Ref. no. 6790-10-36 updated in December 2014

Position statement of the ZKBS: evaluation of genetically modified organisms into which nucleic acid segments with neoplastic transformation potential have been introduced

Introduction

This position statement assesses the risks resulting from genetically modified organisms (GMOs) into which nucleic acid segments with neoplastic transformation potential have been introduced.

Nucleic acid segments with these properties might cause the development of malignant tumors in experimental animals after injection or application to skin lesions. For experimental work with such nucleic acids, the ZKBS released its "Position statement of the ZKBS: Precautionary measures for handling nucleic acids with oncogenic potential" (Ref. no. 6790-10-01) in September 1991.

The current statement complements the one mentioned above from September 1991 by a risk assessment for handling GMO into which nucleic acid segments with neoplastic transformation potential have been introduced, and updates the "Position statement of the ZKBS on the evaluation of genetically modified organisms into which nucleic acid segments encoding proteins with gene-regulatory function have been introduced" (Ref. no. 6790-10-36) from February 1996.

Assessment of the ZKBS

If

- nucleic acid segments, encoding proteins with transformation potential for mammalian cells and standing under the control of promoters, which are active in mammalian cells, or
- noncoding RNA (e.g. siRNA, shRNA, miRNA) and/or noncoding DNA segments, which produce transformation effects by means of a stable deregulation of cellular factors,

are introduced into recipient organisms assigned to risk group 1 (e.g. bacterial cells or established cell lines), the produced GMOs shall be assigned to risk group 1, provided that the vector-recipient systems are acknowledged as biosafety measures according to Annex II of the German Genetic Engineering Safety Regulation (GenTSV).

If such nucleic acid segments are introduced into primary cells, the GMOs shall be assigned to risk group 1, provided that the primary cells have been assigned to risk group 1 and the vectors applied fulfil the conditions of Art. 6 Para. 5 of the German Genetic Engineering Safety Regulation (GenTSV).

Genetic engineering operations with the abovementioned GMOs are to be assigned to biosafety level 1 and carried out in conformity with the safety measures applicable to level 1. When working with such GMOs the same personal safety measures must be observed as are required when working with nucleic acids with oncogenic potential (cf. Position statement of the ZKBS, Ref. no. 6790-10-01):

1. Disposable gloves should be worn when working with nucleic acids possessing the abovementioned hazard potential.

2. The use of sharp, pointed or breakable laboratory equipment should be avoided, whenever possible.
3. Laboratory work sites and laboratory equipment coming in touch with these nucleic acids should be cleaned carefully after cessation of work.
4. Laboratory waste containing such nucleic acids should be denatured by autoclaving or by applying chemical methods.
5. Persons with considerable skin injuries (open eczemas, wounds and infections) or with pronounced verrucosis (multiple wart formation) should not carry out any operations with these nucleic acids.

These additional safety measures are not required if the recipient organisms are established cell lines or primary cells assigned to risk group 1 and the transferred nucleic acid segments are integrated into the host genome and do not exist in a stable episomal configuration.

If the vector-recipient system is not a biosafety measure in the sense of Art. 6 Para. 4 and 5 of the GenTSV, the assignment of the GMO to a risk group must be reviewed in the individual case, unless such assignment may proceed on the foundation of the position statements mentioned further below.

Reasons

GMOs into which the nucleic acids described above have been introduced could represent a hazard potential for humans if these nucleic acids are transferred to body cells where they persist and, if possible, become expressed. In case of an accidental uptake of GMOs by means of inhalation, ingestion, or contact with injured skin, there are in principle two ways how the transfer of nucleic acids into human cells may proceed:

a) DNA is transferred from the GMOs to the body cells by means of cell-cell contacts, microvesicles and/or cell fusions, or

b) the GMOs are lysed and the free DNA is taken up by the body cells.

ad a) Transfer of DNA from GMOs to body cells

A direct transfer of nucleic acid from *Escherichia coli* K12 to body cells under *in vivo* conditions in not known. Besides, *E. coli* K12 is incapable of establishing itself in the body and will be rapidly eliminated by the body's own defense mechanisms like the complement system and/or phagocytosis.

Cell fusion is a prerequisite for a transfer of nucleic acids from eukaryotic cells. This may be induced under experimental conditions *in vitro*, however, under *in vivo* conditions such fusion of body cells is only possible if specific agents not occurring in the body are additionally available. As a consequence, it must not be anticipated that a direct transfer of nucleic acids from GMOs to body cells will occur under normal conditions.

ad b) Uptake of free DNA from lysed GMOs:

Eukaryotic cells are capable of taking up nucleic acids from the environment. This has been demonstrated both in cell cultures and under *in vivo* conditions. However, the efficiency of the uptake by mammalian cells is low *in vitro* and requires a concentration of the DNA as well as special experimental conditions. In general, the nucleic acid taken up under *in-vivo* conditions usually remains only temporarily in the cells. In addition, it must be assumed that extracellular nucleases rapidly decrease the availability of free nucleic acids *in vivo*. Therefore, it may be assumed that under *in-vivo* conditions the probability for nucleic acids released from GMOs by lysis to be incorporated into body cells, to persist and perhaps become expressed there will be low.

The probability of the events mentioned under a) and b) is considered to be very low if the recommended personal safety precautions, which are also recommended for working with nucleic acids possessing oncogenic potential, are observed.

Additional precautionary measures are recommended for episomal plasmids because of their relatively small size and high number of copies and thus resulting potentially increased transferability. The precautionary measures stated above are not necessary when working with eukaryotic cell lines or primary cells containing expressible transforming nucleic acids stably integrated into the genome, as a transfer out of the chromosomal context is less likely to occur.

Further information specific to the respective genetic engineering operations are to be found in the general position statements of the ZKBS below:

- Position statement of the ZKBS on classifying genetic engineering operations where cytokine and apoptosis-regulating genes are integrated into replication-competent microorganisms (Ref. no. 6790-03-05)
- Recommendation of the ZKBS on adenoviral and AAV-derived replication-defective viral particles which transfer a nucleic acid segment with neoplastic transformation potential (Ref. no. 6790-10-83)
- General position statement of the ZKBS on frequently carried out genetic engineering operations based on the criteria of comparability: gene transfer using retroviral vectors (Ref. no. 6790-10-41)
- 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 (Ref. no. 6790-10-34)