COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

BEZOEKADRES:
A. VAN LEEUWENHOEKLAAN 9
3721 MA BILTHOVEN

POSTADRES:
POSTBUS 578
3720 AN BILTHOVEN

TEL.: 030 274 2777
FAX: 030 274 4476
INFO@COGEM.NET
WWW.COGEM.NET

To the State Secretary for
Infrastructure and Water Management
Mrs S. van Veldhoven-van der Meer
P.O. Box 20901
2500 EX The Hague

| | |
|---|---|
| DATE | 21 March 2019 |
| REFERENCE | CGM/190321-02 |
| SUBJECT | Advisory report on the Dutch proposal for exempting certain GM plants |

Dear Mrs Van Veldhoven,

Further to a request by the Ministry of Infrastructure and Water Management for advice on the criteria in the Dutch proposal for discussion on amending Annex IB of Directive 2001/18/EC, COGEM notifies you of the following:

**Summary:**
The Netherlands has submitted a proposal for discussion within the EU on exempting genetically modified (GM) plants that meet certain conditions from the obligations under the GMO legislation on deliberate release into the environment (Directive 2001/18/EC). The intention of the proposal is to exempt those GM plants that have been produced using new breeding techniques and have a safety profile equivalent to traditionally bred plants.

COGEM was asked by the Ministry of Infrastructure and Water Management to advise on the criteria that GM plants should meet in order to be eligible for exemption. COGEM has previously reported that GM plants obtained by the use of certain techniques (such as site-directed mutagenesis and cisgenesis) are eligible for exemption because the risks they pose are comparable with those of traditionally bred plants. The Dutch proposal makes it possible to exempt these plants.

COGEM notes that the proposed criteria are ambiguous and could be interpreted in a way that includes the possibility of exempting GM plants that are not known in advance to be just as safe as traditionally bred plants. COGEM therefore proposes an amendment to the wording of the proposed criteria.

The attached report contains COGEM's advice and a discussion of the underlying reasoning.

Yours sincerely,

Professor Sybe Schaap
Chair of COGEM

c.c. Dr J. Westra, Head of the GMO Office
J.K.B.H. Kwisthout, Ministry of Infrastructure and Water Management

# Proposal for Amending the Exemption in the GMO Legislation: Additional criteria for exempting GM plants

**COGEM advisory report CGM/190321-02**

## 1. Introduction

Human and environmental safety is protected by legislation. As activities involving genetically modified organisms (GMOs) may involve risks, in the European Union (EU) these activities are regulated by various directives and regulations, which are in turn implemented in national legislation.

Since the entry into force of the GMO legislation, various new plant breeding techniques have been developed that blur the dividing line between genetic modification and conventional plant breeding. Some of these techniques lead to plants that are barely distinguishable, if at all, from plants that are obtained by traditional breeding methods. Various scientific advisory bodies, including COGEM, have indicated that the EU GMO legislation has therefore been rendered obsolete and should be amended. Discussions have been ongoing within the EU for some years on whether plants obtained by a range of new breeding techniques fall within the remit of the GMO legislation or whether they should be exempt from the regulations, but no decision has yet been made.

To initiate a discussion about the exemption of certain GM plants, in 2017 the Netherlands submitted a proposal for discussion in the European Union. The principle behind this proposal is that GM plants produced using new breeding techniques and which are at least as safe as traditionally bred plants should be exempt from the obligations under the GMO legislation.

The Dutch Ministry of Infrastructure and Water Management asked COGEM to advise on the exemption criteria described in the proposal. Given that the proposal is for exempting certain GM plants, COGEM has restricted its analysis of the proposed criteria to the GM plants that could be considered for exemption and to the environmental safety of these plants.

## 2. Proposal for extending the exemption to include certain GM plants

The cultivation and import of GM plants is regulated by the EU directive on 'deliberate release into the environment' (Directive 2001/18/EC). This directive contains the following definition of a genetically modified organism (GMO): *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.*[1]

Crops obtained by mutagenesis and protoplast fusion also meet this definition. These techniques were already being used before the relevant directive[a] came into effect and crops that had been obtained using these techniques were already on the market (some for many years) when the directive entered into force. To ensure that these crops would not have to meet the requirements set out in the GMO legislation, an exemption was included for organisms obtained by mutagenesis and protoplast fusion (between cells of plant species which can also exchange genetic material through traditional breeding methods).

The Dutch proposal is to extend the exemption from the obligations under the GMO legislation that has been given to plants obtained by conventional mutagenesis or protoplast fusion to include those GM plants that have been obtained by new breeding techniques and which have a safety profile equivalent to traditionally bred plants.

To this end, the Netherlands has made a proposal for amending Annex IB of Directive 2001/18/EC, which contains descriptions of the techniques that result in organisms that are exempted from the provisions of the legislation. The proposed amendment to this annex contains additional exemption criteria for certain GM plants.

The proposed new text for this annex is given in the text box below.

**Proposal**
**Annex I B**
**TECHNIQUES REFERRED TO IN ARTICLE 3**

I. Techniques of genetic modification as referred to in Article 3, to which this Directive shall not apply, shall only yield organisms resulting from the use thereof in as far as these organisms no longer contain recombinant nucleic acid molecules* that are used for or during modification and do not contain genetically modified organisms other than those produced by one or more of the techniques, methods or applications referred to in this annex.

II. As regards the non-applicability of this Directive to the techniques referred to in this Annex, any person deliberately releasing a genetically modified organism obtained with these techniques, shall, at the request of the Commission or a competent authority of a Member State, provide without undue delay a written justification as regards the fulfilment of the provisions of this Annex.

III. Without prejudice to the above conditions, techniques referred to in Article 3 are:

A) The following techniques, methods or applications thereof:
(1) conventional random mutagenesis methods using ionising radiation or mutagenic chemical agents;
(2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.

[a] Directive 90/220/EEC – Council Directive of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. This directive was repealed when Directive 2001/18/EC came into force.

B) Techniques, methods or applications thereof resulting in plants, provided that:
(1) no other genetic material is introduced into the resulting plant than genetic material from the same plant species or from a plant species with which it can exchange genetic material through traditional breeding methods; and
(2) recombinant nucleic acid molecules* that are used for or during modification are no longer present in the resulting plant that is meant for deliberate introduction into the environment.

IV. Every five years the Commission, following consultation with relevant stakeholders and in collaboration with the competent authorities of the Member States, shall review this Annex. The first review shall be completed by 1 January 2023.

* recombinant nucleic acid molecule: a molecule that is not generated by natural recombination and is generated by joining two or more nucleic acid molecules and which can replicate after its transfer into a living cell. It is created outside the cells through the formation of a new combination of genetic material or nucleic acid molecules.

## 3. Plants that meet the exemption criteria

Under the proposed criteria, several new plant breeding techniques will qualify for exemption from the provisions of the directive. In this section the risks associated with plants obtained by these techniques are compared with the risks associated with traditionally bred plants. Where COGEM has previously published advice on a technique, this advice is explained.

### *3.1 Site-directed mutagenesis (gene editing)*

Gene-editing techniques can be used to alter specific sequences in the genome of a plant. Small insertions and deletions can be made and base pairs can be changed (substitutions).[2,3,4] A gene-editing technique that has been much used in recent years is the CRISPR technology; other examples are oligo-directed mutagenesis, TALENS and zinc fingers.

Plant breeders have made use of conventional mutagenesis for many decades. Plants obtained by conventional mutagenesis[b] were therefore exempt from the obligations under the GMO legislation when this came into force. In conventional mutagenesis, numerous random and unknown mutations are induced throughout the plant genome. Gene-editing techniques, on the other hand, can be used to make a precisely targeted mutation (which could also occur spontaneously or be induced randomly by conventional mutagenesis). These techniques make fewer changes in the genome and therefore involve fewer risks than conventional mutagenesis.

In view of this, COGEM has previously advised that plants obtained by site-directed mutagenesis should be exempt from the GMO legislation – just like plants produced using conventional mutagenesis.[5,6,7,8] The reason for this advice is that plants obtained by different mutagenesis techniques are hard to

[b] conventional mutagenesis: inducing genomic changes by exposing plants, plant parts or plant cells to ionising radiation or chemical mutagens.

distinguish one from another, if at all, and so COGEM questions the current situation in which some plants are regulated and others with the same mutations are not, depending on the technique used in each case.

Although some of these gene-editing techniques make use of recombinant nucleic acid molecules, these molecules are no longer present in the resulting plant. The risks associated with the resulting plants are no greater than those of traditionally bred plants.

### *3.2 Techniques in which no DNA is inserted into the resulting plant*

Various new breeding techniques, such as reverse breeding[5] and agroinoculation[5] make use of genetic modification, but do not alter the genome sequence of the resulting plants and the resulting plants do not contain any new DNA sequences. The resulting plants have no new traits and they pose the same level of risk as traditionally bred plants. COGEM has previously stated that from a risk point of view there is no reason for such plants to fall within the remit of the GMO legislation.

### *3.3 Cisgenesis*

Plants produced by cisgenesis are modified using DNA taken from the same species or from a crossable species and which can also be introduced into the plant by traditional breeding methods. The coding sequences are controlled by their own regulatory signals (promoter and terminator) and must contain their own introns (if present). The DNA is derived entirely from the donor plant and does not consist of multiple fragments.

It is now technically possible to insert DNA at a specific site in the genome. Although this technique is not very efficient yet, the literature contains reports of successful applications in various plant species (including maize, wheat and tomato).[9,10,11] The technique can be used to insert an extra gene at a specific site in the DNA or replace a gene with a gene from a different species (gene replacement).

Many of the methods currently being used to modify plants insert the desired DNA at a random location within the genome. This may alter the expression of a gene already present in that location or inactivate a gene. The introduced DNA could also fuse with a gene already present in the plant genome. In theory, the fusion of different genes (or fragments of genes) can lead to the production of new proteins.

Cisgenic plants cannot obtain any traits derived from the introduced DNA that are not or could not be present in the species or in a crossable species. In principle, any genes created by the fusion of genes or gene fragments, as described above, could arise naturally or through traditional plant breeding.

The degree to which the inserted genes are expressed can vary, depending on the site of insertion. However, because the inserted genes are controlled by their own regulatory signals, the level of expression will remain within the range of expression levels found in traditionally bred plants.

COGEM has previously advised on cisgenic plants, reporting that cisgenic plants present no greater risk to human health and the environment than traditionally bred plants and therefore could be exempted from the GMO legislation.[6,12]

*Transformation methods*

The bacterium *Agrobacterium tumefaciens* is often used to genetically modify plants. In addition to the desired DNA sequence, this bacteria usually also inserts T-DNA border sequences which are of bacterial origin. GM plants that contain bacterial T-DNA border sequences do not meet the proposed criteria and under the Dutch proposal are not eligible for exemption.

In *A. tumefaciens* transformations, the T-DNA border sequences are sometimes by chance not inserted.[i] It is therefore possible to select GM plants that only contain the desired DNA sequences. Also, instead of using the standard bacterial vector with T-DNA border sequences, it is possible to use an 'intragenic' vector with P-DNA border sequences composed of plant sequences.[ii,iii]

In addition, there are other methods for genetically modifying plants that do not involve the insertion of sequences other than the desired sections of DNA. These include the particle bombardment method, in which tiny metal particles are coated with DNA and fired into the plant cells using a 'gene gun' (biolistic particle delivery system), and the more recent magnetofection method in which a magnetic field is used to deliver magnetic nanoparticles coated with DNA into pollen grains.[iv] The CRISPR technology can also be used to genetically modify plants without unwanted sequences being inserted as well.[v]

[i] Zhu S *et al.* (2013). Vector integration in triple *R* gene transformants and the clustered inheritance of resistance against potato late blight. Transgenic Research 22: 315-325

[ii] Conner AJ *et al.* (2007). Intragenic vectors for gene transfer without foreign DNA. Euphytica 154: 341-353

[iii] Zare B *et al.* (2017). United States Patent application publication. Binary vectors with minimized biosafety concerns and high transformation rates by engineered plant-derived transfer-DNA. US 2017/0260537 A1 https://patents.google.com/patent/US20170260537A1 (accessed: 21 December 2018)

[iv] Zhao X *et al.* (2017). Pollen magnetofection for genetic modification with magnetic nanoparticles as gene carriers. Nature Plants 3(12): 956-964

[v] Soyars CL *et al.* (2018). Cutting edge genetics: CRISPR/Cas9 editing of plant genomes. Plant & Cell Physiology 59(8): 1608-1620

## 4. Plants potentially eligible for exemption

COGEM realises that on closer examination the criteria as stated in the proposal can be interpreted in several ways, offering either more or less leeway to exempt GM plants than may have been intended or is desirable.

**Criteria for exemption**

B) Techniques, methods or applications thereof resulting in plants, provided that:

(1) no other genetic material is introduced into the resulting plant than genetic material from the same plant species or from a plant species with which it can exchange genetic material through traditional breeding methods; and

(2) recombinant nucleic acid molecules that are used for or during modification are no longer present in the resulting plant that is meant for deliberate introduction into the environment.

One interpretation of the proposed criteria is that GM plants eligible for exemption may contain inserted DNA sequences that are derived from plant species with which the plant in question is able to exchange genetic material through traditional breeding methods, and also may contain induced mutations. Any recombinant nucleic acid molecules used to induce mutations must not remain in the resulting plant.

If the criteria are interpreted in this way, besides the GM plants described in the previous section, several other types of GM plants could also be eligible for exemption because the introduction of combinations of sequences is not explicitly excluded. This is explained further in the following two sections.

*Unambiguous legal terms*

It is difficult to describe and define biological processes and scientific developments using unambiguous legal terms and criteria. The definition of recombinant nucleic acid molecules used in the proposal illustrates this difficulty well. It is based on a proposal by the European Commission and the response to the proposed definition by the EFSA.

*Recombinant nucleic acid molecule: a molecule that is not generated by natural recombination and is generated by joining two or more nucleic acid molecules and which can replicate after its transfer into a living cell. It is created outside the cells through the formation of a new combination of genetic material or nucleic acid molecules.*

Recombinant nucleic acid molecules that cannot replicate in a cell, such as naked DNA, are not covered by this definition. From this we may conclude that activities in which use is made of such recombinant nucleic acid molecules are not regulated under the GMO legislation. Nevertheless, in the Netherlands consent is still required for such activities.

### *4.1 Intragenesis*

Plants produced by intragenesis, just like cisgenic plants, are modified with DNA sequences derived from plant species with which they can exchange genetic material through traditional breeding methods. However, in intragenic plants the regulatory signals of the inserted genes can be replaced by the regulatory signals of other genes, as long as these are derived from crossable species.[13]

A gene's regulatory signals determine the place, time and degree of expression. The expression of a gene can be changed by replacing the regulatory signals (especially the promoter) with other regulatory signals or by adding regulatory signals. Numerous plant promoters with different expression profiles are known that can be used to change the expression of a gene.[14]

Genes under the control of other regulatory signals can be expressed at different times, in other parts of the plant and at different levels than is normally the case. An example of this are maize plants in which the promotor of the *ARGOS8* gene was replaced with a promoter of a different maize gene (*GOS2*), leading to a level of expression higher than that in 400 conventional breeding lines. The resulting maize plants produced more ARGOS8 protein and were less susceptible to drought.[15]

At the moment it is not known whether or not the risks associated with intragenic GM plants are within the range of traditionally bred plants or exceed that level of risk. COGEM intends to look into this further and provide advice on this subject at a later stage.

### *4.2 Modification with chimeric genes or chimeric regulatory signals*

Plants can also be modified with a chimeric DNA fragment composed of different DNA sequences derived from plant species with which they can exchange genetic material through traditional breeding methods.

Parts of genes and/or regulatory signals can be combined with the intention of creating a chimeric gene that produces the desired protein or creating a chimeric promoter responsible for the desired expression profile. Some examples of chimeric genes and chimeric promoters can be found in the literature. For example, the chimeric SlP14a-PPC20 protein,[c] coded for by a new combination of DNA sequences from tomato, has been expressed in GM tomato plants, conferring resistance to the plant pathogenic bacterium *Ralstonia solanacearum*.[16] In addition, elements of the promoters of various rice genes were used to create a new chimeric promoter which expresses a gene specifically in the green parts of the rice plant.[17] The above examples give an impression of the possible changes that can be made. Incidentally, these GM plants do not fully meet the proposed criteria for exemption because besides the sequences described they also contain some other sequences.

In addition, it is also possible to randomly create new genes or regulatory signals. 'DNA shuffling' is a method for recombining different variants of a single gene, or different genes, in vitro. These are then

[c] SlP14a-PPC20: a chimeric protein consisting of a fusion of the pathogenesis-related P14a protein with the PPC20 alpha-helix antimicrobial peptide. Both the P14a and the PPC20 are produced by the tomato plant.

screened to determine if one of the new chimeric genes expresses the desired traits. If so, this new chimeric gene can be inserted into the plant genome.

DNA shuffling can be used to make new combinations of genes from different plant species. The DNA shuffling technique was used on cDNA from *GST* genes of the common bean (*Phaseolus vulgaris*) and of the soy bean (*Glycine soja*), which were expressed under abiotic stress conditions, to create a new *GST* gene that may function better under certain stress conditions.[18]

The method can also be used to make new variants of a gene. A new gene variant of the bacterial *cry1Ac* gene obtained this way was found to be more toxic to the pest insect beet armyworm (*Spodoptera exigua*), and other variants are toxic for more pest insects (*S. exigua* and *Heliothis zea*).[19]

Creating chimeric genes and changing regulatory signals are much quicker ways of obtaining new proteins and changing expression profiles than is possible in traditional plant breeding. It is not yet known whether or not the risks of plants modified in this way are the same as those of traditionally bred plants.

## 5. Reasoning and advice

The GMO legislation exists to protect human health and the environment from the potential risks of GMOs. Under the proposed exemptions human and environmental safety remains safeguarded. The intention of the Dutch proposal is to extend the exemption from the obligations under the GMO legislation to include those GM plants that have been obtained by new breeding techniques and have a safety profile equivalent to traditionally bred plants.

***Amendment to the criteria***

In the view of COGEM, the criteria for exempting GM plants as proposed are not categorical enough. They are ambiguous and could be interpreted in such a way that exemption is also possible for GM plants for which it is not known in advance that they are just as safe as traditionally bred plants, such as GM plants in which chimeric plant sequences have been inserted.

COGEM thinks that incorrect interpretation of the criteria can be prevented by amending the wording as follows:

*B) Techniques, methods or applications thereof resulting in plants, provided that:*

*(1) no genetic material is introduced into the resulting plant <u>other than non-recombinant DNA sequences</u> derived from the same plant species or from a plant species with which it can exchange genetic material through traditional breeding methods; and*

*(2) recombinant nucleic acid molecules that are used for or during modification are no longer present in the resulting plant that is meant for deliberate introduction into the environment.*

An additional condition has been added to criterion B1 stating that only non-recombinant DNA sequences[d] may be inserted. This addition removes the possibility of exempting GM plants with a combination of DNA sequences that do not occur next to each other in nature. Intragenic plants and chimeric plant sequences must then continue to satisfy the conditions set out in the GMO regulations.

GM plants with non-recombinant sequences, such as cisgenic plants, are not affected by this amendment and remain eligible for exemption. COGEM points out that it may be desirable to introduce multiple genes, for example to obtain a GM plant with multiple resistance genes. Under the proposed amendment it also remains permissible to insert several such genes (at the same time or separately), as long as these genes are intact and come from plant species with which the plant being modified is able to exchange genetic material through traditional breeding methods.

COGEM is of the opinion that with this amendment to the proposed criteria human and environmental safety is safeguarded, while at the same time permitting room for innovation.

## 6. References


1. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC
2. Zong Y *et al.* (2017). Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nature Biotechnology 35(5): 438-440
3. Shimantani Z *et al.* (2017). Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nature Biotechnology 35(5): 441-443
4. Li C *et al.* (2018). Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. Genome Biology 19:59
5. COGEM (2006). New techniques in plant biotechnology. COGEM report. CGM/061024-02
6. COGEM (2009). Adviserende brief onderzoeksrapport 'nieuwe veredelingstechnieken'. CGM/091222-01
7. COGEM (2014). CRISPR-Cas – Revolution from the lab. COGEM report and advice CGM/141030-01
8. COGEM (2017). Advies CRISPR-Cas en gerichte mutagenese bij planten. CGM/170308-01
9. Gil-Humanes J *et al.* (2017). High-efficiency gene targeting in hexaploid wheat using DNA replicons and CRISPR/Cas9. The Plant Journal 89: 1251-1262
10. Čermák T *et al.* (2015). High-frequency, precise modification of the tomato genome. Genome Biology doi 10.1186/s13059-015-0796-9
11. Svitashev S *et al.* (2015). Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiology 169(2): 931-945


[d] In its previous advice on the status of the oligonucleotides in the context of site-directed mutagenesis, COGEM explains how it defines 'recombinant'. COGEM (2010). Advisory and Policy Report CGM/100701-03.

12. COGEM (2006). Vereenvoudiging van regelgeving bij genetische modificatie met planteigen genen, cisgenese, een reële optie? COGEM signalering CGM/060428-05
13. Nuccio M *et al.* (2015). Chapter 2 – Plant trait gene expression cassette design. Azhakanandam K *et al.* (eds.). Recent advancements in gene expression and enabling technologies in crop plants. Springer Science+Business Media, doi 10.1007/978-1-4939-2202-4_2
14. Dutt M *et al.* (2014). Temporal and spatial control of gene expression in horticultural crops. Horticulture Research 1: 14047, doi 10.1038/hortres.2014.47
15. Shi J *et al.* (2017). ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnology Journal 15: 207-216
16. Morais TP *et al.* (2019). The plant-based chimeric antimicrobial protein SlP14a-PPC20 protects tomato against bacterial wilt disease caused by *Ralstonia solanacearum.* Plant Science 280: 197-205
17. Wang R *et al.* (2015). Novel green tissue-specific synthetic promoters and *cis*-regulatory elements in rice. Scientific reports 5: 18256 doi: 10.1038/srep18256
18. Chronopoulou EG *et al.* (2018). Expanding the Plant GSTome through directed evolution: DNA shuffling for the generation of new synthetic enzymes with engineered catalytic and binding properties. Frontiers in Plant Science 9: 1737
19. Lassner M & Bedbrook J (2001). Directed molecular evolution in plant improvement. Current Opinion in Plant Biology 4: 152-156