ZKBS
Zentrale Kommission
für die **Biologische Sicherheit**

Announcement
the work of the Central Committee
on Biological Safety in 2022

BVL 125/2023/04

33rd report after the entry into force of the Gene Technology Act

The aforementioned report on the work of the Central Committee on Biological Safety in 2022 is announced below.

Berlin, 23. May 2023

(BVL ref.: 45040)

Federal Office

for Consumer Protection and Food Safety

On behalf of

Dr Anke S t e i n

Structure

Abbreviations

ATMP	Advanced Therapy Medicinal Products
BfN	Federal Agency for Nature Conservation
BfR	Federal Institute for Risk Assessment
BMEL	Federal Ministry of Food and Agriculture
BVL	Federal Office of Consumer Protection and Food Safety
EFSA	European Food Safety Authority
EC	European Community
EMA	European Medicines Agency
EU	European Union
ECJ	European Court of Justice
FLI	Friedrich Loeffler Institute, Federal Research Institute for Animal Health
GenTG	Genetic Engineering Act
GenTSV	Genetic Engineering Safety Ordinance
GMO	Genetically Modified Organism
JKI	Julius Kühn-Institut, Federal Research Centre for Cultivated Plants
PEI	Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedical Products
RKI	Robert Koch Institute, Federal Institute of Public Health
TRBA	Technical Rule for Biological Agents
ZKBS	Central Committee on Biological Safety

Technical abbreviations are explained in the text.

1 Introduction

1.1 Fundamentals of the ZKBS

The Central Committee on Biological Safety (ZKBS) is an expert commission consisting of 20 members and 20 deputy members. The members are experts from different disciplines and are represented by experts from the same discipline. The fields represented are specified in the Genetic Engineering Act (GenTG). The ZKBS examines and evaluates safety-relevant questions on genetic engineering according to the regulations of the GenTG and advises the Federal Government and the Federal States. It issues statements to the competent authorities, in particular on the risk assessment of microorganisms, on the safety classification of genetic engineering work, on necessary safety measures in genetic engineering facilities and on possible risks of releasing or placing genetically modified organisms (GMOs) on the market. In its recommendations, it takes into account international developments in the field of genetic engineering safety. The members of the ZKBS and their deputies perform their work on an honorary basis in accordance with the GenTG.

The ZKBS has its office at the Federal Office of Consumer Protection and Food Safety (BVL), which is part of the portfolio of the Federal Ministry of Food and Agriculture (BMEL). The members of the ZKBS and their deputies are appointed by the BMEL in agreement with the Federal Ministries of Education and Research, of the Environment, Nature Conservation and Nuclear Safety, of Health, of Labour and Social Affairs, and of Economics and Climate Protection for a period of three years. Reappointment is permissible.

The ZKBS has a chairperson who is assisted by two deputy chairpersons. The Commission takes its decisions either at one of the regular meetings or in a written procedure. The members of the ZKBS and their deputies are bound to secrecy. The meetings are not public, but the ZKBS publishes general statements and reports annually to the public on its work.

1.2 Development of genetic engineering in the Federal Republic of Germany and in other Member States of the European Union

Genetic engineering work and genetic engineering facilities

The term "genetic engineering work" covers in particular the production of GMOs and the handling of GMOs. Depending on their safety level, genetic engineering operations must be notified, registered or approved by the competent Land authority and carried out in a genetic engineering facility, which must also be notified, registered or approved depending on its safety level. Genetic engineering facilities can be laboratories, production facilities, greenhouses and/or animal rooms.

In principle, the competent authority obtains a statement from the ZKBS on the safety classification of the planned genetic engineering work and on the necessary safety measures before deciding on a licence. This usually involves genetic engineering work of safety levels 3 or 4 and genetic engineering facilities with safety measures of levels 3 or 4. Due to the legal requirements, the competent authority also asks the ZKBS for statements on genetic engineering work of safety level 2 that is not comparable with other work or on which the ZKBS has already issued a statement in the past. It also asks for advice on genetic engineering work whose assignment to safety level 1 is uncertain.

Since the GenTG came into force in 1990, 2 227 initial applications for safety classification of genetic engineering work and/or for assessment of the necessary safety measures have been submitted to the ZKBS. In the year under review, 50 initial or extension applications were submitted and the ZKBS issued 49 statements.

In Germany, a total of 6 720 genetic engineering facilities have been notified, registered or approved (as of December 2022). Table 1 lists the genetic engineering facilities according to the level of safety measures. Table 2 summarises the genetic engineering work notified, registered or approved in Germany according to safety level.

Table 1 Notified, registered or approved genetic engineering facilities in Germany (as of December 2022)

Level	Number
S1	4 806
S2	1 868
S3	97
S4	5

Compared to the past five years, the total number of 6 000 to 7 000 genetic engineering facilities of levels 1 to 4 has remained largely stable. The largest proportion is accounted for by facilities of safety levels 1 and 2 (see Figure 1). While the number of genetic engineering operations in the area of safety levels 3 and 4 has changed only slightly within the past five years, a slight increase can be observed in the reported genetic engineering operations of safety level 2. Concrete information on the actual number of genetic engineering activities at safety level 1 cannot be provided, as there are no requirements for official recording.

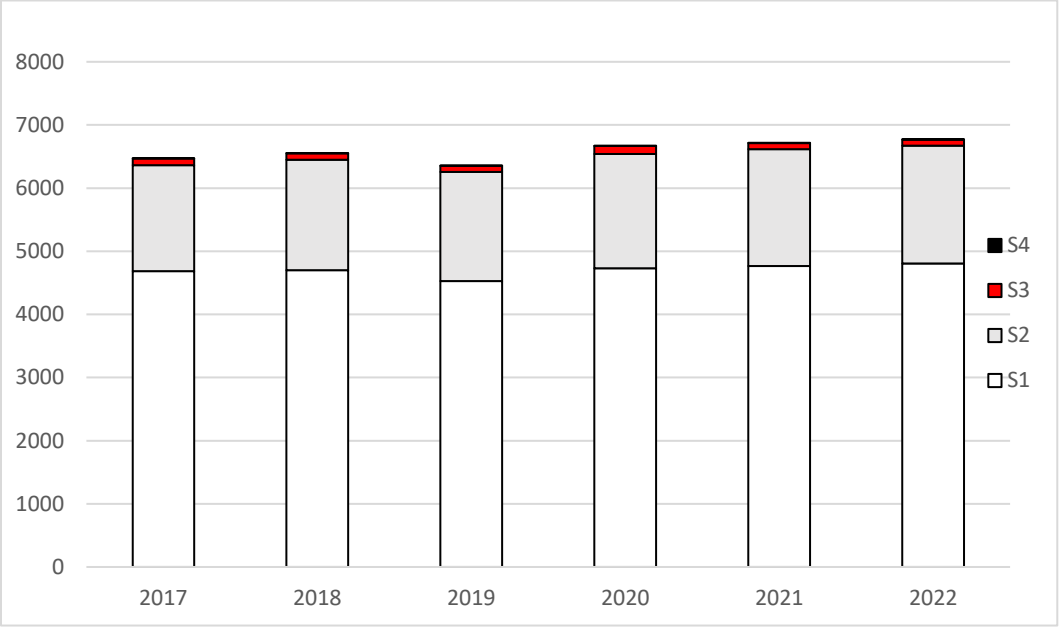

Figure 1 shows the numbers of notified, registered or approved genetic engineering facilities in Germany, differentiated by safety level (S1-S4), for the years 2017 to 2022.

Table 2 Currently notified, registered or approved genetic engineering work in Germany (as of December 2022) *

Level	Number
S2	9 627

S3	431
S4	17

* An exact specification is not possible for the genetic engineering work carried out at safety level 1, since the operators are obliged to record further work at safety level 1 according to § 9 GenTG, but there is no obligation to notify or report to the competent Land authority. Thus, further S1 work is not recorded in the official databases.

Further information on genetic engineering work and genetic engineering facilities is provided on the ZKBS website: https://www.zkbs-online.de.

A comparison of the number of genetic engineering operations or genetic engineering facilities between Germany and other Member States of the European Union (EU) is not possible, as no information is available on this. General information on the implementation of the underlying Directive 2009/41/EC is provided to the European Commission at regular intervals by the Member States in the form of completed questionnaires. The feedback from the Member States is summarised by the European Commission and published on its homepage[1] . In 2022, questions were addressed, among other things, on the official implementation of procedures including public participation and enforcement activities in the years 2018 - 2021, but also on accidents that have occurred with possible effects on the environment and existing experiences with the evaluation of *gene* drive *systems*. Feedback was also requested in case there were difficulties in the interpretation of the law. The summary report was published at the following link: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52023DC0075.

GMO releases

If GMOs are released into the environment for a limited period of time and in a limited area during an experiment, this is a "release". According to the Genetic Engineering Act (GenTG), a permit must be obtained from the BVL for each intended release, which can be granted if the planned release does not have any harmful effects on humans, the environment, animals, plants or material goods. If a marketing authorisation already exists for the GMOs to be released, no separate authorisation is required.

In Germany, the BVL has been responsible for approving the release of GMOs as the higher federal authority since 1 April 2004. The BVL makes decisions in consultation with the Federal Agency for Nature Conservation (BfN), the Federal Institute for Risk Assessment and the Robert Koch Institute (RKI). The ZKBS, the Julius Kühn Institute (JKI) and the competent authority of the federal state concerned each issue statements on the proposed release. In the case of the release of genetically modified vertebrates or of genetically modified microorganisms used on vertebrates, the Friedrich-Loeffler-Institut (FLI) is also involved. The other EU Member States are informed about release applications and can comment on them.

As in the years 2013 to 2021, no GMO releases were applied for in Germany in 2022, nor were any approvals granted for such releases. A comparison of the release applications submitted from the various EU Member States shows that in 2022 only isolated applications were submitted in Spain (1 - tobacco), Belgium (1 - maize) and the Czech Republic (1 - wheat). Details of applications can be found in the register kept by the European Commission[2] . Within Europe,

[1] https://food.ec.europa.eu/plants/genetically-modified-organisms/reports-and-studies_en#implementation_of_legisla

[2] https://webgate.ec.europa.eu/fip/GMO_Registers/GMO_Part_B_Plants.php

release trials of wheat are also being carried out in a *protected site* in Switzerland[3] , and releases of wheat, barley, potato and flax in the UK.[4]

Placing on the market of GMOs

“Placing on the market" means the supply of GMOs and products containing or produced from GMOs to third parties, usually for the purpose of marketing.

The placing on the market of GMOs requires an EU-wide authorisation procedure. This procedure distinguishes whether the GMO is to be used as food or feed [Regulation (EC) No 1829/2003] or not [Directive 2001/18/EC]. Products made from GMOs that do not fall within the scope of the above-mentioned Regulation or Directive, such as cotton clothing, do not require a marketing authorisation. After going through the EU-wide procedure, the authorisation is valid for all EU Member States.

The BVL is the competent German authority and issues a national opinion on applications for the placing on the market of GMOs in consultation with the BfN, the BfR and the RKI. Prior to this, an opinion is obtained from the JKI and, if the GMOs are vertebrates or microorganisms that are to be used on vertebrates, also from the FLI and the Paul Ehrlich Institute (PEI).

In addition, the BVL asks the ZKBS for an opinion on such applications under Directive 2001/18/EC that have been submitted in Germany. In the case of applications under Regulation (EC) No. 1829/2003, the BVL then requests a statement from the ZKBS if Germany has been commissioned by the European Food Safety Authority (EFSA) to carry out an environmental risk assessment of an application for cultivation. In 2022, there were no requests to the ZKBS in this regard.

Currently, *events* (number in brackets) of the following plants are mostly authorised for import as GMOs capable of multiplication and their processing or as food and feed:

- Cotton (15, +1 compared to previous year)
- Maize (82, +2 compared to previous year)
- Rapeseed (18, +2 compared to previous year)
- Soy (28, +3 compared to the previous year)
- Ornamental plants (6, +1 compared to the previous year)
- Sugar beet (1, unchanged compared to previous year)

Further details can be found on the BVL website[5] and in the entries of the EU register[6] . In contrast to local and time-limited release trials, the agricultural cultivation of genetically modified plants is not limited to specific locations or trial years. Cultivation of genetically modified plants by farmers can only take place once the placing on the market of the genetically modified seeds for the purpose of application in the environment has been authorised. An authorisation is usually valid for ten years and must be renewed thereafter. EFSA is responsible for the scientific assessment.

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[3] https://www.bafu.admin.ch/bafu/de/home/themen/biotechnologie/fachinformationen/freisetzungsversuche/freisetzungsversuche-mit-gentechnisch-veraenderten-organismen--g/b20002-gesuch-weizen.html

[4] https://www.gov.uk/government/collections/genetically-modified-organisms-applications-and-consents

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

[6] https://webgate.ec.europa.eu/fip/GMO_Registers/GMO_Part_C.php
or https://webgate.ec.europa.eu/dyna/gm_register/index_en.cfm

Decision of the European Court of Justice (ECJ) that plants produced with new mutagenesis methods are GMOs

Following an action brought by French (agricultural) associations before the French Council of State, the ECJ, which was asked to rule on this matter, stated in its judgment of 25 July 2018 that plants produced using both conventional and new mutagenesis methods are genetically modified organisms. They therefore fall under the regulations of the EU Directive 2001/18/EC. However, GMOs produced by conventional mutagenesis are excluded from the scope of the directive.

The study commissioned by the European Commission on the status of new mutagenesis methods within EU legislation, published in April 2021, came to the conclusion that an adaptation of the current GMO legislation to the state of the art in science and technology was absolutely necessary. The initiative presented by the Commission in 2021 for adapted legislation on plants and derived food and feed products produced by targeted mutagenesis and cisgenesis was followed up. After an intensive discussion involving stakeholders and competent authorities, the Commission worked on a draft law to be presented to Parliament and Council in 2023. In order to fill the knowledge gaps identified in the study in the area of genome-edited microorganisms and genome-edited animals, two mandates were given to EFSA.

Clinical trials with GMO-containing test products and use of GMO-containing medicinal products in humans

According to § 2 Paragraph 3 GenTG, the use of GMO-containing test products on humans is exempt from regulations under genetic engineering law in Germany. The approval of clinical trials with investigational medicinal products consisting of or containing a GMO or a combination of GMOs is regulated by the Medicinal Products Act. In Germany, the PEI, as the upper federal authority, is responsible for granting approval. The BVL is involved as a consultative authority in the assessment of the risk to the environment and in the determination of the demarcation of certain activities from the GenTG and reports to the ZKBS on this. In some cases, the ZKBS evaluates the hazard potential of the GMOs used in a statement.

In 2022, 28 approvals were granted by the PEI in which the BVL was involved. The test products were potential advanced therapy medicinal products (ATMPs), such as the treatment of cancer with reprogrammed endogenous cells for immune stimulation or gene therapy approaches for serious diseases due to monogenetic hereditary defects, or advanced vaccines against infectious diseases. An overview of approved clinical trials in the EU is provided by the European Union Register[7] .

Authorisation for the use of human medicinal products consisting of or containing a GMO or a combination of GMOs is granted through a centralised procedure by the European Commission under Regulation 726/2004/EC. Applications are submitted to the European Medicines Agency (EMA), which, as part of its role, prepares guidance documents for the evaluation and provides scientific coordination of the procedures. For the environmental risk assessment, the competent authorities of the member states according to Directive 2001/18/EC are involved, which in Germany is the BVL. As with clinical trials, there has been an increase in applications for marketing authorisations for ATMPs. The products applied for are increasingly reprogrammed endogenous T cells for the therapy of cancer and vaccines. An overview of ATMPs already approved can be found on the PEI homepage[8] . The ZKBS also receives regular reports on this.

[7] https://webgate.ec.europa.eu/fip/GMO_Registers/GMO_Part_B_Others.php

[8] https://www.pei.de/DE/arzneimittel/atmp/gentherapeutika/gentherapeutika-node.html

2 Composition of the ZKBS

The ZKBS brings together experts from different fields. In this way, a broad range of expertise is institutionalised and made available for the tasks of the ZKBS specified in the GenTG, namely the evaluation of microorganisms as donor and recipient organisms for genetic engineering work, the safety classification of genetic engineering work, the evaluation of safety measures of genetic engineering facilities and the evaluation of releases and placing on the market of GMOs. Table 3 shows the composition of the ZKBS.

In April 2022, Prof. Dr. Dr. Thomas Vahlenkamp, a member of the Virology Division since 2012, was elected Chair of the ZKBS. Prof. Dr. Sigrun Smola and Prof. Dr. Uwe Groß are the deputy chairpersons.

The terms of appointment of Dr Sabine Sydow, member for the area of economics since 2021, and Dr Gerd Neemann, member for the area of environmental protection since 1995, ended in 2022.

The reappointments due in 2022 were only delayed by the Federal Government (Prof. Dr. Dr. T. Vahlenkamp, Prof. Dr. A. Ehrhardt, Prof. Dr. U. Groß) or not made (Prof. Dr. E. Maiß). In a joint letter from the chairman of the ZKBS and the president of the BVL, the Federal Minister of Food and Agriculture was informed of the concern that the expert tasks assigned to the committee could not be fulfilled within an adequate period of time if the expertise required to evaluate the applications was not available. Both pointed out that this could have negative consequences for Germany as a research and science location in the field of biotechnology. The precarious situation was regularly pointed out by the members of the ZKBS to the BMEL representatives in the ZKBS meetings, but was not resolved in 2022.

There were no new appointments.

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

Table 3 Subject areas and members of the ZKBS (as of December 2022)

Subject area	Member	deputy member
Experts according to § 4 paragraph 1 number 1 GenTG		
Microbiology	Prof. Dr. Petra Dersch University of Münster **	Prof. Dr Susanne Hartmann Free University of Berlin
Cell Biology	Prof. Dr Michael Meisterernst University of Münster **	N.N.
Virology	Prof. Dr. Dr. Thomas W. Vahlenkamp University of Leipzig	Prof. Dr. Edgar Maiß University of Hanover *
Virology	Prof. Dr Sigrun Smola Saarland University **	Prof. Dr. Stefan Pöhlmann German Primate Centre GmbH, Göttingen ** Prof. Dr. Anja Ehrhardt University of Witten/Herdecke
Genetics	Prof. Dr Jürgen Wienands University of Göttingen	Prof. Dr Jens Boch University of Hanover **
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	Prof. Dr Michael Pester German Collection of Microorganisms and Cell Cultures, Braunschweig	N.N.
Ecology	Prof. Dr. Rainer Waldhardt University of Gießen	Prof. Dr. Martin Hasselmann University of Hohenheim
Plant breeding	Prof. Dr. Karl Schmid University of Hohenheim	Prof. Dr Maria v. Korff-Schmising University of Düsseldorf **
Security technology	Dr. Sven Deutschmann Roche-Diagnostics GmbH, Penzberg	Dr Holger Lübben GlaxoSmithKline Marburg
Toxicology	Prof. Dr. Edmund Maser University of Kiel	N.N.

Experts according to § 4 paragraph 1 number 2 *GenTG*

Occupational safety and health	Frank Gerschke Potsdam State Office for Occupational Safety **	Dr Daniela Harkensee Employer's Liability Insurance Association for Raw Materials and Chemical Industry, Hamburg **
Trade unions	Prof. Dr. Dr. h. c. Wilfried Wackernagel University of Oldenburg	Dr Brigitte Dreiseikelmann Bielefeld University
Agriculture	Prof. Dr Joseph-Alexander Verreet University of Kiel	Prof. Dr Ulrich Schurr University of Düsseldorf
Nature conservation	N.N.	N.N.
Research funding organisations	Dr. Ingrid Ohlert German Research Foundation, Bonn **	Dr Jan-Wolfhard Kellmann University of Marburg
Environmental protection	N.N.	N.N.
Consumer protection	Sigrid Lewe-Esch Deutscher Evangelischer Frauenbund e. V., Duisburg	Annette Neuhaus District Chief Chemist - Lippe District, Detmold
Economy	N.N.	Dr. Anja Matzk KWS SAAT SE & Co KGaA

* Reappointment procedure not completed by December 2022

** appointed until 2023

3 Consulting activities of the ZKBS in 2022

3.1 Mode of operation

The working methods of the ZKBS are regulated in its rules of procedure.

The year 2022 was also marked by the pandemic caused by infections with the *Severe acute respiratory syndrome-related coronavirus* Type 2 (SARS-CoV-2) virus. In 2022, five ordinary meetings were held in the form of video conferences. Two meetings were held in presence in Berlin, whereby a digital connection was possible.

Nationwide, this year also saw an increase in research into SARS-CoV-2 and the development of therapeutic and preventive approaches.

Most of the opinions of the ZKBS were adopted at the meetings. In addition, however, decisions were also made by written procedure when there were simpler issues that did not require extensive discussion between all members.

3.2 Working groups

In 2022, the work of the working group "Synthetic Biology" was continued under the leadership of Dr. J. Kellmann. Publications were continuously reviewed with regard to the latest developments and the need for an adaptation of genetic engineering regulations was examined. The results of

the continuous monitoring are regularly made available on the homepage of the ZKBS[9] . In addition, the third summary report was adopted and published in July 2022 with the assessment that there is currently no threat to biological safety from the latest developments in the field of synthetic biology[10] .

The working group "New Genomic Techniques" also met regularly to deal with the questions posed by the Commission in the context of public consultations on a possible new regulation of genome-edited plants. The chairman of the working group, Prof. Dr. J. Boch, reported on this to the committee at its meetings.

By resolution of the ZKBS, the preparation of a general statement with criteria for the evaluation of genetic engineering work with prions was prepared. The working group met regularly under the leadership of Prof. Dr. M. Meisterernst. Prof. Dr. I. Vorberg (German Centre for Neurogenerative Diseases, Bonn) was invited to one of the meetings as an external expert to contribute her expertise. Thanks to her expertise, a draft was presented at one of the meetings of the ZKBS, which was adopted and published as a statement after extensive discussion.

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

The following questions from the competent state authorities were discussed and evaluated by the ZKBS:

- Substantial modification of the ventilation and air-conditioning system of a genetic engineering facility of safety level 3, ref. 6790-01-1703
- Testing of the exhaust air filter of the room air conditioning system of a genetic engineering facility of safety level 3, 6790-01-1429
- Administrative assistance for the authorisation of genetic engineering facilities of safety level 3, assessment of the state of the art in science and technology, ref. 45110.2180
- Notification pursuant to § 28 para. 1 no. 2 GenTG concerning an incident in the genetic engineering facility 140/07 of safety level 4 and request for a statement pursuant to § 10 para. 7 GenTG, ref. 6790-01-1568
- Administrative assistance for the evaluation of genetic engineering work with the vaccine candidate Ad26.COV2.S.529 (Omikron vaccine), ref. 45242.0180
- Investigation of genetically modified SARS-CoV-2 particles in an aerosol test rig, Ref. 45110.2104
- Process for the chemical inactivation of *Pseudomonas putida* KT 2440 GMO strains, ref. 45250
- Application for downgrading of SARS-CoV-2 variants, ref. 45110.2106

3.4 Risk assessment of donor and recipient organisms

In 2022, a total of 73 microorganisms used as donor or recipient organisms in genetic engineering work were assigned to a risk group or their classification reviewed in accordance with Section 5 in conjunction with Annex I GenTSV. These included 23 viruses, 42 bacteria or archaea, 7

[9] https://www.zkbs-online.de/ZKBS/DE/SynthetischeBiologie/SynthetischeBiologie_node.html

[10] https://www.zkbs-online.de/ZKBS/SharedDocs/Downloads/Berichte/3._Bericht_ZKBS_Synthetische_Biologie_Juni_2018-Dez_2021.html?nn=15069024#download=1

eukaryotic microorganisms and one agent of transmissible spongiform encephalitis. A risk assessment was carried out for the following microorganisms:

Table 4Newly classified microorganisms, as of 31 December 2022

Organism	Risk group	File reference	Proce dure
Bacteria			
Aeribacillus pallidus	1	45241.0241	E
Anaerobutyricum hallii	1	45241.0231	E
Bacteroides uniformis	2	45241.0230	S
Brucella anthropi	2	45241.0242	S
Brucella ceti	2	45241.0242	S
Brucella inopinata	**2**	**45241.0242**	**S**
Brucella intermedia	2	45241.0242	S
Brucella microti	2	45241.0242	S
Brucella papionis	2	45241.0242	S
Brucella pinnipedialis	2	45241.0242	S
Brucella sp. BO2	2	45241.0242	S
Brucella spp. isolated from amphibians and rodents	2	45241.0242	S
Brucella vulpis	2	45241.0242	S
Caulobacter vibrioides	1	45241.0240	E
Dinoroseobacter shibae	1	45241.0240	E
Dorea formicigenerans	1	45241.0231	E
Enhydrobacter aerosaccus	1	45241.0235	E
Enterobacter sp. SA187	1	45241.0248	E
Herbaspirillum seropedicae	1	45241.0240	E
Hyphomonas neptunium	1	45241.0240	E
Klebsiella grimontii	2	45241.0238	S
Klebsiella michiganensis	2	45241.0238	S
Pantoea eucalypti	1	45241.0234	S
Phaeobacter inhibens	1	45241.0240	E
Photorhabdus temperata	1	45241.0246	E
Pseudomonas lundensis	1	45241.0228	E
Pseudomonas oleovorans	**2**	**45241.0230**	**S**
Ralstonia pseudosolanacearum	2	45241.0243	S
Ralstonia syzygii ssp. *celebesensis*	2	45241.0243	S
Ralstonia syzygii ssp. *indonesiensis*	2	45241.0243	S
Ralstonia syzygii ssp. *syzygii*	1	45241.0243	S

Salmonella enterica ssp. *enterica* Serovar Typhi ZH9	1	45241.0245	S
Stella humosa	1	45241.0240	E
Stella sp. ATCC 35155	1	45241.0240	E
Stella vacuolata	1	45241.0240	E
Stieleria maiorica	1	45241.0239	E
Streptococcus salivarius other than the above strains	2	45241.0229	S
Streptococcus salivarius strains K12, M18, 24SMB and DB-B5	1	45241.0229	S
Streptomyces chartreusis	1	45242.0247	E
Sulfitobacter dubius	1	45241.0240	E
Vibrio coralliilyticus	2	45241.0233	S
Xenorhabdus doucetiae	1	45241.0246	E
Eukaryotes			
Chrysotila carterae	1	45247.0010	E
Chrysotila dentata	1	45247.0010	E
Chrysotila haptonemofera	1	45247.0010	E
Chrysotila roscoffensis	1	45247.0010	E
Emiliania huxleyi	1	45247.0010	E
Trichoderma atroviride	1	45243.0124	E
Trichoderma virens	1	45243.0124	E
Viruses			
Adeno-associated virus serotype rh.74	2	45242.0202	S
Aotine betaherpesvirus 1	2	45242.0205	E
Avian coronavirus	2	45110.1951	S
Avian orthoreovirus	2	45242.0198	E
Bean yellow dwarf virus (BeYDV)	2	45242.0203	S
Chickpea chlorotic dwarf virus	1	45242.0204	E
Dengue virus rDENV1Δ30, rDENV4Δ30, rDENV2/4Δ30(ME), rDENV3Δ30/31	1	45242.0201	S
East African cassava mosaic virus	1	45242.0204	E
Feline morbillivirus	2	45242.0206	S
La Jolla virus (LJV)	1	45110.2183	S
Maporal orthohantavirus	3	45110.2219	S
Motts Mill Virus (MMV)	1	45110.2183	S
Mustelidae endogenous lentivirus of the ferret (MELVmpf)	2	45110.2143	S

Nelson Bay orthoreovirus	2	45110.2204	E
Rice rat hepatitis B virus (RRHBV)	3**	45110.2199	S
Rice tungro bacilliform virus	1	45242.0200	E
Rubivirus ruteetense	2	45110.2216	S
Rubivirus strelense	2	45110.2216	S
Sangassou orthohantavirus	3	45110.2219	S
Severe acute respiratory syndrome-related coronavirus, viruses OTS-206 and OTS-228	2	45242.210	S
Tigray orthohantavirus	3	45110.2219	S
Tomato leaf curl Yunnan virus	2	45242.0204	E
Tomato yellow leaf curl China virus	2	45242.0204	E
TSE agents			
Agent of Chronic Wasting Disease (CWD)	3**	6790-10-75	S

S: Session
E: Simple procedure by e-mail
Bold: Organisms whose classification deviates from that in the respective TRBA (partly only in details).

The assignments to risk groups can be found in the organism database of the ZKBS[11] . General statements on the risk assessment of organisms are published on the homepage of the ZKBS[12] .

3.5 Safety classification of genetic engineering work and assessment of safety measures of genetic engineering facilities

In 2022, the ZKBS issued 49 statements on the safety classification of genetic engineering work and/or on required safety measures. The genetic engineering work and facilities assessed concerned the topics summarised in Table 5. For many genetic engineering activities that were assessed, only a reference to the GenTSV was made for the safety measures. In some cases, however, a detailed assessment was made of the technical and organisational safety measures available or planned in the genetic engineering facility.

Table 5 Safety-assessed genetic engineering works and facilities in 2022. The titles of the genetic engineering works were taken from the submitted application documents.

Security level 1 (1)

- A controlled gene-drive for the maize pathogen *Ustilago maydis*, Az. 45110.2212

Security level 2 (21)

- Recombinant picornaviruses for tumour gene therapy, ref. 45110.2089_1. Extension

[11] https://zag.bvl.bund.de/organismen/index.jsf

[12] https://www.zkbs-online.de/ZKBS/DE/Stellungnahmen/stellungnahmen_node.html

- Use of recombinant replication-competent luciferase/GFP reporter viruses (derivatives of human adenovirus type 5 (hAd5), herpes simplex virus-1 (HSV-1), influenza A virus (FLUAV), murine hepatitis virus (MHV), Semliki Forest virus (SFV), vesicular stomatitis virus (VSV) and Zika virus (ZIKV)) for the study of cell biological processes, in particular the innate immune response, ref. 45110.2181
- Production of recombinant bee and insect viruses, ref. 45110.2183
- Investigation into the role of autophagy receptors in the pathogenesis of cytomegalovirus, ref. 45110.2186
- Infection of cell lines and primary cells with recombinant LCMV in which the LCMV glycoprotein (GP) has been replaced by Lassa virus (LASV) GP, ref. 45110.2188
- Infection of cell lines and primary cells with recombinant LCMV in which the RRLA SKI-1 cleavage site in the LCMV glycoprotein has been replaced by the RRR Furin cleavage site, Ref. 45110.2189
- Investigation of cell binding, cell entry and pathogenicity of respiratory envelope viruses (FLUAV, VSV) and their gene products as well as the contribution of host-specific factors in different cell lines and human cell isolates, ref. 45110.2190
- Identification and characterisation of antibiotic resistance systems and disinfectant resistance systems from various Enterobacteriaceae, ref. 45110.2191
- Development of test methods for a rabies vaccine for dogs and cats containing the rabies virus glycoprotein G (RabG) in the equine herpesvirus vector (EHV-1): EHV-1-RabG.gbl., ref. 45110.2193
- Adenoviral oncolysis with mutated human adenoviruses, ref. 45110.2196
- Production of vaccine vectors or viruses based on HSV1, ref. 45110.2201
- Work with the vaccine candidate sCPD9, a SARS-CoV-2 virus attenuated by genetic modification, ref. 45110.2202
- Use of a plasmid-based reverse genetic system to study basic biological mechanisms of rotaviruses, ref. 45110.2204
- Evaluation of the oncolytic activity (VSV-GP) of virus-based vaccines in a syngeneic mouse melanoma model (B16F10), ref. 45110.2205
- RNA switch for regulating the expression of viral genes and/or therapeutic transgenes of oncolytic measles virus vaccine strains, ref. 45110.2207
- Storage, secondary packaging and dispatch of GMO (NDV) products, Ref. 45110.2210
- Investigation of the activity of a type IV-A CRISPR-Cas system in *Pseudomonas oleovorans*, ref. 45110.2213
- Production of recombinant arteriviruses, ref. 45110.2214
- Identification and characterization of plant genes and proteins involved in the infection by geminiviruses**,** Az. 45110.2215
- Characterisation of vetters of the genus Rubivirus, ref. 45110.2216
- Virus-based transient transformation of maize, Az. 45110.2217

Security level 3 (27)

- Testing of the exhaust air filter of the ventilation system of a genetic engineering facility of safety level 3 of the Ludwig-Maximilians-University MunichRef. 6790-01-1429
- Substantial modification of the genetic engineering facility PEI 13, ref. 6790-01-1658
- Approval of the substantial modification of an existing genetic engineering facility of safety level 3, ref. 6790-01-1676_Substantial modification
- Isolation of HIV-1 and HCV neutralising antibodies - 2nd extension, ref. 45110.1935_2. Extension
- Substantial change: Technical adjustment to the thermal waste water inactivation system (TAI) and the ventilation and air conditioning system (RLT) Ref. 45110.2075_substantial change
- Establishment of an animal model for SARS-CoV-2 and testing of vaccines and therapeutics, ref. 45110.2079_2. Extension
- Studies on the replication cycle of the *Severe acute respiratory syndrome Coronavirus* (SARS-CoV)-2 in eukaryotic cells using recombinantly produced viruses, ref. 45110.2108_1. Extension
- Visualisation and characterisation of cells surviving infection with mouse-adapted SARS-CoV-2 - 1st extension, ref. 45110.2142_1. Extension
- Investigation of recombinant immunodeficiency viruses for pathogenesis and host-virus interactions in human and other mammalian cells - extension application 4, Ref. 45110.2143_1. Extension
- Construction and operation of a genetic engineering facility - amendment to the safety measures applied for, ref.45110.2152_1. Amendment
- Biology and pathogenesis of human immunodeficiency viruses type 1 and type 2 (HIV-1, HIV-2)-extension, ref. 45110.2176
- Characterisation of SARS-CoV2 mutations, ref. 45110.2187
- Investigations of virus-host interactions in co-infection of primary cells and cell lines with pairs of recombinant arthritogenic alphaviruses (Mayaro virus and Chikungunyavirus) as well as chimeric viruses, ref. 45110.2192
- Cryptic reading frames in SARS-CoV-2 ORF3a, ref. 45110.2194
- Characterisation of the evolution and immune evasion of lentiviruses and the pathogenesis of AIDS, ref. 45110.2195
- Tetracycline-dependent expression control of essential genes for drug validation against *Mycobacterium tuberculosis*, ref. 45110.2197
- Characterisation of the pathogen-host interactions of a mouse-adapted SARS-CoV-2 isolate, ref. 45110.2198
- Production and characterisation of subviral hepadnavirus particles, ref. 45110.2199 (indicated as S2)
- Production of recombinant beta-coronaviruses (subgenus SARS-CoV-2), ref. 45110.2200

- Studies on new active substances MJ22 and B6 against *Mycobacterium tuberculosis*, ref. 45110.2203
- Investigations on molecular secretion mechanisms of virulence factors and immunomodulating effector proteins and their modes of action in *Salmonella enterica* serovar Typhi, ref. 45110.2206
- Functional RNA structures of the replication-competent HIV-1 genome, ref. 45110.2208
- Evaluation of attenuated SARS-CoV-2 viruses as potential vaccine vectors in huACE-2 transgenic mice, ref. 45110.2209
- Replication of Mayarovirus (MAYV) variants in *vitro,* ref. 45110.2211
- Genetic engineering work on coronaviruses in connection with the SARS-CoV-2 pandemic, ref. 45110.2218
- Studies on replication and virus-host cell interactions of hantaviruses, ref. 45110.2219
- Influence of open reading frames on the replication of SARS coronavirus 2 in human cells, ref. 45110.2220

Safety level 4

- none

In summary, Figure 2 shows the number of opinions issued by the ZKBS in 2022 in comparison to the number of opinions issued in the past four years. Overall, the significantly increased demand for opinions on genetic engineering work of safety level 3 in 2020 compared to other years is confirmed. This is due to the pandemic-related and federally funded orientation of research approaches towards coronaviruses. The total number of applications submitted to the ZKBS in 2022 corresponds to that of the previous pandemic years. In 2022, the focus of the applications submitted for the implementation of genetic engineering work at safety level 3 was again on research work on coronaviruses (36 %); in some cases, applications were submitted for the expansion or reorientation of work already started with the virus. However, with the entry into force of the amended Genetic Engineering Safety Ordinance in March 2021, an increasing number of applications were also submitted for significant modifications to a genetic engineering facility of safety level 3 in order to comply with the legally required state of the art in science and technology. These applications primarily described the modification of ventilation systems in order to be able to check the sealing and integrity of the H14 HEPA filters contained therein (particle scanner).

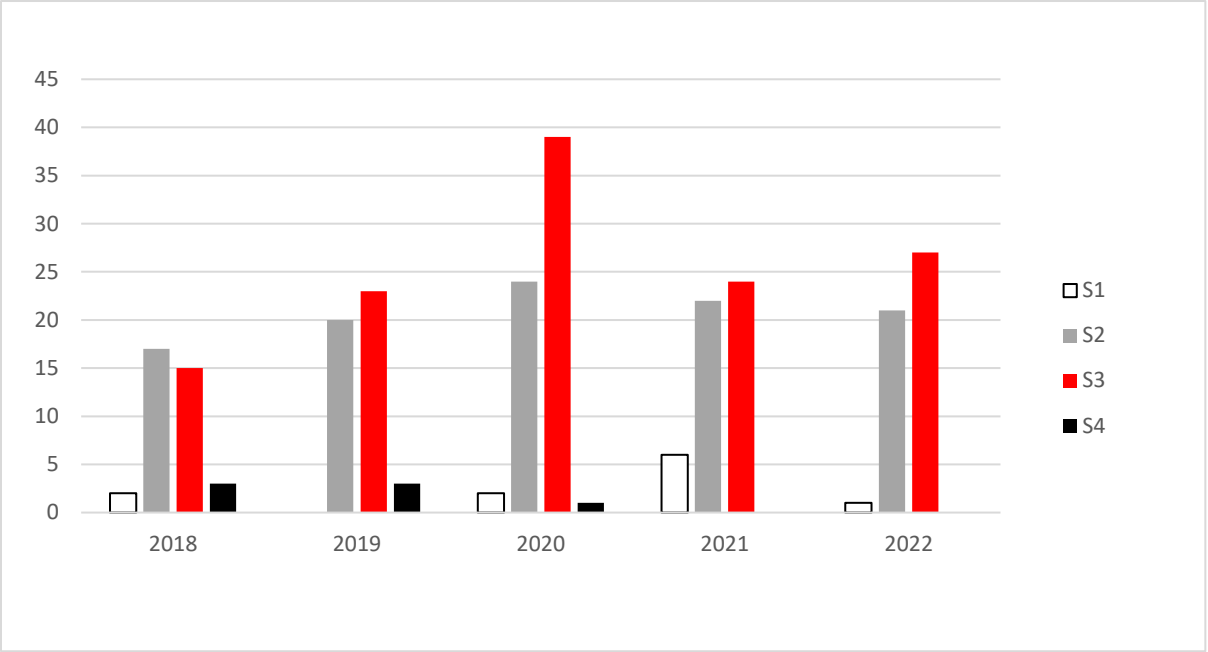

Figure 2 The figure describes the respective number of statements on genetic engineering work in genetic engineering facilities prepared by the ZKBS in the past five years, differentiated according to safety levels 1 to 4 (S1 - S4).

3.6 General statements and reports

The ZKBS has prepared or revised the following general statements in 2022:

- Update of the statement of the ZKBS on the risk assessment of prokaryotic environmental isolates in genetic engineering work, ref. no. 6790-10-43
- Update of the statement of the ZKBS on the classification of genetic engineering work in which genes for immunomodulating proteins are inserted into the genome of replication-competent microorganisms, ref. no. 6790-03-05
- Statement of the ZKBS on the suitability of asporogenic, thymine-dependent mutants of *Bacillus subtilis* 168 as part of biological safety measures according to § 8 paragraph 1 GenTSV, ref. no. 45270_e
- Statement of the ZKBS on the risk assessment of genetic engineering work on the expression of prion proteins, ref. 6790-10-75
- General statement 'Sendaivirus-derived vectors for the generation of pluripotent stem cells (iPSC)' - Disclaimer, ref. 45310.0119
- 3rd Report of the ZKBS on Synthetic Biology

All general statements can be found on the ZKBS website[13] .

[13] https://www.zkbs-online.de/ZKBS/DE/Stellungnahmen/stellungnahmen_node.html

3.7 Overruling of opinions

The ZKBS regularly reviews its statements to ensure that they are up to date. In 2022, the following statements were repealed:

- Statement of the ZKBS on the infection of animals with genetically modified organisms of risk group 2 (ref. 6790-10-31, 1994)
- Statement of the ZKBS on the evaluation of the expression of the murine pelota gene using the vector pTAT-2.2 (ref. 6790-04-0231, 2006)
- Statement of the ZKBS on frequently performed genetic engineering work with the underlying criteria of comparability: Genetic engineering work with SV40 as donor organism (ref. 6790-10-34, 1995)

3.8 Opinions on releases

The ZKBS did not issue any statements on applications for the release of GMOs during the reporting period.

3.9 Opinions on placing on the market

The ZKBS did not issue any statements on applications for the placing on the market of GMOs during the reporting period.

3.10 Reports on topics of general importance

With the help of its own homepage (https://www.zkbs-online.de), the ZKBS would also like to use the opportunity to report to the public in an appropriate manner on topics of general importance, such as developments in the field of synthetic biology. In 2021, the homepage appeared in a new design and with a new structure. In 2022, an explanatory video was posted, in which the working methods of the ZKBS are explained to the interested public by means of an example.