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Position statement of the ZKBS

on classifying genetic engineering operations with primary cells from vertebrates

Definition of terms

Primary cells are defined as explants obtained directly from body fluids or body tissues of multi-cellular organisms. Primary cell cultures are primary cells grown in culture up to the first passage. Regarding the aspect of risk assessment, further passages of primary cell cultures are to be treated as primary cells until they are sufficiently characterized. Cultivation of primary cells for the purpose of virus enrichment requires an individual risk assessment.

Potential risks

Infection or contamination of cell cultures with disease causing agents represents a potential hazard when working with these cell cultures. However, there are only very few reported cases of illness associated with handling cell cultures. In contrast to well-characterized established cell lines, there is the risk of transferring pathogens due to unknown contaminations when handling primary cells from higher organisms.

Based on the list of donor and recipient organisms for genetic engineering operations published according to § 5 para. 6 of the Genetic Engineering Safety Regulations (GenTSV), cells and cell lines are to be assigned to **risk group 1** as donor and recipient organisms, if they do not release organisms of a higher risk group. If they contain organisms of a higher risk group, classification is based on the risk group of these organisms.

Generally, cells and cell lines from higher organisms can only be cultivated using complex media and specific cell culture vessels. Since they are not viable without these conditions, they do not represent a hazard to the environment in principle. Based on this experience it can thus be assumed that assigning primary cells to **risk group 1** generally meets the requirements of the GenTSV.

However, if a reasonable suspicion exists that primary cells or primary cell cultures contain and release transmissible pathogens, it must be assumed that there is a risk for the laboratory personnel. Previous experience in handling such cell material has shown that a hazard emanates from possible contaminations with human pathogenic viruses. There is no evidence for risks associated with contaminations with non-viral pathogens.

The possibility that viruses are present in primary cells largely depends on the species and the state of health of the donor organism as well as on the tissue or body fluid the material is established from. Due to the special situation that primary cells might contain unknown contaminations with viral pathogens, for reasons of precaution it should be checked whether the donor is free from disease symptoms and/or serologically negative for certain viruses in order to retain the basic classification as **risk group 1** for individual cases.

The following section provides several examples serving as a guideline for the differentiated risk assessment of primary vertebrate cells and primary vertebrate cell cultures. Other cells - particularly primary plant and insect cells - are not considered within the framework of this position statement.

A. Risk assessment of primary vertebrate cells

For estimation of the hazard potential of primary cells it is useful to distinguish between cells established from humans, other primates, chiroptera or other vertebrates.

1. Human cells

Risk assessment of primary human cells can be based on experiences gained in transfusion and transplantation medicine. For transfusions/transplantations it is known that there is a high risk of infection for the recipient if the material used is contaminated with pathogens. For this reason donated blood is screened for the human pathogenic viruses HIV (*Human immunodeficiency virus*), HBV (*Hepatitis B virus*) and HCV (*Hepatitis C virus*) using serological and molecular biology methods. Even if the primary cells are contaminated with HIV, HBV or HCV, the risk of infection associated with handling primary cells for the purpose of carrying out genetic engineering operations is much lower compared to the risk of infection following transfusion or transplantation. For clinically symptomless donors it is thus sufficient to exclude the possibility of contaminations with the help of serological tests. This stands in accordance with the procedure of occupational health examinations, where serological tests are regarded as sufficient despite the diagnostic gap, provided that there is no actual suspicion of an exposure to HIV, HBV or HCV.

<u>Risk assessment of primary human cells</u>

- Primary cells from clinically symptomless donors are to be assigned to **risk group 1**, if seronegativity of the donor for HIV, HBV and HCV has been demonstrated by immunological tests, or if it has been shown by other procedures that the cells are free from these viruses. In individual cases, if there is reason to suspect the presence of a certain virus of a higher risk group in the cells to be used, the primary tissue is to be checked for the absence of this virus.
- If the donor or cells are not checked for the absence of the viruses mentioned above, the primary cells – following the experience in handling diagnostic samples in medicine – are generally to be assigned to **risk group 2**.
- If the donor or cells are not checked for the absence of the viruses mentioned above and if the cells are not permissive for the viruses mentioned above, the primary cells can be assigned to **risk group 1**, if it is guaranteed that they are not contaminated with blood or with cells that are permissive for the viruses mentioned above.
- If primary cells are isolated from tissues or body fluids which are expected to release viral pathogens due to an illness of the donor or due to the nature of the diseased tissue, the material is to be assigned to the risk group of the respective virus.

2. Non-human primate cells

Primate cells can be carriers of viral pathogens that can be transmitted between various monkey species and also to humans (inter-species transmission). Several of these transmissible viruses persist in the animals without detectable symptoms, and symptoms only appear after inter-species transmission.

For example, retroviruses like spumaretroviruses (*Simian foamy virus*, SFV), lentiviruses (*Simian immunodeficiency virus*, SIV), *Simian retrovirus-1* type D (SRV-1) or *Simian T-lymphotropic virus* (STLV) are widespread in numerous monkey species. Infection of the animals with these viruses, however, usually remains unrecognized. Infection of macaques with SIV occasionally causes AIDS-like symptoms. Infection with SRV-1 can also cause AIDS-like symptoms and infection with STLV can be associated with lymphoproliferation. Infection with SFV is widespread and usually remains asymptomatic. Numerous transmissions from monkeys to humans are reported for SFV. Infected humans remained asymptomatic as well, and the virus was not transmitted to contact persons. Thus, it can be assumed that handling of primary simian cells contaminated with SFV does not present a hazard for humans.

Cercopithecine herpesvirus-1 (CeHV-1, also called B virus or Herpesvirus simiae, formerly named Herpes B virus) naturally infects macaques and possibly also other old world monkeys. Infection of monkeys remains mostly asymptomatic whereas transmission to humans can result in a fatal encephalomyelitis. The majority of free-ranging (Rhesus) macaques as well as animals kept in breeding facilities are seropositive for CeHV-1. However, the animals are usually not viraemic, and release of CeHV-1 from the donated tissues or cells is not to be expected. Virus release is only to be expected when using cell material from viraemic animals. However, CeHV-1 can persist in monkeys without being recognized by the immune system, so that seronegativity does not reliably rule out an infection.

The Common Marmoset (*Callithrix jacchus*) is a special case. There are no reports of infections of these new world monkeys with STLV, SIV or CeHV-1. Infections of Common Marmosets with inter-species transmissible pathogens cause severe disease associated with high lethality and are thus easily recognized. Since the 1960s Common Marmosets are used for animal experiments or are kept as pets. Diseases that can be transmitted from these animals to humans are not known. Besides Rhesus macaques (*Macaca mulatta*) and Cynomolgus Monkeys (*Macaca fascicularis*), Common Marmosets are one of the primates most frequently used as laboratory animals. However, only specially bred and not wild caught Common Marmosets are used for this purpose.

<u>Risk assessment of primary non-human primate cells</u>

- Provided that no special risk assessment is performed, primary cells from clinically symptomless non-human primates from veterinary controlled breeds are to be assigned to **risk group 2** due to the widespread prevalence of inter-species transmissible viruses.
- Primary cells from clinically symptomless Rhesus Macaques (*Macaca mulatta*) or Cynomolgus Monkeys (*Macaca fascicularis*) from veterinary controlled breeds are to be assigned to **risk group 1**, if they are tested negative for SIV, SRV, STLV and CeHV-1.
- Embryonic stem cells from Rhesus Macaques (*Macaca mulatta*) or Cynomolgus Monkeys (*Macaca fascicularis*) isolated from *in vitro* fertilized embryos of macaques from veterinary controlled breeds are to be assigned to **risk group 1**, if they are tested negative for SIV, SRV, STLV and CeHV-1.

For example, primary cells can be tested for the viruses mentioned above using polymerase chain reaction (PCR) and appropriate controls.

- Primary cells from clinically symptomless Common Marmosets (*Callithrix jacchus*) from veterinary controlled breeds are to be assigned to **risk group 1**.
- Cell material taken from wild caught primates requires an individual risk evaluation, but these cells are at least to be assigned to **risk group 2**.

3. Vertebrate cells (excluding primates)

In most cases, genetic engineering operations use rodents (mouse, rat, hamster, rabbit and guinea pig) as donor organisms for primary cells. In addition, primary cells are often taken from domestic animals and pets, e.g. cow, pig, goat, sheep and dog as well as amphibians and birds. These animals can carry pathogens, in rare cases also human pathogens. For example, bats that do not show any symptoms of disease can be infected with the human pathogenic rabies virus.

Risk assessment of primary cells from vertebrates (excluding primates)

- Primary cells from vertebrates (excluding primates and chiroptera) are to be assigned to **risk group 1**, if the animals do not show any symptoms of disease. This assignment applies to animals from veterinary controlled populations.
- Primary cells from chiroptera that have been shown to be free from rabies virus are to be assigned to **risk group 1**, if the animals do not show any symptoms of disease.
- Primary cells from chiroptera that have not been tested for the absence of rabies virus are to be assigned to **risk group 2**.
- If primary cells are isolated from tissues or body fluids which are expected to contain viral zoonotic pathogens, the material is to be assigned to the risk group of the respective pathogen.

B. Classification of genetic engineering operations using primary cells from vertebrates

1. Primary cells as recipient organisms

If primary cells are used as recipient organisms, the genetic engineering operations are to be assigned to the containment level corresponding to the risk group of the primary cells, provided that the transferred nucleic acids are characterized to such a degree that the hazard potential of the genetically modified organisms, after a preliminary safety assessment according to § 5 of the GenTSV, does not exceed the hazard potential of the primary cells.

2. Primary cells as donor organisms

In the following, genetic engineering operations involving establishment of genomic or cDNA gene banks in *E. coli* K12 from primary cells are assessed.

2.1 If a reasonable suspicion exists that the primary cells are infected with a virus, the cells are to be assigned to the risk group of the expected virus. Genetic engineering operations using these cells as donor organisms are to be assessed as genetic engineering operations using the corresponding virus and are thus to be evaluated individually.

2.2 Primary cells from clinically symptomless persons are to be assigned to **risk group 1**, if seronegativity of the donor for HIV, HBV and HCV has been demonstrated by immunological tests, or if it has been shown by other procedures that the cells are free from these viruses. If the donor or the cells are not tested for the absence of the viruses mentioned above and if the cells are not permissive for the viruses mentioned above, the primary cells can be assigned to **risk group 1**, provided that contamination with blood or cells permissive for the viruses mentioned above can be excluded.

Primary cells from chiroptera that are demonstrably free from rabies virus as well as other primary vertebrate cells (excluding non-human primates) are generally to be as-

signed to **risk group 1**. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using pBR-derived vectors is to be assigned to **containment level 1**. The resulting genetically modified organisms are to be assigned to **risk group 1**.

Reasons:

The primary cells and the nucleic acid fragments transferred from them do not hold a hazard potential. The vector-recipient system corresponds to a biological safety measure according to § 6 para. 4 and 5 of the GenTSV.

2.3 Primary cells from clinically symptomless persons that are not tested for the absence of HIV, HBV and HCV and that are permissive for these viruses or may be contaminated with cells permissive for these viruses are to be assigned to **risk group 2**, if they are used as donor organisms.

a. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using pBR-derived vectors is to be assigned to **containment level 1**, if according to the experimental design it is expected that in case of an actual virus infection either no viral nucleic acid fragments or only sub-genomic nucleic acid fragments will be transferred. The resulting gene bank is to be assigned to **risk group 1**.

b. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using pBR-derived vectors is to be assigned to **containment level 2**, if according to the experimental design it is expected that in case of an actual virus infection complete viral genomes will be transferred. The resulting gene bank is to be assigned to **risk group 2**. If the experimental design includes specific enrichment of genetically modified organisms with viral genomes, the hazard potential of the virus is to be taken into account for the risk assessment of the genetically modified organism.

Reasons:

Primary cells usually do not hold a hazard potential. There could be a low risk due to infection of the cells with the viruses mentioned above. However, only certain human cells including for example hepatocytes or hematopoietic cells are permissive for HIV, HBV or HCV. Since the donors or the cells isolated from them are not tested for these viruses and since the cells are permissive for these viruses or contaminations with blood or permissive cells can not be excluded, these primary cells are to be assigned to **risk group 2** as a precaution.

Nucleic acids isolated from these primary cells are either exclusively of cellular origin or possibly also of viral origin.

to a. Either cellular nucleic acid fragments or possibly also viral sub-genomic fragments are transferred. The vector-recipient system corresponds to a biological safety measure according to § 6 para. 4 and 5 of the GenTSV.

to b. Either cellular nucleic acid fragments or possibly also viral genomes are transferred. Further enrichment of genetically modified organisms containing viral genomes is not performed. The vector-recipient system corresponds to a biological safety measure according to § 6 para. 4 and 5 of the GenTSV.

2.4. Primary cells from clinically symptomless non-human primates (excluding *Callithrix jacchus*) from veterinary controlled breeds or primary cells from chiroptera that are not tested for the absence of rabies virus are to be assigned to **risk group 2**.

a. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using pBR-derived vectors is to be assigned to **containment level 1**, if according to the experimental design it is expected that in case of an actual virus infection ei-

ther no viral nucleic acid fragments or only sub-genomic nucleic acid fragments will be transferred. The resulting gene bank is to be assigned to **risk group 1**.

b. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using pBR-derived vectors is to be assigned to **containment level 2**, if according to the experimental design it is expected that in case of an actual virus infection complete viral genomes will be transferred. The resulting gene bank is to be assigned to **risk group 2**. If the experimental design includes specific enrichment of genetically modified organisms with viral genomes, the hazard potential of the virus is to be taken into account for the risk assessment of the genetically modified organism.

Reasons:

Primary cells usually do not hold a hazard potential. There could be a low risk due to infection of primary non-human primate cells or untested cells from chiroptera with interspecies transmissible viruses. Therefore, these primary cells are to be assigned to **risk group 2** as a precaution.

Nucleic acids isolated from these primary cells are either exclusively of cellular origin or possibly also of viral origin.

to a. Either exclusively cellular nucleic acid fragments or possibly also viral sub-genomic fragments are transferred. The vector-recipient system corresponds to a biological safety measure according to § 6 para. 4 and 5 of the GenTSV.

to b. Either exclusively cellular nucleic acid fragments or possibly also viral genomes are transferred. Further enrichment of genetically modified organisms containing viral genomes is not performed. The vector-recipient system corresponds to a biological safety measure according to § 6 para. 4 and 5 of the GenTSV.

Annotation to the experimental design:

- When cutting the DNA of the donor cell with a restriction enzyme it should be considered that genomes of possibly present DNA viruses (e.g. HBV) can also be cleaved by the same restriction enzyme. Thus, sub-genomic nucleic acid fragments that can be inserted in the vector as well as nucleic acid fragments that can not be inserted could be generated. If the extra-chromosomal genome of the DNA virus contains no restriction sites for the respective restriction enzyme, transfer of the complete viral genome with the help of the vector is not expected.
- Circular viral DNA genomes cut at a single site by a restriction enzyme can be transferred as a complete genome, but the genetic continuity is disrupted at the restriction site. Viral DNA integrated in the host genome (also proviruses) can be completely transferred to the vector via restriction sites in the flanking cellular DNA, if there are no internal restriction sites for the respective restriction enzyme.
- Generation of cDNA banks involves transfer of expression products of genes. In case of a virus infection of the donor cells, it can thus be generally assumed that only sub-genomic nucleic acid fragments will be transferred.
- In case that the expected virus is an RNA virus (e.g. HCV or HIV), the transfer of the cDNA of a complete viral genome can not be excluded, if the primer used for synthesis of the complementary strand matches sequences at the 3' terminus of the viral genome and allows reverse transcription to proceed over the full length of the viral genome. However, it should be noted that the average length of cDNA fragments is approximately 2 kb. Fragment lengths of 6 kb or more are only seldomly achieved. In case of cDNA generation from retroviral genomes, production of infectious nucleic acids is not expected, since there are no complete long terminal repeat (LTR) regions.

2.5. Primary cells from clinically symptomless Rhesus Macaques (*Macaca mulatta*) or Cynomolgus Monkeys (*Macaca fascicularis*) from veterinary controlled breeds are to be assigned to **risk group 1**, if they are tested negative for SIV, SRV, STLV and CeHV-1. Embryonic stem cells from Rhesus Macaques (*Macaca mulatta*) or Cynomolgus Monkeys (*Macaca fascicularis*) isolated from *in vitro* fertilized embryos of macaques from veterinary controlled breeds are to be assigned to **risk group 1**, if they are tested negative for SIV, SRV, STLV and CeHV-1. Primary cells from clinically symptomless Common Marmosets (*Callithrix jacchus*) from veterinary controlled breeds are also to be assigned to **risk group 1**. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using pBR-derived vectors is to be assigned to **containment level 1**. The resulting genetically modified organisms are to be assigned to **risk group 1**.

Reasons:

The primary cells and the nucleic acid fragments transferred from them do not hold a hazard potential. The vector-recipient system corresponds to a biological safety measure according to § 6 para. 4 and 5 of the GenTSV.

2.6. Cell material taken from wild caught non-human primates requires an individual risk evaluation, but these cells are at least to be assigned to **risk group 2**. If the cells are used as donor organisms for genetic engineering operations, bio-safety classification is to be individually performed.