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When is an organism subject to the provisions of the EU GMO legislation? An in-depth analysis

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1. Preface

The EU GMO legislative framework consists of different pieces of legislation. Within this framework EU Directive 2001/18/EC is of great importance. This directive determines which type of organisms have to go through a very strict risk assessment and authorization process before being allowed into the environment or onto the market. What is, or what is not a GMO subject to the provisions of this Directive has become the subject of significant debate since new innovative techniques have been added to the breeders' toolbox in the past decade. Full clarity is important. With this analysis we wish to support efforts to create this necessary clarity.

2. Analysis of the wording of EU Directive 2001/18/EC

2.1. The definition

The scope of EU Directive 2001/18 is determined by articles 2 and 3 and annexes IA and IB to that Directive. In article 2(2) a GMO is defined as follows:

> 'genetically modified organism (GMO)' means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;
>
> Within the terms of this definition:
> a. genetic modification occurs at least through the use of the techniques listed in Annex I A, part 1;
> b. the techniques listed in Annex I A, part 2, are not considered to result in genetic modification;

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2.2. Analysis of the phrase "has been altered in a way that"

The phrase "*……has been altered in a way that does not occur naturally by mating and/or natural recombination"* is of crucial importance. What does "*…has been altered in a way that does not occur naturally…"* actually mean? Does it refer to the method of alteration, to the end result of the alteration, or to both? This is not immediately clear from the wording itself and therefore we have to go for a more systemic interpretation of the directive and also look for other clues in the text itself and consider consistencies with other legal instruments.

The definition of a GMO specifies that *"genetic modification occurs at least through the use of the techniques listed in Annex I A, part I"*. The techniques listed in annex I A, part I are:

> (1) recombinant nucleic acid techniques involving the formation of **new combinations of genetic material** by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation **into a host organism in which they do not naturally occur** but in which they are capable of continued propagation;
>
> (2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;
>
> (3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with **new combinations of heritable genetic material** are formed through the fusion of two or more cells by means of methods that do not occur naturally.

The use of these techniques therefore by definition leads to genetic modification and forms the first trigger. But does the mere use of these techniques lead to the formation of an organism subject to the provisions of the Directive? The answer to this question is given by the wording of Annex 1 A part I itself (see above). From this wording it is crystal clear that only when the use of these techniques leads to the formation of a *"new combination of (heritable) genetic material"* (indicated in bold in the text above), the resulting organism is subject to the Directive. Additionally, recombinant nucleic acid techniques only lead to the formation of an organism subject to the requirements of the Directive if these recombinant nucleic acids are incorporated *"into a host organism in which they do not naturally occur"*. The new combination of genetic material resulting from the application of the recombinant nucleic acid techniques should therefore be 'foreign' to the natural gene pool of that organism.

The way these genetic modification techniques have been described in Annex IA part I clearly indicate that in order for an organism to become subject to the provisions of the Directive two additive criteria need to be fulfilled:

(1) Certain techniques must have been used (this is the 'process trigger'),
(2) As a result of which a new combination of (heritable) genetic material must have been formed (this is the 'product criterion')

To be internally consistent the only possible conclusion therefore is that the phrase "*…has been altered in a way that does not occur naturally…"* in the GMO definition refers to both the method of alteration *AND* the end result of the genetic modification.

2.3. Analysis of the 'product criterion'

In order to further understand the scope of the Directive it is necessary to analyze when the end result of the genetic modification fulfills the product ("newness") criterion. If we combine the wording of the GMO definition and Annex I A part I, it means that we have to determine when a *'new combination of (heritable) genetic material'* is formed beyond what '*does occur naturally by mating and/or natural recombination*'.

In order to have a 'new combination of genetic material' beyond what 'does occur by mating and/or natural recombination', this combination of genetic material should present novelty and be distinguishable in a meaningful way from what is present in nature and existing germplasm, from what can spontaneously arise in nature, or from what can be obtained through conventional breeding methods. The words '*…does not occur naturally…*' should be interpreted as meaning 'cannot occur naturally' or at least as 'is very unlikely to occur naturally'. Because if one would not interpret the product criterion in this way, the directive would become *de facto* unenforceable and would also result in a situation in which exactly the same product (with exactly the same combination of genetic material) – one resulting from a modern technique, and the other resulting from a conventional technique –, would be treated in a different manner. This cannot have been the intention of the legislator and would also present a violation of the legal principle of non-discrimination.

2.4. The interpretation of the exemptions to the directive

EU Directive 2001/18/EC also contains through the interplay between recital 17, article 3 and Annex IB a number of exemptions to its scope. Organisms that have been obtained through the techniques of genetic modification listed in Annex IB are not subject to the provisions of the directive. Annex IB lists the following techniques:

1. mutagenesis,

2. cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.

Mutagenesis
The term mutagenesis is not further defined in the Directive. We therefore have to fall back on what is commonly understood by this term at the time of drafting the definition. Mutagenesis is generally defined as "a process by which the genetic information of an organism is changed in a stable manner, resulting in a mutation". A mutation is "the permanent alteration of the nucleotide sequence of the genome of an organism". Mutagenesis is therefore a process resulting in the permanent alteration of the nucleotide sequence of the genome of an organism.

Mutations can occur spontaneously as a result of natural phenomena such as exposure to UV light, exposure to certain chemicals, or copying errors during the replication of genetic material. Mutations can also be deliberately induced by humans through the application of gamma-radiation, X-rays, and different types of chemicals such as Ethyl Methanesulfonate (EMS). From the wording of recital 17 in conjunction with article 3 and annex IB, we conclude that the term mutagenesis in the Directive refers to the process of inducing mutations, and not to the mutations that occur spontaneously in nature.

The main difference between classical mutagenesis and spontaneous natural mutations is the fact that classical mutagenesis results in much higher frequencies of mutations compared to what spontaneously happens in nature. In nature only a few mutations per generation arise. Classical mutagenesis leads to the formation of hundreds to several thousands of random mutations in one go.

Even though each individual mutation resulting from classical mutagenesis can occur naturally, the deliberate application of radiation and mutagenic chemicals resulting in thousands of mutations was seen as resulting in the formation of organisms that possess alterations to their genetic material that were considered extremely unlikely to occur spontaneously in nature or to occur in conventional breeding. It is for this reason that organisms resulting from the deliberate application of classical mutagenesis were seen as GMOs, and are covered by the GMO definition in article 2(2) of the Directive. The Directive then exempts the organisms obtained using classical mutagenesis from the provisions of the Directive on the basis of familiarity (recital 17 and article 3).

In recent years novel techniques of induced mutagenesis have been added to the breeders' toolbox, such as Oligo-Directed mutagenesis (ODM), and different types of Site-Directed Nuclease (SDN) technology including the so-called CRISPR/Cas9 technology. In contrast to the classical mutagenesis using radiation or mutagenic chemicals, these novel techniques of targeted mutagenesis do not lead to higher frequencies of mutations when compared to spontaneously occurring mutations in nature and conventional breeding. The application of these techniques leads to one or a few precision mutations, and these mutations cannot be distinguished on the DNA level from what is present in nature, is present in existing germplasm, from what can spontaneously arise in nature, or from what can be obtained through conventional breeding methods. The organisms containing such precise, targeted mutations therefore do not contain a 'new combination of (heritable) genetic material' beyond what 'does occur naturally by mating and/or natural recombination', and are therefore not subject to the provisions of the Directive. They do not fulfil the product criterion.

It is further useful to analyze the reasons why the organisms resulting from induced mutagenesis have been exempted from the scope of the directive. Recital 17 states:

> "This Directive should not apply to organisms obtained through certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record"

This recital forms the rationale for article 3 and annex IB. At the moment of conception of the Directive induced mutagenesis using ionizing radiation and mutagenic chemicals had already been used on a wide scale for more than 60 years and resulted in thousands of registered mutant varieties. Currently the IAEA Mutant Variety Database lists 3.240 mutant varieties that have been generated using classical mutagenesis.

To obtain these different varieties a far larger amount of mutants have been created from which these varieties have been selected. In other words: in order to create one registered mutant variety, usually thousands of mutants have been created. Many of these mutants show undesirable product properties, quite some are not viable, and only a few show enhanced traits. Through evaluation of their traits, mutants with undesirable characteristics have been discarded and mutants that appear to have added value have been selected. The final mutants that have survived this process of testing and selection, and which have then been placed on the market, are generally recognized as safe.

This clearly shows that the technique or process of classical mutagenesis does not inherently lead to the formation of safe organisms. And when recital 17 refers to having "a long safety record", it does not refer to the safety of the process of classical mutagenesis. It refers to organisms in which mutations have been generated using mutagenesis, and which have gone through the conventional process of trait evaluation and selection, before they were put on the market. It is this full process that constitutes the safety record, not the process of mutagenesis in itself. To formulate it more clearly once more: the 'long safety record' therefore does not apply to the process of classical mutagenesis, but to the selected mutants with selected traits.

Conclusion
We conclude that the process of classical mutagenesis does lead to GMOs, in view of the high number of genetic changes in the resulting organism, far exceeding the natural mutation rate. However, for targeted mutagenesis we come to a different conclusion. Even though targeted mutagenesis using e.g. CRISPR/Cas9 or ODM is a form of mutagenesis, we conclude that such targeted mutagenesis leading to one or a few mutations only, that can occur spontaneously too, and occur at the same mutation rate as occurs in nature and conventional breeding, does not lead to a GMO in the first place, and that these organisms therefore also do not have to be exempted from the scope of the directive.
We also conclude that the organisms that are the result of classical mutagenesis have been exempted from the scope of the directive, not because classical mutagenesis as a technique in itself is considered safe, but because the process resulting in the selection of specific mutants with selected traits has a long safety record.

3. Consistency with other legal instruments

The 'Cartagena Protocol on Biosafety to the Convention on Biological Diversity' is an international protocol that sets out requirements for the transboundary movement of living modified organisms. In this Protocol 'living modified organisms' are defined as:

> "Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology"

Modern biotechnology is further defined as:

> "The application of:
> a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
> b. Fusion of cells beyond the taxonomic family,
> That overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection;"

The European Union has ratified the Cartagena Protocol on Biosafety and implemented it by means of EU regulation 1946/2003 on Transboundary Movement of Genetically Modified Organisms.

The interpretation of the term 'GMO' within the European legislation should be consistent with the definitions of this term in the Cartagena Protocol on Biosafety. In its wording the definition of 'LMO' is much clearer than the definition of 'GMO'. In order to become a LMO the organisms needs to possess a ***novel combination of genetic material*** which is obtained ***through the use of modern***

biotechnology. In other words: the organism needs to fulfill both the process criterion (the use of modern biotechnology) *AND* the product criterion (the novel combination of genetic material) in order to qualify as an LMO.

This is consistent with the above interpretation of EU Directive 2001/18/EC in which we have elaborated that the GMO definition not only refers to the process, but also contains the product criterion. In other words, the Cartagena Protocol on Biosafety supports our systemic analysis that came to the conclusion that the mere use of a certain technique in itself does not qualify the resulting organism subject to the provisions of the GMO Directive. The resulting organism also must have certain "novelty". It has to have a 'novel combination of genetic material' (Cartagena Protocol on Biosafety), or a 'new combination of (heritable) genetic material' (EU directive 2001/18/EC). And 'novel' or 'new' needs to mean that it should be distinguishable biologically from what can occur in nature, or can result from conventional breeding techniques.

4. The role of the precautionary principle in determining what constitutes an organism subject to the provisions of the European GMO legislation

Recital (8) of EU directive 2001/18/EC states that the precautionary principle has been taken into account when drafting it and must be taken into account when implementing it. Article 1 of the directive reads:

> "**In accordance with the precautionary principle**, the objective of this Directive is to approximate the laws, regulations and administrative provisions of the Member States and to protect human health and the environment when:
> - carrying out the deliberate release into the environment of genetically modified organisms for any other purposes than placing on the market within the Community,
> - placing on the market genetically modified organisms as or in products within the Community"

The application of the precautionary principle has guided the European Union in bringing certain organisms within the realm of regulatory oversight and requiring elaborate risk assessments prior to their release, in the absence of evidence or certainty that either the techniques of genetic modification themselves, or the products resulting from them are inherently risky. At the time of conception of the original GMO Directive the modern techniques of genetic modification were relatively new and there was not that much experience yet with the organisms obtained through their use. These considerations have guided the European Union in setting up the GMO legislation.

The reference in the Directive to the precautionary principle can however not be used to state that organisms that are not within the scope of the Directive, are still covered by the Directive. The scope of the Directive is the scope of the Directive. If one is of the opinion that the precautionary principle directs us to bringing certain organisms within the realm of legislation, which are currently not covered by this legislation, then one should either alter the scope of the current legislation, or develop new legislation.