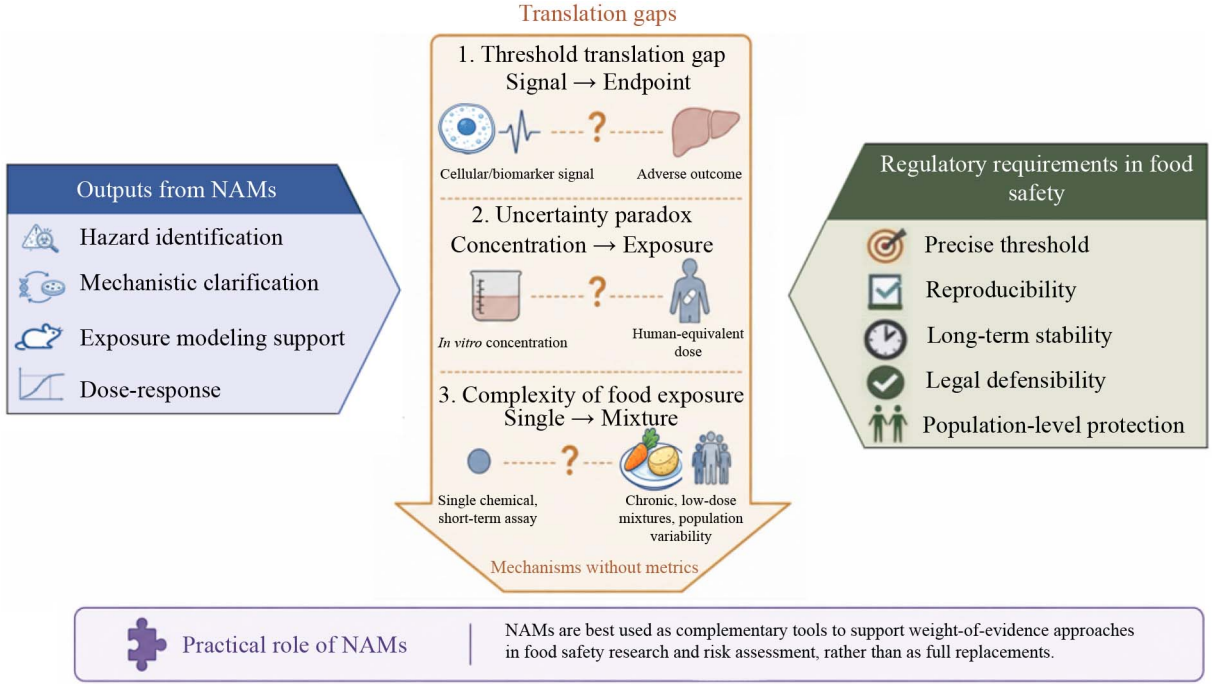



**Fig. 1** Structural misalignment between outputs of NAMs and regulatory requirements in food safety.

Accordingly, this review argues that the limited regulatory integration of NAMs in food safety reflects a structural mismatch between their current outputs and the quantitative, reproducible, and legally defensible evidence required for regulatory decision-making. Rather than pursuing the immediate replacement of conventional toxicological methods, NAMs should, in the near term, be strategically integrated as complementary tools to strengthen mechanistic interpretation, chemical prioritization, exposure modeling, and uncertainty characterization. Such integration may provide a more realistic pathway towards progressively reducing reliance on

animal testing while preserving the scientific reliability of food-safety assessment.

## 2 Core Requirements of Food Regulation: Thresholds, Stability, and Defensibility

Regulatory standard setting is among the most important, yet most challenging areas for the application of NAMs in food safety. Regulatory authorities and other authoritative bodies across countries have continuously expressed strong interest in NAMs. However, the laws and regulations that have been enacted by some institutions suggest that a substantial distance remains before NAMs can be truly implemented in the food-safety domain. The Food and Agriculture Organization of the United Nations (UN FAO), in the interest of safeguarding public health, emphasizes the importance of reviewing and interpreting the meaning and regulatory relevance of safety assessments for complex matrices when such assessments are derived from NAM-based measurements, in order to prevent indiscriminate use. Despite recognizing the potential of NAMs, current assessment processes may still require animal-derived evidence as part of the supporting data package [2]. This cautious approach is also evident among regulatory authorities in the European Union and Asia, which generally continue to rely on established methods while emphasizing that food-safety decisions have direct implications for human health [3]. Accordingly, novel methods are unlikely to serve as independent bases for regulatory decisions until their reliability, relevance, and performance have been demonstrated to be at least comparable, and where appropriate superior, to those of existing approaches [4]. A stakeholder study commissioned by the UK Food Standards Agency further suggests that uncertainty surrounding the regulatory acceptance and practical value of NAM-derived evidence has, in some jurisdictions, constrained public investment and the allocation of specialized scientific expertise. This regional disparity appears particularly pronounced in Asia, where the scale of NAM-related research and institutional support remains smaller than that observed in America.

Meanwhile, research indicates that the primary obstacle is not merely the technological maturity of NAMs, but rather the degree to which the outputs generated by current NAMs align with the evidentiary logic of food regulation. Compared with the cosmetics and pharmaceutical sectors, where NAMs have developed rapidly, food systems involve complex mechanisms and uncertain compositions, and the substances present may simultaneously exhibit synergistic and antagonistic toxicological characteristics. Consequently, as research tools that are mostly applied to relatively single, well-defined substances, NAMs tend to be positioned toward internal product research and development rather than regulatory evaluation [5]. From this pattern, we can observe a mismatch between the design objectives of NAMs and the end goals that regulatory standard setting must ultimately achieve.

Examining the reasons for this mismatch, the first challenge concerns the type of endpoints required by regulators. Food regulation is, in essence, threshold-based. Key metrics depend on determinate numerical values, such as acceptable daily intake (ADI) and maximum residue limits (MRLs). However, most current applications of NAMs in food toxicology still focus on hazard identification and mechanistic bioactivity analyses. For instance, the assessment of certain substances in pharmaceutical contexts may reveal unanticipated toxic effects in humans. These outputs are valuable—they help elucidate biological pathways and modes of action, and they may also support the analysis of certain food extracts, thereby informing potential applications in cosmetics or other domains. Yet they do not automatically provide the quantitative benchmarks required for regulatory standard setting. Because sufficiently validated, physiology-based toxicokinetic and toxicodynamic models are lacking, and because there are no broadly accepted rules for translating *in vitro* signals into regulatory metrics, NAM-derived responses often cannot be converted into points of departure that regulatory agencies can defensibly rely on in guidance documents or legal instruments [6]. This limitation is particularly consequential in food safety, because exposures are typically chronic, low-dose, and population-wide [4]. Under such circumstances, uncertainty factors and extrapolation assumptions become especially sensitive.

The second challenge concerns stability and reproducibility. Food regulation depends on standards that remain stable over long periods of time. Monitoring systems, enforcement practices, and international comparability all rely on consistent reference values. By contrast, NAMs continuously evolve within a rapidly changing innovation environment. Organoids, microphysiological systems, omics workflows, and AI-driven predictors are all changing in platform design, analytical performance, and data interpretation. Such progress has major scientific value, but it also generates friction: regulatory systems tend to favor methods that are validated, standardized, and durable. Japan has actively promoted research on NAMs in recent years and has explored the application of approaches such as the threshold of toxicological concern (TTC) and Ames-based QSAR in the standard setting and safety assessment of food additives [7]. However, a 2026 survey

commissioned by the Food Safety Commission of Japan and involving 30 experts identified the lack of standardization, guideline-based procedures, and adequate validation as factors that may compromise the reliability and scientific validity of evidence generated using NAMs. Expert interviews conducted for the survey further highlighted that, in several areas of food risk assessment, reproducibility and validity have not been adequately evaluated, equivalence with established methods remains to be demonstrated, and common criteria for regulatory acceptance have yet to be developed. These findings do not indicate that Japan rejects NAMs; rather, they suggest that many existing approaches have not yet achieved the methodological robustness, standardization and institutional consistency required for routine use in food-safety decision-making. Therefore, there is an enduring tension between rapid methodological innovation and the slower, formal institutional processes required for regulatory adoption [8].

The third challenge concerns the scope of regulatory questions. Food risk assessment is intrinsically systemic. Pharmaceutical evaluation commonly revolves around a specific compound, a defined dose, a route of administration, and a therapeutic target. Food safety assessment is different: it must address exposures involving chemically diverse constituents, mixture effects, heterogeneous diets, and inter-individual differences in susceptibility. This broader scope makes it more difficult to integrate NAM evidence, because regulatory judgments must simultaneously account for complexity at both biological and exposure levels. In this regard, some scientists have proposed combining multiple NAMs. The cost and operational complexity of multi-NAM strategies may limit their feasibility and reduce institutional incentives for routine adoption [9]. Meanwhile, if toxicological information is needed for substances in certain relatively single-component foods, some high-toxicity or short-term hazard signals relevant to food safety can already be obtained from environmental toxicology or biomedical datasets. This raises a practical question: even if NAMs could be further optimized for food-specific applications, would such investments yield greater regulatory benefit than strengthening exposure assessment, biomonitoring, and uncertainty characterization?

## 3 Structural Misalignments Between NAM Outputs and Regulatory Needs

### 3.1 The threshold translation gap

One major barrier is the gap between mechanistic signal detection and threshold derivation. NAMs are highly effective at detecting cellular perturbations, including receptor activation, mitochondrial stress, and oxidative damage. However, these molecular initiating events do not automatically map onto organism-level adverse outcomes in a way that supports regulatory threshold setting through adverse outcome pathways. A liver chip, for example, may show CYP1A1 induction at 0.1 μM after pesticide exposure. That is useful mechanistic information. But regulation asks a different question: what external dose causes adverse liver effects in humans under chronic exposure, and under what assumptions about inter-individual and population variability?

Currently, technical limitations in *in vitro*-to-*in vivo* extrapolation (IVIVE) further exacerbate the difficulty of threshold translation. Although physiologically based toxicokinetic (PBTK) models are regarded as critical bridges connecting *in vitro* data to *in vivo* doses, their standardized application in food safety regulation still faces severe challenges [10,11].

First, generic PBTK models exhibit significant uncertainty in predictive accuracy. The World Health Organization notes that PBTK predictions within a factor of two of experimental data are generally considered acceptable; however, for food safety assessments covering complex exposure scenarios such as chronic low-dose dietary exposure, this threshold is often too lenient [10]. Second, methodologies for deriving benchmark doses (BMD) or no observed adverse effect levels (NOAEL) based on *in vitro* data remain immature. Although the US EPA's ToxCast program has attempted to derive human *in vivo* benchmark doses from *in vitro* concentration-response data through computational workflows [12], most *in vitro* assays still lack direct linkage to apical endpoints, making it difficult to establish dose–response curves required for regulatory purposes [13].

A third technical barrier, often overlooked, lies in the design and parameterization of the PBTK models themselves. Food-relevant chemicals exhibit wide physicochemical diversity—ranging from hydrophilic mycotoxins to lipophilic persistent organic pollutants—each requiring distinct model structures and input parameters (e.g., tissue partition coefficients, metabolic clearance rates, and intestinal absorption fractions) that are rarely available from *in vitro* systems alone [14]. Moreover, the lack of standardized protocols for integrating NAM-derived kinetic data into PBTK frameworks introduces additional uncertainty, as different laboratories may apply varying assumptions for protein binding, hepatic extraction, or renal clearance, leading to divergent *in vivo* dose predictions from identical *in vitro* concentration-response datasets [15]. This parameterization gap is particularly problematic for food safety,

where chronic, low-dose exposures require high precision in toxicokinetic modeling to support legally defensible health-based guidance values.

Furthermore, regulatory agencies maintain a cautious stance toward NAMs based dose–response relationships [16]. The European Food Safety Authority (EFSA) has explicitly stated that high-throughput *in vitro* assays are primarily designed for screening and mechanistic exploration, resulting in insufficient predictive power for apical endpoints and failing to meet the stringent requirements for chronic exposure threshold setting in food safety regulation [3,17].

In practice, current strategies often rely on a patchwork of methods. Quantitative structure–activity relationship models are used for screening. Physiologically based pharmacokinetic models are used for dose extrapolation. Organoids and chip systems provide mechanistic evidence. Each component contributes something important. However, the integration of these disparate data types remains methodologically fragmented: there is no widely accepted framework for weighting mechanistic evidence against toxicokinetic predictions, nor for propagating uncertainty from *in vitro* concentration-response curves through PBTK models to final point-of-departure estimates. Even so, none of them alone, and often not even in combination at the current stage of validation, can robustly deliver the no observed adverse effect level or benchmark dose lower confidence limit needed to support an acceptable daily intake. As a result, NAM evidence often remains confirmatory rather than substitutive. Animal studies still serve as the final calibration reference. This creates a translation valley, in which promising NAM-derived findings must still be benchmarked against animal data, thereby weakening their replacement potential.

### 3.2 The uncertainty paradox

A second structural problem lies in how uncertainty is handled. Traditional toxicology manages uncertainty through standardized uncertainty factors. A common approach is to apply a tenfold factor for interspecies differences and another tenfold factor for human variability. The resulting thresholds are conservative, but they are also institutionally legible. NAMs alter this structure. Rather than reducing uncertainty in a straightforward way, they often shift it from familiar species-extrapolation problems to more complex epistemic uncertainties. These include the biological representativeness of engineered tissues, sensitivity to culture conditions and microfluidic forces, and the validity of inferring chronic low-dose outcomes from short-term *in vitro* responses.

This creates a regulatory double bind. Accepting NAMs may introduce new uncertainties that are harder to quantify. Rejecting them, however, preserves the long-recognized uncertainties of animal-to-human translation. This uncertainty paradox manifests at the regulatory framework level as severe lag in standardization and validation [2]. Although the OECD has issued guidance documents for PBTK models in chemical regulation, specific guidelines tailored for the food safety domain remain inadequate [3,11].

A critical but frequently overlooked aspect of this paradox is the asymmetric nature of uncertainty perception. In food safety regulation, uncertainties associated with traditional animal studies—despite being well documented—are often treated as familiar and therefore manageable through established uncertainty factors. By contrast, uncertainties arising from NAMs, even when objectively smaller in magnitude, are perceived as novel and thus subject to stricter scrutiny. This asymmetry is not merely psychological; it has concrete institutional consequences. Regulatory agencies require extensive validation datasets for NAMs before acceptance, yet the very novelty of these methods means that such datasets are often unavailable, creating a validation paradox**:** without historical data, NAMs cannot be validated; without validation, they cannot generate historical data [18].

Furthermore, the uncertainty introduced by NAMs is not monolithic—it operates across multiple interconnected layers. At the biological level, uncertainties arise from the incomplete recapitulation of tissue architecture, multicellular interactions, and organ-level physiology in microphysiological systems [6]. At the technical level, inter-laboratory variability in culture protocols, scaffold materials, and microfluidic perfusion rates can produce divergent results from nominally identical experimental designs, yet these sources of variability are rarely quantified in a systematic manner. At the translational level, the extrapolation of *in vitro* concentration-response relationships to *in vivo* exposure scenarios requires assumptions about toxicokinetics and toxicodynamics that are themselves uncertain, and these assumptions propagate through the assessment chain in ways that are difficult to trace or validate [10]. This multi-layered uncertainty structure is fundamentally different from the relatively simple, unidimensional uncertainty factors used in traditional risk assessment, making it difficult for regulators to incorporate NAM evidence into established frameworks.

Additionally, recent EFSA analyses indicate that enhancing regulatory acceptance requires a “dual data package” transition strategy—employing both traditional animal data and novel NAMs data with comparative validation—but this entails substantial temporal and

economic costs [19]. This dual-requirement strategy, while scientifically prudent, inadvertently reinforces the uncertainty paradox: by demanding that NAMs demonstrate equivalence to animal data before acceptance, regulators effectively require NAMs to compete against the very methods they are meant to complement or replace, on terms that inherently favor the established approach. In the current absence of clear health protection baselines, regulatory agencies struggle to establish performance targets for new methods [16], thereby becoming trapped in a vicious cycle of "no data means no acceptance, no acceptance means no data"[18].

This institutional conservatism is particularly pronounced in the food safety sector because food exposure is involuntary and population wide [20,21], leading regulators to exhibit extremely low tolerance for uncertainty [22], preferring to retain traditional uncertainty factors with known deficiencies rather than adopting novel epistemic uncertainties that are difficult to quantify [23]. This preference, however, is not necessarily rooted in empirical evidence that traditional methods are more accurate—rather, it reflects a regulatory culture that values procedural familiarity and legal defensibility over the potential but unproven advantages of NAMs.

### 3.3 Exposure characteristics and biological complexity

A further mismatch arises from the nature of food exposure itself. Food toxicology must often deal with mixtures, repeated exposure, and long latency outcomes. Chronic exposure to multiple chemicals at sub-threshold levels challenges the ability of NAMs to predict additive or synergistic interactions. Many endpoints that now receive increasing regulatory attention, including carcinogenicity, endocrine disruption, and metabolic dysfunction, emerge over years or decades [24,25]. These outcomes are difficult to reproduce in organ-on-chip systems without substantial increases in experimental complexity and cost.

This is where the contrast with pharmaceutical applications becomes particularly clear. Pharmaceutical NAMs usually address more defined scenarios. The dose, duration, route of exposure, and biological target are often known in advance. That makes validation more tractable. In food safety, by contrast, the very advantage often attributed to NAMs—their human relevance—can become harder to operationalize. The challenge is not simply to model human biology, but to do so under heterogeneous, long-term, real-world exposure conditions.

However, the key difficulty is not merely conceptual—it operates at the level of assay design and technical feasibility. The complexity of food matrices further amplifies this mismatch [2]. Unlike single purified compounds in conventional pharmaceutical assessments, real food represents complex mixtures containing thousands of chemicals, including not only contaminants and additives but also naturally occurring bioactive constituents [26–28]. This complexity presents four distinct challenges that directly constrain the design assumptions of current NAM platforms**.**

First, throughput limitations pose a practical barrier at the experimental level. Real-world food matrices contain thousands of chemical constituents, and their combined effects may involve synergistic or antagonistic interactions that cannot be predicted from single-compound data [29]. Comprehensive testing of all possible combinations is computationally and experimentally prohibitive with current organ-on-chip and high-content screening platforms, which are designed primarily for single-compound or limited-mixture assessments [30]. This combinatorial explosion severely restricts the use of NAMs for mixture risk assessment in regulatory contexts, as the number of experimental conditions grows exponentially with the number of mixture components.

Second, culture duration remains a fundamental constraint that is often underestimated. Many food-relevant endpoints of regulatory concern manifest after months or years of chronic low-dose exposure [24,25]. However, current organ-on-chip systems are constrained by short culture durations, high costs, and poor physiological stability, making them incapable of simulating chronic exposure processes lasting months or even years [31,32]. This temporal gap—between the short-term window of NAM observations and the long-term window of chronic dietary risk—makes it exceedingly difficult to simulate the cumulative effects that underpin chronic dietary risk assessment. Even when extended culture is technically possible, the associated costs and resource demands render large-scale chronic screening economically unfeasible.

Third, metabolic characterization capacity is often incomplete, yet this is a critical determinant of toxicokinetic profiling. The bioavailability and toxicity of food contaminants are profoundly modulated by intestinal absorption, hepatic phase Ⅰ/Ⅱ metabolism, and critical gut microbial biotransformation [33,34]. Most current *in vitro* models lack fully competent phase Ⅱ conjugation pathways or integrated microbiome compartments, and those that include microbial components often oversimplify host–microbe dynamics [35]. Moreover, the physicochemical properties of food matrices—such as fat partitioning and protein binding—directly influence contaminant bioaccessibility, yet these factors are rarely incorporated into NAM-based exposure

systems [36]. As a result, NAMs frequently fail to recapitulate the complete toxicokinetic trajectory of foodborne chemicals, undermining their utility for quantitative *in vivo* extrapolation.

A fourth, interconnected challenge concerns the prediction of non-monotonic dose–response relationships. Many endocrine-disrupting chemicals exhibit U-shaped or inverted U-shaped curves that do not conform to the linear or sigmoidal models commonly used in high-throughput *in vitro* screening [24,25]. This non-linearity complicates the identification of a clear point of departure, as the traditional benchmark dose approach assumes monotonicity. NAM platforms that rely on standard curve-fitting algorithms may therefore miss critical low-dose effects, leading to either overestimation or underestimation of regulatory thresholds.

Furthermore, the interaction between food matrix components and the gastrointestinal environment introduces additional complexity that is poorly captured by current systems. For example, digestive processes, including gastric pH variation, enzymatic breakdown, and bile salt emulsification, can significantly alter the bioaccessibility and bioactivity of food contaminants [36]. These dynamic physicochemical conditions are rarely simulated in organ-on-chip platforms, which typically operate under static or simplified fluidic conditions that do not replicate the full biochemical complexity of the human digestive tract.

Consequently, although NAMs possess unique advantages in mechanistic research, their application boundaries must be carefully delineated when confronting the systemic and chronic challenges of food safety. Taken together, these technical constraints limited throughput, short culture windows, incomplete metabolic representation, and poor handling of non-monotonicity and digestive dynamics, mean that NAMs are presently better suited for mechanistic screening and hypothesis generation than for replicating the systemic, long-term, and matrix-dependent realities of human dietary exposure. This does not diminish their scientific value, but it does define clear boundaries for their regulatory applicability. Regulatory agencies prefer to rely on traditional methods capable of providing stable, quantifiable, and legally defensible evidence rather than rapidly evolving innovative technologies that have not yet demonstrated their fitness for these complex exposure scenarios. The core mismatches between NAM outputs and food safety regulatory requirements are summarised in Table 1.

**Table 1** Summarizes the four core dimensions where NAM outputs structurally diverge from food safety regulatory requirements.

| Dimension | NAM outputs | Regulatory needs | Core misalignment |
|---|---|---|---|
| Threshold Translation | Provide qualitative mechanistic signals (e.g., receptor activation, cellular stress) | Require deterministic numerical thresholds (ADI/MRL) and legally recognized points of departure (NOAEL/BMDL) | Mechanistic signals cannot be directly translated into regulatory thresholds; lack standardized dose–response derivation methods |
| Uncertainty | Introduce novel uncertainties difficult to quantify (e.g., *in vitro* system representativeness, culture condition variability) | Rely on standardized uncertainty factors for conservative estimation | Novel epistemic uncertainties lack quantification rules; regulators cannot establish clear health protection baselines |
| Exposure Characteristics | Suitable for short-term, specific mechanistic screening; difficult to simulate chronic low-dose and mixture effects | Must assess population wide chronic exposure, long latency hazards (carcinogenicity/endocrine disruption), and complex food matrices | Fundamental contradiction between technical capabilities and assessment needs |
| Evidence Formality | Rapidly evolving technical platforms (organoids, chips, AI) lack long-term standardization validation | Require methodologically stable, internationally comparable, legally defensible long-term consistent evidence | Rapid innovation conflicts with slow institutional adoption; creating a "promising but not ready" regulatory stalemate |

## 4 Mechanistic Research and Complementary Roles of NAMs in Food Safety

Despite these limitations, NAMs have played a pivotal role in investigating food safety mechanisms. Their current value lies not so much in the total replacement of traditional toxicology, but rather in expanding the scope of scientific questions that can be explored. With the explosive growth of synthetic chemicals, novel food ingredients, and nanomaterials, the constraints of traditional methods—particularly regarding processing throughput, interspecies extrapolation, and the elucidation of modes of action (MoA)—have become increasingly prominent [37]. NAMs provide critical mechanistic insights and improved exposure modeling; this complementary function is especially significant in the formulation and refinement of food safety regulations, enabling scientific evidence to achieve a balance between reliability, efficiency, and human relevance.

One highly active research area involves the study of food-related chemical exposure and contaminant behavior. Computational modeling and human-relevant *in vitro* systems are increasingly applied to the study of veterinary drug residues in food-producing animals. For instance, Physiologically based pharmacokinetic (PBPK)

models can simulate the absorption, distribution, metabolism, and excretion (ADME) processes of drugs within edible tissues. The integration of NAMs facilitates the provision of high-precision, species-specific physiological parameters (such as hepatic metabolic rates, intestinal absorption rates, and transmembrane transport characteristics), offering core input data for the construction of PBPK models. These models provide critical support for predicting residue depletion patterns and establishing withdrawal periods for veterinary drugs used in livestock and poultry [14]. These models provide a reference for regulatory limits, such as MRLs. While adhering to the 3R principles (Replacement, Reduction, and Refinement), the establishment of models across different species—such as pigs, chickens, sheep, cattle, and humans—enables researchers to correlate the residues of veterinary drugs in animal bodies with their subsequent impacts on human health [15]. In cases where experimental pharmacokinetic data are limited, QSAR models are employed to predict the pharmacokinetic and toxicological properties of compounds based on their chemical structures. This expands the data resources available for regulatory reference; a typical application involves integrating these models into residue avoidance databases for food-producing animals to support risk management decisions in the field of food safety. The combined application of these approaches significantly enhances the predictive accuracy regarding the accumulation behavior of unknown compounds in tissues.

NAMs platforms are also employed to investigate exposure pathways directly associated with food systems. Human intestinal and epithelial microphysiological models, including gut-on-a-chip systems and organoid cultures, can recapitulate key features of epithelial transport, metabolism, and barrier functions. Although these models remain simplified compared to the physiological structure of the actual human body, they successfully reconstruct core functions such as intestinal absorption and barrier protection. Such systems provide an effective research platform for studying chemicals that migrate from food contact materials or enter the food chain through environmental pathways. For instance, chemicals such as Bisphenol A (BPA) and certain plasticizers can be tested within these systems to observe the mechanistic toxicity occurring in the intestinal epithelium [38]. In parallel, microbiome-integrated *in vitro* systems are increasingly being used to investigate how gut microbial metabolism alters the bioavailability and toxicity of contaminants such as mycotoxins and processing-derived chemicals [33,39,40].

Currently, researchers have utilized NAMs to achieve modular management and computational assessment of safety evaluations by constructing integrated assessment roadmaps. The process begins with the physicochemical characterization of food components, combined with large-scale toxicological databases and exposure scenarios for target populations, to perform refined characterization of key parameters such as bioavailability and ADME. Furthermore, through the integration of PBPK modeling, high-throughput risk assessment and nutrient synergistic analysis are achieved without the need for animal testing. This provides precise mathematical and physical support for establishing safety limits for food ingredients [41]. Taken together, these approaches show that NAM-based research can generate mechanistic evidence that is highly relevant to food toxicology, particularly for residue behavior, exposure pathways, and contaminant-host interactions.

Therefore, from the perspective of the entire risk assessment process, the role of NAMs should be positioned as complementary rather than as a complete replacement. In the early stages of risk assessment, detection methods based on these new methodologies can utilize high-throughput cellular assays to rapidly screen large numbers of chemicals and identify potential hazards before costly animal testing is undertaken. This facilitates both hazard identification and the prioritization of substances. High-throughput *in vitro* assays and pathway-based screening platforms can detect early biological perturbations associated with food contaminants, enabling researchers to screen for substances that warrant more in-depth evaluation [30]. NAMs can also enhance the interpretation of results in the later stages of toxicological assessment. Human-relevant organoids and microphysiological systems help explain how intestinal transport, microbial metabolism, or tissue-specific sensitivity influence the toxicological outcomes observed in animal trials. In this manner, without displacing the traditional evidentiary framework, NAMs enhance the capacity for mechanistic interpretation, ensuring that final regulatory decisions are grounded in more robust scientific evidence. This also lays the theoretical foundation for the gradual replacement of animal experiments by NAMs in the future.

Another significant contribution of NAMs is the enhancement of the efficiency with which experimental results are translated into practical application scenarios. Researchers can integrate data such as chemical metabolic rates obtained from cellular assays with PBPK models to estimate the actual dose of a substance reaching human tissues. This mode of data integration has become a core component of Next-Generation Risk Assessment. The objective of NGRA is precisely to ensure that safety assessment outcomes are more aligned with reality and highly relevant to actual human exposure conditions [42].

Currently, NAMs have been gradually integrated into

practical applications. The EFSA has been actively promoting the application of these methodologies in its scientific guidance documents, particularly within the field of chemical risk assessment [3]. At a broader international level, organizations such as the OECD have developed frameworks like the adverse outcome pathway (AOP) to systematically organize mechanistic knowledge, making it more applicable to regulatory work. These frameworks are highly compatible with data generated from NAMs, as they link molecular-level changes to actual health effects, and provide relevant data to support regulatory decision-making. Although these methodologies are still in the developmental stage, they have already contributed to enhancing the consistency and transparency of food safety regulation across different countries.

Although current applications demonstrate the complementary value of NAMs in food safety assessment, several critical barriers remain before their broader regulatory implementation. Therefore, future research should focus not only on expanding NAM technologies but also on improving their regulatory applicability, quantitative interpretation, and validation framework.

First, priority should be given to establishing standardized frameworks for quantitative translation between NAM outputs and regulatory endpoints. Current NAM platforms frequently generate molecular or cellular responses that are difficult to directly relate to population-level protection goals, such as ADI or benchmark dose (BMD). Therefore, integrating NAMs with PBPK models, quantitative adverse outcome pathway (qAOP) frameworks, and probabilistic dose–response modeling represents a critical research direction. These approaches may provide a structured pathway for converting mechanistic signals into regulatory-relevant points of departure [43]. Second, future research must prioritize the establishment of validation frameworks for NAMs tailored specifically to food safety. Unlike pharmaceutical safety evaluations, dietary exposure is typically characterized by chronic, low-dose regimes, complex chemical matrices, and inter-individual dietary variations. Consequently, the validation of NAMs must extend beyond establishing biological relevance; it requires rigorous evaluation of reproducibility, inter-laboratory transferability, and predictive capacity under realistic exposure conditions. Pursuing internationally coordinated validation studies and establishing standardized data reporting protocols—while accommodating the personalized nature of food safety assessments—will be pivotal in securing regulatory acceptance.

Third, in the near term, advancing Integrated Approaches to Testing and Assessment (IATA) represents a more pragmatic strategy than aiming for the immediate, total replacement of traditional toxicological models. Synthesizing NAMs with existing toxicological data, exposure assessments, epidemiological evidence, and computational models creates a continuum from short-term utility to long-term translation. This integrative paradigm lowers reliance on animal testing progressively while safeguarding the scientific certainty required for regulatory decision-making.

Finally, rapid technological innovation in NAMs remains indispensable. Current adoption is primarily bottlenecked by limited capacities to simulate chronic exposure, replicate complex food matrices, and predict multifactorial interactions. Future research should prioritize the development of culture systems capable of long-term exposure modeling, the integration of the microbiome and immune components into organ-on-a-chip platforms, and the leverage of artificial intelligence to synthesize heterogeneous biological and exposure data streams. Such advancements will enable NAMs to better recapitulate real-world food exposure environments, thereby enhancing their utility in regulatory contexts.

Overall, the most valuable role of NAMs in the field of food safety in the near term is to optimize experimental design, enhance the level of mechanistic interpretation, and support quantitative modeling analysis within existing assessment frameworks. Therefore, in the future, NAMs will be the subject of more in-depth research in the field of food safety and will be incorporated into regulatory frameworks. This transition does not indicate the disappearance of traditional approaches; rather, by fusing diverse research methodologies, the entire food safety assessment system will become more balanced. This integration enhances the system's capacity to address emerging challenges, leading to assessment results that are more reliable and more closely aligned with actual human conditions.

## 5 Conclusions

NAMs are not limited by a lack of scientific value. Their constrained uptake in food safety instead reflects the evidentiary logic and low risk tolerance built into food regulatory systems. These frameworks prioritize population-protective thresholds, long-term consistency, and legally defensible endpoints. For that reason, NAMs are unlikely to replace traditional toxicological paradigms in food regulation in the near future. That does not make them marginal. On the contrary, their most realistic and impactful role may be as complementary tools within integrated risk assessment. In that role, NAMs can strengthen hazard identification, clarify mechanisms, refine kinetic interpretation, and support priority setting. The path forward, therefore, is unlikely to be one of simple replacement. The future development of NAMs in

food safety is more likely to rely on progressive integration than on the complete replacement of conventional toxicological approaches. Research priorities should include improving the quantitative interpretation of NAM-derived responses, establishing food-specific validation frameworks, and integrating NAM evidence with exposure assessment, computational modeling, and traditional toxicological data. Particular attention should be given to chronic exposure, complex food matrices, and inter-individual variability to facilitate the gradual regulatory adoption of NAMs without compromising the reliability and scientific defensibility of food-safety decisions.

**Data availability** No data was used for the research described in the article.

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