
**BETRIEBSANWEISUNG
(Kurzfassung) GEMÄß
§ 14 GEFÄHRSTOFFVERORDNUNG
UND
§ 12 BIOSTOFFVERORDNUNG**

**SAFETY INSTRUCTIONS (Summary)
ACCORDING TO
§ 14 GEFÄHRSTOFFVERORDNUNG
AND
§ 12 BIOSTOFFVERORDNUNG**

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Institute for Neurophysiology, University of Köln, Germany

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(teilweise entnommen aus:

taken in part from: "Safety Sense. A Laboratory Guide" Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York, 2001.

Introduction: General instructions for safety and waste disposal

The guidance offered here is for coworkers who have received an intensive education in the field of life sciences. All of us are asked to use **common sense** rules which means, that you have to act with “**gesundem Menschenverstand (sanity and reason)**”.

For those of us who started recently in the field of life sciences, one major rule is, to keep to the information and introduction given by the group instructor (Arbeitsgruppenleiter). Furthermore, a detailed list of instructions was summarized in German which can be found in the internet-home-page of the institute of Neurophysiology. Similarly, guidelines in English for the work in laboratories are supplied by:

<http://userpage.chemie.fu-berlin.de/~tlehmann/guidel.html>

Proper safety and proper waste disposal procedures for the laboratory personnel can be found at:

- the local **Safety Office:**

(internal phone numbers)

Dr. Hartmann, 6428

Dr. Sander 6427

<mailto:dietmar.hartmann@uk-koeln.de>

<mailto:ralf.sander@uk-koeln.de>

The best source of toxicity, hazard, storage, and disposal information is our local safety office. Always consult this office for proper use and disposal procedures.

Place the important phone numbers for our local safety office, security office, poison control center and lab emergency personnel in your lab:

Wichtige Rufnummern / Important phone numbers:

Interner Notruf (Werkfeuerwehr)	112	Internal emergency call (plant fire brigade)
Zentrale Notaufnahme	6267	Central emergency admission in the hospital
Notfalltelefon	5555	Emergency phone
Krankenwagenleitstelle	5491, 5492	Ambulance control center
Betriebsärztlicher Dienst	6090, 6091	Company medical officer
Abt. Arbeitssicherheit und Umweltschutz	6428	Department for job and environmental safety
Fachkräfte für Arbeitssicherheit	5236	Specialists for job safety
Fachgruppe Abfallwirtschaft	6442, 5960, 4468	Section for waste control
Fachgruppe Gefahrstoffverordnung	6427	Section on the Ordinance on Hazardous Substances (Dr. Sander)

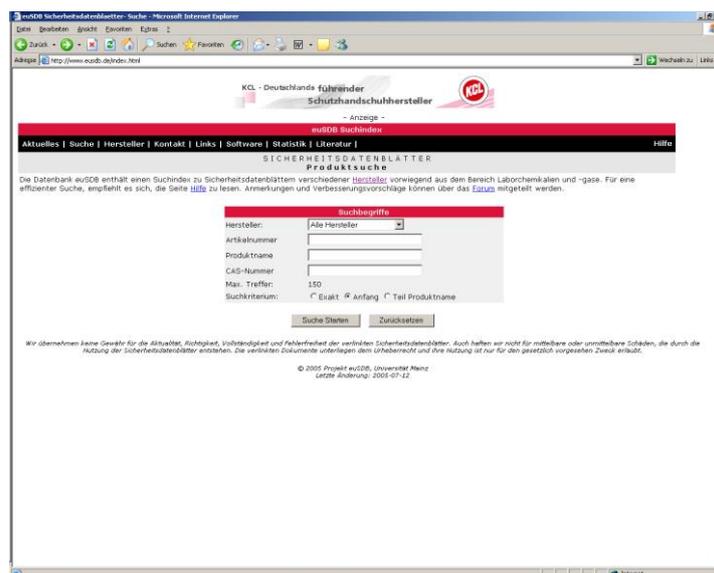
Please, use also the guidelines stored in:

- **the Material safety Data Sheets (MSDS):**

Several government institutions in Europe and elsewhere demand that all hazardous products, which are shipped, are accompanied by a MSDS. These data sheets contain detailed safety information. DSDS should be filed in the laboratory of each working group, in a central location as a reference guide (a very few will be attached to this list of information and instruction). Online, you can get access on the following home page:

<http://www.eusdb.de/index.html>

introduce your request e.g. as product name (Produktname).



Another source, which is more related to toxins and chemical emergencies can be found at the Center for Disease Control and Prevention:

<http://emergency.cdc.gov/chemical/>

It is essential for laboratory workers to be familiar with the potential hazards of materials used in the laboratory experiments **beforehand** and to follow recommended procedures for their use, handling, storage, and disposal.

General Safety and Disposal Cautions:

The following general cautions should always be observed:

- Become **completely familiar** with the properties of the substances **before** beginning the procedure.
- **The absence of a warning** does not necessarily mean that the material is safe, since information may not always be complete or available.
- If **exposed** to toxic substances, contact your local safety office immediately for instructions.

- Use **proper disposal procedures** for all chemical, biological (see below, Disposal of Laboratory Waste), and radioactive waste.
- For specific guidelines on **appropriate gloves**, consult your local safety office (for handling ethidiumbromide containing solutions, use Nitril-gloves).
- Handle concentrated **acids and bases** with great care. Wear goggles and appropriate gloves. A face shield should be worn when handling large quantities. Do not mix strong acids with organic solvents as they may react. Sulfuric acid and nitric acid especially may react highly exothermically and cause fires and explosions. Do not mix strong bases with halogenated solvent as they may form reactive carbenes, which can lead to explosions.
- Never **pipette** solutions using mouth suction. This method is not sterile and can be dangerous. Always use a pipette aid or bulb.
- **Keep halogenated and nonhalogenated** solvents separately (e.g., mixing chloroform and acetone can cause unexpected reactions in the presence of bases). Halogenated solvents are organic solvents such as chloroform, dichloromethane, trichlorotrifluoroethane, and dichloroethane. Some nonhalogenated solvents are pentane, heptane, ethanol, methanol, benzene, toluene, N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and acetonitrile.
- **Laser radiation**, visible or invisible, can cause severe damage to the eyes and skin. Take proper precautions to prevent exposure to direct and reflected beams. Always follow manufacturers' safety guidelines and consult your local safety office.
- **Flash lamps**, due to their light intensity, can be harmful to the eyes. They also may explode on occasion. Wear appropriate eye protection and follow the manufacturer's guidelines.
- **Photographic fixatives and developers** also contain chemicals that can be harmful. Handle them with care and follow manufacturer's directions.
- **Power supplies and electrophoresis equipment** pose serious fire hazards and electrical shock hazards if not used properly.
- **Microwave ovens and autoclaves** in the lab require certain precautions. Accidents have occurred involving their use (e.g., to melt agar or Bacto agar stored in bottles or to sterilize). If the screw top is not completely removed and there is not enough space for the steam to vent, the bottles can explode and cause severe injury when the containers are removed from the microwave or autoclave. Always completely remove bottle caps before microwaving or autoclaving. An alternative method for routine agarose gels that do not require sterile agar is to weigh out the agar and place the solution in a flask.
- Use extreme caution when handling **cutting devices** such as microtome blades, scalpels, razor blades, or needles. Microtome blades are extremely sharp! Take care when sectioning. If you are unfamiliar with their use, have someone demonstrate proper procedures. For proper disposal, use the "sharps" disposal container in your lab. Discard used needles **unshielded**, with the syringe still attached. This prevents injuries (and possible infections) while manipulating used needles, since many accidents occur while trying to replace the needle shield. Injuries may also be caused by broken Pasteur pipettes, coverslips, or slides.

Disposal of Laboratory Waste:

No harmful substances should be released into the environment in an uncontrolled manner. Appropriate waste disposal procedures, as stipulated by your institution's safety office, must be followed at all times.

Some basic rules:

- Only **neutral aqueous solutions** without heavy metal ions and without organic solvents can be poured down the drain (e.g., most buffers). Acid and basic aqueous solutions need to be neutralized before their disposal.
- For proper disposal of **strong acids and bases**, dilute them by placing the acid or base onto ice and neutralize them. **Do not** pour water into them. If the solution does not contain any other toxic compound, the salts can be flushed down the drain.
- Waste from **photo processing and automatic developers** should be collected separately in order to recycle the silver traces found in it.

Biological Safety Procedures:

Biological safety fulfills three purposes:

- To avoid contamination of your biological sample with other species,
- to avoid exposure of the researcher to the sample and
- to avoid release of living material into the environment.

Biological safety begins with the receipt of the living sample, continues with its storage, handling, and propagation, and ends only with the proper disposal of all contaminated materials, as it is outlined in the "Betriebsanweisung" for our S1-laboratories.

Human blood, blood products, and tissues may contain occult infectious materials such as hepatitis B virus and HIV that may result in laboratory-acquired infections. Investigators working with lymphoblast cell lines transformed by Epstein-Barr virus (EBV) are also at risk of EBV infection. Any human blood, blood products, or tissues should be considered a biohazard and should be handled accordingly until proved otherwise. **Wear appropriate disposable gloves**, use mechanical pipetting devices, **work in a biological safety cabinet**, protect against the possibility of aerosol generation, and **disinfect all waste materials** by autoclaving before disposal.

Further information can be found in the *Frequently Asked Questions* catalogue of the

ATCC homepage:

<http://www.atcc.org>

General Properties of Common Chemicals:

Hazardous materials can be subdivided into the following categories:

- **Inorganic acids**, such as hydrochloric, sulfuric, nitric, or phosphoric are colorless liquids with stringing vapors. Avoid spills on skin or clothing. Spills should be diluted with large amounts of water. The concentrated forms of these acids can destroy paper, textiles, and skin, as well as cause serious injury to the eyes.
- **Inorganic bases**, such as sodium hydroxide are white solids that dissolve in water and under heat development. Concentrated solutions will slowly dissolve skin and even fingernails.
- **Salts of heavy metals** are usually colored powdered solids that dissolve in water. Many of them are potent enzyme inhibitors and therefore toxic to humans and to the environment (e.g. fish and algae).
- Most **organic solvents** are flammable volatile liquids. Avoid breathing the vapors, which can cause nausea or dizziness. Also avoid skin contact.
- **Other organic compounds**, including organosulfur compounds such as mercaptoethanol or organic amines, can have very unpleasant odors. Others are highly reactive and should be handled with appropriate care.
- If improperly handled, **dyes and their solutions** can stain not only your sample, but also your skin and clothing. Some of them are also **mutagenic** (e.g. **ethidium bromide**), carcinogenic, and toxic.
- All names ending with “-ase” (e.g., catalase, β -glucuronidase, zymolase) refer to **enzymes**. There are also other enzymes with nonsystematic names like pepsin. Many of them are provided by manufacturers in preparations containing buffering substances, etc. Be aware of the individual properties of materials contained in these substances.
- **Toxic compounds** are often used to manipulate cells. They can be dangerous and should be handled appropriately.
- **Incompatible chemicals** should be kept segregated
(see: <http://bohr.winthrop.edu/chp/appf.html>).
- Pay attention on resistance of some **plastics** to chemicals and sterilization.

Guidelines for phenol, acrylamide, DEPC, ethidium bromide, mitomycin C, and N₂:

Phenol is extremely toxic, highly corrosive, and can cause severe burns. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves, goggles, and protective clothing. Always use in a chemical fume hood. Rinse any areas of skin that come into contact with phenol with a large volume of water and wash with soap and water; do not use ethanol!

A Material Safety Data Sheet (MSDS) for phenol can be found at:

<http://assets.chemportals.merck.de/documents/sds/emd/deu/de/1002/100206.pdf> (dt.)

<http://assets.chemportals.merck.de/documents/sds/emd/int/en/1002/100201.pdf> (engl.)

Acrylamide (unpolymerized) is a potent neurotoxin and is absorbed through the skin (the effects are cumulative). Avoid breathing the dust. Wear appropriate gloves and a face mask when weighing powdered acrylamide and methylene-bisacrylamide. Use in a chemical fume hood. **Polyacrylamide** is considered to be nontoxic, but it should be handled with care because it might contain small quantities of unpolymerized acrylamide.

A Material Safety Data Sheet (MSDS) for acrylamide can be found at:

http://www.alfa-chemcat.com/daten_msds/D/A17157_-_D.pdf (deutsch)

http://www.alfa-chemcat.com/daten_msds/GB/A17157_-_GB.pdf (englisch)

Diethylpyrocarbonate (DEPC) is a potent protein denaturant and is a suspected carcinogen. Point the bottle away from you when opening it; internal pressure can lead to splattering. Wear appropriate gloves and lab coat. Use in a chemical fume hood.

A Material Safety Data Sheet (MSDS) for DEPC can be found at:

<http://www.merckbiosciences.co.uk/msds/English/298711English.pdf>

Chloroform, CHCl₃ is irritating to the skin, eyes, mucous membranes, and respiratory tract. It is a carcinogen and may damage the liver and kidneys. It is also volatile. Avoid breathing the vapors. Wear appropriate gloves and safety glasses and always use in a chemical fume hood.

A Material Safety Data Sheet (MSDS) for DEPC can be found at:

<http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do> (deutsch)

<http://ptcl.chem.ox.ac.uk/MSDS/CH/chloroform.html> (englisch)

Ethidium bromide is a powerful mutagen and is toxic. Avoid breathing the dust. Wear appropriate gloves when working with solutions that contain this dye.

A Material Safety Data Sheet (MSDS) for ethidium bromide can be found at:

<http://www.merckbiosciences.co.uk/msds/German/331564German.pdf> (deutsch)

http://ptcl.chem.ox.ac.uk/MSDS/ET/ethidium_bromide.html (englisch)

Mitomycin C is a carcinogen. It may be fatal by inhalation, ingestion, or skin absorption. Do not breathe the dust. Wear appropriate gloves and safety glasses and use only in a chemical fume hood.

A Material Safety Data Sheet (MSDS) for mitomycin can be found at:

http://www.sigmaaldrich.com/catalog/ProductDetail.do?N4=M4287/SIGMA&N5=SEARCH_CONCAT_PNO|BRAND_KEY&F=SPEC

Liquid nitrogen can cause severe damage due to extreme temperature. Handle frozen samples with extreme caution. Do not breathe the vapors. Seepage of liquid nitrogen into frozen vials (in our elder storage bins) can result in an **exploding tube** upon removal from liquid nitrogen. Use vials with O-rings only, and ensure that they are closed properly before storing them. Wear cryo-protective clothes and a face mask.

*Do not allow the liquid nitrogen to spill onto your clothes. If liquid nitrogen spills onto your clothes, remove it IMMEDIATELY. The low temperatures numb everything so fast that no pain is felt while frostbite is incurred. The damage, which can be **equivalent to second- and third-degree burns**, is only noticed after the clothes are removed and requires treatment in a hospital.*

A Material Safety Data Sheet (MSDS) for liquid nitrogen can be found at:

http://gasekatalog.airliquide.de/sdb/089b_stickstoff_fluessig_tiefkalt.pdf (dt.)

<http://www.mathesonrigas.com/pdfs/msds/MAT16630.pdf> (englisch)

UV light and/or UV radiation is dangerous and can damage the **retina**. Never look at an unshielded UV light source with the naked eye. Examples of UV light sources that are common in the laboratory include hand-held lamps and transilluminators. View only through a filter or safety glasses that absorb the UV light with harmful wavelengths. UV radiation is also **mutagenic and carcinogenic**. To minimize exposure, make sure that the UV light source is adequately shielded. Wear appropriate protective gloves when holding materials under the UV light source.

Safety guidelines for UV light can be found at:

<http://www.safety.rochester.edu/ih/uvlight.html>