When a Toxicology Method Becomes Regulatory Evidence

The 2026 EFSA–FDA shift towards qualifying non-animal methods for food ingredients, nanomaterials and complex formulations

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Abstract

Food-safety innovation faces an evidentiary bottleneck: sophisticated cell models, computational toxicology and high-content assays can generate mechanistic information, yet a technically impressive result does not automatically become regulatory evidence. In 2026, EFSA's NAMs4NANO initiative advanced a proposed qualification framework for new approach methodologies (NAMs), while the US Food and Drug Administration described its food-specific programme for developing and qualifying such methods. This article distinguishes analytical validation, biological relevance, regulatory qualification and formal product authorisation. It examines why particulate ingredients, and complex formulations challenge conventional toxicology, how exposure and dosimetry constrain interpretation, and where human-relevant methods can add decision value without unsupported promises to replace animal studies universally. The European Union, Spain, Great Britain and United States are considered separately. A proposed research agenda tests whether a transparent, context-of-use evidence architecture can reduce avoidable testing while identifying consequential hazards. The central argument is that regulatory confidence depends less on technological novelty than on reproducible decisions under explicitly defined uncertainty.

Keywords: *new approach methodologies; food toxicology; nanomaterials; method qualification; in vitro dosimetry; regulatory science; evidence integration; food ingredients*.

1. The 2026 signal: qualification rather than technological enthusiasm

EFSA's 2026 nanotechnology topic update describes an implementation plan developed through the NAMs4NANO consortium for a proposed qualification system for new approach methodologies. It identifies a distinction between formal validation of broadly standardised test methods and context-specific qualification of methods that may not yet be regulatory guidelines. The initiative remains a proposal and voluntary approach within EFSA's remit; it is not a newly enacted rule requiring all applicants to substitute a particular assay for an animal study. The public consultation period described by EFSA closed on 31 October 2026. [1]

The US FDA's Human Foods Program likewise explains its work to identify priority applications, develop a programme for assessing the suitability of NAMs for regulatory use, and engage external researchers. Its position is conditional: a method must be reliable and relevant to the safety

question before it can support a determination. FDA's September 2026 terminology initiative concerning nonclinical testing for drugs and biologics should not be misrepresented as a blanket change in food-additive approval requirements. [2,3]

This convergence is strategically important but legally limited. Scientific authorities are investing in evidence pathways that may eventually improve safety assessment; they have not issued a universal waiver from existing statutory standards. For manufacturers, the immediate opportunity is better-designed evidence, not a shortcut to market.

2. Four propositions that must not be conflated

2.1 Analytical validation

Analytical validation asks whether a measurement performs reproducibly within a specified matrix, concentration range and operating environment. Precision, accuracy, detection capability, selectivity, stability, batch effects and interlaboratory transferability matter. A transcriptomic assay can be analytically repeatable and still measure a response with no demonstrated relationship to adverse human health outcomes.

2.2 Biological relevance

Biological relevance asks whether the test system captures a mechanism, tissue, exposure route or biological response that matters to the hazard question. An intestinal organoid may be more mechanistically informative than a generic immortalised cell line for epithelial barrier endpoints, yet neither necessarily represents systemic distribution, immune regulation, hepatic metabolism or long-term carcinogenesis.

2.3 Regulatory qualification

Qualification establishes fitness for a defined context of use: the substance class, decision question, exposure scenario, endpoint, decision threshold and evidentiary role. It is not an unrestricted certificate of universal validity. A method useful for screening insoluble particles for inflammatory potential may be inadequate for evaluating reproductive toxicity or chronic dietary exposure.

2.4 Product authorisation

Even a qualified method does not authorise an ingredient. Product approval, where required, follows the applicable legal instrument and risk-management decision. A positive NAM result is one element in a dossier, not a licence to sell.

3. Why food ingredients are an unusually demanding test case

Food ingredients are rarely administered in isolation. The final food matrix modifies dissolution, digestion, aggregation, intestinal residence time and interactions with proteins, lipids, minerals and other dietary chemicals. Manufacturing can change particle-size distributions, impurity profiles or surface chemistry without changing the ingredient's commercial name. Consequently, a toxicity study conducted on a laboratory reference material may not characterise the actual commercial material.

For soluble chemicals, nominal medium concentration may differ from the unbound concentration reaching cells because of binding, volatility, metabolism or degradation. For particulate materials, the discrepancy can be greater: sedimentation, agglomeration, dissolution and adsorption determine the delivered cellular dose. Comparing two materials by nominal mass concentration alone can therefore create misleading rankings.

An evidence package should connect identity and manufacturing history to relevant exposure metrics: mass, particle number or surface area where appropriate; dissolved fraction; bioaccesible concentration; and time-dependent dose at the biological target. The appropriate metric is substance- and mechanism-specific, not an arbitrary universal preference for nanoparticle counts.

4. Nanomaterials expose a regulatory classification trap

The Commission Recommendation of 10 June 2022 defines nanomaterial for broad policy purposes using, among other criteria, a number-based particle-size distribution threshold of 50% and specified dimensional characteristics. It is a recommendation, not a direct replacement for sector-specific definitions. The European Commission expressly notes that the engineered-nanomaterial definition in Regulation (EU) 2015/2283 differs from the general recommendation, including the absence of that same 50% threshold. [4–6]

This matters to mineral ingredients, pigments, carriers and powders that contain a small-particle fraction without having been marketed as 'nano'. EFSA guidance covers technical requirements for establishing the presence of small particles, including nanoparticles, in regulated food and feed applications. The legal classification of a material must be determined under the applicable instrument, not inferred solely from one instrument’s size threshold. [1,6]

A defensible programme begins with the production process, microscopy and suitable complementary particle-sizing techniques, including uncertainty around sample preparation and dispersion. Dynamic light scattering is informative for some dispersions but can be biased toward larger scatterers and is not a universal substitute for electron microscopy or number-based particle characterisation. The toxicological question then follows the material’s relevant form under intended use.

5. The architecture of a decision-grade NAM dossier

5.1 Define the decision before selecting the technology

The first page of the protocol should state the exact decision: for example, whether a defined ingredient batch under a specified intake scenario produces an epithelial injury signal requiring further investigation. The study should state what a positive, negative or indeterminate result would mean, and which conventional evidence it supplements. Without this, assay selection becomes a technology showcase.

5.2 Anchor exposure and toxicokinetics

A realistic programme estimates intake, gastrointestinal transformation, bio-accessibility and relevant tissue exposure. Physiologically based kinetic models may help link in vitro concentrations to plausible human exposure, but their parameters and domain of applicability must be justified. Extreme experimental doses can be valuable for hazard discovery while remaining unsuitable as direct estimates of risk under ordinary dietary conditions.

5.3 Use orthogonal evidence

Orthogonal endpoints reduce dependence on a single mechanistic interpretation. Depending on the question, they might include barrier integrity, cellular stress responses, genotoxicity indicators, inflammatory mediators and metabolite profiling. Apparent agreement is not enough if assays share a common artefact, such as nanoparticle interference with optical readouts or adsorption of assay reagents.

5.4 Predefine reproducibility and failure

Independent laboratories, blinded samples, reference substances, positive and negative controls, acceptance criteria, analytical characterisation of the test material and transparent data-processing pipelines are essential. Failure conditions should include unstable material identity, unacceptable interlaboratory variability, unexplained dose-response reversals and results dominated by cytotoxicity or measurement interference.

6. Artificial intelligence is a supporting tool, not an evidentiary authority

Machine-learning models can prioritise chemicals, detect high-dimensional response patterns and help select informative experiments. But performance within a curated dataset is not evidence of validity for a new ingredient family. Data leakage, class imbalance, batch confounding, incomplete chemical coverage and uncertain labels can inflate apparent accuracy. External validation should reflect the intended regulatory use, including negative and borderline cases.

For auditability, developers should preserve dataset provenance, training/test separation, model version, applicability domain, calibration, uncertainty estimates and the decision rule linking predictions to additional experiments. A proprietary model may be commercially valuable, but opacity cannot replace the information necessary for competent authorities to evaluate the conclusion.

7. Regulatory comparison: the same science, different legal routes

7.1 European Union and Spain

The EU regulates novel foods under Regulation (EU) 2015/2283, food additives under Regulation (EC) No 1333/2008, and feed additives under Regulation (EC) No 1831/2003, alongside general food law under Regulation (EC) No 178/2002. The legal route depends on the product and intended use. EFSA supplies scientific opinions, while the Commission and Member States exercise the relevant risk-management functions. A proposed NAM qualification system does not override dossier requirements in the applicable legislation or guidance.

Spain applies directly applicable EU legislation, with national and autonomous-community authorities exercising their respective control functions. AESAN's role should not be confused with unilateral authority to approve a novel food for the entire EU. Operators must document the actual material identity, use category, legal status and evidence accepted by the relevant procedure.

7.2 Great Britain and Northern Ireland

Great Britain operates domestic regulated-product arrangements, with the Food Standards Agency and Food Standards Scotland performing relevant roles. Applicants must verify the current legislation and guidance for the specific category; an EFSA proposal does not automatically qualify a

method under British processes. Northern Ireland must be evaluated separately under its applicable legal arrangements. Regulatory divergence should be assessed at the date of filing, not inferred from historical alignment.

7.3 United States

The FDA's food-safety framework assesses food additives and other substances according to the legally applicable pathway, including GRAS where justified. Its NAM initiative concerns how evidence can become useful for safety determinations, not a general exemption from establishing safety under intended conditions of use. A European scientific opinion or qualification proposal cannot establish US legal status, and vice versa. [2]

8. Practical implications for manufacturers and laboratories

The near-term commercial advantage is not a claim that animal testing has ended. It is the ability to make more discriminating development decisions earlier: eliminate poorly characterised candidates, prioritise experiments that address consequential uncertainties, and avoid repeating studies whose results do not change the decision. This requires a cross-functional interface among formulation, analytical chemistry, toxicology, data science, quality assurance and regulatory affairs.

A manufacturer introducing a new mineral technology or functional ingredient should maintain a material-identity dossier across pilot and commercial scale; assess whether particle-size or transformation issues are relevant; specify the intended exposure; and determine whether a NAM would answer a concrete unresolved question. Contract laboratories should quote the validation scope and limitations, not simply the assay name. Procurement should ask whether a supplier's safety data correspond to the same material and process being purchased.

For INTABIOTECH's domains of mineral technologies, functional ingredients, protein systems and biotechnology, the opportunity is a rigorous evidence-development service or collaborative research programme, not a claim of established superiority for any named product. Special care is needed when processing modifies solubility or particulate characteristics: chemical identity alone may not establish toxicological equivalence.

9. Research & Innovation Opportunity

9.1 The unresolved problem

Numerous NAM platforms, OECD methods, computational approaches and nano-specific studies already exist. The proposed opportunity is not to invent non-animal toxicology. It is to test whether a transparent, decision-oriented qualification workflow improves the consistency and efficiency of assessments for food-relevant materials whose properties change during digestion or processing. A comprehensive patent and disclosure review would be necessary before claiming a proprietary innovation.

9.2 Hypothesis and development pathway

Hypothesis (unvalidated): for a defined class of food-relevant particulate and soluble ingredients, an evidence package combining material transformation, realistic delivered-dose modelling, two orthogonal human-relevant biological endpoints and prespecified uncertainty thresholds will produce more reproducible expert decisions than conventional single-assay screening.

Stage one would select representative materials with well-characterised identities, including negative and positive controls, and map their applicable regulatory questions. Stage two would quantify changes after simulated digestion and across relevant food matrices. Stage three would compare a preregistered NAM panel with existing reference evidence in independent laboratories. Stage four would convene blinded multidisciplinary reviewers to determine whether the additional evidence changes the regulatory-relevant conclusion, rather than merely increasing the volume of data.

9.3 Validation and constraints

Success criteria should include interlaboratory reproducibility, stability of the decision across realistic exposure assumptions, low false-negative rates for relevant hazards and transparent uncertainty reporting. Failure would include strong performance only on training materials, inability to reconcile delivered-dose estimates, or non-reproducible biological effects. Ethical review, data governance and relevant regulatory consultation would be required. A favourable research outcome would support further qualification discussions; it would not itself constitute regulatory acceptance.

10. Conclusions

The 2026 EFSA and FDA developments indicate a change in the architecture of food toxicology: method developers are increasingly being asked to prove not merely that an assay can measure something, but that its result can reliably inform a defined safety decision. That shift is scientifically more demanding than promotional claims about animal-free innovation suggest.

For industry, the strongest strategy is to start from product identity, exposure and the actual regulatory question, then select the minimum combination of methods that can resolve consequential uncertainty. In the longer term, credible qualification may reduce unnecessary animal use and improve mechanistic understanding. In the present, neither scientific promise nor a voluntary framework should be mistaken for an authorisation.

References and primary sources

[1] EFSA (2026). Nanotechnology: NAMs4NANO qualification system, implementation plan and scientific guidance. https://www.efsa.europa.eu/en/topics/topic/nanotechnology

[2] US FDA (2026). New Approach Methodologies (NAMs) for Food. https://www.fda.gov/food/toxicology-research-food/new-approach-methodologies-nams-food

[3] US FDA (2026). New Approach Methodologies: regulatory-science overview and September 2026 nonclinical terminology initiative. https://www.fda.gov/science-research/science-and-research-special-topics/new-approach-methodologies-nams

[4] European Commission (2022). Recommendation 2022/C 229/01 on the definition of nanomaterial. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32022H0614(01)

[5] Joint Research Centre (2023). Guidance on implementation of Recommendation 2022/C 229/01. https://doi.org/10.2760/143118

[6] European Commission. Nanomaterials in food: legal definitions and scientific guidance. https://food.ec.europa.eu/food-safety/novel-food/nanomaterials_en

[7] Regulation (EU) 2015/2283 on novel foods. https://eur-lex.europa.eu/eli/reg/2015/2283/oj

[8] Regulation (EC) No 1333/2008 on food additives. https://eur-lex.europa.eu/eli/reg/2008/1333/oj

[9] Regulation (EC) No 178/2002 laying down general food law principles. https://eur-lex.europa.eu/eli/reg/2002/178/oj

[10] Regulation (EC) No 1831/2003 on additives for use in animal nutrition. https://eur-lex.europa.eu/eli/reg/2003/1831/oj

[11] UK Food Standards Agency. Regulated products: guidance. https://www.food.gov.uk/business-guidance/regulated-products

Evidence classification: regulatory instruments and published agency programmes are established documentary evidence; assay limitations are established scientific principles; implementation recommendations are expert interpretation; the proposed comparative qualification workflow is an unvalidated research hypothesis.

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