When the Enzyme Is Not the Toxicological Hazard

Reconsidering 90-Day Animal Studies, Manufacturing Impurities and Allergen Bioinformatics in Europe's 2026 Food-Enzyme Debate

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Abstract

Europe is reconsidering how the safety of food enzymes should be demonstrated. In May 2026, EFSA presented preliminary findings from a retrospective review of 258 repeated-dose, 90-day rodent studies; most did not identify safety concerns, while the limited findings reported were associated with local effects or manufacturing-related impurities. A further EFSA stakeholder session scheduled for 25–26 November will examine both systemic toxicity testing and bioinformatic allergen-homology assessment. This article argues that the emerging question is not whether enzymes are intrinsically safe, but how evidence can be allocated to the correct hazard: catalytic protein, production organism, fermentation residues, contaminants, unintended activities, and consumer exposure. It distinguishes an evidence-led reduction of routine animal testing from an unsupported exemption, compares EU, UK and US regulatory routes, and proposes a matrix-sensitive, impurity-centred qualification framework for enzyme producers and industrial users. The proposed framework is a research agenda, not a recognised substitute for applicable authorisation requirements.

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1. The current signal: a regulatory method under examination

On 18 May 2026, the European Food Safety Authority (EFSA) convened a session on the retrospective review of 90-day toxicity studies used in food-enzyme assessment. EFSA reported preliminary results covering 258 rodent studies. Most did not reveal a safety concern; among the relatively few studies showing effects, EFSA described either local gastric effects not considered relevant for humans or findings associated with impurities and manufacturing contamination rather than the enzyme protein itself. These are preliminary observations from a retrospective programme, not a binding decision to remove toxicology requirements. [1]

EFSA has announced a further information session for 25–26 November 2026 in Amsterdam, addressing two related workstreams: possible reduction of repeated-dose animal studies and a more harmonised bioinformatics pipeline for allergen sequence comparisons. The agency says the workstreams are intended to inform public consultation. As of 9 November, their final outcomes cannot be presumed. The important novelty is a prospective change in evidentiary strategy, not a discovery that enzymes are safe by definition. [2]

The timing matters because food enzymes sit at the intersection of industrial biotechnology, food processing and regulation. They may be added at low levels, perform a specific catalytic task, and subsequently be removed or inactivated; yet production-derived constituents can accompany the enzyme preparation, and some residual activity may persist. A safety dossier must therefore identify what is manufactured, what is used, what remains, and what consumers may encounter. The test substance in a 90-day study is usually an enzyme preparation rather than an abstract, chemically pure enzyme. This distinction makes the retrospective impurity signal scientifically consequential.

2. Why the hazard cannot be reduced to the protein name

An enzyme is a catalyst, typically a protein, defined by activity on one or more substrates. Commercial preparations are more complex. Depending on the production process they may contain other proteins, residual biomass components, fermentation-medium constituents, salts, stabilisers, processing aids and trace contaminants. The specification of a preparation must therefore be distinguished from the sequence and identity of its principal catalytic protein. The total organic solids (TOS) convention is useful for exposure calculations in European dossiers, but TOS is not a chemical identity: it groups organic material whose toxicological relevance may differ markedly. [3]

An adverse finding in a study on the whole preparation cannot automatically be attributed to the enzyme molecule. Conversely, a negative study cannot prove that every future preparation from another strain, substrate, plant or purification route is equivalent. The critical question is causal attribution. Was the observed response linked to enzyme activity, another protein, a metabolite, an impurity, or an experimental artefact? A scientifically efficient programme should be designed to discriminate these possibilities before demanding additional animal studies.

This reasoning does not support an unconditional class exemption. Some enzymes or co-purifying proteins may have allergenic relevance; microbial production strains may raise concerns over toxins, antimicrobial-resistance determinants or viable cells; and manufacturing changes may create unexpected impurities. Even if systemic toxicity is uncommon in a

retrospective dataset, the risk assessment must address foreseeable exceptions. The objective should be targeted sensitivity to meaningful hazards, rather than a ritualised choice between “animal test” and “no animal test”.

3. The manufacturing route is part of the safety identity

For a fermentation-derived food enzyme, the production organism is not merely an origin statement. Taxonomic identity, strain-level characterisation, genetic modification where relevant, antimicrobial-resistance information, toxigenic potential and process controls are central to understanding the preparation. EFSA’s qualified presumption of safety (QPS) framework can assist assessment of eligible microorganisms, but QPS is a pre-assessment with qualifications and does not automatically authorise a specific enzyme or preparation. [4]

Consider two hypothetical batches of the same nominal enzyme, one manufactured with a validated downstream purification step and the other produced after a process change that increases residual host-cell proteins. They may have equivalent declared catalytic activity yet different impurity distributions. If the specification records only activity, moisture and microbiological counts, a consequential chemical difference may go unnoticed. The relevant comparison is not simply activity per gram, but activity together with a validated compositional fingerprint, impurity limits and evidence that the change does not undermine the safety basis.

A practical manufacturing hazard map should begin with raw materials and production strain, then follow fermentation, cell separation, concentration, purification, formulation, storage and final use. At each stage the operator should ask what might persist or be generated, what analytical method could detect it, and whether exposure would be plausible. Such a map may prioritise microbial toxins, unwanted enzyme side activities, residual nucleic acids, fermentation metabolites, allergenic proteins or contaminants introduced by process aids. The priority depends on the organism and process; no universal checklist can replace the actual manufacturing history.

4. What 258 studies can, and cannot tell us

Retrospective evidence is powerful when it tests a precise proposition. A collection of 258 studies with few findings relevant to systemic toxicity may suggest that routine testing has low incremental yield for certain well-characterised enzyme preparations. It does not by itself determine the false-negative rate of a proposed replacement strategy. That requires knowledge of study selection, enzyme classes, organisms, manufacturing routes, exposure levels, assay sensitivity and the distribution of rare but important hazards. [1]

There is also a counterfactual problem. If the preparations reaching regulatory assessment had already undergone extensive selection and quality control, low toxicity rates may reflect effective upstream screening. Removing the animal study without retaining those upstream controls would not reproduce the conditions that generated the reassuring dataset. Equally, repeated negative studies on closely related preparations may provide limited new information. The rational response is a risk-stratified design that preserves hazard discovery while reducing low-value repetition.

Any reform should publish transparent eligibility criteria for waiving a study, define triggers for additional testing, and validate alternative evidence against a suitable reference set. Retrospective findings should be stratified by microbial source, enzyme class, production process and impurity profile. Cases in which an impurity caused a signal deserve particular attention: they could reveal which analytical controls would have detected the hazard earlier and whether the same control would work in a different manufacturing context.

5. Allergenicity: a sequence match is a screening result

Enzyme allergenicity assessment requires a separate evidentiary logic from repeated-dose systemic toxicity. Protein sequence comparison with known allergens can identify candidates for closer investigation, but a sequence match is not proof of clinical allergy, while the absence of a database match does not guarantee the absence of sensitisation potential. Sequence databases are incomplete and evolve; alignment methods and thresholds can affect the number and interpretation of hits. EFSA's announced bioinformatics workstream aims to standardise how such homology results are identified and reported. [2]

An effective evaluation should document the exact protein sequence, database version, algorithm, thresholds and treatment of fragments or modified sequences. It should then interpret matches in light of the source organism, relevant allergen families, exposure route, protein abundance, process degradation and available clinical or experimental evidence. Digestibility may inform exposure but should not be used as a binary surrogate for allergenicity. Occupational respiratory sensitisation to industrial enzymes and oral allergy following food consumption also involve different exposure pathways and cannot be conflated.

The industrial opportunity is not to replace expert judgement with an automated red/green score. It is to create reproducible, version-controlled evidence that can be re-evaluated when allergen databases or manufacturing processes change. An AI tool might flag emerging sequence relationships, but its output remains a prioritisation signal until interpreted against relevant biological evidence.

6. A concrete industrial case: enzyme-assisted protein hydrolysis

Consider a hypothetical manufacturer using a microbial protease blend to produce a highly soluble plant-protein hydrolysate for an acid beverage. The enzyme preparation may be added during hydrolysis, then thermally inactivated, followed by filtration and concentration. Three different claims require separate evidence: that the enzyme has achieved the intended hydrolysis, that its residual activity has been controlled, and that the final ingredient is safe under its intended use. A change in peptide size distribution cannot alone establish improved human absorption, and enzyme inactivation does not prove the absence of protein residues or manufacturing impurities.

The analytical plan would measure activity before and after inactivation using a matrix-validated assay, molecular-size distribution and degree of hydrolysis, residual protein or enzyme markers where feasible, microbial criteria, and priority impurities associated with the production strain. Process controls should include a no-enzyme matrix, a heat-treated enzyme control when informative, and independently produced lots. If the finished ingredient is to be used in a

beverage, the final pH, heat treatment, storage, consumption and potential exposure to residual preparation constituents must be considered. A supplier certificate can support but cannot replace validation in the user's process.

The regulatory question is not resolved by the phrase "processing aid". In the EU, food enzymes fall within Regulation (EC) No 1332/2008 and the common authorisation procedure under Regulation (EC) No 1331/2008, subject to the actual status and transitional provisions of the applicable framework. The classification and labelling of residual enzyme constituents require analysis of function, carry-over and the relevant provisions of Regulation (EU) No 1169/2011. A food-enzyme safety opinion should not be represented as permission for every possible downstream use. [5–7]

7. A second case: a multienzyme preparation for encapsulated supplements

Now consider a hypothetical digestive-enzyme blend containing protease, lipase and amylase intended for incorporation into a dried encapsulation matrix. The technological challenge is distinct from protein hydrolysis in manufacturing: enzymatic activity is intentionally preserved until consumption. A dossier built around the assumption that processing removes or inactivates the enzyme would therefore be inappropriate. The product may also contain wall materials, salts and excipients that affect moisture, stability, release and exposure.

The programme should quantify each enzyme activity separately, investigate activity loss during drying and storage, characterise unintended cross-activities, and evaluate exposure at the maximum recommended serving. Batch release specifications should distinguish total mass from enzyme activity units and identify the analytical conditions under which those units were determined. Where allergenicity or sensitisation concerns exist, sequence-based screening should be linked to the actual proteins present, not merely to a trade name for the blend.

This example also illustrates a classification boundary. A food enzyme used as a technological aid during manufacture is not automatically regulated in the same way as an active ingredient deliberately consumed in a supplement. Depending on its intended use, the finished product may require assessment under general food law, national supplement rules, novel-food provisions where relevant, and claims legislation. Any proposed physiological claim must be assessed independently of enzyme manufacturing safety.

8. Jurisdictional comparison: what changes across markets

EU and Spain. Regulation (EC) No 1332/2008 governs food enzymes, Regulation (EC) No 1331/2008 establishes the common authorisation procedure, and Regulation (EC) No 178/2002 provides the general safety framework. EFSA supplies scientific risk assessment, while the Commission and Member States exercise risk-management functions. The applicable Union-list and transitional situation must be checked for each enzyme and use; an EFSA scientific opinion is not itself a universal marketing authorisation. Spain applies directly applicable EU rules and conducts controls through competent national and regional authorities. [5–8]

Great Britain and Northern Ireland. The British regulatory position must be checked under domestic law as amended, including applicable food-enzyme and food-additive provisions, rather than assuming that every subsequent EFSA guidance change is binding. Northern Ireland must be considered separately under its EU-linked regulatory arrangements. Companies supplying both territories should maintain an authorisation and claims matrix with legal source, current status and destination-market limitations. [9]

United States. The FDA framework is organised around the intended conditions of use of substances added to food, food-additive approvals and, where appropriate, generally recognised as safe (GRAS) conclusions. Enzyme preparations may have specific regulatory histories and conditions; a European scientific assessment is not a US authorisation, and a US GRAS determination is not an EU authorisation. Manufacturing specifications, impurity profiles, exposure and intended function remain decisive. The analysis must also distinguish a food-processing use from direct inclusion in a dietary supplement. [10]

The comparative lesson is that toxicological data may be scientifically portable but legal conclusions are not. A manufacturer can maintain a common analytical core—identity, manufacturing, impurity control, exposure, allergenicity—while building jurisdiction-specific appendices for status, permitted uses, labelling and claims. That is more defensible than a single “internationally compliant” certificate.

9. Research & Innovation Opportunity: an impurity-centred evidence architecture

Unresolved problem. Food-enzyme qualification frequently separates protein identity, production-strain safety, chemical impurity testing, allergen sequence analysis and end-use exposure into different documents. EFSA’s preliminary review raises a testable question: could a unified evidence architecture detect relevant hazards at least as reliably as routine 90-day testing for carefully defined classes of preparations, while reducing unnecessary animal use? This is a hypothesis, not a demonstrated result.

Proposed pathway. Build a prospective multicentre dataset covering enzyme classes, production organisms, manufacturing routes, specifications and historical toxicity findings. Predefine high-priority impurity families based on organism and process. Apply validated targeted assays, selected non-target high-resolution mass spectrometry, bioinformatic allergen screening and realistic exposure assessment. Assign every finding a confidence level and maintain raw-data provenance. Where the data indicate a meaningful unresolved hazard, targeted toxicological testing, including animal testing when scientifically and legally necessary, remains available.

Validation. The proposed approach must be benchmarked on blinded historical and prospective cases. Primary endpoints should include detection of consequential hazards, false-negative rate, false alarms, reproducibility across laboratories and the number of unresolved safety questions at dossier submission. Secondary endpoints may include time, analytical cost, animal use and frequency of manufacturing-change requalification. An apparent reduction in testing cost is not a safety success if detection of rare hazards deteriorates.

Legal constraints. Neither a proposed alternative method nor EFSA's consultation activity overrides binding evidence requirements. Applicants should follow current guidance and any specific requests from the evaluating authority. Proprietary strain data and supplier know-how may require controlled disclosure, but confidentiality cannot justify omission of information necessary for safety assessment.

Industrial relevance. An impurity-centred approach would be especially useful for fermentation-derived enzymes, protease blends, functional-protein manufacturing, enzymatic extraction and emerging bioprocesses. For INTABIOTECH, the realistic opportunity is to strengthen qualification of enzyme-containing ingredients and development services through better source-to-finished-product traceability, not to assert a proprietary exemption from testing.

10. Critical conclusions

The preliminary EFSA retrospective review is a timely challenge to regulatory habit, not a licence to presume safety. Its most important signal is causal: when toxicological effects appear, the preparation's manufacturing history and impurities may matter more than the principal catalytic protein. That observation deserves systematic investigation and careful limits of inference.

A robust reform would move from routine repetition toward an explicit decision architecture: identify the enzyme and organism, characterise the preparation and impurities, establish actual exposure, examine allergenicity separately, and require targeted additional evidence when unresolved risks justify it. Such an approach could reduce low-value animal studies without weakening protection, but only if the replacement system is independently validated and retains sensitivity to exceptional hazards.

The wider industrial lesson is that the safety identity of a biotechnological ingredient is inseparable from its production process. The most defensible dossier is not the one containing the greatest number of tests; it is the one in which every consequential hazard is addressed by evidence capable of answering the correct question.

Related Knowledge Areas

Biotechnology; Food Safety; Functional Ingredients; Nutrition; Regulatory Affairs; Processing Technologies; Emerging Research; AI & Food.

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