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To the Minister of
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**DATE** 21 March 2018

**REFERENCE** CGM/180321-01

**SUBJECT** Advice on the pathogenicity classification of AAVs

Dear Mrs Van Veldhoven,

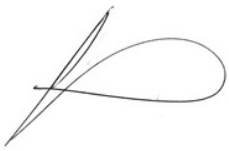
Further to a request for advice concerning the dossier IG 18-028_2.8-000 titled 'Production of and activities involving recombinant adeno-associated virus (AAV) vectors with modified capsids and capsids of wild type AAV serotypes absent from Appendix 4', submitted by Arthrogen B.V., COGEM hereby notifies you of the following:

**Summary:**
COGEM was asked to advise on the pathogenicity classification of five adeno-associated viruses (AAVs): AAV10, AAV11, AAV12, AAVrh10 and AAVpo1. More than 100 AAVs have been isolated from various hosts. AAVs can infect vertebrates, including humans (95% of people have at one time or another been exposed to an AAV). AAVs are dependent on a helper virus to complete their life cycle. In the absence of a helper virus, the AAV genome persists in a latent form in the cell nucleus. So far no mention has been made in the literature of disease symptoms caused by an infection with AAVs. Neither have there been any reports of pathogenicity of AAV10, 11, 12, AAVrh10 or AAVpo1. AAV vectors are regularly used in clinical studies.

Given the above information, COGEM recommends assigning AAV10, AAV11, AAV12, AAVrh10 and AAVpo1 as non-pathogenic to pathogenicity class 1. Based on the ubiquity of AAVs and the lack of evidence of pathogenicity, COGEM recommends that all AAVs belonging to the species *Adeno-associated dependoparvovirus A* and *Adeno-associated dependoparvovirus B* should be assigned to pathogenicity class 1.

The grounds on which COGEM has reached its conclusions and the resulting advice are set out in the enclosed report.

Yours sincerely,



Professor Sybe Schaap
Chair of COGEM

c.c. H.P. de Wijs, Head of the GMO Office
J.K.B.H. Kwisthout, Ministry of Infrastructure and Water Management

# *Adeno-associated dependoparvovirus A* and *Adeno-associated dependoparvovirus B* assigned to pathogenicity class 1

## COGEM report CGM/180316-01

### 1. Introduction

Further to a licence application from Arthrogen B.V. (IG 18-029), COGEM was asked to advise on the pathogenicity classification of five adeno-associated viruses (AAVs): AAV10, AAV11, AAV12, AAVrh10 and AAVpo1.

### 2. Pathogenicity classification under the GMO Regulation

The GMO legislation requires that the pathogenicity classification takes into account the risks to human health and the environment. The Ministerial Regulation on Genetically Modified Organisms (GMO Regulation)[1] assigns microorganisms to one of four pathogenicity classes. This classification runs from pathogenicity class 1, which includes non-pathogenic microorganisms, to pathogenicity class 4, the group of highly pathogenic microorganisms. Each pathogenicity class is coupled with a containment level for activities involving GMOs in that class.

Non-pathogenic microorganisms are assigned to pathogenicity class 1. Such microorganisms must meet at least one of the following criteria:

a) the microorganism does not belong to a species of which representatives are known to be pathogenic to humans, animals or plants;
b) the microorganism has a long history of safe use under conditions involving no special containment and control measures;
c) the microorganism belongs to a species which contains representatives of class 2, 3 or 4, but the strain in question contains no genetic material responsible for the virulence;
d) the non-virulent nature of the microorganism has been demonstrated in adequate tests.

Pathogenicity class 2 is for microorganisms that can cause a disease in humans or animals which is unlikely to spread throughout the population and for which an effective prophylaxis, treatment or control measure is available, as well as for microorganisms that can cause a disease in plants.

Pathogenicity class 3 is for microorganisms that can cause a serious disease in humans or animals which is likely to spread throughout the population and for which an effective prophylaxis, treatment or control measure is available.

Pathogenicity class 4 is for microorganisms that can cause a very serious disease in humans, animals or plants which is likely to spread throughout the population and for which no effective prophylaxis, treatment or control measure is available.

## 3. Taxonomy

Adeno-associated viruses (AAVs) belong to the *Parvoviridae* family and have been assigned to various species in the genus *Dependoparvovirus*.[2] So far more than 100 different AAVs have been isolated from a variety of hosts, such as humans, simians, goats, bats, sea lions, birds and cows. According to the current taxonomic classification (ICTV 2017), 23 AAVs have been assigned to the genus *Dependoparvovirus*.[3] The other AAVs have not yet been classified. *Adeno-associated dependoparvovirus A* is the type species of the genus and contains AAV1-4, AAV6-13 and AAV-S17. *Adeno-associated dependoparvovirus B* contains AAV5, Bovine adeno-associated virus (BAAV) and Caprine adeno-associated virus (CapAAV). Besides these two species, the genus also contains the species *Anseriform dependoparvovirus 1* (contains Duck parvovirus, DPV; Goose parvovirus, GPV; and Goose parvovirus-PT, GPV2), *Avian dependoparvovirus 1* (contains Avian adeno-associated virus, AAAV), *Chiropteran dependoparvovirus 1* (contains Bat adeno-associated virus, BtAAV), *Pinniped dependoparvovirus 1* (contains California sea lion adeno-associated virus, CslAAV), and *Squamate dependoparvovirus 1* (contains Snake adeno-associated virus, SAAV).

## 4. Adeno-associated viruses

For *in vivo* replication, AAVs depend on co-infection with a helper virus, usually an adenovirus or in some cases a herpes virus.[4,5] This characteristic sets AAVs apart from other viruses within the *Parvoviridae* family.[6] Without the presence of a helper virus the viral genome will not replicate, resulting in a persistent 'infection' in which the AAV genome remains latent in the cell nucleus as an intrachromosomal or extrachromosomal episome.[5,7] Integration of the AAV genome into the chromosome is location-specific and usually takes place in the long (q) arm of chromosome 19.[8] The Duck and Goose parvoviruses (belonging to the species *Anseriform dependoparvovirus 1*) do not a helper virus for replication and are therefore the only exceptions in the genus *Dependoparvovirus*.[4]

### *4.1 AAV genomes*

AAVs have a single-stranded DNA genome that codes for two genes: *rep* and *cap*. These genes are flanked by two inverted terminal repeats (ITRs), which are necessary for DNA replication and integration of the DNA into a host chromosome. The DNA contains three promotors for the transcription of the mRNA, and various proteins can be produced through cleavage of parts of the mRNA.[4] The *rep* gene encodes four replicase proteins involved in replicating the virus, expressing the structural proteins and integrating the virus genome into the host genome. The *cap* gene encodes the three capsid proteins. Hypervariable regions in the capsid proteins influence the tissue specificity of AAVs.[9]

### *4.2 Infection and pathogenicity*

AAVs can infect almost all vertebrate animals, including humans, and have a narrow host range.[4] Viral sequences are found in many different types of tissue.[5,10] AAVs display differences in host specificity and tissue tropism. Most AAVs have been isolated as DNA sequences from cells or tissues of a range of animal species (including humans).[11] Only the first six AAVs (AAV1-6) were isolated as distinct viruses from simian and/or human tissue.

Infections with AAVs are frequent and occur worldwide; about 95% of the human population has been exposed to AAV2 at one time or another.[7] The virus is probably transmitted via the respiratory or gastrointestinal route.[5] AAV1 can be transmitted to the fetus via the placenta.[4] So far no mention has been made in the literature of disease symptoms caused by AAVs.[12,13]

Recombinant AAV vectors are used in clinical studies as gene therapies for various disorders. In these vectors the transgene sequence is placed between the ITRs and the *rep* and *cap* genes are supplied *in trans*.[7] AAV2 was the first AAV to be used as a gene therapy vector. AAV2, AAV5, AAV8 and AAV9 vectors have since been used in more than 160 clinical trials on humans.[14]

### *4.3 AAV10, AAV11, AAV12, AAVrh10 and AAVpo1*

The International Committee on Taxonomy of Viruses (ICTV) has assigned AAV10-12 to the species *Adeno-associated dependoparvovirus A*. In 2004, AAV10 and AAV11 were isolated as DNA sequences from the tissue of Crab-eating macaques.[15] The amino acid sequences of the AAV10 and AAV11 capsid proteins resemble those of AAV8 and AAV4. AAV10 and AAV11 have a broader tropism than AAV2.[15,16] AAV12 was isolated from simian adenovirus stock in 2008.[17] The genome sequence of AAV12 most closely resembles that of AAV11 (83%) and AAV4 (81%) and is least like that of AAV5 (63%).[17] AAVrh and AAVpo1 have not yet been classified by the ICTV. AAVrh10 is one of the 37 clones which were isolated from rhesus monkey tissue in 2003 using PCR.[9] AAVrh10 (like AAV9) has the ability to cross the blood-brain barrier, which other AAVs cannot do, allowing it to transduce cells in the central nervous system (including the brain)[18] as well as in many other types of tissues. Because these AAVs come from rhesus monkeys it was assumed that the immune response to AAVrh10 would be less than to AAV9 (isolated from human tissue). However, it was found that 59% of the human population possess antibodies to AAVrh10,[19] from which it can be concluded that antibodies for human AAVs can also be effective against AAVs from rhesus monkeys. AAVpo1 was isolated from various pig tissues in 2009.[20] The AAVpo1 Cap protein showed an 87.1% correspondence in amino acid sequence and 84.7% correspondence in nucleotide sequence with AAV5.[20]

## 5. Previous COGEM advice

COGEM has not previously issued any advice on AAV10-12, AAVrh10 or AAVpo1. COGEM has assigned AAV1-9 to pathogenicity class 2.[21,22]

## 6. Classifications by other assessment agencies

The German Federal Institute for Occupational Safety and Health (BAUA), which assesses pathogenicity to humans, has assigned AAV1, 4 and 6-11 to risk group 2, and AAV2, 3 and 5 as non-pathogenic to the lowest risk group (group 1).[23] Canine, Equine, Avian, Bovine and Ovine AAV have also been allocated to risk group 1. The German Central Committee on Biological Safety (ZKBS), which considers pathogenicity to humans, animals and plants, has assigned AAV2, 3, 3b and 5 to risk group 1, and AAV1, 4, 6, 7-11, 12, po1 and rh10 to risk group 2.[24] The Swiss Federal Office for the Environment (FOEN), which also considers pathogenicity to humans, animals and plants, has assigned

AAV1-6 as human pathogens and Avian, Bovine, Canine, Equine and Ovine AAV as mammal pathogens to risk group 2.[25] This is accompanied by the comment that laboratory strains of AAV2, 3 and 5 which are used as vectors can be assigned to risk group 1. The Belgian Biosafety and Biotechnology Unit (SBB) has assigned human and animal AAVs as human and animal pathogens to risk group 2.[26] The US National Institutes of Health (NIH) has assigned all adeno-associated viruses ('AVV-all serotypes') as non-pathogenic to risk group 1.[27] The determinations of containment level by these foreign agencies serve as reference and background information for risk assessments by COGEM.

**7. Considerations and advice**

AAVs are widespread in nature and have been shown to be present in tissues of many different vertebrates, including humans. About 95% of the world's population have been exposed to AAV2 at one time or another. The literature contains no reports that the AAVs described in this advice (AAV10-12, AAVrh10 and AAVpo1) are pathogenic to humans, animals or plants.

AAVs are replication-deficient viruses and depend on co-infection with a helper virus (usually an adenovirus) for replication. Without the presence of a helper virus, the AAV genome integrates into the chromosome or persists in the cell nucleus as an extrachromosomal episome. Cells may therefore be persistently infected with AAVs in these latent forms. AAVs can only complete their life cycle when there is a superinfection with a helper virus. There are no disease symptoms associated with AAVs and it has never been demonstrated that AAVs aggravate the clinical symptoms of an adenovirus infection.[8] There are indications that AAVs may protect against cancer. [28]

Based on the information given above, COGEM is of the opinion that AAV10, AAV11, AAV12, AAVrh10 and AAVpo1 meet the first criterion for allocation to pathogenicity class 1. Given their dependence on the presence of a helper virus and the lack of evidence of pathogenicity, COGEM recommends that all AAVs belonging to the species *Adeno-associated dependoparvovirus A* and *Adeno-associated dependoparvovirus B* should be assigned to pathogenicity class 1. Due to the limited time available to prepare this advice, the other species in the genus *Dependoparvovirus* have not been considered.

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