COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

VISITING ADRESS:
A. VAN LEEUWENHOEKLAAN 9
3721 MA BILTHOVEN

POSTADRES:
POSTBUS 578
3720 AN BILTHOVEN

TEL.: 088 689 2777
INFO@COGEM.NET
WWW.COGEM.NET

To the Minister for the Environment
Mr. M.G.J. Harbers
P.O. box20901
2500 EX The Hague

**DATE** 17 June 2024
**REFERENCE** CGM/240617-01
**SUBJECT** Advice on the EC decision on the GMO status of viral replicon particles (public version)

Dear Mr Harbers,

The European Commission (EC) has made a decision on the status of viral replicon particles (VRPs). The EC states that VRPs are not genetically modified organisms (GMOs). However, VRPs are derived from viruses that are genetically modified to express a foreign gene in animal or human cells. They can be used for various applications, including vaccines. The EC's decision means that applications for placing on the market of products containing, consisting of or produced from VRPs do not have to be accompanied by an environmental risk assessment. In this letter COGEM points out that this decision raises ambiguities, lacks a sound scientific and legal basis and has negative implications for public safety and public acceptance.

**1. Background information on viral replicon particles (VRPs)**

VRPs are a subset of the broader group of replicons and are obtained by genetically modifying viruses. The virus genome (RNA or DNA, depending on the virus) is packaged inside particles. Viruses can infect cells, replicate in the cell, make new virus particles and spread to other cells. In contrast, after VRPs have infected a cell they are unable to make any new virus particles. This is because the genes that code for the proteins that make up the virus particle (the structural proteins) have been deleted from the viral genome. The viral genome still contains the genes that code for the proteins responsible for the replication of the viral genome in the cell. VRPs are produced by providing the missing structural genes *in trans* in order to package the viral genome inside the structural proteins. This enables the produced VRPs to infect cells (in animals or humans). Following infection, the genes in the viral genome are transcribed, resulting in the replication of this viral genome, which allows it to remain present in the cells for long periods. However, no new virus particles can be formed because the viral structural genes required for this are no longer present in the genome. This means that in principle no more cells can be infected.

VRPs and other replicons are used to express foreign genes (genes from an organism other than the infected organism, also called transgenes) in cells and can be used as vaccines. The transgenes are inserted into the site of the deleted structural genes in the viral genome. Whether the viral genomes in the VRPs can or cannot spread to other cells depends, among other things, on the introduced genes.

## 2. The decision by the European Commission

The EC stated that VRPs do not meet the definitions of an 'organism' and 'genetically modified organism' as included in Directive 2001/18 on the deliberate release into the environment of GMOs.[1] The definitions in this Directive:

1. *'organism' means any biological entity capable of replication or of transferring genetic material;*
2. *'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;*

In summary, the EC states that VRPs are not organisms because after infecting a host cell no new virus particles are formed and that therefore no new infections of other cells can take place. The EC is of the opinion that viral genetic material, either RNA or DNA, on itself is not an organsim. It is not clear whether or not this line of reasoning also applies to other replicons, such as naked replicons or self-amplifying mRNAs which consist of the modified viral genome without a surrounding virus particle as in a VRP.

## 3. COGEM's analysis

COGEM considers that the decision by the EC lacks a scientific basis and is inconsistent with other organisms and biological entities, such as viroids, certain wild-type viruses (which are legally considered to be organisms) and replication-deficient viral vectors (which are considered to be GMOs). COGEM also considers the interpretation by the EC to be legally untenable. These points are explained further below.

### *3.1 A VRP is obtained by genetically modifying a virus*

VRPs are obtained by genetically modifying viruses and meet the legal definition of a GMO, as do replication-deficient viral vectors (see also section 3.3).

In Directive 2001/18[a] a GMO is defined 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.*[1] VRPs are viruses that have been genetically modified by deleting the genes that code for the structural proteins of the virus particle, into which transgenes have been inserted.

---

[a] Directive 2001/18 on the deliberate release into the environment of genetically modified organisms, such as clinical trials and field testing.

According to Directive 2009/41[b], viruses are microorganisms: *any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, and animal and plant cells in culture.*[2] VRPs can therefore be considered to be modified ('stripped down') viruses and therefore GMOs.

The EC states that VRPs are not organisms, and can therefore not be GMOs, because they can no longer form virus particles. To justify this, the Commission refers to the definition of an organism as described in Directive 2001/18: *an organism means any biological entity capable of replication or of transferring genetic material.*[1] However, a VRP is an entity, something that has a real existence, and it can by definition replicate because it contains the genes that code for the proteins responsible for the replication of the viral genome. This is how the modified viral genome replicates in the infected host cell. Moreover, there is a 'transferring of genetic material': VRPs consist of genetic material and the infection of the host cells involves the insertion of the viral genome, which is used to express transgenes in these host cells. VRPs therefore meet the definition of an organism in Directive 2001/18 and consequently they are GMOs.

### *3.2 The decision is inconsistent with the legal status of viroids and certain viruses*

The EC states that the virus particle is the organism and that the viral genetic material on its own is not an organism. This decision is inconsistent with the legal status of viroids and certain viruses. Viroids and some viruses do not form virus particles, and yet in Directive 2009/41/EC they are still considered to be organisms.[2]

Viroids are small circular replicating RNA molecules that do not form particles and contain no genes.[3] Viroids can infect plants and cause economically important diseases in crops.[4] There are also viruses (including the family *Endornaviridae*) that do not form virus particles. They are found as infectious RNA genomes associated with cytoplasmic membrane vesicles, which are similar to VRPs.[5,6] The Hepatitis D virus consists of an infectious DNA genome packaged inside the proteins of another virus (Hepatitis B virus).[7] According to the EC's reasoning, these viruses and viroids should not be organisms because they cannot form virus particles. This contradicts, among others, Directive 2009/41/EC, which states that viroids are organisms.

### *3.3 The decision is inconsistent with replication-deficient viral vectors*

The EC considers that VRPs are not GMOs because genetic material *in itself* cannot be a GMO and no new virus particles are formed. On the other hand, viral vectors incapable of replication (replication-deficient) that are used as gene therapies do fall within the scope of the GMO legislation, for example for marketing authorisation. According to the EC's line of reasoning, replication-deficient viral vectors should also be considered not to be GMOs.

---

[b] Directive 2009/41 on the contained use of genetically modified microorganisms, such as laboratory activities.

Replication-deficient viral vectors frequently used for research or as gene therapies are derived from the adeno-associated virus (AAV). The AAV has a DNA genome that can only replicate in cells infected with another DNA virus, which functions as a helper virus (usually adenoviruses or herpes viruses).[8,9,10,11] In vectors derived from AAVs, all genes have been removed and replaced with transgenes and only the ends of the original virus genome still remain. When an AAV vector infects a cell it can no longer replicate or form a virus particle because all the AAV genes have been deleted. Even in the presence of a helper virus, they are still unable to replicate.

Other frequently used replication-deficient vectors are derived from adenoviruses. The areas deleted from the genome of these adenoviral vectors include, at the minimum, the genes essential for replication. As a result, the vectors are incapable of independent replication and cannot form new virus particles.[12,13,14] Vaccines that consist of adenoviral vectors, such as the AstraZeneca and Janssen COVID-19 vaccines, have already been designated as GMOs.[15,16]

Some replication-deficient viruses are derived from lentiviruses (often HIV-1) and gammaretroviruses. These lentiviral and retroviral vectors are often used in the development of gene therapies because they can insert their genome into the genome of the infected cell.[17,18]

The above-mentioned AAVs and adenoviral, lentiviral and retroviral vectors cannot replicate, even within a cell. These vectors do fall within the scope of the GMO legislation, in contrast to the decision by the EC on the status of VRPs.

### *3.4 It is not clear whether or not the decision is legally tenable*

It is not clear whether or not the decision by the EC is legally correct or tenable. As described in the sections above, the decision is not consistent with the definition of a GMO in Directive 2009/41.[2] In addition, it is also inconsistent with the status of other viruses, viroids and replication-deficient viral vectors. The decision also appears to contradict one of the definitions of techniques for genetic modification, as defined in Annex I A of Directive 2001/18:

*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.*

The decision by the EC appears to assume that this definition does not apply because VRPs are incapable of 'continued propagation'. A VRP is by definition capable of replication and therefore of 'propagation'. Moreover, in some cases VRPs can infect other cells in the same host organism (see also section 4.1).[19,20] This means that the decision by the EC that VRPs are not GMOs would appear to contradict Directive 2001/18. In this situation, the legal status of VRPs can only be determined by the European Court of Justice.

## 4. The decision may have implications for environmental risks, the legislation and public acceptance

The EC's decision that VRPs are not GMOs has various implications. It means that applications to the European Medicines Agency (EMA) for placing on the market of products containing VRPs would no longer have to be accompanied by an environmental risk assessment. COGEM points out that activities involving VRPs can involve environmental risks, which may be overlooked in the absence of a risk assessment. In addition, this decision may have implications for the national legislation and what are considered to be GMOs under Dutch law. Finally, the decision may have implications for public acceptance of activities involving GMOs. The potential consequences of the EC's decision are discussed in more detail below.

### *4.1 Possible environmental risks associated with the use of VRPs are not assessed*

The decision by the EC means that applications for marketing authorisation would no longer have to be accompanied by an environmental risk assessment. COGEM notes that if something is not recognised as a GMO, it does not mean that there are no environmental risks.

VRPs lack essential genes, which prevents them from independently producing the structural proteins needed to form virus particles. If similar genes (or gene fragments) are present in the cells, for example due to an infection with another virus, the deleted traits may be compensated for by complementation and as a consequence the VRP genomes would be able to spread to other cells. In addition, the deleted traits in the VRP genome can be restored by recombination with other viruses, which could lead to the formation of a modified virus capable of spreading. In the absence of an environmental risk assessment, the likelihood of and possible risks arising from complementation and recombination are not assessed. The deleted traits in VRPs can also be partially compensated for by some transgenes. This means that the transgene used has an influence on the safety and nature of the VRP. However, this aspect does not appear to have been considered in the position taken by the EC.

According to the EC's reasoning, not only VRPs, but other viral vectors as well are not GMOs if they cannot spread. How this non-spreading trait (phenotype) arises is not defined. This phenotype can even be created by inducing small deletions (or point mutations) in the viral genome. The decision by the EC ignores the possibility the deleted functions in these viral vectors can also be restored. For example, deletions may be restored by recombination with wild-type viruses or by complementation of the transgene, which would then permit these viral vectors to spread. Induced point mutations can sometimes back mutate. These are scenarios that are investigated in an environmental risk assessment.

The decision by the EC takes no account of the formation of virus-like vesicles (VLVs). VLVs are membrane vesicles containing the VRP genome and they can arise during replication of the viral genome. VLVs can infect cells and spread to other cells.[20] Infectious VLVs can be created by complementation, for example when the transgene consists of sequences that code for envelope proteins of other viruses. Infectious VLVs may also be created if only one structural gene is deleted from the viral genome. The VRP genome can be transmitted to other cells within the organism via infectious VLVs.[21,22]

Research was carried out in 2022 for COGEM on viral replicons – such as VRPs – that are derived from positive-stranded or negative-strand RNA viruses.[23] The research results indicate that a generic environmental risk assessment for replicons other than VRPs derived from alphaviruses and flaviviruses is not possible owing to insufficient scientific data.[23] This means that in the absence of an environmental risk assessment for all possible VRPs, adverse consequences for humans and the environment cannot be ruled out.

### *4.2 The decision may have implications for the legislation*

The EC's decision is not in agreement with the Dutch interpretation of what a GMO is. In the Netherlands, VRPs have so far fallen under the GMO legislation. This inconsistency between the EU and the Dutch interpretation is not welcome. It is to be expected that the EC's decision will have consequences for work with VRPs in Dutch laboratories. Because too little is known about the risks – see above – COGEM is of the opinion that excluding VRPs from the legislation on safe working with GMOs can lead to risky situations.

### *4.3 The decision may have implications for public acceptance*

The EC's decision raises questions about the definition of an organism. A VRP is obtained by genetically modifying a virus by deleting genes. It is not clear whether deleting essential structural genes from the genome of other organisms by genetic modification would result in them no longer being organisms. The decision by the EC may have an influence on people's perception of genetic modification. If the EC's line of reasoning does not align with society's perception of an organism and a GMO, this may have consequences for the level of public acceptance of these applications. In addition, it may raise the question of whether the safety of the activities and products concerned are adequately assessed and guaranteed.

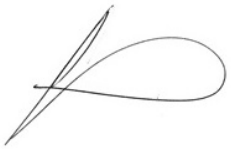
## 5. Conclusion

COGEM observes that the EC's decision implies that environmental risk assessments will no longer be needed for applications for placing on the market of VRPs, while risks to human health and the environment cannot be ruled out. COGEM points out that it cannot have been the intention of the legislature that genetic modification of an organism could lead to a situation in which it no longer falls within the scope of the GMO legislation, while at the same time there may be residual risks to human health and the environment.

COGEM therefore considers that the EC's decision does not have a sound scientific basis, raises ambiguities and may have adverse consequences regarding environmental risks and public acceptance.

Furthermore, COGEM highlights the need for consultation and coordination between the EC and the EU member states on the status of VRPs.

Yours sincerely,



Professor Sybe Schaap
Voorzitter COGEM

c.c.

- Y. de Keulenaar, head of the GMO Office
- Ministry of Infrastructure and Water Management, Environmental Safety and Risks Directorate, Directorate-General for the Environment and International Affairs
- Dr. M.M.C. Gielkens, Gene Therapy Office
- Dr. K.R.J. Vanmolkot, Central Committee on Research Involving Human Subjects
- K.E. Kok, Ministry of Health, Welfare and Sport

## 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. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02001L0018-20210327 (accessed 17/06/2024)
2. Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009. On the contained use of genetically modified micro-organisms. Official Journal of the European Union https://eur-lex.europa.eu/eli/dir/2009/41/oj (accessed 17/06/2024)
3. Wang Y (2021). Current view and perspectives in viroid replication. Curr. Opin. Virol. 47: 32–37
4. Tsagris EM *et al.* (2008). Viroids. Cell. Microbiol.10: 2168–2179
5. Valverde RA *et al.* (2019). ICTV Virus Taxonomy Profile: Endornaviridae. J. Gen. Virol. 100: 1204–1205
6. Lefebvre A *et al.* (1990). The double-stranded RNA associated with the '447' cytoplasmic male sterility in Vicia faba is packaged together with its replicase in cytoplasmic membranous vesicles. Plant Mol. Biol. 14: 477–490
7. Thiyagarajah K *et al.* (2023). Cellular factors involved in the hepatitis d virus life cycle. Viruses 15: 1687
8. Berns KI & Parrish CR (2013). Parvoviridae. In: Fields virology, volume 2, 6th edition. Edited by Knipe DM en Howley PM. Lippincott Williams & Wilkins. Philadelphia
9. Cotmore SF *et al.* (2019). ICTV Virus Taxonomy Profile: Parvoviridae. J. Gen. Virol. 100: 367–368
10. Zinn E & Vandenberghe LH (2014). Adeno-associated virus: fit to serve. Curr Opin Virol. 8: 90–97
11. ICTV report: Dependoparvovirus https://ictv.global/report/chapter/parvoviridae/parvoviridae/dependoparvovirus (accessed 17/06/2024)
12. Wold WSM & Ison MG (2013). Chapter 56 Adenoviruses. In: Fields virology, volume 2, sixth edition. Ed. Knipe DM et al., Lippincott Williams & Wilkins. Philadelphia
13. Bergmans JEN *et al.* (2017). Omlaagschaling van werkzaamheden met replicatie-defectieve AAV- en adenovirale vectoren. COGEM rapport CGM 2017-4 [In Dutch]
14. Kovesdi I. & Hedley SJ (2010). Adenoviral producer cells. Viruses 2: 1681–1703
15. European Medicines Agency. Vaxzevria (previously COVID-19 Vaccine AstraZeneca). https://www.ema.europa.eu/en/medicines/human/EPAR/vaxzevria-previously-covid-19-vaccine-astrazeneca#ema-inpage-item-product-info
16. European Medicines Agency. Jcovden (previously COVID-19 Vaccine Janssen). https://www.ema.europa.eu/en/medicines/human/EPAR/jcovden-previously-covid-19-vaccine-janssen
17. Goff SP (2013). Retroviridae. In: Fields virology, volume 2, sixth edition. Ed. Knipe DM et al. Lippincott Williams & Wilkins, Philadelphia
18. Freed EO & Martin MA (2013). Human Immunodeficient Viruses: Replication. In: Fields virology, volume 2, sixth edition. Ed. Knipe DM et al. Lippincott Williams & Wilkins, Philadelphia
19. COGEM (2023). Inschaling werkzaamheden met VEEV-replicondeeltjes met structurele genen van retrovirussen BLV en FeLV. COGEM advies CGM/231019-01 [In Dutch]

20. Rose NF *et al*. (2014). In vitro evolution of high-titer, virus-like vesicles containing a single structural protein. Proc. Natl. Acad. Sci. U.S.A 111: 16866–16871
21. Ruiz-Guillen M *et al*. (2016). Capsid-deficient alphaviruses generate propagative infectious microvesicles at the plasma membrane. Cell Mol. Life Sci. 73: 3897–3916
22. Jia F *et al.* (2017). Pseudo-typed Semliki Forest virus delivers EGFP into neurons. J. Neurovirol. 23: 205–215
23. Van der Meulen K & Rüdelsheim PLJ (2022). Viral replicon systems and their biosafety aspects. COGEM report CGM/2022-06