

EU regulation of genetically modified enzymes

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In science, enzymes are defined as proteins produced by living organisms that function as biological catalysts to promote or otherwise influence chemical reactions (for example, see www.alz.org/Resources/Glossary.asp). In contrast, the term "enzymes" is not defined in EC law. However, this lack of definition is not mirrored by a lack of regulation.

This chapter gives a general overview of the EU regulatory system applicable to food enzymes produced by processes involving biotechnology, whether obtained from a genetically modified organism (GMO) or by fermentation using a genetically modified micro-organism (GMM).

THE USES OF ENZYMES REGULATED BY EC LEGISLATION

Over the last decades, the food industry has increasingly used enzymes and enzyme preparations in all sectors, including dairy, brewing, alcohol production, baking, wine, fruit juice, meat and starch. EC legislation regulating the use of biotech enzymes in food falls into the following three main categories:

- **Biotech and novel foods legislation.** If genetically modified bacteria or GMMs are used to produce the enzyme (together with the enzyme so produced, if used as a food additive (see below, *Food additives*)), they may fall within the scope of EC biotech legislation as far as the genetically modified part is concerned. In addition, "novel" enzymes may also fall within the scope of EC legislation on novel foods.
- **Food processing aids.** Although enzymes are mainly used as food processing aids, this use is not yet regulated at EU-level. In addition, only two member states currently impose national requirements in relation to the use of enzymes as processing aids (see below, *Food additives, processing aids, flavourings and other food legislation: Food additives and processing aids*).

However, to fill what is perceived as a gap in regulation, specific EC legislation on food enzymes will be presented in the (near) future. The forthcoming proposed regulation will provide for a time-limited authorisation of individual enzymes used, in particular, as food processing aids (see below, *Food additives, processing aids, flavourings and other food legislation: Pending legislation*).
- **Food additives.** The use of enzymes as food additives is much less common in practice, but is fully regulated at EU-level (by a positive-list system) (see below, *Food additives, processing aids, flavourings and other food legislation*).

- **General chemicals legislation.** As well as being regulated under EC legislation specifically aimed at food, the use of genetically modified enzymes in food may also be caught under EC legislation aimed at regulating chemicals in general, because enzymes are chemical substances.

BIOTECH AND NOVEL FOODS LEGISLATION

The applicable biotech rules consist of:

- Directive 90/219/EEC on the contained use of genetically modified micro-organisms (GMMs Directive).
- Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms and repealing Directive 90/220/EEC on the deliberate release into the environment of genetically modified organisms (GMOs Directive).
- Regulation (EC) No. 1829/2003 on genetically modified food and feed (GM Food and Feed Regulation).

GMMs Directive

The GMMs Directive requires that any "contained use" of GMMs to produce an enzyme must be notified. The phrase "contained use" covers:

"...any activity in which micro-organisms are genetically modified or in which such GMMs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment."

Micro-organisms are defined as (*Article 2(a), GMMs Directive*):

"...any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture."

And a GMM is a micro-organism:

"...in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination."

Therefore, genetically modified living bacteria are GMMs as defined in the GMMs Directive, and the use of the bacteria to produce enzymes in laboratory facilities falls within the definition of contained use.

Notification. The GMMs Directive imposes notification of the first use (and, depending on the risk class, subsequent uses also). The notification must be submitted by the user to the national competent authority of the member state where the premises used for contained uses are located.

The competent authority examines the accuracy and the completeness of the notification, and can ask the user to provide further information or to modify the conditions of the proposed use. The competent authority can also limit the time for which the contained use is permitted or subject it to certain conditions.

The proposed use must also be subject to a risk assessment (*Articles 7 et seq., GMMs Directive*) (see below, *Exemptions from notification*).

Exemptions from notification. The use of GMMs to produce the enzymes is exempt from the notification requirement if the genetic modification is obtained through the use of one of the following techniques or methods (*Annex II, Part A, GMMs Directive*):

- Mutagenesis (that is, inducing mutations in genetic material).
- Cell fusion, including protoplast fusion (a technique in which protoplasts, plants or bacterial cells whose cell walls have been removed, are fused into a single cell), of:
 - cells of any prokaryotic species (cells lacking a nucleus and other membrane-bounded organelles, such as bacteria) that exchange genetic material by known physiological processes; or
 - cells of any eukaryotic species (cells containing a nucleus and other membrane-bound organelles), including the production of:
 - hybridomas (hybrid cells produced by fusion of a special type of cell (a lymphocyte) with a type of cancer cell (a myeloma) used in the production of identical antibodies (monoclonal antibodies)); and
 - plant cell fusions.
- Self-cloning, which is defined as (*GMMs Directive*):

"...consisting in the removal of nucleic acid sequences from a cell of an organism which may or may not be followed by reinsertion of all or part of that nucleic acid (or a synthetic equivalent) with or without prior enzymic or mechanical steps, into cells of the same species or into cells of phylogenetically closely related species which can exchange genetic material by natural physiological processes where the resulting micro-organism is unlikely to cause disease to humans, animals or plants. Self-cloning may include the use of recombinant vectors with an extended history of safe use in the particular micro-organisms."

Other exemptions from the scope of the GMMs Directive include the storage, culture, transport, destruction, disposal or use of GMMs which have been placed on the market either:

- In accordance with the GMOs Directive (see below, *GMOs Directive*).
- Under other EC legislation that provides for a specific environmental risk assessment similar to that in the GMOs Directive.

This exemption only applies where the contained use is in accordance with the conditions, if any, of the consent for placing on the market.

Currently, no environmental risk assessment has been formally recognised as equivalent to that required by the GMOs Directive. This means that, for example, authorisation for placing on the market under Directive 98/8/EC on biocidal products and Directive 91/414/EEC on placing plant protection products on the market does not replace notification and consent under the GMMs Directive. Therefore, any contained use of GMMs in premises (which has not been placed on the market in accordance with the GMOs Directive) would have to be notified under the GMMs Directive before commencing the use.

GMOs Directive

If the use of a GMM is not contained, it is considered to be a "deliberate release" and is regulated under the GMOs Directive.

Deliberate release is defined in Article 2(3) as the:

"...intentional introduction into the environment of a GMO or a combination of GMOs for which no specific containment measures are used to limit their contact with and to provide a high level of safety for the general population and the environment."

This mirrors the definition in Article 2(c) of the GMMs Directive. Therefore, depending on whether the use of the genetically modified bacteria is contained or whether no specific containment measures are used, the activity will either fall under the GMMs Directive or under the GMOs Directive.

A GMO is defined in Article 2 as an organism (a biological entity capable of replication or of transferring genetic material) in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

Notification. The GMOs Directive requires notification and explicit consent for the following two types of activities, regulated under Part B and Part C of the Directive:

- **Part B notification.** Under Part B, any deliberate release of GMOs for any purpose other than placing on the market must be notified to the competent authority of the member state in which the release will take place (*Article 6, GMOs Directive*). The notification must include a technical dossier and the environmental risk assessment required in Annex II, section D of the GMOs Directive. The competent authority must respond, in writing, within 90 days either:
 - acknowledging compliance with the GMOs Directive and indicating that the release can proceed;
 - prohibiting the release.

- **Part C notification.** Under Part C, notification and consent is required for the placing on the market of GMOs, whether on their own or contained in products. The definition of placing on the market set out in Article 2(4) contains both a positive definition and also a delimitation of the definition. As under other EC legislation, placing on the market is defined as:

"...making available to third parties, whether in return for payment or free of charge."

However, making available GMMs for activities regulated under the GMMs Directive (including culture collections) is not considered to be placing on the market under the GMOs Directive. Similarly, making available GMOs other than micro-organisms to be used exclusively for activities where appropriate stringent containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment (based on the same principles of containment as under the GMMs Directive), are also not considered to be placing on the market.

As under Part B, notification must be made to the competent authority of the member state in which the release will take place and explicit consent given before placing the GMO on the market in that member state. However, unlike Part B, Part C contains an important EU component in that whenever a notification is filed, a summary of the dossier must be sent immediately to the competent authorities of the other member states and also to the Commission.

In addition, once the notification is complete and, at the latest, when the national authority has completed its (positive) assessment report required under Article 14, the entire file is also sent to the Commission and subsequently to the member states. If a member state raises objections – which they have regularly done in the past under Directive 90/220/EEC on the deliberate release into the environment of genetically modified organisms – the Commission will have the file assessed by one of its scientific committees (composed of independent experts) and a decision is taken by comitology (a process in which the final decision is delegated to the Commission provided it receives a qualified majority vote from a committee composed of member states' representatives) (*Article 18*). The entire procedure takes about two to four years, depending on the complexity of the matter and whether the authorisation is for cultivation or only for import and processing.

Exemptions from notification. Annex IA, Part 1 sets out a non-exclusive list of the techniques which must be considered to result in genetic modification. This list is similar to the list of genetic modification techniques in the GMMs Directive. Annex IA, Part 2 lists the techniques which are not considered to result in genetic modification and which are, therefore, exempt from notification. Again, this list is similar to the list set out in the GMMs Directive (see above, *GMMs Directive: Exemptions from notification*).

GM Food and Feed Regulation

Under the GM Food and Feed Regulation, any food and feed containing or consisting of GMOs, or produced from or containing ingredients produced from GMOs, as well as any GMOs for food

use (defined in Article 2(8) as GMOs used as food or as a source material for the production of food), must obtain pre-marketing authorisation.

The GM Food and Feed Regulation covers food and feed produced "from" a GMO but not food and feed produced "with" a GMO. The determining criterion is whether or not material derived from the genetically modified source material is present in the food or in the feed.

Processing aids which are only used during the food or feed production process are not covered by the definition of food or feed because they are not expected to become an ingredient of the food and, therefore, do not fall within the scope of the GM Food and Feed Regulation. Similarly, food and feed which are manufactured with the help of a genetically modified processing aid do not fall within its scope.

Consequently, if the enzyme is used as a food processing aid, it is not covered by the authorisation requirement of the GM Food and Feed Regulation. If the enzyme is used as a food additive, the genetically modified bacteria is considered as one of the following:

- A source material for food (that is, the enzyme).
- Contained in the food, if the enzyme is marketed together with the live bacteria.
- Consisting of GMOs, if the enzyme is marketed together with the live bacteria.

In these three cases, the pre-marketing authorisation requirements of the Regulation are applicable.

Obtaining pre-marketing authorisation. The new authorisation procedures include the new principles introduced in the GMOs Directive in relation to a specific environmental risk assessment (*recital 9 of the Preamble to the GM Food and Feed Regulation*).

If the GMO itself is used, or used as a source material, it is the user who must obtain authorisation, unless the person marketing the food product does it himself. In the other cases, it is the person placing the food on the market who must obtain the authorisation.

The application for authorisation must be sent to the national competent authority of a member state, who makes the application available to the European Food Safety Authority (EFSA). The application must contain, among other things, all of the following:

- Copies of the studies demonstrating compliance with the applicable requirements.
- An analysis showing that the characteristics of the food are not different from those of its conventional counterpart or a proposal for:
 - labelling;
 - methods of detection;

- o sampling; and
- o identification of the transformation event.
- Where appropriate, a proposal for post-market monitoring regarding use of the food for human consumption.

In the case of GMOs, or food containing or consisting of GMOs, the application must be accompanied by both of the following:

- The complete technical dossier required by Annexes III and IV to the GMOs Directive.
- A monitoring plan for environmental effects.

The EFSA gives its opinion on the application and the Commission prepares a decision on the application taking into account this opinion, any relevant provisions of Community law and other relevant legitimate factors. The final decision is taken in accordance with the comitology procedure (*see above, GMOs Directive: Notification*). The procedure takes about two years to complete.

Which legislation applies and when?

The manufacturer of an enzyme used as a food additive can choose whether to obtain the authorisation for the genetic modification of the bacteria or micro-organism producing the enzyme under the GM Food and Feed Regulation, or both under the GM Food and Feed Regulation and the GMOs Directive (or the GMMs Directive if the use is contained). The manufacturer of the food in which the enzyme is used must obtain an authorisation under the GM Food and Feed Regulation, unless this has been obtained by the manufacturer of the enzyme.

The manufacturer of an enzyme used as a processing aid must obtain authorisation for the genetic modification of the bacteria or micro-organism producing the enzyme under the GMOs Directive (or the GMMs Directive if the use is contained).

In addition, any enzyme used as a food additive must also be authorised under the food additives legislation (*see below, Food additives, processing aids, flavourings and other foods legislation: Food additives and processing aids*) (recital 12 of the GM Food and Feed Regulation specifies that food additives containing or consisting of, or produced from, GMOs also fall within the scope of the GM Food and Feed Regulation for the safety assessment and authorisation of the genetic modification).

Novel foods legislation

Finally, although this is not an agreed-on regulatory conclusion (that is, some reports or analyses state that enzymes will not be covered by Regulation (EC) No. 258/97 on novel foods and novel food ingredients (Novel Food Regulation); these reports are not official and are not issued by a competent authority), if the enzyme can be considered to be a "novel food" or "novel food ingredient", it will fall within the scope of the Novel Food Regulation. Novel foods are defined as foods or food ingredients (*Article 1, Novel Food Regulation*):

"...which have not hitherto been used for human consumption to a significant degree within the Community."

And which, among others:

"...consist of or are isolated from micro-organisms [...] to which has been applied a **production process not currently used**, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances." (Emphasis added.)

The Novel Food Regulation does not apply, in principle, to food additives. Only novel enzymes used as food processing aids are covered.

At a minimum, even if the enzyme is not considered to be a novel food because it is not considered to be "food", the enzymes used in the processing of novel foods would be covered by the authorisation of those novel foods, although indirectly.

Notification. If covered by the Novel Food Regulation, the novel enzymes must be assessed and authorised before being placed on the market. The applicant must submit a request to the member state in which the product is placed on the market for the first time. The member state performs an initial assessment, following which it informs the applicant that it can place the product on the market or that it needs an authorisation. In the latter case, the final decision is taken in accordance with the comitology procedure (*see above, GMOs Directive: Notification*). Processing this type of application takes about one to two years until completion.

FOOD ADDITIVES, PROCESSING AIDS, FLAVOURINGS AND OTHER FOOD LEGISLATION

In addition to requirements under EC biotech and novel foods legislation, the use of genetically modified enzymes in food may also fall within EC legislation on:

- Food additives and processing aids.
- Food flavourings.

In addition, there is some legislation pending which will alter EC regulation in these areas.

It is also necessary to consider how the relevant European bodies evaluate and assess the scientific data presented to them as part of an application under the various pieces of EC legislation.

Food additives and processing aids

Food enzymes produced from genetically modified bacteria can qualify as food additives or as processing aids.

Food additives are defined as (*Directive 89/107/EEC on food additives authorised for use in foodstuffs intended for human consumption*):

"...any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport

or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods."

Processing aids are defined as:

"...any substance not consumed as a food ingredient in itself, intentionally used in the processing of raw materials, foods or their ingredients, to fulfill a certain technological purpose during treatment or processing and which may result in the unintentional but technically unavoidable presence of residues in the substance or its derivatives in the final product, provided that these residues do not present any health risk and do not have any technological effect on the finished product."

The main criteria for the differentiation between a food additive and a processing aid are:

- The intended presence of the substance in the food after use.
- The continuation of the technological function.

Food additives are covered by and can only be used if included on positive lists. The positive lists include:

- Colourants (*Directive 94/36/EC on colours for use in foodstuffs*).
- Sweeteners (*Directive 94/35/EC on sweeteners for use in foodstuffs*).
- Additives used for miscellaneous uses (*Directive 95/2/EC on food additives other than colours and sweeteners*).

Processing aids must meet a general safety requirement and any additional requirement (including positive listing) imposed by national legislation. Currently, only France and Denmark impose pre-marketing approval on processing aids, including enzymes used as such.

The above directives on food additives do not contain specific provisions on genetically modified additives or processing aids. To date, E1103 Invertase and E1105 Lysozyme have been approved as food additives. However, as discussed above, in addition to its authorisation under one of the directives specifically applicable to food additives, a new genetically modified food additive must also be assessed and approved, with respect to its genetic modification, under the GM Food and Feed Regulation (see above, *Biotech and novel foods legislation: GM Food and Feed Regulation*).

In addition, the Commission is currently working to revise the food additives legislation to provide, among other things, for more precise indications as to when a substance is a food additive or a processing aid. Under the present draft, many enzymes as they are currently used will be considered to be food additives under the future legislation. However, this distinction will be largely irrelevant for enzymes, as they (regardless of whether they are additives or processing aids) will be subject to a specific regulation on enzymes (see below, *Pending legislation*).

Flavourings

Enzymes can also be used as flavourings. Flavourings are defined, among other things, as (*Directive 88/388/EEC on flavourings for use in foodstuffs and source materials for their production*):

"...defined chemical substances with flavouring properties which are obtained by appropriate physical processes (including distillation and solvent extraction) or enzymatic or microbiological processes from material of vegetable or animal origin either in the raw state or after processing for human consumption by traditional food-preparation processes."

Flavourings must comply with general safety criteria, such as an obligation not to contain any element or substance in a toxicologically dangerous quantity. Smoke flavourings are covered by separate legislation, which imposes a general safety requirement and listing on a positive list of primary products (primary smoke condensates and primary tar fractions), to be used to the exclusion of all others (*Regulation (EC) No. 2065/2003 on smoke flavourings in foods*).

Pending legislation

The Commission is currently preparing a specific regulation on food enzymes. The regulation is expected to apply to all enzymes, whether used as food additives or processing aids, with the exception of enzymes used in the production of food additives, flavourings and novel foods. It is also expected to impose a positive list of enzymes to be used to the exclusion of all others.

The applications for the authorisation of enzymes will be assessed by the EFSA and will be the subject of a risk management decision by the Commission, taking into account general criteria such as technological need and consumer considerations. Authorisations will be granted for a period of ten years. Enzymes that are currently used will also have to undergo this authorisation procedure.

Food enzymes produced from a GMO or GMM, which fall under the scope of the GM Food and Feed Regulation because they are food additives, will be subject to authorisation both under the GM Food and Feed Regulation and the future regulation on food enzymes.

In addition, a food enzyme that is authorised for use under the future regulation on food enzymes, for which the manufacturing process has been modified and which would, as a consequence, fall under the scope of the GM Food and Feed Regulation, would also have to undergo authorisation under the latter regulation.

Scientific evaluation

In terms of scientific evaluation, enzymes are covered by an 11 April 1991 Opinion of the EU Scientific Committee on Food on "Guidelines for the presentation of data on food enzymes". The opinion split the safety issues related to the use of enzymes into:

- The toxicological properties of the enzyme preparation.
- The quantity of the enzyme consumed.

- Allergies and irritations.
- Unintended reaction products in the food caused by enzymatic reactions in the final foodstuffs.
- The safety of the source organism.

More recently, the Scientific Committee of the EFSA, which is responsible for multi-sectorial issues, issued an opinion on "A generic approach to the safety assessment by the EFSA of micro-organisms used in food/feed and the production of food/feed additives" (adopted on 15 April 2005) after a request from the EFSA to issue an opinion on the introduction of the Qualified Presumption of Safety (QPS) to be applied to selected types of micro-organisms.

The QPS is similar in concept and purpose to the Generally Recognised As Safe (GRAS) definition in the US. Its use would imply a presumption (defined as an assumption based on reasonable evidence) that different strains of micro-organisms falling within a QPS group are not required to undergo further safety assessment. To establish safety, the companies would only need to submit the following:

- Data on the identity of the organism.
- Evidence that strains are not excluded by any qualifications.
- Product-specific safety data.

The Scientific Committee concluded that the QPS approach could, as a concept, provide a generic approval system that could be applied to all requests received by the EFSA for the safety assessment of micro-organisms deliberately introduced into the food chain.

The Final Report "Collection of Information on Enzymes", contracted by the Commission to the Austrian Federal Environment Agency and issued in 2002, provides a general overview of the scientific issues surrounding the safety evaluation of enzymes.

GENERAL CHEMICALS LEGISLATION

Enzymes are considered to be "substances" and, as such, are subject to EC general chemicals legislation. New enzymes (not listed in the European Inventory of Chemical Substances (EINECS)) must be notified before placing on the market in the EU, unless they are used as food additives (*Directive 92/32/EEC amending Directive 67/548/EEC on the classification, packaging and labelling of dangerous substances*). The new substance notification does not apply to enzymes used in, or as, any of the following:

- Feed additives.
- Flavourings.
- Actives for medicinal products (excluding intermediates).

- Actives for biocidal or pesticides products.

The notification must be submitted to the competent authority of the member state where the substance is first placed on the market. The notification must contain a technical dossier, which contains data depending on the yearly tonnage placed on the market. In the absence of any indication to the contrary from the competent authority, the substance can be placed on the market 60 days after receipt of the notification by the authority. Alternatively, the substance can be placed on the market 60 days after receipt by the authority of the information necessary to bring the notification into conformity with the applicable legislation.

In addition, all enzymes, whether used as food additives or as processing aids and whether new or not, must be classified, packaged and labelled if they meet the relevant hazard criteria.

Establishing whether an enzyme is covered by an EINECS entry is not straightforward and the enforcing national competent authorities (CAs) may have differing opinions on the criteria to consider when deciding whether a new enzyme is identical to an EINECS-listed one. For example, all the CAs consider that, in principle, genetically modified enzymes are new substances, unless the notifier can submit analytical proof that the substance is identical to the EINECS-listed entry. Similarly, the genetic modification of the bacteria can be an element against establishing that the enzyme is identical to an EINECS-listed naturally occurring substance.

The GMM or the genetically modified bacteria do not fall under EC general chemicals legislation because they are not a substance, but a live organism. However, if the micro-organism is dead, whether genetically modified or not, it must be notified as a new substance unless it is EINECS-listed or covered by an applicable exemption.

CONSIDERATIONS FOR MANUFACTURERS

The regulation of food enzymes at the EU level is complex to say the least. How to adapt regulatory constraints to the complexity and diversity of enzyme-producing processes is, and remains, a challenge. Coverage and authorisation under one piece of legislation does not guarantee exemptions from other approvals needed under completely different provisions. In addition, it is not clear how complex scientific questions will be addressed in the practical implementation of the rules. For example, it is not yet clear how issues such as the coverage of different strains of micro-organisms by the same authorisation will be addressed (such as whether the QPS concept will gain regulatory acceptance).

Companies should therefore prepare for possible overlaps and an increase in regulatory burden, which, combined with uncertainty on the interpretation of the data requirements, will result in a large increase in additional data requirements and related costs. In particular, this uncertain overarching web of rules and regulations will eventually result in fewer available enzymes and less marketing choices for the companies involved, possibly combined with a loss of returns on heavy investments in biotechnology.

