Safety manual for researchers in biotechnology laboratories

Edited by
Dimitri Sossai, Mariangela Miele, Paola Bet

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FOREWORD

Improvement of safety in work places must be a high priority in any country, and more so in those that have a high rate of industrial development, which includes most of Europe. In recent history, failure to introduce or enforce safety measures have lead to very severe social consequences. Suffice it to mention, for example, the thousands of fatal accidents which have occurred during the construction of bridges, railways and tunnels; the thousands of industrial workers who have died of silicosis in the first half of the 20th century; the innumerable mine disasters all over the world. One might be tempted to dismiss these examples as past history; but we are forced to reject this attitude by just reminding ourselves of the dioxine tragedy in Seveso, Italy (1976) or the methylisocyanate tragedy in Bhopal, India (1984).

By comparison, safety in biological laboratories may seem a minor issue. Instead, it is an important issue for several reasons. First, the biotechnology industry is young compared to the mechanical or chemical industry: as a result, our understanding of safety issues is based on less experience, and this calls for caution. Secondly, the risks associated with biotechnology are qualitatively different from those of more traditional industries. With some 25 years hindsight we are relieved to find that much feared biological time-bombs have not exploded. However, it is a fact that artificial manipulations of genomes from any organism generate a feeling of awe that we cannot simply ignore. In a way, bioterrorism is to microbiology what a nuclear weapon is to physics, but the public perceives the former as more frighteming than the latter.

As for research laboratories, there is a third important point. In theory, it is precisely research scientists who might cause problems: who will watch the watchpersons? It is therefore their duty to be seen to be actively concerned in protecting the public as well as themselves while they are doing experiments. The first explicit statement in this

respect dates back to 1976, when a landmark document was published in the U.S. after the famous Asilomar conference, with the aim to establish high standards of biosafety, whereby scientists showed their willingness to regulate their own activities.

For all these reasons this book is important and timely. The book results from the collaboration of biotechnology investigators and of professionals who deal with all aspects of safety at all levels of public health. Taking into account the recent European Legislation on biosafety, this volume aims to be a useful guide for the chemical, physical and biological safety in research laboratories in the field of biotechnology and any laboratory where genetically modified microorganisms are used.

The authors have developed their professional experience in important European research and therapy centres such as the Istituto Nazionale per la Ricerca sul Cancro (IST - Genova, Italy), the International Agency for Cancer Research (IARC - Lyon, France), the Istituto Superiore Per la Sicurezza del Lavoro (ISPESL - Rome, Italy), l'Azienda Ospedaliera Ospedale San Martino e Cliniche Universitarie Convenzionate di Genova (Genova, Italy), the Centre Nationale pour la Recherche Scientifique et Technique (CNRS - Gif sur Yvette, France), the Centro de Seguridad e Higiene en el Trabajo, Consejeria de Empleo y Desarrollo Tecnologico, Junta de Andalucia (Cordoba, Spain) and the Consorzio Interuniversitario Biologia Molecolare Piante (CIBMP - Genova, Italy).

I am pleased that the book has originated here and I sincerely hope it will be a useful tool in the interest of safety of health workers, the quality of work and our worldly environment.

Prof. Lucio Luzzatto
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National Institute for Cancer Research - Genova, Italy

RISK ASSESSMENT

Dimitri Sossai, Ubaldo Leoncini Paola Bet, Mariangela Miele

Employees that work in research laboratories are exposed to professional risks that are frequently underestimated by the parties concerned who, often, only become aware of the existence of such risks when serious incidents occur, laboratory risks are generally "invisible", and therefore more dangerous with respect to other work activities. It follows that, the dangerousness of the agents, the adequacy of protective devices and the condition of the equipment should not be evaluated separately, but as if they make up part of a single procedure. All risk factors that contribute to a given activity should be identified in order to define standard procedures to limit as much as possible the risk correlated to such activity.

Risk is defined as the probability that a substance, an object or a situation can cause danger in specific conditions.

Danger is a source or a situation that can create potential damage to the health, to the environment or both.

Risk is generally considered a combination of two factors:

- the probability that an incident or adverse event will occur
- the consequence of the incident (damage)

Incident means an unplanned event which has the potential to produce damage to the health, to the environment or to both.

Risk is defined by the following formula:

R (risk) = **P** (probability) x **D** (damage)

Risk assessment is the complex process of estimation of the extent of the risk and of determining whether or not a risk is tolerable or acceptable.

For assessment of R the following elements must be considered:

- characteristics of the danger and/or of its source
- identification of the target and of its importance
- nature of the damage
- target vulnerability
- characterisation of transmission of danger from the source to the target

Risk assessment therefore consists in the process of recognition of the type of danger that exists, quantification of the seriousness of the damage produced by such danger and of the probability that a given damaging event will occur. The process of recognition of the danger and, consequently, risk assessment are not simple actions. Regarding this, use of the **risk matrix** is suggested which was developed precisely as an instrument for risk analysis. (Ozog, 1985; Code of Federal Regulation, 1986). The risk matrix is a simplification of the ISO-risk curves in the given probability/damage (P, D) level, where the co-ordinates individuate judgements regarding the situation under examination.

In a specific event in which the risk present in laboratories is assessed, a matrix is suggested which takes into consideration three levels of probability and three levels of damage severity (fig. 1). Table I shows, as an example, considerations regarding the frequency of incidents, the work environment and the health of the worker which can be useful in order to identify the probability that a given damaging event will occur.

Table I. Probability values associated with the environment and the health

PROBABILITY	FREQUENCY OF THE INCIDENTS	SAFETY ASPECTS RELATIVE TO ORGANISATION AND MANAGEMENT IN THE WORK ENVIRONMENT	SAFETY ASPECTS RELATIVE TO STAFF HEALTH
Improbable	- No incidents noted or they occur with very low frequency. - Occurrence of an incident causes a big surprise. - An incident can be generated by the occurrence of at least two independent low probability events.	- Electrical safety: presence of ground leakage, distance and/or suitable isolation of electrical parts from water. - Fire: existence of general precautions, good installation and maintenance of the equipment, clear emergency procedures, passive extinguishing systems, proper staff training, adequate practices and existence of escape routes. - Chemical agents: absence of reactivity with water and/or with solvents.	- Chemical agents: occasional exposure to concentrations 25% lower than TLV-TWA*, irritant action is unknown. - Non-ionising radiation: irregular exposure to very low doses. - Ionising radiation: irregular exposure to very low doses. - Biological agents: low or unknown transmissibility to man. - Allergenic agents: irregular exposure. - Identification and proper management of stores of dangerous biological agents and radiochemical compounds.
Possible	- Some episodes are known in which an incident has occurred. - The occurrence of an incident causes moderate surprise.	- Presence of heat generating equipment, appliances which need decontamination, solvents used and stored in an unsuitable way, flammable substances in freezers. - Chemical compounds: presence of corrosives, irritants, effervescence reactions with water, generation of heat, reaction to volatile solvents, possibility of intoxication.	- Chemical agents: carcinogenic, teratogenic, mutagenic: discontinuous polyexposure to levels close to the TLV-TWA, possibility of dissemination of powders and aerosols. - Non-ionising radiation habitual exposure. - Ionising radiation habitual exposure to controlled doses. - Biological agents: average transmissibility, host specificity, and infectivity by means of vectors. - Allergenic agents: habitual exposure.

follow Table I

PROBABILITY	FREQUENCY OF THE INCIDENTS	SAFETY ASPECTS RELATIVE TO ORGANISATION AND MANAGEMENT IN THE WORK ENVIRONMENT	SAFETY ASPECTS RELATIVE TO STAFF HEALTH
Probable	- Incidents have already occurred. - Occurrence of an incident causes no surprise.	- Presence of old equipment without regular maintenance, lack of protection of the electrical parts from water. - Chemical agents: reaction with solvents and generation of explosive mixtures.	- Chemical agents: habitual polyexposure to concentrations normally higher than the TLV-TWA. - Non-ionising radiation habitual exposure to high doses. - Ionising radiation: possibility of inhalation, dissemination. - Biological agents: elevated transmissibility, stability.

* TLV: Threshold Limit Value, exposure limit value based on the 8 work hours 5 days a week. TWA: Time Weighted Average, exposure limit value based on the 8 work hours 5 days a week.

The P value becomes associated with a **Probability Index** (PI) in the following manner:

Improbable	PI=1
Possible	PI=2
Probable	PI=3

If doubts exist concerning the number to associate with the calculated P it is suggested that the highest value be chosen.

Table II shows, as an example, the duration and effect of the exposure, which can be useful in order to identify the severity of the Damage.

Table II. Damage Value associated with the effect and the duration of the exposure.

DAMAGE	EFFECT (DURATION)
Light	- acute exposure with temporary invalidity (a few days) - chronic exposure with homeostatic results (psycho-physical stress) - accident with temporary disability
Serious	- acute exposure with serious results - chronic exposure with reversible results and/or partial disability - accidents with permanent disability
Very Serious	- acute exposure with lethal or seriously disabling results - chronic exposure with irreversible results

The severity of the damage becomes associated with a **Damage Index** (DI) in the following manner:

Light	DI=1
Serious	DI=2
Very serious	DI=3

Analysis of the Probability and Damage indexes can lead to calculation of the **Critical Index.**

The Critical Index can be calculated for each type of risk and allows definition of the measures of prevention that should be performed to reduce or in any case control the risk.

The Critical Index can be calculated with the following formula:

Critical Index = Probability Index + Damage Index

In the figure below, a matrix is shown proposed by Sossai and Leoncini (personal communication, 2000), where the co-ordinates are represented by Probability and Damage Index. The square in the top right-hand corner corresponds to maximum probability and greater

damage, while the square in the bottom left-hand corner corresponds to minimum probability and lower damage.

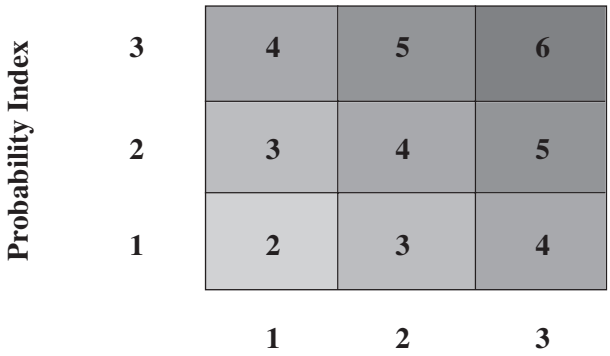

Figure 1. Risk Matrix

On the basis of the result obtained (Critical Index) the necessary interventions are evaluated in order to reduce to the minimum the risk associated with a determined operation, as shown in table III.

Table III. Necessary interventions on the basis of Critical Index

CRITICAL INDEX	NECESSARY INTERVENTION
2	Specific interventions in the programming phase must be evaluated, urgent interventions are not foreseen.
3	It is necessary to keep risks under control by evaluating hypotheses of improved interventions in the programming phase.
4	It is necessary to constantly monitor the risks by evaluating the necessity of improved interventions in a brief/average period.

follow Table III

CRITICAL INDEX	NECESSARY INTERVENTION
5	It is necessary to urgently intervene to individuate and carry out the interventions for prevention and protection which reduce the risk.
6	It is necessary to intervene immediately to eliminate/reduce the danger.

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BIOLOGICAL RISK

BIOLOGICAL AGENTS

Mariangela Miele, Paola Bet, Dimitri Sossai

Biological risk is a risk difficult to perceive and, similar to the risk from radiation or by genotoxic substances that provokes damage over time which is difficult to associate to a particular exposure.

Countless information exists regarding the dangerousness of physical and chemical agents, but the same cannot be said for biological agents.

Biological agents are infectious agents which include bacteria, rickettsia, viruses, yeasts, moulds, single and multicellular parasites and prions. Each species of infectious agent may have subtypes, strains and variants which differ in relation to pathogenic potential, host specificity, transmission, sensitivity to antimicrobial agents etc. Furthermore, biotechnology research laboratories produce a large variety of artificial vectors, with the aim of increasing the probability of genetic transfer between unrelated species. These new DNA fragments can self recombine and give rise to dangerous products.

In conclusion, although biological agents are well defined by current regulations, it is difficult to perform a complete assessment of the risks associated with their manipulation. Numerous factors such as biological diversity, chemical complexity of the molecules, multiplicity of the diffusion pathways and host interaction specificity, as well as the possible production of new coding, must be considered to evaluate the effect of these organisms on the health and the environment.

A **biological agent** is defined, according to current regulations *"as any microorganism, even genetically modified, cell culture or human endoparasite, which could provoke infection, allergies or intoxication"* in exposed workers.

A **microorganism** is defined as a microbiological entity cellular or non-cellular, capable of replication or of transferring genetic material.

The biological agents have been classified into four groups depend-

ing on the level of risk of infection (2000/54/EC):

Group 1	**Biological agent** which presents little probability of causing disease in human subjects.
Group 2	**Biological agent** which can cause disease in human subjects and constitute a risk to workers; it is unlikely to spread to the community; effective prophylactic or therapeutic measures are available.
Group 3	**Biological agent** which can cause severe disease in humans and constitutes a serious risk to workers; it may spread to the community, but effective prophylactic or therapeutic measures are usually available.
Group 4	**Biological agent** which can cause severe disease in human subjects and represents a serious risk to workers; it may present a high risk of diffusion within the community; effective prophylactic or therapeutic measures are not usually available.

The classification of biological agents is shown in the Annex I. Such classification reflects the present knowledge, and should be updated as soon as it no longer reflects this state.

The list of biological agents only includes agents which can provoke disease in human subjects; animal and plant pathogenic agents whose effects in man are unknown are not taken into consideration.

Genetically modified microorganisms

A **genetically modified microorganism** (GMM), according to the 98/81/EC Directive, is *"a microorganism (microbiological entity cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture) whose genetic material has been modified in an unnatural way, through multiplication or natural recombination."*

The following are considered unnatural:

- Recombinant nucleic acid techniques which bring about the formation of new combinations of genetic material by insertion of nucleic acid molecules produced by any means different than an organism, in a virus, in a bacterial plasmid or in other vector systems and their insertion into a host organism in which they do not naturally occur but in which they are able to multiply in a continuative manner.
- Techniques which require the direct introduction of heritable material in a micro-organism prepared outside of the for mentioned, including micro-inoculation, macro-inoculation and micro-encapsulation.
- Cell fusion or hybridisation techniques which produce live cells with new combinations of heritable genetic material by the fusion of two or more cells using methods not present in nature.

In vitro fertilisation, transduction, transformation, polyploidy conjugation and induction are not considered as being the results of genetic modifications.

Theoretically a GMM is separable into: the **host** organism, in which the genetic information is inserted; the **donor** organism, from which the genetic information has been obtained; the **vector** which transfers the information between these organisms; the **insert** which contains one or more genes capable of displaying biological activity.

Each of these parts, together with the final construct must be taken into consideration in order to obtain an accurate and proper risk assessment.

Vectors are, by definition, plasmids, bacteriophages, viruses and other DNA elements with a capacity for autonomous replication, in which a fragment of foreign DNA (insert) is inserted. A vector contains one or more replication sites and genes which confer a few phenotypic characters to the cells that contain it (e.g. antibiotic resistance). The vector may or may not integrate into the host DNA.

The vectors used in biotechnology research laboratories are numerous, any list would therefore be incomplete. Consequently only a few

considerations relative to vectors used in gene therapy experimentation and for the production of transgenic plants are reported since they represent the most recent applications in recombinant DNA technology.

Gene Therapy. The term **gene therapy** defines a series of interventions directed towards the intentional modification of genetic material with the aim of preventing, diagnosing or curing a disease.

The modification serves to correct a genetic defect determined by the absence or alteration of a protein, or to add genetic information which modifies the cell characteristics.

The genetic modification can be obtained in cells "in vitro" or else directly "in vivo" through the use of vectors capable of gene transfer and therefore through modification of the genetic property of target cells (Jones et al., 1995).

Gene therapy products include:

- free or complexed nucleic acids
- genetically modified cells
- viral vectors

At present viral vectors appear to be the most efficient method for gene transfer. They act following real "infection" of the target cell, transferring the therapeutic gene using their natural biological mechanisms (Kay et al., 2001).

The vectors used in gene therapy are predominantly replication defective or else they can only infect once and cannot multiply inside the target cell, unless by an accidental "genetic recombination".

As well as being unable to replicate these vectors have no lytic activity.

The viral vectors used in gene therapy are derived from **retroviruses, adenoviruses, parvovirus, herperviruses and poxviruses.**

The most frequently used vectors originate from avian and murine **retroviruses** (Miller et al., 1990). The main characteristics of retroviruses are:

- relative genetic simplicity

- the ability to infect a vast series of cell types with high efficiency
- genetic material composed of a single RNA strand

The RNA contained within the retrovirus integrates in the host cell genome in the form of DNA (proviral DNA). The transformation of RNA into DNA takes place due to a specific polymerase called reverse transcriptase. The proviral form of a retrovirus has a genome composed of two sequences "long terminal repeats" (LTR) and three important genes, which encode the core proteins (gag gene), the pericapsid proteins (env gene) and reverse transcriptase/integrase/proteases (pol gene). The integrated provirus can be considered in all effects in the same way as any cellular gene: its replication coincides with the transcription process, which is conducted by the cellular polymerases and regulated by cellular transcription factors which bind to the LTR at the provirus 5' extremity.

The **lentiviruses** are a retrovirus sub-group able to infect non-dividing cells or terminally differentiated like neurones (Naldini et al., 1996). The principal lentivirus vectors derive from feline immunodeficiency virus, equine infectious anaemia virus, simian immunodeficiency virus and human viruses amongst which HIV.

The **adenoviruses** are used in gene therapy (Berkner, 1992) when a high level of expression of the transferred gene is requested for brief periods, from the moment that strong immunogenicity of the adenovirus structural proteins induce a potent immune response which limits transfer efficiency of the gene after the initial infection. Adenoviruses are infectious human viruses, which often cause mild respiratory diseases, nevertheless in rare cases severe disease can occur. Consequently the use of vectors derived from them, due to the fact that they are transmitted by air, requires particular attention.

In gene therapy a parvovirus sub-group, the **adeno-associated viruses (AAV)** are used. They are small viruses that replicate in association with adenoviruses, defective and therefore completely dependent on the adenoviruses. The AAV are small single stranded DNA viruses; in cell culture AAV DNA integrates with high frequency in the host cell genome and replicates (Bern et al., 1995).

The **herpesvirus** include infectious human viruses such as herpes simplex virus type-1 (HSV-1), which is the most commonly used vector system. HSV-1 is common in the general population, but in rare cases can cause encephalitis; its utility as a vector system stems from its broad host cell range, ability to transduce neurones, and its capacity to receive large inserts.

The **poxviruses** are highly stable and include avian viruses (avipox) which cannot establish productive infections in humans, as well as mammalian viruses, such as vaccinia virus and modified vaccinia viruses, which can productively infect man. Thanks to their strong immunogenicity, these viral vectors have been successfully used in immunogenetic therapy protocols, allowing the activation of immune responses against tumour antigens transported in dendrite cells.

The laboratory worker who uses the vectors described above is exposed to risk from the moment in which the individual or collective protection devices are not adequately used or when work procedures directed towards protection of the operator from contact with such micro-organisms are not followed.

The problems tied to safety during the use and manipulation of viral vectors are obviously acute at the moment in which virus capable of infecting human cells are used.

However, the dangerousness of a pathogenic agent, endowed with lytic and replicative capacity, also depends on the state of immunological competence of the host.

Transgenic Plants. The elected method for the production of genetically modified plants is transformation using **agrobacteria**. Agrobacteria are Gram-negative common soil-dwellers, able to infect a large number of plant species. *Agrobacterium tumefaciens and A. rhizogenes* are the species most used and therefore the most studied: they cause collar tumours and hairy root disease respectively. The pathogenic effects are caused by Ti (tumour inducing) or Ri (root inducing) bacterial plasmids, relatively large plasmids whose dimensions vary from 140 to 235 Kb (Melchers and Hooykaas, 1987). The importance of agrobacterium lies in its capacity to transfer a section of plasmid DNA to the host plant cell chromosome. The transferred region (T-

DNA) of Ti and Ri plasmid is integrated and expressed by the plant genome; expression of these genes induce the development of collar tumours or hairy root pathology, respectively tumours. T-DNA also induce the production and secretion of opines, amino-acid derivatives which the agrobacterium utilises as a food source.

The T-DNA region ranges in length from 14-42 Kb and is bordered by conserved 25 bp sequences: Left Border (LB) and Right Border (RB). All of the sequences including LB and RB integrate in the plant cell chromosomes. Other genes present within the plasmid are not transferred, but are necessary for the production of transregulatory proteins, essential for transformation. The *vir* (virulence) genes are found amongst these, which produce proteins responsible for excision, transfer and integration of the T-DNA into the plant cell genome and the genes responsible for the catabolism of opines, which allow *agrobacterium* to utilise the opines secreted in tumours and hairy roots. In fig. 2 the schematic structure of the Ti plasmid is represented (Draper et al., 1988).

Another group of genes indirectly involved in transformation is present in the bacterial chromosome and is responsible for the bond between the bacterial and plant cells.

The virulence genes are activated in agrobacterium by phenolic substances (among which acetosyringone) produced by damaged plant cells.

The pathogenic Ti and Ri plasmid genes are not necessary for the transformation process, they can therefore be removed and substituted with the genes which are to be inserted in the plant. The Ti and Ri plasmids are very large and are not suitable as primary vectors. Foreign genes are therefore inserted in these vectors mainly by means of the binary vector system (Fraley et al., 1986; Klee et al., 1987).

Other systems available for the production of genetically modified plants which use viruses as vectors, electroporation and microparticle bombardment are the same as those used for the transformation of animal cells.

The agrobacteria like other phytopathogens are not included in the European biological agents list (Annex I); researchers that work in this field are maybe at less risk, but they definitely have greater difficulty in tracing information about classification of the biological agents used.

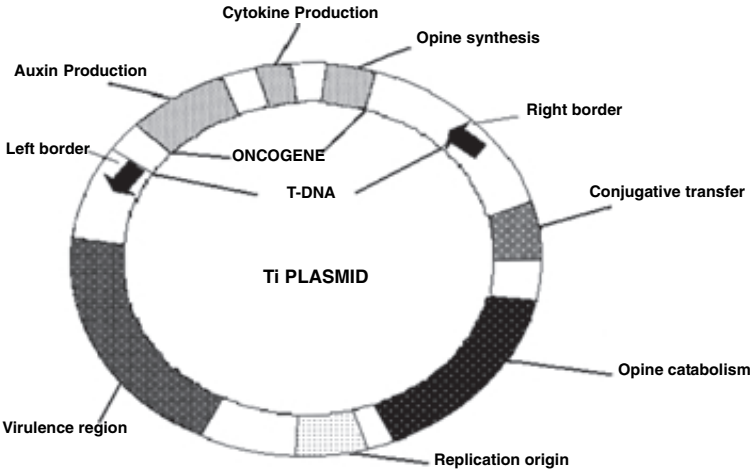

Figure 2. Structure of *Agrobacterium tumefaciens* Ti plasmid

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BIOLOGICAL RISK ASSESSMENT

Paola Bet, Mariangela Miele, Bernardetta Ledda, Dimitri Sossai

The procedures to follow for correct risk assessment for the use of biological agents are defined by the 2000/54/EC Directive.

The 98/81/EC Directive, which amends the 90/219/EEC Directive, instead regulates the **contained use** of Genetically Modified Microorganisms (GMMs).

Contained use means *"any activity in which microorganisms are genetically modified and in which such GMMs are culture, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with the general population and the environment"*.

Risk assessment is a complex process which requires the identification of many factors. In the event of a specific activity which can lead to risk of exposure to biological agents, the nature of the risk, the degree of exposure and the duration of exposure must be determined, in order to be able to assess the risk to the health or safety of the workers and to determine the measures to adopt.

The risks connected with manipulation of biological agents are generally associated to possible contact between the operator, or the general community, and the microorganism. Therefore the following parameters must be taken into consideration:

- the characteristic risk of the microorganism
- the risk of the activity

For **characteristic risk of the microorganism**, in the first instance, classification of the biological agents reported in Annex I must be taken into consideration and the danger subsequently determined. Such danger is influenced by numerous factors amongst which:

- The pathogenic power, that is the capacity of an agent to cause disease which varies according to the subtype, strain or resistance of the biological agent; for example, the *Ebola* virus is considered very dangerous and it is therefore placed in group

4. However the dangerous strain is *Ebola Zaire* while *Ebola Reston* does not seem to cause disease in man. *Escherichia Coli* is a normal saprophyte of intestinal flora; yet the strain 0157H7 is mortal for man.

- The virulence, which represents the degree of pathogenicity. Virulence depends on the infectivity and the severity of the disease produced by the biological agent and it is influenced by the mode of transmission of infection. For example Bacillus anthrax spores can cause fatal pneumonia when inhaled, but if introduced through the skin causes cutaneous lesions. Until the virulence of an isolated strain is known for certain it is worth considering such a strain as pathogenic and virulent.
- The infective dose. Generally diluted samples of low infectious agents are more dangerous than concentrated samples of high infectious agents.
- The severity of the disease and the availability of effective therapeutic treatment. For example staphylococcus aureus, which is a common habitant of human skin and can cause a large variety of diseases generally curable with antibiotics, is classified as a group 2 biological agent; bacillus anthrax even though fatal by inhalation, belongs to group 3 as it is sensitive to antibiotics; viruses capable of causing serious diseases such as HIV and HCV fall into in group 3 as they are little or non-transmittable by air.
- The method of transmission of the infective agent. The transmission pathway of a given agent can be single or multiple.

Furthermore there are other factors which add to the infectious process and they are: the resistance or the susceptibility of the host, the mechanism of exposure and the infectious dose of the agent. Moreover host susceptibility is determined by many factors amongst which age, race, sex, the state of health, pregnancy and vaccinations.

For microorganisms not yet classified, if at least the family to which they belong are known, the risk groups in which other species of the family belong are analysed. If the family is unknown, the possibility can be assessed, based on the work characteristics, of encountering pathogenic species in the substrates used and therefore the risk groups

in which these species belong analysed.

Risk assessment for GMMs must furthermore take into account the dangerousness of the receiving organism (host), of the donor organism, of the insert and of the final construct.

The risk of activity is relative to the type of manipulations carried out with the micro-organism. Workers handle different types of samples (biological fluids, urine, blood, serum, tissues) which can be contaminated with biological agents, cell cultures, bacterial liquids or agar cultures and viruses. The level of risk to which the workers are exposed depends on the nature of the sample. Blood, serum or tissue samples probably contain a lower concentration of infective agent and consequently represent a lower risk. Purified cultures and bacterial or virus cell concentrates in liquid solutions are a much higher risk.

Within the laboratory contamination can take place due to aerosols, ingestion, exposure of the mucous membranes and horizontal inoculation. Aerosols are considered the mode of transmission most at risk of an infectious agent. Aerosols can be generated by manipulation of liquids, tissue fragmentation, preparation of bacterial plates or the improper use of laboratory equipment including centrifuges, or breakage of containers with cell cultures (Collins, 1983).

Other ways in which a worker can enter into contact with a biological agent can be through:

- Accidental inoculation, for example by pricking or cutting of the skin with infected instruments or with sharp objects such as needles, scalpels or broken glassware.
- Accidental ingestion, for example pipetting by mouth or eating or drinking inside the laboratory.
- Direct contact with exposed parts of the body (face, eyes), for example from sprays generated by violent agitation, use of syringes or liquid spills.

Furthermore, in order to perform risk assessment, the activities of genetic manipulation of biological agents, aimed at the transfer of exogenous genes in animals, plants or man during which a large vari-

ety of nucleic acids are produced (constructs), must be considered.

Constructs generally contain marker genes for antibiotic resistance and, depending on the circumstance, a large number of genes derived from pathogenic bacteria, viruses and other parasites belonging to all kingdoms of living organisms (Ho et al., 1998). The majority of constructs that are produced have never existed in nature and can therefore be potential causes of damage (Traavik, 1999) to the environment and to the health.

For a long time it was thought that DNA degraded rapidly in the external environment and therefore was not capable of being absorbed through the skin or the intestinal tract. These assumptions have been surpassed by experimental studies which have demonstrated that DNA persists in all environments and is readily absorbed by the cells of all organisms. High concentrations of naked DNA have in fact been found in all natural environments: in the ground, in marine and freshwater sediments, in the air-water interface, where it retains the capacity to transform microorganisms (Lorenz and Wackernagel, 1994; Ho, 1998; Ho et al., 1998). DNA also persists in the mouth (Mercer et al., 1999) and in the digestive tract of mammals (Schubbert et al., 1994), where it can be absorbed and incorporated by the microbial population and by the host cells. Mercer has reported that partially degraded plasmid DNA is able to transform Streptococcus gordonii, which normally lives in the human mouth and pharynx and that human saliva contains factors which increase the capacity of the bacteria to be transformed (Mercer et al. 1999).

The capacity of DNA to penetrate intact skin has been known since 1990, when a number of researchers demonstrated that the application of DNA cloned from a human oncogene to a mouse's back, induced the development of tumoral tissue (Brown, 1990).

Finally recent studies have demonstrated how nucleic acids can readily enter in all types of human and mammalian cells. Nucleic acids can in fact be successfully administered in aerosols (Yei et al., 1994), by topical eye applications (Noisakran et al., 1999) through the inner ear (Yamasoba et al., 1999), via the scalp follicles (Hoffman, 2000), by direct intramuscular injection (Budker et al., 1998), through the skin (Khavari et al., 1997), or by mouth (During et al., 1998).

The studies reported previously refer to the genetic manipulation

of microorganisms used for the transformation of cells or animal organisms or in gene therapy. Regarding the genetic manipulation of plants the risks which are mainly taken into consideration are relative to the environment and to the horizontal transfer of genes conferring antibiotic resistance to ground microflora. The use of agrobacteria, elected system for the genetic transformation of plants (see previous), is not considered a risk for the worker, since it is of the general opinion that the process which brings about first the excision and then the integration of plasmid DNA only functions in plant cells. A recent study has instead reported that agrobacterium, under certain experimental conditions, is also able to transfer its own plasmid DNA to animal and human cells (Kunik et al., 2001). Further investigations are necessary to confirm whether or not risk effectively exists for workers who use agrobacteria, in the meantime the use of these microorganisms in laboratories with suitable containment levels is suggested, even if they are not included in the European list of biological agents (Annex I).

The process of biological risk assessment, which must necessarily take into account all that has been previously reported, has the aim of reducing to the minimum the risk of exposure during the manipulation of biological agents through the use of specific containment measures. For this purpose the activities which involve the use of biological agents, in particular GMMs are separated into 4 classes:

Class 1	Activities that present no or negligible risk, that is to say activities for which level 1 containment is adequate to protect human health and the environment.
Class 2	Activities of low risk, that is to say activities for which level 2 containment is adequate to protect human health and the environment.
Class 3	Activities that present a moderate risk, that is to say activities for which level 3 containment is adequate to protect human health and the environment.
Class 4	Activities of high risk, that is to say activities for which level 4 containment is adequate to protect human health and the environment.

Where there is doubt as to which class is appropriate for the proposed contained use, more stringent protective measures should be applied, unless sufficient evidence, in agreement with the competent authority, justifies application of less stringent measures.

Notification to the Competent Authority

Notification is the presentation of the required information to the Competent Authority of a Member State and it is foreseen for biological agents as well as genetically modified microorganisms.

Notification for the use of biological agents

Those who intend to work, for the first time, with a biological agent

- from group 2
- from group 3
- from group 4

must send notice to the Member State Competent Authority within the limits foreseen by Directive 2000/54/EC (art. 13).

Notification must be made at least 30 days before the start of work (or in a different manner in accordance with adoption of the European Directive in each country).

Further notice must be made if substantial changes for the health and safety of the workers occurs rendering the previous notice out-of-date.

Notification for the use of GMM

Those who intend to proceed with contained uses for the first time in a given premises are required to submit to the Member State Competent Authority, before commencing such use, a notification within the limits foreseen by Directive 98/81/EC (Attachment V, part A).

Following such notification, subsequent class 1 contained use may proceed without further notification. Users of GMMs in **class 1** contained uses must however keep a record of each risk assessment reports

and must make them available to the competent authority on request.

For **class 2** contained uses in premises already notified, a notification must be submitted on occasion of the first use as well as for subsequent uses according to the indications contained in attachment V, part B.

For **class 3** and **class 4** contained uses in premises already notified, a notification must be submitted on occasion of the first use as well as for subsequent uses according to the indications contained in attachment V, part C.

Contained use of class 3 or a higher class may not proceed without the prior consent of the Competent Authority, which shall communicate its decision in writing:

- No later than 45 days after submission of a new notification, if the premises have been the subject of a previous notification to carry out class 3 or a higher class of contained uses and if the associated consent requirements have been satisfied for the same class or a higher class than the contained use with which it is intended to proceed.
- At the latest 90 days after submission of the notification in other cases.

For the whole duration of the contained use it is the responsibility of the user to ensure that the containment measures or other protection measures for the class assigned to contained use are applied, as well as keeping the notebooks in which the operations performed are registered. Moreover the user should periodically re-examine the assessment of the class to use and compile a re-examination document.

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PREVENTION OF BIOLOGICAL RISK

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The assessment of biological risk has the aim of individuating different types of danger connected to the manipulation of biological agents in order to remove or reduce to an acceptable level, the risk of contamination of workers, of samples, of the environment and of the general community (Rodricks, 1994). Risk control is performed through the definition and the adoption of adequate prevention measures such as:

- adequate containment levels
- adequate equipment
- adequate rules for conduct in the laboratory
- adequate general and/or personal protection measures

Furthermore it appears that for removal or reduction of the risk of contamination, professionalism, training, experience, and common sense is of fundamental importance; therefore the formation and periodical updating of staff and the elaboration of a manual with the indications of appropriate procedures to adopt during activity or in the event of an accident forms an integral part of the prevention program. The biosafety manual should necessarily be presented and given to all who undertake for the first time any activity with biological agents.

Laboratory containment levels

The containment levels represent the requirements necessary to provide adequate personal protection for personnel who work with biological agents and to prevent contamination of the surrounding environment.

Whatever the activity carried out in a laboratory the indications described below must be followed in all events:

- The laboratory must be supplied with sufficient space large

enough to work without the possibility of accidental collisions between staff and against the equipment.
- Walls, ceilings and floors must be flat and easy to clean. Floors in PVC with electrically soldered junctions are recommended.
- External tubes and plumbing should be avoided and, if present, should be separated from the walls.
- The surfaces of workbenches should be waterproof, resistant to disinfectants, acids, organic solvents, and alkalis and have a high degree of fire resistance.
- Laboratory furniture should be tough, securely anchored and easily accessible for outer as well as internal cleaning operations between shelves.
- The storerooms should be used to contain objects for immediate use therefore avoiding disorder on the benches and in the areas of passage.
- A suitable space for long-term storage of the material used in the laboratory, conveniently situated outside of the work area, should be contemplated.
- A foot or elbow operational washbasin, preferably close to the exit, should also be present.
- An eye wash apparatus should be present and readily accessible.
- The doors should be suitably fire resistant, self-closing and have transparent panels.
- An autoclave for the decontamination of infected waste should be available in each laboratory or within the same building.
- Personal garments must be placed in an area separate to that where work garments are kept.

Four containment levels are distinguished according to the operations performed within the laboratory.

Containment level 1

Containment level 1 is indicated when operations arise which present no or negligible risk to human health and the environment. Minimum containment and protection measures must be applied. The room should be separated from the outside by a door which must

remain closed when working. A washbasin is recommended.

Containment level 2

Containment level 2 is indicated when operations are performed which present low risk to human health and the environment. This containment level foresees the presence of a class I or II biological safety cabinets to protect the worker from the eventual formation of aerosols. The biological hazard sign must be displayed on the laboratory door. An autoclave must be present in the laboratory or in the building in order to inactivate waste before disposal.

Containment level 3

Containment level 3 is indicated when operations arise which present a moderate risk to human health and the environment. Access to the laboratory is strictly controlled and the presence of a class I or II biological safety cabinet is necessary. The biological hazard sign must be displayed on the laboratory door. An autoclave must be present in the laboratory or on the same landing. In the latter event procedures must be adopted which allow the safe transfer of material in an autoclave outside of the laboratory. Access to such laboratories must take place through a filter zone and environment separate from the laboratory. The contamination free area, which has no filter zone, must be separated from the limited access part by a changing room or a shower unit, preferably, with self-blocking doors. The pressure inside the laboratory must be negative compared to that of the changing room and of the surrounding laboratories. The air expelled from the laboratory must be submitted to ultrafiltration (HEPA). The work zone must be sealable in order to allow fumigation. In fig.3 Containment level 3 is represented (Richmond 1999; Richmond, 2000).

Containment level 4

Containment level 4 is indicated when operations occur which present a high risk to human health and the environment. The laboratory must be separated from the other zones within the same building or must be placed in another building. Access to the laboratory is strictly controlled and a class I, II or III biological safety cabinet is necessary.

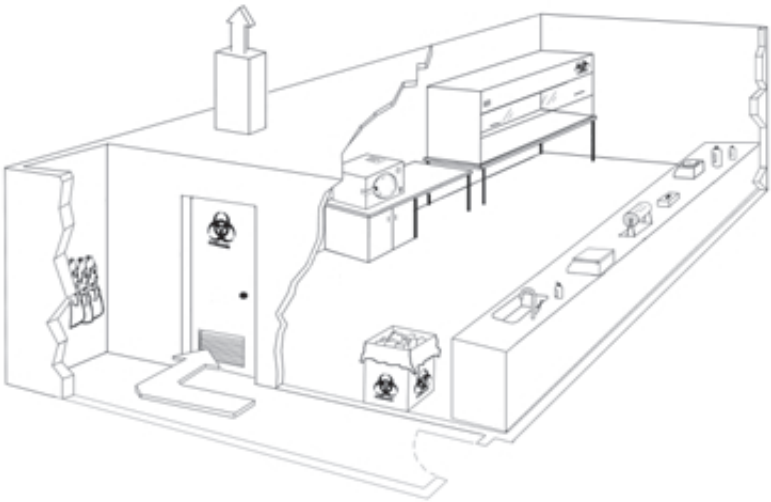

Figure 3. Containment level 3

A double entrance autoclave must be present. Access to such a laboratory must occur through a filter zone inside which a decontamination shower must be present. The pressure inside the laboratory must be negative. The air introduced and expelled from the laboratory must be submitted to ultrafiltration (HEPA).

The containment measures and other protection measures for laboratory activity are described in table 1a of the 98/81/EC European Directive.

Laboratory equipment and main procedures for their use

Laboratory equipment must have adequate CE certification and have the appropriate safety manual written in the language of the country of use with clear information; it should also guarantee easy use, easy maintenance, easy access for cleaning and decontamination.

Biological safety cabinets

Biological safety cabinets are a valid primary prevention system in as much as they impede the diffusion of potentially dangerous biological material. The cabinets are classified in three categories according to the level of protection guaranteed for operators and the surrounding environment. The choice of biological safety cabinet is determined by the risk associated with the biological agents and GMMs that are used.

Horizontal laminar flow cabinets, that are predominantly used in plant biotechnology laboratories, are not considered biological safety cabinets as they protect the sample from eventual contamination, but not the worker.

The equipment present in the work area or positioning of the cabinet in close proximity to heat sources or air currents can alter the functioning of the cabinet giving less protection. Ultraviolet lamps are not necessary in biological safety cabinets. If present, UV lamps should be cleaned weekly to remove dust and dirt which can diminish the germicidal effects of the UV ray. The lamps should always be switched off during activity, they must be left switched on for 15 minutes at the end of activity and for at least 5 minutes before the start of activity. Efficiency of the lamps must be checked periodically.

Class I biological safety cabinets are used when working with low risk biological agents; they protect the worker and the surrounding environment from eventual contamination, but not the sample. External air is aspirated inside the cabinet and expelled outside after being purified by a HEPA filter and if organic solvents are used, by an active carbon filter.

Class II biological safety cabinets are used when working with moderate risk biological agents and protect the worker, the surrounding environment and the sample from eventual contamination. External air is aspirated and transported to the work zone after being purified by a HEPA filter. The exiting air is passed through a HEPA filter again before being expelled outside.

Class III biological safety cabinets are used when working with high risk biological agents; they are hermetically sealed and the internal environment is maintained under negative pressure. All operations in the cabinet work area take place through frontal gloves. This type of cabinet guarantees almost full protection for the operator, for the envi-

ronment and for the sample. The air that enters passes through a HEPA filter, crosses the work surface and subsequently passes through two HEPA filters or else a HEPA filter and an incinerator before being expelled outside. Class III cabinets are generally installed in laboratories with a high level of containment and strictly controlled access.

For good use of biological safety cabinets the following procedures are recommended:

- Switch on the cabinet and the UV lamp, if present, 15 minute before the start of operations.
- If supplied with a hatch, check that it does not allow an opening of more than 20 cm, unless different indications are given by the manufacturers.
- Keep the instruments and reagents in the cabinet to a minimum.
- Place small containers for biological waste in the cabinet and transfer them tightly closed in larger containers.
- Do not use Bunsens or other types of burners in type II and III cabinets, in fact the induced air warm can deviate the normal internal flow, subsequently causing contamination in the work area as well as in the external environment; furthermore it can damage the HEPA filters.
- Always clean the cabinet surface carefully with disinfectant at the end of each work session (e.g. 70% alcohol).

For further information on biological safety cabinets consult the CDC-NIH publication (Primary Containment for Biohazards: Selection, installation and Use of Biological Safety Cabinets, 2000).

Centrifuges

For good centrifuge use it is necessary to note the following:

- Position them in a way that makes them accessible to all staff.
- Follow the indications given in the instruction manual and carryout periodic maintenance.
- Balance containers and centrifuge accessories with non corrosive liquids.

- Use thick-walled glass or plastic centrifuge tubes if biological agents which present a moderate to high risk are to be centrifuged and preferably use screw cap tubes labelled with a fixed code.
- Seal the tubes properly to avoid diffusion of eventual contaminating aerosols.
- Use centrifuges with anti-aerosol rotors or with appropriate anti-aerosol seals in the event of working with group 2, 3 or 4 biological agents.
- Centrifuge liquids containing group 3 and 4 biological agents separately from other materials and open and close the tubes in the biosafety cabinet.
- Inspect the centrifuge rotors and containers after use to verify the absence of corrosion and fine cracks and place them upside down to allow removal of condensation.

Fridges, freezers and liquid nitrogen containers

Fridges, freezers and liquid nitrogen containers are used for storage of biological samples, reagents, solutions etc. For their correct use the indications given below must be followed:

- Do not frequently and unnecessarily open and close them.
- Install fridges and freezers at a distance from heat sources and detached from walls.
- Use containers adapted to sustain the low temperatures requested for storage of the material.
- Avoid excessive filling of the containers intended for freezing.
- Clearly label all containers with information on the contents, worker and date.
- In addition to gloves for biological protection wear gloves for protection from low temperatures for the extraction of samples stored at –80° C and in liquid nitrogen, face masks and aprons to avoid burns from the cold. Furthermore containers with liquid nitrogen must be kept in a well ventilated environment in order to prevent possible incidents of suffocation caused by the movement of oxygen due to the action of nitrogen. Installation of an alarm system for the analysis of atmospheric oxygen is recommended.

- Store dangerous products in a fridge or freezer supplied with a key which must be kept by one person only.
- Store flammable solutions in appropriate ignition free fridges for solvents, without internal lights.
- Display on the outside of each fridge or freezer the name of the person to contact in the event of malfunction.
- Periodically clean them; for fridges and freezers check that the plug has been detached. During this operation wear face protection and rubber gloves; unlabelled material should be eliminated after sterilising.
- Use forceps for transportation of broken containers or fragments of glass and plastic.
- Disinfect the inside surface of fridges and freezers with 70% ethanol.

Incubators

Humidifying incubators are a very high source of contamination for biological samples as well as for the surrounding environment; they should therefore be cleaned regularly. Before cleaning remove the contents (flasks, plates, shelves, trays etc.) from the incubator, then clean the internal part with a non toxic detergent and remove traces of the detergent with 70% ethanol that should be allowed to evaporate completely before replacing the flasks, plates, shelves and trays. If work is carried out using cells that may be dangerous, positioning of the flasks in transparent plastic containers ("Sandwich boxes", Freshney, 2000) with sealable lids is recommended; the containers must be open in the cabinet. In the event of cell culture spillages autoclave the water contained in the incubator before elimination.

For operations using air transmissible class 4 or class 3 microorganisms, incubators with air aspiration systems and relative external expulsion are recommended; or else position incubators inside aspiration systems with air filtration through HEPA filters.

Thermostatic water-baths

Thermostatic water-baths include baths which contain water heated by electrical elements. To render the temperature homogeneous within

the bath a water recycling or agitation system is installed, therefore the containers only need to be immersed in a suitable support and well closed to avoid sprays or accidental sample discharges. Use of thermostatic water-baths with inclined covers is preferable in order to prevent drops of condensed vapour depositing on the immersed containers. Never place the lid near wires, sockets, or electrical apparatus. Furthermore it is recommended that:

- The thermostatic water-bath is installed at a distance from live electrical derivations (sockets, cables, apparatus).
- Fill the thermostatic water-bath with distilled water with the addition of an anti-mould or anti-microbe (do not use sodium azide as it can form highly explosive compounds on contact with copper, brass and lead).
- Change the water at least every 15 days and in the event of sample spillage, in such circumstances dispose of it as biological refuse.
- Periodically proceed with cleaning of the thermostatic water-bath using gloves.
- Avoid immersion of naked hands in the water.
- Always check, before proceeding with an incubation, that the containers are heat resistant, this avoids dispersion of biological or potentially infected material.

In the event of operations with biological agents and cells or tissue of human or animal origin with equipment (e.g. homogenisers, agitators for cell culture, lyophilisers, sonicators) which may generate aerosols, work inside a biosafety cabinet is recommended; when this is not possible use appropriate personal protective devices and open the containers in the cabinet after 10 minutes to allow aerosol to deposit. Containers must be in plastic or thick glass, in each case before use check they are in good condition. When using sonicators wear protective devices for the ears (plugs, headsets).

Rules of conduct in the laboratory

The majority of contamination with infectious agents occurs as a

consequence of human error. To eliminate or limit the risk of contamination a series of work and hygiene rules have been defined that take into consideration all aspects of the work, from organisation of the laboratory, to the conditions in which it is planned and the conduct that each worker must adopt during the activities (CDC and NIH Guidelines, 1976; CDC – NIH Biosafety in Microbiological and Biomedical Laboratories, 1999; Manual of Biosafety in laboratory, 1994).

For general rules of conduct application of "Good Laboratory Practice" is intended or else a series of rules that each worker must follow in order to eliminate or limit the risk present in the work environment and guarantee the quality of each worker.

The main rules are shown below:

General rules

- Access to the laboratory (including the kennels) must be strictly controlled.
- Provide adequate training and information on biological safety to all who carry out activity in the laboratory.
- Use a safety manual or a work protocol which identifies known or potential risks and which specifies practices and procedures to be followed in the event of incidents.
- Suitably inform employees each time new risks are introduced.
- Keep the doors closed during experimentation.
- Display the appropriate biohazard sign on the door to the entrance of the laboratory.
- Keep the laboratory and the animal cages clean, in order and free from any objects not relevant to the work.
- Cover the work surfaces with absorbent plastic covered paper such as "benchcoat".
- Decontaminate the work surfaces at least once a day and each time spillage of potentially dangerous material occurs; remove the contaminated absorbent paper and place it in the appropriate container for biological waste.
- Do not mouth pipette; use mechanical or electrical pipettes.
- Use a pipettor that can be easily decontaminated and if necessary autoclaved.

- Use pipettes or tips with filters to protect pipettors from eventual contamination.
- Perform all technical procedures in a way that reduces formation of aerosols to a minimum.
- Place contaminated pipettes in a container with disinfectant or directly in the container for biological waste.
- Wear disposable protective clothing or personal protective equipment suitable for the operations that are carried out.
- Wear glasses, protective screens or other means for protection from sprays or from bruising objects.
- Avoid use of syringes and needles, when this is not possible follow the procedures shown in paragraph below; hypodermic needles and syringes cannot be used to remix infected fluids.
- Always wear gloves during experiments with biological agents; remove them in the correct manner and deposit them in containers for biological waste.
- Accurately clean containers and equipment contaminated with blood or other potentially dangerous biological material with sodium hypochlorite or other decontamination systems (see table IV).
- Keep animals that are used for experiments separate from those that are untreated.
- Place contaminated solid waste for autoclaving or incineration in robust waterproof biological waste containers, and re-close them before removal from the laboratory.
- Give attention to vials containing lyophilized material, the contents may be under negative pressure and the sudden entrance of air within the vial can cause aerosol production. Vials containing infectious material should never be immersed in liquid nitrogen as if overturned or badly sealed they can explode when removed. If low temperatures are required, the vials should only be stored in the gaseous phase above the liquid nitrogen or in anhydrous carbon (dry ice). The external surfaces of vials stored in this way must be disinfected when removed from storage.
- Wear protective equipment for the eyes and the hands when removing vials from refrigerated storage.
- Use the biosafety cabinet when working with infectious agents,

cell cultures or tissues potentially contaminated with biological and/or carcinogenic agents.
- Immediately notify the head of the laboratory in the event of contamination and presumed or manifested exposure of a worker with infected material and follow the emergency procedures for surveillance and medical treatment foreseen by the Organisation.
- Apply the "work in pairs rule" otherwise no individual should work alone inside the laboratory when working with dangerous agents.

Hygiene rules
- Wear a laboratory coat or protective garments before entering the laboratory.
- The following recommendations can be taken in consideration for one's own safeguard during the use of biological agents: do not eat, drink, smoke, store food, apply make-up, wear rings and bracelets or handle contact lens.
- Wear gloves when working with potentially pathogenic material or material contaminated with toxic, mutagenic or carcinogenic substances (see related section on the risk of gloves when using chemical carcinogens).
- Do not wear laboratory coats or protective garments in the area designated for food consumption and outside the laboratory.
- Wash hands frequently and for at least twenty seconds with neutral soap after handling animals or infected materials, after removing gloves and before leaving the laboratory.
- Place laboratory coats or protective garments in a zone separate to that where personal clothing is kept.
- Wash contaminated laboratory coats or protective clothing separately from those uncontaminated.

Rules for the correct use of biological safety cabinets
- Turn off the UV or fluorescent lamp, if present, and switch on the air flow for at least 5 minutes, before starting work in the cabinet.
- Adjust the latch, if present, in relation to ones own height.
- Wash hands with neutral soap and wear suitable gloves.

- Disinfect the inner surfaces and position a waste container in a way that does not obstruct the air flow.
- Arrange essential objects in a way to create a "clean" and a "contaminated zone".
- Always move from the "clean" to the "contaminated" zone and wait a few minutes before restarting activity, in order to stabilise the air flow.
- Transfer the waste to appropriate biological waste containers and disinfect the inner surface at the end of all operations.

Rules for control of the formation of aerosols

- Use centrifuges with biosafety covers.
- Use self-locking or lockable syringes and needles.
- Slowly refill the syringe in order to reduce the formation of air bubbles and foam in the inoculating fluid.
- Do not use a syringe for mixing infectious fluids and check that only the tip of the needle is immersed below the level of fluid in the container avoiding the necessity of excessive force.
- Wrap the needle and its blocking system in cotton wool soaked in a suitable disinfectant, before extracting the needle from a rubber cap.
- Expel the excess liquid and the air bubbles in cotton wool soaked in a suitable disinfectant or into a small container with sterile cotton wool keeping the syringe in a vertical position;
- Prepare bacterial plates in the cabinet; during such activity potentially dangerous aerosols can form in particular when using air transmissible pathogenic agents.
- Switch on UV light at the end of the experiment.

Personal protective equipment

Personal protective equipment (PPE) is defined by Directive 89/686/EEC as *"any equipment or article destined to be worn or held by a person in order that they are protected from one or more risks that could place their health and safety in danger".*

Selection of the most suitable personal protective equipment can only be made following subsequent analysis of the activities to be performed, the associated risks and the degree of protection necessary.

All personal protective equipment must be put on before commencing any activity considered a risk to avoid contamination of employees and should only be removed at the end of the work session.

It is important to note that the worker who uses such equipment must know their use well and understand the necessity of adopting certain PPE to safeguard ones own physical integrity.

All PPE must show a declaration of EC conformity with the relative EN regulation reference marked directly on the equipment, an information manual on the correct way to be worn and the relative limits of protection; some personal protective equipment are subject to expiration therefore it is necessary to check them accurately before use.

Gloves

Gloves are made to protect the worker from a large variety of dangers amongst which: chemical substances, high and low temperatures, microorganisms, toxins, radioactive material and animal bites and scratches. Gloves that are ideal for any type of intervention or that protect from all risks do not exist; suitable gloves for the type of risk and the level of individual protection and as indicated by european reference rules should be worn each time.

For biological risk disposable latex gloves are normally used tested with ΦX 174 the reference microorganism for the test relative to biological permeation EN374.

It is important to note that to date gloves capable of giving an absolute protection do not exist.

Disposable gloves for biological risk must:

- display the EN374 markings required for operations where elevated exposure to biological microorganisms are foreseen
- not be worn for periods longer than 30'
- be eliminated if they present defects visible to the naked eye
- always be removed when leaving the work area or when touching "clean" objects

- never be washed and/or reused

Sterile gloves should only be used in cases where it is strictly necessary. Remember that the level of protection is diminished by prolonged use, in fact gloves lose their elastic properties and the effect of sweating hands favours permeation from the outside. In order to prevent possible allergies whenever t is possible it is recommended that vinyl or nitrile gloves be used, therefore avoiding latex gloves.

For cleaning operations thick rubber gloves should be worn or else two pairs of disposable gloves.

Gloves should also be worn during the manipulation of tubes in freezers of liquid nitrogen containers covered by adequate gloves for protection from low temperatures.

Protective clothing

Protective clothing protects work garments from contamination and must be worn when carrying out activities with infectious agents, blood or other organic liquids or biological materials. They must always be put on before entering the laboratory and removed before leaving. They must have the following characteristics:

- be sufficiently airy
- have the opening at the back and elasticised wrists to guarantee adequate protection
- be cotton so that they can be sterilised
- be changed daily when working with particularly dangerous microorganisms
- use disposable overalls tested with ΦX 174, shoe covers, caps, masks and glasses when containment level 3 operations occur in laboratories

PPE for the face, the eyes, or the respiratory tract

PPE for the face, the eyes, or the respiratory tract must:

- Limit the least possible the field of vision and the sight of the user.
- The optic systems of these types of PPE must be have a degree

of optic neutrality compatible with the nature of the activity more or less meticulous and/or prolonged by the user. And if necessary, must be treated or supplied with equipment that permits avoidance of the formation of vapours.

- Glasses with lateral protection or facial safety masks must be used in all operations which can cause sprays towards the worker such as for example the opening of containers, the opening of centrifuges, operations of forced aspiration etc.
- PPE for face and eye protection should have the label EN166.

In the event of accidental sprays the eyewash must be used to quickly eliminate all potentially dangerous liquid.

FFP3SL Filtering Face mask

These are shell masks suitable for protection from microorganisms with particles no smaller than 0,02 µm. They must comply with European legislation EN143.

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EMERGENCY PROCEDURES

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In all laboratories emergency procedures must be established for use in the event of accidents (Campi et al., 1998; Manual of Biosafety, 1994).

Each laboratory must define safety rules adapted to their own needs, in such a way as to make them operative as foreseen by the current regulations and therefore an emergency plan should be drawn up. The emergency plan should be made known to all employees and appropriate training courses should be programmed. The emergency plan must contain information on:

- risk assessment
- localization of the risk areas
- precise identification of dangerous biological, chemical and physical agents
- emergency procedures to adopt in the event of accidental exposure and decontamination
- identification of the persons at risk
- identification of the persons in charge of the different sections (biosafety officer, head of the laboratory etc)
- emergency telephone numbers
- a list of sources of emergency supplies, protective garments, disinfectants, and decontamination equipment
- emergency medical treatment in the event of contaminated or wounded persons
- medical surveillance for contaminated persons

Emergency procedures in the event of accidental exposure to biological agents

In the event of discharge of potentially dangerous aerosols from the biosafety cabinet adopt the following procedures:

- all persons must immediately evacuate the contaminated area
- close the room and apply contaminated zone warnings to the door and block entrance
- the head of the laboratory must be immediately informed
- activate procedures foreseen by the institute
- do not enter the room for at least an hour, to allow the aerosol to deposit
- put on protective clothing and suitable protection for the respiratory tract and proceed with decontamination, under supervision of the biosafety officer
- consult a doctor if necessary

In the event of accidental spread of liquid material containing biological agents adopt the following procedures:

- wear two pairs of gloves
- handle fragments of glass with tweezers and place in disinfectant
- cover with a piece of cloth or absorbent paper, soaked in disinfectant, and leave to work for at least 30 minutes
- pick up the cloth or the paper and any damaged material with a dustpan and eliminate in the appropriate containers for biological waste
- clean and disinfect the contaminated surfaces
- autoclave or immerse in disinfectant for 24 hours all material used

Note: if documents are also contaminated, the information must be copied on to another sheet and the original copy thrown in the container for infected waste.

In the event of breakage or suspect breakage of tubes containing potentially dangerous material during centrifugation adopt the following procedures:

- stop the motor and leave the centrifuge closed for at least 30 minutes
- put on gloves if possible thick rubber ones
- open sealed containers in the biosafety
- recuperate glass or plastic fragments with tweezers

- autoclave or immerse in disinfectant for 24 hours all broken tubes, fragments of glass, the containers, the accessories and the rotor; do not use hypochlorite solution to disinfect the metals as it is corrosive
- clean the inside of the centrifuge and leave the disinfectant to work overnight, subsequently wash with water and dry
- treated all contaminated material as biological waste

In the event of accidental injections, cut and abrasions, use the following procedures:

- remove gloves and protective clothing
- rinse the hands and the affected parts abundantly
- apply a suitable skin disinfectant
- go to casualty and inform the on-call doctor of the cause of the wound and, if possible give information regarding the microbiological agent involved

In the event of accidental ingestion of potentially hazardous material one must:

- remove all protective clothing and go to the accident and emergency department
- inform the doctor about the material ingested

In the event of incidents with dangerous biological agents that are transmitted by air, in addition to normal personal protective equipment, the respiratory tract must be protected by wearing filtering FFP3SL model face masks which give protection against solid and liquid aerosols. When wearing the face mask the following rules must be followed:

- hold the mask in the palm of the hand, leaving the harness hanging free
- place the mask under the chin with the nose clasp system turned towards the outside
- bring the lower elastic behind the head fixing it under the ears
- push the mask towards the face with one hand, bring the upper

elastic behind the nape of the neck fixing it above the ears
- regulate the tension by pulling the ends of each elastic (the tension can be reduced by pushing on the back of the buckle) while keeping the mask in position
- model the nose area on the face, running the fingers along both sides of the top of the nose grip system and at the same time pushing inwards

Furthermore the following instructions must be considered:

- wear a face mask and check the seal before starting preparations
- wear a face mask for the duration of exposure to contaminants
- eliminate the face mask and change to a new one when excessive clogging causes respiratory difficulty or discomfort, or if the mask suffers damage
- only use the mask for that which it has been provided
- do not use in an atmosphere lacking oxygen
- the requirements relative to the seal are not satisfied if hair or facial hairs pass under the face mask seal
- the face mask can be used for a number of hours corresponding to a work shift (7,5 hours), taking care to replace it in a sealed container away from contaminated zones
- identify the face mask by writing the owners name on the plastic cover positioned above the expiration valve
- on the above mentioned make a mark for every 30 minutes of use, after 15 marks eliminate it
- substitute the mask when eventual clogging causes respiratory difficulty or when it has been damaged or is visibly dirty

All incidents must be registered in an appropriate manner, following the instructions supplied by the institute in question.

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SHIPMENT OF PERISHABLE AND/OR POTENTIALLY INFECTIOUS BIOLOGICAL MATERIALS

Paola Bet, Silvia Franchello

The activity connected to the transport and shipment of perishable biological material, diagnostic samples and infected substances forms a justified means of preoccupation for all interested subjects, from researchers to analysts, from laboratory staff to those assigned to the transport and postal services.

The International Organisations involved in the problematic relative to the manipulations and transport of biological material and perishable substances (ONU Expert Committee on dangerous substances, World Health Organisation, International Organisation for Civil Aviation, International Air Transport Association, Universal Postal Union) have over time prepared directives which, if on one hand guarantee the quick transport of infected substances and biological samples, on the other hand propose to protect not only the safety of the general population, but also that of professionally exposed workers.

These organisations are also in agreement on common definitions and on requirements for packaging and labelling.

Definitions in use since 1991

- *Infected substances:* substances that contain viable microorganisms, such as bacteria, viruses, rickettsia, parasites, fungi, or recombinant hybrids or genetically mutated organisms, which are known or reasonably suspected to cause disease in man or in animals. Toxins are not included in this group.
- *Diagnostic samples:* human or animal material, for example excretions, secretions, blood and its components, tissues and tissue fluids, that are transported for diagnostic purposes. Live infected animals do not form a part of this category.
- *Biological products:* these can be finished biological products shipped for human or veterinary use, produced in compliance with national authority rules, that travel with special permission

or licence from this authority; or else finished biological products shipped before obtaining the licence for study and research purposes. Unfinished biological products prepared according to specialised government agency procedures also form part of this group. Live animal and human vaccines are considered biological products and not infected substances.

Preparation of packages for shipment of biological agents

For infected substances and diagnostic samples probably from infected sources, triple packing in accordance with the United Nations, the IATA and the ICAO is recommended.

Therefore preparation of the packages must be performed with respect to the following procedures:

- Infected substances and diagnostic material should be placed in a three layer wrapping: first an airtight vessel containing the sample, a second container, this also airtight, from the first by a layer of absorbent material, in a quantity sufficient to retain the liquid present in the sample in the event of leakage, a protective and waterproof outer wrapping, to avoid damage caused by physical agents or water.
- On the outside of the second container a card with information concerning the contents must be attached, in a manner that is not easily removable; a copy of this card must be immediately passed on to the destination laboratory, while a third must be given to the shipment officer. In this way, the assigned carrier as well as those destined to receive the goods will be able to adopt all necessary precautions.
- A label with the symbol for biological risk must be displayed on the outer wrapping of packages containing infected or potentially infected substances.
- In preparation of the pack foresees the presence of material such as liquid nitrogen or dry ice, containers and wrappings resistant to very low temperatures must be chosen; furthermore the first and the second containers should be able to sustain a differential pressure of at least 95 kPa.

- If the substance is perishable it must be identified as such on the accompanying documents.

Shipment of packages

In order to be efficient, transfer of infected substances requires good co-ordination between the sender, the carrier and the destination laboratory.

Therefore infected substances must not be shipped without preliminary agreements between these three interlocutors, or before the consignee has had confirmation from the national authority on the possibility of importing such substances legally and that subsequently there will not be a delay in delivery of the package to the destination.

The sender should therefore regard the following procedures:

- Make agreements in advance with the carriers and the consignee to insure that the sample will be received and analysed quickly.
- Prepare the shipment documents.
- Check the travel route.
- Send an opportune communication to the consignee with all the information concerning the consignment.

It is the responsibility of the consignee to:

- Obtain authorisation from the national authority for importation of the substance.
- Provide the sender with the necessary importation permits, the authorisation letter, or other documents requested by the national authority of the country or origin of the sample.
- Immediately notify the sender once the substance has been received.

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ANIMAL MANAGEMENT

Modesto Román Delgado, Rosa Montero Simó.

Working with animals involves contact with vertebrates which have been inoculated with microorganisms, or material that may contain them, during diagnostic and research activities. It also involves the study of the pathological process for a given animal in order to detect the agent that has caused illness and consequently allow the correct treatment to be applied.

The animal house is the essential working place for all experiments, situated close to the main laboratory, but usually with different staff and environmental conditions. The people who look after the animals must have sufficient information, and they must also be trained to work in safe conditions with animals. They must have suitable containment measures and levels for the risk groups of known or presumed biological agents.

Protection against persistent or latent infections in animal species is an obligatory measure against biological risk. Also, contact with hair, nails, skin and excretions of known sensitizing capacity is an additional risk that must be taken into account while working in the animal house.

Due to the great diversity of animal species used in experiments, establishments have to adapt to each of them. There are three different types of establishments, under the Council Decision 1999/575/EC of 23rd of March of 1998 related to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (OJ EC 24 of August of 1999). They are:

- Breeding and supplying establishments, where animals are bred and maintained with the objective to provide them to the final users.
- Supplying establishments, from which animals are bred only to supply them to other establishments who will use them in experiments.

- User establishments where animals are used and treated in experiments.

The list, although incomplete, of the animal species used in procedures will include:

- Mouse *(Mus musculus).*
- Rat *(Rattus norvegicus).*
- Guinea pig *(Cavia porcellus).*
- Golden hamster *(Mesocricetus auratus).*
- Rabbit *(Oryctolagus cuniculus).*
- Quail *(Coturnix coturnix).*
- Pig *(Sus escrofa familiaris),* preferably the species: pig mic.
- Dog *(Canis familiaris),* especially beagles.
- Cat *(Felis catus)*
- Primates, including: chimpanzee, mandrill, rhesus, green African monkey, mangabey, titi, etc.

It is necessary to know which are their anatomical and physiological peculiarities and their environmental conditions, as well as their different strains and the characteristics they should have for their use in experimental models, in order to design laboratory investigation procedures in appropriate conditions.

Technicians as well as assistant staff must know the risks that their work involves and which are the rules to be followed to avoid them. This is the main objective of health care and work safety training.

The reasons for accidents at work in an animal house are:

1. animals:

   - hosts of experimental biological material
   - attacks
   - transmission of zoonoses
   - allergenic sources

2. environmental conditions

3. activities which take place
4. personnel staff physicological state

The following accidents are more frequently observed:

- skin and mucous alterations caused by formaldehyde, alkalis, toxicants, acids, etc.
- auditory damage caused by excessive noise
- bites, scratches and other accidents caused by animals
- bruises when handling material
- infectious or parasitic processes infected by animals with germs originating zoonoses
- allergy caused by dust, fungi, arthroparasites, etc.

The majority of animal species intended for experimental procedures are a potential source of pathogenic microorganisms. Since personnel are continuously manipulating the animals, they are especially susceptible to infections and consequently development of different pathological processes. The risks derive from daily physical contact with animals, as well as from different practices (blood sampling, surgery, necropsy, etc) and even from the manipulation of cell cultures, as in the case of Simian Immunodeficiency Virus (SIV) or Lymphocyte Choriomeningitis Virus (LCMV), responsible for meningitis processes among those who manipulate these infected cells.

Thus, at present, health control of animals involved in experimental procedures is considered a priority. The early detection of any agents that can cause zoonosic diseases is essential. They must not be present in the animal house, and all possible control measures must be taken to avoid them.

Animal suppliers must draw up a certificate stating that animals do not have diseases such as:

- Mice: choriomeningitis virus.
- Non-human primates: tuberculosis, encephalitis, Malburgo disease, yellow fever.
- Capridos, Bovines and Swine: brucellosis.

- Cats and dogs: vaccination against rabies certificate, free of honeycomb moths and parasites.

It is difficult to distinguish between pathologies transmitted by an animal that has been voluntarily inoculated and zoonoses that are not controlled by the experimenter. Biological risk cannot be calculated and it is very difficult to define "prevention". It is necessary to control the possible variables to determine the appropriate "biosafety level" that is required to work with biological reagents, by selecting the animal's origin, performing an exhaustive health control and applying appropriate preventive measures.

In order to evaluate the biological risk derived from the transmission of pathogenic material from the experimental animal (host), several characteristics should be considered:

- Characteristics of the infected agent (virulence, biological stability, etc).
- Characteristics of the disease (seriousness, immunoprophylactic availability and treatment).
- Host discharge (excrements, urine, saliva, aerosols, etc)
- Host transmission (aerosols, vectors, etc).
- Potential hazards in manipulation (syringes, cuts, contact, aerosols).

Animal manipulation may cause some incidents involving injuries to personnel, such as scratches and bites, which can be prevented by means of:

- Use of sedated animals, if the experiments allow it.
- Cages provided with immobility mechanisms for big animals.
- Knowledge of fastening and immobility techniques.
- Use of appropriate protective gloves.

Furthermore, some workers may be allergic to certain animals. This produces "an exaggerated and inappropriate immune adaptation response when there is contact with the antigen, causing injuries". All

of this starts an allergic process that constitutes a professional disease.

An animal laboratory, as a place where experiments with animals take place, requires an appropriate design in order to allow efficient and economical functioning and, consequently, to provide adequate animal care and surveillance. Design is mainly based on the application of rules of organisation and environmental and microbiological safety in order to provide better results and better care for animals in standard or "defined" laboratories.

Each facility should be exhaustively planned, according to the activities that are going to be performed (breeding, toxicological tests, biomedical research, etc), although all of them should maintain the animals in a constant environment, free of physical, chemical and microbiological agents. There should be service areas, animal areas and interconnecting areas or corridors to provide appropriate circulation of people, animals, materials and equipment.

An animal experimentation laboratory should be provided with, at least, the following facilities:

- animal breeding and animal production area
- animal experimentation area
- annexes used for elaboration of diets, laboratory, treatment, operating room, etc.
- areas where animals are received and from which they are sent, washing facilities for Personnel, waste containers, etc.
- management rooms, library, staff rooms, computer labs, etc.
- large equipment area: air-conditioning, electricity generators, fridges, incinerators, etc.

Animal production areas should be physically separated from personnel areas in order to meet the requirements of public health, personnel welfare, animal care and environmental control rules. Laboratory animal facilities should combine an appropriate quality of animal care (a certain degree of movement, food, water and care, minimum restrictions for their physiological activities, etc) with research procedures, which should guarantee the final fulfilment of the objectives by avoiding or reducing unnecessary animal discomfort. Moreover,

animal laboratories should combine structural organisation, technical resources and personnel training in order to allow procedures and experimentation.

Different areas should be allocated depending on whether or not they have to be near the laboratory, as well as depending on the building structure. The animal laboratory may be situated on an independent floor, or it may occupy several floors, with a lift that can only be used by laboratory personnel. It is also possible to create many independent units under a central one, and to have animal cages situated in different parts of the building.

Furthermore, animal laboratory facilities should meet the following requirements in order to provide good work conditions for personnel and appropriate animal housing:

- Work surfaces should be solid and cleanable.
- Floors should be monolithic, stain- and chemical-resistant, slip-resistant, seamless and of a suitable appearance without the need for sealing products or waxes.
- Walls should have protective handrails, be resistant to knocks with moveable devices; be seamless and resistant to leaning products, brushes, disinfectants and pressurised water. Wall holes should be sealed, as well as floor and ceiling seams, with 15cm of curvature.
- Ceilings should have the same characteristics as walls. Ceilings with fitted plates are not advisable because they are not easily cleanable and allow insects and rodents to hide. Exposed pipes and accessories are not recommended either, if present it must be possible to clean and disinfect them. Unless there are special requirements, ceilings should not be higher than 2.70m.
- Doors should open inwards, except if there is a hall. Doors in animal housing area should not be less than 1.10m wide and 2.15m high and they should be hermetically closed, so that insects and wild rodents cannot enter cages. Metal doors are preferred, and protective strips should be fitted at the lower part of the door and at the edges. Doors should be self-closing and hinges should be built-in. It is advisable to include a small peephole in order to

control the cage without going inside.

- At the warehouse, doors should be of size to allow the entrance of transport. Wide automatic doors (1.80 m) are advisable in busy areas (cages cleaning, animal sending and receipt). For fire safety reasons, doors should close automatically when the alarm goes off.
- Aisles should be, at least, 2.10m. They should be free of objects and should have skirting boards or handrails to protect them, and steal boards, or other resistant materials.
- Lifts should only be for laboratory use. It is advisable to have one for dirty cages and another for clean cages and supplies. Lifts should be big enough to hold 3-5 different types of cages.
- Electricity installation: each cell or room should have one or more plugs, with a minimum power of 2000w. Service areas should hold a general panel from which all the equipment is connected. A generator set should exist, for use in case of a power cut.
- Sockets, fluorescent tubes etc, should be sealed, or be situated at least 10cm away from the ceiling, so that they can be cleaned. It is necessary to have an automatic lighting system to control the daily rhythm of each room.
- Plumbing and drainage: in those areas in which hoses are used for routine cleaning and decontamination, floors should have a minimum inclination of 0.64cm/m, and in dog areas their inclination should be 0.64cm/0.3m minimum. Floor drains should not be less than 10.2 in diameter: in frequently used areas, such as cage cleaning rooms or dog rooms, it is advisable that they are 15.3cm in diameter and on floor level. Those drains that are going to be used intermittently should have tops and be sealed to avoid waste gases.
- Intercommunication systems, such as telephones or public address systems should not disturb animals.
- Security measures are required due to the great value of experimental animals and vandalism. These measures will depend on the layout of the animal laboratory and its relation with other areas.

- Installation should be isolated and doors should be automatically closed when appropriate. Only authorised personnel may enter the laboratory, using identification systems. It is advisable to install locks in order to limit the access to research cells, quarantine animals and breeding rooms.
- Insect and rodent control without using chemical agents, as they should not be present in animal laboratories. Many insecticides are powerful enzymatic inducers and they can alter experimental work. The most effective method is to seal all possible ways of entrance for insects and wild rodents (cracks, seams, etc) and to eliminate all hiding places and nests inside the cells (only animal racks are allowed inside the cells).

In addition to building services and materials used in the animal laboratory, certain environmental requirements must be fulfilled to meet animal needs and to provide appropriate practices for personnel, so that they can face the risks that animal care involves. It may be necessary to provide personnel with appropriate protective equipment and to organise their tasks according to their training. The main requirements to be fulfilled by laboratories are:

- Good ventilation systems, with fresh air supply and with a low level of odours, noxious gases, dust and biological agents. Heat and humidity should also be controlled. The guideline of 15 or 20 fresh-air changes per hour should be used in normal conditions, but if there are not many animals, there could be 10 air changes per hour. It is advisable to avoid air currants and non-filtered air intakes. Do not smoke in animal housing facilities. They must be regularly cleaned so as to avoid unpleasant smells and to allow the correct treatment of recycled air.
- Temperature in animal housing facilities should be appropriate for the experimental animal species, between 20 or 24 degrees Centigrade, although it should be higher if they are young or new-born animals. Rabbits, cats, dogs and pigs can bear temperatures which are higher than 10 or 15 degrees Centigrade. The ventilation system should be provided with a thermostat that

automatically keeps the heating system at a constant temperature, according to thermal changes that animals may experience during experimental work, without changing their metabolism.

- Humidity may have a negative effect on animal's health and well-being. A level of relative humidity within the range of 40 and 70 degrees is recommended.
- Illumination: Animal facilities should not have windows, it is better if they are lit by artificial light so that intensity and wavelength of light are appropriate for the natural habitat of the species. Lighting should also provide safe conditions for personnel.
- Noises may disturb animals. Noise-production support areas should be separated from housing and experimental areas; sound-attenuating materials bounded to walls or ceilings might be appropriate for noise control. A continuous noise of moderate intensity, such as soft music, may be appropriate to overlap sudden noises.

Laboratory personnel must receive appropriate training on the potential hazards associated with the work involved, so that no accidents happen as a consequence of inappropriate practices. Daily tasks, such as animal identification or handling, animal care, etc, together with experimental practices may cause occupational injuries.

The following are suggestions to prevent accidents in animal handling:

- Special training in animal handling. Information about different animal species, courses or experienced workers.
- Do not be scared when handling animals, it is very difficult to work under such conditions.
- Safe and gentle animal handling.
- If animals are badly handled, they may be harmed or stressed, and the experimental work may be affected.
- Procedures should be performed under general or local anaesthesia or with the animal fastened or immobilised.

- It is necessary to wear latex gloves to manipulate laboratory mice. Sudden movements or noises are to be avoided. They should be taken by their tail and put on the palm or cage screen. Their neck should be taken between the handler's thumb and forefinger to immobilise them, so that they cannot turn round and bite the handler. Do not drop them when they are being taken back to the cage because they may injure their backbone. Place them gently on a beal.
- It is necessary to wear latex gloves for work with rats. The handler must put his hand into the cage, to check animal's movements and behaviour. Do not take rats by their head. Take them by their tail, unless they are more than 450 grams in weight because in this case their tail may be harmed. Rats should be handled like this only for cage changing, weighting, and any other brief procedures. If the handler wish to immobilise them, he should take rats by their shoulders and back before performing any immobilisation procedure (injection, for example). Do not hand rats to another person, as they could escape.
- Handlers must also wear gloves when handling rabbits. They do not usually bite handlers, but they can scratch them with their legs. Take rabbits by their back with one hand and by their legs and stomach with the other. Do not take them by their ears. If they have to be held longer, the handler must put their head under his arm.
- For prolonged immobilisation, it is possible to use restraining devices or systems where animals' abdomen and head are not restrained, so that it is possible to take blood samples, to give intravenous injections or inoculations under the skin.
- Marking methods should be selective, brief and painless and should be performed under general or local anaesthesia. Animals involved in experimental protocols should be identified. There should be records containing different factors that have to be considered in order to identify animals, such as species, strain, etc. There are various suitable methods, such as:
  - Cage cards with written information stating animal identification.

- Detectable natural marks or phenotypic characteristics. They should be kept in written records.
- Temporary colourings, such as methyl or gentian violet, fuchsin, etc. They should be used in brief procedures, and should be recorded with a special code.
- Ear notches, made with special pincers. This procedure should be recorded with the appropriate code. Amputation of phalanges is not recommended.
- Earrings. Animals are provided with plastic or metal tags stating their identification numbers or letters. This procedure is not advisable in rodents.
- Tattoos on colourless skin (ear or tail). Electric pencils or marking pincers should be used.
- Microchips under animal skin. A syringe should be used. Microchips are made of biocompatible material. Animals can be identified by means of a special device.

Animal care involves a series of routine practices, such as animal housing, cleaning of cages, waste disposal, etc, that can be hazardous for the health and safety of personnel, due to the materials and animals used. Some considerations about routine procedures should be taken into account:

- Food should be carefully prepared so as to avoid aerosol production. Foodstuffs should be packed in sacks of at least 25 kilograms in weight.
- Cages, cage racks and associated equipment, such as feeders or water devices, should be regularly cleaned and sterilised, specially when wet foodstuffs are used or when they get easily contaminated with water, urine, excrements, etc. In this case, they should be cleaned once a day.
- Cages and equipment can be washed by hand with water (immersion, water hoses), with detergents and brushes, chemical disinfectants, etc. Personnel should wear protective personal equipment to protect themselves from humidity and chemical products (hypochlorite, phenol, ammoniac, formaldehyde, etc).

They will have to follow several basic rules:
- Read instructions carefully.
- Handle volatile products in extractor cabinets.
- Use disposable gloves, goggles or masks when working with corrosive or harmful material.
- People who wear contact lenses have to be especially careful.

- Automatic watering systems are recommended.
- It is essential to sterilise cages and associated equipment in autoclaves or with chemical products, in order to maintain them in the conditions that experimental work requires. Sterilisers should be regularly calibrated and monitored to ensure their safety and effectiveness. Personnel should be informed of the risks involved (burns, intoxication, skin reactions, etc).
- Soiled bedding should be removed and replaced with fresh material as often as it is necessary to keep the animals clean and dry. The frequency depends on such factors as the number and size of the animals, bedding material, quality, etc. Suction pumps are recommended to remove urine, excrements and microorganisms. This avoids aerosol production and inhalations.
- Waste disposal poses important problems. Dead animals are part of that waste. The animal laboratory should have an incinerator; if this is not the case, there should be a dedicated waste-storage area provided with special freezers for dead animals. Waste containers should be leak-proof and equipped with tight-fitting lids. Their disposal should be closely co-ordinated with safety specialists.

Personnel caring for animals should be appropriately trained regarding techniques to be used during their work. They should care for animals well-being, maintain facilities in good conditions, follow-up the experimental work, etc.

The European Council Resolution, 3rd of December of 1993, has established the qualifications of personnel required to work in animal laboratories, according to classes, standard training requirements and specific training requirements. Personnel working with animals are divided in four groups:

Class A: People in charge of animal care.
Class B: People who perform the procedures.
Class C: People in charge of direction and elaboration of procedures.
Class D: Specialists in laboratory animal science.

These categories are defined in accordance with the task they have to perform, and their specific training should be provided in compliance with applicable laws and regulations to accomplish their tasks in an animal laboratory.

However, all of them must receive appropriate training focused on maintaining a safe and healthy workplace.

- Information about potential hazards associated with zoonoses involved.
- Information of the consequences of a deficient use of materials, equipments and chemical products.
- Training in animal handling.
- Personal hygiene:
  - Personnel must shower after work.
  - Clothes must be washed daily.
  - Frequent hand-washing.
  - Personnel should avoid injuries and cover the ones they already have.
- Work clothing:
  - Clothing should be cleanable and resistant to temperature.
  - Comfortable shoes, preferably cleanable.
  - Disposable gloves and masks for cage-cleaning and for procedures involving aerosol production.
- Do not eat or drink in the animal laboratory.
- Do not touch the face or nose while working in the lab.
- Personnel should know how to use the first aid kit, which should be visible and must contain: soap, serum, sterilised needles and syringes, oxygenated water, iodine, cotton swabs, sticking plaster, sterilised gauze and bandages, analgesics, scissors, a pair of tweezers and a scalpel.
- Basic treatment for injuries:

- Wash with water. Use soap if necessary.
- Apply serum and eliminate foreign bodies with tweezers, scissors or scalpel.
- Apply antiseptic.
- Cover the injury.

Risk assessment in animal laboratories should consider the physical and psychological conditions of personnel. A medical surveillance programme should be developed, in order to:

- Identify personnel at biological risk: decreased immunocompetence, pregnant women, susceptible to contamination (for example, for skin injuries) or infections, and allergic to animal products, such as people who suffer from asthma or nasal rhinitis.
- Protect animals from animal germs, do not accept animals suffering from mycosis, tuberculosis, carriers of salmonella or amoebae, etc., so that zoonoses do not enter the laboratory.
- Evaluate psychological conditions of personnel working with animals such as cats, dogs and primates. If they are afraid of the animals or their behaviour is aggressive, they are at higher risk of suffering accidents. Personnel affection for animals should also be evaluated, because it may hinder some of the tasks.
- Annual medical evaluations are advisable, in order to check that personnel have not been infected with zoonoses.

The medical evaluation of personnel should include, at least, the following:

- A health-history evaluation is advisable to assess potential risks. It is advisable to know if the employee is receiving some kind of antiseptic treatment. (This will put the worker more at risk, corticoids…).
- A full physical examination, especially of the skin, in order to detect mycosis.
- A psychological evaluation of personnel connected with the work

they do.
- Basic biological analyses: blood count – count and formula –, biochemical analyses – renal and hepatic function -, urine analysis.
- Serology and cultures of salmonella, shigella, amoeba, brucella and other parasites.
- Lung radiography and spirometry.

In addition to general vaccinations (diphtheria, tetanus, polio), additional immunisation should be offered to laboratory personnel depending on the animals handled:

- BCG is justified when handling animal carriers.
- Immunisation against rabies is recommended for personnel handling wild animals, or cats and dogs of unknown origin.
- Vaccination against hepatitis A is useful when handling simians.
- Vaccination against typhoid is still under discussion. TAB, that immunise against salmonella typhi, is preferred.
- Vaccination against brucella must be applied when working with pigs.
- Vaccination against hemorrhagic leptsopirosis is recommended for personnel working with rodents, cats, dogs and ruminants.
- Vaccination against smallpox is recommended for personnel handling African monkeys, because it immunises against monkeypox virus.

Personnel should receive appropriate training on the procedures to be followed when there are some accidents during animal experimental work, such as bites, scratches, etc. The basic procedures are:

- Wash the injury with soap and water. Apply a skin disinfectant (iodine, etc).
- Go to the first aid.
- Notify the person in charge, who will make an appropriate record of the accident.
- Medical treatment will depend on the animal involved and its

origin. Immunisation schedule will be checked. Antibiotics are not necessary, unless the injury is rough or has been checked too late. An operation is required if the injury suppurates, with pus inside or around it, or if there are adenopathys, oedemas or lymphangitis, or fever or septicaemia.

- Notify the veterinarian of the accident. He or she will have to control the animal evolution and will take serum samples.

Laboratories must have written protocols regarding procedures to be followed after accidents involving hazards. The main pathological processes must be checked according to animals, their origin, state of health and their use in experimental work. Serum samples of personnel and animals must be taken.

Animal experimental work entails risk of infection. This kind of risk has special features, different from those of chemical or physical risk, because it is not possible to know if an animal is infected or not without a prior investigation. There are many biological agents that do not affect animals. Not very much is known about the ways of transmission of an infectious germ, there is not a relation between dose and effect. It is all or nothing: either the individual get infected, or not. The effects of an infectious pathology are not foreseeable, they depend on the individual. Finally, these agents, especially viruses, can mutate, changing their composition and their pathogenicity.

However, biosafety criteria and health protection of exposed workers can be applied in order to improve the procedures performed in animal laboratories and the conduct of personnel when handling laboratory animals.

Prevention measures against biological agents have general rules of safety, structured in different containment levels and specific precautions depending on the kind of task performed. In establishments where people work with animals the following, at least, is necessary:

1) Design of the facilities.

- Access to the animal laboratory must be restricted and a specific sign must be posted.
- Walls and floors must be seamless, waterproof and easily cleanable. Resistant to solvents, detergents and disinfectants.

- Good ventilation. If the ventilation is mechanical, air must be sent to the atmosphere, the internal flow of air must be regular and continuous, without turbulence.
- Measures for vector control (insects and rodents).
- Drainage trapdoors in the floor must contain water and be regularly cleaned and disinfected.
- Contaminated areas must be separated (isolation area, residue area) from the clean ones (rooms for breaks or for administrative work)

2) Working procedures.
- Reduction of aerosol production.
- It is necessary to have specific disinfectant procedures available.
- It is necessary to decontaminate all waste material before its elimination.
- Use of a steriliser for contaminated material and an incinerator for dead animals.
- Appropriate cleaning of cages before a new use (fumigation, heat treatment).
- The person in charge must collect all the information about accidents or incidents, even bites or scratches.

3) Personal hygiene and individual protection.
- Use of appropriate protective clothes and shoes to work in the animal laboratory. They must be removed when the person leaves the lab.
- Do not smoke, drink, eat or chew gum. Do not apply cosmetics.
- Hands must be washed with bactericide soap in the sink after each contact.
- Use of rubber shoes and waterproof pinafore for wet work.
- Personal protective equipment must be kept in a specific place, and must be regularly cleaned and replaced when necessary.
- Workers must wash their hands and change their clothes before meals and at the end of the working day.

4) Medical programmes.
- Preventive medical examinations to check physical condition,

to detect who is susceptible to allergic agents and evaluate their natural resistance.

- Obligatory vaccination and passive immunisation after an accident and specific chemical prophylaxis.

5) Staff training.

- It is essential to inform personnel of risks at work and favour good working habits.
- It is required to elaborate a safety plan including the most usual incidents and the procedure to be followed when they happen.

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CHEMICAL RISK

CHEMICAL AGENTS IN A BIOTECHNOLOGY LABORATORY

André Picot

Introduction

Chemicals are not chemists' privilege and many biologists may need to use them continuously, in fundamental biology or in applied fields such as biotechnology.

A *chemical* may be a source of hazard, and *the related risks* have to be controlled. At *work*, we must try to improve this control in order to increase the quality and the safety of laboratory experiments.

Safety is associated with a *high quality of work*. It is important to know precisely the risks associated to the chemicals which are handled in the laboratory.

In *biology*, as in *chemistry* or *physics*, any work with chemicals must start by *risk assessment and prevention*, risks being physical-chemical, chemical or toxic in nature.

In the field of risks related to chemicals, the *'language'* is so important that a brief review of the most relevant terms will be done.

How to define a chemical?

In *chemistry*, the smallest individualised species is the *atom*. Atoms can be associated to form more complex structures: *molecules*.

The Biological environment consists mainly of water (75 % for humans); therefore *atoms* act as negatively or positively *charged ions* which correspond to a gain or a loss of one or several electrons on their peripheral layer.

As represented in diagram 1, *cations* are positively charged ions

and *anions* are negatively charged ions.

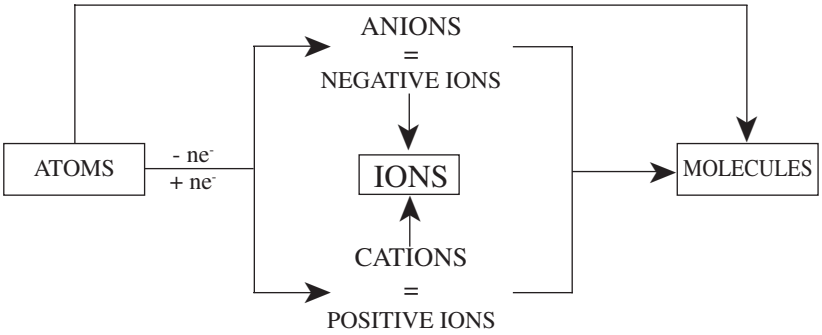


Diagram 1. From atoms and ions to molecules

Since Lavoisier (1743-1794), one of the founders of chemistry, we distinguish *mineral compounds* (from the inert mineral world) and *organic compounds* (discovered in living organisms). Organic compounds are based on *carbon*; and the *carbon atom* can be *linked* to another *carbon* (C-C) or to *hydrogen* (C-H), such as *hydrocarbons*, the simplest family of organic compounds.

Chemicals (*atoms, ions or molecules*) can show a more or less important *chemical reactivity* that is essential and must be taken into account.

Concentrated nitric acid is highly nitrating (able to add one or several nitrogen groups to a molecule) and strongly *oxidising* (able to transform a molecule by oxidation). For example, melting a very reactive mineral product like concentrated nitric acid with an *easily oxidisable organic product* like *alcohol* (methanol, ethanol, glycerol) is hazardous and leads to explosions followed by fire!

We must especially take care of *chemical storage*: we must avoid putting very reactive *incompatible products* together.

Currently, more than 21 million chemicals are listed in the Chemical Abstract (CAS on line, January 2001) amongst which 100.000 are

currently used and under order to be *labelled* by the European Union.

Obviously, most of the chemicals used in biotechnology are already *labelled*. Nevertheless, we must be very careful in the case of *reagents*. They can be more or less sophisticated, their real behaviour is not known and their impact on human health has not yet been studied.

The chemical, properties and risks

For a *laboratory experiment, safety* depends on *the use of appropriate equipment*, on *the nature of the chemicals* and on their handing *conditions*.

In order to evaluate risks, several parameters must be taken into account: *physical-chemical* properties, *reactivity, damage to health* (human or veterinary toxicity), or to the *environment* (ecotoxicity) (diagram 2).

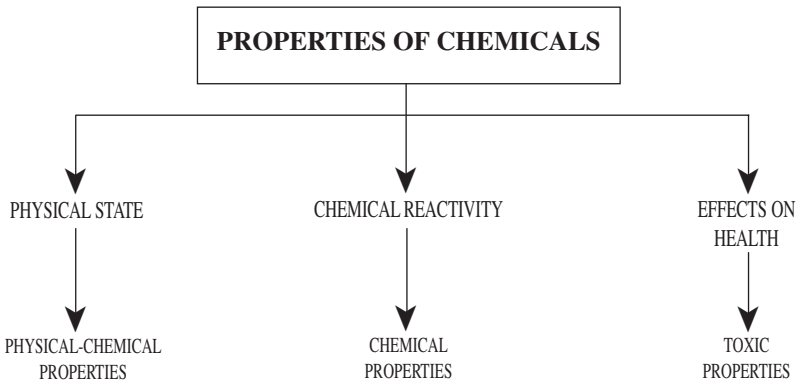


Diagram 2. Main properties of chemicals

A. Phisical-chemical properties and associated risks

Physical-chemical properties depend on the *physical form* of the product: *gaseous* (or vapour phase), *liquid or solid*.

Particular forms such as *aerosols* (solids or liquids greatly divided in suspension in the surrounding air) are very important considerations as they enter the organism via the respiratory tract extremely quickly. Spraying reagents may present considerable risks.

Concentrated sulphuric acid is a powerful broncho-pulmonary carcinogen for humans if sprayed.

Physical-chemical risks depend on the one hand on *physical-chemical properties* such as *instability, flammability, volatility* and on the other hand on their *chemical reactivity* or their ability to react by themselves or in mixtures.

Explosions, fire and some *toxic risks* (related *directly to toxic chemicals* sufficiently reactive to allow their interaction with biological constitutive elements) are dependent on physical-chemical properties as shown in diagram 3.

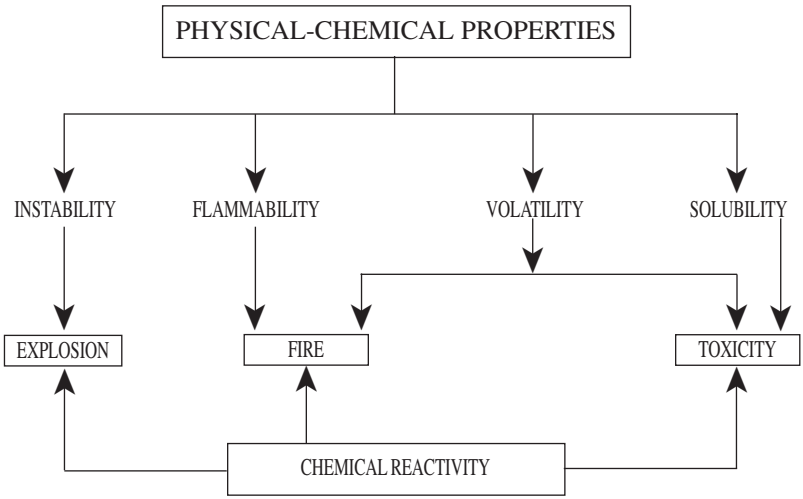


Diagram 3. Main risks associated to chemicals

1. Risks related to chemical instability

The *instability* of a chemical or a mixture of chemicals can lead to two kinds of accident:

- *explosions*
- *fire*

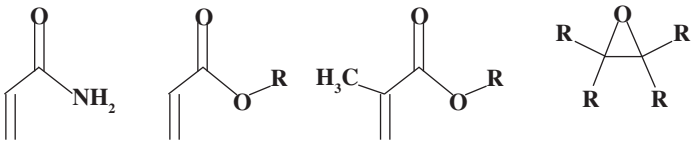
Explosions and fire are major risks, often very destructive for substructures and for the employees involved in these accidents.

Thus *explosion risks* depend essentially on three parameters:

- *stability* of compounds
- their *reactivity*
- the importance of their *explosivity domain* which is situated between the *Lower Explosivity Limit* (LEL) and the *Upper Explosivity Limit* (UEL). LEL and UEL are given in tables.

In *chemistry*, there are numerous *sources of explosion*. In a *biology laboratory*, they are limited to the *careless use of unstable products or mixtures* due to dangerous operations like *brutal polymerisation of monomers*, which are used for making inclusions, gels, etc.

Thus some chemicals can self react: as is the case for some *organic monomers* whose polymerisation may be brutal. Therefore, it is better *not to work on very important units* with chemicals like *acrylamide* (used for making polyacrylamide gels), *acrylates* (or *methacrylates*) and *epoxides*, which are used for preparing corresponding resins, etc.



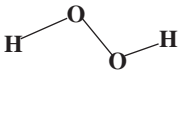
Acrylamide Acrylates Methacrylates Epoxides

Some monomers such as *acrylamide* or *styrene* polymerise with a violent reaction when they are heated. But usually, polymerisation is initiated by trace impurities (acids, bases, peroxides, metals).

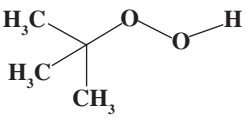
Monomers must be *stored* in small quantities after stabilisation by an *antioxidant* (phenols, amines).

Among especially unstable or explosive compound families, there are:

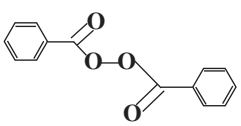
- *hydrogen peroxide* derivatives such as *hydroperoxides* (terbutyl hydroperoxide), *peroxides* (benzyl peroxide), *peracids* (peracetic acid), *persalts* (ammonium persulphate).



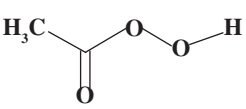
Hydrogen peroxide



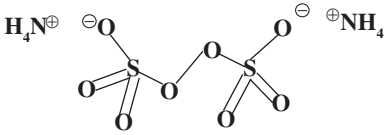
Terbutyl-hydroperoxide



Benzyl peroxide



Peracetic acid

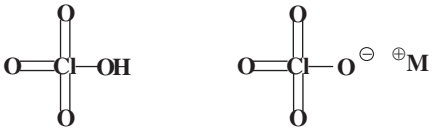


Ammonium persulphate

We must beware of the formation of *peroxide compounds by self-oxidation* of some *monomers in the air* (acrylates, methacrylates, styrene) or of some *solvents* such as *etheroxide* (diethylic etheroxide, 1,4-dioxane) because these peroxidic derivatives are highly thermally unstable.

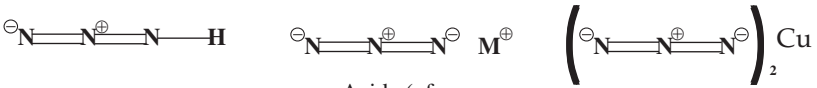
Hydroperoxides can be detected in these products by a lightly acidified potassium iodide aqueous solution (CH_3 COOH) or with the use of a paper strip impregnated with iodide starch.

- *Perchloric acid derivatives* ($HClO_4$) such as *perchlorates* (ClO_4^-): most of which are unstable and can explode by quick heating or by just scraping with a metallic spatula.



Perchloric acid — Perchlorate (of a monovalent metal)

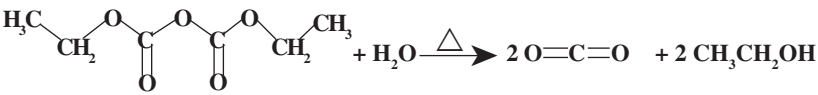
- *Azothydric acid derivatives* (HN_3) like many *azides* (N_3^-) may be very unstable. Thus aqueous solutions of *sodium azide* (NaN_3) used as a bactericide must not be stored in metallic containers (especially avoid copper), because *explosive azides* such as *copper azide* ($(N_3)_2Cu$) can be formed.



Azothydric acid — Azide (of a monovalent metal) — Copper azide

Sometimes biologists use unstable chemicals. They do not know the risks because the information is often difficult to find. *Reading of the label* and the *Safety Data Sheet* (SDS) is totally necessary.

For example, if *diethyl pyrocarbonate* (DEPC) is stored in a fridge with no air-conditioning, a slow increase in temperature will induce decomposition by hydrolysis with a brutal escape of carbon dioxide.



Diethyl pyrocarbonate — Carbon dioxide — Ethanol

Diagram 4. Diethyl pyrocarbonate hydrolysis

As a recommendation, a *bibliographic search* must be done before using: an *unstable chemical or* a chemical with *unknown stability*, or before *preparing an unstable mixture*. Nevertheless, the search is not always easy to do because of the data dispersion.

2. Risks related to chemical flammability

Flammability of a chemical or a mixture is an important characteristic. The corresponding hazard is *ignition and propagation of fire.*

Three elements are needed:

- *fuel* or combustible
- an *oxidizer* which is generally *dioxygen* (21% in the air)
- an *ignition source* to initiate the *combustion reaction*. It can be a *spark,* a *flame*, or *excessive heating*, etc.

Several *physical-chemical parameters* must be taken into account to evaluate flammability risks. Three of them are essential:

- *flammability limits*
- the *flash point*
- the *auto-ignition temperature*

Moreover, to evaluate the flammability risk and the toxic risk of a chemical, the *volatility* and the *maximum vapour pressure* have to be considered. These two parameters give information on the *quantity of vapour emitted* and the *rate of evaporation.*

Flammable chemicals can be *gaseous, volatile liquid vapours or solids, solid chemicals* such as *divided metals* (zinc, magnesium, nickel).

a) Vapour pressure

The *vapour pressure* of a liquid is an important parameter in safety. It corresponds to the *pressure* exerted on a *liquid* by its *vapour*. At this pressure, a *dynamic equilibrium* between the *liquid*

and the *gaseous phase* is obtained.

From the vapour pressure into the air at the *equilibrium*, the operator can deduce if there is a risk of ignition or intoxication.

For ignition risk, the concentrations (in volume) are indicated in percentages and for toxic risk, the concentrations are in ppm or parts per million.

$$1\ \% = 10\ 000\ \text{ppm}$$

b) Evaporation rate

The *evaporation rate* can be quantified by comparing the *evaporation speed* of liquid compounds with that of *diethylic etheroxide* (ordinary ether) which is considered the most volatile solvent (v = l).

$$\text{Volatility} = \frac{\text{chemical evaporation speed}}{\text{diethylic ether-oxide evaporation speed}}$$

c) Flammability limits

To have ignition and fire propagation of a *gas* or *vapours* (from a volatile liquid), the *volatile chemical* (fuel) has to be mixed with *air* (oxidizer) in a special percentage.

The concentration must be between two limits:

- the *Upper Flammability Limit* (UFL)
- the *Lower Flammability Limit* (LFL)

Values of *flammability limits* (FL) and *explosivity limits* (EL) are quantified in *percentages of gas or vapours in the air* (diagram 5).

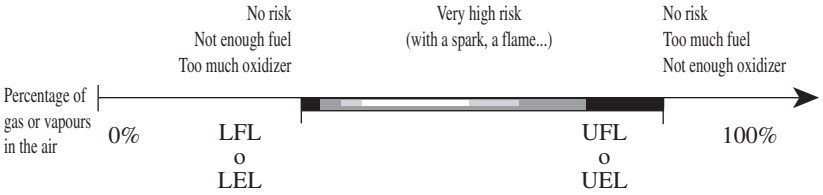


Diagram 5. Flammability or explosivity domains

Explosivity limits (LEL and UEL) of *gaseous mixtures* are not very different from *flammability limits* (LFL and UFL).

d) Flash point

The *flash point* is an *experimental parameter*. This is the *temperature* at which the vapour emission is sufficient to reach a composition corresponding to the *lower flammability limit (LFL).*

At a concentration corresponding to the LFL, a spark can provoke an explosion.

The *temperature measured* in an *open cupola is 5 to 10 °C* higher than the temperature obtained with a *closed cupola*.

The *flash point* is the *essential parameter* to determine the *fire risk*.

In the risky domain (diagram 5), a mechanical or electrical *spark* or *static electricity* is especially hazardous.

The lower the flash point, the more flammable the liquid, and the more hazardous it is.

FLASH POINT	FLAMMABILITY CHARACTERISTICS
Less than 0 °C	Extremely flammable
Between 0 °C and 25 °C	Easily flammable
Between 25 °C and 55 °C	Flammable

For example *isopentane* (solvent used in biology – liquid for cryoscopy) has a flash point of - 49°C. That makes it an *extremely flammable chemical,* more hazardous than *diethylic etheroxide* (ethyl ether) which has a flash point of - 44°C.

e) Auto-ignition temperature

For a *gas/air percentage* in the *flammability limit, ignition* can be done *with a flame* or *a spark* and also through an *increase in temperature*.

A chemical can become flammable without any ignition source due to the auto-ignition temperature.

For example, *carbon disulfide* (CS_2), formerly used for extracting

lipids, becomes spontaneously flammable when heated at a temperature higher than 90°C whereas diethylic ether becomes spontaneously flammable above 160°C.

S = C = S
Carbon disulfide

f) Some recommendations for handling and storing chemicals

In a laboratory where *flammable chemicals* are handled, *fire risks* must be continuously taken into account. Moreover, some simple principles must be applied:

- *Never handle flammable chemicals near a flame or a hot point.* Thus, using a badly protected *hair dryer* is particularly hazardous. Very flammable chemicals must be used in small quantites and whenever possible, under a well ventilated chemical hood.
- *Do not store containers of flammable solvents* (flasks) *on racks or shelves or on the top of a workstation*. They must be placed in a ventilated cupboard or cabinet which will be conveniently located at a distance from the emergency exits.
- *Do not store very large quantities of flammable chemicals in the laboratory*. The flammable chemicals stock should not exceed *1 to 2 days of consumption* to reduce risks in case of fire.
- *Do not store volatile flammable liquids in containers in a refrigerator.* All the laboratory refrigerators must have external thermostats, and no internal lights (spark free refrigerators).

In case of *fire*, appropriate extinguishers have to be used (e.g. carbon dioxide). It is necessary to know where *fire extinguishers* are located and what kind of extinguisher should be used.

It is important to *organise fire exercises* to particularly master the use of fire extinguishers.

B. Chemical properties and associated risks

Chemical reactions *which are sources of accidents are called* hazardous reactions**,** *they are due to the reactivity of the* chemical *involved* (violent polymerisation of monomers**,** incompatible chemicals *mixed together).*

Some chemicals will react violently with water *or* dioxygen *that are omnipresent in the environment. Other chemicals will interact in an uncontrollable and often violent way.*

Diagram 6 summarises possibilities of incompatible reactions between two or several chemical compounds.

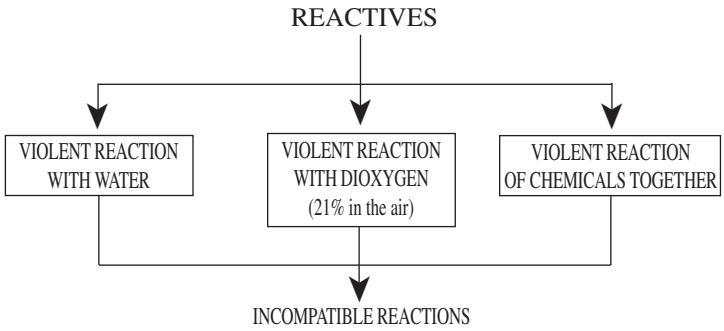


Diagram 6. Main kinds of incompatible reactions

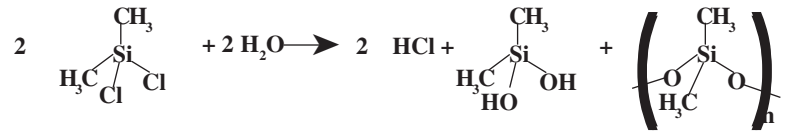
Putting together incompatible chemicals must be avoided in the laboratories. If this has to be done, *small quantities* should be used and safety procedures must be reinforced.

A *chemical storage* has to be safe and well organised taking into account the chemical compatibility of the various products and insuring that this the space will be conveniently divided.

1. Reactions with water

Some *reactive chemicals* can violently react with *water* thus leading to *hydrolysis*. *Hydrolysis* gives off little or a lot of *heat* and sometimes a *flammable gas* such as dihydrogen is produced. This *dihydrogen* (H_2), can burn in some cases. Hydrolysis of some reactive chemicals can release *corrosive chemicals* named *hydracids* (hydrochloric acid is an example).

Dimethyldichlorosilane, is used as an anti-adhesive treatment for laboratory glassware (haemolysis tubes) or as a waterproofing agent. With water there is hydrolysis, hydrochloric acid (very corrosive) and some organic oxygenated derivatives of silicium (dimethylsilanol) and polymers (methyl or dimethylsiloxane) are released (diagram 7).



Dimethyldichlorosilane — Dimethylsilanol — Polymer Dimethylsiloxane

Diagram 7. Dimethyldichlorosilane hydrolysis

Some *unstable compounds* can give off *very toxic chemicals in the presence of water* (i.e. some *alkylating agents* used in molecular biology can release some *very reactive entities*).

In case of penetration in the organism, the water from the biological environment (about 75 % in the human organism) will hydrolyse these molecules, this involves the formation of some *intermediate reactive agents.*

At cellular scale, these *reagents* can bind with *proteins* and *nucleic acids* such as *DNA*. In the case of DNA binding, *mutations***,** sometimes irreversible, can initiate a *tumour growth process.*

Thus with *dimethyl sulphate or DMS* (used to identify interactive sequences as DNA-proteins in the Mobility Shift Assay technique), there are two competitive reactions: on the one hand, a methylation reaction which can affect the DNA; on the other hand a hydrolysis reaction (diagram 8).

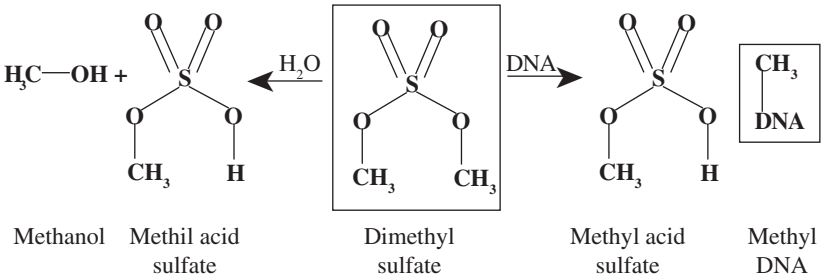


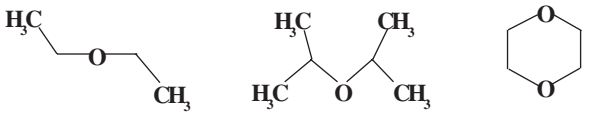
Diagram 8. Reaction of dimethyl sulphate with DNA or water

2. Reactions with dioxygen

There are two kinds of reactions with *dioxygen* (O_2): *brutal* reactions (they can lead to *explosions and fire)* or *slow* reactions as it is for *auto-oxidation reactions*.

Explosive reactions are quite rare in biology laboratories. Usually, they concern *perchloric acid* derivatives ($HClO_4$, ClO_4^-), *azothydric acid* derivatives (HN_3, N_3) and *hydrogen peroxide* derivatives (H_2O_2, $^-O\text{-}O^-$, R-O-OH, R-O-O-R) as explained in chapter A. 1.

However, *auto-oxidation reactions* which involve a radical type of mechanism (only one electron is transferred at a time) are quite frequent and can be source of explosion. Thus *etheroxide solvents* such as *diethylic etheroxide* (ordinary ether), *diisopropylic ether* (iso ether), *1,4-dioxane* (cyclic etheroxide present in some scintillating liquids) can be hazardous: if they are stored in air and in the presence of light, they may peroxidise and form more or less complex peroxidic products (hydroperoxides, peroxides, polymers). These products are very unstable and can explode when solvent purification by distillation is performed (the explosion hazard takes place especially at the end of the distillation, when these unstable peroxidic derivatives are concentrated).



Diethylic ether-oxide Diisopropylic ether-oxide 1,4-Dioxane

Thus *diisopropylic etheroxide* is a very peroxidable solvent. It first auto-oxidises into a moderately stable dihydroperoxide. Then, it slowly transforms into cyclic peroxides of acetone (dimer and trimer) and into extremely unstable polymers. These polymers are responsible for the explosions observed during the handling of old batches of this etheroxide (diagram 9).

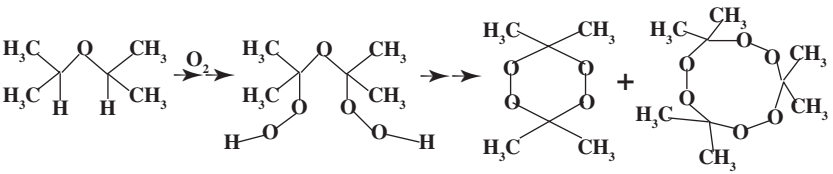
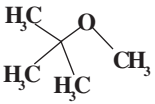


Diagram 9. Self-oxidation of diisopropylic etheroxide into unstable peroxidic compounds

Peroxidable etheroxides can advantageously be replaced by the *methylterbutyl-ether* (MBTE) which is much less sensitive to dioxygen.



Methylterbutylether (MTBE)

3. Hazardous reactions, mixture of incompatible chemicals

Chemicals are *incompatible* if they induce *uncontrollable* and *violent reactions* when they are put together.

As mentioned before, there are four kinds of reactions that lead to especially hazardous mixtures:

- chemicals reacting with water
- powerful oxidising agents

- powerful reducing agents
- monomers easily polymerisable

a) Violent reactions with water

This kind of exothermic reaction is particularly common in laboratories and is often the source of projections of corrosive chemicals on the skin or into the eyes.

Brutal addition of water to *concentrated strong acids* (H_2SO_4) or to *strong bases* (NaOH, KOH, NH_4OH) is hazardous and leads to violent projections.

In order to avoid such a problem slowly pouring the concentrated acid (or base) into chilled water is recommended, never the opposite. When pouring the acid (or base) into the water agitation of the mixture is normally done in order to insure homogenous mixing.

Dissolution in water of *mineral anhydrides* (phosphoric anhydride) or *organic anhydrides* (acetic anhydride) but also *halogenides* (acidic chlorides and acidic bromides, phosphorus and sulphur halogenide, cyanogen bromide) and *inorganic esters* (dimethyl sulphate, methanesulphonates) is often very hazardous (diagram 10).

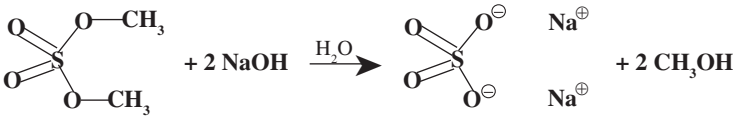


Diagram 10. Destruction of dimethyl sulphate by alkaline hydrolysis

In this kind of reaction, we must work with small quantities and chill the water beforehand. We must work under a well-ventilated chemical hood with all the individual and adapted equipment (safety glasses with lateral protection, appropriate protective gloves etc).

b) Violent oxidation reactions

Strong oxidising chemicals, especially when they are solid and divided, are very strong *oxidising agents* when they are in contact with *reducing agents (fuels*) such as hydrocarbons.

The same can be said for *hexavalent chromium compounds* (chromic acid, chromates, dichromates) or *potassium permanganate* ($KMnO_4$).

Hexavalent chromium compounds are powerful mutagenic and carcinogenic agents for humans. By comparison, potassium permanganate is moderately toxic and its use as a cleaning agent is spreading, despite its risks.

Thus adding *concentrated sulphuric acid* to a *concentrated potassium permanganate solution* (crystals would even be more dangerous) can initiate a violent explosion. Indeed, there is a transient formation of *permanganic acid* ($HMnO_4$) and of its anhydride, *dimanganese heptaoxide* (Mn_2O_7) which are especially unstable (diagram 11).

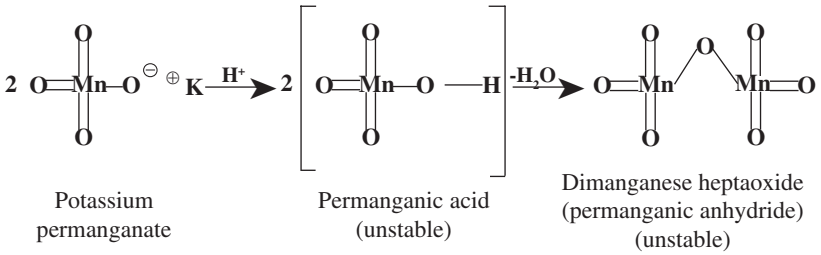


Diagram 11. Formation of permanganic anhydride with potassium permanganate in a strong acid environment

c) Violent reducing reactions

Strong reducing agents, less usual in biology laboratories than strong oxidising agents, can initiate an explosion in the presence of oxidising agents.

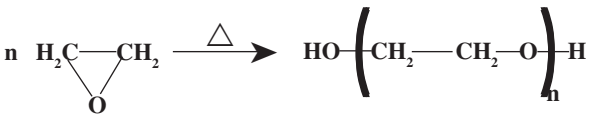
Thus *a strong oxidising agent like potassium permanganate must not directly destroy hydrazine* (H_2N-NH_2) *under hydrate form* (H_2N-$NH_2 \cdot H_2O$), *used for sequencing DNA*. This highly exothermic reaction is very violent; moreover some very toxic gaseous chemicals can be formed (nitrogen oxides).

d) Explosive polymerisation reactions

Many *monomers* (especially those that are not stabilised) can constitute *very unstable mixtures.*

Particularly when there are some impurities (acids, bases, other initiators of radicle reactions such as hydroperoxides, peroxides, transition metal salts) that can initiate *violent polymerisation* of *monomers* such as some *ethylenic compounds* (ethylene, styrene, acrylates, methacrylates, acrylamide) or their derivatives (epoxide).

Thus, *ethylene oxide*, used for disinfecting biological materials, can polymerise explosively, simply by heating or in the presence of trace mineral bases (diagram 12).



Ethylene oxide Polyethylene oxide

Diagram 12. Polymerisation of ethylene oxide into polyethylene oxide

Thereby, ethylene oxide must be handled at concentrations lower than 10 %, diluted with carbon dioxide ($C0_2$).

Several *incompatible chemicals* that may be present in a biotechnology laboratory are presented in table 1. This list is not exhaustive so consulting specialised books should be performed: their lists are more complete.

Table 1. Main groups and incompatible chemicals

MAIN CHEMICALS OR GROUPS	INCOMPATIBLE CHEMICALS	VIOLENT EXOTHERMIC REACTION	SPONTANEOUS IGNITION	TOXIC GAS RELEASED
Strong mineral acids (HCl, H_2SO_4, HNO_3)	Water Strong bases	++		
	Cyanides Azides Sulphurs Hypochlorites			++++
$HClO_4$	Combustible organic materials (wood, cotton, paper) Alcohols (methanol, ethanol, glycol, glycerol)	++	++	
Strong mineral bases (NaOH, KOH, NH_4OH)	Water Strong acids	++		
Powerful oxidising agents ($KMnO_4$, CrO_3, O_3)	Ethylenic hydrocarbons Strong reducing agents	++	(++)	
H_2O_2 (concentrated)	Combustible organic materials (fat) Alcohols Acetone	++	(+)	
NaClO	Acids Amines Formaldehyde	+		++

C. RISKS ASSOCIATED WITH TOXIC PROPERTIES

For a *living organism*, chemicals can be *constitutive elements*: they are *endogenic compounds*. In this category, we find small and medium size molecules that are inorganic such as water, urea, sodium chloride or organic such as glucose, fatty acids, vitamins.

Living organisms also have large molecules called macromolecules, such as *proteins, unsaturated phospholipids* and *nucleic acids* like *DNA*.

Other chemicals do not belong to living organism and do not act in their biological activity: *exogenic compounds*, also called *xenobiotic compounds* i.e. foreigners to life.

Basic chemistry chemicals (raw materials, monomers, solvents) but also more sophisticated chemicals for fine chemistry such as laboratories reactive agents, medicines, pesticides, etc. are xenobiotic.

Thus we will only consider *toxic xenobiotic agents*, especially solvents and chemicals used in biology.

1. How to define a poison?

A chemical is *toxic* when it is *harmful* for one or several *physiological functions* of *living beings.*

Toxicology is the science of toxic chemicals. It must be pluridisciplinary to be associated with notions of *chemistry* and *physics*.

Due to *toxicochemistry,* a new approach that we offer, we can study the interaction between a chemical, for example a *toxic xenobiotic agent*, with a *biological target*.

In favourable cases, we can even deduct the reason why this interaction releases a toxic effect on health.

Any xenobiotic chemical must be taken into account in its environment. Moreover, any impact on the different ecosystems must be integrated in the resulting global toxic effects.

This global consideration of the information given not only by chemistry but also by biology leads us to define *toxicochemistry* as a new *molecular approach* of *toxicology* summed up on diagram 13.

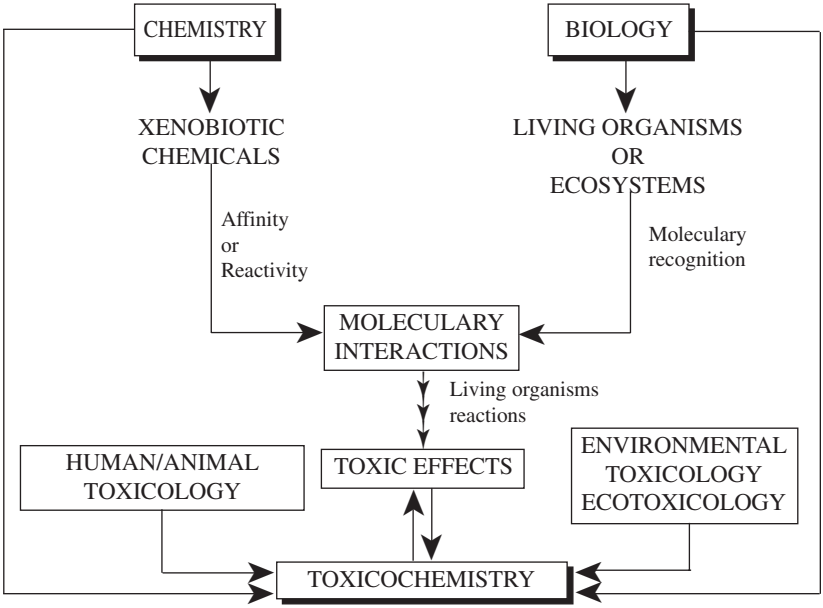


Diagram 13. Appearance of toxic effects by molecular interaction between a xenobiotic chemical and its biological target

Except for local effects (corrosive or irritating effects) at the site of contact of an aggressive chemical, the first condition indispensable to an action of a xenobiotic on a living organism is its possibility to enter the organism.

2. How does a xenobiotic enter the organism and how does it spread?

Volatility and *solubility* are two very important physical-chemical properties for evaluating the toxic risks of compounds (diagram 3).

a) Role of solubility

About *solubility*, we classically distinguish *insoluble* chemicals (in water and fats) from chemicals soluble in *water* (hydrosoluble chemicals) or in *fats* (liposoluble chemicals) (diagram 14).

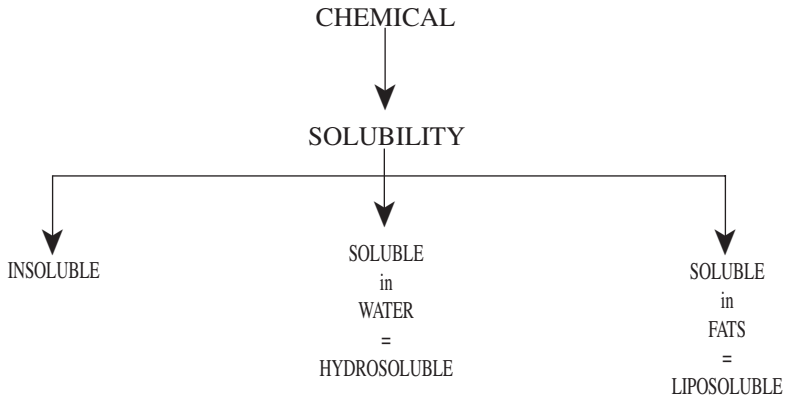


Diagram 14. Main kind of solubility for a chemical

Most xenobiotic agents divide their solubility between water (major constituent of biologic fluids) and lipids (important molecules of biomembranes) and this can be quantified by the *partition coefficient* between those two environments.

$$\text{Partition coefficient} = \frac{\%\ \text{soluble in water}}{\%\ \text{soluble in a fat}}$$

The partition coefficient can give information on the destiny of a xenobiotic agent in a living organism from its entry until its elimination.

b) Main ways of penetration of xenobiotic agents in the organism

Usually, a *xenobiotic agent* can enter the *human organism* by *three* main *ways*: *oral*, *pulmonary* and *through the skin*. More rarely, it enters by the *nasal* or *ocular* way.

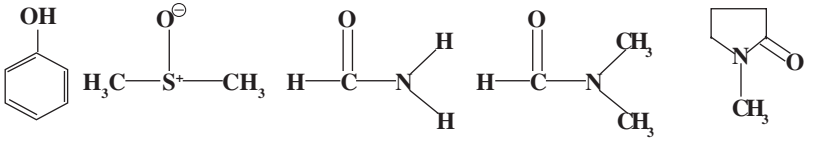
In a laboratory, the main way is via the *respiratory tract.* In fact a large number of chemicals can enter the organism under:

- *gas* or *vapour* form (released particularly by *volatile liquids* such

as organic solvents)
- *divided solids* form (dusts)
- *aerosols* (air + solid or liquid finely divided)

We must not neglect *penetration through the skin* (and mucous membranes) because some chemicals enter more easily this way than by inhalation. In this category of chemicals we find solvents such as:

- phenol
- dimethylsulphoxide (DMSO)
- formamide and dimethylformamide (DMF)
- N-Methylpyrrolidone (NMP)



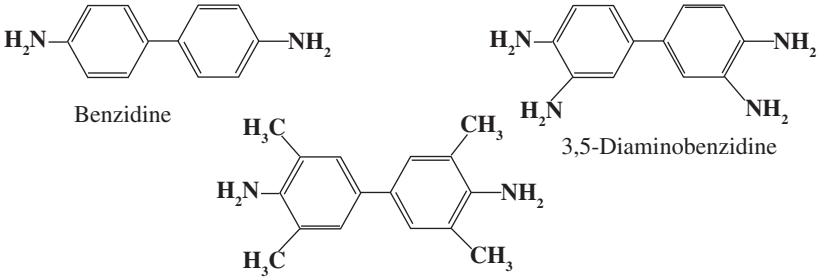
Phenol | Dimethylsulphoxide (DMSO) | Formamide | Dimethylformamide (DMF) | N-Methylpyrrolidone (NMP)

Some reagents used in the biology laboratory are particularly hazardous because they are easily absorbed through the skin and they are very toxic. This is case for *aromatic amines* of the *benzidine family*.

In the past *benzidine* was often used for revealing *peroxidasic activity* (hydrogen peroxide/peroxidase) and today it is used for *immunorevelation* in the Elisa Test. It is a *powerful carcinogenic molecule for the bladder* in humans; several cases of this kind of cancer have been described in relation with laboratory employees working with this reactive chemical.

In some cases, *benzidine* can be replaced by *3,3',5,5'-tetramethylbenzidine* (TMB) a molecule neither mutagenic nor carcinogenic. In other applications, the *3,5-diaminobenzidine* (DAB) (a carcinogenic agent in animal experiments) is an acceptable substitute for benzidine but it must be handled carefully.

Handling such chemicals which are easily absorbed through the skin



requires adequate protection and procedures: adapted disposable gloves, protective glasses, careful cleaning of hands, destruction of residues after use.

c) Destiny of a xenobiotic agent within the organism

Once the xenobiotic compound passes into the organism, it spreads usually through the *blood circulation.*

The chemical destiny depends on its solubility in water or in fats.

Generally, *hydrosoluble chemicals* are *rapidly eliminated* from the organism by the *kidneys* through urine before or after biotranformation.

Very liposoluble xenobiotic chemicals are generally stored in a lipid compartment (nervous system, liver, kidney, bone marrow, fat) from where they will be released after a variable period of time, probably depending on the xenobiotic agent.

Usually, *lipophilic xenobiotic chemicals* must be metabolised to be eliminated from the organism. For *organic lipophilic solvents*, the *metabolisation* will take place in the *liver*. They will be transformed into *water soluble metabolites*, excreted in *urine* through *kidneys*.

Some xenobiotic compounds with high molecular weight (above 300) are eliminated from the *liver* through the *bile*. Due to the *entero-hepatic cycle*, a proportion can be reabsorbed. This cycle can increase toxic effects as it is for nitro *aromatic products*: they are metabolised into aromatic amines in the liver, eliminated through the bile and reabsorbed by the intestine. When they are in liver, they result as hepatotoxic effects.

Diagram 15 sums up the different possibilities of elimination of

hydrosoluble or liposoluble xenobiotic agent from the organism.

Such a diagram is well with an *organic chemical* such as a *lipophilic*

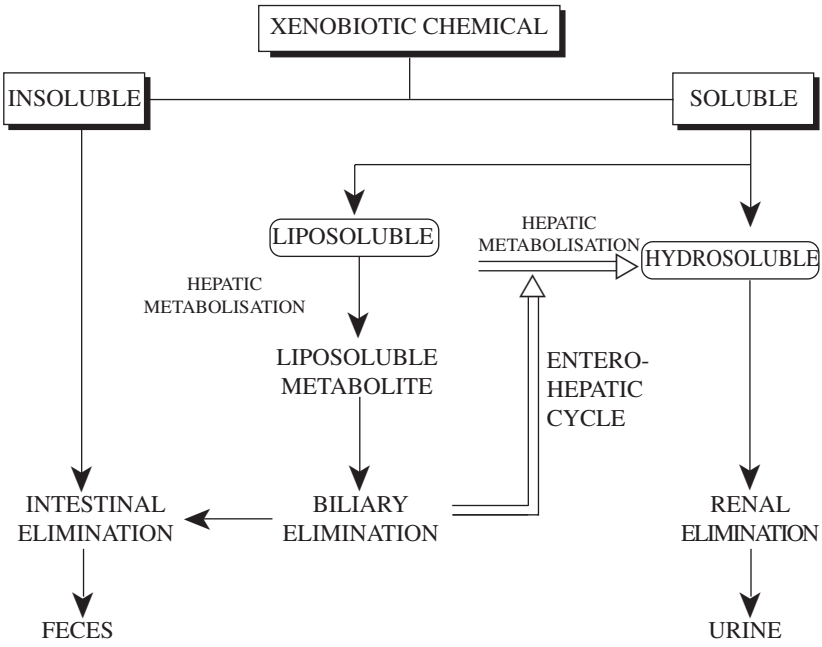


Diagram 15. Elimination of a xenobiotic compound out of the organism

solvent but also with an *inorganic chemical* such as *elementary mercury* which is quite common in laboratories. Mercury is found in measurement devices (thermometer, manometer). Under the *vapour* form, it can be easily absorbed by inhalation. As it is lightly *lipophilic* it can go through the *alveoli* membranes *in the lung*.

Mercury is able to cross the *hemato-encephalic barrier* and to go in the *grey substance of the brain*. There, it can be oxidised into water soluble *mercuric salt* and eliminated through the *kidneys*.

Mercuric ion can induce *inflammatory processes* in the *nervous system* (encephalitis, polynephritis) and also in the kidney to the *renal glomeruli* (glomerulonephritis).

In the *intestine, mercuric ions* may be methylated into *methylmercury cations* (CH_3-Hg^+) which allows their *intestinal absorption* thus making their transfer to nervous system easier (diagram 16).

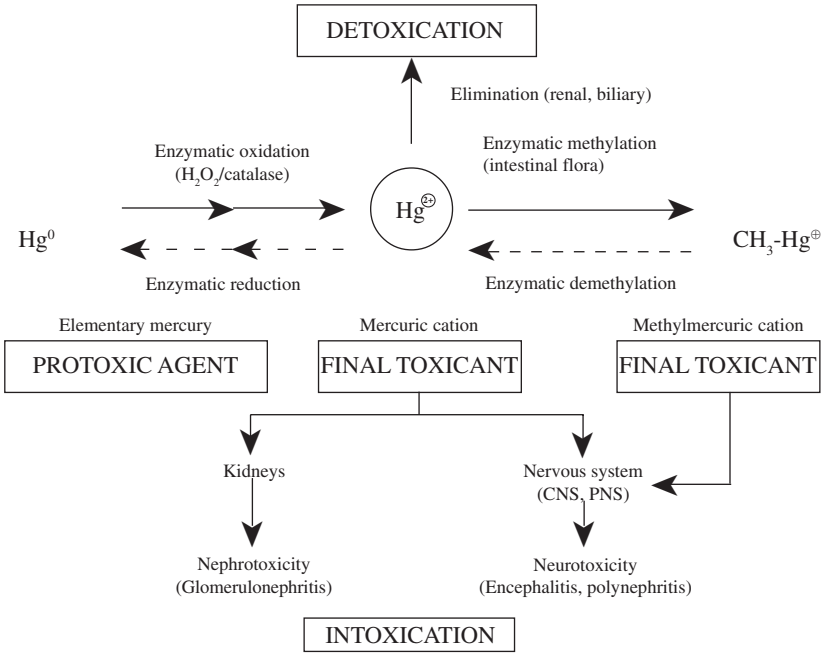


Diagram 16. Different mercuric chemical species, related targets and toxic effects

In biology laboratories, *mercury* can be under *elementary form* (thermometers), under *ion* form (mercuric salts), sometimes under *organometallic compound* form such as *dimethylmercury* (CH_3-Hg-CH_3). Dimethylmercury is a volatile molecule that can result in death even after a simple cutaneous penetration. Use of mercury and its compounds have to be limited as the hazards are very high.

3. What are the main forms of toxicity?

A *xenobiotic chemical* can result in *toxic effects* with *two types of interactions:*

- *direct action* of the chemical on its *target* (mainly dependent on the *chemical reactivity*)
- *indirect action* of an *intermediate* called *final or ultimate toxicant*.

The final toxicant is a derivative from the *first xenobiotic agent* (or *protoxic agent), hydrolysed* in the *biological environment* or from the *biotransformation process* (with *metabolisation enzymes).*

From *experimentation* it is possible to distinguish *immediate effects* that result in *acute effects* (sometimes lethal) from *delayed effects* that lead to *more or less long-term toxic effects.*

Diagram 17 sums up these different possibilities of action.

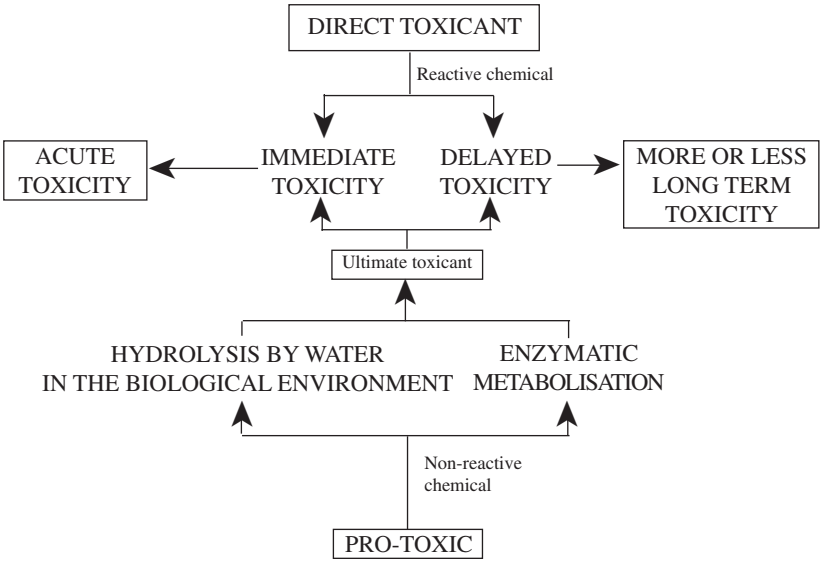


Diagram 17. Main forms of toxicity and toxic effects of a xenobiotic agent

a) Acute toxicity

Acute toxicity is the consequence of a *unique dose* (or several doses in 24 hours) which results in the death of 50 % of experimentation animals.

The value of *lethal dose 50* (LD_{50}) or of *lethal concentration 50* (LC_{50}) are very useful parameters for *informative labelling*. LC_{50} is related to exposure by inhalation during a period of time at various concentrations.

Associated pictograms (very toxic, toxic or harmful) must be on the *labels*.

We have to keep in mind that *acute toxicity* is strongly dependent on animal species sex, route of exposure and as a consequence simple extrapolation to humans is very risky.

Alternative techniques can allow the determination of *acute toxicity* on *animal cells* (cytotoxicity tests) avoiding excessive use of animals.

Acute intoxications are rare in laboratories and are usually due to *mixtures of incompatible chemicals that* release *very toxic gases*.

For example adding some *acid,* even if it is weak, to a *potassium cyanide* aqueous solution (KCN) releases *cyanhydric acid* (also named hydrogen cyanide). This gas, which smells like bitter almonds, is very toxic (diagram 18).

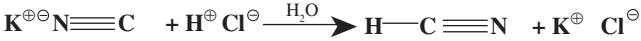


Diagram 18. Formation of hydrogen cyanide by action of hydrochloric acid on potassium cyanide

Sodium azide (NaN_3) with acid forms the following gaseous molecule (HN_3) which is as dangerous as hydrogen cyanide.

Adding some *acid,* even one as weak as *citric acid* (from lemons) to a *sodium hypochlorite* solution releases *dichloride* (chlorine, Cl_2). This type of problem can occur when cleaning toilets (at home or elsewhere) if you use an acidic cleaning agent and bleach (sodium hypochlorite) at the same time. Chlorine is a *very corrosive* gas. It can rapidly become fatal in an enclosed space (toilets) (diagram 19).

In *biology laboratories*, handling some acute toxicants such as *tox-*

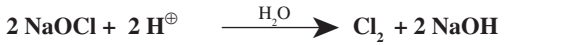


Diagram 19. Formation of chlorine by sodium hypochlorite in acid environment

ins (botulic toxin, mycotoxins), *alkaloids* (strychnine, brucine), *pharmacologically active molecules* (digitoxin) must be performed very carefully.

These toxins, classed as *poisons* and labelled with a *death head pictogram***,** must be stored in a *locked cupboard.*

b) Medium-term toxicity

In animal experimentation, *medium term toxicity* called *subacute toxicity* (in a period shorter than three months) can give information about the target organ which is preferentially touched.

Carbon tetrachloride (CCl_4) is a *solvent* formerly used for the extraction of lipids. Now it is forbidden because of its *destructive action* on the *ozone layer and its effect on human health*. After inhalation, it reaches the *liver* very quickly resulting in hepatitis followed by cirrhosis. *Chloroform* ($CHCl_3$) is often used in biology for the extraction of DNA (mixture phenol/ $CHCl_3$). Depending on the animal species, chloroform has two target organs which are *liver* and *kidneys*. As a rule, be careful with all *chlorinated solvents* (even *methylene chloride* also named dichloromethane) because these solvents are all *irritating and neurotoxic chemicals*. Moreover, they are often *hepatotoxic and nephrotoxic and harmful for the environment*.

c) Long-term toxicity

Long-term toxicity is evaluated after *repetitive exposure* to a *low chemical concentration* during the *whole life of the animal* (2 years for rodents such as mice or rats).

Long term effects generally depend on the total dose absorbed and *threshold doses* can be fixed. The threshold dose is the highest dose permitted which avoid the occurrence of irreversible health troubles.

Occupational exposure limits have been determined, they are published and have to be respected at work.

We can notice that generally, there is *no relation between acute toxicity and long term* toxicity.

Most of the *toxic effects* are due to an *interaction* with *cellular constitutive elements* especially with *essential macromolecules: proteins* (enzymes, structure proteins, transport proteins) and *nucleic acids* (RNA

and especially DNA). *Unsaturated phospholipids*, which are the major constitutive elements of *biomembranes* are also targets sensitive to aggressions (especially oxidising aggressions) from toxic xenobiotic agents.

Harmful effects on an *organ* (liver, kidney, lung, bone marrow, skin) or on a *system* (nervous system, digestive system, reproductive system) can lead to an *organotoxicity* often quite specific.

Immunotoxic effects can appear after an aggression of the *immune system*. These aggressions include: *hypersensitivity reactions* (allergies), *immunodepressive* effects or immunosuppressive and *auto-immune processes*.

Early detection of these immunotoxic reactions by specific tests is hard; in the work environment *allergies* are usually detected by occupational medicine.

Genetic heritage modification, essentially *DNA*, by *genotoxic xenobiotic agents* leads to *mutations* that can result in developing an *uncontrolled proliferation of cells* (cancer) if they are not repaired (or badly repaired). If *reproductive cells* are affected, *malformations in descendants* can appear. Those *teratogenic effects* are very important to track. Unfortunately, very few chemicals are really considered responsible for such effects on humans.

Diagram 17 summarises the main *toxic effects* that *direct poisons* or *protoxic agents* can give after *enzymatic metabolisation.*

Some *chemicals* used in *biology* laboratories and *biotechnology* can *affect the health*: from *acute* to *long term effects***.**

There are many examples but only one will be described here.

Formaldehyde is a very reactive *aldehyde (carbonylated compounds* family with an *aldehyde function*). It is commonly used in laboratories as a *fixative agent in histology* (*in situ* hybridisation of genes) or as a *disinfecting agent*.

Aldehyde Formaldehyde

It can be found as an *aqueous solution* (in France) with concentrations varying from 30 to 56 % *formaldehyde* in weight. It contains variable quantities of *methanol* (CH_3OH) that stabilises the solution. It can also be under solid form like para-formaldehyde ($HO(CH_2O)_nH$). Para-formaldehyde is a linear polymer used for preparing formaldehyde free from methanol. Under *vapour* form, *formaldehyde* is an *ocular* and *respiratory irritating agent* that can become very *corrosive* in high concentrations.

According to individuals, irritating concentrations start in the range of a few ppm (1 to 5 ppm) and becomes very aggressive above 10-20 ppm.

Concentrated aqueous solutions are *caustic* for *skin*. Their *ingestion* can be very severe with a *polyvisceral attack* (gastrointestinal, hepatic, renal, and also pulmonary) and can lead to death after *coma*.

Classic irritations of *nasal* and *ocular mucous membrane* are commonly observed**,** but more severe long term damage such as *chronic respiratory pathologies* (bronchitis) have been reported. Formaldehyde is recognised as a *powerful allergen* that can result in *cutaneous hypersensitivities* (eczema, urticaria) and *respiratory* hypersensitivities (rhinitis, asthma). A dramatic anaphylactic shock sometimes follows these hypersensitivities.

Effects on reproduction, especially complicated pregnancies, have been mentioned.

Experimentally, this is a *genotoxic* compound, *mutagenic in several systems and carcinogenic in rats* (cancer of nasal fossa). In *human epidemiology*, survey results are variable. *Tumour localisations* are very different (haematopoietic organs, brain, colon, larynx, prostate). Formaldehyde is classified as a *probable carcinogen for humans* (group 2A of IARC, Lyon, 1995).

A *hazardous use* has been described in hospital: addition of hydrochloric acid to an *aqueous solution of formaldehyde* in order to increase its *disinfectant power leads to bis- chloromethylether*, a volatile *carcinogen for humans* (classified in group 1 by IARC).

Formaldehyde examples sums up the multiplicity of biological targets for a unique toxic xenobiotic agent. Thereby, *prevention* to apply for such a volatile chemical must include not only *collective measures*

(ventilation etc.) but also *individual protection* (masks, protective gloves and glasses etc.).

Prevention applied to chemical risk

One of the essential objectives of *prevention of chemical risk* is avoiding any interaction of one *chemical* with another (water, dioxygen, incompatible substances) or with vital constitutive elements of the organism. As a rule, the chemical must be considered a *potentially dangerous substance.*

We must *establish collective measures* and *respect individual measures* of *general safety* in order to *control the risk.*

First of all *collective protection measures* must be taken, they will have priority on individual protection measures.

A *first preventive step* is to *identify hazards and evaluate risks*, reading *safety data* about *handled chemicals* (*pure* or under *preparation* form).

General rules of chemical risk prevention are written in *directives 80/1107/EEC* and *88/632/EEC*.

The *main principles of prevention* are applied to *chemical risk* and they are described in the chapter on handling genotoxic agents by M. Castegnaro in this document.

A. Collective measures

In *biotechnology, general rules dedicated to laboratory organisation* must be applied.

1. General rules of functioning

Elaboration of functioning rules must be precisely and rigorously studied. Different partners must be associated: *responsible scientists, safety and occupational medical services, hygiene and safety committees and laboratory employees.*

Any important transformation of a laboratory must be fully studied taking into account a reinforced safety.

Laboratory managers are responsible for the safety of their employees. They must insure that the employees *respect safety instructions*. The necessary *protection material* must be *available* for employees. Every employee of a laboratory assumes a *personal responsibility* for his own safety and for the safety of the other people present in the laboratory.

2. Safety instructions

Safety instructions (general or special, evacuation plan, emergency phone numbers) *must be displayed* in the laboratory in a visible and accessible place. These instructions must be clear and short.

On the *door of the laboratory, pictogram(s)* corresponding to *specific danger symbols* must be visible. For example, biological danger if the laboratory handles genetically modified organisms.

Any accident or incident must be reported, even if it is considered minor. If possible, it must be written in a special document dedicated to accidents. This document must be available for consultation by every employee.

3. Aeration devices

A laboratory where chemical manipulation takes place must be equipped with *aeration devices* in order to guarantee the *air quality* of the laboratory environment.

Ventilation devices are essential for safety in the laboratory. They include: *ventilated chemical hoods*, laminar flow hoods, *glove boxes,* etc.

Ventilation remains the best way of protection against *pollutants* whatever their origins.

In laboratories, *ventilation installations* must not result in discomfort to the workers, especially noises and vibrations.

Whatever the kind of ventilation, the material must be maintained and controlled at least once a year.

Devices with *filters* such as chemical hoods (ETRAF) must have filters compatible with the pollutants to be trapped.

The control of this kind of device must also be done at least once a year.

4. Safety equipment location

Location of safety equipment of the laboratory must be *known*:

- *fire extinguishers* (that must be operational and checked at regular intervals)
- *gas masks* (with adapted cartridges)
- *fireproofed blankets* (free from asbestos)
- *safety showers* and *eye wash systems* (clean and operational)

Devices *to stop fluid* (water, gas) and *electricity delivery* must be *accessible* and *easily localisable.*

Sources of hazards associated to fluids (gas equipments, Bunsen burner) and to *electricity* (imperfect devices, plugs not in accordance with rules) must be limited as much as possible.

It is important to participate at *fire training sessions* and to *learn how to handle fire extinguishers* and *gas masks.*

5. Good laboratory practises

- *A laboratory must be clean and tidy*; installations, devices and instruments must be maintained in perfect functioning and a clean state.
- *Spaces reserved for manipulations, circulation ways* and *emergency exits* must remain *clear*.
- *Fireproof doors* must remain closed.
- *Floors* must be *clear* from any obstruction or storage.

- *Work spaces* must be maintained in *perfect clean state*.
- *Workstations* must be constantly free from devices and containers that are not necessary for the current manipulation. At the end of the manipulation, everything must be cleaned and each operator has to take care personally of the resulting contaminated or hazardous material or waste.
- *Containers* must be arranged in *storage cupboards* or on *shelves* reserved for this use.
- *Ventilated or exhaust chemical hoods* must not be used to store chemicals containers or small laboratory equipment.
- *Chipped or broken glassware* must be immediately replaced.
- *Needles* and *sharp items* (scalpels, cutters) must be put into *containers* reserved for this use.
- *Needles* must never been recapped.

6. Chemical storage

Chemical storage in a laboratory must be submitted to *rigorous instructions* especially *limiting the stored quantity*:

- of *flammable liquids* (avoid glass containers upper than 1 L of very flammable solvent), *corrosive chemicals* (strong bases and acids, powerful oxidisers and acid halogenides)
- of *corrosive chemicals* (strong bases and acids, powerful oxidisers and acid halides)
- of *chemicals that react vigorously with water* (alkaline metals, hydrides, organometallic compounds)
- of *chemicals that react vigorously with dioxygen* (white phosphorus, phosphine, boranes)

Incompatible chemicals must be separated and when it is possible, *chemicals must be regrouped in large* families:

- strong mineral acids
- strong mineral bases

- powerful oxidising agents
- powerful reducing agents
- chemicals sensitive to water
- chemicals sensitive to dioxygen

For *chemical storage*, the following must be used:

- *trays* made of a *resistant plastic*
- *steel* shelves covered with a protection against chemicals

All *containers* must have *statutory labelling* in good condition.

All stored chemicals must be listed. The state of stocks must be verified regularly.

Do not store large quantities of *flammable liquids* in the laboratory: the stored quantity must be lower than the consumption corresponding to one or two days of work.

Flammable solvents must be stored in a specially equipped *solvent cupboard* (non-combustible materials, retention trays, permanent ventilation, spark free electric equipment, automatic extinction mechanism).

Large research institutes must have a *central solvent storage system* made and installed according to the legal standards.

7. Hygiene measures

Elementary hygiene measures must be respected:

- in the laboratory, *wear an overall that is* not made of synthetic material
- *wash your hands* after each manipulation
- *tie back hair* if it is long or loose
- *do not store food* and *drinks* in *refrigerators* reserved for chemical storage

It is prohibited to eat, drink (especially using laboratory glassware) or *smoke* at work. If possible, *a special room should be set apart for breaks* and *a smoking space* must be *reserved.*

Daily cleaning of the floors avoids the accumulation of dust charged of hazardous chemicals.

B. Individual measures

To *work with maximum safety in a laboratory*, different *measures for individual protection* must be respected.

1. Eye protection

Projections and explosions are usually unexpected. Among laboratory accidents, the ones that touch eyes are the most frequent and the most serious. In a laboratory, we must wear *protection glasses.*

In a case of manipulation which implies an important risk, it is necessary to work under a *ventilated chemical hood* switched on and with a sliding front *screen of polycarbonate* for operator protection.

2. Hand protection

Corrosive chemicals (strong bases and acids, powerful oxidising agents), *compounds that easily enter trough skin* (nitrated derivatives, aromatic amines, aprotic dipolar solvents) must be handled with *gloves* corresponding to the kind of chemical used (gloves of cotton, latex, vinyl, fluorocarbonate).

The *choice of gloves depends on the kind of the chemical handled*. However, it is not sure that they insure total protection against cutaneous penetration. *Glasswork* often results in accidents (section of the tendon). You must be efficiently protected during these manipulations (gloves and rags).

3. Respiratory tract protection

Working with *toxic gases* (phosgene, chlorine, dihydrogen sulphur, carbon monoxide) must be done with a *self contained respiratory protection mask* if possible. Do not use gas masks with cartridges when the percentage of toxic vapours in the air is beyond 2 % you must know the location of the protection masks and you must know how to use them.

To *improve working conditions* and *prevent working diseases, exposure limit values* (American TLV-TWA, German MAC, French VME and VLE) have been set.

Nevertheless, it concerns particularly *pure chemicals.*

These conditions are quite far from the working reality in laboratories where chemical and other harmful effects (physical, radioactive, microbiological, psychological) are often associated.

4. General protection of body

You must wear a *clean cotton blouse* (not synthetic) and respect a strict corporeal hygiene (washing hands).

As a rule, *never work alone* especially during the *night* or on public holidays.

HANDLING GENOTOXIC, MUTAGENIC, CARCINOGENIC COMPOUNDS

Marcel Castegnaro

*Special precautions must be exercised when storing, handling, or transporting genotoxic, mutagenic and/or carcinogenic chemicals. They should be stored separately from other substances in locked cabinets which should be identified by the warning "**DANGER – CHEMICAL CARCINOGEN**". The rooms where these compounds are stored and/or handled and those in which the wastes generated by their handling are kept should be identified by the same warning "**DANGER – CHEMICAL CARCINOGEN**". In addition a specific logo for these compounds can be added.*

Aquiring and storage of bulk chemical carcinogens

In an institute or a building which is part of a large institute, all stocks of carcinogenic chemicals must be stored in the same place. Unnecessary dispersion of storage places will greatly increase the risk. A safety officer must be nominated in each institute to follow up the orders and recording of these compounds, their handling and the safe disposal of wastes and of useless compounds. The storage place must be as close as possible to the place where the primary solutions are prepared in order to limit the risk of spillage during transportation.

In order to avoid unnecessary duplication of the same compound in an institute, all compounds should be recorded upon arrival and an inventory must be maintained by the safety officer. Each compound must be identified by a number (in order to facilitate its retrieval) and recorded in the inventory on a special card which must contain at least the following information:

- the common name
- the commercial origin

- the date of delivery
- the quantity delivered
- the name of the person who ordered the compound
- the amount taken out from the stock, when and by whom
- the number given to the compound and its location in the storage cabinet

In addition, the card may contain the following information:

- the official name, the chemical formula, and the CAS number in order to facilitate its computerised recording
- the physicochemical, toxicological and ecotoxicological properties
- the storage conditions and the method of disposal

After receipt and recording, the compounds (under solid or liquid form) in their original containers should be stored in shock-resistant containers which must also be resistant to the contents. Stainless steel containers may be used which, in the event of accidents, present the advantage of being easily treatable for decontamination with chemical reagents.

Storage of the compounds should be performed in locked cabinets (ventilated cupboard with exit air filtered, refrigerators or freezers) located in suitably ventilated areas where access is limited to persons who have been trained to handle these compounds. The area must be classified as a **"CONTROLLED AREA"** and must bear the warning "**DANGER - CHEMICAL CARCINOGEN**" with the specific logo for carcinogenic chemicals.

The officer responsible for the key to the area and/or cabinet must only hand it to authorised persons and must ensure that the utilisation sheet is fully completed.

In the event that the institute is equipped with a code bar system for access to different zones, access to the storage and weighing areas or these compounds should also be equipped with such a system. This will facilitate the follow up of persons who have accessed the area. In all cases, a special sign, for potential users and persons outside the laboratory, should draw attention to the hazards involved.

Gaseous carcinogenic/mutagenic/genotoxic and, more generally toxic compounds, should only be used in laboratories equipped to handled toxic gases.

After receipt and recording, these compounds should be stored in their original condition in, adequately ventilated special rooms, equipped with an automatic air extraction system which allows trapping of gaseous substances and whose efficiency has been fully tested and confirmed. All electrical equipment (including refrigerators and freezers if they are used for storage of such compounds) must be earthed. The containers must be tightly close and periodically checked for tightness. They must be kept away from all heat sources and static electricity.

As for the solid and liquid carcinogens, the area should be classified as a **"CONTROLLED AREA".** The safety officer should only give the key to authorised persons and ensure that the utilisation sheet is fully completed. A special sign, for potential users and persons outside the laboratory, should draw attention to the hazards involved .

Preparation and storage of the mother solutions

Only the persons authorised by the safety officer can enter the area where the carcinogens are stored in their undiluted formed. They must be suitably protected (laboratory coats, gloves, masks). The suitability of the protective equipment will depend on the physical state of the compound handled as discussed below.

After taking the compounds out of the storage cupboard they should be transported to the working area where the solution will be prepared (chemical hood preferentially or laminar flow hood with charcoal filter). A sample should then be removed for preparation of the mother solution. If this area is not close to the storage place, the compounds must be transported in unbreakable and leak-proof containers. The flasks containing the compounds should be securely placed in the containers with an adsorbing agent (i.e. vermiculite or similar).

If the compounds are stored in refrigerators or freezers, they must be taken out in advance and allowed to reach room temperature before the flasks are opened. This will prevent moisture or condensation problems (for hygroscopic or wet compounds) which will result in weighing errors and decomposition problems for certain compounds unstable in the presence of humidity. In order to allow the compound to reach room temperature, the flask should be placed in a retention basin (Stainless steel basins can be used as the surface is easy to decontaminate, enamel may also be used but one must ensure that the enamel is intact) covered with "bench coat" type paper (water proof side on the basin and adsorbing side on the top where the flask will be placed). If the compound is photosensitive, the flask must be protected from light. During this period, the front hatch of the fume cupboard must be closed.

When sampling an aliquot, no attempt should be made to sample an exact weight. An approximate aliquot should be sampled and the volume of solvent adjusted accordingly to obtain the required concentration. Trying to adjust the weight of the aliquot by removing or adding the compound will increase the risk for the manipulator.

The aliquot should be placed in a flask in a volume sufficient for the first dilution. This flask must be equipped with a screw cap. The flask, including the screw cap, should be tared on a balance, it should then be opened and placed on the bench coat in the basin. Some laboratories propose placing the balance inside the hood. There are two majors disadvantages to this approach:

1) it is very difficult, if not impossible to weigh accurately under a working hood
2) in the event of an accident (e.g. spillage) the balance may become contaminated and it will be very difficult to decontaminate. It is therefore advisable to place the balance as close as possible to the hood (adjacent if possible) and not inside it

After sampling and transfer of the aliquot to the flask, it should be capped immediately. The weight of the aliquot should then be determined by double weighing. The amount of solvent necessary to obtain the concentration of the first dilution should then be calculated (safety

procedures must be adapted according to the type of compound as described below).

The flask should then be labelled. The label must contain the following information: the name of the compound, the nature of the solvent, the concentration, the date of preparation and name of the person who prepared the solution.

Once aliquoting has been performed, the flask of undiluted product should be recapped immediately and transferred to the storage cabinet using precautions similar to those taken when taking it out of the cabinet.

After preparation of the mother solutions, the duly labelled flasks should be secured in unbreakable and leakproof containers with an adsorbing agent (e.g. vermiculite or similar). They should then be transported to the laboratories where they are to be used and must be stored in these containers, in refrigerators protected against deflagration and equipped with an alarm system in case of malfunctioning.

TRANSPORTATION CARCINOGENS

Two sub-sections are envisaged. The transportation of carcinogens and mutagens inside the buildings where they are used, and from one institute to another by car, rail or plane.

Transportation of carcinogens inside laboratories

Transportation should be to a laboratory which is fully equipped and designed to handle such compounds and where the employees have been warned about the danger of the carcinogenic/mutagenic compounds.

Dissemination of the compound during transportation must be avoided by confinement. In this respect, the flask containing the compound should be placed in a leakproof container that will not open even if dropped. It should be secured in the container by an adsorbing material such as vermiculite. Stainless steel transportation vessels should be favoured as in the event of accidental breakage of the flask during transportation they have the advantage of allowing direct in situ decontamination by available chemical techniques.

Transportation of carcinogens outside laboratories

Transportation will endanger a population which has not been informed about the risks and is thus more vulnerable. All precautions must therefore be taken to avoid accidental breakage during transportation, or in the event of breakage, to minimise the risk of contact between these persons and the compound. The following packing precautions should be adopted:

- The compound should be placed in a leak proof screw cap tube and, in order to prevent unscrewing of the cap during transportation it should be fixed with an adhesive tape or parafilm placed counter current of the screwing mode.
- The tube should then placed in a plastic flask with a large opening filled with adsorbing agent which will serve two purposes: to check adsorbing and adsorption of the product in the event of breakage of the tube.
- The flask is then placed in a shockproof box (of polystyrene or cardboard filled with polystyrene beads).

Transportation by road must be in accordance with the European agreement dated 30 September 2000 concerning the transportation of dangerous goods by road.

Transportation by air must follow IATA rules.

PRECAUTIONS WHEN HANDLING CHEMICAL CARCINOGENS

The handling procedures for carcinogenic chemical should be adapted to the physicochemical properties of the compounds. Three cases should be envisaged:

1) Volatile compounds (work under a hood and use in incubators)
2) Non volatile compounds
3) Electrostatic powders

Sampling volatile carcinogens

Sampling of a volatile carcinogenic chemical from a primary container should always be performed under a hood [1], equipped with a charcoal filter and maintained under negative pressure in comparison to the laboratory. A minimum air velocity of 0.5 m/s [2] is recommended by the "U.S. Department of Health Education and Welfare". It is very important to ensure that the working surface of the hood contains only the minimum equipment necessary for sampling. To much equipment may generate turbulence which can cause the re-entry of carcinogenic vapours into the laboratory atmosphere and thus expose the worker to their noxious effects.

The worker should wear protective clothing of a colour different from that used in other parts of the building. Safety goggles should also be worn during sampling as well as gloves suitable for the type of compound used [3].

The flask containing the carcinogen should be placed in a retention basin (stainless steel basins can be used because the surface is easy to decontaminate, enamel may also be used but one must ensure that the enamel is intact) covered with "bench coat" type paper (water proof side on the basin an adsorbing side on the top where the flask will be placed).

When working with compounds with high vapour tension, it is advisable to wear automated respiratory equipment.

Use of volatile carcinogens in an incubator

When using volatile carcinogens in an incubator (i.e.: incubation of Petri dishes for mutagenicity testing), it must first be verified that the

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1 The hood should preferably be left permanently working. If this is not the possible, aspiration must be left on for at least one hour after the sampling.

2 One may be of the opinion that the hood is working due to hearing the engine or fan, however it should be noted that certain incidents such as a broken or stretched fan belt will cause the same sound to be made while in fact there is no ventilation. In order to ensure that they are efficiently working, a device to measure the vacuum in the chimney of the hood can be installed, linked to a visual or auditory alarm system.

3 See the section below regarding the precautions concerning the use of gloves.

incubator is linked to an aspiration system. Before opening the incubator, the air should be aspired for at least 30 minutes before opening the doors in order to eliminate any compound which may have evaporated in the atmosphere and thus contaminate it. The aspirator should be left on during the collection of the dishes. The water should be treated as contaminated waste.

Sampling non-volatile compounds

Sampling an aliquot of a non-volatile chemical carcinogen from a primary container should always be performed under a hood [1], equipped an HEPA filter and maintained under negative pressure in comparison to the laboratory. A minimum air velocity of 0.5 m/s [2] is recommended by the "U.S. Department of Health Education and Welfare". To much equipment may generate turbulence which can cause re-entry of carcinogenic vapours/particles into the laboratory atmosphere and thus expose the worker to their noxious effects.

The worker should wear protective clothing of a colour different from that used in other parts of the building. Safety goggles should also be worn during sampling as well as gloves suitable for the type of compound used [3].

The flask containing the carcinogen should be placed in a retention basin (Stainless steel basins can be used because the surface is easy to decontaminate, enamel may also be used but one must ensure that the enamel is intact) covered with bench coat type paper (water proof side on the basin an adsorbing side on the top where the flask will be placed).

Sampling of electrostatic powders

Sampling of an aliquot of powdered chemical carcinogen with elec-

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1 The hood should preferably be left permanently working. If this is not possible, the aspiration must be left on for at least one hour after the sampling.

2 One may be of the opinion that the hood is working due to hearing the engine or fan, however it should be noted that certain incidents such as a broken or stretched fan belt will cause the same sound to be made while in fact there is no ventilation. In order to ensure that they are efficiently working, a device to measure the vacuum in the chimney of the hood may be installed, linked to a visual or auditory alarm system.

3 See the section below regarding the precautions concerning the use of gloves.

trostatic properties (i.e.: aflatoxins) from a primary container should always be performed under a hood [1], equipped an HEPA filter and maintained under negative pressure in comparison to the laboratory. The air velocity must be reduced in accordance with the 0.5 m/s [2] recommended by the "U.S. Department of Health Education and Welfare" as important air flow will favour the dispersion of the powder. To much equipment may generate turbulence which can cause re-entry of the carcinogenic particles into the laboratory atmosphere and thus expose the worker to their noxious effects.

The worker should wear protective clothing of a colour different from that used in the other part of the building. Safety goggles should also be worn during sampling as well as cotton gloves and a face mask. The use of vinyl or latex gloves will favour the dispersion of the powder by electrostatic effects. After dissolution of the powder, gloves adapted to the type of compound must also be used [3].

The flask containing the carcinogen should be placed in a retention basin (Stainless steel basins can be used because the surface is easy to decontaminate, enamel may also be used but one must ensure that the enamel is intact) covered with bench coat type paper (water proof side on the basin an adsorbing side on the top where the flask will be placed).

Risks linked to the type of glove material

Numerous studies have demonstrated the permeability of gloves to certain chemicals. This permeability depends on the nature of the material used for the gloves. The protection time is linked to both the resistance of the glove material to the chemical/solvent used and the thickness of the gloves.

Mutagenic and/or carcinogenic products can also permeate through gloves as demonstrated in the following studies. This permeation is de-

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2 One may be of the opinion that the hood is working due to hearing the engine or fan, however it should be noted that certain incidents such as a broken or stretched fan belt will cause the same sound to be made while in fact there is no ventilation. In order to ensure that they are efficiently working, a device to measure the vacuum in the chimney of the hood may be installed, linked to a visual or auditory alarm system.

3 See the section below regarding the precautions concerning the use of gloves.

pendent on the compound itself but also on the solvent used to handle it.

As early as 1977, Weeks and Dean demonstrated that aromatic amines can transfer through glove material as such or in solution in methanol (Table 1). After ½ hours contact with undiluted aniline, 67 µg/ml of aniline was found in the solution inside thick latex surgical gloves.

N-nitrosamines have been studied by several groups (Gough et al., 1978; Sansone & Tewari, 1981; Walkers et al., 1978). They diffuse through latex gloves either as undiluted compound or as solution in methylene chloride (Walkers et al., 1978). In 4 minutes, 5-15 µg of N-nitrosodimethylamine (NDMA) or N-nitrosodiethylamine deposited on the outside of a latex glove have been transferred into a saline solution placed inside the glove. To improve the protection, two pairs of gloves separated by a layer of talc (Table 2) or barrier cream can be used. When studying the transfer of undiluted NDMA, two pairs of gloves separated by a layer of talc reduce its transfer to a saline solution in 4 minutes to about 0.5 µg (i.e.: by a factor > 10). No NDMA is detected if the two gloves are separated by a layer of barrier cream. Similarly, transfer of NDMA in solution in methylene chloride is reduced from about 8% in 10 minutes to about 0.5% by using two pairs of latex gloves separated by a layer of barrier cream.

This allows ample time, when the outside glove is contaminated, to take it of and replace it. Gough et al. (1978) discovered that N-nitrosamines in solution in hexane diffuses through vinyl and latex gloves, the N-nitrosopyrrolidine being transferred most rapidly in all cases. Sansone and Tewari (1978) have also demonstrated that nitrosamines diffuses through latex, PVC, neoprene gloves. The solution in methylene chloride diffuses through all type of gloves. For those in acetone, latex gloves afford better protection, although not complete. For solutions in ethanol, nitrile and neoprene gloves are the most efficient. Finally, for aqueous solutions, the least penetration occurs through nitrile gloves.

While N-nitrosamines are small molecules in liquid form, one may wonder whether there is a risk of transfer for larger molecules. Castegnaro et al. (1982) investigated the transfer of aflatoxins dissolved in dimethylsulfoxide or chloroform. When dissolved in dimethylsulfoxide, the aflatoxins do not permeate through latex gloves but do through vinyl gloves. When dissolved in chloroform, the

aflatoxins transfer through both types of gloves due to swallowing of the glove material and reduction of the mechanical properties.

Two US studies have demonstrated that a series of antineoplastic agents (several of them are or possibly/probably carcinogenic for humans) diffuse through gloves. That of Connors et al. (1984) demonstrates using two methods (chemical analysis and mutagenicity testing) that carmustine diffuses through both latex and vinyl gloves and confirms that two pairs of gloves provide better protection. Laidlaw et al. (1984) investigated the transfer of 20 antineoplastic agents (carmustine, cyclophosphamide, ifosfamide, mercaptopurine, melphalan, thiotepa, mithoxantrone, dacarbazine, étoposide, teniposide, 5-Fu, floxuridine, cisplatine, daunorubicine, mechlorethamine, doxorubicine, bléomycine) through 4 types of gloves. The test was performed for a period of 90 minutes using solutions as recommended by the suppliers. The results are presented below:

- Thin PVC gloves: Permeable to all compounds tested.
- Thick PVC gloves: Permeable to 4 products (Carmustine, thiotepa, méchloretamine and daunorubicine). Start of transfer of doxorubicine and mercaptopurine.
- Latex examination gloves: Permeable to 4 products (carmustine, thiotepa, méchlorethamine and cyclophosphamide).
- Latex surgical gloves: Permeable to thiotepa.

Connor et al. (1999) have demonstrated the efficiency of protection of nitrile gloves (thickness 0.1-0.15 mm), latex gloves (thickness 0.18-0.22 mm), polyurethane gloves (thickness 0.13-0.15 mm) against the transfer of 18 reconstituted solutions of antineoplastic drugs prepared according to the specifications of the suppliers. The test was performed for 30, 60, 90 and 120 minutes. For nitrile gloves more than 5% of thiotepa was transferred in 30 minutes but this was attributed to micro holes. It is therefore very important to verify the gloves visually to avoid this type of problem. For the 11 other nitrile gloves no drug transfer was noted. For a sample of each type of glove (latex, polyurethane and vinyl) <1% permeability was noted. One sample of latex was permeable to carmustine in 90 minutes. Paclitaxel diffused through polyurethane in 60

minutes and through neoprene in 120 minutes. It can therefore be concluded that, apart from the problem of micro holes, the 4 types of gloves are acceptable for handling the 18 investigated drugs over a period of 30 minutes, which must the maximum time before changing gloves.

These studies demonstrate that all the compounds, including the carcinogens, can diffuse through gloves when they are dissolved in a solvent which reacts with the glove material. In addition, several compounds which exist in liquid form can diffuse through gloves. It is therefore necessary to exert a maximum of precautions when handling these compounds and to change gloves as soon as contamination is observed.

In 1987, Turjanmaa reported serious allergic reactions to latex in Finnish hospital personnel. This report was the start of a long series covering several medical professions (doctors, physicians, nurses, laboratory technologists) but also patients in the US, Canada, France, Belgium, Spain, Australia, Holland, Israel, Taiwan. The clinical manifestations included urticaria, dermatoses, anaphylaxis, respiratory disorders such as asthma, rhinitis and conjunctivitis. Prevention may include, the use of cotton or vinyl undergloves, desensitisation, use of non-powdered gloves or changing job for some personnel (Hudgins et al., 1993; Salkie, 1993; Yassin et al., 1994; Tarlo et al., 1994; Swanson et al., 1994; Moneret-Vautrin et al., 1994; Rustmeyer et al., 1994; Vandenplas et al., 1995; Ortiz et al., 1995; Hunt et al., 1999; Estlander et al., 1996; Katelaris et al., 1996; Liss et al., 1997; Ylitalo et al., 1997; Lai et al., 1997; De Groot et al., 1998; Vila et al, 1999; Levy et al., 1999; Charous et al., 2000; Tarlo, 2001).

Table 1. Transfer of aromatic amines through gloves (from Week & Dean, 1987)

* MOCA : Methylenebis (2-chloroaniline)

Aromatic amine	Concentration (µg/ml)	First measurement of concentration after transfer (µg/ml)	Transfer time (hours)
Anilin	2.400	22	18,0±2,0
Anilin	11.000	8	1,75±1,0
Anilin	Pure	67	0,50±0,25
MOCA*	1.900	29	2,00±0,50
MOCA*	9.800	37	1,5±0,5

Table 2. Gloves and N-nitrosamines, transfer through two pairs of latex gloves separated by a layer of talc.

Time (min)	Nitrosamine tested (% transfer)						
	NDMA	NDEA	NDPA	NDBA	NMPA	NPYR	NPIP
2	0,1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	0,4	0,1	n.d.	n.d.	n.d.	n.d.	n.d.
15	2,3	0,4	n.d.	n.d.	n.d.	0,3	0,15
20	2,5	0,4	n.d.	n.d.	n.d.	0,4	0,2
30	4,1	0,7	n.d.	n.d.	n.d.	1,9	0,3

NDMA: N-nitrosodimethylamine
NDEA: N-nitrosodiethylamine
NDPA: N-Nitrosodipropylamine
NDBA: N-nitrosodibutylamine
NPIP: N-nitrosopiperidine
NPYR: N-nitrosopyrrolidine
n.d. Not done

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ACCIDENTAL CONTAMINATION BY A CHEMICAL CARCINOGEN

Marcel Castegnaro

In the event of spillage, the first objectives are to protect the worker and to isolate the area and then to intervene for decontamination. In the case of spillage, two alternatives must be considered: 1) spillage of a volatile compound, 2) spillage of a compound in powder form. In all cases the person who provoked the accident should maintain self-control.

All persons working with carcinogenic agents must receive training concerning the possibility of accidents. A person who is frightened is more subject to accidents than a person who has been positively trained to overcome the consequences of an accident.

Note: The strategies described below are theoretical and must be adapted to each case. Some time should be allowed during the planning period of an experiment to prepare a strategy of intervention.

Spillage of a volatile compound

In the event of spillage of a volatile compound, the main risks for the worker is breathing the polluted atmosphere and being contaminated by dermal penetration of the compound. In this case, the worker must leave the polluted area immediately removing all contaminated clothes in an adjacent area.

- A*ccess* to the contaminated area and the adjacent areas in which contaminated clothes have been left should then be *restricted*.
- The worker should then *advise* the persons from the *safety group* of the accident, its location, and its circumstances.
- The *report* should include the following items:
  - Name of the spilled compound.
  - Amount of spilled compound.
  - The potentially polluted area.

  - Exact location of the accident (for example: whether the spillage is in a middle of a room or close to a refrigerator or a solid piece of equipment) and of the place where the contaminated clothes have been left.
  - Presence or absence of a solvent and if present, the nature of the solvent (this information is absolutely necessary in the event of chemical decontamination of the polluted area.
- The team in charge of the decontamination must *acquire* all the necessary *equipment* before intervening. This may include:
  - Large wide mouth collection flasks, to place all the broken pieces of the container, gloves, paper-cloths for cleaning the area, etc.
  - A stock of paper cloths.
  - A stock of gloves.
  - Aqueous solutions which may be slightly acidic or alkaline depending on the properties of the spilled compound.
  - Decontamination solutions.
- The person in charge of the decontamination must *protect themself* before starting the decontamination. Protection should include:
  - Overalls.
  - Spectacles.
  - Helmet.
  - Gloves (during decontamination, always wear 2 pairs of gloves and, discard and replace the top pair after each action).
  - Respiratory protection (this will depend on the type of compound, the amount spilled and knowledge of its absorption on activated charcoal).
  - Protective shoe-covers.
  - Adhesive plastic tape (c.a.: 0.5m wide).
- The first step when entering the contaminated area is to exactly *locate* the *contamination* and draw a circle on the floor of the area to be decontaminated. The circle should be much larger than the area itself (for a small spill, it must be at least the size of two arm lengths and for larger spills, as large as necessary). During this period of delineation, furniture (cupboards or refrigerators)

must be checked to see whether they need to be decontaminated.
- **Decontamination of the area:** collect all broken pieces of glass and place them in the wide mouth collection flask. Change the outer glove at each step of glass retrieval and in the event of an overt sign of damage of the outer glove change both gloves.

Note: If the area is too large to allow access to all parts using an extended arm, adhesive plastic tape can be placed on the floor in order to improve access. The area in front of the adhesive tape strip can then be cleaned with paper-cloths. The top surface of this tape should be considered a clean surface on which to walk.

- Remove liquid spills with a paper-cloth, starting each collection in the least contaminated area and stopping at the most contaminated area. Change the paper cloth and outer glove at each movement. For a large spill volume use an adsorbing agent such as vermiculite and collect, changing gloves at collection step.
- Continue cleaning the area with paper-cloths which may be wetted with an alkaline or acidic solution, changing the cloth at each step as described above.
- Check the surface for complete decontamination by wiping the most contaminated area with a solvent, extracting the wipe and performing physicochemical analysis.
- If the area is contaminated, place paper-cloths over the whole area and pour the decontaminating solution on it until the paper-cloths are completely wet but without flooding the area.
- Allow to react for the necessary amount of time, remove the paper-cloths and dry the area.

Note: All cloths at this step must be considered decontaminated.

- **Decontamination of the equipment:** if spills were noted on the equipment, these must be decontaminated using the same procedure as for the floor, using paper-cloths wetted with acidic or alkaline solutions if necessary.
- **Decontamination of the wastes:** these should be treated either

by incineration or placed under a hood for treatment by an appropriate chemical degradation technique.
- The area can only be re-*opened to staff* after these steps.

Spillage of a volatile compound

In case of spillage with a compound in powder form, the main risks for the worker are contamination of clothes by small particles and dissemination of the powder in the atmosphere through ventilation systems. The worker must leave the polluted area immediately taking all contaminated clothes to an adjacent area.

- A*ccess* to the contaminated area and adjacent areas in which the contaminated clothes have been left should then be *restricted*,
- The worker should then *notify* the persons from the *safety group* of the accident, its location, and its circumstances,
- The *report* should include the following items:
  - Name of the spilled compound.
  - Amount of spilled compound.
  - The potentially polluted area.
  - The exact location of the accident (for example: whether the spillage is in the middle of a room or close to a refrigerator or a solid piece of equipment, whether the location is close to a ventilation system which will favour dissemination of the powder) and of the place where the contaminated clothes have been left.
- The team in charge of the decontamination must *acquire* all the necessary *equipment* before intervening. This may include:
  - Large wide mouth collection flasks, to place all the broken pieces of the container, gloves, paper-cloths for cleaning the area, etc..
  - A stock of paper-cloths.
  - A stock of gloves.
  - Aqueous solutions which may be slightly acidic or alkaline depending on the properties of the spilled compound.
  - Decontaminating solutions.
- The person in charge of the decontamination must *protect them*

self before starting the decontamination. The protection should include:

- Overalls.
- Spectacles.
- Helmet.
- Gloves (during the decontamination, always wear 2 pairs of gloves and discard and replace the top pair after each action).
- Respiratory protection (in most cases, a cotton respiratory mask will be sufficient).
- Protective shoe-covers.
- Adhesive plastic tape (c.a.: 0.5m wide).

- The first step when entering the contaminated area is to *locate* exactly the *contaminated area* and draw a circle on the floor of the area to be decontaminated. The circle should be much larger than the area itself (for a small spill, it should be at least of the size of two arm lengths and for larger spills, as large as necessary). All furniture (cupboards or refrigerators) close to the spill area must be considered contaminated.
- **Decontamination of the area:** collect all broken pieces of glass and place them in the wide mouth collecting flask. Change the outer glove at each step of glass collection and in case of an overt sign of damage of the outer glove, change both gloves.

Note: If the area is too large to allow access to all parts using an extended arm, adhesive plastic tape can be placed on the floor in order to improve access. The area in front of the adhesive tape strip can then be cleaned with paper-cloths. The top surface of this tape should be considered a clean surface on which to walk.

- Retrieve the powder with a wet paper-cloth, starting each collection in the least contaminated part and stopping at the most contaminated part. Change paper-cloth and outer glove at each movement.
- Continue cleaning the area with a paper-cloth which may be wetted with an alkaline or acidic solution, changing cloth at each step as above.
- Check the surface for complete decontamination by wiping the

most contaminated area with a solvent, extracting the wipe and physicochemical analysis.
- If the area is contaminated, place paper-cloths over the whole area and pour the decontaminating solution on it until the paper-cloths are completely wet but without flooding the area.
- Allow to react for the necessary amount of time, remove the paper-cloths and dry the area.

Note: All cloths at this step should be considered decontaminated.

- **Decontamination of equipment:** clean the equipment with a wet paper-cloth. The wetting solution can be acidic or alkaline if necessary.
- **Decontamination of wastes:** these should be treated either by incineration or placed under a hood for treatment by an appropriate chemical degradation technique.
- The area should only be re-*opened to staff* after these steps.

RADIOLOGICAL RISK

Marcel Castegnaro

Introduction

Activity in the biological, biomedical or biotechnology laboratory often requires the use of numerous radioactive substances. The most common of which are:

- Carbon-14 (^{14}C), used in research to ensure that potential new drugs are metabolised without forming harmful by-products.
- Tritium (^{3}H), used for life science and drug metabolism studies to ensure the safety of potential new drugs.
- Iodine-125 (^{125}I), used in *"in vitro"* diagnostic kits.
- Phosphorus-32 (^{32}P), used in molecular biology and genetics research.
- Phosphorous-33 (^{33}P) and Sulfur-35 (^{35}S), are often used instead of ^{32}P for safety reasons because as they emit Beta ions of lower energy.

Handling radioactive material

Before initiating work with radioisotopes, all workers should obtain the authorisation to handle such substances from the Radiation Safety Officer who will register them with the National Radiological Safety Service, provide them with the manual of safety for work with radioisotopes and also provide them with training when appropriate (see below).

This manual must advise on the following specific points:

- The special precautions necessary to prevent personal and structural contamination and to minimise any radiation effects.
- Personal monitoring and health checks (medical examinations and blood counts).
- The disposal of radioactive waste.

- The purchase and storage of radioactive materials.

It should be adapted to each institute and provide specific information about the authorised areas for the use and disposal of radioactive materials and the activity which can be performed in each area together with a list of the authorised compounds.

All radioisotopes must be ordered through the Radiological Safety Officer and should conform with the current radiological legislation from the country in question. Individual workers are responsible for cleanliness of the working area, the decontamination of equipment and glassware and the safe disposal of radioactive waste as described in the safety manual and in accordance with the National regulation.

Detailed records of use and disposal of radioactive materials must be maintened and should be kept up-to-date by the user.

When working with radioactive materials, a laboratory coat, gloves and a film badge, which can be obtained from the official national control body must be worn. Eye protection should also be worn if necessary.

Each authorised laboratory should have specific areas set aside for handling isotopes. The maximum quantities used in these areas must be displayed. These areas should be clearly labelled and should not be used for any other purpose. Bench coat can be used to cover the bench surface, absorbent side up. The use of large amounts of ^{32}P (i.e. > 100 μCi) should be confined to a special room. The access of this room must be limited to a very small number of staff members thus avoiding the hazard of crowded environment. Each operator who initiates an experiment must check contamination of the area and the equipment he will be using when signing in. The check must be repeated at the end of the experiment and decontamination performed, if necessary, before signing out. Accident/incident reports must be displayed on a safety panel.

All wet operations involving isotopes should be carried out over trays to contain any spills. Work low over the tray, so that any accidental drips do not bounce off.

When ^{32}P, ^{33}P, ^{14}C or ^{35}S are used, they should be held behind protec-

tive screens made of 1cm plexiglass (see individual data sheet for specifically adapted information). Do not hold a tube containing ^{32}P in your fingers, but always hold it in a rack or specially adapted protecting device (Castegnaro et al., 1993) to limit the dose to the fingers. Automatic pipettes should be fitted with radiation guards.

Do not pipette by mouth during experiments involving radioactive materials.

During the working operation, regular checks of hand / finger contamination should be performed and gloves changed immediately in case of contamination.

In every case, after completion of work involving the use of a radioisotope, the user should check the laboratory area, any equipment used and perform a personal control for possible contamination using a monitor suitable for the isotope in use (see individual data sheets for specifically adapted information).

Staff Training

Before undertaking work with radio-labelled compounds, especially those which use ^{32}P or large amounts (mCi or more) of other radioactive elements, all staff should undergo a training period using solutions of fluorescent compounds as substitutes for radio-labelled compounds. The objective of these training periods are:

- to help the staff monitor the different types of accidents and contamination that can occur and thus better control them;
- to improve their dexterity while working behind a screen and thus reduce the duration of exposure;
- to improve their accuracy in sampling the various solutions, while maintaining a high working rate, thus increasing the reliability of the results.

Storage

Storage of radioactive materials shall be in secured or locked cabinets, refrigerators, freezers or waste areas located in rooms authorised for the use of radioactive compounds. Radioactive materials must be stored in sealed containers in such a way as to prevent accidental spill-

age or breakage, and to prevent release into the air. If the nuclide requires shielding (see individual data sheets for specifically adapted information), it must be stored in shielded containers in order to prevent exposure to personnel accessing the storage areas.

Carcinogenic radioactive compounds must be stored separately, with the carcinogenic substances, duly labelled **'Carcinogenic radioactive substances'.**

For both carcinogenic and non-carcinogenic radioactive compounds, a list of contents should be posted on the refrigerator/freezer, marked with the radioactive label.

Aerosols from stored radioactive materials may cause contamination of adjacent areas and personnel if not handled in the proper way after storage. It is therefore imperative that the radioactive material that has been stored in a freezer or ultra freezer, be thawed before opening and that all manipulations are carried out in a certified fume hood or biological safety cabinet. All radioactive materials, including those stored in waste areas must be labelled with the radioactive warning symbol, the words **"Caution: radioactive substances"**, the isotope, the date and the amount of radioactivity in DPM or microcuries. The ORCBS will store radioactive materials for principal investigators upon request. Contact a Health Physicist to arrange for storage of radioactive materials.

Biological Effects of Ionizing Radiation

Injury due to irradiation is mainly caused by ionisation within the tissues of the body. When radiation interacts with a cell, ionisations and excitations are produced in either biological macromolecules or in the medium in which the cellular organelles are suspended, predominantly water. Based on the site of interaction, the radiation-cellular interactions may be termed as either direct or indirect.

Direct action occurs when an ionising particle interacts with and is absorbed by a macromolecule in a cell (DNA, RNA, protein, enzymes, etc.). These macromolecules become abnormal structures which initiate the events that lead to biological changes.

Indirect action involves the absorption of ionising radiation in the medium in which the molecules are suspended. The molecule which

most commonly mediates this action is water. Through a complex set of reactions the ionised water molecules form free radicals that can cause damage to macromolecules.

The most important target for radiation in the cell is DNA in the nucleus. Biological effects result when DNA damage is not repaired or is improperly repaired. Extensive damage to DNA can lead to cell death. Large numbers of cells dying can lead to organ failure and death for the individual. Damaged or improperly repaired DNA may result in lymphoma and cancers in somatic cells. Two kinds of effects may result.

The **non random effects** are those for which a given exposure will produce , within a small range of variation, the same type of effect. In the case of global irradiation at levels of 1-2 grays (Gy), a modification of the blood counts occurs (i.e.: a decrease in the level of leukocytes and platelets and, to a lower extent, of erythrocytes). In the case of local irradiation of the skin, erythema is observed at doses between 4 and 8 Gy, dry dermatitis at levels exceeding 5 Gy, exudative dermatitis between 12 and 20 Gy and necrosis after 25 Gy. In the case of the eye irradiation opacification of the crystalline lens is observed above 10 Gy of X-rays, or 0.8 Gy of neutrons and cataracts may occur. For testis irradiation, reduction of spermatozoa is observed 0.3 Gy and complete disappearance may occur above 2 Gy, effects which are reversible. In the case of ovaries, irradiation doses of 12-15 Gy will cause irreversible sterility in a of 25 years old woman, while only 7 Gy will produce the same effect in a woman of about 40 years old.

Random effects are reflected in the increase of relative risk of death from cancer or the occurrence of severe hereditary effects in the first two generations after uniform whole body exposure. For these effects, it is recognised that the same dose may not produce the same effect in different persons. The international commission has expressed probability of the risk of developing a cancer after uniform whole body irradiation on radiological protection (1977, 1980) in the following formula:

$$\text{Expected number of cancer cases from irradiation development} = \text{Calculated risk for cancer development} \times \text{Exposure} \times \text{Size of population}$$

where the calculated risks can be expressed for the whole body, by sex, (1.65×10^{-2} Sv^{-1} for female or 1.40×10^{-2} Sv^{-1} for male) or for the individual organs whose susceptibility to irradiation is different (see table1). It should be noted that a new calculated risk value for women (5×10^{-2} Sv^{-1}) has been proposed (International Commission on Radiological Safety, 1990), that is likely to be adopted.

Table 1. Random risk of damage at various exposed sites

Organ exposed	Random risk x 10^{-2} Sv^{-1}
Gonad	0.4
Bone marrow	0.2
Lung	0.2
Breast	0.25
Thyroid	0.05
Bone	0.05

For example, in a population of 1200 women who have been exposed to 50 nSv, one case of cancer or genetic disorder due to radiation can be expected.

For the above list, the major risk lies with the use of ^{32}P.

Monitoring

Each bay or laboratory area must be monitored weekly for the build-up of radioactivity. One person should be nominated to do this for each area and a record kept of the monitoring. For isotopes such as ^{125}I, ^{32}P, ^{35}S and ^{14}C, the appropriate monitor may be used, but for ^{3}H, check by taking swabs from working areas and counting by liquid scintillation.

In particular, the areas to be checked include:

- the bench areas, sinks including U bends and pipes
- equipment such as Gilsons, knobs on power packs, fridge/freezer handles
- communally used equipment, e.g. microfuges, benchtop centrifuges, gel systems

- the monitors themselves

Any areas found to be contaminated should be cleaned with 5% Decon and the area rechecked.

Emergency procedures

In the event of spillage of a large quantity of radioisotope (e.g. breakage of an entire source or vial), follow this procedure:

- Alert all other workers in the immediate vicinity who are not contaminated, and if possible they should leave the area which should be isolated.
- Remove any contaminated clothing as quickly as possible and leave it at the site of the incident.
- Inform the radiological safety officer.
- Treat contaminated personnel (skin should be washed under running water and gentle rubbing with a soapy solution; do not abrade the skin; any wounds should be irrigated with plentiful running water; monitor to check decontamination).
- Specialist medical treatment can be given.
- Decontaminate the affected area once contaminated individuals have been treated.

References

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- Duke University safety manual (http://www.safety.duke.edu/SafetyManuals/)
- International Commission on Radiological protection. Recommendation of the International Commission on Radiological Protection. Annals of ICPR, publication N° 26. (1977).
- International Commission on Radiological protection. Recommendation of the International Commission on Radiological Protection. Annals of ICPR, publication N° 30, part 2, volume 4. (1980).
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Annexes
HYDROGEN-3 [^{3}H]

Physical Data

- ***Beta Energy*:** 18.6 keV (maximum), 5.7 keV (average) (100% abundance).
- ***Physical half-life*:** 12.3 years.
- ***Biological half-life*:** 10-12 days.
- ***Effective half-life*:** 10-12 days. Forcing liquids to tolerance (3-4 litres/day) will reduce the effective half-life of ^{3}H by a factor of 2 or 3. (Relatively easy to flush out of system with fluids.).
- ***Activity*:** 9640 Ci/gram.
- ***Maximum Beta Range in Air*:** 6 mm.
- ***Maximum Beta Range in Water*:** 0.006 mm.
- ***Penetrability in Matter or Tissue: Insignificant*** [0% of beta particle energy transmitted through dead layer of skin].

Radiological Data

- The least radiotoxic of all radionuclides.
- Critical Organ: Body Water or Tissue.
- Routes of Intake: Ingestion, Inhalation, Puncture, Wound, Skin Contamination (Absorption)
- External exposure: from weak ^{3}H beta energy - not a radiological concern.
- Internal exposure & contamination: primary radiological concerns.
- Committed Dose Equivalent (CDE): 64 mrem/mCi (ingested), 64 mrem/mCi (inhaled) 64 mrem/mCi (puncture).
- Committed Effective Dose Equivalent (CEDE): 90 mrem/mCi (ingested), 63 mrem/mCi (inhaled).
- Annual Limit on Intake (ALI): 80 mCi (ingestion or inhalation) [$3H_2O$] [1.0 ALI = 80 mCi (^{3}H) = 5,000 mrem CEDE].
- Skin Contamination Exposure Rate: 57,900 mrad/hr/mCi (contact).

- Exposure rate to dead layer of skin only.
- Skin contamination of 1.0 μCi/cm^2 = 0 mrad/hr dose rate to basal cells.
- Rule of Thumb: 0.001 μCi/ml of ^{3}H in urine sample is indicative of a total integrated whole body dose of approximately 10 mrem (average person) if no treatment is instituted (i.e., flush with fluids).

Shielding

- None required.

Survey Instrumentation

- **Cannot** detect ^{3}H using a G-M or NaI survey meter.
- Liquid scintillation counter (indirect) is the only monitoring method.

Radiation Monitoring Dosimeters

- Whole Body Badge or Finger Rings: Not Needed (beta energy too low).

Radioactive Waste

- Solid, liquids, scintillation vials, pathological materials, animal carcasses.

Regulatory Compliance Information

- Derived Air Concentration (DAC): 2.0 E-5 μCi/cc (occupational).
- Airborne Effluent Release Limit: 1.0 E-7 μCi/cc [Applicable to the assessment & control of dose to the public (10 CFR 20.1302). If this concentration was inhaled continuously for over one year the resulting TEDE would be 50 mrem].
- Controlled Area Removable Contamination Limit: 2,200 dpm/100 cm^2.
- Urinalysis (By-product License): required when handling greater than or equal to 100 mCi ^{3}H.

General Radiological Safety Information

- Inherent Volatility (at STP): Substantial.
- Experimental uses include: total body water measurements & *in vivo* labelling of proliferate cells by injection of tritium-labelled compounds (i.e., thymidine).
- Tritium labelling is also used in a variety of metabolic studies.
- Oxidation of ^{3}H gas in air is usually slow.

CARBON-14 (^{14}C)

Physical Data

- ***Beta Energy*:** 156 keV (maximum), 49 keV (average) (100% abundance).
- ***Physical half-life*:** 5730 years.
- ***Biological half-life*:** 12 days.
- ***Effective half-life*:** 12 days (Bound).
- ***Effective half-life*:** 40 days (Unbound).
- ***Specific Activity*:** 4460 mCi/gram.
- ***Maximum Beta Range in Air*:** 24.00 cm.
- ***Maximum Beta Range in Water/Tissue*:** 0.28 mm.
- ***Maximum Range in Plexiglass/Lucite/Plastic:*** 0.25 mm.
- ***Fraction of ^{14}C beta particles transmitted through dead layer of skin:*** At 0.007 cm depth = 1%.

Radiological Data

- Critical Organ: Fat Tissue.
- Routes of Intake: Ingestion, Inhalation, Skin contact.
- External exposure: deep dose from weak ^{14}C beta particles is not a radiological concern.
- Internal exposure & contamination: primary radiological concerns.
- Committed Dose Equivalent (CDE): 2.08 mrem/μCi (ingested) (Fat Tissue), 2.07 mrem/μCi (puncture), 2.09 mrem/μCi (inhalation).
- Committed Effective Dose Equivalent (CEDE): 1.54 mrem/μCi (ingested).
- Annual Limit on Intake (ALI): 2 mCi (ingestion of labelled organic compound), 2000 mCi (inhalation of labelled carbon monoxide), 200 mCi (inhalation of carbon dioxide) [1.0 ALI = 2 mCi (ingested C-14 organic compound) = 5,000 mrem CEDE].
- Skin Contamination Dose Rate: 1090-1180 mrem per 1.0 μCi/

cm^2 (7 mg/cm^2 depth).
- Dose Rate to Basal Cells from Skin Contamination 1.0 μCi/cm^2 = 1400 mrad/hour.
- Immersion in ^{14}C Contaminated Air = 2.183E7 mrem/year per μCi/cm^3 at 70 um depth of tissue and 4.07E6 mrem/year per μCi/cm^3 value averaged over dermis.

Shielding
- None required (< 0.1 mm plexiglass or glass).

Survey Instrumentation
- Thin-window G-M survey meter can detect ^{14}C but, the meter probe must be at about 1 cm. G-M survey meters have very low counting efficiency for ^{14}C (5%).
- Liquid scintillation counter (indirect counting) may be used to detect removable ^{14}C on wipes.

Radiation Monitoring Dosimeters
- Not Needed (beta energy too low).
- ^{14}C Beta Dose Rate: 6.32 rad/hr at 1.0 in air per 1.0 mCi ^{14}C.
- Skin Contamination Dose Rate: 13.33 mrad/hr per μCi on skin.
- Dose Rate from a 1 mCi isotropic point source of ^{14}C: Distance Rad/Hr 1.0 cm, 1241.4; 2.0 cm, 250.4; 15.2 cm, 0.126; 20.0 cm, 0.0046.

General Radiological Safety Information
- Urinalysis: Not Required; however, prudent after a ^{14}C radioactive spill or suspected intake.
- Inherent volatility (at STP): Not Significant.
- Possibility of organic ^{14}C compounds being absorbed through gloves.
- Care should be taken **Not** to generate $^{14}CO_2$ gas which could be inhaled.
- Internal Dose is the concern: skin contamination, ingestion, inhalation, and puncture.
- Always wear a lab coat and disposable gloves when working

with ^{14}C.

- The concentration of carbon in adipose tissue, including the yellow marrow, is about 3 times the average whole body concentration. No other organ or tissue of the body concentrates stable carbon to any significant extent. The fractional absorption of dietary carbon (uptake to blood) is usually in excess of 0.90.
- Three main classes of carbon compounds may be inhaled: organic compounds, gases (CO or CO_2), and aerosols of carbon containing compounds such as carbonates and carbides.
- Organic Compounds - most organic compounds are **Not** very volatile under normal circumstances; the probability of these being inhaled as vapours is therefore small. In circumstances where such substances are inhaled, it would be prudent to assume that once they enter the respiratory system they are instantaneously completely translocated to the systemic circulation without changing their chemical form.
- Gases - the inhalation of CO and its retention in body tissues has been studied extensively. Since gas has a relatively low solubility in tissue water, doses due to absorbed gas in tissues are insignificant in comparison with doses due to the retention of CO bound to haemoglobin. CO_2 in the blood exists mainly as a bicarbonate. Carbonates and Carbides - it is assumed that inhaled or ingested ^{14}C labelled compounds are instantaneously and uniformly distributed throughout all organs and tissues of the body where they are retained with a biological half-life of 12-40 days.

PHOSPHOROUS-32 (^{32}P)

Physical Data

- ***Beta energy*:** 1.709 MeV (maximum), 0.690 MeV (average, 100% abundance).
- ***Physical half-life*:** 14.3 days.
- ***Biological half-life*:** 1155 days.
- ***Effective half-life*:** 14.1 days (bone) /13.5 days (whole body).
- ***Specific activity*:** 285,000 Ci/gm.
- ***Maximum range in air*:** 610 cm.
- ***Maximum range in water/tissue*:** 0.76 cm.
- ***Maximum range in plexiglass/lucite/plastic*:** 0.61cm.

Radiological Data

- Critical organ (biological destination) (soluble forms): Bone.
- Critical organs (insoluble forms or non-transportable ^{32}P compounds): Lung (inhalation) and G.I. tract/lower large intestine (ingestion).
- Routes of intake: ingestion, inhalation, puncture, wound, skin contamination (absorption) external and internal exposure from ^{32}P.
- Committed Dose Equivalent (CDE): 32 mrem/mCi D/bone marrow.
- Committed Effective Dose Equivalent (CEDE): 7.50 mrem/mCi (ingested/WB) 5.55 mrem/mCi (inhale/Class D), 13.22 mrem/mCi (inhale/Class W).
- Skin contamination dose rate: 8700-9170 mrem/mCi/cm^2 (7 mg/cm^2 or 0.007 cm depth in tissue).
- Dose rate to basal cells from skin contamination of 1.0 mCi/cm^2 (localised dose) = 9200 mrad/hr.
- Bone receives approximately 20% of the dose ingested or inhaled for soluble ^{32}P compounds.

- Tissues with rapid cellular turnover rates show higher retention due to concentration of phosphorous in the nucleoproteins.
- ^{32}P is eliminated from the body primarily via urine.
- Phosphorus metabolism: see ^{33}P Fact Sheet.

Survey Instrumentation

- GM survey meter and a pancake probe.
- Low-energy NaI probe is used only to detect Bremsstrahlung x-rays.
- Liquid scintillation counter (indirect counting) may be used to detect removable surface contamination of ^{32}P on smears or wipes.

Dose Rates (From Unshielded 1.0 mCi Isotropic Point Source)

- Distance Rads/hr: 1.00 cm 348; 15.24 cm 1.49; 10.00 ft 0.0015 780,000 mrad/hr at surface of 1.0 mCi ^{32}P in 1 ml liquid; 26,000 mrad/hr at mouth of open vial containing 1.0 ml ^{32}P in 1.0 ml liquid.

Shielding

- 8 mm thick plexiglass/acrylic/lucite/plastic/wood.
- Do not use lead foil or sheets because penetrating Bremsstrahlung x-ray will be produced.
- Use lead sheets or foil to shield Bremsstrahlung x-rays only after low density plexiglass/acrylic/lucite/wood shielding.

General Precautions

- Because it is a bone seeker, special precautions must be taken to minimise any chance of introduction into the body.
- Airborne contamination can be generated through drying (dust), rapid boiling, or expelling solutions through syringe needles and pipette tips, due to aerosols.
- Personnel radiation monitors (whole body and finger rings) are required when handling greater than 1.0 mCi of ^{32}P at any time.
- Never work directly over an open container; avoid direct eye exposure from penetrating ^{32}P beta particles.

- Always wear a lab coat and disposable gloves when handling ^{32}P.
- Monitor personnel work areas and floors using a GM survey meter equipped with a pancake (beta) probe, for surface contamination.
- Monitor for removable surface contamination by smearing, or wiping where ^{32}P is used.
- Use low-density (low atomic number) shielding material to shield ^{32}P and reduce the generation of Bremsstrahlung x-rays. The following materials are low atomic number materials: plexiglass, acrylic, lucite, plastic, wood, or water.
- Do **NOT** use lead foil, lead sheets, or other high density materials (metals) to shield ^{32}P directly. Materials with atomic number higher than that of aluminium (Z =13) should **NOT** be used. Penetrating Bremsstrahlung x-rays will be generated in lead and other high density shielding material. Safety glasses or goggles are recommended when working with ^{32}P.
- Typical GM survey meter with pancake probe efficiency is greater than or equal to 45%. Typical liquid scintillation counter counting efficiency for ^{32}P (full window/maximum) greater than or equal to 85%.
- Typical detection limit of ^{32}P in urine specimens using a liquid scintillation counter = 1.1 x 10-7µCi/ml.

PHOSPHORUS-33 (^{33}P)

Physical Data

- ***Beta energy*:** 0.249 MeV (maximum, 100% abundance), 0.085 MeV (average).
- ***Physical half-life*:** 25.4 days.
- ***Biological half-life*:** 19 days (40% of intake; 30% rapidly eliminated from body, remaining 30% decays).
- ***Effective half-life*:** 24.9 days (bone).
- ***Specific activity*:** 1,000 - 3,000 Ci/millimole.
- ***Maximum beta range in air*:** 89 cm = 35 inches = 3 feet.
- ***Maximum range in water/tissue*:** 0.11 cm = 0.04 inch.
- ***Maximum range in plexiglass/lucite/plastic***: 0.089 cm = 0.035 inch.
- ***Half-Value Layer (HVL)*:** 0.30 mm (water/tissue).

Radiological Data

- Critical organ (biological destination) (soluble forms): Bone marrow.
- Critical organs (insoluble forms or non-transportable ^{33}P compounds): Lung (inhalation) and G.I. tract/lower large intestine (ingestion).
- Routes of intake: ingestion, inhalation, puncture, wound, skin contamination (absorption).
- Internal exposure & contamination are the primary radiological concerns.
- Committed Dose Equivalent (CDE): 0.5 mrem/mCi (inhalation).
- Skin contamination dose rate: 2,910 mrem/hr/μCi/cm^2 (7 mg/cm^2 or 0.007 cm depth in tissue).
- Fraction of ^{33}P beta particles transmitted through the dead skin layer is about 14%.
- Tissues with rapid cellular turnover rates show higher retention due to concentration of phosphorus in the nucleoproteins.

- ^{33}P is eliminated from the body primarily via urine.
- Phosphorus metabolism:
  - 30% is rapidly eliminated from body
  - 40% has a 19-day biological half-life
  - 60% of ^{33}P (ingested) is excreted from body in first 24 hrs.

Shielding

- Not required; however low density material is recommended, e.g., 3/8 inch thick plexiglass, acrylic, lucite, plastic or plywood.

Survey Instrumentation

- GM survey meter with a pancake probe.
- Liquid scintillation counting of wipes may be used to detect removable surface contamination.

Personnel Dosimeters

- Not required, since they do not detect this low energy nuclide.

General Precautions

- Inherent volatility (STP): insignificant.
- Skin dose and contamination are the primary concerns.
- Drying can form airborne ^{33}P contamination.
- Monitor work areas for contamination, using smears or wipes to check for removable contamination.

SULFUR-35 (^{35}S)

Physical Data

- ***Beta energy*:** 167 keV (maximum), 53 keV (average) (100% abundance).
- ***Physical Half Life*:** 87.4 days.
- ***Biological Half Life*:** 623 days (unbound ^{35}S).
- ***Effective Half Life*:** 44-76 days (unbound ^{35}S).
- ***Specific Activity*:** 42,400 Ci/g.
- ***Maximum Beta Range in Air*:** 26.00 cm. = 10.2 in.
- ***Maximum Beta Range in Water or Tissue*:** 0.32 mm. = 0.015 in.
- ***Maximum Beta Range in Plexiglass or Lucite*:** 0.25 mm. = 0. 01 in.
- ***Fraction of ^{35}S betas transmitted through dead layer of skin* =** 12%.

Radiological Data

- Critical organ: Testis.
- Routes of Intake: ingestion, inhalation, puncture, wound, skin contamination (absorption)
- External exposure (deep dose) from weak ^{35}S beta particles is not a radiological concern.
- Internal exposure & contamination are the primary radiological concerns.
- Committed Dose Equivalent (CDE): 10.00 mrem/µCi (ingested), 0.352 mrem/µCi (puncture).
- Committed Effective Dose Equivalent (CEDE): 2.6 mrem l/µCi (ingested) (Assumes a 90 day biological half life).
- Annual Limit on Intake (ALI)*: 10 mCi (ingestion of inorganic ^{35}S compounds); 6 mCi (ingestion of elemental ^{35}S) ; 8 mCi (ingestion of sulphides or sulphates/LLI)**; 10 mCi (inhalation of ^{35}S vapours) ; 20 mCi (inhalation of sulphides or sulphates); 2

mCi (inhalation of elemental ^{35}S) .

* 1.0 ALI = 10 mCi (inhaled ^{35}S vapours) = 5,000 mrem CEDE.

** 1.0 ALI = 8 mCi (ingestion sulphides/sulphates LLI) = 50,000 mrem CDE.

- Skin Contamination Dose Rate: 1,170 - 1,260 mrem/1.0 μCi/cm^2 (7.0 mg/cm^2 depth).
- Beta Dose Rates for ^{35}S: 14.94 rad/h (contact) in air per 1.0 mCi, 0.20 rad/h (6 inches) in air per 1.0 mC.

Shielding

- None required (1 cm plexiglass will completely block the radiation; 0,3 mm plexiglass and 0.2 mm glass shields will provide enough protection but do not have the mechanical properties).

Survey Instrumentation

- G-M survey meter (pancake) can detect using a thin window however, probe **Must** be at close range, 1 cm distance is recommended. G-M survey meter has low efficiency, usually 4 - 6%.
- Liquid scintillation counter (wipes, smears) may be used for secondary, but will **Not** detect non removable contamination!

Radiation Monitoring Devices

- Badges: Not needed, because ^{35}S beta energy is too low, and not an external radiation hazard.
- Dose Rate from a 1 mCi unshielded isotropic point source of ^{35}S: distance rad/Hr 1.0 cm, 1173.6 ; 2.5 cm, 93.7 ;15.24 cm, 0.2 and 20.00, cm 0.01.

General Radiation Safety Information

- Urinalysis: not required, but may be requested by Health Physics staff after a spill or personnel contamination involving ^{35}S.
- Inherent volatility (STP): **Significant** for ^{35}S methionine and cysteine.
- Radiolysis of ^{35}S amino acids (cysteine and methionine) during storage and use may lead to the release of volatile impurities. Volatile impurities are small.
- Metabolic behaviour of organic compounds of sulphur (cysteine

and methionine) differs considerably from the metabolic behaviour of inorganic compounds.

- Organic compounds of sulphur (cysteine and methionine) become incorporated into various metabolites. Thus, sulphur entering the body as an organic compound is often tenaciously retained.
- The fractional absorption of sulphur from the gastrointestinal tract is typically > 60% for organic compounds of sulphur. Elemental sulphur is less well absorbed from the GI tract than are inorganic compounds of the element (80% for all inorganic compounds and 10% for sulphur in its elemental form).
- Elemental sulphur is retained for weeks in the body. Sulphur entering the lungs in the form of gases (SO_2, COS, H_2S, and CS_2) is completely and instantaneously translocated to the transfer compartment and its metabolism is the same as that of sulphur entering the transfer compartment following ingestion or inhalation of any other organic compound of sulphur.
- Contamination of internal surfaces of storage and reaction vessels may occur (rubber stoppers, gaskets or rings).
- Vials of ^{35}S labelled cysteine and methionine should be opened and used in ventilated enclosures (exhaust hoods).
- The volatile components of ^{35}S labelled amino acids should be opened and used in ventilated enclosures (exhaust hoods).
- The volatile components of ^{35}S labelled cysteine and methionine are presumed to be hydrogen sulphide (H_2S) and methyl mercaptan (CH_3SH), respectively.
- ^{35}S vapours may be released when opening vials containing labelled amino acids, during incubation of culture or cells containing ^{35}S, and the storage of ^{35}S contaminated wastes.
- Excessive contamination can be found on the inside surfaces and in water reservoirs of incubators used for ^{35}S work. Most notable surface contamination can be found on rubber seals of incubators and centrifuges.
- Radiolytic breakdown may occur during freezing processes, releasing as much as 1.0 μCi of ^{35}S per 8.0 mCi vial of ^{35}S amino acid during the thawing process.

- ^{35}S labelled amino acids work should be conducted in an exhaust hood designated for radiolytic work.
- Vent ^{35}S amino acid stock vials with an open-ended charcoal-filled disposable syringe. Activated charcoal has a high affinity for ^{35}S vapours.
- Place an activated carbon or charcoal canister, absorbent sheet, or tray (50-100 grams of granules evenly distributed in a tray or dish) into an incubator to passively absorb ^{35}S vapours. Discard absorbers which exhibit survey meter readings above normal area background levels in the solid radioactive waste.

IODINE-125 [^{125}I]

Physical Data

- ***Gamma Energies*:** 35.5 keV (7% abundance/93% internally converted gamma) (No betas emitted), 27.0 keV (113%, x-ray), 27-32 keV (14%, x-ray), 31.0 keV (26%, x-ray).
- ***Specific Gamma Ray Constant*:** 0.27 to 0.70 mR/hr per mCi at 1 meter (current literature indicates 0.27 mR/hr per mCi at 1 meter).
- ***Physical Half-Life*:** 60.1 days.
- ***Biological Half-Life*:** 120-138 days (unbound iodine)-thyroid elimination.
- ***Effective Half-Life*:** 42 days (unbound iodine)-thyroid gland.
- ***Specific Activity*:** 17,400 Ci/gm (theoretical/carrier free).
- ***Intrinsic Specific Activity*:** 22.0 Ci/millimole.

Radiological Data

- Critical Organ (Biological Destination): Thyroid.
- Routes of Intake: ingestion, inhalation (most probable), puncture, wound, skin contamination (absorption).
- External and internal exposure & contamination concerns exist in use of ^{125}I.
- Committed Dose Equivalent (CDE): 814 mrem/mCi (thyroid/inhalation/class "D") (Organ Doses), 1185 mrem/mCi (thyroid/ingestion/NaI form), 910 mrem/mCi (thyroid/inhalation), 1258 mrem/mCi (any organ/puncture/adult).
- Committed Effective Dose Equivalent (CEDE): 24 mrem/mCi (whole body/inhalation).

Shielding

- Lead foil or sheets (1/32 to 1/16 inch thick): 0.152 mm lead foil.
- Half Value Layer: 0.02 mm - 0.008 inches.

Survey Instrumentation

- Survey meter equipped with a low energy NaI scintillation probe is necessary.
- Survey meters equipped with GM pancakes or end window GM probes are inefficient. These probes are not useful for contamination monitoring; they are only about 0.1% efficient.

Dose Rates (from unshielded 1.0 mCi Isotropic Point Source)

- Distance mrads/hr 1.00 cm 156 – 275; 10.00 cm 15.5 - 27.5, 100.00 cm 0.156 - 0.28 6.00 in 6.5 (some literature indicates 0.7 mrad/hr per mCi at 100 cm.).
- Individuals who will be using ^{125}I in the NaI or KI chemical form are required to obtain a thyroid scan to be used as a baseline reference prior to use.
- The thyroid gland accumulates 20-30% of the soluble radio-iodine taken in by the body. All radio-iodine in the body can be assumed to be eliminated quite rapidly via the urine.
- Thyroid Bioassay is required by law when handling greater than or equal to 1 mCi in the NaI or KI chemical form. In accordance with the NRC license and MSU's commitment to ALARA, the threshold amount is taken to be 0.1 mCi. The thyroid scan is to be obtained not less than 24 hours but not more than one week after the handling or use of that quantity and form of ^{125}I. In addition, all workers who assist or observe in manipulations of the above mentioned quantity and type of ^{125}I, or are sufficiently close to the process so that exposure is possible (within a few meters and in the same room) are required to obtain thyroid scans under the same conditions listed above. Fume hood sash glass provides adequate shielding for most iodination's. Extra shielding is not recommended, since it impedes air flow into the hood.
- Shielding is not required for most uses of this nuclide due to the low energy and low amounts typically used.
- Use a cannula adapter needle to vent stock vials of ^{125}I for iodination's. This prevents puff releases.
- Segregate free and bound ^{125}I waste and store in the fume hood, in tightly sealed ziploc bags (solid waste) or screw top contain-

ers (liquid waste) prior to disposal.

- Cover test tubes used to count or separate fractions from iodination with parafilm or other tight caps to prevent release while counting or when transporting outside the fume hood.

GLOSSARY

Absorbed Dose: the amount of energy imparted to matter by ionising radiation per unit mass of irradiated material. The unit of absorbed dose is the rad, which is 100 ergs/gram.
Absorption: the phenomenon by which radiation imparts some or all of its energy to any material through which it passes.
Activity: the number of nuclear disintegrations occurring in a given quantity of material per unit time.
Acute Exposure: the absorption of a relatively large amount of radiation (or intake of radioactive material) over a short period of time.
Acute Health Effects: prompt radiation effects (those that would be observable within a short period of time) for which the severity of the effect varies with the dose, and for which a practical threshold exists.
Alpha Particle: a strongly ionizing particle emitted from the nucleus during radioactive decay having a mass and charge equal in magnitude to a helium nucleus, consisting of 2 protons and 2 neutrons with a double positive charge.
Alpha Ray: a stream of fast moving helium nuclei (alpha particles), a strongly ionising and weakly penetrating radiation.
Annual Limit of Intake (ALI): the derived limit for the amount of radioactive material taken into the body of an adult worker by inhalation or ingestion in a year. ALI is the smaller value of intake of a given radionuclide in a year by the reference man that would result in a committed effective dose equivalent of 5 rems (0.05 Sv) or a committed dose equivalent of 50 rems (0.5 Sv) to any individual organ or tissue.
Attenuation: the process by which a beam of radiation is reduced in intensity when passing through some material. It is the combination of absorption and scattering processes and leads to a decrease in flux density of the beam when projected through matter.
Airborne Radioactive Material: any radioactive material dispersed in the air in the form of dusts, fumes, mists, vapours, or gases.

Becquerel: the international unit (SI) for radioactivity in which the number of disintegrations is equal to one disintegration per second. A charged particle emitted from the nucleus of an atom during radioactive decay.
Beta Particle: charged particle emitted from the nucleus of an atom during radioactive decay. A negatively charged beta particle is identical to an electron. A positively charged beta particle is called a positron.
Beta Ray: a stream of high speed electrons or positrons of nuclear origin more penetrating, but less ionising than alpha rays.
Bremsstrahlung: electromagnetic (x-ray) radiation produced by the deposition of charged particles in matter. Secondary photon radiation (x-ray) produced by the deceleration of charged particles through matter. Usually associated with energetic beta emitters, e.g. ^{32}P.
Chronic Exposure: the absorption of radiation (or intake of radioactive materials over a long period of time), i.e., over a lifetime.
Committed Dose Equivalent: the dose equivalent to organs or tissues of reference that will be received from an intake of radioactive material by an individual during the 50-year period following the intake.
Committed Effective Dose Equivalent: the sum of the products of the weighting factors applicable to each of the body organs or tissues that are irradiated and the committed dose equivalent to these organs or tissues.
Contamination Radioactive: deposition of radioactive material in any place where it is not desired, and particularly in any place where its presence may be harmful. The harm caused may be a source of excessive exposure to personnel or the validity of an experiment or a procedure.
Controlled Area: an area, outside of a restricted area but inside the site boundary, access to which can be limited by the licensee for any reason.
Critical Organ: the organ or tissue, the irradiation of which will result in the greatest hazard to the health of the individual or his descendants.
Curie: A unit of measurement of radioactivity. One curie (Ci) is that quantity of radioactive material which decays at the rate of 3.7×10^{10} disintegrations per second (dps). Commonly used submultiples of the curies are the millicurie (mCi) (1×10^{-3} Ci or 3.7×10^{7} dps) and the microcurie (μCi) (1×10^{-6} Ci or 3.7×10^{4} dps).
Decay Radioactive: disintegration of the nucleus of an unstable nu-

clide by the spontaneous emission of charged particles and/or photons.
Decontamination: the reduction or removal of contaminating radioactive material from a structure, area, object, or person. Decontamination may be accomplished by
(1) treating the surface to remove or decrease the contamination,
(2) letting the material stand so that the radioactivity is decreased as a result of natural decay,
(3) covering the contamination to shield or attenuate the radiation emitted.
Deep Dose Equivalent: applies to external whole-body exposure and is the dose equivalent at a tissue depth of one centimeter (1000 mg/cm^2).
Delayed Health Effects: radiation health effects which are manifested long after the relevant exposure. The vast majority are stochastic, that is, the severity is independent of dose and the probability is assumed to be proportional to the dose, without threshold.
Derived Air Concentration (DAC): the concentration of a given radionuclide in air which, if breathed by the reference man for a working year of 2,000 hours under conditions of light work (inhalation rate 1.2 cubic meters of air per hour), results in an intake of one ALI.
Disintegration: see decay radioactive.
Dose commitment: The total radiation dose to a part of the body that will result from retention within the body of radioactive material. For of estimating the dose commitment, it is assumed that from the time of intake the period of exposure to retained material will not exceed 50 years.
Dose Equivalent (HT): the product of the absorbed dose in tissue, quality factor, and all other necessary modifying factors at the location of interest. The units of dose equivalent are the rem and the sievert (Sv). The ICRP defines this as the equivalent dose, which is sometimes used in other countries.
Dose or Radiation Dose: a generic term that means absorbed dose, dose equivalent, effective dose equivalent, committed dose equivalent, committed effective dose equivalent, or total effective dose equivalent, as defined in other paragraphs of this section.
Dose Rate: the radiation dose delivered per unit of time. Measured, for example, in rem per hour.

Dose (can mean either): absorbed dose which is the energy imparted to matter by ionising radiation per unit mass of irradiated material at point of interest. The special unit of absorbed dose is the rad (see Rad), or dose equivalent which is the quantity for all radiation that expresses a measure of the postulated effect on a given organ. It is defined as the absorbed dose in rads times certain modifying factors. The unit of dose equivalent is the rem (see Rem).

Dosimeter: a portable instrument for measuring and registering the total accumulated exposure to ionising radiation (see dosimetry).

Dosimetry: the theory and application of the principles and techniques involved in the measurement and recording of radiation doses. Its practical aspect is concerned with the use of various types of radiation instruments with which measurements are made (see film badge; thermoluminescent dosimeter; Geiger-Mueller counter).

Effective Dose Equivalent: the sum of the products of the dose equivalent to the organ or tissue and the weighting factors applicable to each of the body organs or tissues that are irradiated.

Electron: negatively charged elementary particle which is a constituent of every neutral atom. Its unit of negative electricity equals 4.8 x 10-19 coulombs. Its mass is 0.000549 atomic mass units.

Exposure rate: the exposure per unit time such as R/min, mR/h, etc. High energy, short wavelength electromagnetic radiation emitted from the nucleus. Gamma radiation accompanies alpha and beta emissions frequently. Gamma rays are very penetrating and best shielded by lead or uranium. Gamma rays are more energetic than x-rays. E.g., Iodine-125 (I-125).

Exposure: (1) Being exposed to ionising radiation or radioactive material. (2) A measure of the ionization produced in air by x or gamma radiation. It is the sum of the electrical charges on all ions of one sign produced in air when all electrons liberated by photons in a volume element of air are completely stopped in air, divided by the mass of air in the volume element. The special unit of exposure is the Roentgen (see Roentgen).

External Dose: that portion of the dose equivalent received from radiation sources outside the body.

Extremity: hand, elbow, arm below the elbow, foot, knee, or leg below

the knee.

Eye Dose Equivalent: applies to the external exposure of the lens of the eye and is taken as the dose equivalent at a tissue depth of 0.3 centimetre (300 mg/cm^2).

Film Badge: a packet of photographic film used for the approximate measurement of radiation exposure for personnel monitoring purposes. The badge may contain two or more which shield parts of the film from certain types of radiation

Gamma Ray: very penetrating electromagnetic radiation of nuclear origin. Except for origin, identical to x-ray.

Gray: the international (SI) unit of absorbed dose in which the energy deposited is equal to one Joule per kilogram (1 J/kg).

Half Value Layer: the thickness of any specified material necessary to reduce the intensity of an x-ray or gamma ray beam to one-half its original value.

Half Life Biological: time required for the body to eliminate 50 percent of a dose of any substance by the regular processes of elimination. This time is approximately the same for both stable isotopes and radionuclides of a particular element.

Half Life, Effective: time required for a radioactive nuclide in a system to be diminished by 50 percent as a result of the combined action of radioactive decay and biological elimination.

$$\text{Effective half-life} = \frac{\text{Biological half-life x Radioactive half-life}}{\text{Biological half-life + Radioactive half-life}}$$

Half-Life Radioactive: time required for a radioactive substance to lose 50 percent of its activity by decay. Each radionuclide has a unique half-life.

Half-life: the time in which half the atoms of a particular radioactive substance disintegrate to another nuclear form.

High Radiation Area: an area, accessible to individuals, in which radiation levels could result in an individual receiving a dose equivalent in excess of 0.1 rem (1 mSv) in one hour at thirty centimetres from the radiation source or from any surface that the radiation penetrates.

Ionising radiation: any electromagnetic or particulate radiation capable of producing ions, directly or indirectly, in its passage through mat-

ter. Ionising radiation includes gamma rays and x rays, alpha and beta particles, high speed electrons, neutrons, and other nuclear particles.
Ionisation: the process by which a neutral atom or molecule acquires either a positive or a negative charge.
Isotopes: nuclides having the same number of protons in their nuclei, and hence having the same atomic number, but differing in the number of neutrons, and therefore in the mass number. Almost identical chemical properties exist between isotopes of a particular element.
Occupational dose: exposure of an individual to ionising radiation: (1) in a restricted area, or (2) in the course of employment in which the individual's duties involve exposure to ionising radiation, provided that occupational dose shall not be deemed to include any exposure of an individual to ionising radiation for the purpose of diagnosis or therapy of the individual.
Personal monitoring equipment: devices (e.g., film badges, pocket dosimeters, and thermo-luminescent dosimeters (TLDs)) designed to be worn or carried by an individual for the purpose of estimating the dose received by the individual.
Rad: the special unit of absorbed dose. One rad equals 0.01 joule per kg (100 ergs per g) of material; for example, if tissue is the material of interest, then 1 rad equals 0.01 joule per kilogram of tissue.
Radiation: a radiation is the emission of energy in the form of waves or particles. Radiation waves are described by their wavelength and frequency.
Radioactive decay: the decrease in the amount of any radioactive material with the passage of time due to the spontaneous emission from the atomic nuclei of either alpha or beta particles, or gamma radiation.
Radioactive material: any material (solid, liquid, or gas) which emits radiation spontaneously.
Radioactive waste: solid, liquid, and gaseous materials from nuclear operations that are radioactive or become radioactive and for which there are no further uses. Radioactivity. The disintegration of unstable atomic nuclei with the emission of radiation.
Rem: the special unit of dose equivalent. One millirem (mrem)= 0.001 rem. Generally speaking, any of the below is considered to be equal to one rem: (1) an exposure of 1 R of x-ray or gamma radiation; (2) an

absorbed dose of 1 rad due to x-ray, gamma, or beta radiation; (3) an absorbed dose of 0.05 rad due to particles heavier than protons and with sufficient energy to reach the lens of the eye; or (4) an absorbed dose of 0.1 rad due to neutrons or high energy protons.

Restricted area (controlled area): any area access to which is controlled by the licensee or registrant for purposes of protection of individuals from exposure to radiation and radioactive material.

Roentgen: the special unit of exposure. One roentgen (R) equals 2.58 x 10^{-4} coulombs/kilogram of air.

Shielding Material: any material which is used to absorb radiation and thus effectively reduce the intensity of radiation, and in some cases eliminate it. Lead, concrete, aluminum, water and plastic are examples of commonly used shielding material.

Sievert: the international unit (SI) of dose equivalent (DE, human exposure unit), which is equal to 100 rem. It is obtained by multiplying the number of grays by the quality factor, distribution factor, and any other necessary modifying factors

Specific Activity: total radioactivity of a given nuclide per gram of a compound, element or radioactive nuclide.

Thermo-luminescent Dosimeter (TLD): crystalline materials that emit light if they are heated after being they have been exposed to radiation.

Total Effective Dose Equivalent (TEDE): the sum of the deep dose equivalent (for external exposures) and the committed effective dose equivalent (for internal exposures).

Unrestricted area (uncontrolled area): any area access to which is controlled by the licensee or registrant for purposes of radiation protection of individuals from exposure to radiation and radioactive material.

Wipe sample: a sample made for the purpose of determining the presence of removable radioactive contamination on a surface. It is done by wiping, with slight pressure, a piece of soft filter paper or a cotton swab over a representative type of surface area (normally about 100 cm^2).

X-rays: penetrating electromagnetic radiation (photons) having a wavelength that is much shorter than that of visible light. These rays are usually produced by the excitation of the electron field around certain nuclei. Similar to gamma rays.

WORKERS' HEALTH SURVEILLANCE

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Introduction

Workers' health surveillance is a generic term which covers procedures and investigations to assess workers' health in order to detect and identify any abnormality. The results of surveillance should be used to protect and promote the health of all the workers and the health of other individuals at the workplace. Health assessment procedures may include - but are not limited to - medical examinations, biological monitoring, radiological examinations, questionnaires or a review of health records.

Workers' health surveillance should be an essential component of programmes aimed at the protection of workers, and such programmes should provide the medical examinations prescribed by law. A comprehensive system of workers' health surveillance includes individual and collective health assessments, occupational injury and disease recording and notification, sentinel event notification, surveys, investigations and inspections.

Principles of health surveillance

Aims and organisation

Health surveillance is considered a tool aimed at maintaining workers' health and protecting them by assessing exposure and detecting any early biological effects.

Assessment of workers' health is one of the main components of any programme of prevention in the workplace. Medical examinations are the most common method of health assessment for individual workers.

Medical examinations and consultations, either as part of screening programmes or on an as-needed basis, serve five main purposes:

- Evaluation of the effectiveness of control measures in the workplace.
- Detection of pre-clinical and clinical abnormalities at a point when intervention is beneficial to individuals' health.
- Prevention of further deterioration in workers' health.
- Reinforcement of safe methods of work and of health maintenance.
- Assessment of fitness for a particular type of work, the present concern being the adaptation of the workplace to the worker.

Health surveillance is widely considered a composite activity of secondary prevention, and is based on periodic health checks for workers with a view to protecting their health and preventing work-related illnesses. The aim is to identify any changes to the state of health early – if possible still at the sub-clinical stage – by checking the function of organs and systems that might be affected by risk factors in the work environment. It also aims to detect any changes in health that, although they are not consequences of exposure, might be aggravated by specific types of work or might interfere with normal working activity.

Health surveillance should be an integral part of broader occupational health promotion schemes designed to identify risk factors and modalities of exposure. Risk assessment is essential for planning health checks, as underlined by the European Union (EU) directives on this subject.

The aims of health surveillance (Table I) comprise health protection and prevention of occupational disease in the widest sense, including prevention of damage but also prevention of discomfort, and when this is done using adequate means it can provide a basis for future well being. The goal is achieved when a worker is assigned a job that fits his abilities, with no negative repercussions on his own or others' health. This can be achieved by:

- establishing general fitness for the job, bearing in mind the possibility of it beng changed
- assessing any conditions that might constitute contraindications to jobs involving specific risks

- assessing any conditions that might get worse in the future as a result of the job assigned
- drawing up an initial picture against which to compare future conditions

Table I – General principles of Health Surveillance

- Health surveillance is part of the discipline of occupational health and safety.
- Its main aim is primary prevention of work-related accidents and occupational diseases.
- It should be related to the specific risks involved in each type of work.
- The procedures should be periodically reassessed and must not be considered mere routine.
- It should preferably be part of an occupational health service, organized according to Convention no. 171 (which deals with occupational health services) adopted by the ILO in 1985.

Health surveillance follows in the footsteps of risk assessment. Before a worker is hired a check should be made whether s/he is likely to be suitable for the specific job in mind and – apart from subsequent risk assessments – it may already become evident at that visit that further health checks will be necessary. In any event workers medical examinations should be scheduled on the basis of risk assessments (Table II). Changes in the occupational risks in a workplace may influence the health surveillance program (schedule of medical visits, types of clinical tests, vaccinations, etc.).

Table II – Organization of health surveillance

- Medical examinations to check workers' health.
- Biological tests and other medical investigations.
- Notification and data recording system.
- Workplace inspections.
- Epidemiological research.

The occupational physician has various tools besides visits and clinical examinations for checking workers' health. These include:

- checks to be made before a worker is hired (pre-hiring check);
- before a worker is transferred to a new job (pre-transfer check);
- periodical checks after hiring (periodic checks);
- checks when a worker leaves.

All health data must be collected, processed and communicated in full respect of confidentiality and personal privacy. Health findings are intended to be used to protect workers' health and suitability is assessed only in relation to a specific job.

Contents of workers' health surveillance

The occupational physician who has to establish, at pre-hiring visits or subsequent periodic checks, whether a worker is fit to be exposed to certain risk factors applies the principles of occupational medicine. These involve verifying that the worker's psychophysical conditions are compatible with the specific individual risks involved in his task and workplace.

Depending on the type of risk involved, the physician will take particular account of the following physio-pathological conditions when assessing whether a worker is suitable for a job:

- Conditions that might be activated or aggravated by exposure to the risk factor.
- Conditions that might enhance the absorption of chemical, physical or biological agents or reduce physiological detoxification and/or excretion mechanisms.
- Conditions that might be confused with pathologies resulting from exposure to chemical, physical or biological agents and attributed to their action.

Depending on the nature and entity of the risk and the type of work concerned, any mental or physical conditions that might create problems as regards safety at work must be taken into account, as well as any anomalies or pathologies that could limit the use of personal protection measures.

During medical examinations and consultations, it may be appropriate for the occupational physician to:

- Inform workers of potential injuries or diseases and the measures necessary for their prevention.
- Inform workers of potential diseases and conditions of work or exposures which are medically contraindicated, and advise them

where they can get help in the treatment or correction of their condition.
- Inform workers and their employers of the effectiveness or otherwise of control measures.
- Help the employer place workers in occupations that take into account their capacity for particular work.
- Draw young persons' attention to their physical and mental aptitudes, in order to facilitate appropriate vocational guidance.
- Prevent the total exclusion of any worker from employment, and provide for the employment of each worker, despite any contraindications, in work s/he is capable of doing, taking into account the employment opportunities available.

Medical examinations and tests should not be carried out as perfunctory routine and their value and relevance should always be given due consideration. They should be governed by a set of principles including:

- selecting appropriate tests that are acceptable to workers
- discarding tests that cannot meet requirements for relevance, specificity and sensitivity
- periodically reviewing health surveillance programs as a whole and modifying them in the light of improved working conditions

The procedures for medical examinations comprise a personal history and a clinical examination. They may include questionnaires, diagnostic tests, function measurements and biological tests of exposure levels to environmental agents in the workplace. These examinations should be relevant to the nature of the hazards. Occupational health physicians or medical practitioners engaged in occupational health practice should retain overall responsibility for biological tests and other medical investigations, as well as for the interpretation of results, although tests can be done by nurses, technicians and other trained personnel under their supervision.

Medical examinations should be done, where appropriate, before or shortly after employment or assignment, to collect information and to serve as a baseline for future health surveillance.

Medical examinations may be planned at periodic intervals during employment and should be appropriate to the occupational risks of the

enterprise. These examinations may also be scheduled:

- on resumption of work after a prolonged absence for health reasons, for the purpose of determining any possible occupational causes, recommending appropriate action to protect workers, and determining suitability for the job or the need for reassignment and rehabilitation;
- at a worker's request, for example, when s/he changes work and, in particular, when a worker changes work for medical reasons.

In some cases, occupational health physicians may be required to carry out a medical examination of workers on or after the cessation of their assignment or employment, in order to establish a final bill of health and, taking into account the information provided by previous periodical examinations, to assess the effects job assignments may have had on the workers' health. Continued post-employment surveillance using medical examinations may be desirable for people who have been exposed to agents with delayed effects, for the purposes of ensuring early diagnosis and treatment of such diseases as skin, lung, or bladder cancer.

Medical examinations should serve for prevention and protection purposes which include not only the protection and promotion of workers' health, but also the protection of access to work, entitlement to compensation, health insurance benefits and social protection. Under no circumstances should medical examinations for employment be used as a substitute for measures to prevent and control hazardous exposures. Medical examinations should be used to improve working conditions in such a way that they will facilitate the adaptation of work to workers.

Results of periodic examinations, in combination with information on environmental exposure levels, can be used to verify the level of protection provided by exposure limits and to contribute to their revision. In addition, such examinations may often be used to identify possible health effects of changes in working methods, work organization, working conditions, new technology, or working process or new products.

In the case of exposure to specific occupational hazards, in addition

to the health assessments described above, the surveillance of the workers' health should include, where appropriate, any examinations and investigations which may be necessary to detect exposure levels and early biological effects and responses.

When a valid and generally accepted method of biological monitoring of the workers' health exists for the early detection of the effect on health of exposure to specific occupational hazards, it may be used to identify workers who need a detailed medical examination, subject to individual consent.

Biological tests and other investigations

Specifically designed biological tests and other investigations to detect any signs of organic disorders or potentially harmful exposures as early as possible are available and are widely used. In most cases, they are an integral part of the medical examination. Such investigations are subject to the workers' informed consent and must be conducted according to the highest professional standards, with the least possible risk.

Biological tests and other medical investigations must be carried out under the supervision of a physician and are subject to medical confidentiality; they must be relevant to the protection of the health of the worker concerned, with due regard to their sensitivity, specificity and predictive value.

When it is possible and appropriate to make a choice, preference must always be given to non-invasive methods and to examinations which do not pose any danger to the worker's health. An invasive investigation or an examination which involves a risk to a worker's health can be recommended only after an evaluation of the benefits to the worker and the risks involved, and cannot be justified in relation to insurance claims.

Biological monitoring tests, which are simple and have the best validated action levels, are particularly useful in workers' health surveillance when properly used, and are cost-effective when used for the individual or collective monitoring of exposed workers. However, they should not be a substitute for surveillance of the working environment and the assessment of individual exposures. Priority should

be given to environmental criteria (exposure limits) over biological ones (biological exposure and limits). Values commonly found in the general public should be taken into account when assessing the significance of results of biological monitoring.

Workers' health surveillance may be prescribed by law (Table III) or not, and may be compulsory or voluntary.

TABLE III - Main directives concerning workers' health surveillance

NUMBER	TOPIC	Section No.
89/391	Occupational safety and health improvement	14
90/394	Cancerous risk	14 (App. II)
2000/54	Biological risk	14 (Annex IV)
97/42	Cancerous risk	
90/394	Cancerous risk modified for the first time	14 (App. II)
98/24	Chemicals	10
99/38	Cancerous risk	
90/394	Cancerous risk modified for the second time	14 (App. II)

Health surveillance in biotechnology research

Potential hazards in a biotechnology laboratory or manufacturing facility include exposure to biological agents (including recombinant and non-recombinant microorganisms), chemicals and a variety of physical hazards.

Biological hazards

Biotechnology research workers can come into contact with numerous biological agents. Work processes often require them to use – or expose them to – organic materials contaminated with infectious or potentially infective organisms (viruses including oncogenic strains; bacteria; parasites; fungi; cell cultures; human or biological samples; laboratory animals; recombinant DNA, etc.).

Directive 2000/54/EEC concerns protection of workers handling microorganisms, cell cultures and human endoparasites capable of causing infections, toxic effects and allergy, including those employed in biotechnologies. Section 14 prescribes health surveillance of workers exposed to biological agents, without providing for specific medical examinations. Appendix IV specifies that the physician responsible for occupational health surveillance should be aware of each worker's conditions and circumstances of exposure and that health surveillance should comprise the assessment of workers' health and, if necessary, biological monitoring in order to ascertain early and reversible effects.

Risks for human health related to occupational exposure to genetically modified microorganisms concern three main areas:

- immunopathologies, especially allergies
- toxic effects, especially those linked to the production of complex species- specific proteins
- infections related to wild-type microorganisms handled

Risk assessment

Although workers today are generally highly aware of biological risk, particularly since the spread of HIV pathologies, they tend to be much less sensitive about what can be done to prevent it. This is especially true in comparison with other occupational risks such as chemical and physical hazards, or with the risk factors for common chronic degenerative disorders.

Some comments on the characteristics and mechanisms of infectious diseases in general will be useful to understand the importance of risk assessment and the measures for checking and preventing biological risk. An **infection** gains ground when the number of infectious agents reaches a level where either directly, or through the production of toxins, it affects a large enough number of cells – usually with special or unique functions – to induce illness.

The number of infectious agents needed to turn a minor or local infection into overt disease can be expressed as the **infectious load** or burden, or as the minimum amount of the biological agent entering the organ needed to trigger the pathogenic process. It varies in any depending on the degree of pathogenicity of the causal agent, and on

the strength of the host's immune defences. A minor infection becomes overt disease whenever the causal agent is pathogenic enough to overcome the host's defences, or if those defences are impaired to the extent that they cannot fight off even a light infectious burden.

How **contagious** an infection is depends mainly on the amount of pathogens eliminated by the infected organism, or present in the environment, on the routes of elimination and transmission, and how long the pathogen can survive outside a suitable host. It also depends on the presence and concentration of the pathogen in biological fluids - usually blood – which are transmissible by certain carriers.

The process of infection involves several steps: contamination of skin or mucous membranes by the microrganism; penetration of the microorganism into deep tissues and access to the bloodstream or lymphatic system; localization in certain organs and/or tissues; infection; dynamic relation between the pathogen and the host organism, and active response from the host's immune system. Clinical symptoms indicative of the start of the disease are usually only detectable when the infection has completed its incubation period and affected a certain number of cells or vital organs.

The **routes of transmission** that can increase the risk of infection for health workers include:

- **direct contact or by droplet particles**
- **airborne droplet nuclei**
- **parenteral transmission**
- **oro-fecal transmission**

Risk assessment is therefore essential for health surveillance and is in fact one of its basic criteria. The assessment of biological risk poses different problems from chemical or physical risk, which are both measurable.

Problems of health surveillance

To cover these various points, a health surveillance program needs to take account of several factors:

- The intrinsic dangers of the biological agent. These may vary widely depending on how it is transmitted, its degree of

infectiousness, pathogenicity, transmissibility, neutralizability.

- The objective difficulty of measuring exposure to biological agents. It is generally complicated to quantify and identify bacterial, viral or fungal species dispersed in the air, and there are no standard methods or reference parameters. In addition, these microrganisms not only present a wide biochemical and morphological variety but also tend to be ubiquitous, adding to the difficulties of environmental monitoring.
- No dose-response curves are available so this relation cannot be established for biological agents, for their infective, toxic or allergic effects. It is thus impossible to quantify the risk and the damage it might produce.
- We do not know the threshold dose below which there is no health risk. For many microorganisms the minimal infectious dose is around unity, meaning that mere contact with the biological agent is enough to trigger infection and disease.
- There are no exposure limits against which to assess risk.
- Some factors are intrinsic to the worker, who may present conditions that raise the risk of contracting infectious diseases, such as congenital or acquired immune deficiency. Such conditions must obviously be carefully identified, and their severity evaluated, following each case individually.

It is clear therefore that no set schedule of periodic medical examinations can be established for infectious conditions. Problems must be tackled case by case as they arise, using the range of "tools" available as a basic part of health surveillance:

a) Surveillance of accidental exposure of workers

This implies assessing the incidence and follow-up of any accidents involving biological risk. An enquiry can be made to obtain details of the accident, recording how it happened – prick, cut, contact with healthy or broken skin, or with mucous membranes.

b) Information from workers' health surveillance

This is the pivot for preventive measures. At this stage further or broader investigations may be necessary, either when the worker is first hired, or when s/he is being checked for transfer to a post

involving exposure to biological agents. Such situations arise, for instance, in health care. In other circumstances, where the biological risk is more circumscribed and easier to identify, investigations can be targeted more precisely.

c) Medical history

A targeted medical history is of course the first step in an investigation of this sort and serves to complete the family, physiological and pathological data already collected in the worker's general health record. We find questionnaires useful in these situations, for a close assessment of:

- the **working history,** with a description of the type of job or activity at biological risk, the work methods, classified on the basis of their level of risk – high, medium, low – and frequency – occasional, frequent, continuous. Each worker can therefore be classified on the basis of the real risk to which s/he is exposed;
- **extra-occupational activities** that might involve biological risk. Examples include farming, animal breeding, first aid work, hunting and fishing;
- **lifestyle habits** or situations involving biological risk. Examples include intravenous drug use, piercing, tattooing, visits to foreign countries in zones considered at risk;
- the worker's **history of infectious diseases**. This involves checking whether the worker has suffered infectious diseases in the past, has undergone major surgery or invasive diagnostic procedures, dialysis, blood transfusions, acupuncture, major dental work;
- checking for hypersusceptibility states. This is fundamental in preventive examinations. Generally speaking, conditions of individual hypersusceptibility to biological risk include pathologies of the skin and mucous membranes which reduce their barrier properties, active inflammation, congenital or acquired immune deficiencies, pregnancy, lack of vaccination.

d) Assessment of immune status

A worker's immune status can be assessed by laboratory tests, calibrated to take account of the specific working environment. Specific vaccines are now available (Tables IV and VII) for workers who are not already immune to the biological agent(s) they are likely to encounter in their job, which can be administered by the health

surveillance doctor. The physician must examine each case individually, in relation to the biological risk involved, to establish whether the worker needs a vaccine, assessing its efficacy, the duration of immunity it confers, and any contraindications.

e) Medical examinations

These are certainly important for assessing the worker's general health, checking the various organs and systems that might be targets for biological agents, and investigating clinical signs of pathologies that might cause individual hypersusceptibility.

However, clinical examination is known to have limited sensitivity for early detection of biological effects.

f) Laboratory tests

At the prevention stage, tests can be made to assess general health. These include a complete blood count with leucocyte formula, blood glucose, serum creatinine, transaminases, gamma-GT, serum protein electrophoresis, complete urinalysis.

g) Periodic medical examinations

Current legislation sets no specific requirements for health visits for workers at biological risk. The physician must therefore decide which workers should be put under surveillance and how.

Conclusions

Health surveillance is clearly one of the factors which, together with risk assessment as described above, serves to control biological risk in the workplace.

Epidemiological surveillance is a cornerstone of this approach, and an "observatory" on these lines should be set up in every workplace. This involves risk identification and quantification, and classification of work areas, jobs or activities by level of risk.

With this sort of structure in place better use can be made of risk assessment and health surveillance data, health surveillance will be more efficient and of its quality control will be better. Useful information will also be obtained for establishing the priority of preventive measures and implementing them.

For work involving handling genetically modified microorganisms (GMMs), diagnostic tests indicating exposure, contact and possible colonization and/or infection by GMMs and their products should be

taken into account. For example, for workers handling class II GMMs, infection caused by retroviral vectors can be detected by monitoring the expression of retroviral proteins, seeking infectious viruses and identifying the immunological response to them. Handling class I GMMs, though at low risk for adverse effects on human health, could involve a risk of contamination with genetic products and, since some of these may be employed as vectors for gene transfer into eukaryotic cells, they could – though it is highly improbable - become inserted in the genome of exposed workers.

Table IV – Biological agents for which a vaccine is available

Bordetella pertussis	Yellow fever
Clostridium tetani	Viruses
Corynebacterium diphtheriae	hepatitis A, B, D
Haemophilus influenzae	influenza A,B
Mycobacterium africanum	measles
Mycobacterium bovis	parotitis
Mycobacterium tuberculosis	poliomyelitis
Neisseria meningitis	monkeypox
Salmonella paratyphi A, B, C	smallpox (major)
Salmonella typhi	whitepox (variola minor)
Yersinia pestis	rabies
Rift Valley fever	Eastern American equine
Central European tick virus encephalitis	encephalomyelitis
Japanese B encephalitis	Venezuelan equine encephalomyelitis
Kyasanur forest	Western American equine encephalomyelitis
Omsk	Rubivirus (*rubella* - German measles)
Russian summer-winter encephalitis	

Chemical hazards

Workers engaged in biotechnology research are exposed to a variety of potentially harmful chemicals. The types of exposure differ from that in other occupational environments in several respects. First, they include many agents known for their genotoxic action. Second, the combined or individuaI toxicity of these agents for humans may not be fully appreciated, and adequate decontamination or destruction techniques are not always available or routinely used. Finally, techniques change fast in biotechnology research, especially with the development

of molecular biology, and the consequent changes in the products used. These features, combined with a very competitive work environment, explain why the objective surveillance of work conditions, essentially based on the degree of compliance to "Good Laboratory Practice" and specific health surveillance, is extremely difficult.

The range of agents handled today in biotechnology research encompasses solvents, alkylating, intercalating, and promoting agents; aromatic amines; hormones; heavy metals, etc. Many substances such as solvents were once thought to be innocuous and were handled without precautions, before it was suggested at the end of the 1970s that they may have long-term adverse effects. In contrast, substances with known high immediate toxicity were manipulated with greater care, but their long-term effects at low levels of exposure were unknown.

The use of hoods in laboratories became widespread from the 1970s, as did the development of instrument-based methods that require smaller amounts of chemicals.

These developments, and probably better skin protection with gloves, have reduced levels of laboratory exposure substiantially, but the sources are numerous.

The multiple exposures encounted today introduce an additional methodological difficulty in the design and implementation of epidemiological studies. In contrast to chemical industry process workers, people working in laboratories are exposed to a larger variety of agents from different sources - biochemical, physical, or biological - but probably at lower levels. This may explain why no study has yet quantitatively assessed specific exposure. In fact, most studies did not assess exposures at all. Another methodological difficulty arises from the use of mortality data, instead of incidence data.

The substances most widely used in laboratories act either acutely or chronically. The effects are related to the exposure time and the amount of the substance in the ambient air, the route of penetration, and the physico-chemical properties of pollutants present. The effects can be influenced by the presence of other chemicals, by the use of medicines or substances of abuse.

The characteristics of multiple low-dose exposure to chemicals make it extremely complicated, in a health surveillance program, to identify specific indicators to show up the preclinical signs induced by any single substance.

For some chemicals estimation of exposure through monitoring is based on a lot of studies with uniform results and is reliable, while for others there is still wide uncertainty. Health risk assessment using biomarkers of exposure is only possible for a few chemicals. Table V lists some of the chemicals used in biotechnology research laboratories and the biomarkers available.

One particular point is the possibility of using specific tests known as "indicators of integrated exposure", particularly when one does not know the exact composition of the substances to which a worker is exposed, or when the concentrations of some of them are too low to be measured. Tests for assessing the function of hepatic microsomal enzymes, for instance, provide indicators of integrated exposure for biological monitoring of workers exposed to solvents.

Table V - Main chemical agents used in laboratories, their carcinogenicity and biomarkers of exposure

CHEMICALS IN THE LABORATORY	BIOMARKERS OF EXPOSURE
Acetone	Acetone in urine
Arsenic*	Arsenic inorganic metabolites in urine
Benzene*	S– henyl mercapturic acid in urine
Luminol	
M - xylene	Methyl hippuric acid in urine
Mercury	Total mercury in blood and in urine
Methanol	Methanol in urine
O - xilene	Methyl hippuric acid in urine
P- xylene	Methyl hippuricacid in urine
Phenol	Phenol in urine
Phenylmethylsulfonyl fluoride	Fluoride in urine
Tetrahydrofuran	
Toluene	O – cresol in urine, hippuric acid in urine and toluene in blood
Trichloro ethylene*	Trichloroacetic acid and trichloroethanol in urine, trichloroethanol in blood, trichloroethylene in expired air, trichloroethylene in blood
Xylene	Methil hyppuric acid in urine

*** Carcinogenic**

Carcinogenic risk

Some of the chemicals used in the laboratory are carcinogenic. Health surveillance with regard to these substances is one step in a strategy of prevention (Table VI) which is only effective, however, if all measures are applied to the full and simultaneously. From the point of view of prevention, if only some of them are implemented the result will not be satisfactory.

Table VI – Prevention strategies for workers exposed to carcinogens

- Assessment of the degree, nature and duration of exposure
- Replacement or reduction of the use of the carcinogenic substance
- Adoption of closed systems
- Reduction of exposure to the lowest level technically possible
- Limitation of the amounts of a carcinogenic substance
- Limitation of the numbers of workers exposed
- Evacuation of carcinogens at source
- Application of adequate working procedures and methods
- Collective and/or personal protective measures
- Prohibition on eating, drinking and smoking in all areas where there is any risk of contamination by carcinogenic substances

An appropriate health surveillance schedule for all workers must be established before exposure and at regular intervals during the work.

No specific tests can be recommended for early biological effects since their predictive value as regards the subsequent appearance of a tumor is doubtful, and there are no valid criteria for attributing any change from normal to specific agents present in the workplace. In addition, even if the tests give negative results there is no reliable way of assuring there are no other potential effects.

Nevertheless, there are regulations obliging employers to maintain health surveillance on workers exposed to carcinogens. General indications specify the need for thorough health examinations, at least once a year, with supplementary tests as appropriate, mainly useful as an opportunity to explain to workers the significance and limits of the health surveillance procedures and the general and specific rules for prevention.

Clearly this approach rightly gives priority to primary prevention, with perhaps too little attention to secondary measures.

It seems "ethical", once a worker has been identified as exposed to carcinogens, to provide for the best surveillance science can offer. It therefore seems reductive to discourage outright the use of early tests as a basis for prompt action in the early stages of carcinogenesis, or at least in the preclinical stages. It is more important to evaluate their true significance.

In recent years various means have been proposed, and some are now feasible, to detect "early effects", and it is worth looking at their theoretical basis before considering their practical utility.

A series of markers, referred to generally as "cytogenetic", reflect alterations to the cell's genetic equipment; the tests mainly detect chromosomal aberrations, sister chromatid exchange (SCE), and micronuclei. These are all non-specific markers of biological response.

SCE is used mainly for chemical carcinogens and was first studied in patients receiving antitumoral drugs. SCE is induced acutely and is no longer seen once the patient completes the therapy. These local aberrations – in the chromatids – are affected by confounding factors, particularly cigarette smoking, but are considered more sensitive than chromosomal aberrations, and can be assessed faster and more simply. They are markers of early lesions, not specific for any particular chemical, so they cannot help identify, even less quantify, the causal agent. They therefore have "group value", as long as confounding factors are taken into due consideration.

Counting micronuclei in peripheral lymphocytes is an even faster test than SCE and chromosomal aberrations, and the alteration persists. However, it is still hard to quantify it accurately.

To give an overall judgement of the three most widely employed cytogenetic indicators, it is essential to bear in mind that in each individual they are at the least a marker of personal exposure. *In vitro* and in animals positive correlations have been found between their appearance and cytotoxicity, mutations, cell transformation and tumor formation. They have predictive value for a tumor if they are found in stem cells, as happens with rare tumors such as retinoblastoma. It is advisable to use them in association with adducts.

It has been suggested that a definite finding of chromosomal aberrations – when these can be detected – could serve as an indicator

not only of exposure, but also of an effect on health, but there is still controversy about this. These aberrations reflect cumulative exposure and indicate a group genotoxic effect. If they are confirmed repeatedly in comparison with the indispensable control group, they should be considered an alarm signal. This is mainly because in practice such effects are rarely very marked. In addition, in occupationally exposed populations no significant correlation has ever been reported with the "response", i.e. tumor onset.

What therefore is the best way to approach this wide range of risks and targets? Health surveillance can take one or more of several paths. If we know which oncogen is involved and which tumor it may induce, we can plan a specific approach, comprising measurement of internal doses, including molecular dosimetry, and can rationally select indicators of a biologically effective dose, and of the early biological effects in somatic or germinal cells. If, however, we do not know which specific agent is responsible for which "occupational" tumor the problem is much more complicated and no practical solutions are yet available. The use of aspecific indicators such as urinary mutagenicity and chromosomal aberrations is suggested, but the results can easily be interpreted ambiguously.

Physical hazards

Ionizing and non-ionizing radiation are among the physical risks that may be present in a biotechnology laboratory.

Ionizing radiation

Medical surveillance in persons exposed to sources of ionizing radiation such as X-rays, ^{32}P, etc. involves – very briefly – a series of specific diagnostic, prognostic and therapeutic interventions and various assessments, such as the following:

- preventive examinations and periodical verification of the person's suitability for a particular job, on the basis of the specific risk involved; planning and evaluation of specialist visits and biological

tests; transmission of the conclusions on the person's suitability for that job to the employer;
- diagnostic and therapeutic intervention in cases of over-exposure or accidental contamination;
- legal-medical work regarding health files, measurements of exposure levels, as required by law, assessments, reports and notifications of accidents and occupational diseases.

There are at least three approaches to the rational organization of a program for preventive and periodical medical surveillance of workers exposed to the risk of ionizing radiation.

a) clinical, designed to identify any change in the state of health of each worker that might be attributed to radiation, using specific diagnostic tests;
b) epidemiological, based on clinical observation of homogeneous groups of workers exposed to similar risks in similar ways;
c) preventive, designed to assess each worker's suitability for the job, and bring to light any clinical contraindications to occupational exposure to the specific risk involved.

Radioprotection is increasingly becoming a multidisciplinary field, with heavy emphasis on physics, medicine, and biology, and on technical and ecological factors, and an important interface with the social sciences. The ICRP recommendations are generally acknowledged as the most authoritative means of reaching conclusions in this area, where there is a pressing need for studies of the fundamental aspects of the interactions between radiation and biological materials. A "modern" radiobiology is needed, taking account of progress in related areas, including the latest findings related to molecular biology.

Non-ionizing radiation

Under this heading we shall focus on ultraviolet radiation rather than on radiofrequencies and magnetic fields, which are not particular risks in a laboratory.

Ultraviolet rays are the part of the spectrum of electromagnetic waves that lies between X rays, which are the longest, and visible light, whose

wavelengths are short. As the frequencies in the UV spectrum rise, four zones can be distinguished:

- the "near" zone, which is close to the visible, where the UV wavelengths are between 320 and 400 nm. This is the UV light from the sun that is absorbed by the ozone in the upper atmosphere;
- the "middle" zone, between 320 and 280 nm, where the light can pass through glass;
- the "far" zone, between 280 and 160 nm, where the light is absorbed by glass but transmitted through the air and through quartz;
- the "vacuum" zone, below 160 nm, where the rays can only exist in a vacuum or in a confined inert gas.

In the laboratory, persons may be exposed to UV rays in various situations: in premises being sterilized with UV lamps; when detecting chemicals under UV lamps (at a wavelength of 366 nm) in thin-layer or paper chromatography, and during electrophoresis.

The most common effects of occupational exposure are skin rash, keratoconjunctivitis, chronic keratosis of the exposed parts, skin cancers.

Medical surveillance for optical or laser-related radiation risk basically involves oculistic and dermatological inspection, and checking the person's general health applying the normal criteria of preventive medicine. The oculist should check visual acuity and the fundus of the eye, making a slit-lamp examination; retinography – especially in color - is a worthwhile supplement to the clinical documentation. The dermatologist can include a photographic record, and skin examination under Wood's light.

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PRECAUTIONS FOR THE USE OF BIOLOGICAL AND CHEMICAL AGENTS

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In biotechnology research laboratories a large variety of biological agents and chemical compounds are manipulated which can be dangerous for the health and for the environment. A list of all the agents and compounds used and therefore of the risks present in a laboratory would result in many doubts. The present manual limits itself to providing information which allows researchers to define the precautions to take even if precise indications do not exist in the current legislations.

In tables IV the principle chemical and physical agents used for decontamination, disinfection and sterilisation are shown and in tables V and VI the principal sources of biological and chemical risk are reported respectively. The tables provide useful indications in order to avoid or diminish contamination during operations that are performed more frequently in laboratories (David, 1997; Sambrook et al. 1989):

- amplification and Polymerase Chain Reaction (PCR) for DNA and RNA
- analysis and manipulation of DNA, RNA and oligonucleotides
- creation of recombinant DNA banks-preparation of inserts for cloning
- extraction of DNA from animal, plant and bacterial cells
- extraction of plasmidic DNA
- introduction of DNA in mammalian cells, plant and bacterial cells
- genetic sequencing
- hybridisation techniques
- use of vectors in the preparation of DNA from phage lysates

However remember that before using a chemical compound it is necessary to carefully read the toxicological cards and get information on the precaution to use.

Table IV. Principle chemical and physical agents used for Decontamination* (De), Disinfection (Di) and Sterilisation*** (St)**

CHEMICAL OR PHYSICAL AGENT	ACTIVITY	ADVANTAGES	USE	OBSERVATIONS
Alcohols (ethanol, isopropyl alcohol) *De, Di*	- denatures proteins - inhibits cell metabolism	- low cost - low toxicity - high bacterial and mycobaterial activity - little activity on viruses - no interaction with detergents	- excellent skin (intact) antiseptic	- inactivated by dirt - poorly inactivated by proteins, natural and synthetic materials - easily flammable - low toxicity for the eyes - no activity on fungi and spores
Aldehydes *St*	- denatures proteins - alkylates DNA and RNA	- active on bacteria , mycobacteria, spores (above 40°C Formaldehyde, above 20°C Glutaraldehyde) and fungi - very low viral activity	- sterilisation of heat labile instruments	- temperature, contact time and pH dependent - high toxicity (**formaldehyde is carcinogenic**) - poorly inactivated by proteins, natural materials, synthetic materials, hard water (**do not use Glutaraldehyde with proteins**) - low toxicity for the skin, eyes, lungs; possible allergic and irritant effects
Chlorhexidine *De, Di*	- cell membrane interactions	- low toxicity - only active on spores	- local antiseptic for wounds and abrasions	- incompatible with anionic detergents
Sodium hypochlorite *De, Di*	- oxidises peptide bonds - denatures proteins	- low cost - wide range - easily found - high activity on bacteria - discrete activity on mycobacterium and spores - scarce activity on viruses and fungi	- surface disinfectant - treatable with water	- pH sensitive - binds to organic substances such as amines - low toxicity for the skin, eyes, lungs - inactivated by proteins - poorly inactivated by natural and synthetic materials, hard water and cationic detergents - **cannot be used with acids and strong bases**

EDTA (Ethylendiaminetetra-acetic acid) *De, Di*	- increases cell wall and membrane permeability	- increases antibiotic efficiency	- Pseudomonas - Proteus - Staphylococcus	- Low bactericidal activity - Does not have a wide spectrum - Irritant
Ethylene oxide *St*	- alkykates nucleic acids (DNA)	- high bactericidal activity	- sterilisation of heat labile instruments	- carcinogenic - toxic - mutagenic - flammable - explosive
Iodine *Di*	- interrupts the oxygen transport mechanisms	- sporicidal - cysticidal	- surgical skin antiseptic	- toxic - only active in certain forms
Peroxides (hydrogen peroxide, plasma gas) *St*	- dissolves the cell wall - denatures proteins	- low cost (hydrogen peroxide) - doesn't contaminate the environment - not toxic for biological tissues	- sterilisation of laboratory instruments and small apparatus	- cytotoxic - low stability - expensive instruments (for plasma gas) - low efficacy in the presence of organic materials
Ozone *Di*	- native "super oxygen" - oxidises proteins, unsaturated lipid	- dissolves in water - doesn't leave residues	- water disinfection - topical antiseptics	- unstable - irritant
Phenols *Di*	- penetrates the cell wall - denatures proteins	- penetrates wood and porous surfaces - high bacterial and fungal activity - moderate mycobacterium activity - low viral activity	- environmental disinfectants	- toxic, corrosive - minimal sporicidal activity - moderately inactivated by natural and synthetic materials - poorly inactivated by proteins and hard water - penetrates skin very easily, irritant
Quaternary ammonium and quaternary ammonium phenolic salts *De, Di*	- interacts with the cell membrane and impairs permeability	- surfactants that destroy lipids - usable on surfaces - generally non toxic for mammalians	- environmental disinfectants	- inactivated by lipids

High pressure steam (autoclave) *St*	- denatures proteins and nucleic acids	- active on bacteria, mycobacteria, fungi and viruses - very efficient sporicidal system	- small instruments - equipment resistant to high pressure (1-3 bar) and high temperatures (120-130°C)	- destroys heat labile materials - inefficient against steam resistant organisms
Dry heat (Hot air ovens) (100°C for 1 h, 160°C for 2 h, 170°C for1 h) *St*	- denatures proteins and nucleic acids	- active on bacteria, mycobacteria, fungi and viruses	- sterilization of waterproof or steam damageable materials (glass, sharp instruments, metal)	- destructive for materials that cannot withstand high temperatures for long periods of time
Ultraviolet light *St*	- acts on DNA and creates thymidine dimers	- inexpensive - broad spectrum	- sterilisation of surfaces, water, air	- acts on surfaces, operates in an extremely space and requires correct maintenance with frequent cleaning of the lamp; a thin layer of dust will cause malfunctioning - skin cancer
Gamma irradiation *St*	- denatures DNA, proteins and the cell wall	- rapid - broad spectrum	- instruments and small equipment	- requires expensive equipment

*Decontamination: destruction of the majority of microorganisms; it is always integrated with sterilisation or disinfection of materials that have been in contact with pathogens.

**Disinfection: elimination of the majority or all pathogenic microorganisms (with the exception of spores) from inanimate objects. The agent used is called a *disinfectant,* when applied to objects and the environment, *antiseptic* for live tissues (skin, mucous membranes) and generally, has a specific target organism. Normally the disinfection process uses chemical substances or pasteurisation.

***Sterilisation: complete elimination or destruction of all forms of microbial life. It has a broad spectrum acting on: pathogenic and non-pathogenic microorganisms, spores and vegetative forms. The agent used is called a *steriliser* (bactericidal, germicidal). It is used in hospitals as well as research laboratories with chemical or physical processes. The most common applications are: steam sterilisation (autoclave), dry heat (hot air ovens), gas sterilisation (ethylene oxide, ozone), chemical agents.

Table V. Principle sources of biological risk in research laboratories

ACTIVITY	RISK	PREVENTION
Hybridisation	Nature of the probes and tissues (human, animal or plant). The risk tied to the use of probes is limited to use of the oligonucleotides	Classic precautions for potentially infectious samples apply. To avoid possible infections, manipulate in sterile conditions
Cell immortalization using viral vectors	Skin contact can cause tumours. Formation of aerosols	Precise determination of the risk. Use the main safety level available. Reduce aerosol formation
Manipulation of biological liquids (blood, plasma, serum) and cells or tissues from infected materials	Risk tied to sample collection. Aerosol formation	Manipulate everything as if it is infected. Avoid skin contact with cells or tissues. Reduce aerosol formation. Handle waste in a suitable manner
Nature of the bacterial colonies and the human, animal and plant cells used	Risk of contamination (bacterial colonies) and of possible infection (human, animal and plant cells). The risks can be due to the nature of the insert, the cells or the serum or other agents which can stimulate undesirable proliferation of the culture. Aerosol formation	Handle with gloves that have been tested for protection from microorganisms, face masks (partial or total, for respiratory tract protection) and automatic respiratory masks*
Nature of the DNA (bacterial, animal, plant, plasmids, genes coding for toxins, sequences of unknown nature, DNA for sequencing) and vectors	Aerosol formation. Avoid contact with the skin and ingestion	Handle in a cabinet with gloves and a mask. Eliminate waste in an adequate way to protect the environment

PER CORTESIA RISCRIVETEMI IN INGLESE LA PARTE MANCANTE GRAZIE

ACTIVITY	RISK	PREVENTION
Nature of the RNA	Cells: infection possible Animals: infections possible Bacteria: contamination risk	Avoid contact with the skin (handle with gloves) and ingestion. Eliminate waste in an adequate to protect the environment
Nature of the vector and of the insert	Dangerous by contact and for the environment	Avoid contact with the skin and ingestion. Adopt all precautions to protect the environment
Specific danger for the use of retroviruses	Possibility of contamination by aerosols	Use adequate containment levels
Bacteriophage vectors carrying a modified insert: risks due to the insertion of new sequences, with new and unforeseeable methods of self-propagation	Possibility of contamination by aerosols	Precise determination of the containment level (> L1), to avoid diffusion. Maintain immunodeficiency under control. Avoid skin contact and aerosols. Verify the existence of special measures, in relation to the organism treated. Create of an emergency plan

* Regarding the masks, the efficiency in confront of submicron particles (ultrafine powders and aerosols), the filtration systems used must have an efficiency of at least 99,99% for 0,3 micrometer particles (class P3), according to the EN 143 regulation; this deals with unique masks efficient for protection from biological risk. Gloves must be of the type tested with ΦX-174 for protection from microorganisms (according to EN 374)

Table VI. Principle sources of chemical risk in research laboratories

Compound	Risk	Prevention
Acetic anhydride	Corrosive Flammable Emits highly irritant vapours Avoid inhalation and contact with the skin and eyes	1, 2, 4
Acetonitrile	Flammable, toxic	1, 2, 3
Acrylamide	Neurotoxic, allergenic, carcinogenic, neurotoxic when in the non-polymerised form	1, 2, 3
Actinomycin D	very toxic	1, 2, 3
Adenine hemisulfate	Irritant	1, 2, 4
Aluminium chloride	Irritant	1, 2, 4
Amiprophos methyl	Harmful	1, 2, 4
Ammonium acetate	Irritant	1, 2, 4
Ammonium chloride	Irritant	1, 2, 4
Ammonium dihydrogen phosphate	Irritant	1, 2, 4
Ammonium hydrogen carbonate	Harmful	1, 2
Ammonium nitrate	Irritant. Oxidising Provokes violent reactions with reducing substances	1, 2, 4
Ammonium peroxodisulfate (ammonium persulfate, APS)	Harmful, oxidising Allergenic Provokes violent reactions with reducing substances	1, 2, 3
Ammonium sulfate	Irritant	1, 2, 4
Amphotericin B	Harmful	1, 2, 3
Amsacrine	Very toxic	1, 2, 3
Antibiotics	Harmful Irritant Allergenic Risks tied to antibiotic-resistance	1, 2, 3
L-Arginine	Irritant	1, 2, 4
BES (N,N-bis[2-hydroxymethyl]-2-aminoethanesulfonic acid)	Irritant	1, 2, 4

Compound	Risk	Prevention
Bleomycin sulfate	Harmful	1, 2, 3
Boric acid	Harmful Irritant	1, 2, 3
5-Bromo-4-chloro-3-indolyl-phosphate (BCIP), X-Phos	Toxic	1, 2, 3
Bromophenol blue (3',3",5', 5"- tetrabromophenolsulpho-nephthalein) sodium salt	Harmful	1, 2, 3
Caesium Chloride	Harmful	1, 2, 3
Calcium chloride	Irritant	1, 2, 4
Calcium nitrate	Irritant Provokes violent reactions with reducing substances	1, 2, 4
Carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilid)	Harmful	1, 2, 3
Cellulase-powder	Harmful	1, 2, 3
N-Cetyl-N,N,N- trimethylam-monium bromide	Harmful	1, 2, 3
CHAPS (3-[(3- cholamido-propyl)dimethyl-ammonio]-1-propanesulfonate)	Harmful	1, 2, 3
p-Chlorophenoxyacetic acid	Harmful	1, 2, 3
Chloroform	Danger by inhalation and contact, especially when found in mixes with phenol	1, 2, 3
Cobalt chloride	Harmful	1, 2, 3
Colcemid® (N-deacetyl-N-methylcolchicine)	Toxic	1, 2, 3
Colchicine	Very toxic	1, 2, 3
Copper sulfate pentahydrate, or anhydrous	Harmful Dangerous for the environment, irritant	1, 2, 3 Organise a suitable method for waste disposal

Compound	Risk	Prevention
Destomycin A	Irritant	1, 2, 4
Diethyl ether	Highly flammable Can form explosive peroxides Has antiseptic effects (narcotic effect)	1. Store in a well ventilated place, away from flames and sparks. Do not smoke. Avoid accumulation of electrostatic charges. Do not throw waste in the sewage system
Diethylpyrocarbonate (DEPC)	A very unstable substance that can self-decompose, exploding and liberating CO_2. It is a suspect carcinogen	1, 2, 4 Organise a suitable method for waste disposal
Dimethyl ether	Highly volatile Extremely flammable	1. Handle under a chemical hood. Store in a ventilated, explosion proof area.
Dimethylformamide (DMF) and Formamide	Toxic and teratogenic, easily penetrates across the skin causing malformations in animals	1, 2, 3. Use glass or polypropylene containers, dimethylformamide dissolves common plastics. Arrange disposal of the waste in an appropriate manner. Store in a well ventilated and dry place.
Dimethyl Sulfate (DMS)	Mutagenic Corrosive Carcinogenic	1, 2, 3. Collect the waste in bottles containing 5N NaOH.
Dimethyl sulfoxide (DMSO)	Avoid all contact, penetrates skin very easily No Toxic	1, 2, 3
3,5- dimethoxy -4-hydroxy-acetophenone (Acetosyringone)	Eye, respiratory tract and skin irritant	1, 2, 4

Compound	Risk	Prevention
Doxorubicin HCl	Toxic	1, 2, 3
Ethidium Bromide (ETB)	Mutagenic and moderately toxic powder. Avoid inhalation Irritant	1, 2 Decontaminate solutions with a strong oxidiser.
Ethylendiamine ferric (Fe-EDDHA)	Harmful	1, 2, 3
Ethylendiaminetetraacetic acid (EDTA)-iron, sodium and disodium salts	Irritant	1, 2, 4
Ethyl alcohol (ethanol)	Easily flammable Irritant Neurotoxic	1
Fluorescein isothiocyanate (FITC)	Harmful	1, 2, 3
5-Fluoro orotic acid (5-FOA)	Harmful	1, 2, 3
5-Fluorouracil	Harmful	1, 2, 3
Folinic acid (calcium salt)	Irritant	1, 2, 4
Formaldehyde	Very toxic Provokes nasal tumours if inhaled in rats Very irritant Very allergenic (eczema, asthma) Mutagenic	1, 2, 3. Collect the waste and arrange an appropriate means of disposal
Formic acid	Corrosive Toxic It is easily absorbed through the skin	1, 2. Collect the waste and arrange disposal. Store in a dry and well ventilated place
Giemsa-solution (Azure/eosin methylene blue)	Flammable Toxic	1, 2, 3
Acetic acid	Irritant Emits highly irritant vapours Avoid inhalation and contact with the skin and eyes	1, 2, 4
Glyphosate (N-phosphono-methylglycine)	Irritant	1, 2, 4
Griseofulvin	Harmful	1, 2, 3
Guanidine hydrochloride	Irritant	1, 2, 4
Guanidine isothiocyanate	Harmful	1, 2, 3

Compound	Risk	Prevention
HEPES (N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]])	Irritant	1, 2, 4
Hydrochloric acid	Corrosive Emits highly irritant vapours	1, 2, 3, 4
Hydrazine	Toxic Carcinogen Explosive in the anhydrous state	1, 2. Collect waste in bottles containing 3M iron chloride.
p-Hydroxybenzoic acid ethyl ester	Irritant	1, 2, 4
8-Hydroxyquinoleine	Harmful	1, 2, 3
Hygromycin B	Very toxic	1, 2, 3
Iodoacetamide	Dangerous if inhaled	1, 2, 3
Iron chloride: anhydrous, hydrated tetrahydrate, hexahydrate	 Harmful Corrosive	 1, 2, 3 1, 2, 3
Isoamyl alcohol	Dangerous by inhalation and through contact Irritant	1, 2
Isopropyl alcohol (isopropanol)	Explosive Irritant	1, 2
Jasmonic acid(±) ([±]-1 α, 2β-3-oxo-2-[*cis*-2-pentyl] cyclopentaneacetic acid)	Irritant	1, 2, 4
Lysis buffers: Tris/Glucose/EDTA	See TRIS, EDTA	
Magnesium chloride-solution	Irritant	1, 2, 4
Maleic hydrazide	Very toxic	1, 2, 3
Malic acid-(DL)	Irritant	1, 2, 4
Manganese sulfate	Harmful	1, 2, 3
MES (2-(N-Morpholino) ethanesulfonic acid)	Irritant	1, 2, 4
Methanol (methyl alcohol)	Flammable Toxic for the optic nerve, retina	1, 2, 3
Methotrexate ((+)-Amethopterin)	Very toxic	1, 2, 3

Compound	Risk	Prevention
Methyl jasmonate cyclopentane acetic acid (3-oxo-(2-pentemethyl ester))	Harmful	1, 2, 3
Mitomycin C	Very toxic	1, 2, 3
MOPS (4-Morpholino propanesulfonic acid)	Irritant	1, 2, 4
MTT-Thiazol blue (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)	Harmful	1, 2, 3
Nicotinamide (vitamin PP)	Irritant	1, 2, 4
Nicotinic acid (vitamin B)	Irritant	1, 2, 4
Nitric acid 65% >65%:	Corrosive Oxidising	1, 2
NP40	It is advisable to avoid skin contact	1, 2
Pectinase-powder	Harmful Avoid skin contact and inhalation	1, 2, 3
Pectolyase-powder	Avoid skin contact and inhalation	1, 2, 3
Phenol	Danger by inhalation and skin systemic contact (especially dangerous when found mixed with chloroform) Caustic	1, 2, 3. Collect waste in suitable containers and arrange disposal.
Phloroglucinol (1,3,5-Trihydroxybenzene)	Irritant	1, 2, 4
Phosphate buffered saline-pH 7,4	Irritant	1, 2, 4
6-Phosphonogluconic acid (6-Phospho-D-gluconate, 6-Pg)	Irritant	1, 2, 4
Photographic material	Dangerous if inhaled and through contact with the skin and eyes Irritant Allergenic	1, 2, 4
Phyto-hormones (auxin, cytokinin, auxin-like and cytokinin-like), synthetic or not.	Harmful	1, 2, 3
PIPES (Piperazine-N,N'-bis –2-ethanesulfonic acid]1,4-piperazine diethanesulfonic acid)	Irritant	1, 2, 4

Compound	Risk	Prevention
Piperidine	Toxic if inhaled Irritant	1, 2, 3
Polyethylene glycol (PEG, monomethylether mesylate (2.000 e 5.000))	Irritant	1, 2, 4
Potassium acetate-solution	Irritant	1, 2, 4
Potassium carbonate anhydrous	Harmful	1, 2, 3
Potassium chloride-powder	Irritant	1, 2, 4
Potassium hydroxide	Corrosive	1, 2
Potassium iodide	Harmful	1, 2, 3
Potassium nitrate	Oxidising Irritant	1, 2
Potassium permanganate	Oxidising Harmful Enviromental contaminant Corrosive Toxic by inhalation, skin contact and ingestion	1, 2, 4 Keep distant from combustible materials.
Proteinase K-powder	Harmful if inhaled Can also provoke sensitisation through skin contact Eye, skin and respiratory tract irritant	1, 2, 3
Putrescine (1,4-Diaminobutane dihydrochloride)	Irritant	1, 2, 4
Ribavirin	Harmful	1, 2, 3
Salicylic acid (2-Hydroxybenzoic acid)	Harmful	1, 2, 3
SDS (Sodium dodecyl sulphate, Sodium lauryl sulfate)	Harmful Irritant Dangerous by inhalation (can cause sensitisation) and ingestion Eye (can cause serious damage), respiratory system and skin irritant	1, 2, 3, 4. Collect the waste and arrange an appropriate means of disposal. Do not inhale the powder.
Silver nitrate	Corrosive Environmental contaminant	1, 2, 4
Sodium carbonate	Respiratory system and skin irritant Causes very serious damage to the eyes Causes serious irritations	1, 2, 4 Do not inhale the powder
Sodium dihydrogen phosphate	Irritant	1, 2, 4

Compound	Risk	Prevention
Sodium hydroxide	Corrosive Hydrates very easily with atmospheric humidity On contact with water produces exothermic reaction: give particular attention when preparing solutions, especially if very concentrated	1, 2
Sodium Nitrate	Oxidising	1, 2
Iron sulfate	Harmful	1, 2, 3
Spermidine (N-(3-amino-propyl)-1,4-diaminobutane)	Corrosive Irritant	1, 2
TE, TAE, TBE buffers:	See TRIS, Boric acid, Acetic acid, EDTA	
N,N,N',N'- tetramethylenethi-lendiammine (TEMED)	Harmful by inhalation and ingestion Causes burns	1, 2, 4 Do not smoke
Thimerosal	Very toxic	1, 2, 3
Tomato-powder	Irritant	1, 2, 4
Trichloroacetic Acid (TCA)	Risk of skin irritations Corrosive	1, 2
Triethanolamine	Irritant	1, 2, 4
2,3,5- Triiodobenzoic acid (TIBA)	Harmful	1, 2, 3
TRIS (Tris(hydroxymethyl) aminomethane, 2-Amino-2-hydroxyl-methyl-1,3-propa-nediol) and TRIS HCl	Irritant	1, 2, 4
Urea	Harmful by inhalation, skin contact and if swallowed Eye, respiratory system and skin irritant	1, 2, 3 Do not inhale the powder
Xylene	A dangerous flammable and toxic solvent (with long term manifestations) Harmful by contact	1, 2, 4 Maintain distant from oxidising agents, in a well ventilated area
Zinc sulfate	Irritant	1, 2, 4

1 Use under a chemical hood
2 Wear gloves
3 Wear a mask
4 Wear glasses or face masks

References

- David J.C. Éléments de sécurité en biologie moléculaire. Flammarion Médicine-Sciences. (1997).
- Sambrook J., Fritsch E.F., Maniatis T. Molecular cloning - A laboratory manual, 2nd edition. (1989).

ANNEX I

Biological agents classification

(Directive 2000/54/EC, annex III)

1. Biological agents which have not been classified for inclusion in Group 2 to 4 of the list are not implicitly classified in Group 1.
2. Where a strain is attenuated or has lost known virulence genes, then the containment required by the classification of its parent strain need not necessarily apply, subject to assessment appropriate for risk in the workplace.
3. Member States are to ensure that all viruses which have already been isolated in humans and which have not been assessed and allocated in this Annex are classified in Group 2 as a minimum, except where Member States have proof that they are unlikely to cause disease in humans.
4. Certain biological agents classified in Group 3 which are indicated in the appended list by two asterisks (**), may present a limited risk of infection for workers because they are not normally infectious by airborne route. Member States shall assess the containment measures to be applied to such agents, taking account of the nature of specific activities in question and of the quantity of agent involved, with a view to determining whether, in particular circumstances, some of these measures may be dispensed with.

BACTERIA
and similar organisms

NB: For biological agents appearing on this list, "spp" refers to other species which are known pathogens in humans.

Biological Agent	Classification	Notes
Actinobacillus actinomycetemcomitans	2	
Actinomadura madurae	2	
Actinomadura pelletieri	2	
Actinomyces gereneseriae	2	
Actinomyces israelii	2	
Actinomyces pyogenes	2	
Actinomyces spp.	2	
Arcanobacterium haemolyticum (Corynebacterium haemolyticum)	2	
Bacillus anthracis	3	
Bacteroides fragilis	2	
Bartonella bacillifornnis	2	
Bartonella quintana (Rochalimaea quintana)	2	
Bartonella (Rhochalinea) spp.	2	
Bordetella bronchiseptica	2	
Bordetella parapertussis	2	
Bordetella pertussis	2	V
Borrelia burgdorferi	2	
Borrelia duttonii	2	
Borrelia recurrentis	2	
Borrelia spp.	2	
Brucella abortus	3	
Brucella canis	3	
Brucella melitensis	3	
Brucella suis	3	
Burkholderia mallei (Pseudomonas mallei)	3	
Burkholderia pseudomallei (Pseudomonas pseudomallei)	3	
Campylobacter fetus	2	
Campylobacter jejuni	2	
Campylobacter spp.	2	
Cardiobacterium hominis	2	
Chlamydia pneumoniae	2	

Biological Agent	Classification	Notes
Chlamydia trachomatis	2	
Chlamydia psittaci (avian strains)	3	
Chlamydia psittaci (other strains)	2	
Clostridium botulinum	2	T
Clostridium perfringens	2	
Clostridium tetani	2	T,V
Clostridium spp.	2	
Corynebacterium diphtheriae	2	T,V
Corynebacterium minutissimum	2	
Corynebacterium pseudotuberculosis	2	
Corynebacterium spp.	2	
Coxiella burnetii	3	
Edwardsiella tarda	2	
Ehrlichia sennetsu (Rickettsia sennetsu)	2	
Ehrlichia spp.	2	
Eikenella corrodens	2	
Enterobacter aerogenes/cloacae	2	
Enterobacter spp.	2	
Enterococcus spp.	2	
Erysipelothrix rhusiopathiae	2	
Escherichia coli (with the exception of non-pathogenic strains)	2	
Escherichia coli, verocytotoxigenic strains (e.g. 0157:H7 or 013)	3 (**)	
Flavobacterium meningosepticum	2	
Fluoribacter bozemanae (Legionella)	2	
Francisella tularensis (type A)	3	
Francisella tularensis (type B)	2	
Fusobacterium necrophorum	2	
Gardnerella vaginalis	2	
Haemophilus ducreyi	2	
Haemophilus influenzae	2	
Haemophilus spp.	2	
Helicobacter pylori	2	
Klebsiella oxytoca	2	
Klebsiella pneumoniae	2	
Klebsiella spp.	2	
Legionella pneumophila	2	

Biological Agent	Classification	Notes
Legionella spp.	2	
Leptospira interrogans (all serovars)	2	
Listeria monocytogenes	2	
Listeria ivanovii	2	
Morganella morganii	2	
Mycobacterium africanum	3	V
Mycobacterium avium/intracellular	2	
Mycobacterium bovis (except BCG strain)	3	V
Mycobacterium chelonae	2	
Mycobacterium fortuitum	2	
Mycobacterium kansasii	2	
Mycobacterium leprae	3	
Mycobacterium malmoense	2	
Mycobacterium marinum	2	
Mycobacterium microti	3 (**)	
Mycobacterium paratuberculosis	2	
Mycobacterium scrofulaceum	2	
Mycobacterium simiae	2	
Mycobacterium szulgai	2	
Mycobacterium tuberculosis	3	V
Mycobacterium ulcerans	3 (**)	
Mycobacterium xenopi	2	
Mycoplasma caviae	2	
Mycoplasma hominis	2	
Mycoplasma pneumoniae	2	
Neisseria gonorrhoeae	2	
Neisseria meningitidis	2	V
Nocardia asteroides	2	
Nocardia brasiliensis	2	
Nocardia farcinica	2	
Nocardia nova	2	
Nocardia otitidiscaviarum	2	
Pasteurella multocida	2	
Pasteurella spp.	2	
Peptostreptococcus anaerobius	2	
Plesiomonas shigelloides	2	
Porphyromonas spp.	2	
Prevotella spp.	2	

Biological Agent	Classification	Notes
Proteus mirabilis	2	
Proteus penneri	2	
Proteus vulgaris	2	
Providencia alcalifaciens	2	
Providencia rettgeri	2	
Providencia spp.	2	
Pseudomonas aeruginosa	2	
Rhodococcus equi	2	
Rickettsia akari	3 (**)	
Rickettsia canada	3 (**)	
Rickettsia conorii	3	
Rickettsia montana	3 (**)	
Rickettsia typhi (Rickettsia mooseri)	3	
Rickettsia prowazekii	3	
Rickettsia rickettsii	3	
Rickettsia tsutsugamushi	3	
Rickettsia spp.	2	
Salmonella arizonae	2	
Salmonella enteritidis	2	
Salmonella typhimurium	2	
Salmonella paratyphi A, B, C	2	V
Salmonella typhi	3 (**)	V
Salmonella (other serovars)	2	
Serpulina spp.	2	
Shigella boydii	2	
Shigella dysenteriae (type 1)	3 (**)	T
Shigella dysenteriae (other than type 1)	2	
Shigella flexneri	2	
Shigella sonnei	2	
Staphylococcus aureus	2	
Streptobacillus moniliformis	2	
Streptococcus pneumoniae	2	
Streptococcus pyogenes	2	
Strepfococcus suis	2	
Streptococcus spp.	2	
Treponema carateum	2	
Treponema pallidum	2	
Treponema pertenue	2	

Biological Agent	Classification	Notes
Treponema spp.	2	
Vibrio cholerae (including El Tor)	2	
Vibrio parahaemolyticus	2	
Vibrio spp.	2	
Yersinia enterocolitica	2	
Yersinia pestis	3	V
Yersinia pseudotuberculosis	2	
Yersinia spp.	2	

VIRUSES (*)

Biological Agent	Classification	Notes
Adenoviridae	2	
Arenaviridae		
LCM-Lassa-virus complex (old world arena viruses):		
Lassa Virus	4	
Lymphocytic (strains)	3	
Lymphocytic choriomeningitis virus (other strains)	2	
Mopeia Virus	2	
Other LCM - Lassa complex viruses	2	
Tacaribe - Virus - Complex (new world arena viruses):		
Guanarito Virus	4	
Junin Virus	4	
Sabia Virus	4	
Machupo Virus	4	
Flexal Virus	3	
Other Tacaribe complex viruses	2	
Astroviridae	2	
Bunyaviridae		
Belgrade (also known as Dobrava)	3	
Bhanja	2	
Bunyamwera Virus	2	
Germiston	2	
Oropouche Virus	3	

Biological Agent	Classification	Notes
Sin Nombre (formely Muerto Canyon)	3	
California encephalitis virus	2	
Hantaviruses:		
Hantaan (Korean haemorrhagic fever)	3	
Seoul virus	3	
Puumala virus	2	
Prospect Hill virus	2	
Other hantaviruses	2	
Nairoviruses:		
Crimean-Congo haemorrhagic fever	4	
Hazara virus	2	
Phleboviruses:		
Rift Valley fever	3	V
Sandfly fever	2	
Toscana virus	2	
Other Bunyaviridae known to be pathogenic	2	
Caliciviridae		
Hepatitis E virus	3 (**)	
Norwalk Virus	2	
Other Caliciviridae	2	
Coronaviridae		
Filoviridae		
Ebola virus	4	
Marburg virus	4	
Flaviviridae		
Australia encephalitis (Murray, Valley encephalitis)	3	
Central European tick-borne encephalitis virus	3 (**)	V
Absettarov	3	
Hanzalova	3	
Hypr	3	
Kumlinge	3	
Dengue virus type 1-4	3	
Hepatitis C virus	3 (**)	D
Hepatitis G virus	3 (**)	D
Japanese B encephalitis	3	V

Biological Agent	Classification	Notes
Kyasanur Forest	3	V
Louping ill	3 (**)	
Omsk (a)	3	V
Powassan	3	
Rocio	3	
Russian spring-summer encephalitis (TBE) (a)	3	V
St. Louis encephalitis	3	
Wesselsbron virus	3 (**)	
West Nile fever virus	3	
Yellow fever	3	V
Other flaviviruses known to be pathogenic	2	
Hepadnaviridae		
Hepatitis B virus	3 (**)	V,D
Hepatitis D virus (Delta) (b)	3 (**)	V,D
Herpesviridae		
Cytomegalovirus	2	
Epstein-Barr virus	2	
Herpesvirus simiae (B virus)	3	
Herpes simplex virus 1 and 2	2	
Herpesvirus varicella-zoster	2	
Human B-lymphotropic virus (HBLV-HHV6)	2	
Human herpes virus 7	2	
Human herpes virus 8	2	D
Orthomyxoviridae		
Influenza viruses types A, B and C	2	V (c)
Tick-borne orthomyxoviridae:		
Dhori & Thogoto	2	
Papovaviridae		
BK and JC viruses	2	D (d)
Human Papillomaviruses	2	D (d)
Paramyxoviridae		
Measles virus	2	V
Mumps virus	2	V
Newcastle disease virus	2	
Parainfluenzae viruses types 1 to 4	2	
Respiratory syncytial virus	2	

Biological Agent	Classification	Notes
Parvoviridae		
Human parvovirus (B 19)	2	
Picornaviridae		
Acute haemorrhagic conjunctivitis virus (AHC)	2	
Coxsackie viruses	2	
Echo viruses	2	
Hepatitis A virus (human enterovirus type 72)	2	V
Polioviruses	2	V
Rhinoviruses	2	
Poxviridae		
Buffalopox virus (e)	2	
Cowpox virus	2	
Elephantpox virus (f)	2	
Milkers' node virus	2	
Molluscum contagiosum virus	2	
Monkeypox virus	3	V
Orf virus	2	
Rabbitpox virus (g)	2	
Vaccinia virus	2	
Variola (major & minor) virus	4	V
White pox virus ("Variola virus")	4	V
Yatapox virus (Tana & Yaba)	2	
Reoviridae		
Coltivirus	2	
Human Rotaviruses	2	
Orbiviruses	2	
Reuviruses	2	
Retroviridae		
Human immunodeficiency viruses	3 (**)	D
Human T cell lymphotropic viruses (HTLV) types 1 and 2	3 (**)	D
SIV (h)	3 (**)	
Rhabdoviridae		
Rabies virus	3 (**)	V
Vescicular stomatitis virus	2	
Togaviridae		

Biological Agent	Classification	Notes
Alphaviruses:		
Eastern equine encephalomyelitis	3	V
Bebaru virus	2	
Chikungunya virus	3 (**)	
Everglades virus	3 (**)	
Mayaro virus	3	
Mucambo virus	3 (**)	
Ndumu virus	3	
O'nyong-nyong virus	2	
Ross River virus	2	
Semliki Forest virus	2	
Sindbis virus	2	
Tonate virus	3 (**)	
Venezuelan equine encephalomyelitis	3	V
Western equine encephalomyelitis	3	V
Other known alphaviruses	2	
Rubivirus (rubella)	2	V
Toroviridae	2	
Unclassified viruses:		
Equine morbillivirus	4	
Hepatitis viruses not yet identified	3 (**)	D
Unconventional agents associated with the transmissible spongiform encephalopathies (TSEs):		
Creutzfeldt Jakob disease	3 (**)	D (d)
Variant Creutzfeldt Jakob disease	3 (**)	D (d)
Bovine spongiform encephalopathy (BSE) and other related animal TSEs (i)	3 (**)	D (d)
Gerstmann-Sträussler-Scheinker syndrome	3 (**)	D (d)
Kuru	3 (**)	D (d)

PARASITES

Biological Agent	Classification	Notes
Acanthamoeba castellani	2	
Ancylostoma duodenale	2	
Angiostrongylus cantonensis	2	

Biological Agent	Classification	Notes
Angiostrongylus costaricensis	2	
Ascaris lumbricoides	2	A
Ascaris suum	2	A
Babesia divergens	2	
Babesia microti	2	
Balantidium coli	2	
Brugia malayi	2	
Brugia pahangi	2	
Capillaria philippinensis	2	
Capillaria spp.	2	
Clonorchis sinensis	2	
Clonorchis viverrini	2	
Cryptosporidium parvum	2	
Cryptosporidium spp.	2	
Cyclospora cayetanensis	2	
Dipetalonema streptocerca	2	
Diphyllobothrium latum	2	
Dracunculus medinensis	2	
Echinococcus granulosus	3 (**)	
Echinococcus multilocularis	3 (**)	
Echinococcus vogeli	3 (**)	
Entamoeba histolytica	2	
Fasciola gigantica	2	
Fasciola hepatica	2	
Fasciolopsis buski	2	
Giardia lamblia (Giardia intestinalis)	2	
Hymenolepis diminuta	2	
Hymenolepis nana	2	
Leishmania brasiliensis	3 (**)	
Leishmania donovani	3 (**)	
Leishmania ethiopica	2	
Leishmania mexicana	2	
Leishmania peruviana	2	
Leishmania tropica	2	
Leishmania major	2	
Leishmania spp.	2	
Loa loa	2	
Mansonella ozzardi	2	

Biological Agent	Classification	Notes
Mansonella perstans	2	
Naegleria fowleri	3	
Necator americanus	2	
Onchocerca volvulus	2	
Opisthorchis felineus	2	
Opisthorchis spp.	2	
Paragonimus westermani	2	
Plasmodium falciparum	3 (**)	
Plasmodium spp. (human and simian)	2	
Sarcocystis suihominis	2	
Schistosoma haematobium	2	
Schistosoma intercalatum	2	
Schistosoma japonicum	2	
Schistosoma mansoni	2	
Schistosoma mekongi	2	
Strongyloides stercoralis	2	
Strongyloides spp.	2	
Taenia saginata	2	
Taenia solium	3 (**)	
Toxocara canis	2	
Toxoplasma gondii	2	
Trichinella spiralis	2	
Trichuris trichiura	2	
Trypanosoma brucei brucei	2	
Trypanosoma brucei gambiense	2	
Trypanosoma brucei rhodesiense	3 (**)	

FUNGI

Biological Agent	Classification	Notes
Trypanosoma cruzi	3	
Wuchereria bancrofti	2	
Aspergillus fumigatus	2	A
Blastomyces dermatitidis (Ajellomyces dermatitidis)	3	
Candida albicans	2	A
Candida tropicalis	2	
Cladophialophora bantiana (formerly:		

Biological Agent	Classification	Notes
xylohypha bantiana, cladosporium bantianum or trichoides)	3	
Coccidioides inunitis	3	A
Cryptococcus neoformans var. neofonnans (Filobasidiella neofonnans var. neoformans)	2	A
Cryptococcus neoformans var. gattii (Filobasidiella bacillispora)	2	A
Emmonsia parva var. parva	2	
Emmonsia parva var. crescens	2	
Epidermophyton floccosum	2	A
Fonsecaea compacta	2	
Fonsecaea pedrosoi	2	
Histoplasma capsulatum var. capsulatum (Ajellomyces capsulatus)	3	
Histoplasma capsulatum duboisii	3	
Madurella grisea	2	
Madurella mycetomatis	2	
Microsporum spp.	2	A
Neotestudina rosatii	2	
Paracoccidioides brasiliensis	3	
Penicillium marneffei	2	A
Scedosporium apiospermum (Pseudallescheria boydii)	2	
Scedosporium prolificans (inflatum)	2	
Sporothrix schenckii	2	
Trichophyton rubrum	2	
Trichophyton spp.	2	

(*) See introductory notes 3
(**) See introductory notes 4

(**a**) Tick-borne encephalitis
(**b**) Hepatitis D virus is pathogenic in workers only in the presence of simultaneous or secondary infection caused by hepatitis B virus. Vaccination against hepatitis B virus will therefore protect workers who are not affected by hepatitis B virus against hepatitis D virus (Delta).
(**c**) Only for types A and B
(**d**) Recommended for work involving direct contact with these agents.

(**e**) Two viruses are identified: one a buffalopox type and the other a variant of the Vaccinia virus.
(**f**) Variant of cowpox virus.
(**g**) Variant of Vaccinia.
(**h**) At present there is no evidence of disease in humans caused by the other retroviruses of simian origin. As a precaution containment level 3 is recommended for work with them.
(**i**) There is no evidence in humans of infections caused by the agents responsible for other animal TSEs.
Nevertheless, the containment measures for agents categorised in risk group 3 (**) are recommended as a precaution for laboratory work, except for laboratory work relating to an identified agent of scrapie where containment level 2 is sufficient.

A: Possible allergic effects
D: List of workers exposed to this biological agent to be kept for more than 10 years after the end of last known exposure
T: Toxin production
V: Effective vaccine available

ANNEX II

LEGISLATIONS

SAFETY AND HEALTH AT WORK

Council Directive 82/605/EEC of 28 July 1982, on the protection of workers from the risks related to exposure to metallic lead and its ionic compounds at work (first individual Directive within the meaning of Article 8 of Directive 80/1107/EEC).
Council Directive 83/477/EEC of 19 September 1983, on the protection of workers from the risks related to exposure to asbestos at work (second individual Directive within the meaning of Article 8 of Directive 80/1107/EEC).
Council Directive 86/188/EEC of 12 May 1986, on the protection of workers from the risks related to exposure to noise at work.
Council Directive 89/391/EEC of 12 June 1989, on the introduction of measures to encourage improvements in the safety and health of workers at work.
Council Directive 89/654/EEC of 30 November 1989, concerning the minimum safety and health requirements for the workplace (first individual directive within the meaning of Article 16 (1) of Directive 89/391/EEC).
Council Directive 89/655/EEC of 30 November 1989, concerning the minimum safety and health requirements for the use of work equipment by workers at work (second individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC).
Council Directive 92/85/EC of 19 October 1992, on the introduction of measures to encourage improvements in the safety and health at work of pregnant workers and workers who have recently given birth or are breastfeeding (ten individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC).
Council Directive 95/63/EC of 5 December 1995, amending Directive 89/655/EEC concerning the minimum safety and health requirements for the use of work equipment by workers at work (second individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC).
Communication from the Commission (2000) 466 of 5 October 2000, on the Guidelines on the assessment of the chemical, physical and biological

agents and the industrial processes considered hazardous for the safety and health of pregnant workers and workers who have recently given birth or are breastfeeding (Council Directive 92/85/EC).
Directive 2001/45/EC of the European Parliament and of the Council of 27 June 2001, amending Council Directive 89/655/EEC of 30 November 1989 concerning the minimum safety and health requirements for the use of work equipment by workers at work (second individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC).

Safety signs

Council Directive 92/58/EEC of 24 June 1992, on the minimum requirements for the provision of safety and/or health signs at work (ninth individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC).

Personal protective equipment

Council Directive 89/656/EEC of 30 November 1989, on the minimum health and safety requirements for the use by workers of personal protective equipment at the workplace (third individual directive within the meaning of Article 16 (1) of Directive 89/391/EEC).
Council Directive 89/686/EEC of 21 December 1989, on the approximation of the laws of the Member States relating to personal protective equipment.

Good laboratory practice

Council Directive 88/320/EEC of 9 June 1988, on the inspection and verification of Good Laboratory Practice (GLP).
Commission Directive 90/18/EEC of 18 December 1989**,** modifies the Annex to Council Directive 88/320/EEC on the inspection and verification of Good Laboratory Practice (GLP).
Commission Directive 1999/11/EC of 8 March 1999, adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on

chemical substances (Text with EEA relevance).
Commission Directive 1999/12/EC of 8 March 1999, adapting to technical progress for the second time the Annex to Council Directive 88/320/EEC on the inspection and verification of good laboratory practice (GLP) (Text with EEA relevance).

Dangerous substances

Council Directive 67/548/EEC of 27 June 1967, on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Commission Directive 76/907/EEC of 14 July 1976, adapting to technical progress the Council Directive of 27 June 1967 concerning the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Council Directive 79/831/EEC of 18 September 1979, amending for the sixth time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Council Directive 80/1107/EEC of 27 November 1980, on the protection of workers from the risks related to exposure to chemical, physical and biological agents at work.
Council Directive 88/379/ECC of 7 June 1988, on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous preparations.
Council Directive 88/642/EEC of 16 December 1988, amending Directive 80/1107/EEC on the protection of workers from the risks related to exposure to chemical, physical and biological agents at work.
Council Directive 90/394/EEC of 28 June 1990, on the protection of workers from the risks related to exposure to carcinogens at work (Sixth individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC).
Commission Directive 91/155/EEC of 29 May 1991, defining and laying down the detailed arrangements for the system of specific information relating to dangerous preparations in implementation of Article 10 of the Council Directive 88/379/ECC.
Commission Directive 91/322/EEC of 29 May 1991, on establishing indicative limit values by implementing Council Directive 80/1107/EEC on the protection of workers from the risks related to exposure to chemical, physical and biological agents at work.

Council Directive 92/32/EEC of 30 April 1992, amending for the seventh time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Commission Directive 96/94/EC of 18 December 1996, on establishing indicative limit values by implementing Council Directive 80/1107/EEC on the protection of workers from the risks related to exposure to chemical, physical and biological agents at work.
Council Directive 97/42/EC of 27 June 1997, amending for the first time Directive 90/394/EEC on the protection of workers from the risks related to exposure to carcinogens at work (Sixth individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC).
Council Directive 98/24/EC of 7 April 1998, on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual Directive within the meaning of Article 16(1) of Directive 89/391/EEC).
Council Directive 99/38/EC of 29 April 1999, amending for the second time Directive 90/394/EEC on the protection of workers from the risks related to exposure to carcinogens at work and extending it to mutagens.
Commission Directive 2000/39/EC of 8 June 2000, establishimg a first list of indiative occupational exposure limit values in implementation of Council Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work.

Biological agents

Council Directive 90/679/EEC of 26 November 1990, on the protection of workers from risks related to exposure to biological agents at work.
Commission Directive 93/88/EEC of 12 October 1993, adapting, for the third time, to technical progress Council Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work.
Commission Directive 95/30/EC of 30 June 1995, amending Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work.
Commission Directive 97/59/EC of 7 October 1997, adapting to technical progress Council Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work.
Commission Directive 97/65/EC of 26 November 1997, adapting, for the third time, to technical progress Council Directive 90/679/EEC on the

protection of workers from risks related to exposure to biological agents at work.
Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000, on the protection of workers from risks related to exposure to biological agents at work.

Contained use of GMMs

Council Directive 90/219/EEC of 23 April 1990, on the contained use of genetically modified micro-organisms.
Commission Decision 91/448/EEC 29 July 1991, concerning the guidelines for classification referred to in article 4 of Directive 90/219/EEC.
Commission Directive 94/51/EC of 7 November 1994, adapting to technical progress Council Directive 90/219/EEC on the contained use of genetically modified micro-organisms.
Commission Directive 96/134/EC of 16 January 1996, amending Decision 91/448/ECC concerning guidelines for classification referred to in article 4 of Council Directive 90/219/EEC on the contained use of genetically modified micro-organisms.
Council Directive 98/81/EC of 26 october 1998, amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms.
Commission Decision 2000/608/EC of 27 september 2000, concerning the guidance notes for risk assessment outlined in Annex III of Directive 90/219/EEC on the contained use of genetically modified micro-organisms.
Council Decision 2001/204/EC of 8 March 2001, supplementing Directive 90/219/EEC as regards the criteria for establishing the safety, for human health and the environment, of types of genetically modified micro-organisms (Text with EEA relevance).

Radioprotection

Council Directives 59/221/CEE laying down the basic standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiations.
Council Directive 80/836/Euratom of 15 July 1980, amending the Directives laying down the basic safety standards for the health protection of the general public and workers against the dangers of ionizing radiation.

Council Directive 84/466/Euratom of 3 September 1984, laying down basic measures for the radiation protection of persons undergoing medical examination or treatment.
Council Directive 84/467/Euratom amending the Council Directive 80/836/ Euratom of 15 July 1980, amending the Directives laying down the basic safety standards for the health protection of the general public and workers against the dangers of ionizing radiation.
Council Directive 89/618/Euratom of 27 November 1989, about health protection measures to be applied and steps to be taken in the event of a radiological emergency.
Council Directive 90/641/Euratom of 4 December 1990, on the operational protection of outside workers exposed to the risk of ionizing radiation during their activities in controlled areas.
Commission Decision 92/3/EEC of 9 December 1991, establishing the conditions governing the notification of chemical substances existing on the market of the former German Democratic Republic prior to 18 September 1981 which do not appear on the inventory provided for in Article 13 of Directive 67/548/EEC (Only the German text is authentic).
Council Directive 96/29/Euratom of 13 May 1996, laying down the basic safety standards for the protection of the health of workers and the general public against the dangers of ionising radiation.
Council Directive 97/43/Euratom of 30 June 1997, on health protection of individual against the dangers of ionising radiation in relation to medical exposure.

Transport

Council Directive 92/118/EEC of 17 December 1992, laying down animal health and public health requirements governing trade in and imports into the Community of products not subject to the said requirements laid down in specific Community rules referred to in Annex A (I) to Directive 89/662/EEC and, as regards pathogens, to Directive 90/425/EEC.
Council Directive 93/75/EEC of 13 September 1993, concerning minimum requirements for vessels bound for or leaving Community ports and carrying dangerous or polluting goods.
Council Directive 94/55/EC of 21 November 1994, on the approximation of the laws of the Member States with regard to the transport of dangerous goods by road.
Council Directive 96/35/EC of 3 June 1996, on the appointment and vocational

qualification of safety advisers for the transport of dangerous goods by road, rail and inland waterway.
Commission Directive 96/39/EC of 19 June 1996, amending Council Directive 93/75/EEC concerning minimum requirements for vessels bound for or leaving Community ports and carrying dangerous or polluting goods.
Council Directive 96/49/EC of 23 July 1996, on the approximation of the laws of the Member States with regard to the transport of dangerous goods by rail.
Commission Directive 96/86/EC of 13 December 1996, adapting to technical progress Council Directive 94/55/EC on the approximation of the laws of the Member States with regard to the transport of dangerous goods by road (Text with EEA relevance).
Commission Directive 96/87/EC of 13 December 1996, adapting to technical progress Council Directive 96/49/EC on the approximation of the laws of the Member States with regard to the transport of dangerous goods by rail (Text with EEA relevance).
Commission Directive 97/34/EC of 6 June 1997, amending Council Directive 93/75/EEC concerning minimum requirements for vessels bound for or leaving Community ports and carrying dangerous or polluting goods (Text with EEA relevance)
Directive 2000/61/EC of the European Parliament and of the Council of 10 October 2000, amending Council Directive 94/55/EC on the approximation of the laws of the Member States with regard to the transport of dangerous goods by road.

Waste

Council Directive 75/442/EEC of 15 July 1975, on waste.
Council Directive 76/403/EEC of 6 April 1976, on the disposal of polychlorinated biphenyls and polychlorinated terphenyls.
Council Directive 78/319/EEC of 20 March 1978, on toxic and dangerous waste.
Council Directive 91/156/EEC of 18 March 1991, amending Directive 75/442/EEC on waste.
Council Directive 91/689/EEC of 12 December 1991, on hazardous waste.
Directive 94/62/EC of the European Parliament and of the Council of 20 December 1994, on packaging and packaging waste.
Council Directive 96/59/EC of 16 September 1996, on the disposal of polychlorinated biphenyls and polychlorinated terphenyls.

Animals

Council Directive 86/609/EEC of 24 November 1986, on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes

Council Decision 1999/575/EC of 23 March 1998, related to the European Agreement about the protection of vertebrated animals used for experimental and other scientific purposes.


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