ZKBS
The Central Committee on Biological Safety

Publication
of the work of the Central Committee
on Biological Safety in 2019

(BVL-ref.: 45040)

30th report after entry into force of the Genetic Engineering Act

Dated April 28, 2020

The aforementioned report on the work of the German Central Committee on Biological Safety (ZKBS) in 2019 will be published below.

Berlin, April 28, 2020

Federal Office

of Consumer Protection and Food Safety

By order

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Outline

Abbreviations

ATMP	Pharmaceutical Drugs for Novel Therapies
BfN	Federal Agency for Nature Conservation
BfR	Federal Institute for Risk Assessment
BMEL	Federal Ministry of Food and Agriculture
BVL	German Federal Office of Consumer Protection and Food Safety
EFSA	European Food Safety Authority
EC	European Community
EMA	European Medicines Agency
EU	European Union
CJEU	European Court of Justice
FLI	Friedrich Loeffler Institute, Federal Research Institute for Animal Health
GenTG	Genetic Engineering Act
GenTSV	Genetic Engineering Safety Regulations
GMO	genetically modified organisms
JKI	Julius Kühn Institute
PEI	Paul Ehrlich Institute, Federal Institute for Vaccines and Biomedicines
RKI	Robert Koch Institute
ZKBS	Central Committee on Biological Safety

Technical abbreviations are explained in the text.

1 Introduction

1.1 Basics of the ZKBS

The Central Committee on Biological Safety (ZKBS) is an expert committee composed of 20 members and 20 deputy members. The members are experts in various disciplines and are represented by experts in the same discipline. The areas of expertise represented are specified in the Genetic Engineering Act (GenTG). The ZKBS reviews and assesses safety-related questions about genetic engineering in accordance with the provisions of the GenTG and advises the Federal Government and the federal states. It provides position statements to the competent authorities, in particular on the risk assessment of microorganisms, the biosafety classification of genetic engineering operations, the safety measures required in genetic engineering facilities and the possible risks of deliberate release or placing on the market of genetically modified organisms (GMOs). It considers international developments in the field of genetic safety in its recommendations. The members of the ZKBS and their deputies volunteer their work in accordance with GenTG.

The ZKBS has its office at the Federal Office of Consumer Protection and Food Safety (BVL), which is part of the Federal Ministry of Food and Agriculture (BMEL). Members of the ZKBS and their representatives are appointed by the BMEL in agreement with the Federal Ministry of Education and Research, the Federal Ministry for Economic Affairs and Energy, the Federal Ministry of Labour and Social Affairs, the Federal Ministry of Health and the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety for the duration of three years. Reappointment is permissible.

The ZKBS has a chairperson who has two deputies. It makes its resolutions either at a meeting or by written procedure. The members of the ZKBS and their representatives are obligated to maintain confidentiality. The meetings are not public, but the ZKBS publishes general position statements and reports annually about their work to the public.

1.2 Development of genetic engineering in the Federal Republic of Germany and in other member states of the European Union

Genetic engineering operations and genetic engineering facilities

The term "genetic engineering operations" refers in particular to the production and handling of GMOs. Genetic engineering operation, depending on its class, must be reported, registered or approved by the competent state authority and carried out in a genetic engineering facility, which must also be reported, registered or approved depending on the class. Genetic engineering facilities may be laboratories, large scale facilities, greenhouses and/or animal facilities.

Generally, before deciding on license permission, the competent authority shall obtain a position statement from the ZKBS on the safety-related classification of the planned genetic engineering operation and on the required safety measures. This mainly refers to genetic engineering operations of safety levels 3 or 4 and genetic engineering facilities with safety measures of class 3 or 4. However, the competent authority also asks the ZKBS to provide position statements on class 2 genetic engineering operations that are not comparable to other operations for which the ZKBS has already issued a position statement in the past, or on genetic engineering operations whose assignment to class 1 is uncertain.

Since the Genetic Engineering Act (GenTG) went into effect in 1990, the ZKBS has had 2070 applications submitted for the biosafety classification of genetic engineering operations and/or evaluations of the required safety measures. In the year under review, 51 applications were submitted and the ZKBS issued 46 position statements.

In Germany, a total of 6,553 genetic engineering facilities are reported, registered or approved (as of December 2019). Table 1 lists the genetic engineering facilities based on the level of safety measures. Table 2 summarizes the reported, registered or approved genetic engineering operations in Germany by class.

Table 1 Reported, registered or approved genetic engineering facilities in Germany (status: December 2019)

Level	Quantity
S1	4,664
S2	1,784
S3	100
S4	5

Compared to the past five years, the total number of 6,000 to 7,000 genetic engineering facilities of class 1 to 4 has remained largely stable. The greatest part is occupied by class 1 and 2 facilities (see Figure 1).

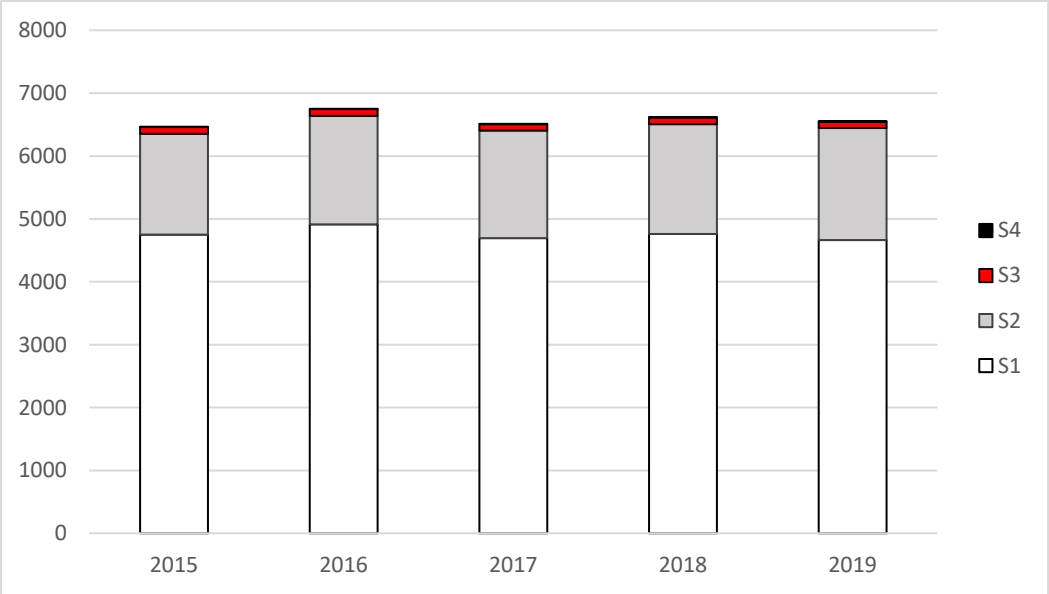

Figure 1 Number of reported, registered or approved genetic engineering facilities in Germany from 2014 to 2019

Table 2 Currently reported, registered or approved genetic engineering operations in Germany (as of December 2019) *

Level	Quantity
S2	8,085
S3	378
S4	16

* Precise specification is not possible for the performed genetic engineering operation of class 1, since the operators are obligated according to § 9 GenTG to record subsequent class 1 projects, however they are not obligated to notify or report to the competent state authority. Thus, further S1 work is not recorded in the regulatory databases.

Further information on genetic engineering operations and genetic engineering facilities is available on the ZKBS website: http://www.zkbs-online.de/ZKBS/EN/Home

It is not possible to compare the number of genetic engineering operations or genetic engineering facilities between Germany and other Member States of the European Union (EU) as there is no information available in that regard. General information on the implementation of the underlying Directive 2009/41/EC will be made available to the European Commission at regular intervals by the Member States. They are summarized by the European Commission and published on its website[1].

Deliberate releases of GMOs

If GMOs are released into the environment for a limited time and space during an experiment, this is referred to as "deliberate release". For each intended deliberate release, permission must be obtained from the BVL in accordance with GenTG, which can only be granted if the planned deliberate release does not have any harmful effects on humans, the environment in its causal network, animals, plants and material assets. If the GMOs to be released already have permission to be placed on the market, no separate permission is required.

In Germany, the BVL, as the higher federal authority, has been responsible for permission for deliberate releases of GMOs since April 1, 2004; previously it was the RKI. The BVL makes the decisions in consultation with the BfN, the BfR and the RKI. The ZKBS, the JKI and the competent authority of the respective federal state issue position statements on the deliberate release intent. In case of a deliberate release of genetically modified vertebrates or genetically modified micro-organisms used on vertebrates, the FLI will also be involved. The other EU Member States are notified about deliberate release applications and can comment on them.

As in the years from 2013 to 2018, no deliberate releases of genetically modified organisms were requested in Germany in 2019, nor was permission granted for such releases. A comparison of the submitted release applications from the different EU Member States shows that in 2019 individual applications were submitted in Great Britain, Spain, Sweden, Rumania and Belgium. Details can be found in the register maintained on behalf of the Commission[2].

[1] https://ec.europa.eu/food/plant/gmo/reports_studies_en

[2] http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx

Placing genetically modified organisms on the market

"Placing on the market" refers to the distribution of GMOs and products containing or produced from GMOs to third parties, usually for marketing purposes.

The placing on the market of GMOs requires an EU-wide approval procedure. This procedure distinguishes whether the GMO should be used as food or feed [Regulation (EC) No. 1829/2003] or not (Directive 2001/18/EC). Products from GMOs that fall outside the scope of the aforementioned regulation or directive, such as cotton clothing, do not require permission to be placed on the market. After processing the EU-wide procedure, the permission applies to all EU Member States.

The BVL is the competent German authority and issues a national position statement on applications for the placing on the market of GMOs in consultation with the BfN, the BfR and the RKI. Prior to this, a position statement of the JKI and, if the GMOs are vertebrates or micro-organisms to be applied to vertebrates, a position statement from the FLI and the PEI will be obtained.

In addition, the BVL asks the ZKBS for a position statement on such applications under Directive 2001/18/EC, which were filed in Germany. For applications under Regulation (EC) No. 1829/2003, the BVL will seek the position statement of the ZKBS if Germany has been commissioned by EFSA to carry out an environmental risk assessment of an application for cultivation. In 2019 there were no requests in this regard made of the ZKBS.

Currently, events (number in parentheses) of the following plants are mostly approved for importation as a GMO capable of reproduction and its processing or as feed and food.

- Cotton (12)
- Maize (ca. 150)
- Rapeseed (9)
- Soya (20)
- Ornamental plants (5)
- Sugar beet (1)

Further details can be found on the BVL[3] website and in the entries of the register of the European Union[4].

In contrast to local and time-limited deliberate release experiments, the agricultural cultivation of genetically modified plants is not limited to specific locations or trial years. Cultivation of genetically modified crops by farmers can only take place once the placing on the market of the genetically modified seed has been approved for the purpose of exposure to the environment. A permission is usually valid for ten years and must be renewed/extended thereafter. The EFSA is responsible for the scientific evaluation.

Decision of the European Court of Justice (CJEU) that plants produced with new mutagenesis processes are considered to be GMOs

Following a complaint by French (agricultural) associations to the French State Council, the CJEU in its judgement dated 25/07/2018 found that plants produced by both conventional and new mutagenesis methods are genetically modified organisms. They thus fall under the regulations of EU Directive 2001/18/EC. However, GMOs produced by conventional mutagenesis are excluded

[3] https://www.bvl.bund.de/DE/06_Gentechnik/02_Verbraucher/03_Genehmigungen/01_Inverkehrbringen/gentechnik_GenehmigungenInverkehrbringen_node.html

[4] http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

from the applicability of the directive. As a result, genomically identical organisms are regulated differently.

In 2019 there was also intense argumentation at the ZKBS about the effects of the decision of the CJEU. As previously, the ZKBS is of the opinion that the European genetic engineering law, which is essentially based on the 1990 state of knowledge, urgently needs to be adapted to the current state of knowledge.

In a disclaimer before the general ZKBS position statements with reference to the use of the new molecular technology, the ZKBS refers to the fact that organisms produced with the new procedures for mutagenesis, which were mainly developed only after the Deliberate Release Directive was adopted in 2001, are to be classified, according to the decision, as GMO and are subject to the Deliberate Release Directive. The ZKBS makes clear, however, that there is not yet a judicial clarification as to the extent to which this decision also applies to the closed system. The disclaimer was prepended to the General Position Statement of the ZKBS on new technology for plant breeding dated June 2012 (ref. 45310.0140) and to the use of zinc finger nuclease technology 1 dated December 2011 (ref. 6790-10-103).

In early 2019 a contribution appeared in the book, "Encyclopaedia of Food Chemistry" in which the author presented the thesis that there can be unequivocal identification of whether mutations in plant genomes originate from natural mutagenic processes or whether they are produced by the new mutagenesis procedures (often described as gene editing). Only the corresponding procedures would have to be determined for that. The ZKBS clearly distanced itself from this statement and commented on the statement publically on its homepage with the summary that the possibility suggested by the author, of identifying gene editing in plants and the thereby to retroactively identify the technology used, does not exist. The suggested methods are not based on the current state of scientific knowledge and additionally involve highly variable biological parameters (such as epigenetic changes), which do not represent a reliable basis for identification[5].

Clinical trials with GMO containing investigational drugs and use of GMO containing medicines in humans

According to § 2 (3) GenTG, the application of GMO containing investigational drugs in humans is excluded from genetic engineering regulations in Germany. The permission for clinical trials with investigational medicinal products consisting of or containing a genetically modified organism or a combination of genetically modified organisms is regulated in the German Regulation on the application of good clinical practice in the conduct of clinical trials with medicinal products for human use (GCP-V). In Germany, the PEI is responsible for granting permission as the upper federal authority. The BVL is involved as an advisory authority in the assessment of the risk to the environment and in determining the delineation of certain activities to the GenTG and reports to the ZKBS. In some cases, the ZKBS assesses the hazard potential of the GMOs in a position statement.

In total, 24 permissions were issued by the PEI in 2019 involving the BVL. The investigational drugs were potential advanced therapy medicinal products (ATMPs), such as in the treatment of cancers with reprogrammed endogenous cells for immunostimulation or gene therapy approaches to serious diseases due to monogenic genetic defects, or novel infectious disease vaccines. An overview of approved clinical trials in the EU can be found in the Register of the European Union[6].

The authorisation for the use of human medicinal products consisting of, or containing, a genetically modified organism or a combination of genetically modified organisms is subject to a

[5] http://www.zkbs-online.de/ZKBS/DE/01_Aktuelles/Kommentar%20zu%20Bertheau%20(2019)/Kommentar%20zu%20Bertheau_node.html;jsessionid=16BA27ABE9379E97E87E8780C726E0E4.1_cid322

[6] http://gmoinfo.jrc.ec.europa.eu/gmo_browse.aspx

centralized procedure by the European Commission in accordance with Regulation 726/2004/EC. The application is submitted to the European Medicines Agency (EMA), which prepares guidelines for evaluation as part of its mission and handles the scientific coordination of the procedures. The environmental impact assessment must involve the competent authorities of the member states in accordance with Directive 2001/18/EC, which is the BVL in Germany. As with clinical trials, there has been an increase in applications for market authorizations for ATMPs. The requested products increasingly concern reprogrammed endogenous T cells for the treatment of cancer and vaccines. An overview of already approved ATMPs is provided on the website of the PEI[7]. Again, the ZKBS is regularly updated.

2 Structure of the ZKBS

Experts from various areas of expertise meet in the ZKBS. In this way, a broad range of expertise is institutionalized and made available for the tasks of the ZKBS, which are specified in the GenTG, namely the evaluation of microorganisms as donor and recipient organisms for genetic engineering operation, the biosafety classification of genetic engineering operation, the assessment of safety-related measures of genetic engineering facilities, and the evaluation of deliberate releases and placing on the market of GMOs. Table 3 shows the composition of the ZKBS.

Prof. Dr Sigrun Smola, Member of the Virology Division since 2012, Chair of the ZKBS since June 2016. Vice Chairmen are Prof. Dr Uwe Groß and Prof. Dr Thomas Vahlenkamp (as of December 2019).

The appointment periods for Prof. Dr Kai Matuschewski, who has been a member of the microbiology area since 2016, and Prof. Dr Alfons Gierl, deputy member for the field of genetics since 2010, and Prof. Dr. Ilona Leyer, deputy member for the field of ecology since 2016, ended in 2019.

No new appointments were made.

The composition of the ZKBS shown in Table 3 corresponds to the specifications of the GenTG in the currently valid version.

[7] https://www.pei.de/DE/arzneimittel/atmp-arzneimittel-fuer-neuartige-therapien/atmp-arzneimittel-fuer-neuartige-therapien-node.html

Table 3 Areas of expertise and members of the ZKBS (as of December 2019)

Area of expertise	Member	Deputy member
Experts according to § 4 (1) No. 1 GenTG		
Microbiology	Prof. Dr Petra Dersch University of Münster	N.N.
Cell biology	Prof. Dr Bernd Gänsbacher Munich	Prof. Dr Michael Meisterernst University of Münster
Virology	Prof. Dr Thomas W. Vahlenkamp University of Leipzig	Prof. Dr Edgar Maiß University of Hannover
Virology	Prof. Dr Sigrun Smola Saarland University	Prof. Dr Stefan Pöhlmann Deutsches Primatenzentrum GmbH Göttingen Prof. Dr Anja Ehrhardt University of Witten/Herdecke
Genetics	Prof. Dr Jürgen Wienands University of Göttingen	N.N.
Genetics	Prof. Dr Uwe Sonnewald University of Erlangen-Nuremberg	Prof. Dr Uwe Völker University of Greifswald
Hygiene	Prof. Dr Uwe Groß University of Göttingen	Prof. Dr Werner Solbach University of Lübeck
Ecology	Dr Walter Durka Helmholtz-Zentrum für Umweltforschung GmbH Halle	N.N.
Ecology	Prof. Dr Rainer Waldhardt University of Gießen	Prof. Dr Martin Hasselmann University of Hohenheim
Plant cultivation	Prof. Dr Karl Schmid University of Hohenheim	N.N.
Security technology	Dr Sven Deutschmann Roche-Diagnostics GmbH Penzberg	Dr Jürgen Vorlop Marburg
Toxicology	Prof. Dr Andrea Hartwig Karlsruhe Institute of Technology (KIT)	Prof. Dr Edmund Maser University of Kiel
Specialists according to § 4 (1) No. 2 GenTG		
Occupational safety	Frank Gerschke State Office for Occupational Safety Potsdam	Dr Beatrice Spottke Trade Association for Raw Materials and Chemical Industry, Hamburg
Unions	Prof. Hon. Dr Wilfried Wackernagel University of Oldenburg	Dr Brigitte Dreiseikelmann University of Bielefeld
Agriculture	Prof. Dr Joseph-Alexander Verreet University of Kiel	Prof. Dr Ulrich Schurr University of Düsseldorf
Nature conservation	N.N.	N.N.

Area of expertise	Member	Deputy member
Research funding organizations	Dr Ingrid Ohlert German Research Foundation (Deutsche Forschungsgemeinschaft DFG), Bonn	Dr Jan-Wolfhard Kellmann University of Marburg
Environmental protection	Dr Gerd Neemann BLaU-Umweltstudien (environmental studies), Göttingen	N.N.
Consumer protection	Sigrid Lewe-Esch Deutscher Evangelischer Frauenbund e. V., Duisburg	Annette Neuhaus District Chemicals Council – District Lippe Detmold
Economy	Dr Siegfried Throm vfa Die forschenden Pharmaunternehmen, Berlin	Dr Anja Matzk KWS SAAT SE Einbeck

3 Advisory activities of the ZKBS in 2019

3.1 Mode of operation

The working method of the ZKBS is set out in its rules of procedure, which was adapted in 2018 to current standards for legislation. In 2019, seven meetings of the ZKBS (219th - 225th meeting sessions) took place at the BVL in Berlin. Most of the position statements of the ZKBS were adopted at these meetings. However, further decisions were also made using the written procedure, mainly if there were simpler questions that did not require a detailed discussion between all members.

3.2 Task forces/work groups

The efforts of the "influenza viruses" work group, which had already developed criteria for a risk assessment in the previous years, were continued in 2019. The outcome of the discussions was the adoption of an update of the position statement on the risk assessment of genetic engineering operation on recombinant influenza A viruses, ref_ 45310.0113, including its classification aid for influenza-A-virus mutants.

The "synthetic biology" work group was also continued. Publications relating to the newest developments are continually being sifted through and checked for a requirement to adapt the genetic engineering regulations. The results of continuous monitoring are regularly made available on the ZKBS homepage.

3.3 Advising the Federal Government, the competent state authorities and the BVL

The following questions of the responsible state authorities were discussed and evaluated by the ZKBS:

- Request for administrative assistance on the risk assessment of an AAV derived vector with oncogenes under the control of inducible promoters

- Transfusion of CAR-T cells in HIV-infected mice (the ZKBS was asked to check whether, by eliminating HIV-infected cells through CAR-T cells, a homologous recombination and thus the formation of recombinant HIV particles of risk group 3**, or the formation of recombinant, replication deficient lentivirus particles of risk group 2 can arise.)
- Query on downgrading recombinant influenza A viruses of the WSN strain with mutations in genes of the polymerase complex
- Query on a GMO/non-GMO *Actinobacillus pleuropneumoniae*
- Request for advice on repeal of an incidental provision for handling scrapie
- Fixture for leakage testing the room exhaust filter for a genetic engineering facility of a class 3 genetic engineering facility
- Request for administrative assistance for single case assesment of the risk group for influenza A viruses
- Request for administrative assistance for the classification of EBV transformed IPS
- Request for administrative assistance for the classification of the expression of alpha-synuclein in HEK293 cells
- Query on the scope of the position statement of the ZKBS on risk assessment of adeno-associated viruses from primates and vectors derived therefrom
- Application for administrative assistance to assess the calculation of the inactivation time in a waste water sterilization installation
- Request for advice on the use of rotation autoclaves for the sterilization of animal cadavers in a S3 installation

3.4 Risk assessment of donor and recipient organisms

The following microorganisms, which are used as donor or recipient organisms in genetic engineering operations, were assigned to a risk group in 2019 in accordance with § 5 in conjunction with Annex I GenTSV, or their classification was checked:

Table 4 Newly classified microorganisms

Organism	Risk group
Viruses	
Pestivirus B	2
Pestivirus E	2
Pestivirus F	2
Pestivirus G	2
Pestivirus H	2
Pestivirus I	2
Pestivirus J	1
Pestivirus K	2
*Bat pestivirus 1 a*nd *2*	1
Rodent pestivirus 1 to 4	1
Lateral-shaking inducing neurodegenerative agent (Linda) virus	2
Bombali ebolavirus	4
Cyprinid herpesvirus 1	2

Organism	Risk group
Cyprinid herpesvirus 2	2
Cyprinid herpesvirus 3	2
Salmonid herpesvirus 1	2
Salmonid herpesvirus 3	2
Beet curly top Iran virus	2
Cedar henipavirus	2
Snake HDV-like virus (sHDV)	2
Avian HDV-like virus (avHDV)	2
Fish HDV-like virus (fiHDV)	2
Toad HDV-like virus (tfHDV)	2
Newt HDV-like virus (amHDV)	2
Termite HDV-like virus (tHDV)	2
Rodent HDV-like virus (rHDV)	2
Bacteria	
Clostridium carboxidivorans	1
Clostridium ljungdahlii	1
"Clostridium autoethanogenum"	1
Synechococcus spp.	1
Arthrospira platensis	1
Eubacterium limosum	2
Clostridium beijerinckii	1
Clostridium scatologenes	1
"Clostridium aminobutyricum"	1
Bacillus anthracis, strains Sterne, Pasteur, STI-1, CDC1014, BH490, BH500, BH510	2
Komagataeibacter rhaeticus	1
Mycolicibacterium neoaurum	2
Escherichia coli 131/07, 2772a, 3234/A, D6-117.07, D6-117.29, O157:H43 T22, RiKo 2299/09, RiKo 2305/09, RiKo 2308/09, RiKo 2331/09, RiKo 2340/09 und UVM2	1
Escherichia coli 1303, AA86, D6-113.11, ECA-727, ECA-O157, ECC-1470, ECC-Z, MPEC4839, MPEC4969, O32:H37 P4, P4-NR, RiKo 2351/09 und W26	2
Aquifex aeolicus	1
Bartonella birtlesii	2
Bartonella schoenbuchensis	2
Sulfurospirillum barnesii	1
Bacillus cereus bv. *anthracis* CAR-H	2
Staphylococcus pettenkoferi	2
Aeromonas caviae	2
Curvibacter spp.	2
Bdellovibrio bacteriovorus	1
Micavibrio aeruginosavorus	1
Parasites and eukaryotic protozoa, except fungi/oomycetes	
Amphora coffeaformis	1
Craspedostauros australis	1
Cyclotella cryptica	1
Cylindrotheca fusiformis	1

Organism	Risk group
Fragilariopsis cylindrus	1
Seminavis robusta	1
Thalassiosira pseudonana	1
Thalassiosira oceanica	1
Euplotes crassus	1
Loxodes magnus	1
Loxodes striatus	1
Blepharisma japonicum	1
Blepharisma stoltei	1
Blepharisma undulans	1
Stentor spp.	1
Chlorogonium spp.	1
Auxenochlorella protothecoides	1
Chlorella sorokiniana	1
Schizochytrium spp.	1
Tetratrichomonas gallinarum	2
Trypanosoma brucei gambiense	3**
Fungi and oomycetes	
Sporothrix stenoceras	1
Capronia mansonii	1
Hortaea werneckii	1
Yarrowia sp.	1
Thermothelomyces thermophilus	2
Thermothelomyces thermophilus HC and derived production strains	1
Termitomyces spp.	1
Pseudoxylaria spp.	1
Melanopsichium pennsylvanicum	1
Sporisorium reilianum	1
Sporisorium scitamineum	1
Ustilago avenae	1
Ustilago nuda	1
Ustilago striiformis sensu lato complex (species existing in Germany and bordering nations or species, the host plants of which do not exist in Germany or bordering nations)	1
Ustilago striiformis sensu lato complex (species that do not exist in Germany and bordering nations, the host plants of which exist in Germany or bordering nations)	2
Ustilago tritici	1

The allocation to risk groups can be found in the ZKBS organism database[8]. General position statements on the risk assessment of organisms are published on the ZKBS website[9].

[8] http://apps2.bvl.bund.de/organismen/organisms.jsf

[9] http://www.zkbs-online.de/ZKBS/DE/05_Allgemeine_Stellungnahmen/Allgemeine_stellungnahmen_node.html

3.5 Biosafety classification of genetic engineering operations and assessment of safety measures of genetic engineering facilities

In 2019 (as of December), the ZKBS issued 46 position statements on the biosafety classification of genetic engineering operations and/or required safety measures. The evaluated genetic engineering operations and facilities concerned the topics listed in Table 5. For most of the genetic engineering operation that was evaluated, only a reference to the GenTSV was made for the safety measures. However, a detailed assessment was issued for a few of them regarding the technical and organizational security measures available or planned in the genetic engineering facility.

Table 5 Risk assessed genetic engineering operations and facilities in 2019. The titles of the genetic engineering operations were taken from the submitted application documents.

Class 2

- Delivery, storage and inactivation of solid and liquid wastes arising from genetic engineering laboratory facilities of class 1 and 2 and containing GMO or comprised of such, as well as HEPA filters of microbiological safety work benches that originate from laboratories or animal facilities up to class 2
- Production of flavivirus replicon particles as a tool for basic research, antiviral screening and the establishment of diagnostic tests
- Incorporation of genes for anti-tumour peptides in tumour colonizing bacteria
- Recombinant zika viruses for studying viral replication cycle, innate and acquired immunity and virus inhibitors
- Development of oncolytic adenoviruses with microRNAs against anti-apoptotic Bcl-2 proteins in combination with the death ligands TRAIL for improved efficiency with malignant melanoma
- Production of pseudo-rabies virus mutants by means of BAC technology in *E. coli* and characterisation of the recombinants *in vitro* and *in vivo*
- Genetic engineering operations with *Eubacterium limosum*
- Conjugation and transfection of recombinant virus plasmids from and to avirulent strains of *Bacillus anthracis* and other *Bacillus cereus* sensu lato
- Targeted immunomodulation through oncolysis with modified measles virus vaccine strains
- Expression of a fusion protein from charybdotoxin, SUMO and an His-Tag in *E. coli*
- Process development and GMP production operations with a replication competent, oncolytic, recombinant HSV-1
- Recombinant expression of chlorotoxin and chlorotoxin-fusion proteins in *Escherichia coli* (B, BL21; K12 derivatives, RG1) and yeast *Kluyveromyces lactis/Saccharomyces cerevisiae* (both GRAS organisms, RG1)
- Identification of infection-relevant Leishmania genes by gene deletion by means of CRISPR/Cas9 technology
- Onco-suppressive protoparvovirus isolate: Production of replication competent plasmids
- Combination tumour antigen expressing ORF virus vectors and VSV-GP pseudotype vectors for cancer immunotherapy
- Investigations of replication and pathogenesis of cedar viruses (CedPV)
- ConVIRgens: De- and reconstructing virulence strategies of fungal plant pathogens
- Determination of the immune response against a Rift-Valley-Fever-Virus vaccine

- Investigation of hepatitis B virus replication and pathogenesis in vivo
- Investigation of DNA repair mechanism in the immune system of *Drosophila melanogaster* during infection with *Mycobacterium marinum*

Class 3

- Discovery of novel inhibitors of *Chikungunya virus* (CHIKV)
- Construction and operation of a class 3 genetic engineering facility, stable
- Influence of HIV infection, antiretroviral therapy and restriction factor expression on the infectability of T-cell subpopulations
- Approval for the operation of a genetic engineering facility of class 3
- Production of reverse genetic systems for TBEV-EU viruses
- Investigation of the function of cellular lipid droplets in hepatitis C virus replication
- Interaction of cellular and viral proteins and antiviral active substance development
- Infection experiments with recombinant orthomyxoviruses
- Investigation of the role of metabolic processes in the replication and pathogenesis of flaviviruses
- *In vitro* characterisation of lentiviral luciferase-reporter viruses *
- Setting up a genetic engineering facility of class 3 *
- Producing recombinant West Nile early summer meningoencephalitis and yellow fever viruses
- Lentiviral reporter vectors for the resistance and neutralisation test
- Use of GFP-MERS coviruses for establishing serological verification procedures and for infection of mice
- Construction of mutants of *Bacillus anthracis* and *Bacillus cereus* biovar *anthracis* (Bcbva)
- Use of genetically modified flaviviruses: Reverse genetics of FSME viruses RG3 in eukaryotic cells and production of reporter viruses
- Investigations of recombinant Mayaro viruses (MAYV) for host virus interactions in humans and other mammalian cells and insect cells
- Characterisation of the hepatitis C virus replication cycle and the use of host factors - comparison of cell culture viruses and natural HCV isolates (relocation) *
- Characterisation and optimisation of a pan-flavivirus inhibitor
- Immune response of myeloid mammalian cells and experimental animal models against *Mycobacterium tuberculosis*
- Production of recombinant *Rift Valley Fever* viruses, replacement of the RVFV NSs gene by NSs-genes of other phleboviruses (relocation) *
- Molecular biological analyses of coronaviruses by means of reverse genetics (relocation) *
- Isolation of HIV-1 and HCV neutralising antibodies *

Class 4

- Functional characterisation of nipahvirus proteins
- The role of cellular and viral factors in the life cycle of filoviruses and identification of the molecular determinants of pathogenesis for filoviruses
- Replication and morphogenesis of filoviruses - subsequent projects 2019, infection of new genetically altered target cells

* updated position statements on already requested/implemented genetic engineering operations or facilities

3.6 General position statements and reports

The ZKBS issued or revised the following general position statements in 2019:

- General position statement by the ZKBS on the classification of genetic engineering operations in which genes for immunomodulating proteins are inserted in the genome of replication-competent microorganisms, ref. 6790-03-05 *
- Revision of the general position statement on classifying recombinant rabies- and vesicular-stomatitis viruses, ref. 45310.0117 *
- General position statement on the risk assessment of *E. coli* K12 derivatives with a plasmid containing the (c)DNA of the genome of a replication-competent virus, ref. 6790-10-89*
- Position statement of the ZKBS on the risk assessment of genetic engineering operations with recombinant influenza A viruses, ref. 45310.0113*

* Updates

All general position statements can be found on the ZKBS website[10].

In summary, Figure 2 displays the number of position statements issued by ZKBS in 2019 graphically compared to the number of position statements issued in the past five years. Overall, it can be seen that the annual number of position statements during this period has been largely constant.

[10] https://www.zkbs-online.de/ZKBS/DE/04_Allgemeine_Stellungnahmen/Allgemeine_stellungnahmen_node.html

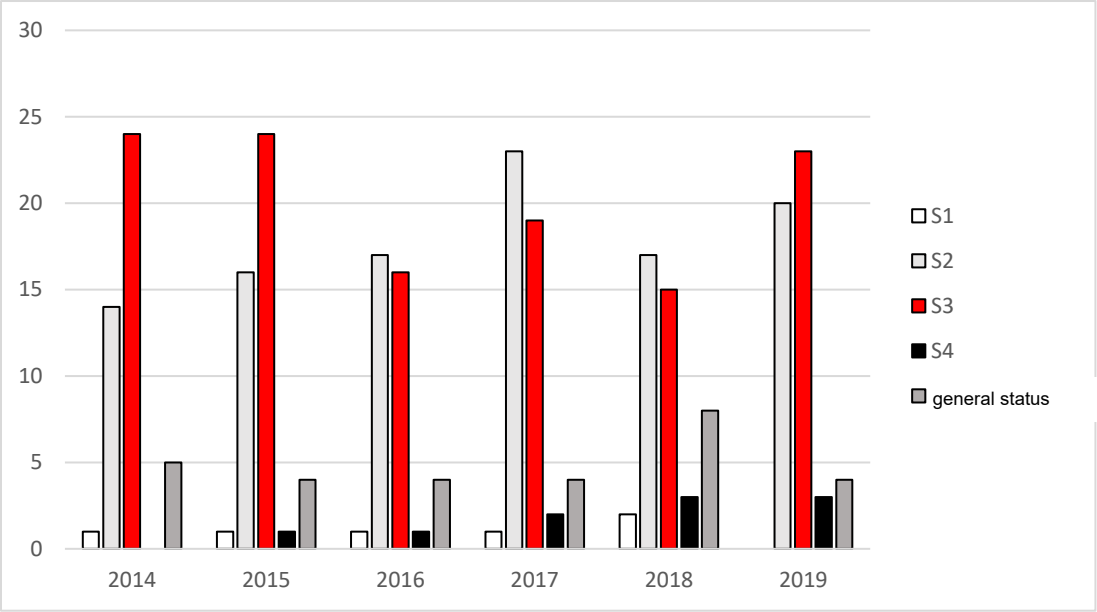

Figure 2 Number of position statements issued by the ZKBS in the past six years on genetic engineering operations in the genetic engineering facilities of the corresponding class and the general position statements

3.7 Position statements on deliberate releases

Position statements on deliberate release applications for GMOs were not submitted by the ZKBS in the reporting period.

3.8 Position statements on placing on the market

Position statements on applications for the placing on the market of GMOs were not submitted by the ZKBS in the reporting period.

3.9 Reports on topics of general importance

With the help of the newly established website (http//www.zkbs-online.de), the ZKBS also wants to use the opportunity to inform the public in a suitable way about topics of general importance. In 2019, the following topics were presented and commented on by the ZKBS[11].

1. Overview *Influenza viruses* with the following points:

- Trigger of the "real" flu
- Master of mutation
- Genetic engineering operations with influenza viruses
- Risk assessment of genetic engineering operations with influenza viruses
- Collection of general position statements by the German Central Committee on Biological Safety (ZKBS) on influenza A viruses
- Flu vaccine infographic
- Infographic on mutation experiments with influenza viruses

[11] https://www.zkbs-online.de/ZKBS/DE/03_Fokusthemen/DIY-Biologie/DIY-Biologie_node.html

2. Continuous monitoring of this research field was undertaken following the publication of the second report of the ZKBS on Synthetic Biology. The results are regularly presented in summary under the arranged section of "Synthetic Biology" on the homepage under the topic of "Developments in Synthetic Biology".

4 International networking

The 9th meeting of the European Advisory Committees on Biosafety (MEACB) was organized by the ZKBS and the BVL in November 2019. On two days, 65 participants from 15 European nations exchanged information on their working focus and discussed the newest scientific knowledge and the resulting challenges to risk assessment and the recommendations on safety measures in a closed system as well as for developments that are to find application outside of the lab. Thus, for example, the polio virus eradication programme of the World Health Organisation (WHO) was presented, gene-drive systems and their application potential were explained, medical usage of GMOs for humans and animals was introduced, and new developments in the field of plants were presented. The decision of the CJEU on new mutagenic technology in plant breeding was also made a subject of the discussion. As a result of a podium discussion, the majority of the representatives of the Committees on Biosafety agreed that an adaptation of the European GMO regulations to the state of the art is necessary. In follow-up, a common declaration by the ZKBS, the Swiss EFBS and the Netherlands COGEM was issued on a scientifically based future regulation of the new molecular technologies and was sent to the European Commission. The programme and impressions of the meeting are available on the homepage.