Application of the EU and Cartagena definitions of a GMO to the classification of plants developed by cisgenesis and gene-editing techniques

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Contents

Summary

In the EU, regulations have been devised to mandate the assessment of risks to the environmental, human food and animal feed safety arising from genetically modified organisms (GMOs). However, there is currently debate over whether, or which of, the new plant breeding techniques (NPBTs) under development would be classified as producing GMOs and whether any exemptions might apply. Here, we examine whether the NPBTs collectively termed "gene-editing" techniques, i.e. oligonucleotide directed mutagenesis (ODM) and site-directed nuclease (SDN) techniques fall into the classification of a GMO within the EU. The grounds for any possible exemption of GM plants developed through cisgenesis from the EU GMO regulations are also discussed.

The techniques used to create a GMO are not exhaustively defined by the EU regulations. In the regulations, examples are given of techniques used at the time the regulations were devised in 2000/1. Although recombinant DNA has, to date, been the agent of direct modification in all commercially grown GM crops, in both the EU and Cartagena Protocol definitions, there is also an emphasis on the use of *in vitro* techniques where the modification is induced by heritable material that has been prepared outside the organism. In this analysis, we find that ODM and SDN techniques fall into the category of direct modification using *in vitro* techniques, and hence would be classified as a GMO according to the EU and Cartagena definitions. We find little similarity with traditional mutagenesis in the process of the modification, and argue that exemption based on a similarity to mutagenesis is not valid.

Like traditional genetic engineering techniques (including those used for cisgenesis/ intragenesis), unintended changes to plant chemistry arising from the use of gene-editing techniques may result from: unforeseen interactions between the new or altered gene(s) and the plant's endogenous genes; genomic irregularities arising from the genetic engineering process itself and unintended alterations to plant biochemical pathways arising from the changed or new function(s) of the altered or novel gene(s). Maintaining the EU process-based approach to GMO regulation (as distinct from product- or trait-based regulations) is important because it provides a basis for assessing any potential risks to food, feed and environmental safety arising from both intended and unintended changes to the plant arising from the genetic engineering process. Unintended changes could impact food, feed and environmental safety but there would be no requirement for these to be detected and assessed under a product-based approach, or if such plants are exempt from the GMO regulations. Exemption from the EU GMO regulations would also exempt products of NPBTs from GMO labelling requirements, which could restrict, or remove, consumer choice.

Introduction

National, regional and international regulation of genetically modified organisms (GMOs) has been developed in response to concerns regarding the ability of modern biotechnological techniques to directly alter genetic material (usually DNA) in order to change an organism's characteristics. For GM crops, concerns that have been raised include potential adverse effects arising through their use as food and feed[1], as well as environmental impacts including those on biodiversity[2] and GM contamination of neighbouring non GM crops or wild/feral relatives[3]. In many countries and regions, including the EU[4], labelling of GM food ingredients, seed and feed is required to enable consumer and farmer preference.

Current commercial GM crops have been generated using genetic engineering technologies developed during the 1970 and 1980s. These involve the introduction of a cassette containing the modified gene(s) of the commercial trait and associated operational genetic elements, e.g. promoter/terminator, although changes can potentially be directed at other genetic material, e.g. changes to some forms of RNA (ribonucleic acid) can also induce heritable changes in the engineered organism[5].

In the past decade, new, additional biotechnologies have been developed, collectively termed new plant breeding technologies (NPBTs) [6]. There is debate in both scientific[7] and political circles[8] over whether, or which of, these NPBTs would be classified as producing GMOs. NPBTs generally involve targeted changes to genetic material, sometimes small in nature. The crux of the debate is whether such small changes should be regarded as genetic modification and, if this is the case, whether they should be considered similar to traditional mutagenesis, which is exempted from the EU GMO regulations. In addition, there is a long running discussion about whether GMOs developed through the transfer of genes from one organism to another but within the same or closely related species (cisgenesis), could be exempt from the EU GMO regulations[9].

Although there are a range of NPBTs under development[10], discussions are currently focussed on "gene-editing" techniques comprising oligonucleotide directed mutagenesis (ODM) and site-directed nuclease (SDN) techniques (also called site-specific nucleases (SSN), These discussions have been precipitated by the regulatory approval for commercialisation of a herbicide-tolerant oilseed rape (or canola) developed using ODM, in North America[11]. The developer, Cibus has also approached national regulatory bodies in the EU, e.g. UK (2011) and Germany (2014/5) to request advice on whether authorisation for a deliberate release of a GMO is required for pre-market field trials, requiring these bodies to determine whether plants developed using ODM fall under the GMO regulations. Although these national authorities considered ODM equivalent to chemical mutagenesis and therefore exempt from the GMO regulations[12], more recently the EC Commission has advised EU Member States to await, where possible, the outcome of the Commission's legal interpretation of ODM before proceeding to field trials of the oilseed rape[13]. SDN techniques are becoming widespread in the laboratory and it is generally thought that a commercial application to cultivate or market a plant variety developed through SDN will be made to the EU regulatory authorities in the near future[14].

The implications of whether a plant with directly modified genetic material is considered a GMO within the EU are significant. If the plant is covered by the GMO regulations, then any

potential risks it poses, including those arising from any unanticipated effects of the genetic modification, to human and animal health, and to the environment if deliberately released (e.g. cultivated in open fields), have to be evaluated prior to commercialisation. In addition, if an organism is regulated as a GMO, then seed, food and feed products derived from that organism are required to be labelled as derived from GMOs. Conversely, if the plant is deemed not covered by the GMO regulations, no risk assessment or labelling is required.

Here, we examine the terminology and spirit of the definition of a GMO within the EU GMO regulations and the UN Cartagena Protocol on Biosafety. Finally, we examine the applicability of the EU definition and regulations to plants with directly modified genomes arising from cisgenesis and those NPBTs which are the subject of most discussions at present, ODM and SDN techniques. Although we restrict our considerations to plants, some of these techniques, e.g. TALEN, CRISPR/Cas, can be applied to animals[15] and similar considerations would apply.

Definitions of a GMO in the UN Cartagena Protocol on Biosafety and the EU

The UN Cartagena Protocol on Biosafety has defined a living modified organism (LMO)[16] (Annex 1) and the EU (which is a signatory to the Cartagena Protocol) has defined a genetically modified organism (GMO) in a similar way in Directive 2001/18[17] (Annex 2). An LMO is broadly equivalent to a GMO but the scope of the Cartagena Protocol is concerned solely with the import/export of living GMOs (or other living products of modern biotechnology), whilst the EU regulations are also concerned with food and feed products from GMOs.

The EU has adopted a slightly different definition of a GMO to the Cartagena Protocol, but the two rely on similar concepts. A key element of both definitions of a GMO is that the genetic material has been altered by direct intervention, rather than by mating or natural recombination. The Cartagena Protocol explicitly states that direct intervention is through "modern biotechnological techniques" whilst in the EU the use of modern biotechnology is implicit as traditional or established biotechnological techniques, such as mutagenesis by chemical or radiation and cell fusion (between plant cells with can exchange genetic material through traditional breeding), are considered as resulting in GMOs but exempt from the scope of GMO regulations.

The techniques used to create a GMO are not specifically defined by the EU regulations. Instead, examples are given of techniques used at the time the regulations were devised in 2000/1. The regulation states "techniques of genetic modification… are, *inter alia:"* and then provides an indicative, but not exhaustive list of techniques that are considered to result in a GMO. The wording "*inter alia*" implicitly acknowledges the existence of (and potential for) other modern biotechnological approaches that can directly modify genomes.

In the EU definition of a GMO, two of the techniques identified in the list of examples (Annex 1A, Part 1) that are most relevant to plant breeding are: (1) recombinant nucleic acid techniques involving nucleic acid molecules prepared outside the organism and introduced via a vector system (e.g. *Agrobacterium*-mediated techniques) and (2) direct injection of heritable material prepared outside the organism. This second technique does not specify whether the material is recombinant, only that it is heritable. The third technique, cell fusion, is not discussed here as it does not relate to NPBTs[18]. Certain techniques are specifically exempted on the grounds that they do not involve the use of recombinant nucleic acids, or use GMOs.

Implicit in these exclusions is the concept that the genome has not been directly modified by a technique involving material prepared outside the organism. Exempted techniques include *in vitro* fertilisation, natural processes such as conjugation, transduction and transformation and polyploidy induction. Mutagenesis is also exempted from the regulations on the grounds that it does not involve the use of recombinant nucleic acids or GMOs. At the time the regulations were devised, chemical or radiation mutagens were the only commercially viable way of performing mutagenesis, and the intent of the exemption was to ensure that these 'traditional' or established mutagenesis methods were excluded from coverage by EU law[19].

In the UN Cartagena Protocol on Biosafety, "modern biotechnological techniques" are defined in a general way, as "*in vitro* nucleic acid techniques, including the use of recombinant DNA and direct injection of nucleic acid into cells or organelles" and cell fusion both of which "overcome natural physiological reproductive or recombinative barriers and are not techniques used in traditional breeding and selection". An important point is that both the EU and Cartagena Protocol definitions (excepting cell fusion) place an emphasis is on the use of *in vitro* methods. "*In vitro*" refers to the whole technique that causes the modification to the genome because of the intervention of this technique, rather than the cell being contained within glass whilst being modified[20] (as would be the case with *in vitro* fertilisation). Thus, a central concept defining a GMO in both the EU and Cartagena Protocol is that the genome has been directly altered by techniques that include a step where the material introduced to the organism has, for part of the procedure, been outside the organism and handled by people in ways that do not occur in nature. However, under the EU definition, GMOs are not limited to those produced by *in vitro* techniques because the list of techniques is not exhaustive.

Process-based assessment of GMOs in the EU

In the EU, the food, feed and environmental safety assessment of a GMO is "process-based". That is, along with the specifics and characteristics of the newly created plant (e.g. herbicide tolerance), the assessment requires additional consideration of the process by which it was created, notably an assessment of any unintended modifications to the plant genome and/or any unintended changes to plant composition (e.g. nutritional status). An alternative way of assessing GMOs is via a "product-based" approach, such as that used by the US and Canadian regulatory authorities[21]. In a product-based approach, assessment focuses primarily on the resulting trait (e.g. herbicide tolerance) and the specifics of both the direct modification (e.g. the source of any inserted material) and any novel protein produced. With a product-based approach, there is either limited or no requirement to detect or assess any unintended changes. This absence of knowledge regarding unintended changes is likely to severely limit any meaningful safety assessment[22].

The fundamental concern regarding the direct modification of genetic material is that it can unintentionally interfere with the functioning of an organism's genome, namely gene expression. In existing commercial GM crops, events are selected where the genetic material inserted into a chromosome performs well enough to confer the desired trait. However, the precise way in which the plant's regulatory network functions is poorly understood. That the inserted material confers the trait does not itself preclude interactions within the network, nor does it provide insight into what the outcome of those interactions might be. This is underlined by recent advances in our understanding of the genome regulation functions of certain non-coding RNAs[23] and the current debate on whether all of the "junk" DNA in the human genome

is actually “junk” or performs regulatory functions[24]. Because of this lack of understanding of genomic regulation, it is not possible to predict the nature and consequences of all interactions of the altered genetic material within the host genome. GM plants may exhibit unexpected and unpredictable effects as a result of unforeseen interactions between the new or altered gene(s) and endogenous genes; genomic irregularities arising from the genetic engineering process itself and/or unintended alterations to plant biochemical pathways arising from the changed or new function(s) of the altered or novel gene(s).

Alongside the intended direct modification of plant genomes, unintended alterations of the host genomes have been observed in GM crops that are currently grown commercially. To date, these genomic alterations have arisen from the unintended insertion of multiple copies and fragments of the genetic cassette at different locations[25] and rearrangements of host DNA flanking the intended genetic insert. For example, additional, unintended fragments of the inserted genetic cassette have been found in Roundup Ready soya after its commercialisation[26] and at least one coding fragment has been transcribed to the RNA level[27], potentially an intermediary step along the pathway to producing an unintended protein. Rearrangements or deletions of sections of host DNA have been detected in the flanking regions surrounding the inserts in both Roundup Ready soya[28] and insect resistant maize, MON810[29].

A primary function of the genome is to produce proteins. Unintended genomic irregularities or unforeseen genomic interactions could disrupt protein production. This disruption could result in the production of unintended novel proteins, including altered host plant proteins, in terms of either chemical composition or structure. Although any intended novel protein arising from the genetic modification is likely to be characterised, altered host or unintended novel proteins may not be. The character of proteins produced by a plant is important for environmental, food and feed safety, especially as some proteins are immunogenic, potentially even allergenic.

Changes to plant genetic material, both intended and unintended, could unexpectedly alter the levels and composition of plant metabolites[30]. Plant secondary chemistry is complex. Plants produce secondary metabolites (chemicals) for many purposes e.g. defence against herbivory or to attract pollinators. Concerns regarding alterations to secondary metabolites relate to any differences in gene expression between the native and modified plant. Such alterations to secondary metabolites could affect the toxicity or palatability of these plants to wildlife. For example, certain lines of GM Bt maize unexpectedly displayed an increased susceptibility to aphid infestation[31]. Differences in both amino acid composition and secondary metabolites were found between the GM lines and non-GM counterparts and it was suggested that these could have contributed to the increased susceptibility[32]. Changes in secondary metabolites could also affect the fitness of a GM crop, an important environmental concern should outcrossing to wild or weedy relatives be possible. Changed metabolite composition could also affect the nutritional quality or even the toxicity of the GM food/feed product. Unintended changes in plant secondary chemistry can also occur in conventional breeding. However, the direct modification of genetic material can substantially alter plant chemistry, e.g. by engineering whole new biochemical pathways as in GM ‘Golden’ rice. In such GM plants there is potential for more radical unintended alterations of plant chemistry than there is with conventional breeding.

Unintended effects from directly modified plant genomes could have consequences for the environment, food and feed safety. A process-based approach for the assessment of plants

with directly modified genomes is important because it requires detection of any unintended changes to be assessed for their implications to the environment, human and animal health. This is in addition to assessing the consequences of the novel characteristics of the newly created plant.

Transfer of genes within the same species ("cisgenesis")

Cisgenesis involves the direct modification of genetic material and, to date, has used recombinant nucleic acids. Cisgenesis is sometimes confused with intragenesis. In cisgenesis, intact genes, together with associated promoter/terminator from one species are inserted into the genome of the same or a closely related (i.e. sexually compatible) species. By contrast, in intragenesis the functional gene may be partial and the promoter/terminator may not be associated with the functional gene in the native plant, although all components are derived from the same or a closely related species[33]. Cisgenesis and intragenesis are not new plant breeding techniques per se and differ only in source material from the more established transgenesis (or intergenesis), where genes from a different species are inserted into the engineered organism. Cisgenesis and intragenesis can, however, be performed with SDN-3 type gene-editing techniques (see "ODM and other gene-editing techniques").

It has been suggested[34] that GM plants produced by cisgenesis do not carry the same risks as transgenic GM plants because the components are derived from the same or closely related species and, further, that they should be granted an exemption from the EU GMO regulations. The processes of directly modifying genetic material, however, are the same for cisgenesis, intragenesis and transgenesis, regardless of the origin of the inserted genes. Therefore, the concerns regarding unintended changes to the plant genome and unforeseen genomic interactions remain the same as with transgenesis.

The potential for the number of unintended changes in secondary metabolites levels and composition may be reduced for plants developed through cisgenesis compared to transgenesis as the genes are endogenous, but the potential for unintended changes, including those with a potential adverse effect on health or environment is not necessarily reduced. Insertion of the genetic construct can cause unintended genomic alterations in the same way as transgenesis (e.g. multiple copies and fragments of the genetic cassette and/or deletion or rearrangement of endogenous DNA flanking the intended genetic insert). In both cisgenesis and intragenesis, the expression pattern (i.e. when and where expression occurs) of the inserted gene may be different due to its changed location on the genome (position effects) [35]. Thus, cisgenesis and intragenesis could still alter plant biochemical pathways in similar ways to transgenesis, potentially giving rise to unexpected effects.

The European Food Safety Authority (EFSA)[36] considers similar hazards to be associated with cisgenic GM plants as conventionally bred plants. This argument is largely based on the assumption that similar changes can also be caused by "random movement of numerous mobile genetic elements such as transposons and retrotransposons". However, evidence that these movements are random is scant[37]. On the contrary, the importance of transposon movement for evolution and any associated deletions/rearrangements are highly active areas of enquiry, especially for plants[38].

There does not appear to be a strong scientific basis for assuming that the hazards and risks of cisgenic (or intragenic) plants are markedly less from transgenic plants with regard to unexpected and unpredictable effects[39]. This is because such changes can occur irrespective of the origin of the novel genetic material. Unintended changes to either genetic material and/or plant metabolism in the resulting cisgenic or intragenic plant could be important in terms of plant's impact on the environment and human and animal health. Exemption of cisgenesis from the EU GMO regulations would mean there would be no requirement to either detect or assess any untended changes. Therefore, it would neither be logical nor desirable to exempt them from the risk assessment procedures for GMOs in the EU.

Oligonucleotide directed mutagenesis (ODM) and other "gene-editing" techniques

Gene-editing (or "genome-editing") techniques generally use nucleases, often called "molecular scissors", which cleave DNA at specific sites and trigger the plant's own repair mechanisms. These include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases (MN) and the clustered regularly interspaced short palindromic repeat (CRISPR/Cas) system. Depending on whether a repair template is used or not, these methods can induce random (non-specific) changes to one or more base pairs via non-homologous recombination (SDN-1) or specific changes via homologous recombination (SDN-2) changes to nucleotide sequences. These changes can be substitutions, deletions or insertions of one or more base pairs. More extensive changes involving whole genes (including gene-stacking) are also possible, involving donor DNA (SDN-3)[40]. Whilst SDN-3 techniques clearly result in a GMO as the insertion of genes is involved, it is not yet clear whether SDN-1 and SDN-2 techniques would be considered to result in a GMO within the EU[41].

ODM is a gene-editing technique that does not use molecular scissors. Instead, short DNA (or DNA-RNA) fragments (oligonucleotides) are introduced into cells where they trigger the cell to modify its own DNA to match the introduced DNA fragments. This technique can change, insert or delete one or a few base pairs of DNA[42]. There are suggestions that ODM[43] could be exempt from the EU GMO regulations because it result in a similar changes to the plant genome as traditional mutagenesis. A similar argument has also been made for SDN-1 and SDN-2 genome-editing techniques[44]. It is true that they involve changes to a small number of DNA bases but the techniques used are considerably different from traditional mutagenesis. In determining whether the resulting plant is classified as a GMO under the EU regulations or Cartagena Protocol, the extent of change to the plant's DNA is irrelevant. It does not matter whether only one or two DNA bases have been inserted, changed, deleted or whole novel gene sequences inserted, the critical question is whether plant genetic material has been directly modified. The answer to this is yes. Plant genetic material has been directly modified using modern biological techniques that include an *in vitro* step.

Reviews of the application of ODM and other gene-editing techniques have identified many possibilities for unintended changes to the host's DNA[45]. For example, ODM and "molecular scissors" can have off-target effects (meaning they might cut and/alter DNA in places additional to those intended)[46]. Many of these gene editing techniques are new so it is not yet possible to fully evaluate the potential for unintended changes. However, it is evident that unintended changes to genetic material cannot be excluded, and indeed, might even be expected. Although

more targeted than the current random insertion of genes into plant genomes, the potential for unforeseen genomic interactions, genomic irregularities and unintended biochemical alterations still remains with gene-editing techniques. As with plants developed through traditional genetic engineering technologies, both intended and unintended changes could be important in terms of plant protein production and metabolism. Thus, it is possible that, like traditional techniques of genetic engineering, ODM and other gene-editing techniques can give rise to plants displaying unexpected and unpredictable effects with implications for food, feed and environmental safety[47].

There is discussion whether ODM falls within the scope of the EU regulatory regime for GMOs because some consider it a mutagenesis technique that does not involve the use of recombinant nucleic acid molecules and is therefore exempt under Annex 1B as mutagenesis is[48]. Expert opinion is divided on whether ODM involves *recombinant* nucleic acid molecules[49]. Those who consider ODM not to involve *recombinant* nucleic acid molecules consider the size of the recombinant nucleotide sequence to be critical, and that the small number of nucleotides changed in ODM less than that required to be considered as recombinant nucleic acid[50]. There are two counter arguments to this: (1) it is not possible to physically distinguish a DNA oligonucleotide from 'recombinant DNA'. Whilst a single nucleotide, particularly a base analogue, can be a chemical mutagen, once nucleotides are polymerised along their phosphodiester backbones to form DNA, they behave like DNA in all conceivable contexts. There is no distinguishing the DNA based on the number of polymerised nucleotides nor whether it was synthesised or excised by restriction endonucleases from a larger molecule. As such, the outcome of its interaction with another DNA molecule is recombination: it is an inevitable outcome whether or not the DNA molecule was called recombinant; (2) ODM is an *in vitro* technique, and organisms developed through the use of *in vitro* techniques are classified as GMOs. ODM invokes a change in the genetic material of the plant via heritable material which has been prepared outside the organism. Even if the oligonucleotide is not permanently inserted into the genome, genomic changes still result from material prepared outside the organism and introduced to cells.

ODM and other gene-editing techniques, if applied sequentially or in combination, could result in greater genomic changes than a few base pairs. It's conceivable that these changes could result in substantial changes to the genome. For bacteria, these could fall within the scope of "synthetic biology", where the whole genome is modified [51]. As the Netherlands Commission on Genetic Modification (COGEM) state, in their consideration of ODM, that "a successive cycle of directed mutagenesis could introduce an entirely new sequence" [52]. Although only plants are considered here, it's clear that the inclusion or exemption of gene-editing techniques within the EU regulations could have far reaching consequences.

Unintended effects can only be assessed under GMO legislation

Regulations on GMOs have been devised so that there would be an assessment of the food, feed and environmental safety of organisms developed using modern biotechnological techniques. For example, the EC food safety website for GMOs states: "In order to ensure that the development of modern biotechnology, and more specifically of GMOs, takes place in complete safety, the European Union has established a legal framework regulating genetically modified (GM) food and feed in the EU. This framework pursues the global objective of

ensuring a high level of protection of human life and health and welfare, environment and consumer interests, whilst ensuring that the internal market works effectively"[53].

Within the current assessment regime of GMOs in the EU, there are concerns that GMOs are not adequately assessed, particularly for long-term health and health impacts[54]. To exempt plants produced by cisgenesis, ODM or other gene editing techniques from the EU GMO regulations would mean that there would be no requirement to detect and assess any unintended changes or to assess any potential effects on food, feed or environmental safety. Even so, any risk assessment is limited. The preference would be to employ the precautionary principle and not to let crops developed using gene-editing (or 'traditional' genetic engineering techniques) enter the environment or food chain.

As Araki et al 2014 state: "*If organisms modified with genome editing in which a gain of function unintentionally arises are released without rigorous risk assessments, they may rapidly affect the local ecosystem by seriously threatening native species. Even if they do not pose a serious threat to native species, the released organisms may negatively affect the environment owing to cross breeding.*"[55]

The exemption of plants produced by cisgenesis, ODM or other gene editing techniques from the EU GMO regulations would also have implications for consumer choice. EU legislation requires labelling of food and feed derived from GM organisms, if they are present above a threshold value of 0.9%[56]. If any of the plants resulting from cisgenesis/intragenesis, ODM or other gene editing technologies were to be exempted from the GMO regulations, they would also be exempt from GMO labelling regulations for GMO seeds, crops and food/feed products. This would mean that farmers, producers and consumers who choose not to make use of, or eat, foods derived from genetic engineering technologies could be restricted in the choices available to them.

Detectability is not a pre-requisite for classification as a GMO

Detection and identification of a GMO are essential for the labelling of food and feed ingredients derived from the GMO. They are also essential for traceability, which allows for detection of any GM contamination and post-market monitoring of GMOs, a cornerstone of the EU GMO legislation. It is the legislation that requires GM foodstuffs to be detectable, rather than detectability being a trigger for labelling or for defining an organism as a GMO.

Under the EU regulations, a company seeking authorization to market or cultivate a GM plant is required to provide a unique identifier for the GM plant and detection methodology for the GM event. To date, these detection methodologies have principally been based on polymerase chain reaction (PCR) techniques, with protein-based methods (typically employing an enzyme-linked immunosorbent assay, ELISA) primarily used for screening purposes[57]. The same type of PCR methodologies would be applicable to the event-specific detection of plants developed by SDN-3 techniques (including those classed as cisgenesis and intragenesis). The detection of genetic changes induced by ODM and SDN-1 and SDN-2 techniques would also be possible using current PCR technologies, if information regarding DNA sequences of the areas immediately adjacent to the genetic deletion, alteration or insert (i.e. the flanking regions) were supplied by the company (as it is currently for GM plants). However, the detected changes may not be distinguishable from a genetic change produced by traditional mutagenesis or even

a natural mutation[58], precluding event-specific detection. Plants developed using SDN-1, 2 or ODM may require additional, different or more complex methodologies of detection (e.g. whole genome sequencing)[59]. Quantification of the amount of a GMO present in a foodstuff, essential for determining whether GMOs are present above the labelling threshold, may also present challenges. High–throughput DNA sequencing technologies are being rapidly developed for a broad range of applications. It is a rapidly moving field so such challenges are not insurmountable, especially if there is a necessity for additional detection methodologies for plants developed using NBPTs.

Conclusions

A fundamental concept in defining a GMO within both the Cartagena Protocol and the EU is that the genetic material has been directly modified using modern biotechnological techniques, with an emphasis on the use of *in vitro* techniques. Cisgenesis, ODM and other gene-editing techniques are modern biotechnologies which directly alter genomes utilising *in vitro* techniques and should be classified as GMOs. It has been argued here that there is little similarity between the processes of traditional mutagenesis and gene-editing techniques and therefore comparisons with traditional mutagenesis do not provide a basis to exempt plants produced by these techniques from the EU GMO regulations.

ODM and other gene editing techniques may be more precise in their positioning of the intended alteration to genetic material than traditional genetic engineering techniques but there is still potential for the newly created plants to display unexpected and unpredictable effects. These effects could arise from unforeseen genomic interactions associated with the novel genetic material, genomic irregularities and changes to the secondary chemistry of the plant. Maintaining the current process-based approach to GMO assessment in the EU provides a basis for assessing any potential risks to food, feed and environmental safety arising from any unexpected and unpredictable effects. If these techniques were exempted from the EU GMO regulations, it is possible any such unexpected effects would remain undetected. If such effects are not detected, they cannot be assessed for their implications to food, feed and environmental safety.

Exemption from the EU GMO regulations also would also mean exemption from GMO labelling requirements for GMO seeds, crops and food/feed products. This could restrict the choices available to the clear majority of European consumers and organisations that wish to avoid food and feed derived from plants produced with genetic engineering techniques. The GMO regulations in the EU should be interpreted in their intended sense, to encompass all modern biotechnological processes that directly modify genomes. Otherwise, the EU would be failing European citizens.

Annex 1: Definition of a GMO in the Cartagena Protocol

Article 3 of the Protocol states:

(g) "Living modified organism" means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;

(h) "Living organism" means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids;

(i) "Modern biotechnology" means 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;

Annex 2: Definition of a GMO in the EU

Article 2(2) of Directive 2001/18/EC (OJ L 106: 1-138) defines a GMO as:

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;

Further, Annex 1A, Part 1 defines the techniques through which a GMO is produced, and Part 2 techniques that do not produce a GMO.

Techniques of genetic modification referred to in Article 2(2)(a) are inter alia:
(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.

PART 2
Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex I B:

(1) in vitro fertilisation,
(2) natural processes such as: conjugation, transduction, transformation,
(3) polyploidy induction.

Article 3 and Annex 1B gives exemptions

Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are:

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

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