The Next Evidence Standard for Food Enzymes

Why EFSA's November 2026 reassessment of animal toxicology and allergen bioinformatics could reshape enzyme development, manufacturing specifications and cross-border market access

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Abstract

The European Food Safety Authority is preparing to revisit two pillars of food-enzyme risk assessment: repeated-dose systemic toxicity studies and the interpretation of amino-acid sequence matches to known allergens. Its stakeholder meeting of 25–26 November 2026 is a timely signal of methodological change, not an enacted relaxation of safety requirements. This article examines the scientific case for reducing redundant animal studies, the limitations of conventional allergen-homology screening, and the more demanding question of whether enzyme preparations can be characterised sufficiently to support reliable, proportionate decisions. It connects production-strain genomics, total organic solids, residual activity, exposure modelling, fermentation impurities, process validation and post-market governance. The comparative analysis distinguishes the European Union and Spain from Great Britain and the United States, where evidence can travel but authorisations do not automatically follow. A research agenda proposes a transparent, uncertainty-calibrated assessment architecture that could reduce unnecessary testing without transferring hidden risks to consumers or manufacturers.

Keywords: *food enzymes; toxicology; allergenicity; bioinformatics; total organic solids; alternative methods; regulatory convergence; fermentation.*

1. The November signal: consultation is not a change in law

EFSA has scheduled a technical stakeholder session for 25–26 November 2026 on two related self-task initiatives: a standardised allergen-homology bioinformatics pipeline and the possibility of reducing repeated-dose 90-day oral toxicity studies for food enzymes. The event materials describe outputs intended for consultation in November. This is a methodological development under discussion, not a new binding rule, a blanket exemption from toxicology, or a decision that sequence similarity is clinically irrelevant [1,2].

The distinction is crucial because food-enzyme safety assessment operates at the intersection of biological source, manufacturing process, final preparation and dietary exposure. The regulatory question is not whether an enzyme class is intrinsically "safe", but whether a defined preparation,

produced by a defined organism and process, is safe at specified uses and exposure levels. Better methods may reduce unnecessary studies while making this definition more demanding.

The 2021 EFSA guidance already incorporated criteria for characterising production microorganisms, improved exposure estimation and circumstances in which toxicological testing may not be required. The 2026 initiative should therefore be read as a possible refinement of an existing risk-based approach, not the invention of a previously absent waiver concept [3].

2. What exactly is being assessed?

A commercial enzyme preparation is not identical to its named catalytic protein. It may contain active enzyme, other proteins, fermentation-derived constituents, residual medium components, stabilisers, carriers and other formulation ingredients. Regulatory identity is consequently relational: source organism, production strain, genetic modifications where relevant, manufacturing process, purification, activity, total organic solids (TOS), impurities and intended technological function must be considered together [3,4].

The enzyme may be removed, inactivated or remain active in the consumed food. These outcomes are not interchangeable. For example, a starch-processing enzyme removed during purification creates a different residual-exposure profile from an enzyme added late in production to a minimally processed food. A heat-treatment claim requires matrix-specific evidence that catalytic activity is sufficiently reduced; inactivation does not automatically mean removal of enzyme protein or allergenic epitopes.

Quality control must also distinguish batch-release identity from dossier identity. A supplier can meet a nominal activity specification yet shift the TOS-to-activity ratio through changes in fermentation yield or downstream purification. That shift matters because exposure is frequently expressed in milligrams TOS per kilogram body weight per day, not merely enzyme units per kilogram of food.

3. The case for rethinking 90-day toxicity studies

Repeated-dose rodent studies have historically supplied a no-observed-adverse-effect level for systemic toxicological assessment. The conventional margin of exposure compares a toxicological point of departure with modelled dietary exposure. A high margin can be reassuring within a defined dossier, but it cannot correct a nonrepresentative test article, poorly characterised impurities or an exposure estimate that ignores major uses [3,5].

The scientific case for reducing animal testing is strongest where evidence from production-strain history, genome analysis, manufacturing controls, compositional characterisation, genotoxicity assessment and dietary exposure collectively makes a repeated-dose study unlikely to change the decision. This is a conditional argument about information value, not a claim that enzymes are harmless because they are proteins. Systemic exposure, immunological effects and process-related contaminants still require attention.

An improved waiver framework should therefore be explicit about exclusion criteria. Unusual production organisms, unresolved toxigenic potential, novel fermentation substrates, material changes to impurity profiles, poorly characterised genetic constructs or unexpected exposure may justify additional testing. The aim is proportionality: a study should be commissioned because it resolves a consequential uncertainty, not simply because it has become a ritual in a dossier.

Conversely, eliminating a study without strengthening analytical and manufacturing evidence could merely relocate uncertainty. A scientifically credible reform should publish decision trees, evidence thresholds, counterexamples and transparent treatment of uncertainty. Ideally, it should test its decisions retrospectively against cases where animal studies revealed effects that would otherwise have been missed.

4. Allergen homology: from screening thresholds to biological interpretation

Bioinformatics compares the amino-acid sequence of a food enzyme with proteins recognised as allergens. Matches can flag potential cross-reactivity, but sequence similarity is not a clinical diagnosis. Risk depends on structural conservation, epitope accessibility, protein abundance, processing, route and magnitude of exposure, and the immune status of the exposed population. False reassurance and excessive alarm are both possible when a sequence hit is treated as a binary verdict [1,3].

A harmonised pipeline could improve reproducibility by documenting database versions, search parameters, alignment windows, identity thresholds, treatment of low-complexity regions and reporting of full-length versus local similarities. Its real value would lie in calibrated interpretation and auditability, not simply in increasing the number of flagged matches.

Allergenicity also has at least two separable questions: whether an enzyme could sensitise a previously non-allergic individual and whether it could elicit a reaction in someone already sensitised to a related protein. Data supporting one question do not automatically answer the other. Occupational respiratory sensitisation among enzyme workers is an additional exposure context that must not be confused with dietary risk, although it remains relevant to industrial hygiene.

For formulation and quality teams, the practical requirement is to connect the bioinformatics result to the actual marketed preparation. Processing can denature proteins without necessarily destroying every immunologically relevant determinant. A dossier that reports only the source organism or an uncontextualized sequence hit is scientifically incomplete.

5. Exposure modelling and the total-organic-solids problem

TOS is an operational measure of organic matter in an enzyme preparation, often used as a conservative proxy for material potentially carried into food. It is not a direct measure of active enzyme, intact enzyme protein, a particular impurity or systemic bioavailability. Accordingly, a TOS-based margin of exposure is useful only when the analytical basis and assumptions about removal or carry-over are explicit [4,5].

For an ingredient supplier, the most valuable dataset may be a process-specific mass balance: enzyme preparation added, activity after each step, protein and TOS carried into the final product, and the influence of pH, temperature, filtration and matrix composition. This can turn an overly conservative default estimate into a justified exposure estimate, provided that analytical detection limits and sampling uncertainty are reported.

Exposure must be aggregated where the same enzyme preparation or closely related material is used across multiple food categories. High-consumer subgroups and children can drive the upper tail. A supplier who expands from one application into several categories should revisit the assessment even if the enzyme itself has not changed. This is especially relevant when the

commercial proposition shifts from industrial processing aids to consumer-facing functional formulations.

6. Production-strain genomics and manufacturing control

Microbial production is attractive because fermentation enables scale, consistency and targeted enzyme design. Yet genomic characterisation is only one component of a manufacturing safety case. Sequence-based searches for antimicrobial-resistance determinants, virulence factors and toxigenic pathways depend on reference databases, assembly quality, thresholds and expert interpretation. Negative findings do not prove that all possible hazards have been excluded [3].

For genetically modified production organisms, developers must establish the relevant genetic changes and address whether viable production organisms and production-organism DNA remain in the final preparation, as required by the applicable assessment framework. Contamination control, fermentation media, antifoams, purification aids and cleaning agents can create process-specific hazards even where the intended enzyme is well characterised.

Change control is consequently a regulatory instrument as much as a quality-management practice. A new supplier of nitrogen source, a different fermentation vessel, altered harvest timing or a revised filtration membrane may affect impurities or the TOS-to-activity ratio. The correct response is a documented comparability assessment proportionate to the change, not automatic repetition of every study nor automatic reliance on the old dossier.

7. Regulatory geography: evidence travels more easily than permissions

European Union and Spain. Regulation (EC) No 1332/2008 governs food enzymes, alongside the common authorisation procedure in Regulation (EC) No 1331/2008. The EU system includes transitional provisions associated with establishing the Union list. Operators must check the applicable transitional regime, specific uses and other relevant food law rather than assume that every EFSA-reviewed enzyme is already listed and unrestricted. EFSA evaluates scientific risk; Commission-level legal measures and national enforcement are separate functions. Spain operates within the directly applicable EU framework, with competent national and regional authorities involved in official controls [6].

Great Britain. The post-Brexit regime requires an independent check of assimilated law, current domestic measures and relevant FSA/FSS guidance. An EFSA opinion can inform a scientific dossier but does not itself create British authorisation. Northern Ireland must be evaluated separately under the arrangements applicable there. A single UK-wide status should not be inferred from a supplier certificate or an EU assessment [7].

United States. Enzyme preparations may be regulated through food-additive provisions or a substantiated generally recognised as safe (GRAS) conclusion for defined conditions of use. FDA guidance emphasises source, manufacturing, enzyme preparation composition, intended uses, reaction products and dietary exposure, including the fate of TOS. An FDA inventory is explicitly partial and cannot be treated as a comprehensive list of all legally acceptable enzyme uses [4,8].

Other markets. Local food-enzyme rules, processing-aid classifications and labelling obligations differ. A robust international dossier should maintain a shared scientific core and jurisdiction-specific modules with dated legal-status checks. Scientific comparability does not remove the need to establish each market's legal route.

8. Industrial consequences: reform can increase the value of good data

If EFSA eventually reduces reliance on 90-day studies for appropriate enzyme dossiers, smaller developers may benefit from lower costs and shorter development cycles. But the gains will not be automatic. Stronger demands for strain identity, exposure modelling, process comparability and bioinformatics reproducibility could shift expenditure from animal studies toward analytical science, data governance and quality engineering.

This creates an opportunity for enzyme suppliers that can demonstrate robust process characterisation. Procurement teams should ask for activity units with defined assay conditions, TOS, source organism, genetic-modification status where relevant, batch comparability, intended uses, allergen assessment, contaminant limits and evidence of residual activity in relevant applications. An attractive price per enzyme unit can be economically misleading when the application requires additional purification, thermal treatment or verification.

Industrial customers should also distinguish an EFSA safety conclusion from the authorisation and labelling position for their exact application. An enzyme used to improve dough handling, extract plant proteins or clarify beverages may affect the final product differently. Safety, technological efficacy and permission to market are three separate questions.

9. Research & Innovation Opportunity

The underexplored opportunity is not to invent a new enzyme-safety framework from nothing: EFSA, FDA and researchers have long worked on tiered risk assessment, bioinformatics and alternative methods. The more specific research gap is whether an integrated, independently benchmarked decision-support system can combine sequence-based allergen screening, production-strain evidence, process impurity profiles, TOS mass balances and use-specific exposure into a transparent estimate of residual uncertainty.

Hypothesis (proposed, not established): an uncertainty-calibrated evidence architecture would identify more dossiers in which a 90-day study is unlikely to alter the regulatory decision, while flagging more reliably the subset requiring targeted additional evidence. This is plausible because current evidence streams are often reviewed separately, yet the decision depends on their interaction. It remains unproven until evaluated against real dossiers and independent outcomes.

Validation should begin with a systematic literature and patent/public-disclosure landscape review; the present article does not claim novelty or patentability. A multicentre retrospective dataset should include enzyme dossiers with and without toxicological findings, varied production organisms and different food uses. The project should preregister decision rules, test sensitivity to database versions and exposure assumptions, and report false-negative as well as false-positive classifications.

Prospective work would compare conventional dossier assessment with the integrated approach, using blinded independent experts. The system should produce an auditable rationale, not an opaque safety score. Explicit failure criteria include missed biologically consequential hazards, unstable results when databases update, unreliable residual-exposure estimates and recommendations that depend on unverified process claims.

Regulatory constraints are substantial: no algorithm can itself waive a legally applicable data requirement, and any dossier must follow current authority guidance. Industrial relevance would lie in earlier identification of decisive experiments, improved supplier qualification and more efficient

design of internationally reusable evidence packages. The concept is a research programme, not a validated INTABIOTECH product.

10. A practical implementation roadmap

In the near term, enzyme developers should catalogue existing dossiers by strain, manufacturing process, intended application and exposure model. A second step is to identify where evidence is transferable and where a material process or use change breaks that transfer. This prevents the common mistake of treating all preparations sharing an enzyme activity name as interchangeable.

Next, organisations should build a controlled evidence register linking raw analytical data, batch genealogy, bioinformatics software versions, allergen databases, process parameters and regulatory submissions. Scientific teams should define what constitutes a consequential deviation before scale-up, while legal teams maintain dated country-specific authorisation assessments.

Finally, governance should require that commercial claims be reviewed against actual evidence. “Food-grade”, “enzyme-free after processing”, “non-allergenic”, “approved in Europe” and “sustainable” are different statements with different proof requirements. The best commercial advantage of regulatory reform may be improved trust in claims that are narrow, precise and reproducible.

11. Conclusions

The November 2026 EFSA initiative may mark a significant step toward more proportionate food-enzyme safety assessment. Its significance lies not in a promise to dispense with animal studies or allergen evaluation, but in the prospect of assigning evidence requirements according to the questions that matter for a defined enzyme preparation and use.

The decisive scientific challenge is integration: production-strain identity, manufacturing residues, TOS exposure, sequence similarity, biological interpretation and change control must form a coherent case. Regulators should publish transparent criteria and validate them against adverse as well as reassuring examples. Industry should invest in traceable analytical evidence rather than assume that methodological reform will automatically ease compliance.

For the food and biotechnology sectors, the strongest innovation opportunity is a more informative, internationally portable scientific dossier, without confusing portability of evidence with portability of legal authorisation.

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Evidence classification: EFSA meeting and guidance are verified institutional sources; legal descriptions concern the cited instruments; industrial recommendations are expert interpretation; the proposed integrated benchmark is a hypothesis and research agenda, not established performance.

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