



Biological Risk Assessment: An Explanation Meant for Safety Advisors in Belgium

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1. Introduction to Biological Risk Assessment

Biosafety is one of the most recent safety disciplines. It is intended to eliminate or prevent the risks related to the use of “biological agents” and is based on the analysis of the work started by Pike in the last century to assess the risk of occupational infection in clinical and research laboratories.

In a compilation of his works, in 1976 Pike (9) counted 3,921 cases of infection resulting from laboratory work, of which 164 were fatal. In only 20% of the cases can the infection be attributed to a known accidental exposure such as inoculation, contact with infectious substances, skin lesion [caused] by contaminated material, animal and insect bites, etc. The remaining cases could have stemmed from the use of animals, clinical specimens, the handling of the agent involved, autopsy work, aerosols, etc.

In 1979, Pike (10) added 158 cases of infection, including four fatal cases, to those recorded in 1976, with more hepatitis cases but fewer cases of infection than the number recorded since 1968. On the basis of that study, the author determined what hazards are specific to laboratory work and suggested some solutions to contain them. The conclusion of his article is somehow the pillar of biological safety “common sense, good work practices and using the appropriate equipment should protect workers from the risks related to the use of hazardous biological agents.”

The first European Biosafety directives were published in 1990. They will be summarised in the

following text, except for directive 90/220/EEC (22) dealing with the deliberate release of genetically modified organisms (GMOs) since it does not deal with the protection of workers.

We will then address the identification of the dangers or “risk factors” and the assessment and management of the biological safety risks.

This text is meant to help safety advisors. Indeed, in Belgium, there is a law, that follows from the 89/391/EEC directive (25), about the well-being of workers at work: employers are obliged to ensure health and safety of workers in every aspect related to the work. To achieve this goal, they are helped by safety advisors. The competency and the mission of the safety advisor is clearly defined in the law. As many of them do not have a biological background, we have intentionally detailed things that may be obvious to Biosafety Officers and borrowed terms commonly used in different safety management systems.

2. Legal Foundations

The prevention advisor is confronted with a variety of legal texts with regard to biosafety.

First, the Royal Decree of 4 August 1996 (M.B., 1 October 1996) refers to the protection of workers from risks related to the occupational exposure to biological agents (transcription of European Directive 90/679/EEC). This decree has a very broad scope since, in addition to situations in which biological agents are used, it anticipates all cases of exposure even if there is no deliberate intent to work

with or use a biological agent. This is the case for workers in health services, waste disposal facilities, wastewater treatment facilities (1).

In compliance with this decree, the employer is to previously notify the occupational hygiene and medicine administration that biological agents will be used for the first time.

Second, directive 90/219/EEC (16) regarding the contained use of genetically modified micro-organisms has been incorporated in Belgium as part of the general regional environment-related laws applicable to classified facilities (environmental permits). More specifically, these are:

- the decree by the Walloon Government of 4 July 2002 (6), which defines sectoral conditions with respect to the contained use of genetically modified micro-organisms or pathogens (M.B., 21 September 2002);
- the decree by the Government of the Brussels-Capital Region (26) of 8 November 2001 with respect to the use of genetically modified organisms and/or pathogens and the classification of the facilities involved (M.B., 26 February 2002);
- the decree by the Flemish Government of 6 February 2004 (24) which defines the general and sectoral provisions regarding hygiene and environment.

These regional texts are intended to protect health and the environment. They, like the directive from which they arise, regulate not only the contained use of genetically modified micro-organisms (GMM) but they have also been extended to GMOs and pathogens.

By their implementation, authorisation must be requested from the competent authority and obtained prior to any contained use of GMOs or pathogens.

Through the cooperation agreement of 25 April 1997, which binds the three Regions and the Federal State (14), the Regions agree to standardise technical biosafety criteria, classification of GMOs, pathogens and their risk classes.

The “risk class” concept is defined hereunder (point 3.3.2.). In the meantime, there is a need to offer some clarification of the terminology used in regional and federal legislation so this matter can be properly understood.

Actually, the regional legislation defines two

kinds of “risk class.” The first concerns contained use, while the second refers to the biological risk of pathogens or genetically modified organisms. In both cases, four risk classes were defined, which leads to some confusion. Furthermore, the federal Royal Decree uses the terms respectively for the same concepts “containment level” and “hazard group.”

To avoid confusion about the terms and to make the text easier to read, the term “risk class” will be used exclusively for the classes of risk related to contained use and the term “hazard group” for the classification of biological risk of pathogens or genetically modified organisms.

Risk analysis is the key element in all these statutes, making it possible to determine *in fine* the prevention and protection measures to be implemented (for workers, for the community, and for the environment), as well as to establish the necessary medical surveillance.

Lastly, there are different regulations in effect for transporting biological agents according to whether shipment is by road (Accord européen relatif au transport international des marchandises dangereuses—ADR), by air (Dangerous Goods Regulations—IATA)...Natural or genetically modified pathogens are included as infectious substances that are subject to certain packaging, labelling, quantity restrictions and documentation requirements. Some instructions are also given for non-pathogenic GMOs.

3. Risk Analysis

3.1 Legal Definitions

In the case of any activity likely to involve a risk of exposure to biological agent, the nature, the degree and duration of worker’s exposure must be determined in order to make it possible to assess any risk to the worker’s health and safety to lay down the measures to be taken and to identify the workers from whom special measures are necessary. (R.D., 4 August 1996, Section 5).

3.2 Legal Identification

3.2.1. Definition of Hazards

A hazard is the presence of an inherent characteristic that threatens human health. This character-

istic may be attributed to an object (machine, chemical agent, biological agent...to a process (movement, transport, chemical process, biotechnological process...) or to a situation (climate, storage). A hazard may have adverse effects on the product (quality), on the environment and on the health of workers (safety-health). We will focus on the effects on human health and on the environment (4).

3.2.2. Biological Agents and Hazard Groups

Biological agents are classified under four hazard groups according to the severity of the harmful effects they may have on workers' health. Consideration is given to: the ability of the biological agents to cause disease in "healthy" people; the mean severity of the disease; the likelihood of the biological agents causing epidemics, and the existence of effective treatments and suitable prophylactic measures.

According to the EEC directives and the Belgian regulations, hazards groups are defined in the follow-

ing manner:

Hazard group 1—biological agent unlikely to cause disease in humans.

Hazard group 2—biological agent that can cause a disease in human and could be a hazard to directly exposed workers. It is unlikely to spread to the community. Effective prophylaxis and treatment are usually available.

Hazard group 3—biological agent that can cause severe human diseases and is a serious hazard to directly exposed workers. It may present a risk of spreading to the community. Effective prophylaxis and treatment are usually available.

Hazard group 4—biological agent that can cause severe human diseases is a serious hazard to directly exposed workers. May present a risk of spreading to the community. Effective prophylaxis or treatment are usually not available.

Table 1 is a collection of a few examples of biological agents classified into these hazard groups. A

Table 1

Example of the classification of biological agents into hazard groups (European Directives and Belgian regulations)

Biological Agent	Disease	Hazard Group
<i>Bordetella pertussis</i>	Whooping Cough	2
<i>Clostridium botulinum</i>	Botulism	2
<i>Clostridium tetanii</i>	Tetanus	2
<i>Legionella pseudophilia</i>	Legionnaire's Disease	2
<i>Listeria monocytogenes</i>	Listeriosis	2
<i>Streptococcus pneumoniae</i>	Pneumonia	2
<i>Vibrio cholerae</i>	Cholera	2
<i>Bacillus anthracis</i>	Anthrax/Blackleg	3
HBV	Hepatitis B	3
HCV	Hepatitis C	3
HIV	AIDS	3
<i>Mycobacterium tuberculosis</i>	Tuberculosis	3
<i>Salmonella typhi</i>	Typhoid Fever	3
Marburg virus	Hemorrhagic Fever	4
Smallpox virus	Smallpox	4

few comments are necessary.

1. By disease, we mean: infection, allergy, poisoning by bacterial toxins. The risks associated with the use of oncogenic viruses and genetically modified organisms are not covered in the Royal Decree.

2. From a practical point of view, there are numerous reference lists covering the natural biological agents (not genetically modified) as well as the group under which they are classified. These lists originate from international authorities (WHO, CDC (13), ...) and the Belgian lists (Appendices to the R.D. of 4 August 1996, Appendix to the W.G.D. of 4 July 2002, Appendices to the Bxl-Cap. Reg. D. of 8 November 2001, ...). There are also similar lists for zoopathogens and phytopathogens.

3. These reference lists provide approximate hazard levels of natural biological agents. authorities in the field assess the "overall risk" as low for Group 2 agents, moderate for Group 3 and high for Group 4. This classification is one reference for risk assessment. Also, a comparison of the different lists reveals some different classifications for some microorganisms: *Salmonella typhi* is classified in hazard group 2 in the CDC lists but in group 3 in the European and Belgian lists.

4. Not all biological agents are dangerous to humans. In fact, those in group 1 are not likely to cause disease, for example:

- Bakers' yeast (*Saccharomyces cerevisiae*) used in making bread, beer;
- Naturally-occurring intestinal bacteria such as *Bacillus acidophilus* and bifidobacteria are necessary for the proper functioning of the digestive system;
- The lactobacilla used in dairies; and
- Attenuated viruses used as vaccines: Measles, Rubella, Mumps, Varicella.

5. Agents not covered in groups 2, 3, or 4 do not necessarily belong to Group 1 (not dangerous). They will need to be examined on the basis of their inherent characteristics. This is also the case for GMOs or GMMs or cell cultures for which a hazard group will have to be defined.

6. Furthermore, hazard and harm must not be separated. We make a distinction between two categories of effects.

- *Immediate effects*: transmission of the biological agent, resulting in the contamination of a person:

- ♦ contamination by needle prick in the case of accidental inoculation,

- ♦ contamination by contact with the skin or with the ocular, mouth or nasopharyngeal mucous membranes, the tracheobronchial tree and the pulmonary alveoli, the digestive and intestinal tracts. This contact can be direct or the result of inhaling or ingesting the agent.

- *Delayed effects*: resulting from contamination:

- ♦ depending on the exposed worker's immune status, the infection can assume a variety of forms: infectious disease, permanent disability resulting from the infectious disease, and death;

- ♦ allergy: the signs and symptoms of allergies vary quite widely from person to person. Some allergens that are not dangerous to most people cause certain phenomena that can be extremely serious in a sensitized subject. Later on, we will discuss the crucial role of occupational physicians in protecting workers against biological risk, especially during the pre-employment check-up. It is that much more important for them to identify the workers exhibiting signs of hypersensitivity because repeated allergic reactions are the source of a higher risk of infection: constantly irritated, the membranes become more permeable to infectious agents (7);

- ♦ oncogenic effects that favor tumor development, for example, type 16 and 18 humanpapilloma viruses are frequently associated with cervical cancer.

7. Lastly, almost all the hazard group 2, 3 or 4 biological agents can cause fatal illness. Why then are they classified in different groups? What justification is there for considering some more dangerous than others?

We find one response element when studying the probabilities, the frequencies of the occurrence of delayed harm. Actually, when a given biological agent is transmitted to a person, there is immediate harm (the contamination) and potential delayed harm (infection, disease, death). For a given biological agent, probability is associated with each of these types of harm. For example in contamination, $1 \geq P_{\text{infection}} \geq P_{\text{disease}} \geq P_{\text{death}}$

For example, the infection resulting from the poliomyelitis virus can express itself in a variety of

signs and symptoms. In the vast majority of cases (90 to 95%), the infection remains unseen. When it expresses itself clinically (4 to 8%), it starts in the form of a non-specific, acute febrile flu-like syndrome. In 1 to 2% of the infections, this syndrome is accompanied by an aseptic lymphocytic meningitis. All these signs can spontaneously improve toward a full recovery within 10 days, but in less than 1% of the infections, acute flaccid paralyses can occur, placing the prognosis for survival in doubt when they involve the respiratory and/or swallowing muscles (20).

Each probability and frequency is specific to the biological agent (Table 2).

It is certain that the hazard groups are not determined solely by the frequency of the most harmful effect. For example, for *Neisseria meningitidis*, the agent responsible for meningococcal meningitis, the fatality rate can reach 50%. But it is reduced to less than 10% by early diagnosis and modern treatment methods (antibiotics). This is probably why this pathogenic agent is in hazard group 2.

3.2.3. Classification Criteria

A. Micro-organisms

Table 3 presents the interpretation principles used in determining a hazard group based on the different characterisation factors of the natural biological agents. The first step is differentiating between biological agents that are not dangerous (Group 1) and other agents (groups 2, 3, and 4) on the basis of their infectious or pathogenic nature. The second step uses the other characterisation fac-

tors to determine classification in hazard group 2, 3, or 4.

Data concerning inherent characteristics of natural biological agents are catalogued in databanks. Canada's Office of Laboratory Security publishes "Material Safety Data Sheets" (MSDS) from which are drawn the items described hereunder (23). The public health department of the Centers for Disease Control and Prevention published a compendium on biosafety for workers in microbiological and biomedical laboratories "BMBL," in which we can also find safety summary sheets concerning most pathogenic biological agents (13).

The epidemiological data can be consulted on the WHO web site (21).

For many of the micro-organisms used in the biotechnology industry, the information necessary for designating a hazard group will be unavailable or incomplete. This is particularly the case for cell cultures or for GMMs/GMOs for which other approaches have been developed to rank the hazards.

B. Cell Cultures

A distinction must be made between the primary cultures taken directly from the tissue or the organ of interest and the cell line culture that have been immortalised through the use of oncogenes or by viral transformation. We have summarised the process to follow hereunder. Analysis may sometimes prove complex and this is why we recommend the prevention advisor consult specialists.

For primary culture, the factors to consider in de-

Table 2
Examples of fatality rates

Contamination by	Hazard Group	Fatality Rate
HAV (Hepatitis A virus)	2	low - rare case of mortality
<i>Haemophilus influenzae</i> type B (bacterial meningitis)	2	2 to 5%
Yellow Fever virus	3	5 to 50% depending on the population infected
<i>Yersinia pestis</i> (Plague)	3	50% in the absence of treatment
Ebola virus (Hemorrhagic Fever)	4	50 to 90% depending on the epidemics

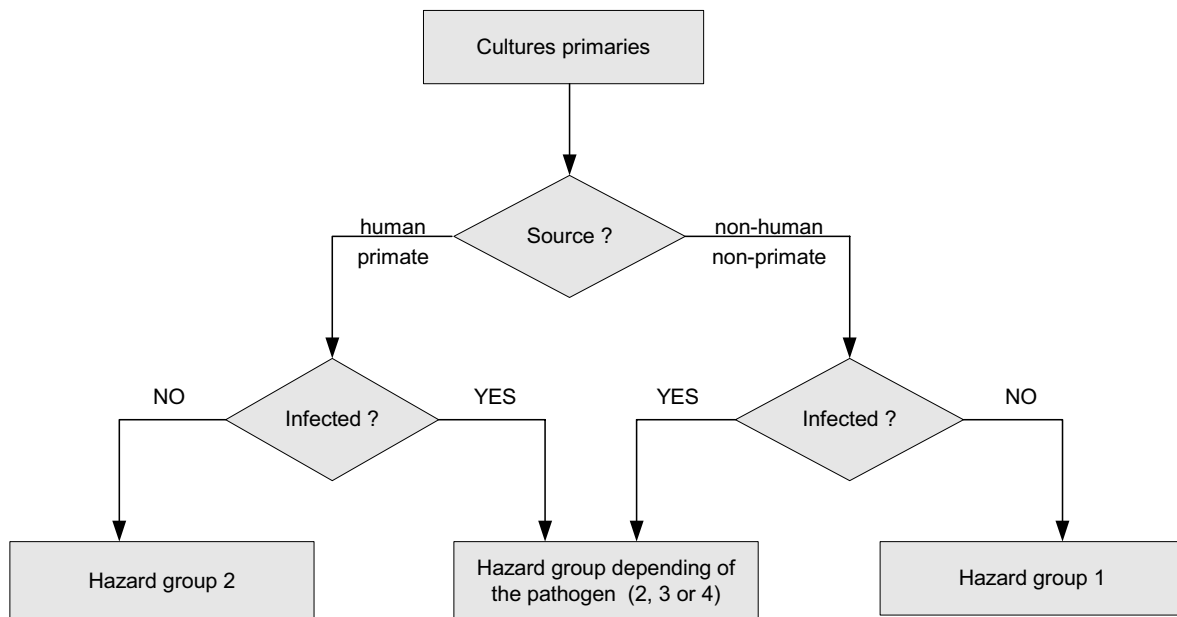
Table 3

Interpretation principles: Attempt to attribute hazard group

Characteristics of the Biological Agent		Interpretation	
<u>Step 1</u>		<u>Hazard Group</u>	Hazard Group
		1	2 to 4
-Infectious (colonizes)		no	yes
-Pathogenic		no	yes
<u>Step 2</u>		<u>Hazard Group 2</u>	→→→→
Virulence (probability of harmful effects)		Low	Medium
Epidemiology (populations' immunity)		Indigenous	
Host range		Single host	
Reservoir (possible outside humans)		No	
Vectors		No	
Infectious dose		High	Medium
Mode of transmission (probability of transmission by)		Inoculation	Ingestion Contact
Survives outside hosts		No	
Sensitive to drugs, antibiotics...		Yes	
Inactivated by physical means		Yes	
Inactivated by chemical means		Yes	
			Hazard Group 4
			High
			Foreign
			Multiple hosts
			Yes
			Yes
			Low
			Inhalation
			Yes
			No
			No
			No

Figure 1

Classification of primary cultures



termining hazard group are: the nature of cells sampled (normal cells, tumor cells); their source (potential existence of infectious agents); the conditions for taking and handling the explants to be grown, where applicable; the nature of the genetic modification contemplated, and the anticipated usage.

In terms of cell line culture, besides the factors to be considered for the analysis of the primary cultures from which the cells come, it is necessary to take into account the potential harmful effects resulting from immortalization and the type of usage envisaged. They could be considered GMMs. Figure 2 summarises the process to follow for classifying cell line cultures as described in the Belgian regulation.

C. GMMs/GMOs

Genetically modified microorganisms and organisms (GMMs/GMOs) are defined as pathogenic or non pathogenic microorganisms or organisms whose

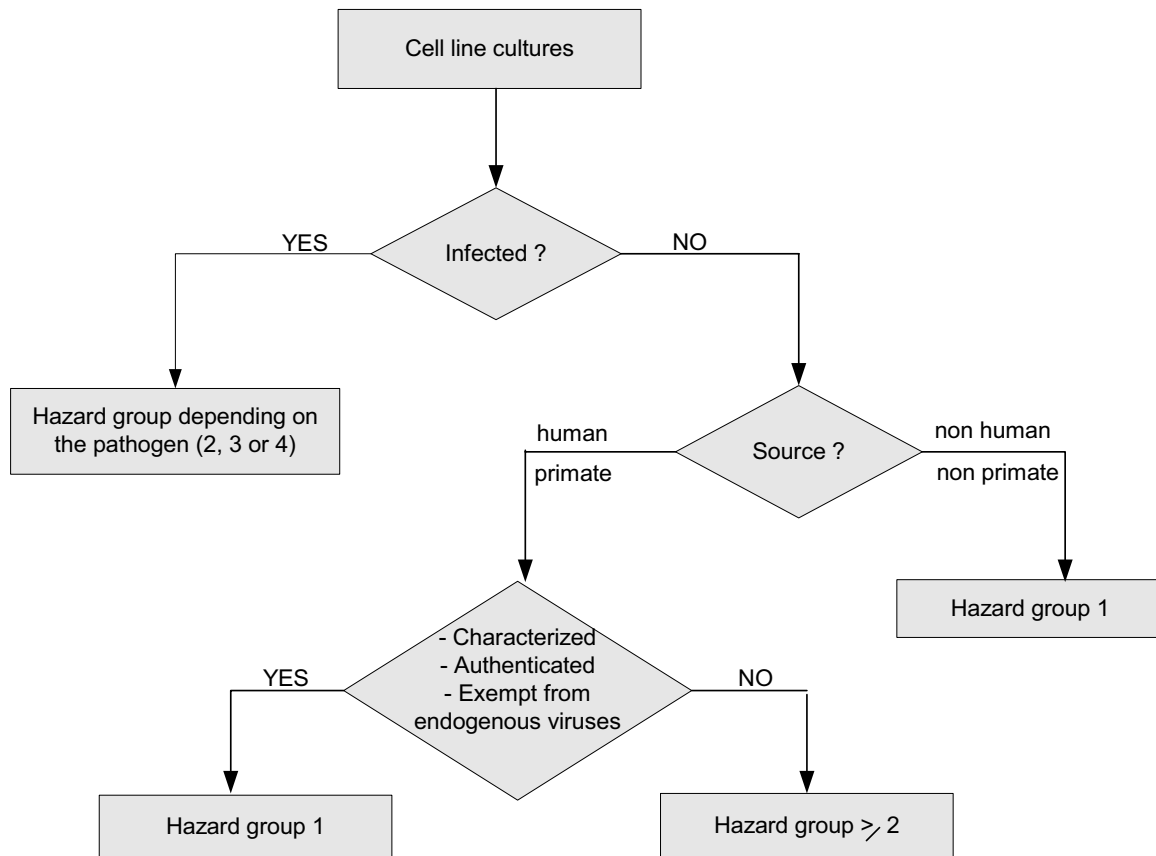
genetic material has been modified in a manner that does not occur in nature by natural multiplication and/or recombination.

3.3 Exposure

3.3.1. Definition of Exposure

A hazard only exists insofar as the operator is exposed to it (3). In Van Hemelen (4), exposure always refers to the extent to which the worker may enter into contact with a hazard. However, Malchaire writes that exposure is evaluated in terms of the duration of or the frequency with which the operator is exposed to the hazard (3). It may be rare (once a year or 0.1% of the time), unusual (once a month or 0.1-1% of the time), occasional (once a week or 1 to 5% of the time), frequent (once a day or 5 to 10% of the time), very frequent (once an hour or 10 to 50% of the time), continuous. This suggests that one may be exposed continuously to a

Figure 2
Classification of cell line cultures



biological agent without it necessarily reaching the right specific point of entry for it to adhere, multiply and produce harmful effects.

The different points of entry are:

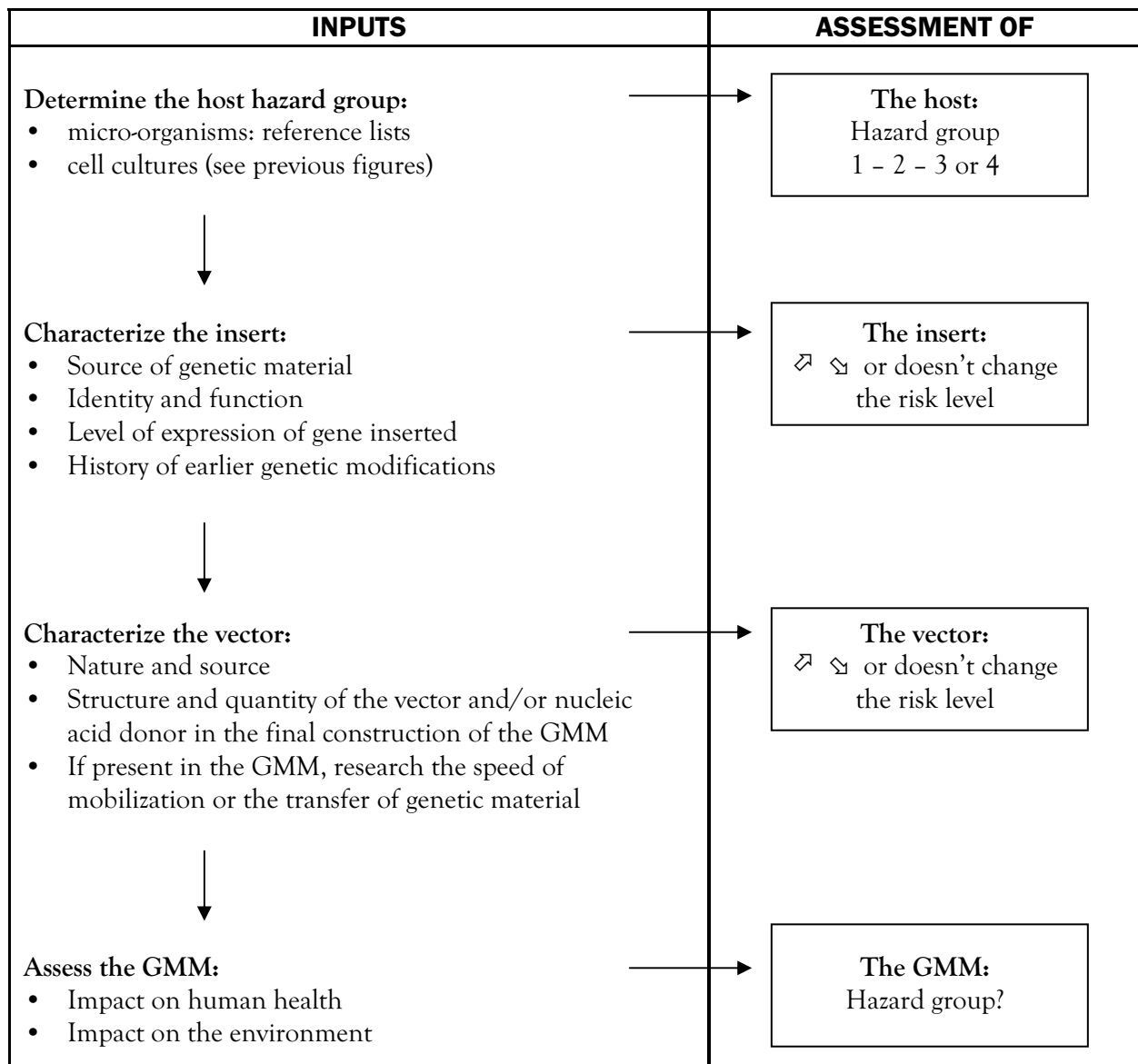
- unprotected, injured or weakened skin;
- the natural orifices: eyes, nose, mouth, urogenital area.

How likely you are to not just be exposed, faced with a hazard, but be in contact with a given biological agent depends on how the latter is used. So, what should be done is determine in which cases there

can be transmission of the biological agent to exposed people and what immediate and possibly delayed damage (contamination, infection, disease, death) can follow. In other words, how likely a given biological agent is to get transmitted and arrive at a specific port of entry depends on what kind of activity or operation is performed.

For some agents, such as chemical agents or noise, the recommendation is to quantify the exposure.

Figure 3
Classification of GMOs



3.3.2. Quantification of the Exposure

The quantification of exposure to biological agents is a topic that has not received much study by biosafety experts. To attempt to understand the reasons for this and to ensure that such an approach is not definitive for guaranteeing the safety of exposed workers, we propose to review the concepts used in the cases of exposure to chemical agents and evaluate the relevance of their application to biosafety.

The tolerable levels of exposure to a chemical agent are determined by analysing dose-effects and dose-response curves (27). This presupposes:

- specifying the biological effects that occur and their intensity when the intensity of the exposure is raised;
- defining or qualifying the unacceptable effect (for example: for a substance acting on the respiratory tract, are we to deem unacceptable: an unpleasant odor, a sensation of irritation of the nasal mucous membranes, chronic bronchitis, ...) and quantifying it (determining the intensity of effect deemed unacceptable);
- deducing, on the basis of the dose-effect curve, what is unacceptable exposure;
- considering inter-individual variations, i.e., characterizing the relationship between the exposure dose and the percentage of individuals exhibiting a biological effect of a given intensity: that is the dose-response curve;
- setting the tolerated percentage of individuals surpassing the acceptable effect threshold. This last point is undoubtedly the jurisdiction of the competent authorities who must structure their decisions on the basis of objective data provided by the scientific community.

The practical establishment of a dose-effect curve requires:

- measuring the pollutant concentration at the workstation and in surroundings of the respiratory tracts of the exposed subjects. This concentration reflecting real exposure is usually compared to the levels of so-called acceptable exposure (value below which the probability of altering the workers' health is considered acceptable) as in the TLV (Threshold Limit Value = reflects the concentration that the majority of individuals can tolerate daily for an 8-hour shift without the appearance of health prob-

lems). These thresholds, determined by the competent authorities, change with the growth of toxicological knowledge;

- determining the quantity of poison absorbed (internal dose);
- estimating the concentration in the vicinity of the target organ;
- looking for early biochemical or physiological lesions at a stage when they are still reversible.

Is this approach applicable to biological agents and, that being the case, can we infer from it data concerning acceptable exposure or contact?

Start by evaluating whether the preceding concepts can be transposed to biosafety and if so, let us see how they can be concretely applied during occupational exposure.

Making the dose-effect curve implies a qualification of the dreaded harmful effect and verification of whether it intensifies when exposure to, and therefore contact with, the biological agent increases. It assumes modes and routes of transmission are known beforehand.

In the workplace, the main routes are, in order of frequency, by air through infective aerosols, contact with the mucous membranes and weakened skin (lesions, cuts, pricks). Having specified these parameters, it is then time to sample the environment of interest and to measure the concentration of the biological agent in it. This requires:

- either carrying out detection and quantification measurements on the material and samples used when performing the work, for example, detecting the presence of the hepatitis B or AIDS virus in the blood handled in transfusion centers;
- or measuring the concentration of the viable biological agents in the workstation atmosphere. The first difficulty with this measurement is managing to differentiate the viable and non viable biological agents. Next, it is important to realize that the concentration of viable biological agents in the workstation atmosphere does not necessarily reflect the concentration of viable agents transmitted to the host's specific point of entry. Furthermore, the distribution of pathogens in the workstation atmosphere is not uniform. That distribution will vary intermittently in time and space with, for example, the

production of infectious aerosols.

It is therefore possible to measure concentrations of biological agents but the measurements do not generally reflect the real work conditions so that it is tricky to draw useful information from them regarding the workers' real exposure.

We still need to deal with the following question: does the feared harmful effect (usually infection) intensify with a rise in the concentration of the biological agent in the environment, in other words, is there a correlation between the dose measured in the samples used or in the ambient air of the workstation and that found at the specific point of entry and in the target tissues of the host?

The appearance of harmful effects on workers' health is modulated by their immune status and general state of health.

With regard to dose-response curves, in biosafety we have a datum analogous to the TLV; it is the minimum infective dose (MID), which represents a tolerance threshold vis à vis an infectious germ. Below this threshold there is no infection of the exposed individual. Above it, it is probable that the infection (or other harmful effect) will emerge. The MID is a characteristic of the biological agent and is not always known. It is the result of studies conducted on "healthy" volunteers. Therefore, we have little information on agents in hazard groups 3 and

4. Indeed, we could not even conceive of conducting a study to obtain the MID of the Ebola virus, knowing that the virus is classified in group 4, is transmitted by contact, spreads rapidly in the community and that there are no effective means of prophylaxis or treatment. A few minimum infectious doses are presented for information in Table 4.

Of the examples cited, only *Bacillus anthracis* (responsible for anthrax), *Salmonella typhi* (responsible for typhoid fever) and *Plasmodium falciparum* (Malaria) are classified in group 3 by Belgian authorities.

The highest minimum infectious doses are found in bacteria. That would suggest, for the examples used, that the probability of developing an infection after viral contact is higher.

Establishing the dose-effect and dose-response curves is, admittedly, an interesting exercise, provided one has the organizational and financial means to do so. On the other hand, we must be aware that they only reflect a snapshot of the reaction of the exposed worker and will differ with relation to the worker's general health at the time of the assessment.

Conscious of the limitations of such an undertaking, the international and Belgian authorities do not require the quantification of the exposure but do require that its nature, the degree and the dura-

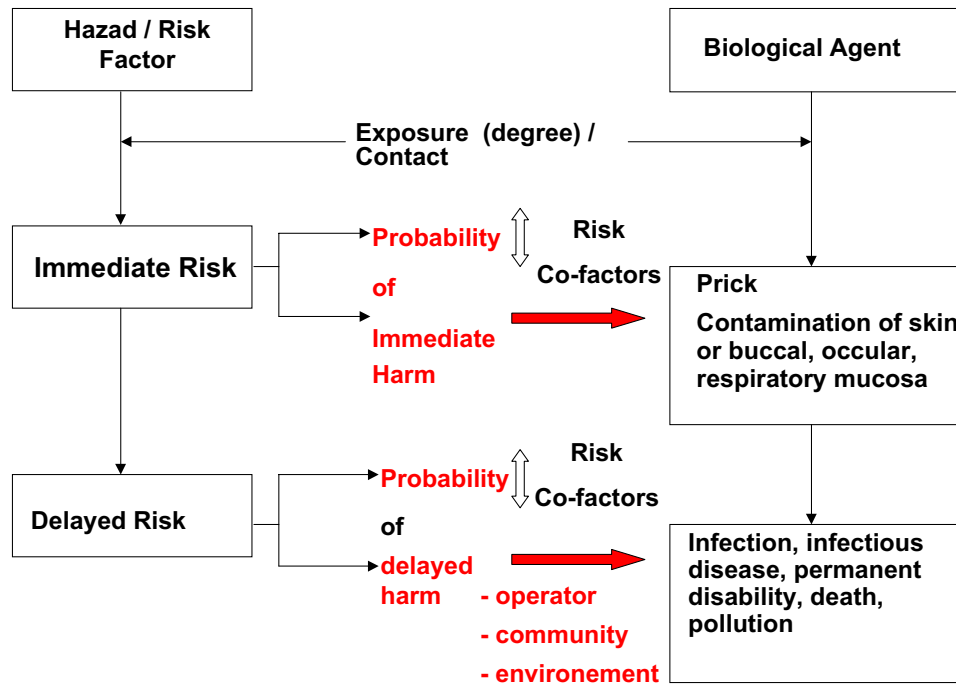
Table 4

A few minimum infectious dose values

Infectious Disease or Pathogenic Agent	Transmission Mode and Route	Minimum Infective Dose (number of organisms)
Malaria	Intravenous	10
Syphilis	Intradermal	57 57
Anthrax	Inhalation	1,300
Typhoid	Ingestion	100,000
Cholera	Ingestion	100,000,000
Rubeola	Intranasal (spray)	0.2
Rhinovirus	Intranasal (spray)	1
Poliovirus 1	Ingestion	2
Rubella	Subcutaneous	30

Figure 4

Risk assessment according to a model created by Van Hemelen (4)



tion of the workers' exposure be determined in order to assess any health and safety risk and to define measures to be taken. It is more an appraisal of the exposure.

3.4 Assessing Biosafety Risk

3.4.1. Principle of Biosafety Risk Assessment

What is the risk related to some degree of exposure to biological agents? We will suggest some answers in Figure 4.

Let us refer to the definition by Malchaire (3) in which risk is the probability of harm of some severity in view of exposure to a risk factor (synonymous with hazard) and the probability of the occurrence of harm during that exposure.

We identified the hazards (risk factors) and defined the associated harmful effects in point 3.2: when a given biological agent is transmitted to a person, there is immediate harm, contamination of specific points of entry, and potential delayed harm (infection, disease, permanent disability, death).

The probability of the occurrence of immediate effects or the immediate risk is a function of the

work situation parameters, the nature and/or means of collective protection, work conditions and characteristics of the operator (age, general health, skill, ability, behavior). These are what Malchaire (3) calls risk co-factors, i.e., characteristics likely to increase the risk or the probability of the occurrence of a harmful effect.

Probability is evaluated using a qualitative scale: it can be virtually impossible, possible and very unlikely, subject to unusual circumstances, very possible and expected.

The occurrence of delayed effects is determined by the probability of immediate effects and may be influenced by other co-factors such as the worker's general state of health, the existence of care infrastructures, effective therapeutic means.

Appraising a "degree of exposure" to evaluate risk boils down to determining the risk co-factors likely to raise the probability of the agent's transmission from a reservoir (patient, water reservoir, animal, bioreactor, culture, human or primate bodily fluids...) and the probability of contact at the host's specific point of entry. That probability can vary

with work situations:

- it varies between “**virtually impossible and expected**” when the activity does not involve deliberate intent to work with or use a biological agent. In this context, however, the workers may be more or less exposed depending on what the circumstances are and more or less often (periodically or haphazardly) since the source of contamination or the activity change. For example, at water treatment facilities, the load in microorganisms pathogenic for the human being found in discharged wastewaters depends on how healthy the population is.
- it can be “**very possible and expected**” when there is deliberate intent to use biological agents: pharmaceutical industries, the use of transgenic plants and gene therapy, for example. In such situations, in the absence of preventive measures, the worker is deliberately and continuously exposed to risk—an unacceptable situation. Because of this, the authorities have defined some containment measures to minimize or prevent the release of biological agents: this is contained use. The larger the risk to the workers, the community and the environment, the more stringent the containment measures will be. Hence, the residual risk for the exposed worker should be assessed.

In summary, assessing biosafety risk boils down to:

- characterizing the biological agent and classifying it in one of the four hazard groups;
- identifying the potential immediate and delayed harmful effects for humans and the environment;
- defining the work situation and looking for procedures that favor the agent’s transmission or contact with it;
- evaluating the probability of the occurrence of immediate harm in view of the work situation;
- for contained uses: defining their usage risk class (maximum risk class of usage). See the following for the concept of usage risk class.

Although essentially qualitative, this assessment is no less effective in defining, with reference to the legislative requirements, the prevention or containment measures for protecting exposed workers.

3.4.2. Risk Assessment for Activities Without Deliberate Intent to Work with a Biological Agent

These activities (following) are summarily listed in section 19 of the Royal Decree of 4 August 1996:

- work in food processing facilities;
- activities in which there is direct contact with food products or substances;
- agricultural work;
- work where there is contact with animals or animal products;
- work in health services, including isolation units and postmortem examination units;
- work in clinical, veterinary and diagnostic laboratories, excluding microbiological diagnostic laboratories;
- activities in social services, emergency intervention and in penal institutions;
- work in waste-disposal facilities;
- work in wastewater treatment facilities.

By way of example, a description of risk assessment in health services is presented hereunder.

This is a typical situation in which workers are exposed by their activity but without deliberate intent. Care givers in hospitals are exposed almost continuously to patients, but patients are not all hospitalized for infectious diseases. However, it is prudent to consider all of them as potentially infected before there is proof to the contrary through microbiological analyses.

For the patients exhibiting obvious signs of infection, what is important to us is the probability of transmission to the health care personnel and the pathogen’s contact with the specific point of entry. This will vary according to the patient’s pathology, the transmission’s period of the agent and the effectiveness of the treatment that will be administered. The probability is therefore variable for the entire hospitalization period.

So assessing risk is not easy. Besides there is a prerequisite: a diagnosis has to be performed (observation of the clinical symptoms and microbiological analyses) so as to identify and characterize the hazard. During this entire period, the health care personnel is, in some cases, at high risk.

How likely the immediate effects are to occur, during the whole care period, can be minimized by an adequate training of the health care staff, their expertise, their compliance with work practices and their acceptance of the constraints that are effective

barriers to the transmission of biological agents (Travail et Sécurité, January 2003, INRS).

When the diagnosis reveals an infection by group 3 or 4 agents, complementary measures to general hygiene measures are required: these are containment measures to protect personnel and prevent the spread of an epidemic in the community.

A case study was recently published in *Travail et sécurité* No 625 (January 2003, INRS) and addresses risk assessment for activities other than those previously presented.

3.4.3. Risk Assessment for Activities with Deliberate Intent to Work with a Biological Agent

The activities deemed as intentional or deliberate are as follows:

- laboratories, including diagnostic laboratories and facilities with laboratory animals that have been deliberately contaminated with group 2, 3, or 4 biological agents;
- laboratories that perform work involving the handling of group 2, 3, or 4 biological agents for research, development, teaching or diagnostic purposes;
- laboratories that handle substances wherein there exists the possible presence of biological agents may lead to illness in humans but whose purpose is not to work with biological agents as such (no concentration, no culture);
- industrial processes that use group 2, 3, or 4 biological agents;
- health care treatments involving the use of vaccines or therapy using GMOs;
- greenhouses that use transgenic plants or plants experimentally infected with genetically or non-genetically modified (micro) organisms, etc.

These activities must be performed in a contained environment. The containment level is assigned in accordance with the risk assessment and is divided into four classes, as defined in the Belgian regulation:

Class 1—contained uses with negligible or no risk, i.e., uses in which level 1 containment is recommended to protect human health and the environment;

Class 2—contained uses presenting a low risk, i.e., uses in which level 2 containment is recom-

mended to protect human health and the environment;

Class 3—contained uses presenting a moderate risk, i.e., uses in which level 3 containment is recommended to protect human health and the environment;

Class 4—contained uses presenting a high risk, i.e., uses in which level 4 containment is recommended to protect human health and the environment.

3.4.4. Assignment of Risk Class of Contained Use

Assigning the containment level necessary to protect human health and the environment requires the classification of contained use, which leads to the assessment of risk of use.

If biological agent is unknown or not characterized, use class 3.

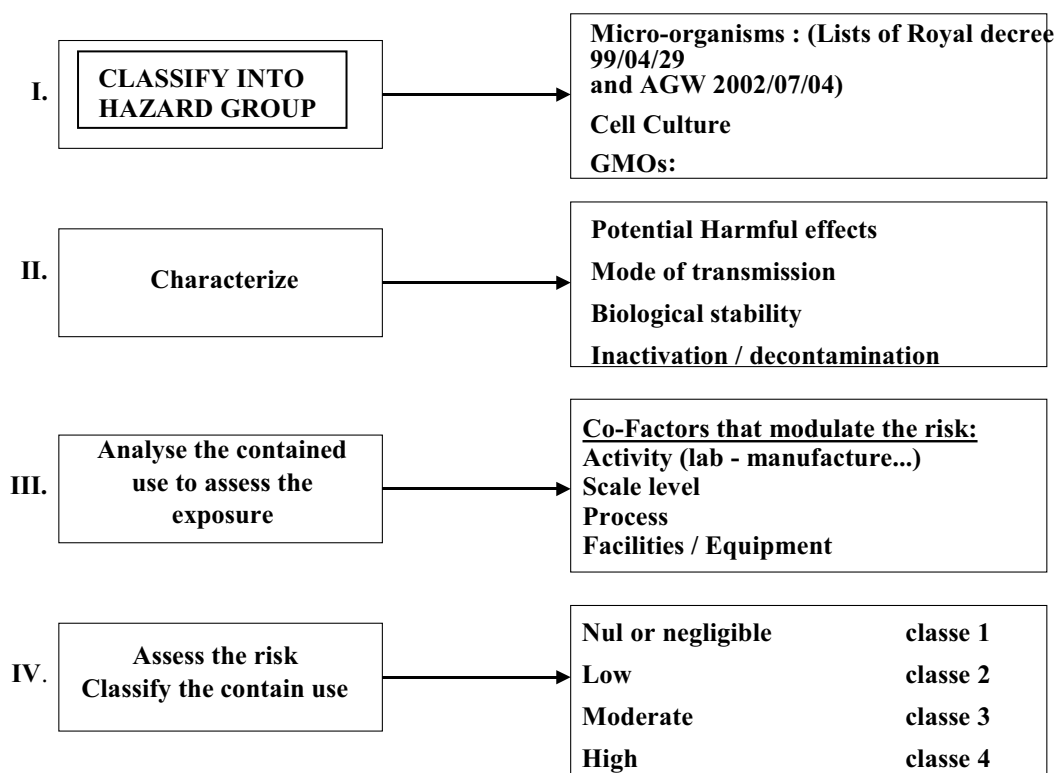
Biological agents belonging to different hazard groups, the contained use category will be defined based on the biological agent of the higher group.

An analysis of contained use calls for the definition of the following:

- Which sector of activity is in question, indeed legal requirements are adapted to the sector of activity
 - ◆ research, teaching, diagnostic laboratory.
- Note:* Laboratories that handle substances wherein the possible presence of biological agents may lead to illness in humans for identification purposes and whose purpose is not to work with biological agents as such (i.e., no concentration, no culture), the minimal use risk class of 2 is assigned, regardless of the pathogens potentially contaminating the samples. This applies to diagnostic laboratories with no microbiological activity. However, if an assessment identifies additional risk due to the origin of samples for example (region where an epidemic has been reported) the risk class of use may be 3 or 4;
- ◆ animal supply facilities;
 - ◆ hospital rooms where vaccines or therapies using GMMs are administered;
 - ◆ industrial processes.
- *Magnitude of activity:* the volume and concentration of biological agents involved may increase the risk for workers and the environment. The minimal volume for large-scale use is not specifically defined

Figure 5

Assigning a risk class to a contained use



in Belgian statutes. We can reasonably state that large-scale processes are involved when the equipment used (bioreactor/fermenter) can no longer be contained inside collective protection equipment, when “cleaning in place” (CIP) or airlock decontamination is required. Fermenters are often used in the biotechnology industry for volumes between 1,00 and 1,000 litres, a use defined as a large-scale production activity.

- *Processes, methods, and equipment* involved may increase or decrease risk. Processes which increase the risk of exposure or equipment reducing it are described in the WHO orange book.

The following two examples illustrate the steps to assigning contained use risk class, using the hepatitis A virus and the *Neisseria meningitidis* bacteria.

Risk class of contained use: class 3 for large-scale operations due to means of transmission, potential harmful effects to exposed personnel and laboratory-acquired infections (8 reported cases in 1974; 2 deaths in 1988; 2 deaths in 1991).

Laboratory work was assigned a risk class of 2.

As a precaution, we also classified as risk class 3 liquid culture use at the laboratory level.

These examples demonstrate that determining risk class of contained use is not easy; it often requires consulting with specialists. It is also involves verification on the part of Belgian experts responsible for issuing notices to those competent authorities who allocate operating permits.

One of the objectives of this chapter was to share information with you to enable you, as a prevention advisor, to dialogue with experts or workers involved in these uses.

4. Preventive and Protective Measures

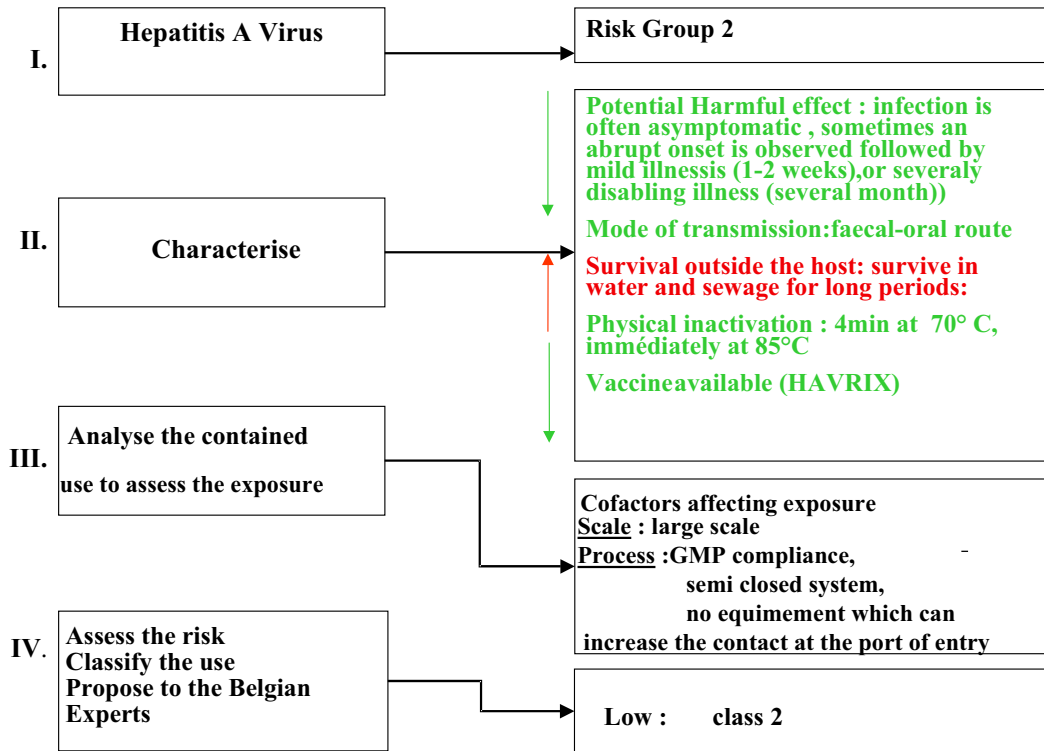
4.1 Physical Containment

4.1.1. General Principles

Containment entails all of the measures implemented to limit biological agent contact with workers, the public and the environment.

Figure 6

Risk class of the contained use of the Hepatitis A Virus



Example 1: Hepatitis A Virus: HAV

The upward arrow indicates that the intrinsic characterization increases risk while the downward arrow indicates those that decrease risk.

However, HAV production is a large scale process, this use has been classified class 2 by the Belgian biosafety technical experts (Service of Biosafety and Biotechnology of the Scientific Institute of Public Health, Louis Pasteur, Brussels) because:

- workers' exposure is quite low
- in case of accidental release there is little risk to the population due to its sanitary level and because of the quality of the care infrastructures.

An assessment of biological risks related to contained use, in other words, the maximum risk class of the operation, determines the appropriate containment measures that guarantee optimal protection of human, animal and plant health as well as protection of the environment. The appropriateness of these measures in a given facility results in the following on a case-by-case basis:

- technical characteristics of premises involved in a contained operation and their layout,
- safety (biosafety) equipment,
- professional work practices, including the wearing of personal protective equipment,
- personnel training,

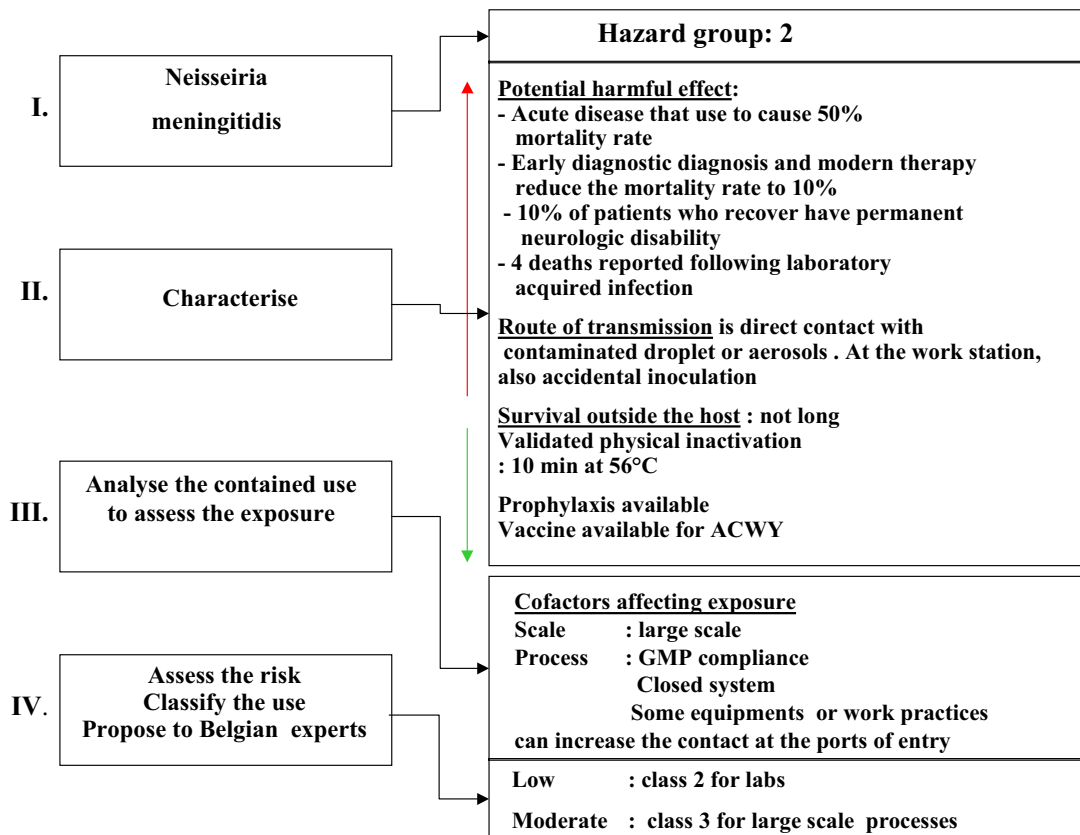
- management of waste and of residual biological substances.

Usually, primary barriers isolate or contain the hazard and secondary barriers stop the release of biological agents outside of the facilities. Distinction will also be made between collective protection equipment and individual protective equipment.

Figure 8 presents a few examples of containment measures.

4.1.2. Containment Implementation

Various containment measures are combined to form the containment levels (levels 1, 2, 3, and 4: from the simplest to the most complex). The ulti-

Figure 7Risk Class of the contained Use of the Meningitis Bacterium (*Neisseria meningitidis*)

Example 2: Meningitis Bacterium

mate goal of level 1 or 2 containment is to limit the spread of biological agents while the goal of level 3 or 4 is to stop any release. It is the maximum risk class of an operation that determines which containment level is to be adopted (a maximum operational risk class of 1, 2, 3, or 4 equals a containment level of 1, 2, 3, or 4, respectively).

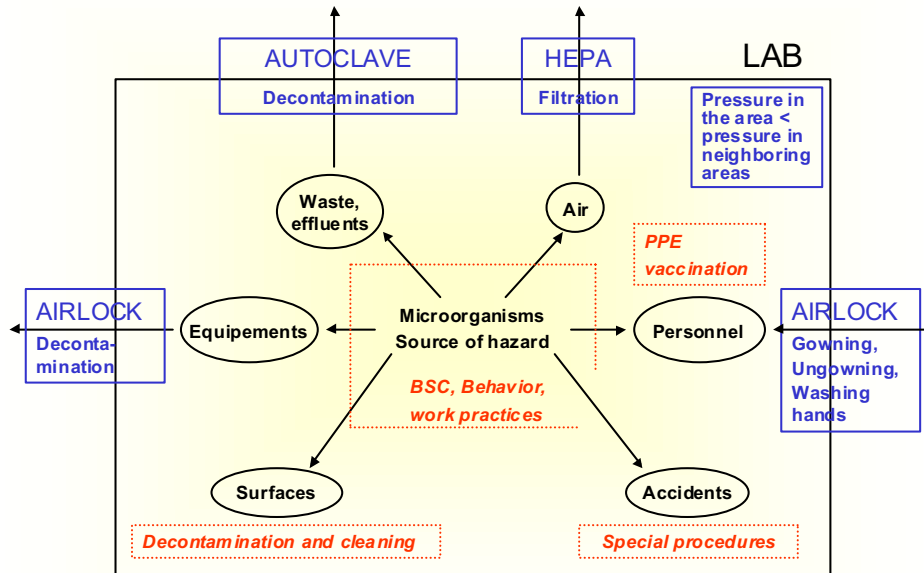
Furthermore, each containment level varies depending on the type of facility or on the activity being performed: laboratories (research, diagnostic, quality control, teaching, etc.), animal supply facilities, greenhouses, hospital rooms (gene therapy) and industrial facilities (large-scale processes). Therefore, it would be more accurate to refer to them as containment levels 1, 2, 3, or 4.

The various containment processes are described in international documents (13), European directives and in Belgian legislation (federal and regional).

In the 1996 Royal Decree, as in its ensuing directive, there is no mention of level 1 containment. In fact, those documents only cover pathogenic biological agents (hazard groups 2, 3, or 4) whose contained use is at least a level 2 (3 or 4, where required).

In Belgium, level 1 containments were introduced through regional legislation and only apply to the use of GMMs/GMOs deemed to be non-hazardous to human, animal or plant health or to the environment. Natural biological agents (not genetically modified) that belong to group 1 (non-hazardous) are exempt from those laws. However, according to the 1996 Royal Decree, an employer is required to adhere to principles of good occupational health and safety when the agents are in use (no containment required but adherence to the provisions of RGPT [General regulations for work pro-

Figure 8
Examples of Containment Measures



primary barriers
secondary barriers
BSC = Biological Safety Cabinet
HEPA = High Efficiency Particulate Air filter

tection]/Code du Bien-être [Welfare code] is required).

Containment levels 2, 3, or 4 ensure the decreased probability of transmitting a pathogenic agent to workers; the higher the containment level, the more effective the protection. Furthermore, where effective protective means are available (vaccines, antibiotics, other therapeutic means) or where agent involvement is such that transmission is almost impossible (absence of spreading, discordance between how the agent is likely to be spread and available ports of entry in workers) for pathogens from hazard group 2 or 3, they are likely to be handled in level 2 containment, depending on the risk class of the operation. There is a reduced probability of delayed effects and may in fact be close to zero. In this case, containment and the application of protective means therefore work to lower the overall risk of contained use to a level that is accept-

able to workers, the community and to the environment. To illustrate this point, we have seen that the hepatitis A virus, classified as hazard group 2, will be used at a containment level 2, regardless of the activity. However, HIV-contaminated blood belongs to risk group 3 yet containment level 2 is generally accepted for diagnostic activities.

In level 3 containment (contained use, risk class 3), pathogens belong to hazard group 2 or 3 (see risk class of use).

For group 2 agents, it is obviously the manner in which they are used that will determine this containment level, especially the magnitude of the operation (see preceding bacterial meningitis case).

In this case also, containment is selected in order that the overall residual risk for use be acceptable.

For hazard group 4 biological agents, any possibility of transmission to workers or spread within the population or in the environment is totally unac-

ceptable since there are no means of controlling those pathogens and the illnesses caused (with the possible exception of quarantine, as practiced during Ebola epidemics in Africa). This is why level 4 containment is designed so that no contact between the agent and the worker is possible (e.g., wearing a full pressure suit). Technical redundancies are also in place to prevent any spread to the outside (e.g., air filtration from the containment area through two HEPA filters, installed in series). To date, there are few level 4 containment facilities. These are research laboratories or animal supply facilities, often attached to a research laboratory.

All containment measures provided for in legal documents must be enforced unless it can be demonstrated, with the consent of the official technical expert, that one or more are of no use or are inoperable. Also with consent of the technical expert, alternate containment measures may be established so long as they are just as effective.

4.2 Biological Containment

If a strain of a pathogenic parasitic, fungal, bacterial or viral species is attenuated either by spontaneous emergence, selection or where the known virulent genes have been described or made non-functional through deletion or stable mutation, the user may propose a lower hazard group than that of the non-attenuated or virulent species. This proposal must be motivated and approved by official technical experts.

Therefore, the purpose of biological containment is to prevent the use of hazardous biological agents by replacing them with agents that, depending on work conditions and the current knowledge situation, are not hazardous or are less hazardous to worker health. Examples include attenuated live vaccines such as vaccines for measles, rubella, mumps and chicken pox.

The construction of genetically modified microorganisms can also lead to a decrease in the risk of pathogen use.

5. Conclusions

Regardless of the activity contemplated, a contained use or activity without deliberate use of biological agents, the occupational health physician's

role is essential although little mention has been made in this document. He or she works toward the establishment of preventive as well as protective measures. During hiring, the occupational health physician ensures that workers are in good health and that their immune systems are working efficiently in order to determine their aptitude for exposure to biological risks. Bear in mind that any risk analysis is based on the established hazard group concept for a "healthy worker" or "an immunocompetent person." The physician will also determine which treatments to give and which behaviors to adopt during pathogen contamination in order to prevent the appearance of delayed damage (illness, handicap, death).

We have demonstrated that biological risk assessment cannot be quantified. Preventive measures can nonetheless be drawn from systematic qualitative analysis. Containment levels are designed for the deliberate use of biological agents so that the overall residual risk is acceptable for exposed persons and for the environment. In the case of non-deliberate use, strict hygienic measures remain the best and principal means to prevent the risk of transmission and of infection.

We have illustrated the complexity of the risk assessment approach, which can only be performed by an expert. The elements of comprehension essential to the prevention advisor have been presented.

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Fund Donations

ABSA thanks the many of you have generously contributed to the Richard C. Knudsen Memorial Fund. The proceeds from this fund will be used to establish an award to honor Rich's memory. Those wishing to make donations to this fund should make their checks payable to the American Biological Safety Association. Please add a notation to the memo line that the check is to be used for the Richard C. Knudsen Memorial Fund. Checks should be mailed to ABSA, 1202 Allanson Road, Mundelein, Illinois 60060-3808.