The Toxicology of a Manufacturing Process

What EFSA's 2026 retrospective review of food-enzyme studies reveals about impurities, exposure, biological relevance and the next generation of safety dossiers

José M. López, BSc, MD, PhD, LLD (C)

Abstract

A 2026 European Food Safety Authority retrospective examination of 258 ninety-day rodent studies submitted for food-enzyme assessments offers a consequential but easily misread signal. EFSA reported that most studies revealed no safety concern and that the comparatively few observed effects were associated with local gastric phenomena of limited human relevance or manufacturing-related impurities rather than the enzyme protein itself. This article asks what such a finding can legitimately change in industrial toxicology, and what it cannot. The discussion connects microbial strain identification, fermentation control, total organic solids, dietary exposure, process-specific removal, allergenicity, non-animal methods and the legal architecture of food-enzyme authorisation. Two recent EFSA opinions provide contrasting quantitative examples. A proposed research programme would link process fingerprints and impurity profiles to exposure-informed toxicological decisions, without treating an exploratory retrospective pattern as a waiver of statutory evidence. The industrial opportunity is not to dispense with safety testing, but to build better, more discriminating evidence about where hazards originate and how they reach consumers.

Keywords: *food enzymes; toxicology; fermentation impurities; total organic solids; alternative methods; exposure assessment; regulatory science; process control; allergenicity*.

1. A retrospective result with prospective consequences

In May 2026, EFSA convened a public information session around a self-tasked retrospective review of ninety-day rodent studies in food-enzyme regulatory submissions. Its preliminary account covered 258 studies and reported that most had disclosed no safety concern. Where effects appeared, EFSA described a small subset attributable either to local gastric effects in rodents not considered relevant to humans or to impurities and manufacturing contamination rather than to the enzyme itself [1]. This is not a final general exemption, a finding that all enzyme preparations are harmless, or a conclusion that the identity of the production microorganism is immaterial. It is a signal about the distribution of observed toxicological findings in a particular historical regulatory dataset.

The result matters because food-enzyme assessment occupies an unusual scientific position. The biologically active substance is often a protein produced by fermentation; the marketed preparation can also contain residual fermentation medium, metabolites, host-cell proteins, processing aids, salts and other organic matter. Industrial practice then subjects that preparation to processing

conditions that may remove, denature or transform its constituents. The consumer is exposed not to an abstract enzyme name but to whatever fraction of that particular preparation survives in a particular food at the intended use level. Hazard identification without manufacturing and exposure context can therefore be analytically incomplete.

The question for regulators is not whether an animal study is intrinsically valuable. It is whether its marginal information gain can be predicted and whether alternative evidence can detect the same relevant risks more directly. For industry, the question is how to improve dossiers without interpreting an early retrospective observation as permission to lower standards. Both questions demand careful separation of evidence from inference.

2. What a ninety-day study actually tests

The test article is not a pure biochemical concept

A repeated-dose oral study typically evaluates the toxicological behaviour of the administered test material over a defined exposure period. In fermentation-derived preparations, the test material may be characterised through total organic solids (TOS), enzyme activity, batch identity and composition. TOS is an exposure and dose convention, not a direct measure of active enzyme molecules or a universal measure of toxicological potency. Two preparations with the same TOS can differ in enzyme activity, residual proteins, secondary metabolites and other constituents.

This distinction becomes crucial when a study identifies an effect. A high-dose response might reflect a constituent of the fermentation broth, a local physical-chemical effect of the concentrated test preparation, an impurity carried through downstream processing, or an intrinsic property of the enzyme. Without compositional data and mechanistic follow-up, the observation cannot automatically be assigned to one of these causes. Conversely, absence of a systemic effect does not establish absence of allergenic potential or guarantee safety under every intended use.

The margin of exposure is conditional arithmetic

The margin of exposure (MoE) commonly compares a toxicological reference point, such as a no-observed-adverse-effect level (NOAEL), with estimated human dietary exposure. The quotient is useful only insofar as the reference point, the material tested, the exposure estimate and the assumed use pattern are appropriate. An MoE of 1,000 is not a biological constant; it is a ratio conditioned on assumptions. A change in proposed food processes, use levels, consumption patterns or residual TOS can change the denominator without any alteration in enzyme identity.

The 2026 EFSA assessment of a preparation containing oryzin and leucyl aminopeptidase from Aspergillus sp. FL 72-230 is illustrative. EFSA estimated exposure up to 0.532 mg TOS/kg body weight/day and identified a NOAEL of 585 mg TOS/kg body weight/day for the native preparation, producing an MoE of at least 1,100. A heat-treated preparation containing only leucyl aminopeptidase was assessed separately; the panel reported an MoE of at least 4,398 on its stated test and exposure assumptions [2]. These are assessments of defined materials and uses, not a generic safety certificate for every fungal protease.

A second 2026 opinion considered an extension of use for triacylglycerol lipase produced by a genetically modified Aspergillus oryzae strain. The panel estimated dietary exposure up to 0.494 mg TOS/kg body weight/day and used a previously established NOAEL of 340 mg TOS/kg body weight/day, resulting in an MoE of at least 688 [3]. The assessment illustrates why authorisation and

extension of use are exposure-sensitive questions. A substance already assessed for two manufacturing processes may require fresh analysis when six additional processes are proposed.

3. Why the manufacturing process can become the hazard source

Fermentation creates a complex chemical environment

A microbial production process has a biological starting point, but its output is a chemical mixture shaped by strain genetics, substrate composition, oxygen transfer, pH, feed strategy, process duration and downstream purification. Carbon and nitrogen sources may introduce impurities; metabolic responses can vary with stress; harvesting and cell removal can alter residual biomass; ultrafiltration may concentrate desired macromolecules while allowing smaller species to pass. No single specification such as enzyme activity or total protein describes this entire risk space.

Process changes are consequently not merely engineering optimisations. Substituting a nutrient supplier, changing the antifoam, increasing fermentation titre or modifying membrane cut-off can alter the impurity spectrum. A robust control strategy should link such changes to targeted analytical questions rather than relying exclusively on an unchanged nominal activity specification. For example, a higher product yield may coincide with increased host-cell protein burden or different low-molecular-weight residuals. That possibility must be measured, not assumed.

Identity is a scientific control variable

The June 2026 EFSA opinion on strain FL 72-230 reports that the production microorganism could not be unambiguously assigned to a particular Aspergillus species and therefore identifies it as Aspergillus sp. [2]. This is a notable example of taxonomic caution in a commercial assessment. A deposited name is not necessarily the last word in species identity; the reliability of genomic characterisation and its relationship to toxigenic potential matter. Strain-level evidence, sequence analysis, production history and contaminant testing must be considered together.

The important distinction is between a production organism, a viable organism remaining in the final preparation and any substances the organism may have produced. Demonstrating absence of viable production cells is a meaningful control, but it does not by itself establish absence of unwanted metabolites. Likewise, an enzyme derived from a genetically modified strain requires appropriate characterisation of the modification and final material; the regulatory question cannot be reduced to whether the word “GMO” appears on a supplier sheet.

Downstream purification can change the exposure pathway

A process may remove enzyme-TOS from the final food, substantially changing dietary exposure. EFSA’s lipase extension-of-use assessment excluded two manufacturing processes from its dietary TOS calculation because residual enzyme-TOS was removed in those processes [3]. The scientific claim here is process-specific: the extent and reproducibility of removal depend on the manufacturing operation. A removal assumption needs a documented basis, not a generic statement that processing destroys enzymes.

Thermal denaturation and physical removal should not be conflated. Heat can reduce catalytic activity while leaving protein fragments or other organic constituents present. Filtration, adsorption and separation may remove material without necessarily inactivating it. These mechanisms have different analytical signatures and implications for exposure. The dossier should specify which is claimed, under what conditions, and by what validated measurements it is supported.

4. Allergenicity remains a separate line of evidence

One of the most consequential limitations of a reassuring ninety-day systemic toxicity study is that it does not settle allergenicity. Food enzymes are proteins, and their sequences may show similarities to known allergens. In the FL 72-230 assessment, EFSA identified sequence matches involving food, contact and respiratory allergens and concluded that the possibility of allergic reactions upon dietary exposure could not be excluded, while finding no safety concern under the intended conditions of use [2]. These statements are compatible, not contradictory: the overall safety conclusion and residual uncertainty about allergic reactions address different endpoints.

Sequence homology screening is a hazard-identification tool rather than a clinical prediction of reaction frequency. It needs interpretation alongside protein stability, exposure, relevant clinical literature and the identity of matched allergens. Respiratory occupational sensitisation also differs from ingestion risk, though it remains pertinent to plant-worker protection. An industrial safety dossier that treats allergenicity as an appendix to systemic toxicity is scientifically unbalanced.

For formulators, this matters in bakery, dairy alternatives, beverages, egg products and nutritional products, where enzymes may be used to modify texture, protein hydrolysis or processing behaviour. The appropriate communication should distinguish occupational controls, residual enzyme exposure and any consumer allergen obligations under the applicable food-information framework. No favourable MoE should be represented as proof that hypersensitivity cannot occur.

5. The methodological problem behind retrospective reassurance

Selection and historical comparability

A retrospective set of submitted studies is not a random sample of all possible fermentation products. Applicants may have preselected strains, eliminated unsuitable production routes and conducted extensive quality control before submitting dossiers. The dataset therefore reflects an already filtered industrial and regulatory population. A high prevalence of negative systemic toxicity findings may indicate effective preselection, limited sensitivity for certain endpoints, genuinely low systemic hazard among accepted candidates, or a combination of these factors.

Study designs, analytical capabilities and reporting standards may also vary across the period represented. If the review is to support future policy, transparent inclusion criteria, endpoint definitions, impurity classification rules and sensitivity analyses will be needed. The public summary alone does not permit independent reclassification of every observed effect. Any claim that ninety-day testing is broadly redundant would exceed the evidence currently available from the preliminary information-session account.

Attribution requires causality, not correlation

A finding attributed to impurities invites a further question: how was the attribution established? Possible evidence includes compositional comparison between batches, dose-response alignment with a specific impurity, purification experiments, analytical detection of a known toxicant or mechanistic studies. Without these, “manufacturing-related” may be a plausible interpretation rather than a demonstrated causal assignment. A modern retrospective analysis should disclose how confidence levels were assigned and whether independent reviewers reached concordant conclusions.

The industrial implication is constructive. Instead of viewing impurities as incidental deviations, manufacturers should treat impurity profiles as measurable process outputs and potential

predictors of safety performance. Such a strategy can focus analytical resources where they are most likely to detect meaningful hazards. It does not eliminate the need for toxicological judgement; it makes that judgement more chemically informed.

6. The legal architecture: European rules and territorial divergence

European Union and Spain

Regulation (EC) No 1332/2008 establishes the European legal framework for food enzymes, including definitions, authorisation principles and conditions of use. Regulation (EC) No 1331/2008 establishes a common authorisation procedure for food additives, enzymes and flavourings. Implementing Regulation (EU) No 562/2012 lays down dossier requirements for food-enzyme applications. Regulation (EC) No 178/2002 supplies overarching food-law principles, including operator responsibility and the prohibition on unsafe food. These instruments must be read alongside the applicable authorisation and transitional provisions rather than assuming that a favourable EFSA opinion alone constitutes unrestricted commercial permission [4–7].

EFSA performs scientific risk assessment; the European Commission and Member States operate within the relevant risk-management and authorisation architecture. Spain applies directly applicable EU regulations and enforces them through competent authorities, with national administrative structures relevant to official controls. An EFSA opinion does not replace verification of the enzyme's current legal status, intended technological function, food category, manufacturing source and any applicable conditions of use.

The proposed shift toward non-animal evidence must also be distinguished from existing legal requirements. EFSA's 2026 retrospective exercise is a scientific-policy development, not itself an amendment of Regulation 1332/2008 or of the dossier rules. Applicants should not interpret it as an automatic waiver of study expectations. Where a different evidence package is proposed, the scientific justification and current authority guidance become decisive.

Great Britain and Northern Ireland

Great Britain operates a separate food-law and authorisation context following EU withdrawal, with the Food Standards Agency and Food Standards Scotland relevant to food-enzyme regulatory matters. Historic retained or assimilated provisions and subsequent domestic changes require jurisdiction-specific verification. Northern Ireland must be assessed separately under the applicable post-withdrawal arrangements; a British market decision cannot be presumed to determine Northern Ireland status. For an exporter, the practical requirement is a product-and-use-specific legal review rather than treating "UK approval" as a single undifferentiated concept.

United States

The US legal analysis follows a different pathway. Depending on the intended use and substance, food enzymes and enzyme preparations may be assessed under food-additive provisions or a generally recognised as safe (GRAS) basis, subject to the statutory and regulatory requirements and the evidentiary standard appropriate to the use. A European EFSA conclusion is useful scientific information but is not an FDA determination. US dossier strategy may place different emphasis on production-organism history, manufacturing controls, compositional specifications, exposure and expert consensus. The absence of a required FDA premarket approval in a particular GRAS situation must not be misrepresented as an absence of safety obligations.

International dossier modularity

For companies supplying multiple territories, the most efficient architecture is not a single generic "global compliance" declaration. It is a shared scientific core—identity, production, compositional characterisation, impurity controls, toxicology, allergenicity, stability and exposure—supplemented by jurisdiction-specific legal analyses. Changes to strain, fermentation substrate, purification or intended use should trigger documented reassessment of both scientific and regulatory assumptions.

7. Implications for industrial development and procurement

The retrospective findings strengthen the case for a process-centred approach to enzyme quality. Procurement teams often compare products by declared activity units and price per kilogram, yet two products with comparable activity can differ in impurity burden, stability, residual protein composition, water content and performance in the food matrix. The relevant commercial metric is frequently cost per validated technological effect under the customer's processing conditions, accompanied by an acceptable and reproducible safety profile.

Manufacturers should develop a critical-quality-attribute framework that distinguishes identity and activity from impurity control. The framework may include enzyme activity and stability, total organic solids, microbial limits, production-strain identity, absence of viable production organisms where required, relevant metabolites, heavy metals, allergen-related information and process-change notification. The exact specification must reflect the particular enzyme and process rather than becoming a universal checklist copied between unrelated products.

At the formulation stage, the same enzyme may behave differently in acidic beverages, protein-rich foods, lipid emulsions or high-temperature processing. pH, ionic strength, substrate accessibility, water activity and heat history alter activity and residual exposure. A pilot study should therefore measure the intended technological endpoint and residual activity or material as appropriate. A supplier's generic statement that an enzyme is inactivated at a certain temperature cannot substitute for validation in the actual product matrix.

For INTABIOTECH's broader biotechnology and ingredient-development activities, the relevant opportunity is methodological: integrating enzyme engineering, fermentation analytics, process design and regulatory dossier construction. This is not a claim that any specific INTABIOTECH preparation has been assessed by EFSA, nor that the 2026 retrospective findings establish safety for an individual product. The potential advantage lies in building a more precise evidence workflow for suppliers and industrial customers.

8. Research & Innovation Opportunity

A process-to-impurity-to-exposure evidence architecture

The unresolved problem is that conventional safety dossiers often organise evidence by product identity and toxicological endpoint, whereas the origin of certain adverse findings may lie in variable manufacturing residues. The preliminary EFSA review suggests that manufacturing impurities deserve particular attention, but it does not establish which analytical fingerprints predict toxicological relevance or whether such predictions generalise across enzyme families [1]. This gap is technically important because an evidence model that identifies problematic batches or process states before animal testing could improve both safety assurance and research efficiency.

A plausible, explicitly unvalidated hypothesis is that a combination of targeted metabolomics, non-targeted high-resolution mass spectrometry, proteomic profiling, production-strain genomics and process metadata can identify impurity patterns associated with toxicological concern more reliably than total organic solids alone. The objective would not be to replace toxicology with a black-box algorithm. It would be to create interpretable evidence linking fermentation inputs and operating states to measurable outputs and, where data permit, biological responses.

Proposed experimental pathway

The first stage would define a diverse reference set of enzyme preparations, including different production organisms, purification schemes and deliberately documented process variations. Each lot would undergo harmonised compositional characterisation and quantitative uncertainty assessment. The second stage would use fit-for-purpose in-vitro assays to examine cytotoxicity, barrier integrity, relevant metabolic effects and genotoxicity where justified. These results would be compared against existing toxicological dossiers without treating any single in-vitro assay as a universal surrogate for systemic safety.

The third stage would construct a mechanistically constrained statistical model. Inputs would include strain and substrate information, processing conditions, residual metabolites, proteomic signatures and anticipated consumer exposure. Outputs would be predefined risk flags and confidence intervals, with strict separation of training and external validation datasets. The model would require negative controls, batch-level replication, prospective challenge samples and tests for distribution shift when a new organism or production route is introduced.

The fourth stage would integrate exposure. A compound detected at a high concentration in the enzyme preparation may be absent after processing, while a persistent trace impurity could remain in a widely consumed food. Risk prioritisation should therefore incorporate transfer, transformation and consumption, not merely analytical detectability. Where the production process claims removal, mass-balance and residual measurements should validate that claim across relevant worst-case conditions.

The final stage is regulatory translation. Any proposal to use such evidence in place of conventional studies would need dialogue with competent authorities and alignment with the current guidance. Proprietary strain data, trade secrets and intellectual-property rights must be managed without compromising auditability. The programme should publish sufficiently detailed analytical and validation methods to allow independent scrutiny, while protecting genuinely confidential manufacturing information where legally justified.

Potential industrial value and failure criteria

If successful, this approach could improve batch release, identify contamination mechanisms, support more efficient process change-control and reduce unnecessary testing. It might also help prioritise the studies that genuinely change a risk conclusion. Failure criteria are equally important: poor external predictive performance, confounding by organism type, unstable analytical fingerprints, insufficient detection of rare hazards or inability to connect findings to realistic exposure would invalidate strong regulatory claims. Novelty must be demonstrated against existing quality-by-design, omics and new-approach-methods literature before patentability or scientific originality is asserted.

9. Decision framework: from retrospective observation to credible dossier

A useful decision sequence begins with the precise identity of the production strain and preparation, followed by manufacturing and impurity characterisation. It then establishes the intended food processes and estimates consumer exposure to residual relevant material. Toxicological and allergenicity evidence should be evaluated against those defined conditions, including uncertainty in both analytical measurements and exposure estimates. Finally, the legal status and dossier requirements must be confirmed for each destination market. A change at any earlier stage can invalidate assumptions at a later one.

Management should resist three shortcuts. First, a favourable EFSA assessment of one enzyme is not transferable to a different enzyme simply because both are produced by fermentation. Second, the absence of systemic effects in most historical ninety-day studies does not mean contaminants or allergic reactions can be ignored. Third, the promise of non-animal methods is not a licence to use unvalidated assays as definitive safety evidence. The correct objective is a proportionate, transparent and scientifically discriminating evidence package.

10. Conclusions

EFSA's 2026 retrospective findings point toward an important rebalancing of food-enzyme safety science: from treating the enzyme preparation as a chemically undifferentiated test article toward understanding the production process, its residual constituents and their actual routes to consumer exposure. The preliminary evidence is encouraging for more targeted assessment and the responsible development of alternative methods, but it is not a regulatory exemption and does not erase the distinct problem of allergenicity.

For biotechnology businesses, the strongest practical lesson is that product quality and toxicological credibility are jointly manufactured. Strain selection, substrate control, purification, exposure modelling and legal dossier strategy are not independent administrative steps. They form a connected system in which process design can create, remove or transform the hazards that risk assessment must identify. The next innovation frontier is to make those connections measurable, reproducible and independently reviewable.

References and primary sources

[1] European Food Safety Authority (2026). Information session: Retrospective review of 90-day toxicity studies for regulatory assessment of food enzymes, 18 May 2026. https://www.efsa.europa.eu/en/events/information-session-retrospective-review-90-day-toxicity-studies-regulatory-assessment-food

[2] EFSA Panel on Food Enzymes (2026). Safety evaluation of a food enzyme containing oryzin and leucyl aminopeptidase activities and a heat-treated food enzyme containing only leucyl aminopeptidase activity from Aspergillus sp. FL 72-230. EFSA Journal 24, e10132. https://doi.org/10.2903/j.efsa.2026.10132

[3] EFSA Panel on Food Enzymes (2026). Safety evaluation of an extension of use of the food enzyme triacylglycerol lipase from the genetically modified Aspergillus oryzae strain NZYM-FL. EFSA Journal 24, e10012. https://doi.org/10.2903/j.efsa.2026.10012

[4] Regulation (EC) No 1332/2008 on food enzymes. https://eur-lex.europa.eu/eli/reg/2008/1332/oj

[5] Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. https://eur-lex.europa.eu/eli/reg/2008/1331/oj

[6] Commission Implementing Regulation (EU) No 562/2012 on submission of applications for authorisation of food enzymes. https://eur-lex.europa.eu/eli/reg_impl/2012/562/oj

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

[8] EFSA. Food enzymes topic portal, including November 2026 scientific opinions. https://www.efsa.europa.eu/en/topics/topic/food-enzymes

[9] US FDA. Generally Recognized as Safe (GRAS). https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras

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

Related Knowledge Areas

Biotechnology · Food Safety · Regulatory Affairs · Processing Technologies · Functional Ingredients · Emerging Research

© 2026 José M. López. All rights reserved.