Bundesamt für
Verbraucherschutz und
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General position statement of the ZKBS on frequently carried out genetic engineering operations based on the criteria of comparability:

Genetic engineering operations with SV40 as donor organism

1. Description of Simian Virus 40 (SV40)

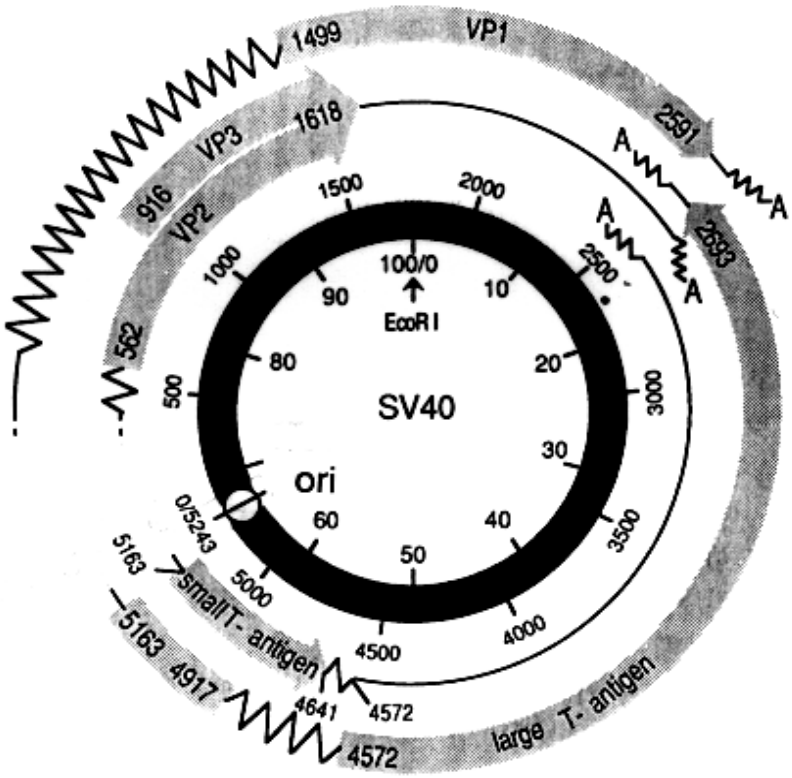
1.1. General introduction

The natural hosts of SV40 are monkeys, in particular Asian macaques and rhesus monkeys. Nearly all adult rhesus monkeys are infected with SV40. In its natural host SV40 causes no clinical symptoms. An infection of monkey cells is productive, rodent cells are non-permissive and human cells are semi-permissive for SV40 replication (1, 6).

The icosahedral SV40 virion contains a circular, double stranded covalently closed genome of 5243 bp with a bidirectional origin of replication (ori) and an early and late gene region (see Figure). In the virion the genome is wrapped around in host cell histones. Early and late transcription start near the replication origin and proceed in divergent directions. The early region codes for two tumor antigens, the small t-antigen ant the large T-Antigen, whose coding regions partially overlap, and which are generated by alternative splicing. The late region codes for the capsid proteins VP1, VP2 and VP3, whereby the coding region for VP2 and VP3 have the same reading frame (1, 7).

The role of small t-antigen is not yet clear. It appears not to be required for productive infection, and it possesses no transforming potential (1, 9).

The large T-Antigen is a multi-functional phosphoproten. It is localized in the cell nucleus of the host cell and binds to specific DNA sequences in SV40 ori as well as to a number of cellular proteins. Its biochemical properties include ATPase and DNA helicase activities. In permissive cells it participates in viral DNA replication as a complex with the host cell DNA polymerase α. In non-permissive cells it can cause uncontrolled cell proliferation through binding the tumor suppressor proteins pRb and p53. *In vitro* the expression of T-Antigen in primary human cells can delay cell senescence by 30 to 40 passages, in rare cases leading to immortalization, whereby the T-Antigen gene is integrated into the host cell genome (1, 7, 9, 10).



Map of SV40 genome with replication origin, early and late genes

The single *Eco*R I site is given as a reference point for the physical map. The relative map units (0-100), the nucleotide positions and the replication origin (ori) are shown. The five virus-encoded proteins are represented by shaded arrows, non-translated mRNA regions are indicated by smooth lines. Introns are shown by zigzag lines; zigzag lines with A mark the 3'-end of the polyA segment (from Fields, B.N. and Knipe, D.M. (1990). Virology, 2. Raven Press, New York).

Kidneys from rhesus monkeys can be latently infected with SV40. In 1960, SV40 was discovered as an impurity in cell culture from monkey kidneys. This discovery led to concern for two reasons:

1. SV40 was an unknown and wide-spread contaminant in viral vaccines (poliomyelitis and adenovirus vaccines) that were produced in monkey kidney cells and administered to millions of people worldwide between 1955 and 1963. In the USA alone, 98 million people were injected with contaminated poliomyelitis vaccine, 10 000 received it orally and 100 000 people were injected with contaminated adenovirus vaccine (6).
2. SV40 has oncogenic potential. Following injection in newborn hamsters it can cause tumors. Its immortalizing potential for human cells *in vitro* is documented (1, 7, 9, 10).

   Therefore, SV40 came under scrutiny as an agent causing disease in humans. In particular, the fate of the vaccinated population received greater attention.

   SV40 related human pathogenic papovaviruses have been described, such as the neurotropic JC virus, which can cause progressive multifocal leukoencephalopathy (PML) (2), or BK virus whose involvement in diseases is not yet clarified (3). But there are only slight

indications of a human pathogenic potential for SV40 (6). Papoviruses with homology to SV40, that are not JCV, were isolated from several patients with progressive multifocal leukoencephalopathy (4). An SV40 related virus was isolated from a human melanoma (5). SV40-like DNA sequences were found in ependymomas and chorid plexus tumors from children (8) and in human mesotheliomas (11). However, a statistically significant indication of a higher incidence of tumors is as yet not apparent. But since a report on the detection of DNA sequences that match SV40 DNA in human tumors (11) was published recently, it seems the time period after the vaccine contamination is still too short to conclusively assess the human pathogenicity potential of SV40 at this point in time, particularly its involvement in human tumors. In contrast, its causing acute or more frequent diseases with shorter latency can be excluded.

1.2. Genetic engineering operations with SV40

SV40 is frequently used as a model system for eukaryotic DNA replication because it replicates its DNA in the nucleus of infected host cells as a “mini chromosome” and uses proteins of the host cell for replication and for chromatin assembly. In addition, its immortalizing/transforming properties are not only a tool for establishing cell lines from a whole range of primary cells from various species, including human cells, but scientifically they are also very important for investigating cellular proliferation control.

Increasingly, instead of infecting cells with virus, either the SV40 genome or only the T-Antigen gene or another sub-genomic nucleic acid fragment are transferred. To prepare for transfecting cell cultures, genetic engineering operations with SV40 as the donor organism involved inserting either the complete genome or sub-genomic nucleic acid fragments into pBR-based vectors and introducing these into *E. coli* K12 derivatives. The amplified recombinant plasmids are then transfected into mammalian cells, where they express the proteins.

A sub-genomic nucleic acid fragment expressed in an *E. coli* K12 derivative is also frequently used for *in vitro* experiments.

2. Summary of relevant criteria for assigning containment levels for genetic engineering operations with SV40 as the donor organism

Safety assessment of genetic engineering operations involving the transfer of the complete genome of SV40, also when it is interrupted by vector sequences, to *E. coli* K12 derivatives or to eukaryotic cells, must take into account the full hazard potential of SV40 in the risk assessment if the SV40 genome is present as an extrachromosomal copy in the genetically modified organism (GMO). Then one has to bear in mind that permissive or semi-permissive cells can possibly form infectious virus particles. With other recipient organisms that themselves cannot replicate the virus, the risk assessment has to consider the possibility that the SV40 genome can be transfered to human cells following inhalation of GMO-containing aerosols, this because after transfer of the SV40 genome to human cells it is possible that infectious particles could be formed, which can spread throughout the human organism. Therefore, a low risk for humans cannot be excluded with such genetic engineering operations.

A hazard potential for humans is not expected with sub-genomic nucleic acid fragments that encode capsid proteins or small t-antigen.

If the gene for large T-Antigen is associated with promoters that function in mammalian cells, one assumes a low hazard potential for humans. But this hazard potential in genetic engi-

neering operations with the large T-Antigen gene can be effectively countered if the vector-recipient system used corresponds to a biological safety measure. Similarly, no hazard potential is assumed if the T-Antigen gene is integrated into the genome of a recipient organism from risk group 1.

If the large T-Antigen gene is has promoters that do not function in mammalian cells, or there is no promoter, one can assume no hazard potential for humans.

3. Criteria for comparability of genetic engineering operations with SV40 as the donor organism

The following sections summarize the general criteria for the comparability of genetic engineering operations with SV40 as the donor organism.

2.1. If a sub-genomic or a sub-gene nucleic acid fragment from SV40 is introduced into a recipient organism from risk group 1 (also with additional nucleic acid fragments with no hazard potential) and this is not able to complement for the missing SV40 sequences, then the genetically modified organism is allocated to **risk group 1**, if the vector-recipient system corresponds to a biological safety measure. Genetic engineering operations with genetically modified organisms that fulfill these criteria are comparable to each other and assigned to **containment level 1**.

2.2. If a sub-genomic nucleic acid fragment from SV40 that codes for the large T-Antigen under the control of promoters active in mammalian cells is introduced into a recipient organism from risk group 1 (also with additional nucleic acid fragments with no hazard potential) and this nucleic acid fragment from SV40 is present extrachromosomally, then the genetically modified organisms are allocated to **risk group 2**, assuming the vector-recipient system corresponds to a biological safety measure. Genetic engineering operations with genetically modified organisms that fulfill these criteria are comparable to each other and assigned to **containment level 2**.

2.3. If a sub-genomic nucleic acid fragment from SV40 that codes for the large T-Antigen is not under the control of promoters active in mammalian cells, and is introduced into a recipient organism from risk group 1 (also with additional nucleic acid fragments with no hazard potential) and this nucleic acid fragment from SV40 is present extrachromosomally, then the genetically modified organisms are allocated to **risk group 2**, even if the vector-recipient system does not correspond to a biological safety measure. Genetic engineering operations with genetically modified organisms that fulfill these criteria are comparable to each other and assigned to **containment level 2**.

2.4. If a sub-genomic nucleic acid fragment from SV40 that codes for the large T-Antigen (also with additional nucleic acid fragments with no hazard potential) is introduced into a recipient organism from risk group 1 not able to complement the missing SV40 sequences, and the nucleic acid fragment of SV40 is integrated in the host's genome, then independently of the expression control of T-Antigen, the genetically modified organism is to be assigned to **risk group 1**, even if the vector-recipient system does not correspond to a biological safety measure. Genetic engineering operations with genetically modified organisms that fulfill these criteria are comparable to each other and assigned to **containment level 1**.

2.5. If a sub-genomic nucleic acid fragment from SV40 that codes for capsid proteins or the small t-antigen, or represents a sub-genomic nucleic acid fragment (also with additional nucleic acid fragments with no hazard potential), is introduced into a recipient organism from risk group 1 not able to complement the missing SV40 sequences, the genetically

modified organism is to be assigned to **risk group 1**, even if the vector-recipient system does not correspond to a biological safety measure. Genetic engineering operations with genetically modified organisms that fulfill these criteria are comparable to each other and assigned to **containment level 1**.

2.6. If the complete genomic information of SV40 is introduced into a recipient organism from risk group 1 (also with additional nucleic acid fragments with no hazard potential) and this nucleic acid fragment from SV40 is present extrachromosomally, the genetically modified organism is to be assigned to **risk group 1**, even if the vector-recipient system does not correspond to a biological safety measure. Genetic engineering operations with genetically modified organisms that fulfill these criteria are comparable to each other and assigned to **containment level 1**.

2.7. If the complete genomic information of SV40 is introduced into a non-permissive recipient organism from risk group 1 (also with additional nucleic acid fragments with no hazard potential) and the SV40 genome integrates into the host genome, then the genetically modified organism is to be assigned to **risk group 1**, even if the vector-recipient system does not correspond to a biological safety measure. Genetic engineering operations with genetically modified organisms that fulfill these criteria are comparable to each other and assigned to **containment level 1**.

2.8. If recipient organisms from risk group 1 are co-transfected with various plasmids described in section 3.1, and there is a possibility that virions could be formed through recombination of the nucleic acids SV40, then the genetically modified organism is to be assigned to **risk group 2**. Genetic engineering operations with genetically modified organisms that fulfill these criteria are comparable to each other and assigned to **containment level 2**.

4. References

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