Bundesamt für
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Position statement of the Central Biosafety Commission on the risk assessment of human adeno-associated viruses and AAV-derived vectors

Adeno-associated viruses (AAV) are widespread among animals and humans. They belong as a subgroup of the defective viruses (genus Dependovirus) to the family Parvoviridae. The single stranded DNA genome consists of the two open reading frames *rep* and *cap*. The four non-structural proteins Rep78, Rep68, Rep52 and Rep40, encoded by *rep*, are important for viral replication and integration into the host cell genome. The three capsid proteins Vp1, Vp2 and Vp3 are encoded by *cap* and generate the icosahedral nucleocapsid. Both the 5' and 3' termini of the genome end in an inverted terminal repeat (ITR). The ITRs contain the packaging signal and the *cis*-active elements for replication of the virus genome and integration of the virus into the host genome. For productive (lytic) infection the AAV require helper functions, which are provided by helper viruses (adenovirus, herpes simplex virus types I and II, cytomegalovirus or human herpes virus 6). In the absence of helper functions in the target cells a cell is infected by AAV, but the transferred AAV genome becomes quiescent after specific integration in the host genome (latent infection). The latent virus can be remobilized by superinfection with adenoviruses or herpes viruses [1, 2].

As human AAV, the ZKBS allocated the known serotypes 2, 3 and 5 to **risk group 1** [3]. The simian serotypes 1, 4 and 6 were allocated to **risk group 2**, because their non-pathogenicity has not yet been demonstrated.

Recently, AAV-9 was isolated by PCR from human tissue and described as a new serotype by means of serological cross-experiments [4]. AAV-7, AAV-8, AAV-10 and AAV-11, isolated from monkeys, were also identified as new serotypes. They can infect human cells *in vitro*; but until now they have not been isolated from humans.

Risk assessment:

The risk assessment for the previously evaluated AAV is not affected by new scientific data so that the risk group allocation remains unchanged.

Nothing is known about the spread of AAV-9 and its possible association with disease symptoms. Although natural infections with AAV-7, -8, -10 and -11 have not yet been detected in humans, there is not enough evidence to consider these serotypes isolated from monkeys as truly non-pathogenic. Therefore, according to § 5 paragraph 1 in conjunction with Appendix I of the GenTSV the ZKBS recommends the following classification:

AAV-2, AAV-3 and **AAV-5:**	**risk group 1**
AAV-1, AAV-4, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10 and **AAV-11:**	**risk group 2**

AAV-derived vector systems:

A common AAV vector system consists of two (mostly pBR322-derived) plasmids and one helper virus. The vector plasmid contains only the inverted terminal repeats of AAV upstream and downstream of the nucleic acid fragment to be transferred. The AAV reading frames *rep*

and *cap* are located on the helper plasmid. No overlap of homologous AAV nucleotide sequences between the vector and helper plasmids has been described, so homologous recombination between the AAV nucleotide sequences carried on the plasmids is not expected.

To generate recombinant, AAV-derived vector particles host cells are co-transfected with the vector and helper plasmid and superinfected with a helper virus that encodes the essential viral helper functions for AAV replication.

In improved AAV vector systems, the viral helper functions are provided independently of the helper virus, which means it is no longer necessary to co-infect a replication-competent helper virus. The generation of AAV particles requires the proteins E1a, E1b, E2a, E4 and VA of the adenoviruses used as helper viruses [7].

If the HEK293 cell line, which provides the adenoviral E1 proteins, is used as a host cell, the helper plasmid only has to contain the genes for the adenoviral proteins E2a, E4 and VA in addition to the *rep* and *cap* nucleotide sequences [8]. This also prevents the production of replication-competent helper viruses.

Risk assessment:

1. Recombinant, AAV-derived vector particles that contain no nucleic acid sequences of AAV except for the ITRs and no potentially hazardous nucleic acid fragment, even if these are pseudotyped, are allocated to **risk group 1**. The classification is irrespective of which AAV the ITRs used originate from. Genetic engineering operations with genetically modified organisms that comply with these criteria are assigned to **containment level 1**.
2. Cells or cell lines of risk group 1, which are infected with the recombinant, AAV-derived vector particles mentioned in the previous paragraph are allocated to **risk group 1**. Genetic engineering operations with genetically modified organisms that comply with these criteria are assigned to **containment level 1**. When using cells of higher risk groups the potential hazard of the respective organisms has to be fully considered in the risk assessment.

Explanation:

AAV has a broad host range, including humans. A clinical study with AAV-2 vectors in humans showed that the vectors are not transferred into the germline. In addition, vector sequences are no longer detectable in the blood and urine after 48 hours, meaning the propagation of infectious AAV vector particles is restricted [9]. The vector DNA remains extrachromosomal within the host cell, and since Rep proteins are absent it integrates only very rarely into the host cell genome. The vectors are replication-deficient, and except for the ITRs of AAV no further genes of AAV or of the helper viruses are present in the vectors. Furthermore, the vector-recipient systems described above (point 2) comply with biosafety measures according to § 6 para. 4 and 5 of the GenTSV.

Note:

1. In the case of contamination of the recombinant, AAV-derived vector populations described above under point 1 with helper viruses, the hazard potential of these viruses has to be fully considered in the risk assessment.

2. If there is a possibility that overlapping AAV nucleic acid sequences on the vector and helper plasmid lead to complete, or perhaps chimeric AAV, or if such AAV are generated on purpose, the risk assessment must be based on the AAV from which the nucleic acid sequences of the Rep proteins were derived.

References:

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