ÚOCHB AV ČR
IOCB PRAGUE

Handling genetically modified organisms (GMOs)

Training course

What are GMOs?

A GMO is an organism, **other than a human**, whose hereditary material has been altered by **genetic modification** carried out in one of the ways listed in the relevant law, mostly using techniques of targeted modification.

GMOs do not arise from the following:

natural recombination, random mutation, bacterial conjugation, transformation and transduction (where artificially prepared recombinant DNA is not used), natural cell fusion and induction of haploidy and polyploidy.

Genetically modified organisms

Micro-organisms (including cell lines)

Plants

Animals

The handling of GMOs is governed by legislation

Act 78/2004 Coll.
of 22 January 2004
on the use of genetically modified organisms and genetic products

Decree 209/2004 Coll.
of 15 April 2004
on detailed conditions for the use of genetically modified organisms and genetic products

AMENDMENT: ACT 346/2005 Coll. Of
29 July 2005

How do GMOs arise?

1) Techniques involving **recombinant DNA** by which hereditary information is inserted into a vector that is transferred into a recipient organism:

Most frequently, a plasmid-based construct is transferred by transformation (of a micro-organism) or transfection (of a eukaryotic cell) or transduction (wrapped in a pseudovirus particle) into a host cell, where it then multiplies as a plasmid or where the introduced genetic information is incorporated into a chromosome.

(2) Techniques introducing **artificially prepared hereditary material directly into the recipient organism** – microinjection, macroinjection, biolistic methods, microencapsulation and artificial chromosomes

(3) **Cell fusion** techniques by which viable cells with a new combination of hereditary material are produced using artificial methods

Risk category

Categories of risk management relate to specific activities.

First category: activities carrying no or negligible risk (e.g. cloning and cultivation of *Escherichia coli*, *Saccharomyces cerevisiae,* cell lines).

Second category: activities carrying a low risk of harmful effects that can easily be undone (e.g. the cloning and cultivation of the pathogenic bacteria *Staphyloccocus aureus, Pseudomonas aeruginosa*, *Bordetella pertussis*, transduction of cell lines using pseudoviral particles with the use of retroviral vectors).

Third category: activities carrying risks that can be removed only by sophisticated measures (e.g. the preparation of HIV mutants)

Fourth category: activities carrying high risk (e.g. involving the ebola virus)

Working with GMOs at the IOCB of the CAS

Authorization to handle GMOs subject to notification:

1st risk category

Linum, *Arabidopsis thaliana*, *Nicotiana*, *Escherichia coli*, *Komagataella pastoris* (*Pichia*), *Saccharomyces cerevisiae*, *Leishmania tarentolae*, *Bacillus subtilis*, cell lines, *Mus musculus*, *Homo sapiens* (cDNA)

2nd risk category

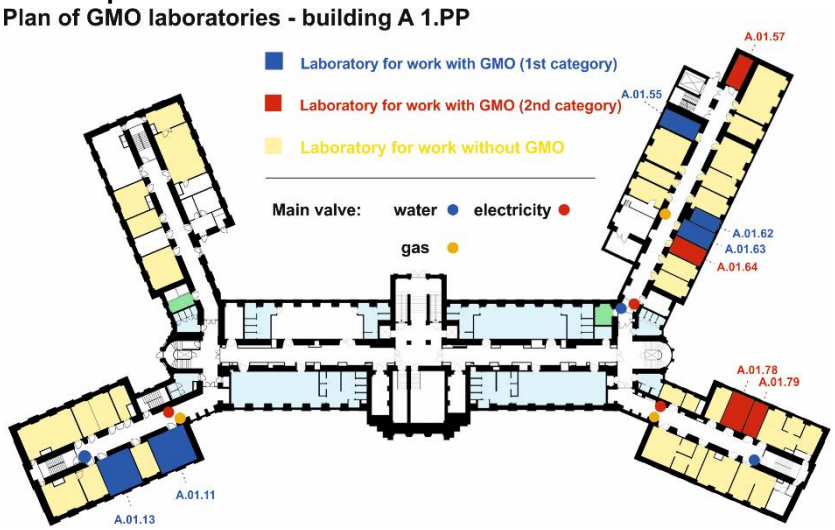
Baculovirus, lentivirus vectors, HEK293(T) cell lines

If any scientific work requires the use of a particular GMO, it is necessary to find out whether the institute holds a **permit for working with this GMO**. If it does not, the new organism has to be included in the '**notification document**' submitted to the Ministry of the Environment.

Documents necessary to lodge a GMO handling notification

Cover letter
Notification of confined handling
Risk assessment
Operating Regulations
Emergency Response Plan.
Enclosed space requirements
Plan of the premises and facilities

Example:



Dealings with the Ministry of the Environment are conducted by the **Expert advisor for the handling of GMOs at the IOCB of the CAS**.

Requirements on the advisor are education in the field, experience and a blank criminal record.

Expert advisors for the IOCB of the CAS, p.r.i..:

2005–2021	Ing. Juraj Sedláček. DrSc.
2021–present	RNDr. Milan Kožíšek, Ph.D.

Content of notifications and notification of changes

- Organization
- Contact persons and persons in charge
- Organisms
- Risk assessment
- Risk category
- Rooms for working with GMOs
- Staff
- Enclosed space requirements (comparison table)
- Import

Certificate of Authorization to Handle GMOs

Notifications and other documents (Emergency Response Plan, Operating Regulations, Risk Assessment) submitted to the Ministry of the Environment are assessed by opponents and returned for possible correction and supplementation. The organization then receives the following:

Certificate of Authorization to Handle GMOs to the extent and in the circumstances described in the Notification.

For the **1st category:** The handling of GMOs is permitted **from the date of the submission of the notification.**

For the **2nd category:** The handling of GMOs is permitted **after 45 days from the delivery of the notification to the Ministry of the Environment.**

A copy of the **Certificate of Authorization to Handle** GMOs is attached to some grant proposals submitted to the Czech Science Foundation (GA CR).

The Ref. No. of the notification (e.g. 75721/ENV/15) shall be provided as proof of an authorization to handle GMOs when importing or exporting GMOs.

Ten rules for working in a GMO laboratory

1. Posted in each room or enclosed space for working with GMOs is the **Emergency Response Plan** and **the Operating Regulations** signed by an expert advisor.
2. Access is allowed only to trained staff, and the door is marked with a **BIOHAZARD sticker** (mandatory from the 2nd category up).
3. Gowns marked **with the emblem BIOHAZARD** are used (for activities falling under the 2nd category), protective gloves, bag for used gowns.
4. **Disinfectant (Ajatin, SAVO)** is in the marked place
5. GMOs are rigorously inactivated by autoclaving or the use of disinfectant.
6. **Waste** is discarded into labelled containers and disposed of safely.
7. It is necessary to keep protocols. Inspections of premises are carried out.
8. The staff undergoes training once a year.
9. Records of areal inspections in the operating logbook (kept by the expert advisor).
10. **Secure windows** (non-openable or with a net).

Accident mitigation

- Visibly mark the **site of contamination** so that the GMO do not spread outside the laboratory.

- Upon spilling a bacterial culture immediately **decontaminate the space** with chemicals; the SAVO and Ajatin disinfectants are available in each room.

- Report any unexpected event or **accident to a supervisor** or to the GMO expert advisor, who will ensure the monitoring of the GMO to prevent its spread outside the guarded area.

- If internal contamination of persons has occurred, provide **first aid** (rinse the mouth, nose and eyes, or the injured area). Secure medical help if necessary.

GMO waste

- **1st category:** all waste containing GMOs (solid – dishes, plasticware) **is to be inactivated by autoclaving** and then **handed over to a specialized company for disposal** – GMO waste is taken away by the company Recovera. The liquid waste (media) is inactivated by the use of disinfectant and then transferred **into a sink.**

- **2nd category:** all waste containing GMOs (liquid, solid) **is to be inactivated by autoclaving or the use of disinfectant** and then **handed over to a specialized company for disposal** – GMO waste **is taken away by the company Recovera.**

Recovera by VEOLIA

- **Moving GMOs and waste between rooms:**

  in an unbreakable, **closed box (e.g. made of plastic)** marked as GMO, e.g. with a BIOHAZARD sticker, waste in marked closed bags

Inspection by the Czech Environmental Inspectorate

Triennial During an inspection, the following shall be submitted:

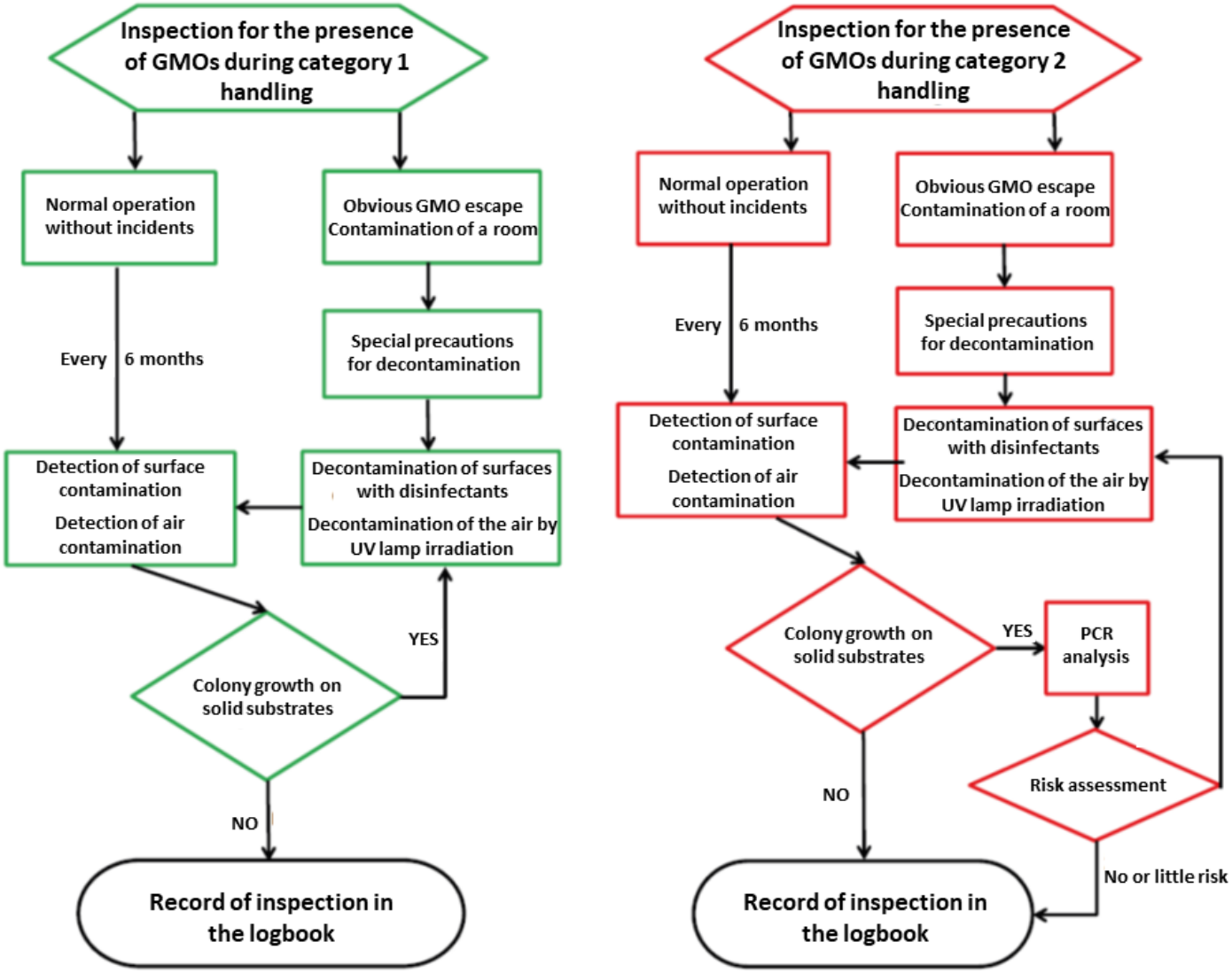
- A copy of the **Certificate of Authorization to Handle GMOs**
- Notifications of Confined Handling submitted
- **List of staff**
- annual **staff training**
- An agreement with a company disposing of GMO waste
- **Operational log book**
- **Emergency Response Plan and Operating Regulations for Laboratories**
- **Annual report (signed by the advisor)**
- Environmental Damage Risk Assessment form
- **Notification of the Import of a** GMO
- Confirmation of the sending of the Emergency Response Plan to the (regional) **fire brigade** and to the **regional authority**

Verification of measures inside rooms

The inspection is carried out by the expert advisor with an assistant: once a year (1st category)
4 times a year (2nd category)

1st	BIOHAZARD emblem on the door	Mandatory from the 2nd category up Access to the room is permitted only to staff trained to work with GMOs.
2nd	Refrigerators and freezers containing GMOs are marked (with the emblem **GMO** or **BIOHAZARD**).	
3.	**Operating Regulations** and **Emergency Response Plan** (bearing the signature of the advisor) are posted in each room where GMOs are handled.	Annexes to the **Operating Regulations**: Up-to-date list of trained staff. Designated persons: advisor, contact person and person responsible. Annex: Plan of the premises
4.	Secure **windows**	non-openable or with a net
5.	**Gowns** marked with the **BIOHAZARD** emblem available	protective gloves, bag for used gowns
6.	**Disinfectant (Ajatin, SAVO) in a conspicuously marked place**	
7.	**Waste bins** marked with the GMO emblem.	
8.	Secure system of rigorous GMO **inactivation**	autoclaving or chemical disinfection (SAVO, Ajatin)
9.	Closed unbreakable containers (cases, boxes) available for **carrying GMOs** outside the enclosed space	
10.	Functional biohazard box and air conditioning	2nd Risk category of handling

Experimental detection of GMOs



Import, export and transport of GMOs

- GMOs can be imported, exported or transported **only by users included on the list of users.**
- Containers or packaging containing GMOs must be visibly labelled **with the words ‘genetically modified organism’.**
- To **inform the MoE at least five days before the import**

Transport GMM: Go Express & Logistics, World Courier, KRD, PMTrans

Mice: AnLab, SEMED, VELAZ

It is not permitted to bring GMOs from an internship abroad without coordination with the Ministry of the Environment!

Useful information

Expert advisor for the handling of GMOs at the IOCB of the CAS:

RNDr. Milan Kožíšek, Ph.D. (milan.kozisek@uochb.cas.cz, telephone line 518)

Advisor's assistant:

Ing. Mária Čechová (ria.cechova@uochb.cas.cz, telephone line 289)

Information pertaining to waste

Petr Šimek (petr.simek@uochb.cas.cz, telephone line 228)

On the intraweb of the IOCB of the CAS, under section **Science/GMO Documents**, you can find the following:

- IOCB Emergency Response Plan for Working with GMOs
- IOCB Operating Rules for Working with GMOs
- Annually updated List of Staff Trained to Handle GMOs
- Certificate of authorization to handle GMOs

More information:

Ministry of the Environment of the Czech Republic

https://www.mzp.cz/cz/formulare_metodicke_pokyny_gmo