SWEDISH ENVIRONMENTAL PROTECTION AGENCY

REPORT

Baltic Biosafety 1st workshop at Hotel Karolina, Vilnius 22-23 of April 2003.

"Contained use of genetically modified micro organisms"

Background:

The workshop was the first of a series of 4 workshops to be held in the Baltic Countries concerning GMOs. Together they constitute the project Baltic Biosafety that is the result of a request from the Baltic States to Sweden. In addition to the Baltic States the Baltic Environmental Forum (BEF) and the Swedish Environmental Protection Agency (SEPA) represents the project stakeholders.

Aim of the workshop

To share experience between Nordic authorities and authorities of the Baltic States concerning the implementation of legislation regarding the use of genetically modified microorganisms. The topics highlighted were: Current EU- and national legislation. Risk assessment of contained use of GMM and Inspection of sites harbouring contained use of GMMs

Various

The name and other data of the 22 participants and 6 facilitators one could find in the Appendix I. 10 Lithuanians, 7 Latvians and 5 Estonians participated in the workshop.
All the workshop participants were invited to put forward ideas or wishes for the coming workshops.
All the participants were granted a certificate to verify the participation in the workshop.

Financing
BEF covered the cost for the hotel accommodation for the Baltic participants and the meals for all involved. The ministries in each country covered the transport in minibuses from Latvia and Estonia respectively. The Lithuanian counterpart covered the rent of conference rooms. The Finnish Ministry of Environment covered the travelling and accommodation costs of the Finnish expert. And finally SEPA contributed with the travelling costs and accommodation for the Danish expert, the Swedish expert and the project coordinator.

Summary of the workshop themes

Legislation
Directive 90/219 as amended by 98/86 is implemented in Estonia and Latvia. In Lithuania the implementation is almost completed.
The Nordic countries Finland, Denmark and Sweden have implemented the directive and the amendments. In Denmark and Sweden an order and different provisions are made to regulate the contained use of GMM in more detail.

The competent authorities (CA) for contained use of GMM:
Lithuania: The Ministry of Environment
Latvia: The Ministry of Health
Estonia: The Ministry of Social Affairs
Finland: The Board of Gene technology
Denmark: National Working Environment Authority
Sweden: Swedish Work Environment Authority

Notifications made to date:
Sweden: 450 (65 notifiers)
Denmark: 250 per year (average for last 4 years)
Finland: 440
Estonia: 2
Latvia: 0
Lithuania: 0

Notification and Risk Assessment
A common problem is that the users do not notify! It maybe is worthwhile discussing how to make them do that. Developing guidelines or the like for the users could be one way.
It was stated clearly that the risk assessment is a job of the notifier not of the CA.
The CA may often have to ask for further information and the questions aught to be precise.
The decisions of the CA may vary. Deciding what risk class (I, II, III or IV) should be applied is central. Potentially harmful activities can get a permit if it is done within a high-risk class.

The risk is the combination of the magnitude of the harm the activity could bring in the worst case AND the possibility that the harm will arise.
Notifications should be made in DIALOG with the notifier.
All CA judgement should be made case-by-case
A notifier may want and be granted a high degree of confidentiality for the information in the notification (but not all!)

Inspection
The three Nordic countries Finland, Denmark and Sweden all have national inspectors. Inspectors have at least a master's degree in natural science. The inspection procedures vary considerably between Finland and Denmark, but the starting point for the inspection is always the risk assessment and the conditions for approval in the notification.
Also inspection should be done in dialog!

The workshop events

Kristina Veidemane (BEF) and Mette Svejgaard (SEPA) introduced the participants to the workshop and the Baltic Biosafety project in general.

The legislation status of the Baltic States

Lithuania

Lecturer: **Odeta Pivorienė**. The Ministry of Environment, the GMO unit.

The Lithuanian law of GMO came into force December 2002. But the implementation of the EU directive 90/219 as amended by 98/81 only exists as a draft. It will concern all GMOs in contained use not only GMMs.
The ministry of Environment is competent authority for all GMOs but share some of its responsibility with the Ministry of Health, State Food and Veterinary Service. Two bodies are asked for opinions on applications: The GMO Steering Committee and The GMO Expert Committee is giving advice on risk assessment and monitoring issues, the members are from the scientific world. A third state body is the GMO Laboratory of the National Veterinary Laboratory. It is under construction and will take care of GMO analysis.

Estonia

Lecturer: **Silja Soon**. The Estonian Labour Inspectorate.
The bill on contained use of GMM went into force in January 2002 it implements the EU directive in question. In case users fail to announce they can be fined up to 50 000 EEK. The steering Committee of GMO consists of 4 members appointed by the government, 4 members appointed by the academic world, universities, a member of the Consumer

Protection Board and an officer of the Ministry of Environment. A public register of GMO activities is soon to be published on the Internet. It is the only place it will be published because the need of the public to know of class I activities is deemed to be low.

Latvia

Lecturer: **Rasa Atauga**. The Latvian Food Center.
The EU directive concerning contained use of GMM is transposed into the Latvian Regulation of the Cabinet Ministers No.323 Procedures for the Use and Distribution of Genetically Modified Organisms (the regulation also contains the regulation of deliberate release and placing on the market). This regulates all GMOs in contained use. The Monitoring Council of Genetically Modified Organisms and Novel Food is responsible for risk assessment and propose if an application should be approved of. Members of the Council are

- Ministry of Environment
- National Plant Protection Service, Ministry of Agriculture
- Veterinary and Food Department, Ministry of Agriculture
- Latvian Food Centre, Ministry of Health
- Ministry of Economy
- Latvian Academy of Science (two representatives)
- Faculty of Biology of the University of Latvia
- National Bureau for Impact Assessment on the Environment
- Latvian Association of Specialists in Genetics and Breeding
- Latvian Institute of Organic Synthesis.

The competent authority for contained use and placing on the market is the Ministry Health (From the 1st of February the Ministry of Welfare has been divided into the Ministry of Health and the Ministry of Welfare). The CA for deliberate release is the Ministry of Environment. It is the responsibility of the Food Centre to inform the public about which GMOs are being used and the like.
There have been very few applications and certainly work with contained use of GMMs is going on without being notified.

GMO legislation in EU, Sweden and Denmark

Lecturer: **Katarina Eskils**. The Swedish Working Environment Authority and **Dorte Harning**. The Danish Working Environment Authority (DWEA)
The first discussion on state level took place in the US 1975, scientists and state officers tried to identify the problems concerning risk assessment in the GMO-field. In 1986 the first law in the world on GMOs was passed in Denmark. A Statuary order of Gene Technology and Working Environment was put into place a year later. In 1990 the first two EU directives of GMO was put into force, both of them minimum directives (90/219 on contained use and 90/220 on deliberate release). The contained use directive was amended 98: EU 98/86. Another part of legislation with implications for the contained use of GMMs is the EU 93/679/EEC on Biologically agents.

Contained use is any activity in which microorganisms are genetically modified or where GMM are

- Cultured, stored, transported, destroyed, disposed of or
- Used in any other way where specific containment measures are used to limit contact with and provide high safety for the general population and the environment;

if not this is deliberate release or placing on the market and a different legislation.

GMM is defined as:

- A microbial entity capable of replication or gene transfer
- Altered by means of certain techniques, that are at least:
  - Recombinant nucleic acid techniques
  - Direct introduction of certain material
  - Cell fusion or hybridisation techniques

The following methods do not give rise to GMM:

- In vitro fertilization
- Natural processes such as conjugation, transduction or transformation
- Polyploidi induction

- that is unless recombinant molecules or organisms (GMM) are used!

Due to the directive the user is defined as a natural or legal person who is responsible for the contained use. In practice the user will be a company or a university. In order to get a permit the user has to present a notification to the competent authority (CA).

In the directive the responsibility of the user and the CA is specified.

The risk assessment is the job of the notifier not of the CA. The CA will judge if it is complete. The CA may often have to ask for further information. It is of importance to be precise on what information is needed. The information should be sufficient to decide what risk class should be applied.

The 4 risk classes:
Class 1 activity: no or negligible risk
Class 2 activity: low risk
Class 3 activity: moderate risk
Class 4 activity: high risk

The risk is the combination of the magnitude of the harm the activity could bring in the worst case and the possibility that the harm will arise.

In addition to the risk assessment the user is responsible for

- Classification and selecting containment and other protective measures
- Notification to the CA
- Review assessment, classification and measures regularly
- Inform the CA if changes or in case of accident

The CA will have to:

- Examine the notifications including all information concerning risk assessment and emergency plans
- Make sure the activity is in accordance with the directive
- Ensure the emergency plans are relevant and sufficient
- If needed communicate emergency plans to relevant stakeholders.
- Organise inspections and other control measures
- Report accidents to the Commission
- Report certain issues to the Commission every year, from June 2003 the experience of the directive must be reported to the Commission every third year on.

In handling the notification the CA may

- ask the user for more information
- require the user to change the conditions and/or the classification for the activity as a prerequisite for a permit
- require the contained use to be suspended
- require the contained use to be terminated
- limit the time for the contained use
- apply binding provisions for a permit to be given.

Handling of notifications should be made in dialog with the notifier.
The notifier is always responsible for the notification, both making it and what is in it. CA should examine the notification and make sure the activity is in accordance to the directive. Therefore, the CA will often have to ask for more information, or that the notifier changes the use, which is most successfully done in dialogue with the notifier.

According to the Directive 90/219/EEC as amended by 98/91/EC, the CA may ask the public if appropriate. In Sweden the CA doesn't do that on a regular basis, even though the possibility to do so exist for new activities.

In principle all information going into a Swedish authority is public. But applicants will be granted a high degree of confidentiality in order to secure that actual persons working with GMMs is not harmed, that companies competitiveness is not harmed and so on. But according to the directive some data must always be relieved, these are:

- General characteristics of GMM
- Name and address of the notifier
- Location of the contained use
- Classification of the contained use
- Measures of containment
- Evaluation of foreseeable effects, in particular harmful effects

It has not been tested what general characteristics of GMM should be relieved. No one will get information about specific GMM activities in Sweden without asking for it. For

its own use, the Swedish Work Environment Authority has a database containing all information on GMM use and activities, but it isn't public.

As a minimum EU directive it is not specified what the different measures for containment should be. It is specified in the tables what containment and other protective measures should **at least** be used at the different safety levels/risk classes. It is allowed though, to have more stringent measures or legislation. In Sweden containment is understood as physical containment perhaps added a 'genetically' containment.

The judgement of the CA has to be made case-by-case.

Risk assessment

Lecturer: **Dorte Harning**. The Danish Working Environment Authority (DWEA)
The legal paper regulating the area is the "Executive order on Genetic Engineering and the Working Environment" (Executive Order No.642 of 29 June 2001). Amongst other things it contains a comprehensive table of the different requirements for Class 1-4 containment levels. Due to historic reasons the approval process is divided in two: Approval of the facilities (called classification) and approval of projects and production (i.e. the actual work and handling). Applications will be made to DWEA, in case of contained use of large-scale, GM animals and GM plants, a copy of the application will be forwarded to the Nature and Forest Agency (NFA). In order to have a facility classified the user must in the application include:
–Directions for cleaning
–Directions for safety measures
–Directions for how work has to be carried out
–Procedures for repair and maintenance
–Plans of the building and facility
In order to get a permit for a project or production with GMM the application must include a risk assessment with a least the following elements:
• A finite lists of organisms (donors and recipients), inserts etc.
• An assessment of the biology (e.g. worst case risk assessment)
• A comparison with the classification of the facility

A key question is: What level of information do you need to approve the risk assessment?

It was asked how one should perform a risk assessment when the genes involved in the activity are unknown. The answer was to compile the information you actually have: Where does the sample come from? Is it hospital waste or soil? What does the notifier want to do with the genes? For example work with rRNAs is placed in risk class I. Whereas making gene library and clones might be class II because you don't know so

much about them. Later on it can be possible to go to level I, when more is known about the material, due to the precautionary measures.

In Denmark the working with naked DNA is not part of the order, it is not until it is put inside a bacteria (or other organism) that the work has to be approved of, i.e. is defined as GMM-work.

It was asked how one could get permission to a c-DNA project. The answer was that it is not how but which level is required. In order to safeguard the project in itself a class II containment level anyhow may be needed.

Part of the risk assessment is to assess if there will be problematic genes and the probability that they will get into humans.

Teamwork.
Real life examples of applications notified at the Swedish CA.
The group task was to make a reply to the notifier containing if necessary a request for further information, conditions for a permit, the arguments for the decision. The groups were divided by countries. See appendix II for examples.

Risk assessment example 1
Lithuanian conclusion: Information missing, what genetically material is inserted? Is there more than one antibiotic resistance? They do not estimate the harm of and therefore cannot estimate the risk that is harm times probability. They say there is no harm at all. No risk management plan.

Latvian conclusion. They just specify that the genes are not harmful. It would be nice to see the list of genes to be used. They mention they have an observation window but it is not needed.

Lithuanian conclusion: The group missed information about number of clones and expressed cDNA. The application wrote nothing about allergic reactions. From the application it was not possible to decide if it should be level I or II.

Katarina Eskils: We asked the notifier the questions: What kind of cDNAs do you have? People think there is no risk if you can buy the strains and the plasmids, i.e. if they are commercialised. Common that the risk is not defined in this kind of applications.

In the following discussion several questions were raised at the workshop:
Do you know the product of the expression? If not, you need a higher containment level.
Choose between the risk levels?
Where does the material come from?

Can use of ampicillin resistant markers be a level I activity? Yes

If you know the level of effect for a product you can calculate if there is a risk for the working personal.
Is there a promoter?

Risk assessment example 2
Estonian comments: A lot of information missing: What is the vector? What will be inserted? How was the vector transformed? Harm is defined but no probability of the allergenic effects. No data of inactivating the organisms. We can not be sure it is class I

Lithuanian comments: We miss a specific security measure.

Katarina Eskils, SWEA. A lot of information is missing. It is doubtful at all to give permission under these circumstances. The evidence is lacking. Enterotoxins are not relevant in this application. What inserts do you have? The fermentor can be in another room because you want it isolated for other reasons than containment. We want the measures for containment specified. Often focused on the host and nothing on the other parts, what genes and inserts. The large-scale limit in Denmark depends on the facility. The limit for labs could said to be 15 litres (in Denmark). The scale can be important for what happens if something escapes to the environment.

Insert names, strain names and more are in principal not confidential. The notifier can wish that parts should be confidential due to commercial and patent reasons. It should be stated in the notification what the notifier wants to be confidential and the reason why.

Risk assessment example 4
Lithuanian comments: We need more information. What is the resistance marker? What are the routines for accidents? The staff only know routines for class I not for class II.

Latvian comments: There has to be level III. Because the vector can give allergenic reaction in the lungs when you are exposed to it for a longer time. Aerosols can build and make these reactions. Need more information on the gene, if it can be expressed in humans. What is the purpose of the experiment, they inject animals with a virus, but why?

Estonian comments: Missing information on the recipient, the vector and the replication technique. A doctor should survey the workers now and then. Maybe a vaccination. It is big amounts but we don't know about the concentration. What is the effective amount to be infectious? We need more time to investigate.

Katarina Eskils: I don't think level III is needed. Because two of the genes, which make the vector infectious, has been deleted. A microorganism which is allergenic is in Sweden a level I organism. This particular virus is under debate. Everybody agreed it to be a level II containment. It was human genes. I miss the cell lines, what are they? We were worried about the volumes. During concentration (centrifugation) there could be a risk

for aerosols. You have to be aware if the company will have class I and II activities in the same room at the same time. We do not accept that. Class II and I activities must be separated either in space or in time.

Risk assessment example 5
Lithuanian comments: We miss information. What are the lab animals? What are the antibiotic resistance markers? It does not say what are the measures to prevent animals from escaping. It doesn't say anything about floors only about tables. The animals cannot spread GMM but they can spread the antibiotic resistance.

Latvian comments: We miss the purpose and what are the animals?

Estonian comments: Just ad some few things: How are the viruses transported between rooms?

Katarina Eskils: We asked about the animals, what would they do to them? How is their bedding? We discussed about autoclaving. 3 day after injection the animal will not shed any of the GMM anymore. It is definite a class II activity.

It is important to know what you want to know when you ask for more information.

Inspection

Lecturer: **Jyrki Pitkajärvi**. The Finnish Environment Institute (FEI)

Inspections of contained use: National Product Control Agency for Welfare (NPCAWH). Supervised and coordinated by the Board for Gene Technology.

<u>Preparation of the inspection:</u>

- Written material is send beforehand to the notifier (blanc minutes of the inspection, recommendations, checklist for the inspection etc.)
- Relevant personnel of the notifier should be present at the time of inspection
- Checking of the documents (notification, risk assessment, decision of the Board, layout etc.)
- Inadequate or unclear information noted and evaluated during the earlier inspection

<u>Inspection procedure in practice</u>
1. Initial discussion
    - Checking of the documents of the notifier
    - Is new GMMs and new facilities taken in use by the notifier (risk assessment, recording)
    - Any defaults and corrective measures will be documented into the minutes of inspection

- Discussion about unclear questions of the notifying and/or inspection process
- Possible recommendations by the inspector

2. Inspection
- Facilities and containment levels; e.g.:
  - Restricted access (class 2)
  - Isolation; negative pressure (class 3)
- Waste treatment: GMMs, chemicals
- Equipment: e.g. validation, maintenance
- System of work: e.g. aerosol dissemination, protective clothing, biohazard signs
- Possible accidental situations

3. Final discussion
- Observed problems --- measures to be taken
- Timetable for the measures
- Preliminary minutes of inspection

4. Minutes of inspection
- During one month after inspection
- Inspection will end after the submission of the final minutes

Most common problems noted
- Lack of the risk assessment document (only summary available)
- Non-comprehensive training of the personnel (documentation)
- Inadequate recording of the activities (lack of updating, new GMMs)
- Inadequate validation and maintenance of the equipment

Tools of the authorities
- Remarks and suggestions for the measures to be taken
- Prohibitions and restrictions (inspectors, Board for the Gene Technology), or threat of a fine and threat of performance (Board) – not used
- International exchange of information and training: European Enforcement Groups of GMO Inspection - Contained use of GMOs; EEG-CU (http://eep-mon.iitb.fhg.de/)
  - Finnish inspectors joined the EEG-DR/CU in 1998
  - Joint inspection visits (2 organized in Finland)

Lecturer: **Dorte Harning**, The Danish Working Environment Authority (DWEA).
In order to have an activity approved the actual site has to be classified in advance. This classification includes an inspection, and the notifier agrees to the time. The classification is done in order to prevent harm to humans and the environment. Later inspections will be made without warning. A newly build lab will without problem be a class I lab.

200 companies and universities in Denmark have classified labs.

4 Danish inspectors is working halftime with GMO. They will do around 50 inspections per year per inspector.

In order to get facilities classified the application must include:
Directions for cleaning, for safety measures and for how work have to be carried out.
Procedures for repair and maintenance and plans of the building and facility.

Important to have all the papers before the inspection. In the beginning of the inspection, the inspectors explain why they do it. During the inspection there is a continuing discussion.

In the order (Executive order on Genetic Engineering and the Working Environment) there is a table page 11-23 describing the requirements for the different classes. The main difference between I and II is the waste treatment (class II always autoclaved, things transported out of the room has to be disinfected). The requirements in that table are higher than in the EU directive.

Labs and the universities with old classifications do not need to fulfil new requirements for lab classification. On the other hand there is no political will to slacken the requirements.
But problems will arise if a university with old buildings want to be classified, though a solution will be found normally through dialog.

In Denmark there is no fee for inspection but there is in Sweden (ca 2000 SEK) and in Finland.

Inspectors are M.Sc. in chemistry or chemical engineering. And there is a continuing education and discussion among them.

Inspectors wear a lab coat during inspection. But some labs do not offer it, which they should.

Fines have been given if a lab has started working without an approval used, but seldom.

It might from the outside look hard to get an approval in Denmark. But the field is growing. The law allows for more time to decide on an approval than is often needed.

Katarina Eskils. SWEA
There is a fee for each notification and each inspection. 4 inspectors situated in Stockholm, has the whole country as region. But the local inspectors can inspect biological agents, and that is for free. The GMO inspection is agreed with the company. We do not send a checklist beforehand but tell what themes will be taken up. The fee is 2000 SEK for the first 4 hours and the following 800 SEK per hour, so it is in their own interest to have all papers in order before the inspectors arrive.

60-70 notifiers in the database, but many more activities. Universities can have up to a 100 activities.
In Sweden the approvals are divided by activity and (specific) contained use of GMM. All activities must be notified or have a consent. An activity is defined by:
The building,
The lab facilities,
The type of work (i.e. lab work),
The work leader,
The work unit,
The containment level (I-V).
One contained use is defined by the specific use of a single type of GMM. If the company or university has an approval for the activity it can begin working with level I without having the (specific) use of GMM approved.

Dorte Harning. DWEA.
Often companies will contact us before they start building to assure that the facilities will be OK.

Jyrki Pitkajärvi, FEI
Often the inspectors will lecture the personal about legislation, risk assessment and other things.

Discussion after teamwork.
How to perform the inspections on example 4 and 5:
Discuss the lacking information. Walk around the lab. Clothes, waste (also dead animals), autoclaving, inactivation of GMM, aerosol dissemination how is it solved, shared equipment how is it done, what will you do if you had a certain accident, do people drink or eat in the lab.

Final discussion.

In Latvia there will be a fee for notification, but for I class. But not in the other countries, not for contained use in Estonia, but for del release and placing on the market. There seems to be no fee in Lithuania.

A discussion of separating the CA and the inspection authority.

It is a very good idea to ask other than the manager what are the routines for the lab, for example the cleaner.

Waste.
GMMwaste is UN class 9. In Sweden everything from class 1 or 2 should be inactivated before leaving the lab if it is not kept in a two-layer container and brought directly to the fire (incineration). GMM from a class 3 or 4 activity cannot be transported as waste; the

GMM must be autoclaved or incinerated at the site. In Denmark class II organisms should be inactivated before leaving the facility.

The end

All participants received a certificate for their engagement in the workshop.

The next Baltic Biosafety workshop will be in Tallinn October 2003 and concern the Cartagena Protocol on Biosafety.

International web-sites

EU-rules: www.europa.eu.int/eur-lex/
Belgian Biosafety server: http://biosafety.ihe.be/
The competent British authority: www.hse.gov.uk
ACGM compendium of guidance, guidance from the health and safety commission's advisory committee on genetic modification:
www.hse.gov.uk/hthdir/noframes/acgmcomp/acgmcomp.htm

Appendix II. Examples discussed at the Biosafety Workshop.

Risk assessment example 1

1. About the activity, user and premises
The user is a unit at a company where laboratory scale research includes cloning and expression of human cDNA sequences. 5-50 persons are involved working with GMM. Advice in biosafety issues can be obtained by experts connected to the company.

Names, addresses and information on responsibilities, knowledge and education of persons supervising the GMM activity and responsible for safety are provided in notification, as well as a sketched plan and a description over the parts of the building where the activity will take place.

Waste containing GMM is chemically inactivated or subject to autoclaving before leaving the building.

2. About the contained use
GMM before modification is (specified) *E. coli* K 12 derivative strains (class 1-organism according to Biological Agents) that is transformed with (specified) standard *E. coli*-vectors
(class I-organism). Vectors carry cDNA from human, that is not containing toxic, cell growth
stimulating nor otherwise potentially harmful genes. Some (specified) antibiotic marker genes
such as ampicillin resistance are used. The resulting GMM is not expected to possess any increased risk than each component, and is classified as class 1-organism according to. Biological Agents.

Maximum volumes handled in one container is approximately 1 liter
Maximum volumes handled simultaneously are approximately 5 liters

3. Identification or risks
No harmful effects are expected on human health, animals, plants or the environment. GMM has no higher risks than the biological material before modification. Spread of antibiotic resistance is not likely to increase because of the contained use. The activity is limited, in a functional facility with functional routines for waste treatment.

4. Final risk assessment

The contained use fulfils the requirements to F-activity (that is class 1 at containment level 1).

5. Table and security measures
1. a - laboratory activity. Precautionary measures chosen:

- Hand-washing facilities provided
- Bench resistant to water, acids, alkalis, solvents and disinfectants and easy to clean
- Observation window or the equivalent provided so that occupants can be seen
- Autoclave provided
- Protective clothing used within the working area and removed when leaving it
- Secure GMM storage
- Routines exist for the prevention of exposure and for dealing with spillage, accidents and incidents
- Specific measures to control aerosol dissemination
- Used material containing GMMs is decontaminated before being washed, re-used, discarded (waste included)

Risk assessment example 2

1. About the activity, user and premises
The user is a research group in a university laboratory, subject is tumour research. GMM work is ranging from cloning genes in *E. coli* K12 strains to the use of replication incompetent retroviral Moloney Leukemia virus vectors for expressing cancer related genes in cell cultures. Less than 5 persons are involved in GMM work at containment level 2. The university has a biosafety committee.

Names, addresses and information on responsibilities, knowledge and education are provided for persons supervising the activity and persons responsible for safety. A thorough description of the laboratory is provided with a plan over the building where rooms used for GMM are marked.

Waste containing GMM is chemically inactivated or subject to autoclaving before leaving the building. Equipment that contained GMM is treated with chemicals or autoclaved before discarded or washed.

2. About the contained use
Two activites with premises are notified, one for class 1 activity (F-activity) and one for class 2 activity (L-activity). This risk assessment is for the contained use of GMM for expression in human cell lines of genes involved in control of human cell growth in amphotropic pseudotyped retroviral vectors (based on Moloney Murine Leukemia Virus).

Recipient organism (GMM before modification): human cell lines, class 1 (class 2)
Vector used: amphotropic retroviral vector, replication deficient, class 2. This vector system is based on replication deficient Moloney Murine Leukemia Virus (MoMuLV).
Antibiotic markers: ampcillin resistance (etc.)
Insert: marker gene other than antibiotic resistance marker gene, genes controlling cell growth, both up-regulating and down-regulating (but no known or suspect oncogene) *(specific gene functions and identities were provided)*
Resulting GMM: a system where retroviral MoMuLV-based vector is multiplied in one human cell line and transfected in another human cell line for expression of inserts controlling cell growth and marker genes, class 2

Maximum volume in one container is 1 litre.
Maximum volume handled simultaneously is 5 l (includes cell culture volumes)

3. Identification of risks

Both humans and animal may theoretically be harmed by the GMM. If known oncogenes are used, the risk increases. Risks are mainly:

a) infection through blood or mucous membrane, where replication incompetent viral vector may act as a mutagen by insertion mutagenesis, regardless of insert
b) infection through blood or mucous membrane, where replication incompetent viral vector containing oncogene may act as carcinogen by production of growth stimulating protein
c) rearrangement of genetic material may give rise to replication competent virus that may act as more potent carcinogens

Risks a) and b) are controlled by sample handling and risk c) is extremely low because of the cell line constructions.

4. Final risk assessment

Risk of GMM escaping from containment is very low with precautionary measures taken. Spreading of GMM is probably not possible due to its limited possibility of infection. Classified as L-activity (class 2 activity, containment level 2).

5. Table and security measures

1a – laboratory activity. Precautionary measures chosen:

- Isolation (segregated from other activities)
- Entry to lab by airlock only (comes with the facility, not by security reasons)
- Biohazard sign
- The laboratory is sealable for fumigation
- Hand-washing facilities provided
- Bench, floor resistant to water, acids, alkalis, solvents and disinfectants and easy to clean
- Autoclave provided
- Laboratory's own equipment inside the controlled area
- Microbiological safety cabinet with HEPA filter or corresponding enclosure provided within the working area

- Alarm system provided to indicate whether technical safety equipments are out of order (safety cabinet)
- Restricted access
- Protective clothing used within the working area and removed when leaving it
- Gloves used
- Secure GMM storage
- Routines exist for the prevention of exposure and for dealing with spillage, accidents and incidents (written)
- Specific measures to control aerosol dissemination
- Used material containing GMMs is decontaminated before being washed, re-used, discarded (waste included)
- Effective pest control (e.g. for rodents and insects)

Risk assessment example 3

1. About the activity, user and premises
Research and development unit at a university, using GMM in both laboratory scale and large scale fermentation. The purpose is to produce recombinant proteins for further studies. Less than 5 persons work with the large scale fermentation.

Names, education, addresses are provided for supervising and safety responsible persons. A plan over the building used is provided.

GMMs are chemically inactivated, autoclaved or heat inactivated in the fermentor before considered as waste. No active GMM leaves the building as waste.

2. About the contained use
E. coli K12 strains (specified; class 1) and *E. coli*-plasmid for protein production is used. Inserts are coding for antibiotic resistance marker and recombinant human protein with no potentially harmful genes. The GMM created means no increased risk.

The maximum culture volume in the fermentor is about 500 litre.

3. Risk identification
All *E. coli* may release endotoxins as other Gram-negative organisms and may give rise to allergic reactions. No known diseases are caused by *E. coli* K12.

4. Final risk assessment
Since the recipient organism (*E. coli* K12 derivative) is classified as class 1 and GMM only contains known fragments from human DNA, the contained use will not harm health or the environment. The classification is F (class 1, containment level).

5. Table and security measures

2 – large scale activity. Precautionary measures chosen:

- Viable organisms handled in a system witch separates the process from the environment
- Bench resistant to water, acids, alkalis, solvents and disinfectants and easy to clean
- The controlled area is designed so that spillage and the entire contents of the closed system can be contained and decontaminated in the event of an accident (by heat treatment in fermenter or other container, spillage with Jodopax)
- Exhaust gas from closed systems is handled in such a way that emissions are minimised (sterile filter, hygiene reasons)
- Seals are designed so that release does not harm health or the environment
- Hand-washing facilities provided
- Restricted access when fermentation, security reasons
- Special protective clothing used
- Inactivation of GMMs in contaminated material and waste including those in. process effluent before final discharge

Risk assessment example 4

1. About the activity, user and premises

A research unit in a company with already notified class 1-activities. Notification for the class 2-actitvity concerns protein expression in cell cultures at laboratory scale. About 20 persons will work with GMM.

Names, education, addresses are provided for supervising and safety responsible persons. A plan and description over the building used is provided. A biosafety committee exist.

GMMs are chemically inactivated or autoclaved before considered waste. No active GMM leaves the building as waste.

2. About the contained use

Adenovirus 5 is well characterized and commonly used as vector in expression systems. Virus particles are replication incompetent. Insert is human protein (*specified - not toxic, cell growth stimulating or oncogene*). When infected in cells, it is transient (does not integrate in genome). The protein obtained is studied.

Maximum volume for largest container is 0.5 litre.
Maximum culture volume handled simultaneously is 5 litres.

3. Risk identification

Wild-type Adenovirus 5 can cause disease, mostly among small children (cold, rhinitis). Exposure could cause a mild cold shortly. GMM containing insert with active protein could in theory cause problems in large amount, as inhalation of aerosol. But the amounts protein is expected to be too low to cause problems.
Produced virus particles are not capable of replication or spreading and does not survive well in the environment.
Antibiotic resistance marker genes could in theory maybe spread to the bacterial flora in the intestine if ingested, but is considered as very low risk.
Proteins are expected to be functional in normal cells.
Cells used are not considered as risk source because of the specific growth requirements.
Aerosol producing steps could lead to exposure. All open handling of vector and infected cell cultures will be performed in microbiological safety cabinet. Methods and equipment will be selected in order to minimize risk. Routines in case of accidents are well known by staff.

4. Final risk assessment

GMM have limited survival potential in the environment and will not cause harm if escaping from the containment.
Exposure for GMM could in worst case cause a mild, temporary disease in the upper aerial tracts. Virus particles are incapable of replication and can not spread to other persons.
Exposure for expressed proteins will probably not cause harm because the amount is too low to reach target cells.
The small scale is risk limiting.
Contained use was found to belong to class 2. Protective measures and containment at containment level 2 were selected.

5. Table and security measures

1 – laboratory scale activity. Precautionary measures chosen:

- Isolated location (Standard at all cell labs)
- Entry to lab by airlock only (Standard at all cell labs)
- Biohazard sign
- The laboratory has negative pressure relative to the pressure of the immediate environment (Standard at all cell labs)
- The laboratory is sealable for fumigation
- Hand-washing facilities provided
- Surfaces resistant to water, acids, alkalis, solvents and disinfectants and easy to clean
- Observation window or the equivalent provided so that occupants can be seen (Glass door is standard)
- Autoclave provided
- Laboratory's own equipment inside the controlled area (Practical reasons and for avoiding contamination)

- Microbiological safety cabinet with HEPA filter or corresponding enclosure provided within the working area (Avoid aerosols an to avoid contamination)
- Alarm system provided to indicate whether technical safety equipments are out of order (Standard)
- Restricted access (Only authorised personnel)
- Protective clothing used within the working area and removed when leaving it (Labcoat, gloves)
- Gloves used
- Secure GMM storage
- Routines exist for the prevention of exposure and for dealing with spillage, accidents and incidents (*Provided and found satisfying*)
- Specific measures to control aerosol dissemination (*Routines provided and found satisfying*)
- Used material containing GMMs is decontaminated before being washed, re-used, discarded (waste included)
- Effective pest control (e.g. for rodents and insects)

Risk assessment example 5

1. About the activity, user and premises
A research unit in a company with already notified class 1-activities and the activity in example 4. Information is virtually the same. Notification for the new class 2-actitvity concerns use of the same vector in animals. About 20 persons will work with GMM.

Names, education, addresses are provided for supervising and safety responsible persons. A plan and description over the building used is provided. A biosafety committee exist.

GMMs are chemically inactivated or autoclaved before considered waste. No active GMM leaves the building as waste.

2. About the contained use
Adenovirus 5 is well characterized and commonly used as vector in expression systems. Virus particles produced are replication incompetent. Insert is human protein *(specified -not toxic, cell growth stimulating or oncogene).* GMM will be injected in laboratory animals.

Maximum volume for largest container is 0.01 liter. (by dilutions)
Maximum culture volume handled simultaneously is 0.007 liters

3. Risk identification

Wild-type Adenovirus 5 is a human subtype capable of causing disease in upper and lower respiratory tracts, mainly among small children. Exposure could cause a mild, temporary infection. GMM containing insert with active protein could in theory cause problems in large amount, as inhalation of aerosol. But the amounts protein is expected to be too low to cause problems.
Produced virus particles are not capable of replication or spreading and does not survive well in the environment. Escaping animals can not spread GMM.
Antibiotic resistance marker genes could in theory maybe spread to the bacterial flora in the intestine if ingested, but it is considered a very low risk.
Animals are put to sleep during injection. Injection means risk for unintended skin puncture with contaminated needles.
Virus are transported between rooms.
Protective measures and routines are provided.

4. Final risk assessment
GMM have limited survival potential in the environment and will not cause harm if escaping from the containment.
Exposure for GMM could in worst case cause a mild, temporary disease in the upper aerial tracts. Virus particles are incapable of replication and can not spread to other persons. Exposure for expressed proteins will probably not cause harm because the amount is to low to reach target cells.
Risk is limited by the small scale and by putting animals to sleep before injection.
Contained use was found to belong to class 2-activity (L-activity). Protective measures and containment at containment level 2 were selected.

5. Table and security measures
1 a - laboratory scale activity. Precautionary measures chosen:
- Isolated location (Separated from other animal facilities)
- Entry to lab by airlock only (Entry to the unit)
- Biohazard sign
- The laboratory has negative pressure relative to the pressure of the immediate
  environment (Negative pressure in airlock to minimize spread of animal allergen)
- The laboratory is sealable for fumigation (Standard)
- Hand-washing facilities provided
- Surfaces resistant to water, acids, alkalis, solvents and disinfectants and easy to clean
- Observation window or the equivalent provided so that occupants can be seen (To avoid unnecessary disturbance)
- Autoclave provided
- Laboratory's own equipment inside the controlled area (Practical reasons and to avoid contamination)
- Microbiological safety cabinet with HEPA filter or corresponding enclosure provided. within the working area (Avoid aerosols and contamination)
- Alarm system provided to indicate whether technical safety equipments are out of order (Standard)

- Restricted access (Only authorised personnel)
- Protective clothing used within the working area and removed when leaving it (complete change and hair protection in order to minimize allergen spreading) Gloves used
- Secure GMM storage
- Routines exist for the prevention of exposure and for dealing with spillage, accidents and incidents (Provided and found satisfying)
- Specific measures to control aerosol dissemination (instruction provided and found satisfying) Used material containing GMMs is decontaminated before being washed, re-used, discarded (waste included)
- Effective pest control (e.g. for rodents and insects)

1b – Additional measures for work with animals deliberately infected with GMMs

- Isolator or other HEPA-filtered containment provided
- Animal facilities separated by lockable doors
- Measures taken to limit the possibility of the animals escaping from the controlled area
- Material and equipment designed for easy cleaning and decontamination
- Surfaces easy to clean
- Incineration of animal cadavers (External. *Routines provided)*
- Clothes changed daily and if needed. *(Routines provided)*
- Bedding and waste decontaminated (Autoclave)*

*Bedding is changed every 3 or 4 day (animals are stressed by changes) and first change after injection is autoclaved, as no GMM is expected later than 3 days after injection. If contamination is expected, the bedding will be treated as containing GMM and autoclaved.