Feasibility Study for a European Mars Sample Receiving Facility

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Preface

Sample return missions from Mars are classified according to COSPAR guidelines as Category V, restricted Earth return, to protect the terrestrial system, the Earth and the Moon. The highest degree of concern is expressed by the absolute prohibition of destructive impact upon return, the need for containment throughout the return phase of all returned hardware which directly contacted the target body or unsterilized material from the body, and the need for containment of any unsterilized sample collected and returned to Earth. Post mission there is a need to conduct timely analysis of the unsterilized sample returned to Earth under strict containment, and using the most sensitive technique.

Following this policy and in light of Mars sample return studies as part of the Aurora exploration programme, the ESA Planetary Protection Working Group (PPWG) recommended in its 2nd meeting on January 22nd 2004 to initiate a feasibility study for a Mars sample return receiving/containment facility to understand the budgetary and schedule implications for such a facility on a Mars sample return project. Following this recommendation, the Aurora executive has initiated this study contract in September 2004.

The study report gives a first indication of the kind of containment necessary for samples returned from Mars in agreement with COSPAR guidelines, minimal requirements for the elements for such a containment facility, cost and schedule estimates, and recommendations for further activities.

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Acronyms

ACH               Air Changes per Hour
BSL               Bio Safety Level
BSC               Biosafety Cabinet
BMBL              Biosafety in Microbiological and Biomedical Laboratories
CMS               Central Monitoring System
COSPAR            Committee on Space Research
DERA              Defence Evaluation and Research Agency
DSTL              Defence Science and Technology Laboratory
ESA               European Space Agency
FMDV              Foot-and-mouth Disease Virus
HEPA              High Efficiency Particulate Air
HVAC              Heating, Ventilation and Air Condition
IATA              International Air Transport Association
IVI                Institute of Virology and Immunoprophylaxis
LAN               Local Area Network
MSRF              Mars Sample Receiving Facility
MRSH              Mars Returned Sample Handling
NASA              National Aeronautics and Space Agency
NIH               National Institutes of Health
PPWG              Planetary Protection Working Group of the ESA
P4                Protection 4, French definition of BSL-4
RAMV              Risk Adjusted Mission Value
RNA               Ribonucleic Acid
TBD               To Be Determined
TSE               Transmissible Spongiform Encephalopathy
UTMB              University of Texas Medical Branch
UPS               Uninterrupted Power Supply
WHO               World Health Organization
Chapter 1

Introduction

It is known that there are an infinite number of worlds, simply because there is an infinite amount of space for them to be in. However, not every one of them is inhabited. Therefore, there must be a finite number of inhabited worlds. Any finite number divided by infinity is as near to nothing as makes no odds, so the average population of all the planets in the Universe can be said to be zero. From this it follows that the population of the whole Universe is also zero, and that any people you may meet from time to time are merely the products of a deranged imagination.


The samples returned from Mars must be kept in quarantine until their biological character and safety is determined. Contrary to conventional microbiological safety laboratories where biological samples must not be cross-contaminated with other biological agents, the Mars samples must be pristine for scientific studies to a high degree similar to electronics production in clean rooms. Therefore, biosafety as well as clean room technology must be applied simultaneously. The mission considerations for receiving, handling and analyzing Mars samples are known as Mars Returned Sample Handling (MRSH) and must be considered as a single entity for all of the different stages [1]. Therefore, criteria for transportation to Earth, transportation on Earth, modes of manipulation and capabilities for destructive and nondestructive testing must show consistent measures to avoid any contamination of the Earth biosphere as well as compromise of science analyses such false positive detection of organic molecules.

The Space Studies Board of the National Research Council provided a series of recommendations to NASA on planetary protection requirements for future Mars sample return missions [7]. From a series of four workshops 2000 and 2001 a draft test protocol for detecting possible biohazards in Martian samples returned to Earth resulted in October 2002. That document is the first complete protocol as a consensus of all workshops until that date. It underwent revision by a special oversight and review committee and a reading by the NASA Planetary Protection Advisory Committee. It is our understanding that this Draft will be subject to further review and debate by the ESA Planetary Protection Working Group.

According to COSPAR category V missions [3][8] comprise all Earth return missions: „The concern for these missions is the protection of the terrestrial
system, the Earth and the Moon. For solar system bodies deemed by scientific opinion to have no indigenous life forms, a subcategory „unrestricted Earth return” is defined. Missions in this subcategory have planetary protection requirements on the outbound phase only, corresponding to the category of that phase (typically category I or II). For all other category V missions, in a subcategory defined as „restricted Earth return”, the highest degree of concern is expressed by the absolute prohibition of destructive impact upon return, the need for containment throughout the return phase of all returned hardware which directly contacted the target body or unsterilized material from the body, and the need for containment of any unsterilized sample collected and returned to Earth. Post-mission, there is a need to conduct timely analyses of the unsterilized sample collected and returned to Earth, under strict containment, and using the most sensitive techniques. If any sign of the existence of a nonterrestrial replicating entity is found, the returned sample must remain contained unless treated by an effective sterilizing procedure. Category V concerns are reflected in requirements that encompass those of category IV plus a continuing monitoring of project activities, studies and research (i.e., in sterilization procedures and containment techniques)."

The reasons for doing research within containment are manifold:

1. An employee of the facility could get infected or sick.
2. The employee could transmit a disease to another employee of the facility.
3. Someone outside the research facility could get infected or sick.
4. The environment could suffer from adverse effects or ecological changes.
5. If one of the above occurs, you might be sued.
6. The Mars sample might be contaminated by terrestrial materials.
7. You might violate the Outer Space Treaty of the UN.
8. You might violate the COSPAR Planetary Protection Policy.

Possibility number three might today be regarded as the most severe and possibility number six the most likely risk. The term „containment” is used in describing safe methods to reduce or eliminate exposure of persons and the outside environment to potentially hazardous agents. Experience has demonstrated the prudence of biosafety level 4 for manipulations of even the most dangerous agents including exotic unknown infectious agents. The backbone of the practice of biosafety is risk assessment. This works well when there is adequate information available. Known risk factors must be identified and explored before the containment level can be lowered. However, in the case of the Mars samples the information is insufficient to perform an appropriate risk assessment and universal precautions are advisable for the first investigations. Concerning the highest risk of the mission - the contamination of the sample by terrestrial material which would put a sample in a higher safety category for a longer period of time and therefore make it costly and reduce the science that can be performed on that sample - a strict containment is also helpful.
Figure 1.1: *This is an artistic view of the Mars Sample Receiving Facility according to the proposed outlay in figure 11.16. The facility is designed after the box-in-the-box concept including an airtight concrete building surrounded by a second barrier of a glass wall. This space between the concrete wall and the outer shell serves as a safety corridor which is at a negative air pressure in relation to the outside. In the entrance region all the shower boxes, material air locks and autoclaves are located.* (Picture: © Fabienne Muni)

It has to be kept in mind that technical as well as organizational methods are equally important in containment technology and that the most important thing in building a perfect laboratory are the people, not the equipment!
Chapter 2

Containment technology

Various types of biosafety level 4 laboratories have been developed over the years. In the United States the scientific era of Fort Detrick began in 1943 with the development of biological weapons and defensive mechanisms against biological attack. Because livestock were always considered as a prime target for possible biological warfare and the economic impact even today is extremely severe in case of outbreaks of e.g. Foot-and-Mouth disease, large animal facilities were developed very early and are at a very high level of reliability. The Engineering Division at Fort Detrick was heavily involved in the development, construction and use of the Lunar Receiving Laboratory in Houston. The Lunar Receiving Laboratory was built for the samples from the Apollo program.

2.1 Definitions

Primary barriers pertain to equipment such as gloves, gowns, masks, biosafety cabinets, respiratory protection, and positive-pressure ventilation suits as well as the use of good laboratory techniques.

Secondary barriers are addressed through facility design with airtight rooms, air handling and filtration, air locks, showers, laundry, sewerage treatment, waste disposal, sterilizers, redundant services, and equipment and material finishes.

Tertiary barriers deal with the physical operation with items such as walls, fences, security, quarantine and animal exclusion zones. Due to the varying risk of biological agents, the facilities that handle these agents need to be designed and classified accordingly.

2.2 Biosafety laboratories

Biosafety laboratories are categorized as one of four types, designated as levels 1, 2, 3, and 4. In his 1979 review, Pike [9] concluded that „the knowledge, the techniques, and the equipment to prevent most laboratory infections are available”. In the United States, however, no single code of practice, standards, guidelines, or other publication provided detailed descriptions of techniques, equipment, and other considerations or recommendations for the broad scope of laboratory activities conducted with a variety of indigenous and exotic infectious agents.
The booklet „Classification of Etiologic Agents on the Basis of Hazard” served as a general reference for some laboratory activities utilizing infectious agents. This booklet, and the concept of categorizing infectious agents and laboratory activities into four classes or levels, served as a basic format for earlier editions of Biosafety in Microbiological and Biomedical Laboratories (BMBL)[10]. The fourth edition of the BMBL continues to specifically describe combinations of microbiological practices, laboratory facilities, and safety equipment, and to recommend their use in four categories or biosafety levels of laboratory operation with selected agents infectious to humans.

The descriptions of biosafety levels 1-4 parallel those in the NIH Guidelines for Research Involving Recombinant DNA, and are consistent with the general criteria originally used in assigning agents to classes 1-4 in Classification of Etiologic Agents on the Basis of Hazard. Four biosafety levels are also described for infectious disease activities utilizing small laboratory animals. Recommendations for biosafety levels for specific agents are made on the basis of the potential hazard of the agent and of the laboratory’s function or activity.

Today the classification is accepted worldwide. The European Standard includes many normative references like the EN 12128: Biotechnology - Laboratories for research, development and analysis - Containment levels of microbiology laboratories, areas of risk, localities and physical safety requirements.

A biosafety level (BSL) 1 or 2 facility would not necessarily have any special ventilation requirements. BSL 1 laboratories work with strains of microorganisms not known to consistently cause disease in healthy adult humans, while BSL 2 laboratories deal with moderate-risk agents that are present in the community and are associated with human disease.

BSL 1 facilities rely on containment based upon standard microbiological practices without special primary or secondary barriers, other than perhaps a sink for hand washing. Agents at BSL 2 facilities can be used safely in activities on an open bench, granted that the potential for producing splashes and aerosols is low. Hepatitis B virus, HIV, and salmonellae are representative of the organisms assigned to these containment areas.

BSL 3 facilities require placing more emphasis on primary and secondary barriers in order to protect the community and the environment, as well as the laboratory personnel. Ventilation systems consequently play a major role in these facilities. This is because work is done with indigenous or exotic agents with a potential for respiratory transmission, which may cause serious and potentially lethal infection. Tuberculosis, the St. Louis encephalitis virus, anthrax and prions are representative of the microorganisms assigned to this containment level. Laboratory manipulations are performed in a biological safety cabinet or other closed equipment, which constitute primary barriers. Secondary barriers include controlled access to the space and ventilation systems that minimize the release of infectious aerosols from the laboratory.

BSL 4 laboratories work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted by aerosol and for which there is no available vaccine or therapy. Viruses such as Congo-Crimean hemorrhagic fever are manipulated at this biosafety level. The primary hazard to personnel working with BSL 4 agents are respiratory exposure to infectious aerosols, mucous membrane, or broken skin exposure to infectious droplets, and autoinoculation. Work with these agents poses a high risk of exposure and infection to laboratory personnel, the community, and the environment. Only
2.3 Containment Laboratory Ventilation

BSL 3 and 4 facilities are often referred to as containment laboratories. They contain airtight rooms and duct systems to ensure that the agents being studied remain in the laboratory. Consequently, all mechanical and electrical service penetrations through the containment barrier are equipped with containment seals.

Airflow must be directional, moving inward toward the containment zone and must be nonrecirculated. Air device placement should work to minimize dead air spaces where contaminated air can accumulate. Supply of 100% outdoor air is required, as recirculation of even HEPA-filtered air is not permitted.

The cabinet is tested and inspected at least every 12 months. This would also be considered as sufficient for unknown agents like potential life forms from Mars. Used HEPA filters would have to be fumigated and then incinerated for disposal. The supply air system must be interlocked with the exhaust ventilation system to prevent positive pressurization. Room air supply and exhaust ductwork should be equipped with bubble-tight dampers to permit sealing for decontamination of individual containment areas.

For instance, the decontamination procedure for a BSC may include sealing off the cabinet and boiling a formalin solution to generate formaldehyde gas. The cabinet fan would recirculate this gas for an hour, and then the cabinet would sit idle for another 15 hours before purging. This illustrates how important it is for the containment zone and ductwork to be sealed not only at the perimeter but also between individual areas within the containment zone.

Exposed ductwork should stand clear of walls to allow access for maintenance of equipment filters and lighting, and to permit leak testing. HEPA filters should be installed as close as possible to the source of the hazard to minimize the length of contaminated ductwork, and they should be monitored by manchelic gauges or other appropriate devices.

2.4 Biosafety level 4: maximum containment

There are two models for BSL 4 laboratories. One is the cabinet laboratory where all handling of the agent is performed in a class III BSC, which is discussed later. The other model is the suit laboratory where personnel wear a protective suit. Use of class I and II cabinets are permitted in the suit laboratory. The BSL 4 laboratory in San Antonio recently replaced its cabinet laboratory with a
suit laboratory. A suit laboratory was depicted in the movie Outbreak, starring Dustin Hoffman who portrayed an Army medical expert sent to combat a deadly virus that destroyed a small town in California and threatened the world.

The BSL 4 containment zone should be located in a separate building or sealed room with independent air supply and exhaust systems. These air systems and components need to be sealed airtight and be accessible from outside the containment area. A ventilated air lock is required for the separation of higher and lower containment areas. The air lock is to have interlocking pneumatic or compressible sealed doors with manual overrides.

The cabinet laboratory is arranged to ensure passage through a minimum of two doors prior to entering any room containing the class III BSC. The personnel working in the suit laboratory wear a one-piece, positive-pressure suit that is ventilated by a life-support system protected by HEPA filtration. The life-support system includes redundant breathing air compressors, alarms, and emergency backup air tanks. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area.

An emergency power source is provided for the exhaust system, life-support systems, alarms, lighting, entry and exit controls, and BSCs. The air pressure within the personnel suit is positive to the surrounding laboratory. The air pressure within the suit area is lower than that of the adjacent area. The entry to the containment area is equipped with magnehelic gauges or pressure-monitoring devices to prove directional flow. The containment area is equipped with audible alarms to detect depressurization.

Supply air to the cabinet room or suit area and associated decontamination shower and air lock is protected by passage through a HEPA filter. The exhaust of the laboratory’s suit area, decontamination shower, and decontamination air lock is treated by passage through two HEPA filters in series prior to discharge to the outside. Redundant supply fans are recommended, and redundant exhaust fans are required.

2.5 HEPA filtration

The development of the HEPA filter during World War II was critical in providing the necessary containment in the modern biosafety laboratory. These filters are constructed of fiberglass “paper” that is pleated to maximize the surface area of the filter and have a minimum particulate removal of 99.97% for particles of 0.3μm. The randomly oriented microfibers cause the particles in the airstream to move in a circuitous path, forcing even the smallest particles to collide with and adhere to the filter. (see figures 11.5 and 11.6).

The filter housings on contaminated exhaust streams should be constructed to facilitate decontamination or should be of the bag-in-bag-out design. This design should also be of the fluid-seal type that incorporates a knife edge that mates into a fluid-filled perimeter channel on the face of the filter. There should be a safety feature where the filter locking arm and the access door interface in such a manner that minimizes the possibility of the door being closed until the filters are correctly seated in the housing.

Prior to leaving the factory, each knife edge should be checked with an alignment gauge to ensure proper alignment with the filter. The filter-sealing mechanism should be replaceable and should be operated through the changeout
bag by a locking handle. The mechanism should exert equal force at the top and bottom edge of the filter when engaging and disengaging the filter from the knife edge.

Three glove sleeves are provided in each bag to facilitate the handling of the filter during changeout. For visibility during this procedure, the bag includes clear PVC at the mouth with an elastic shock cord hemmed into it, so that it fits securely when stretched around the bagging ring. To prevent the bag from sliding off the bagging ring during the changeout process, a nylon security strap is provided at each filter access port. A nylon-cinching strap is also provided at each port to tie off the slack in the bag while the system is in operation.

2.6 Biological safety cabinets

The BSC design has come a long way, with three main classifications: Class I, II, and III.

Class I cabinets are defined as ventilated cabinets for personnel and environmental protection, with unrecirculated airflow away from the operator. Class I cabinets have a similar airflow pattern to a fume hood, except that the class I cabinet has a HEPA filter at the exhaust outlet, and it may or may not be connected to an exhaust duct system. Class I cabinets are suitable for work with agents that require BSL 1, 2, or 3 containment.

Class II cabinets are ventilated cabinets having an open front with inward airflow for personnel protection, downward HEPA-filtered laminar flow for product protection, and HEPA-filtered exhaust airflow for environmental protection. Class II cabinets are suitable for work with agents that require BSL 1, 2, or 3 containment. Class II cabinets are differentiated into various types based on their construction, air velocities, and patterns, and by their exhaust system. For instance, class II type A cabinets may have contaminated plenums under positive pressure that are exposed to the room while type B cabinets must surround all contaminated positive pressure plenums with negative pressure ductwork. Type A cabinets can be exhausted into the lab or outside by way of a canopy connection, whereas type B cabinets must have a dedicated, sealed exhaust system with remote blower and appropriate alarm system.

Class III cabinets are totally enclosed and ventilated of gas-tight construction. Operations are conducted through attached rubber gloves. The cabinet is maintained under negative air pressure of at least 125 Pa. Supply air is drawn into the cabinet through HEPA filters. The exhaust air is treated by double HEPA filtration. The cabinet also has a transfer chamber capable of sterilizing work materials before exiting the glove box containment system. Class III cabinets are suitable for work with agents that require BSL 1, 2, 3, or 4 containment.

2.7 Animal facilities

Many biosafety facilities require the use of laboratory animals, consequently incorporating features and concepts associated with vivariums. The animals are kept in holding rooms, and they require between 12 to 18 Air Changes per Hours (ACH). The purposes of the high ventilation rate include: supplying adequate
oxygen; removal of thermal loads caused by animal metabolism and respiration, lights, and equipment; diluting gaseous and particulate contaminants; adjusting the moisture content of room air; and creating static-pressure differentials between adjoining spaces.

Small animals, such as rodents, are usually housed in ventilated cage racks. These racks do not necessarily act as a containment barrier, but they limit odors and exposure of personnel to allergens. These cage systems vary in manufacturer design, with certain models requiring both central supply and exhaust system connections. Other models require only connection to a central exhaust system and derive the supply air from the holding room itself. Supply and exhaust to these racks are HEPA-filtered.

Regulation of body temperature within normal variation is necessary for the well being of mammals. Generally, exposure of unadapted animals to temperatures above 30°C or below 5°C, without access to shelter or other protective mechanisms, might produce clinical effects which could be life threatening. It is often appropriate to have reheat valves fail closed. Relative humidity should also be controlled, but not nearly as narrowly as temperature. The acceptable range is between 30% and 70% RH.
Chapter 3

Modern laboratories

In order to understand why there are no general standards for biocontainment facilities one has to accept that there is not yet an established agreement among international organizations about the necessary requirements. An exception are regulations for biosafety cabinets and HEPA filter installations and maintenance.

The need for a standard is usually expressed by an industry sector, which communicates this need to a national member body. Once the need for an International Standard has been recognized and formally agreed, the first phase involves definition of the technical scope of the future standard. This phase is usually carried out in working groups which comprise technical experts from countries interested in the subject matter. Once agreement has been reached on which technical aspects are to be covered in the standard, a second phase is entered during which countries negotiate the detailed specifications within the standard. This is the consensus-building phase. The final phase comprises the formal approval of the resulting draft International Standard.

Unfortunately, the situation in certain parts of high containment installations such as airtightness of buildings, doors, air locks and ducts is not yet as far but is in its first phase which means definition of the technical scope. In other fields as concerns autoclaves, HEPA filters and other conventional equipment, standards and test criteria are usually available. A number of proposals of how to measure a certain value and to what limits have been made elsewhere. However, all of these methods and values are of national origin and not accepted by international and sometimes not even by national institutions. At least they could be taken as some sort of state-of-the-art. However, most of them are rather based on what can be achieved than on sound basis of risk assessment.

For this reason the procedure in building a new biocontainment facility is to visit a few of the most current facilities and discuss the best and worst features with the goal to include the former one and prevent the latter one. Sometimes the origin or the „template“ for a facility could easily be seen by a careful study of the concept. E.g. the Swiss Institute of Virology and Immunoprophylaxis (IVI) has certain clear concepts of the Australian Animal Health Laboratory, and the Winnipeg Laboratory has many features of the Swiss facility whereas the new FMD facility in Barcelona is a clear copy of the Swiss IVI. Therefore it is common to study current facilities in order to copy some of the well designed concepts and improve others which did not turn out to work as designed.
3.1 The Canadian Science Centre for Human and Animal Health

The Government of Canada’s laboratory complex in Winnipeg (see figure 11.1) is the first facility in the world to combine laboratories for human and animal disease research at the highest level of biocontainment. The complex is located at 1015 Arlington street in Winnipeg and is adjacent to the Health Sciences Centre and University of Manitoba’s Faculty of Medicine.

The facility contains Canada’s first biosafety level 4 laboratory, providing the capability to work safely with the most serious human and animal diseases. The biosafety level 4 laboratories permit Canada’s research and diagnostic programs to be expanded into crucial new areas such as research into the Ebola virus, Marburg virus and Lassa Fever to identify just a few.

The biosafety level 4 laboratories represent about one percent of the laboratory area, which also includes level 2 and 3 laboratories. Currently about 220 federal government employees work at the complex. The staff figure is expected to rise to approximately 250. Biosafety level 3 and 4 laboratory areas contain airtight rooms and ductwork, and feature interlocking and airtight bio-seal door and damper systems. Air-locks for entry and exit maintain negative air pressure to direct air inward, ensuring organisms being studied remain in the laboratory.

The levels 3 and 4 containment laboratories are specially constructed using „box-within-a-box” negative air pressure zone principles. In addition, specific building materials and techniques have been used to ensure all systems and surfaces are sealed. Air exiting the laboratories is filtered using High Efficiency Particulate Air (HEPA) filtration. HEPA filters can filter out particles 85 times smaller than the smallest known disease-causing agent. Laboratories feature 100% sterilization of solid and liquid waste, accomplished by a 20,000-litre liquid sterilization system and a specially-designed autoclave to heat and break down solid waste. All mechanical, electrical and structural penetrations through the containment barrier are equipped with special containment seals. Three 1000-kilowatt generators handle emergency power back-up to all heating, ventilation and air conditioning systems, and to essential life safety systems. State-of-the-art Direct Control is utilized for control and digital energy management throughout the building.

This laboratory is designed for work with exotic and dangerous agents that usually produce very serious and often fatal human and animal diseases. These agents are transmitted readily from person to person or from animal to human (and vice versa) through the air or casual contact. Researchers must follow the same entry and exit protocols as level 3. In addition, they wear positive air pressure protective suits connected to filtered air lines. The suits are chemically treated after each session and always remain in the secured area of the laboratory.

3.2 The Galveston Lab

The 1,200 m² maximum containment facility is dedicated to the study of tropical and emerging infections and is designed to allow researchers to safely and effectively work with the most hazardous organisms. The level 4 laboratory itself, which is sometimes compared to a submarine inside a giant bank vault,
is just 200 m$^2$. The other 1,000 m$^2$ of the facility contain high-tech support equipment designed to capture and destroy any microbes before they can exit the structure. The full-sized level 4 laboratory of the University of Texas Medical Branch (UTMB) in which researchers work inside the laboratory in pressurized suits - is the first on an American university campus, but it isn’t the first such facility on a campus anywhere in the world. That distinction belongs to a full-sized level 4 at a university in Marburg, Germany.

There is one other full-sized level 4 already in operation in Texas, located at the private Southwest Foundation for Biomedical Research in San Antonio. Meanwhile, Texas Tech University in Lubbock, the University of New Mexico in Albuquerque, and the University of California at Davis reportedly also are looking into building their own full-sized level 4 laboratories. Construction of the level 4 laboratory was largely funded by a grant from the Sealy & Smith Foundation of Galveston, a philanthropy solely dedicated to benefiting UTMB, with additional support for the facility from the National Institutes of Health (NIH), among other sources.

### 3.3 The P4 in Lyon

The P4 Laboratory (see figure 11.3) is a glass and metal structure on four levels: the reception zone, the actual P4 zone, and two technical zones from which the technical systems are operated. The reception zone is in an adjacent two-storey building and it comprises, on the lower level, the control room (where the technical systems controls are centralized) and, on a level with the level 4 laboratory, a reception area and two traditional laboratories for a certain number of preparations.

The P4 zone has a circular viewing corridor, which allows the visitor to see the different areas of the laboratory through portholes. Access to this viewing corridor is from the reception zone, through two doors operated by security badges.

The laboratory entrance zone consists of a series of air locks (airtight cloakroom, airtight shower, airtight changing-room, and airtight decontamination shower). The air locks have interdependent doors which cannot be opened simultaneously.

And inside there are three level 4 work areas: two independent laboratories of 60 to 70 m$^2$, and a communal animal room.

The two technical zones are situated one above and one below the P4 laboratory and handle the operating systems: generators, air supply and treatment systems, heating and air-conditioning, water supply and the waste treatment.

The P4 building is built on the Russian doll principle, a „box-within-a-box“. The laboratory zones are separated from the exterior facades of the building by the circular viewing corridor. The building materials were selected and assembled in such a way as to ensure that the P4 zones are totally airtight. Furthermore, a continuous state of decompression is maintained between the laboratory and the exterior, in order to prevent any leakage of particles. A further environmental protection is ensured by the disposal of all solid waste matter in autoclaves, and by the addition of decontamination chemicals to liquid waste prior to steam sterilization.
The research workers wear specially designed airtight suits and helmets with an air supply and are connected permanently to the control station.

3.4 Porton Down

The Defence Science and Technology Laboratory (DSTL) at Porton Down is a United Kingdom government bio-chemical research facility, famous for its connection with chemical weapons and biological weapons research. It is near Salisbury.

The Defence Evaluation and Research Agency (normally known as DERA), then Britain’s largest science and technology organization, was a part of the UK Ministry of Defence until July 2001. DERA was then split into two organizations: a commercial firm, QinetiQ, and the Defence Science and Technology Laboratory (DSTL).

DSTL has at the Porton Down site new, state-of-the-art, high level containment facilities which are built to the highest standards of design and construction. The microbiological containment facility contains 36 biosafety cabinets of class III (BSC III) and one large level 4 cabinet line as well as 13 plastic isolators.

3.5 Do’s and don’ts

- All people involved in the project must be made to understand biosafety concepts
- All people involved in the project must understand that containment facility projects are no routine projects
- Each bioccontainment facility should be designed to fit to the needs of the research program
- The „cookie cutter” approach does not work with these facilities
- Allowances should be made for logical future expansion, especially in the HVAC system
- Seek advice by people who have been involved in similar systems
- Design commissioning and test system during the design phase already
- The end users have to be involved at the very first stage in design concept
- End user and biosafety specialists have to trust their instincts, architects and engineers often have wrong biosafety concepts
- Review the plans several times during the design development
- Visit the site during construction
- Work with the community on their concerns
- Establish communication with all stakeholders
3.5. **DO’S AND DON’TS**

- Establish single decision makers in all fields: biosafety, science, architect and engineering, management
- Negotiate a realistic development schedule
- Budget for a 10% contingency
- Consult authorities having jurisdiction
- Adopt a consistent language
- Establish a formal and timely response process for queries
- Avoid prototypical design solutions
- Don’t be the guinea pig for the industry
- Design for adequate space requirements for technical installations
- Avoid over-alarming
- Design for flexibility and diversity
- Don’t design the failure into the system
- Design for realistic failure scenario
- Do adequate pretesting and commissioning
- Start the commissioning process right from the first day of design and never stop until operation
- Establish a tiered commissioning process on component, system and integrated system
- Include training program in the overall cost
- All hygienic measures help the biosafety measures
- Never say never
Chapter 4

Essential elements

Handling risk group 4 agents is inherently hazardous because the agents cause serious human disease and can readily be transmitted from one individual to another. The absolute absence of any knowledge about the risk of an agent could lead to the definition to an additional risk group 5 as has been done elsewhere. For example it is believed that Australia has never had a Foot-and-Mouth outbreak and therefore the government decided that FMD virus should never be allowed to enter the country on purpose. Therefore FMDV has been classified as risk group 5. Also some exotic disease agents could be classified as risk group 5. Of course there are no stronger safety measures known than biosafety level 4. If work has to be done with an agent where nothing is known at all and it has been decided to use biosafety levels as a precautionary measure, then biosafety level 4 has to apply. The choices are either to contain the operator in a full encapsulated suit or restrict the micro-organism to a primary device, usually a class III biological safety cabinet. The decision to contain the operator or the bug depends upon the work to be done in each laboratory situation.

4.1 Element 1: Suit laboratory

The first full body suits were ordered by the U.S. Army at the company ILC Dover in 1976. In the US and in many other countries the blue suits are still in use, however, the production has been stopped recently. But there are plans to start the production again.

The polyvinyl-chloride suits have a weight of 6 kg and are positive pressured with breathing air from the outside. Medical breathing air is filtered through a charcoal filter and then through a HEPA filter before entering the protective suit. A HEPA filter is placed in-line between the air supply and protective suit to preclude potential contamination of the researcher while connecting and disconnecting from the air supply. The HEPA filter housing is constructed of stainless steel. All metal connections are threaded with Teflon tape to maintain an airtight and waterproof seal. It could be argued that the breathing air is only once HEPA filtered in comparison to the two HEPA filters in series otherwise. However, in addition to the very small probability of contamination at the breathing air connection, all work with organisms in the laboratory is done in biosafety cabinets of class II which build the first level of HEPA filtration.
The noise level of air continuously entering the suit is about 87 decibels. While wearing the positive-pressure protective suit, personnel are required to wear earplugs or earmuffs to reduce the noise level. All personnel qualified to wear a positive-pressure protective suit are enrolled in a hearing conservation program.

Entry into the suit area is through an airlock with airtight doors. This airlock is actually a chemical disinfectant shower. A shower of the protective suit is required upon exit from the research laboratory. The shower contains air supply lines. The shower begins with a one minute water wash followed by a 3 minute wash with 5% Microchem plus detergent disinfectant. Any residual disinfectant is then removed with a one minute water wash. Effluent from the shower is transported to the sewage water system and steam sterilized.

Full body suits are constructed of a reasonably flexible chlorinated polyethylene material with a clear polyvinyl-chloride (PVC) visor. The suit opening is mechanically fastened with a zipper. Two moulded plastic closures with interlocking lips provide a watertight seal. The suits operate at a nominal positive pressure of 180 Pa with respect to the surrounding atmosphere. The exhaust air valves are a nonreturn design. Movement of the operator in the suit, such as crouching or standing from a crouch, alters the pressure significantly and causes of concern if the pressure differential becomes reversed, even momentarily. During use, flow rates in the suit vary from high (260 l/min) at ambient temperature to low (138 l/min) at cooler temperature when some of the supply air is lost from the vortex cooling tube.

The suits are integrity pressure tested periodically. The integrity test is performed at 1250 Pa. The suit is required to remain above 1000 Pa during the 3 minute test. A cuff at the wrist gloves of a variety of material and various sizes needed to be attached. Flexible adhesive tape, rather than a retaining O-ring is used to keep the glove secured to the cuff.

Two main factors are essential for a biocontainment: first of all an airtight building which guarantees that no aerosols can escape from the facility. Second a negative pressure inside the containment which supports the containment in a dynamical way so that no aerosols can escape if there is a leak in the physical barrier. The whole system is supported by an air change rate which keeps the contamination rate of aerosols as low as possible. The number of air changes must be higher than 10 times and should be about 20 to 30 times the volume of the room per hour. About the airtightness much less information is available. However, one important aspect must be taken into consideration: the higher the airtightness of a volume the more complex a control system for the pressure has to be. A simple example shall demonstrate this. We assume the following situation: an absolutely airtight room shall be ventilated with 20 air changes per hour. Fluctuations of the pressure of 10% shall be acceptable:

- Volume of the room = 200 m$^3$
- Negative air pressure = -100 Pa
- Pressure fluctuation = 10 Pa
- Supply air = 4000 m$^3$·h$^{-1}$
- Exhaust air = 4000 m$^3$·h$^{-1}$
If in an airtight room of 200 m$^3$ 20 liters of air are more supplied than exhausted or more extracted than provided a pressure difference of 10 Pa is resulting. Now, regarding the air flow of 4000 m$^3$ this means a fluctuation of the flux of air volume of only $0.02/4000 = 5 \cdot 10^{-6} = 0.0005$ % per hour! Such a high volume flux balance is absolutely unrealistic. Even the opening of doors and pass-through boxes produce relatively large disturbances, a more realistic value could be 1%. This would mean that a deviation of 1% of $4000 \ m^3 h^{-1} = 40 \ m^3 h^{-1} = 11$ liter per second is reached in less than 4 seconds. In practice this means that the pressure in an airtight room could only be regulated at extremely small airflow. As high air fluxes are demanded in laboratories, additional buffer systems must be foreseen. Leaks operate as such buffering systems. Figure 11.7 shows the effect of leaks in containment buildings. The uppermost curve shows what happens in a room with a low airtightness when the airflow varies from $60 \ m^3 h^{-1}$ to $70 \ m^3 h^{-1}$: the pressure increases by only 1 Pa. The second curve shows a pressure difference of about 5 Pa for the same situation. At a very airtight room (the curve at the bottom) the resulting pressure difference would be extremely large.

What would the required airtightness be? According to figure 11.8 in order to have a consistent system, the airtightness should have at least the same quality as a HEPA filter. If aerosols could leave the building by leaks then the installation of a single HEPA filter in the air supply duct would be questionable.

In the following a theory of the containment is developed.

A leakage $q$ is proportional to the pressure difference; the approximation for $n = 1$ for the exponent $\delta p$ is not a source of an error:

$$q = \lambda \cdot \delta p$$

leading to the equation:

$$\int_{p_a}^{p_e} \frac{dp}{p} = \frac{\lambda}{P} \int_{0}^{t_e} dt$$

With the solution:

$$\lambda = \frac{V}{t_e \cdot P} \cdot \ln \left( \frac{p_e}{p_a} \right)$$

The parameter $\lambda$ is a measure for the airtightness of a barrier and has the dimension of $m^3 \ sec^{-1} Pa^{-1}$. Alternatively the time could be defined within the pressure is decreased to its half value:

$$t_{1/2} = \frac{\ln 2}{\tau}, \ \text{with} \ \tau = \frac{\lambda \cdot P}{V}$$

A value of $\lambda = 10^{-5} \ m^3 \ sec^{-1} Pa^{-1}$ corresponds therefore to a time value $t_{1/2}$ of 69 seconds, assuming a Volume of 100 m$^3$

$$\lambda = 10^{-7} \ m^3 \ sec^{-1} Pa^{-1}$$

to a value $t_{1/2}$ of 2 hours.
A realistic value for $\lambda$ might be between $10^{-6}$ and $10^{-7} \, m^3 \, sec^{-1} \, Pa^{-1}$. That is a leakage in the order of a milliliter per Pascal and second only.

There is, however, a fundamental difference between the functioning of a filter and a wall. A filter could be regarded as a „wall” having many small holes enabling the transfer of large air volumes but holding back efficiently aerosols. A wall might only have a small number of „holes” enabling the transfer of small air volumes but not holding back aerosols. Therefore the following assumptions could be made:

- HEPA filters operate at relatively large pressure differences in order to transfer large volumes of air.
- The wall comes in operation at a small overpressure of a few Pascal caused by heat production inside the containment.
- The penetration efficiency of a HEPA is about $10^{-5}$ to $10^{-6}$.
- Assuming holes in the wall which are large in comparison to the aerosol size, the penetration efficiency for aerosols could be assumed to be close to 1.0 (which means the leakage has no filter effect at all as the aerosol does not collide within the channel).

Comparing the number of aerosols released by a single HEPA filter by the air volume $Q$ and a leakage in the wall by the air volume $q$:

$$Q \cdot \epsilon_{HEPA} = q \cdot \epsilon_{wall} \quad (4.7)$$

and including the relation above for $\lambda$ the airtightness for a wall comparable to a single HEPA filter is defined as:

$$\lambda = \frac{Q \cdot \epsilon_{HEPA}}{\Delta p \cdot \epsilon_{wall}} \quad (4.8)$$

Assuming a laboratory space of 200 $m^2$, 2.5 m room height and 20 air changes per hour leads to $10^4 \, m^3 h^{-1}$ or about $3 \, m^3 s^{-1}$

$$\lambda = \frac{3 \, m^3 s^{-1} \cdot 10^{-5}}{3 \, Pa \cdot 1.0} = 10^{-5} \, m^3 s^{-1} Pa^{-1} \quad (4.9)$$

In other words, after closing the dampers and switching off the ventilator the room pressure of the laboratory of about 500 $m^3$ volume should decrease the pressure difference to 50% not faster than in 14 seconds. A pressure difference of 1/10 should even be found after 46 seconds. Of course much longer times could be reached if a compartment of small airtight rooms within the containment would be available. This effect is due to the changing ratio of the surface to the volume making larger rooms much more susceptible to pressure loss.

A few examples shall illustrate the principle:

**Example 1:** Common test conditions for laboratories of new high containments of biosafety level 4 are $p = 500 \, Pa$, $\Delta p \leq 12.5 \, Pa \, min^{-1}$. Assuming a laboratory volume of 100 $m^3$ we calculate $\lambda = 4.3 \cdot 10^{-7} \, m^3 \, Pa^{-1} \, s^{-1}$
or at the other hand 0.4 \text{ml Pa}^{-1} \text{s}^{-1}. Concerning the half life time of the negative pressure in a room that means 27 min.

**Example 2:** Common test conditions for HEPA boxes (see figure 11.4 are \( p = 2500 \text{ Pa}, \Delta V \leq 0.2\% \). This results in a maximal allowable pressure loss of only 5 Pa per minute.

\[
\lambda = \frac{V}{t \cdot F} \cdot \ln \left( \frac{p_0}{p} \right)
\]

This gives the small value of \( \lambda = 2 \times 10^{-11} m^3 \text{ Pa}^{-1} \text{s}^{-1} \).

**Example 3:** Air ducts: test conditions are \( p = 500 \text{ Pa}, \Delta V \leq 0.1\% \) resulting in a maximal allowable pressure loss of only 0.5 Pa per minute.

\[
\lambda = \frac{V}{t \cdot F} \cdot \ln \left( \frac{p_0}{p} \right)
\]

For a duct of a length of 20 meters this gives a \( \lambda = 10^{-9} m^3 \text{ Pa}^{-1} \text{s}^{-1} \).

This analysis of containment allows the definition of a containment quality without a pressure decay test at high negative or positive pressures as e.g. 500 Pa.

Those values must be compared with the aerosol load of the corresponding air volumes. For this calculation, a virus source within the room is assumed to: \( \dot{n}_{\text{in}} \). The exhaust air transports therefore the amount \( \dot{n}_{\text{out}} \) and the mass conservation law gives:

\[
\dot{n}_{\text{in}} \cdot \delta t - \dot{n}_{\text{out}} \cdot \delta t = V \delta c
\]

and this leads to the differential equation:

\[
\frac{dc}{dt} + \frac{Q}{V} c = \frac{\dot{n}_{\text{in}}}{V}
\]

where \( Q \) represents the air exchanged and \( V \) the volume of the room. The solution is:

\[
c(t) = \frac{\dot{n}_{\text{in}}}{Q} \cdot \left( 1 - e^{-\frac{Q}{V} t} \right)
\]

Assuming a permanent particulate release in aerosol form of \( 10^{12} \) particle per second in a room and assuming a uniform distribution in the room, then a constant particle concentration will result within a relatively short time. This concentration exists therefore in the room and in the ductwork. As long as the building perimeter is absolutely airtight respectively the negative pressure guarantees the containment no particle can escape uncontrolled to the outside. However, by including a leakage flux and at the same time with a failure of the
negative pressure system, the following situation can be calculated:

$$\lambda = 10^{-5} \, m^3 Pa^{-1} s^{-1}$$

$$\Delta p = +10 \, Pa$$

$$q = 10^{-5} \, m^3 s^{-1}$$

**Comparison of static versus dynamic containment**

We have estimated the airtightness of a building to be between $10^{-5}$ and $10^{-7}$ for very high quality containments. However, for this calculation only the permeability for air was considered, no attempt was made to understand the filter effect of e.g. small cracks in the concrete. It can be assumed that in a concrete building the very tiny cracks could even have a filter effect, letting the air molecules penetrate but not larger particles like aerosols containing infectious agents. On the other hand a building constructed by HEPA filters only would let the air molecules penetrate freely but would probably catch aerosols which are either too large or containing electric charged particles. But also here we do not know the filter characteristic for a HEPA house with no active ventilation. However, in a first approximation we assume that the concrete house with a $\lambda$ of $10^{-6}$ could be compared with the HEPA filter house in regard of aerosols only, assuming a HEPA filter quality H14 (for definition see table 11.2).

As long as the building is ventilated with 10 ACH by two HEPA filters in the exhaust air we expect a filter effect of $10^{-10}$ to $10^{-11}$ and as long as the containment is supported with a negative air pressure the building is safe even when releasing some $10^{10}$ aerosol particles inside the building. In case of a power failure the filter effect is only $10^{-5}$ as soon as the negative pressure has decreased to the outside pressure or in case of strong winds inverting the pressure scheme. Without negative pressure during a power loss the quality of the building therefore becomes crucial, especially on the side of the post mortem room with a wall to the outside.

Therefore, we conclude:

**Method 1:** Air tightness of the building. Advantage: No power needed for maintenance, not very vulnerable to power loss. Disadvantage: Expensive in construction. Negative pressure control is more complex as the air tightness increases.

**Method 2:** Air changes per hour. Advantage: Inside aerosol contamination is decreased the higher the ACH. Disadvantage: The higher the ACH the higher the power consumption especially if the air must be conditioned. At extremely high contamination levels (e.g. large animal rooms) the higher the ACH the more aerosols could pass a HEPA filter per time unit because the filter efficiency is always $\leq 1$. 

22
Method 3: Negative pressure inside. Advantage: Supports the static containment as imperfections in the barrier are compensated. Disadvantage: The higher the negative pressure the higher the energy consumption. Very vulnerable to power loss.

4.2 Element 2: BSC class III „glove box”

A risk group 4 microbiological agent may be manipulated in a class III isolator or class III biological safety cabinet. Although the concept is similar, the construction and operation of isolators and cabinets differ.

There exist hard-walled, aluminium and Perspex class III biological safety cabinets (BSC) with arm-length thick, robust rubber gloves. More modern construction is stainless steel and glass. The cabinet is a leaktightly construction. The European Standard EN 12469 notes a minimum leaktightly of equal to or less than a 10% loss of test overpressure of 500 Pa in the whole enclosed system after 30 minutes.

Cabinets operate at negative pressure with respect to room pressure. EN 12469 requires a 200 Pa negative differential pressure. The large negative pressure differential accommodates movement of the glove sleeve volume into the cabinet that alters the cabinet volume and pressure. Additionally, failures such as a detached glove leave a 200 mm diameter hole in the barrier, and to maintain containment an inward airflow of at least $0.7 \text{ m/s}$ is required through the open glove port, with only one glove removed. EN 12469 requires a measured minimum volumetric airflow rate through the inlet filter of $0.05 \text{ m}^3\text{s}^{-1}$ ($3 \text{ m}^3/\text{min}$) for each cubic metre of cabinet volume when the gloves are attached and the cabinet is at negative pressure of 200 Pa. The turbulent airflow within the cabinet may be a drawback if cross contamination within the cabinet is to be avoided. Also for handling dust and very small particles of the Mars samples neither turbulent nor laminar flow of air or inert gas might be appropriate. However, after closing the dampers at the supply side no or only negligible air flow would be required once the negative pressure inside is reached after bringing in the material. Because of loosing the safety requirement of strong air inflow in case of a disrupted glove, the situation of gloves must be considered in a risk analysis and the fixing of gloves on the box as well as the invulnerability of the gloves must be adjusted accordingly.

EN 12469 notes that gloves should have a fit conforming to the diameter and shape of the glove port and it should be possible to replace gloves from outside the cabinet, while it is operating, in such a way that the old gloves can be pushed inside the cabinet and new ones fitted. This is a task if a glove develops a leak.

Negative-pressure flexible-film isolators are airtight like hard-walled cabinets. The integrity pressure test requires that leakage into the isolator must not reduce a pressure of 100 Pa by more than 20 Pa in 1 hour. In use, flexible-film isolator envelopes are held under tension created by a 20 to 40 Pa air pressure differential across the envelope which is significantly lower than hard-wallet cabinets.

Flexible film isolators are designed to operate at a maximum airflow of 350 litres per minute and nominal air changes of 14 to 20 per hour. Gloves are fitted to a triple groove cuff on the plastic sleeve. The cuff design allows them
to be readily changed without broaching the envelope. Flexible film isolators may be linked together with a flexible sleeve connected between large transfer ports approximately 700 mm in diameter. A metal bridge can be installed between the isolators, within the sleeve, to support the weight of the materials and equipment during transfer but place limitations on the bulk and mass of such items that could be handled easily.

The main difference of option 1 and option 2 is the much smaller and therefore better controllable primary volume. An example shall be used to demonstrate the effect. The biosafety cabinet has a working volume of 0.5 $m^3$ and an air change rate of up to 1000 $m^3/h$. Assuming the volume contains $10^8$ aerosols. The time until all aerosols are removed from the air of the chamber can be calculated according to:

$$t = \frac{1}{ACH} \left( \frac{ln(c_0)}{ln c(t)} \right)$$

(4.13)

The result is about a minute. After this short time all aerosol would either be absorbed by the HEPA filters or released. About $25 \cdot 10^3$ of the aerosol would pass the first HEPA filter of the biosafety cabinet. They would be absorbed at the second passage or released into the laboratory. Would the $10^8$ aerosols be released within an air volume of 200 $m^3$ 2 hours would be required to absorb the aerosol in the HEPA filter of that particular room. This calculation demonstrates the advantage of a small air volume and the enormous air change rate of a biosafety cabinet of class III.

Due to the extremely small leak rate of the primary containment, the secondary containment has a much lower air tightness to fulfill. A biosafety cabinet of class III can be installed in a laboratory of biosafety level 3. In general, however, a slightly enhanced biosafety level 3 is chosen in order to have a higher safety margin in case of a break in the primary containment (e.g. a loss of a glove during operation).

### 4.3 Element 3: Clean room technology

A clean room is a work area with controlled temperature and humidity to protect the samples from contamination. It contains plastic walls and ceilings, external lighting, and there is a continuous influx of clean, dust-free air. The room should be cleaned daily to prevent contamination.

As a first analysis step it would be worked inside glove-boxes containing a clear defined air or gas quality. Class III biological safety cabinets have been used for decades to achieve a contained space within which work can be performed. They protect the operator and the environment. Although they are not designed expressly to provide product protection; however, since both inflow and exhaust air pass through HEPA filters, the interior of the cabinet is relatively particulate free.

Recent modifications include working in half-suit that is sealed to the working surface of the cabinet. The goal is absolute containment.

Inside a glove-box there are three possibilities to work in an inert gas environment:

- negative pressure
4.4 Controlled penetrations

4.4.1 Chemical shower

A chemical shower cabin is a hermetically closed and ventilated cubicle by which people come in and out wearing their protective suit. It is absolutely essential to decontaminate the suit before returning to the clean space, and that is done in the chemical shower. The shower is automatically controlled - a button is punched and disinfectants are sprayed out through a series of nozzles. The suit is saturated and then rinsed off. Since anything that is automatic can fail, there is also a handles to provide dumping of chemicals onto the suit to assure disinfection. It is heavily discussed whether the decontamination is primarily done by a disinfectant process or by actually rinse-off of the material from the suit surface. It has to be considered, however, that contact time with the disinfectant is extremely short in any case.

4.4.2 Shower

A shower has two functions: firstly as an airlock as entry to an airtight laboratory and secondly as a shower in order to decontaminate a person. In the following description the function of a shower is illustrated: after entry to the changing room, all clothes must be taken off and the shower cubicle is crossed naked without taking a shower. In the inner change room laboratory clothes are put on. At the exit from the laboratory all laboratory clothes are taken off and left inside the containment; they must be laundered inside or autoclaved out and sent to a laundry. The person enters the shower cubicle and takes a thorough shower for a pre-defined period. An intensive air ventilation removes all the aerosols created during the showering.

Personnel showers are just special airlock systems to guarantee that the aerosols are removed efficiently before the outer door is opened. The shower represents the containment barrier.

For the ventilation scheme there are several possible options shown in figure 11.10. Option A contains two airtight doors. Both changing rooms are ventilated independently and kept at different air pressures. This option is the most sophisticated and is standard for level 4 laboratories.
Option B has one airtight door on the outer side of the shower cubicle. The air flows through the nonairtight door to the inner changing room and is sucked off from there. This option is common for laboratories working with organisms not pathogenic for men.

Option C is contrary to option B with an airtight door on the inner side of the shower cubicle. The air flows through the nonairtight outer door into the shower cubicle and sucked away from there.

Option D contains no airtight doors and the shower cubicle is not ventilated actively. Air flows through both doors from the outer changing room to the inner changing room.

Option E is similar to option D, however, there is no active ventilation of the airlock at all. The air flows passively from the outside to the laboratory by the negative air pressure inside only.

4.4.3 Air locks

Controlled transfer in a level 4 area requires an airlock system in which personnel or material is being decontaminated. Common airlocks are shower systems for personnel, dunk tanks, formaldehyde and ethylenoxid fumigation chambers for material which is not heat stable and autoclaves with a door to the inside and one to the outside of the containment. Other systems are sewage sterilisators. Air filtration systems are normally not described as airlock systems. Typically, an airlock system consists of a room which separates in from out and is actively ventilated. If not ventilated actively it is generally described as an ante-room.

4.4.4 Autoclaves

Autoclaves must be used properly to effectively decontaminate potentially biohazardous materials. The following elements all contribute to autoclave effectiveness.

- **Temperature**: Adequate chamber temperature is at least 121°C.
- **Time**: Adequate autoclaving time is a minimum of 20 minutes, measured after the temperature of the material being sterilized reaches 121°C and 2 bar. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load.
- **Contact**: Steam saturation of the load is essential for effective decontamination. Air pockets or insufficient steam supply will prevent adequate contact. To ensure adequate steam contact, leave autoclave bags partially open during autoclaving to allow steam to penetrate into the bag. Add a small amount of water inside the bag to help ensure heat transfer to the items being decontaminated (do not add water if it will cause biohazardous materials to splash out of the bag).
- **Containers**: Use leak-proof containers for items to be autoclaved. Place plastic bags inside a secondary container in the autoclave in case liquids leak out. Plastic or stainless steel containers are appropriate secondary containers. Make sure plastic bags and pans are autoclavable to avoid having to clean up melted plastic.
4.4. CONTROLLED PENETRATIONS

- **Indicators:** Tape indicators can only verify that the autoclave has reached normal operating temperatures for decontamination. Most chemical indicators change color after being exposed to 121°C, but cannot measure the length of time spent at 121°C. Biological indicators (such as Bacillus stearothermophilus spore strips) and certain chemical indicators (such as Sterigage) verify that the autoclave reached adequate temperature for a long enough time to kill microorganisms.

  Use a chemical indicator in every load to monitor the effectiveness of individual autoclave runs (temperature only).

  Once a month, use either a biological indicator (such as Bacillus stearothermophilus spore strips) or a chemical indicator that measures both time and temperature (such as Sterigage). Bury the indicator in the center of the load to validate adequate steam penetration. Keep a log book to record the results.

- **Safety:** Autoclaves are classified as pressure vessels, and must be inspected at least annually.

4.4.5 Transfer boxes

Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from both the class III biological safety cabinet and the cabinet room.

4.4.6 pipes and cables

All penetrations in these structures and surfaces are sealed, see e.g. figure 11.11. All drains in the floor of the suit area contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters. Other liquid and gas services to the cabinet room are protected by devices that prevent backflow.

4.4.7 Suit versus biosafety cabins

To contain the operator or contain the bug, is a question which can be answered in many ways depending on the aim of the operation and the required flexibility. The table in figure 11.4 shows some advantages and disadvantages of both systems.
Chapter 5

The European MSRF

5.1 Sample canister

In order to discuss requirements of a sample container, clear definitions are essential.

Definition 1: Clean

Clean could mean free of molecular organic earth sourced contaminants and free of any molecular organic mars sourced contaminants. The first requirement is essential for the life form detection. Free is defined as less than TBD $pgcm^{-2}$ organic material measured by the method TBD.

Definition 2: Gastight, airtight

The quality of the gastightness or airtightness is quantitatively measured by the leak rate of the system. Requirements are: less or equal to TBD $mls^{-1}$ at a negative pressure of 20 mbar (2 kPa).

Definition 3: Biohazard

Known hazards: these are biohazards according to the EU law and include also nonreplicating substances (e.g. toxins). Not only planetary protection is regarded here, but also occupational health.

Unknown hazards: an extraterrestrial life form that has the capacity to produce deleterious effects on the terrestrial biosphere.
Definition 4: Evidence of life

Life should be understood to mean „Life as we know it“. This could be a biomolecule or a gas which may contain information about important biological processes in the sample. If referring to possibilities outside terrestrial experience it shall be called „new life forms“. There is consequently no definition for that.

Definition 5: Biocontainment

Biocontainment means to contain a biohazard inside a certain volume and not release it uncontrolled. Controlled release would be a release either in an inactive form - sterilized or inactivated, so that it would not anymore have deleterious effects - or alternatively properly packed in a transport container in order to be transported into another biocontainment.

Definition 6: Containment violation

A containment is violated if a biohazard could be released uncontrolled into the environment. This is however not necessarily fulfilled when loosing one particular biosafety barrier or biosafety measure. This distinction is very important. For example, a containment could be a hermetically closed physical barrier plus a negative air pressure inside the containment. Loosing one of these two containment measures is not yet a containment violation. Loosing the pressure difference inside does not affect the hermetically closed barrier and loosing the barrier partly by a crack in the wall does not violate the containment as long as the negative pressure inside can be guaranteed, e.g. by exhausting actively the air through HEPA filters. Therefore, an increase of the gas pressure inside a sample canister from 20 mbar to around 1000 mbar is not necessarily a biohazardous situation. As long as no particle was released but only terrestrial atmosphere was entering the canister, the gas analysis would not be possible anymore and the sample might have been affected by oxygen, however, as long as a particulate transfer is prevented - for example by a Filter device - there is no biohazardous situation yet.

A sample container would have two requirements:

- Serve as a biocontainment (see definition above)
- Keep the sample pristine until analysis.
The first requirement is more important as it would be mission critical. The second one however, is relevant for the science. Both requirements are quite different. The first only requires the prevention of a release of a unknown bio-hazard.

### 5.1.1 Keep the sample pristine

The question is about the necessary tightness of a container for Mars samples. As mentioned above, it must be considered that the samples be pristine and thus not be contaminated on Earth or by the atmosphere in the space craft by organics or water vapour. The other requirement is that the sample must be stored in such a way that it does not release any of its parts on Earth through a leakage of the container and can be regarded as a much weaker requirement as the pressure inside is only around 6 mbar. As a first approach the contamination by Earth bound \( N_2 \) shall be calculated.

On Earth we have 78% \( N_2 \), 21% \( O_2 \) and 1% Ar at 1013 mbar. On Mars we expect to have 95% \( CO_2 \), 3% \( N_2 \) and 2% Ar at around 6 mbar (600 Pa). Further we assume a container of 1000 \( cm^3 \). The level of acceptable inflow of air from the spacecraft or down at Earth shall be less than or equal to 1 ppt (\( 10^{-12} \)).

In order to estimate the requirements on a monitoring system the following question must be answered: how many molecules of the abundant \( N_2 \) would have to be detected inside the container?

\[
p_{CO_2} \cdot V = n_{CO_2} \cdot RT \quad (5.1)
\]

\[
n_{N_2} = \frac{0.18 \text{ mbar}}{22.4 \cdot 10^3 \text{ mbar mol}^{-1}} = 8 \cdot 10^{-6} \text{mol} \quad (5.2)
\]

These are \( 5 \times 10^6 N_2 \) molecules. At the same temperature as at filling site on the Mars the pressure inside would be increased by this additional molecules by \( 10^{-12} \) mbar only\( (10^{-19} Pa) \). The temporary pressure increase due to temperature rise during the entry of the space craft into the atmosphere is not considered here.

In chapter 4 a theory of the containment was developped.

Assuming a travel time of 6 month we get with equation 4.5 and the above values:

\[
\lambda = \frac{10^3 \text{ cm}^3}{1.5 \cdot 10^7 \text{ s} \cdot 10^5 \text{ Pa} \ln \left( \frac{6 \text{ mbar}}{6 \text{ mbar} + 6 \cdot 10^{-12} \text{ mbar}} \right)} = 10^{-21} \text{ cm}^3 \text{ s}^{-1} \text{ Pa}^{-1} \quad (5.3)
\]

This value seems extremely small and rather close to a hermetic containment e.g. a welded container made by polypropylene or similar material which could be explosive-welded.

The original question was to define a biological containment, however, with the requirement for a containment against single molecule influx it seems obvi-
ous that only a hermetic containment can fulfill such a strong requirement.

An alternative shall be shown here: a „box-within-the-box“ system, consisting of two boxes as shown in figure 11.12. Both, the inner as well as the outer box are containing the lower pressure as existent on the Mars. If the boxes can be made gastight with a high quality, the outer box will only be contaminated with a very small number of gas molecules from the outside and the pressure would therefore only be raised by a tiny amount having almost no effect on the inner box.

If the quality conditions defined above for both containments could be reached (pressure increase in the outer box of only $10^{-12}$ mbar, r. $10^{-10}$ Pa), it may be assumed that almost no molecules would enter the inner box. As an additional safety measure the composition in the outer box would not be very different from the composition inside the inner box and therefore even a leakage into it would have almost no effect.

Transport on Earth has to follow the WHO requirements \cite{10} as applicable, see for example figure 11.13. However, this package is designed to make liquid samples safe for transport, therefore the absorbing material could have to be replaced by another more appropriate material.

### 5.1.2 Contain a potential biohazard

What kind and what size of objects must be taken into account? The Space Studies Board of the National Academies of Science published a report to that issue in 2000 and came to the following conclusions:

**How small can a free-living organism be?** On the surface, this question is straightforward - in principle, the smallest cells can be identified and measured. But understanding what factors determine this lower limit, and addressing the host of other questions that follow on from this knowledge, require a fundamental understanding of the chemistry and ecology of cellular life. The recent report of evidence for life in a martian meteorite and the prospect of searching for biological signatures in intelligently chosen samples from Mars and elsewhere bring a new immediacy to such questions. How do we recognize the morphological or chemical remnants of life in rocks deposited four billion years ago on another planet? Are the empirical limits on cell size identified by observation on Earth applicable to life wherever it may occur, or is minimum size a function of the particular chemistry of an individual planetary surface?

These questions formed the focus of a workshop on the size limits of very small organisms, organized by the Steering Group for the Workshop on Size Limits of Very Small Microorganisms and held on October 22 and 23, 1998. Eighteen invited panelists, representing fields ranging from cell biology and molecular genetics to paleontology and mineralogy, joined with an almost equal number of other participants in a wide-ranging exploration of minimum cell size and the challenge of interpreting micro- and nano-scale features of sedimentary rocks found on Earth or elsewhere in the solar system. This document contains the proceedings of that workshop. It includes position papers presented by the individual panelists, arranged by panel, along with a summary of extensive roundtable discussions that involved the panelists for each session as well as other workshop participants.
The discussions forming the basis of this document sought to address three distinct but related issues:

1. What are the theoretical, observable, and empirically testable limits on the minimum size of organisms living on Earth today?

2. What, in theory, are the size limits on organisms not constrained by the biochemistry of extant cells? and

3. How can we recognize traces of ancient and potentially unfamiliar life in samples from other bodies in the solar system?

As is evident from the summaries, there was strong consensus on the first issue, but the others remain open. The six geneticists and cell biologists in Panel 1 reached consensus on the smallest size likely to be attained by organisms of modern biochemical complexity. Free-living organisms require a minimum of 250 to 450 proteins along with the genes and ribosomes necessary for their synthesis. A sphere capable of holding this minimal molecular complement would be 250 to 300 nm in diameter, including its bounding membrane. Given the uncertainties inherent in this estimate, the panel agreed that 250 ± 50 nm constitutes a reasonable lower size limit for life as we know it. At this minute size, membranes have sufficient biophysical integrity to contain interior structures without the need for a cell wall, but only if the organism is spherical and has an osmotic pressure not much above that of its environment.

Panel 2 consisted of microbial ecologists asked to elucidate the smallest sizes actually observed in free-living organisms. Once again, consensus emerged from the panel's discussion. Consistent with the theoretical limits articulated by Panel 1, members of Panel 2 reported that bacteria with a diameter of 300 to 500 nm are common in oligotrophic environments, but that smaller cells are not. Nanobacteria reported from human and cow blood fall near the lower size limit suggested by cell biologists; however, the much smaller (ca. 50 nm) bodies found in association with these cells may not, themselves, be viable organisms. Observations on Archaea indicate that, in general, they have size limits similar to those for Bacteria.

Two problems constrain discussions of minimal cell size in natural environments. Commonly used methods of measuring cell size have inherent uncertainties or possibilities of error. Perhaps more important, most cells found in nature cannot be cultivated. Thus, ignorance about biological diversity at small sizes remains large. These problems notwithstanding, it appears that very small size in modern organisms is an adaptation for specific environmental circumstances, including stress and scarcity of resources. Primordial organisms may or may not have been tiny, but the smallest organisms known today reside on relatively late branches of the RNA phylogeny.

Whereas Panels 1 and 2 indicated that a cell operating by known molecular rules—with DNA or maybe RNA, ribosomes, protein catalysts, and other conventional cell machinery—would have a lower size limit of 200 to 300 nm in diameter, Panel 4 suggested that primitive microorganisms based on a single-polymer system could be as small as a sphere 50 nm in diameter. There is no assurance that primordial cells would have been this small or, if they were, that such minute cells would have been more than transitory features of early evolution. Nonetheless, unless one is willing to posit that everywhere it has arisen,
life has evolved a biochemical machinery comparable to that seen on Earth, the rules that govern minimum cell size may not be universal.

In fact, as explored by Panel 3, there are a number of ways that living cells or fossils might fall below the minimum size deemed likely by cell biologists and ecologists. On Mars or Europa, fossils might preserve a record of biological systems different from those we understand - perhaps early products of evolution that made do with a small complement of functional molecules. Organisms of modern biochemistry might become small by being pathogens or living in consortia - that is, by using the products of another organism's genes. Or, fossils might preserve remains that shrank after death, or parts of organisms rather than complete cells - both are common in the terrestrial record.

Of course, fossil morphologies are but one of several types of biological signature preserved in rocks. Experience with ancient terrestrial rocks shows that extractable organic molecules, minerals, fractionation in isotopic or elemental abundances, and distinctively laminated sedimentary structures can all provide indications of past life. Many of these features, however, can be mimicked by physical processes. Panel 3 concluded that a much better understanding of biological pattern formation is needed before intelligently chosen martian samples are returned to Earth. The panel also emphasized that this must go hand in hand with improved knowledge of the limits of morphological and chemical pattern formation by non-biological processes. Indigenous features of extraterrestrial samples can be accepted as biogenic only if they are incompatible with formation by physical processes.

- In 1990, Bob Folk at UT, using an electron microscope, observed 50 nm "nanobacteria" in rocks from hot springs. Nanobacteria were later found in blood and in kidney stones. But other researchers have not been able to find DNA or protein in nanobacteria, and it may be that the objects seen in the electron microscope are mineral microcrystals. Because of this, many experts doubt the existence of nanobacteria (see Nature (2000) 408, p. 394). But supporters continue looking for evidence that nanobacteria are living.

- The smallest virus are about 20 nm in diameter.

- Viroids are small circular single strands of RNA that lack a protein coat. To date, they have been shown to only cause plant diseases. Their RNA can be as short as 248 nucleotides long (80,000 molecular weight), which can be less than 10 nm diameter.

- The size of TSE agents or prions have been evaluated by ultra-filtration to be between 15 and 40 nm, although these agents aggregate easily because of their hydrophobicity. There are also values given in the literature between 7 and 12 nm.

In order to know that the Mars sample is still pristine and to know its status it is important to have a fail-safe monitoring system to detect breaches on the sample container.
5.2 Laboratory work

The main scientific task is to search for chemical indicators of past and/or present life. The subsystems to perform this are [12]:

- elemental analyses
- mineralogical determination
- isotopic and molecular analyses of inorganics and organics in connection with structures and life processes

Most of the investigations must be done at University institutes outside the Mars Sample Receiving Facility. Only very few analysis must be done inside the MSRF. The experiments that have to be conducted within the MSRF have been defined in the draft test protocol for detecting possible biohazards in Martian samples returned to Earth, written by the NASA and NRC in 2002. This has yet to be discussed in Europe and possibly up-dated and adapted. The size and complexity of this instrumentation define the primary containment in case of option 2 and 3.

5.2.1 Work with laboratory animals (rodents, nonhuman primates)

Animal experimentation with nonhuman primates would require an animal room and a necropsy table. For the care and use of laboratory animals, the primary enclosure provides the limits of the immediate environment for housing animals. In the context of biological safety, primary enclosure must be constructed of materials that provide the most appropriate housing for maintaining an animal’s normal physiological and behavioral states, but also for safe animal handling and ease in equipment cleaning, sanitation and disinfection. For these reasons stainless steel or heat resistant plastics such as polycarbonate is often recommended.

Biological safety includes safety for the animals as well as for the humans who work in the surrounding environment. A wide variety of housing systems for laboratory animals is currently available.

For rodents microisolator systems exist of a shoe-box style with a solid bottom and a filter top which is an integral part of the lid. For the most parts, the filter tops and cages are fabricated of clear polycarbonate plastics. With the filter tops these cages serve to isolate animals from each other. Air exchange takes place only very slowly in this kind of microisolators and moisture, ammonia and other gases remain in the cage and reach excessive levels after a few days. Therefore cage bedding must be replaced every 4 to 5 days.

A better option are ventilated racks which are typically constructed of multiple stainless steel shelves with each shelf fitted with an access door. Rodents are housed on these shelves in conventional shoe-box style microisolators as described above. The racks provide a discrete air supply to animals on each shelf and then exhausting the air directly out of the rack. Positive pressure ventilated racks normally supply HEPA filtered air to he housing unit and the exhaust air is usually filtered through a less efficient filter. Negative pressure racks are set
up to draw the air supply through coarse filters and then exhaust the air through HEPA filters.

Nonhuman primates are often kept in primary enclosures that are fitted with rear panels or walls that can be moved forward, securing or squeezing the animal against the front wall to facilitate the administration of injectables for chemical restraint. This type of restraint apparatus precludes the need for manual restraint, facilitates the administration of chemical immobilizing agents such as ketamine and markedly reduces the risk of handlers being bitten or scratched by the animal.

Successful design of necropsy areas include high ceilings, ample lighting and good ventilation. For centralized necropsy support, the use of fixed stainless steel tables with fixed surgical-type lighting, grinder for waste disposal and down-draft ventilation is recommended. Portable stainless steel tables with side rails and a sloping surface for drainage provide utility and are versatile.

5.2.2 Alternative solutions taking into account existing facilities

There is always the option to move the samples to another facility for further studies. If for example the first evaluation inside the MSRF shows some concern and requires a further investigation in nonhuman primates, the sample or a portion of it could be moved to an animal holding facility and the experiment could be executed there.

5.2.3 Possible outline

According to the graph in picture 11.14 the following schematic layout could be realized:

Element 1: First the transport container must be transferred from the outside to the BSC III containing defined atmosphere at 1000 mbar ($10^5$ Pa). This can be done by means of a simple dunk tank.

Element 2: Inside the BSC III the transport container must be connected to the gas analysis station for analyzing the mars atmosphere and exchanging the Mars atmosphere with the not yet defined atmosphere of the BSC III.

Element 3: After the analysis within the BSC III the sample can be transferred through another dunk tank to the clean room environment within the MSRF.

Element 4: From the clean room the sample might be transferred to the animal facility or accordingly packed exported and sent to another facility with either the same clean room conditions or an animal room. For the export another dunk tank must be provided.

Element 5: From the animal facility the sample or animals or tissue of the animals must be exported and sent to other facilities with the same biosafety level. A dunk tank has to be provided.
Possible description of the biosafety levels are given in figure 11.15.

From the above list it becomes clear that a high containment facility is needed which contains a glovebox system and a clean room. Figure 11.16 shows a sketch of a possible arrangement. The whole biocontainment is surrounded by a safety corridor opening into an entrance sector. That allows on the first hand to take care of any strong wind on the outer surface which could easily compromise the pressure scheme inside (see figure 11.17), and on the second hand it serves as a safety corridor which could hold back any particulates released accidentally from the inner containment. Both, the inner containment as well the safety corridor as the outer containment are intensively ventilated with supply as well as exhaust air HEPA filtered. Access to the safety corridor is controlled, so no unauthorized persons can enter that part.

From the entrance section separate changing rooms for men and women can be accessed either for entry to the clean room suite or to the biocontainment suite:

- **Clean room:** to work in the clean room respectively at the biosafety cabinet of class III thorough showering including shampooing the hair is required. After the shower complete clean room clothing has to be donned.

- **Suit room and animal room:** entrance through the change room and the showers without showering. Complete change of clothes. Underwear is donned in inner change room men and women respectively. Exit in underwear into the large suit room. The suits are tested and donned. Entrance into the chemical shower and exit either in the laboratory or the animal room.

The exit procedure is the following:

- **Clean room:** exit through the shower without showering. According to the biohazard risk assessment shower can be made mandatory.

- **Suit room:** entering the chemical shower. A one minute water rinse will be followed by a three minute disinfection rinse and by a final three minute water rinse. Exit into the suit room and taking off the suits. Going to the shower for hygenic reasons only.

- **Animal room:** entering the chemical shower. A three minute water rinse will be followed by a three minute disinfection rinse and by a final three minute water rinse. Exit into the suit room and taking off the suits. Going to the shower for hygenic reasons only.

All three rooms are equipped with a large air lock to transfer large equipment in and out. Equipment needs thorough disinfection including fumigation by formaldehyde or alternative gas like $H_2O_2$ before removal from biocontainment. A dunk tank leads from the three rooms into the according air lock. Finally there is a double door autoclave leading from each room out into the safety corridor area.

All three areas are biocontained. However, it has to be kept in mind that we might be dealing with unknown biohazards, requiring new methods of decontamination or altered parameter settings of conventional methods.
One problem would be the singular point connecting three different areas, two of them with the same entrance region. This causes space problems. Such a situation could be solved by a similar design shown in figure 11.18.

There are basically two scenarios for the MRSF: either the samples are to be found nonhazardous or can be inactivated or the samples are found hazardous and may therefore not be released for further studies at the Universities. In the first case the samples are for a limited time within the MSRF only and the biosafety level is lowered then and the sample can be released for further analysis. For this the MSRF is appropriate and sufficient. In the second case however, the sample may not be released for at least a longer period and more research has to be done within the facility. In this case, the MSRF might not be sufficiently equipped and available space must be added. An option would then be to have a mobile container adjusted to the facility to a specially forseen docking station. The single mobile unit would then serve as a corridor to a mobile container system which could be adjusted to the specific needs for future research.
Chapter 6

Risk-based approach

A hazard scenario results from the hazard analysis and from there the necessary safety measures are derived. The resulting safety factor is the sum of safety measures and risk management. Whether the risk is acceptable or not can only be estimated by analysis of the possible damages and the probability of its realization. By increasing the safety measures resp. improving the risk management the possible damages can be directly influenced. To arrive at a risk-based containment analysis the iterative procedure according to figure 11.19 can be applied.

Basically two different safety requirements must be regarded: firstly the integral safety measure, i.e. the overall safety factor of all safety measures, and secondly the redundancy of the safety measures. The former is a requirement for safe working conditions in closed systems and the latter is a requirement of the contingency planning system. In practice this means to optimize rather than maximize safety measures. If there would be a demand to maximize safety factors, there would also be a demand to double the safety measures. But as every serial system could in principal fail, that would consequently imply to repeat the safety barrier ad infinitum. According to such a safety philosophy no criteria to exit the loop could be defined and the question „how safe is safe enough” could not be answered.

In order to define the necessary safety factor the risk must be estimated. Risk is generally defined as product of damage (D) and probability (P):

$$\text{risk} = D \cdot P$$ (6.1)

However, the damage itself is a product of a maximal possible damage potential $D_v$ and probability $P_v$ of its realization. An example shall demonstrate this idea. If a certain amount of infectious substance is spilled, a portion of it will be aerolized while the major part will stay on a surface. Our daily experience tells us that if we spill a glass of milk almost the whole content will unfortunately land on the floor and only a few percent will be aerolized. To complicate the situation even further the aerolized particles will have a statistical size distribution. The larger droplets will condensate in a very short time on a surface. Many of the smaller droplets however, will rapidly dry into droplet nuclei which are the typical vehicles of airborne infections. In still air, terminal
setting velocities for 1 $\mu$m to 5 $\mu$m particles range from $3 \cdot 10^{-3}$ to $10^{-2}\text{cms}^{-1}$ and are therefore truly airborne.

According to each particular distribution the risk must be defined for all existing barriers.

$$ risk = \prod_{\nu=1}^{n} D_{\nu} \cdot P_{\nu} = \prod_{\nu=1}^{n} \varphi(\tilde{D}_{\nu}, w_{\nu}) \cdot P_{\nu} \quad (6.2) $$

This means that the risk must be estimated anew for each containment barrier (see figure 11.20). It is not a reasonable hypothesis to assume a „worst case“ scenario with a constant maximal damage potential at each barrier. This would be in complete contradiction with our daily experience.

A more realistic approach is to apply a risk management for each barrier failure in order to minimize the damage potential. Of course also the risk management could fail, however, this would be a failure of the second order with an according smaller probability. As the realistic probability is less than 1 the risk potential must be decreasing.

Redundant safety systems guarantee that no agent can escape during a failure of the system. Therefore in a risk analysis a failure of different systems in series must be assumed. By means of the model calculation the necessary quality of a barrier can be estimated by taking infection dose and survival time of the particular agent into account.

The concept of risk analysis demands calculation of the rational risk. The risk group gives only a hint on the possible risk. What is important is the resulting true risk. The definition of containment is a general one; not only an absolute isolation but also a gradual measure of containment is regarded.

The situation is even different in an emergency case. Several incidents have to be studied:

- Fire in the laboratory
- Earthquake
- Heavy storm
- Vandalism and sabotage

6.1 Fire in the laboratory

Fire in a laboratory is still one of the highest risks. CO$_2$ fire extinguishers, fire hoses, sprinklers and inert gas are possibilities to study as fire fighting measures in the laboratory. The quantity of burnable liquids inside the containment has to be minimized and only small quantities should be stored in the laboratories. All electric equipment which is not used should be turned off and all electric equipment must be carefully maintained and controlled regularly for any insulation or other problems. No liquids which might evaporate burnable gases may be stored in the freezers.
6.2 Earthquake

A steel fortified concrete construction box is very sound. In addition the highly contaminated areas like animal rooms are designed according to the „box-within-the-box” principle.

6.3 Heavy storm

During a heavy storm the pressure conditions in- and outside the containment could become very complicated and unpredictable. Figure 11.21 shows a possible scenario. However, the situation does not consider the special situation of other buildings around the high containment building. Any building, trees and structures in the near neighbourhood will influence the conditions in a highly nonpredictable way, therefore, the model calculation can only be regarded as a hypothetical possibility and will not be calculated as a risk assessment taking into account probabilities of such a scenario. The purpose is just to increase the awareness for the leakage possibility during a stormy situation which could possibly result in an additional safety measure.

6.4 Vandalism, sabotage and human error

Because of today’s increased concern about bioterrorism and sabotage, public institutions are paying increased attention to the physical security of facility sites, storage areas, and research processes. Institutions should have some measure of site security in place for facilities that handle hazardous substances. Most of these efforts focus on ensuring that the facilities are designed and operated safely on a day-to-day basis, using well-designed equipment, preventive maintenance, up-to-date procedures, and well-trained staff. This contributes to an excellent base from which to plan bioterrorism security measures. The following threats must be considered:

- Theft of hazardous materials
- Research objects as targets
- Facilities as targets

Most research facilities security measures are intended to prevent from gaining access to the site.

- Preventing intrusion
- Limiting damage
  - Site-specific decisions
  - Facility design
  - Procedures and policies
The cost of providing adequate security should be balanced against the worth of the intended research. Any misstatements to the general public should be avoided.

Human factors have to be taken into account in complex technical systems also. In the chemical industry 80 to 90% of all incidents include also human factors. In 60% of incidents in nuclear energy power stations human errors are involved. Therefore, human reliability analysis must consider interaction of human-machine-systems; must include human errors in modelling; can be used to evaluate the most important effects on human factors on technical systems. Techniques for human error prediction must be used to localise systematically sources of *error of omission* as well as sources of *error of commission*. Typical methods of analysis are:

- Human Reliability Analysis (HRA)
- Technique of Human Error Rate Prediction (THERP)
- Work Safety Analysis (WSA)
- Action Error Analysis (AEA)
Chapter 7

Minimal requirements

7.1 Landing site

ESA’s 15 Member States are Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Italy, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom. Greece and Luxembourg are expected to become members of ESA in 2004. In addition, Canada and Hungary participate in some projects under cooperation agreements. The landing site for a Mars Sample Return Mission is not yet discussed, however, it seems obvious that it can probably not be in one of these states. In all studies it has been assumed that the landing site will be in Australia. The legal situation with regard to constructing and accepting complex facilities has also to be taken into account.

7.2 Structure

The design of the structure must be based on the need to withstand forces caused by extreme natural events such as earthquakes for a probability of an annual occurrence of TBD or a TBD year return and hurricanes with wind velocities between TBD kmh$^{-1}$ with a probability of about one time per year.

- Hurricane TBD kmh$^{-1}$
- Earthquake magnitude TBD on Richter scale
- Flood after local rainfall of TBD cmh$^{-1}$

The design of the structure is such that if a disturbance of TBD magnitude on Richter scale occurred on site, only minor fractures and dislocations of components would occur. The silicone sealants and the surface finishes must be chosen for the flexibility in coping with such dislocations. Wind velocities of up to TBD kmh$^{-1}$ may not lead to a significant pressure inversion for longer than a few tens of seconds. The building is to be constructed in three floors at least, in order not to have containment walls to the outside. The building must be constructed according to the so-called box-in-the-box principle.

- Level – 1 (basement) sewage treatment and technical installations
• Level 0 (ground level) work floor
• Level 2 (first floor) air handling system

All walls are to be poured in situ reinforced concrete. Surface treatment also contributes to the containment. There are rubber seals between concrete joints; epoxy paint on the walls, ceilings and floors of laboratory rooms and corridors. The basic concept is achieved by the box-in-the-box principle for all biocontained rooms. Adoption of this principle in three dimensions ensures a fail-safe facility in which a failure of a single item, or indeed multiple items, will not compromise the overall security of the facility.

The building consists of a large number of static elements like walls, floors, ceilings, provided with an array of dynamic elements such as air handling systems, sewage treatment system, autoclaves, etc. The box-in-the-box principle applies equally to static and dynamic elements.

### 7.3 Air treatment

All air supplied to the containment areas is passed through one HEPA filter of at least type H14. Exhaust air from all secure areas passes through two HEPA H14 filters in series. Fans and control equipment are designed to allow automatic start of standby units in event of failure of an operating unit.

Materials for HEPA filters vary, but generally these are made of synthetic fibrous materials. The principle of HEPA filtration is not to restrict the passage of particulate matter by the gap between fibers, but by altering the airflow streamlines. The airflow will slip around the fiber, but any higher-density bioaerosols or particulate matter will not change direction so rapidly and, as a result of their inertia, will tend to impact the fiber. Once attached, most particulates will not be re-entrained in the airstream. Although HEPA filters can theoretically remove particles down to about 0.01 \( \mu m \) in size.

### 7.4 Air pressures

To ensure a directional flow of air, the rooms are provided with a gradient of negative pressures with those areas where the highest potential of hazards is generated being at the greatest negative pressure. Should there be leaks in the building structure, air will always pass from outside to inside. A microprocessor-based control system is being used to control air pressures.

### 7.5 Breathing air

For the guarantee of respiratory air (in level 4 laboratories), the generation of the best available compressed air is provided. Compressed breathing air will meet the quality regulations „medically used compressed (respiratory) air“. Two independent compressed-air blowers as well as a bottling-station shall be provided. Respiratory air distribution needs to be constructed in a ring system with two buffer storages.
7.6 Liquid waste treatment

Batch treatment is used to sterilize the sewage. Sewage is heated in a batch vessel to a temperature of $121^\circ C$ at 2 bars for 20 minutes before discharge. The maximum achievable temperature is $143^\circ C$ which can be held up to 2 hours. After treatment the liquid waste is cooled to $40^\circ C$ and discharged to the public waste water system.

7.7 Solid waste treatment

Solid waste (solvents, waste paper and cartons, discarded HEPA filters) are autoclaved and then burned in the public incineration system. Animal carcasses are either autoclaved or treated in a tissue digester and then released to the sewage. Nonburnable waste is also autoclaved out of the containment area and removed for disposal as industrial waste.

7.8 Fire protection

Injection of an inert gas must be studied. In general, biosafety containments are regarded as burn-out areas, having only the airtight valves closed and minimized the oxygen by this means as fast as possible. However the pressurizing of the room has to be studied, otherwise windows will be broken and sinks could be blown out easily as the temperature and with it the pressure raises. On the other hand a low water sprinkler system could be chosen, however, potential water damage to the expensive equipment and to the samples must be considered.

7.9 Movement of materials

Limited to items which can easily be handled by personnel in suits, no sharps, nothing which could cause a hazardous spill if broken.

Safe and possible only after the autoclave has been run so that the chamber is sterile. However as mentioned before, we might be dealing with unknown biohazards. Further on we might be able to have new sterilization procedures.

Large items and animals can be placed into the air lock from the outside door when air lock is sterile. After locking the outer door, the inner door can be unlocked and personnel can take in material into the containment. Air lock must be fumigated either by formaldehyde or \( H_2O_2 \) afterwards.

The large air locks are rooms which must be regarded as part of the containment barrier, therefore both enclosures to the inside as well as to the outside must be of the same quality as the containment wall. Main use of the air lock is the transfer of material and animals into the containment. The air lock is in general in a clean and decontaminated status. This allows to use the air lock in case of an emergency to take out a person which is unable to pass the chemical shower system (heart attack, broken legs, etc.). The decontamination of the person takes place in the air lock by means of some disinfectant to decontaminate the suit of all persons in that room. After a short decontamination the suit will be taken off and the patient will be transferred to hospital. In very
special cases a piece of large equipment could alternatively be decontaminated by formaldehyde fumigation and/or other appropriate treatment to take out. A careful risk assessment is however necessary for each such process. The air lock may not be used for storing material of any kind. The air lock room must have a minimal air change per hour. Room ventilation is only running by opening a door and remains running for some minutes after that time. There must be an installation and procedure to decontaminate the room by formaldehyde or another appropriate method each time the inner door has been opened. Needless to say that the outer door may only be opened from the outside and the inner door only in the suit from the inside. Both doors must be interlocked and connected to an electronic alarm system. Doors may not stay open and there will be a time limit until an alarm goes off.

As a special safety feature, a dunk tank from the level 4 laboratory could lead into the air lock rather than directly to the safety corridor. The disinfection tank must be emptied from the inside and disinfection goes to the sewage collection tank. After refill and functional control of the dunk tank, the air lock must be fumigated before clearing it from the outside again.

7.10 Physical security

The access is controlled at all entrances. The site is surrounded by a security fence and the entrance of the main building is manned during working hours.

Further monitoring is done at the biocontainment laboratory by an electronic access control system. This could include biometric data. Out of working hours the entry gate is guarded. There could be a video monitoring system.

7.11 Personnel

Personnel entering the contained area must leave all personal effects and clothing in change rooms, pass through a shower without showering, and don laboratory clothing. For entrance into the level 4 laboratories, a full body protecting suit must be worn. Only laboratory clothes are necessary for working at the glove boxes.

7.12 Standby systems

It is recognized by all that any equipment will fail at some time. It is necessary to design the facility so that its microbiological security will not be compromised by the failure of any component in any system. Critical elements will be duplicated or be so designed that the standby system component will come into operation automatically.

Two general design principles are crucial: redundancy and robustness (see figure 11.22).

Definition redundancy: redundancy is additional, sometimes not essential information and ressources of a system. For example, the following text can be read despite the omission of the vocals: This text cannot be read if the vocals.
Definition robustness: robustness stands for the ability of a system to keep its function even by external influences and disturbances.

7.13 Communications

All components of the central monitoring system (CMS) are connected to the UPS.

The system has to be built in such a way that a high electromagnetic compatibility as well as a high availability and a fully developed self-diagnosis function is achieved and that it is protected against data loss. The processing velocity and processing capacity shall be adjusted to the manufacturing process and the interface has to be designed to the process in such a manner that it is able to process all current systems and protocols.

The communication in the level 4 laboratory as well as between the level 4 laboratory and the control room is ensured via a radio system which is integrated in the suit. In emergency situations, communication in the level 4 laboratory occurs via notification panels with running display (one way communication). For signalling, flash lights are used in the level 4 laboratory.

A personnel paging system must be installed at the site. Transmitters are placed in the passages.

7.14 Central monitoring system

The central monitoring system (CMS) is a computer-assisted system designed to monitor the various engineering systems. All equipment essential for biosafety and security will be monitored by CMS and alarms raised.

1. Fault detection

A fire alarm system is to install. In the entrance area, an indicator board for fire brigade is mounted. The transmission of the alarm is sent to the manned main station of the site from which the fire brigade is notified. Extension occurs as full protection. Alarm notification control is integrated into the system via output components. In the case of fire, controls are integrated into the system via output components. A superior building monitoring system enables the transmission of the alarm notification.

Due to personnel safety, video cameras should be installed in the level 4 laboratory as well as in the decontamination shower. The central video technical unit as well as the controls of the facility are both located in the control room. Cameras are made accessible by both a 230V as well as a coaxial transmission line. Cameras commence recording of images as soon as movement is registered within the room. Transmitted images are displayed on a monitor in the control room. Depending on the number of cameras, the images are displayed continuously or via an image splitter on a single monitor only. Manual interventions are possible via a control
panel in the control room. Images may be recorded if so desired and/or may be sent at intervals via LAN. It is possible to connect the video system to other security systems via an overriding management system.

Access control or access authorization is controlled as follows:

- **Zone 1:** The outside doors at the periphery of the building are secured with a batch reader. All important rooms such as technical units and the safety passage (for visitors) are additionally equipped with a batch reader.

- **Zone 2:** The biosafety level 4 laboratories as well as the related control room are surveilled by a biometric reader. To gain access without triggering an alarm, two authorized people must identify themselves within a given time frame. Access may be individually time- and location-specific configured. A control of the whereabouts of all personnel in the building is provided for. The central access control unit is located in the control room. The system is extendable and may be cross-linked and extended over the entire area. Interfaces to other safety systems (video, fire alarm, alarm) and to the building automation are also available.
Chapter 8

Risk-adjusted mission value

From the decision theory point of view, it is essential to have a way to rank the options. If it doesn’t matter what happens, then there’s no reason to think about the choice. But in order to choose, it is needed to know what to accomplish. That means understanding the likely results of each option and then choosing between options to achieve those results. To rank outcome values is essential. So part of deciding better is knowing the values.

It is in general a difficult problem to trade-off between mission risk and mission value (see figure 11.23). A mission is a decision alternative with uncertain outcomes. It is because systems engineering analysis as well as risk analysis aspire to a holistic philosophy in their problem-solving approaches. This means that in order to understand the system’s nature, functional behavior and interaction with its environment we have to identify, quantify and evaluate risks, uncertainties and variability before we are able to improve decision-making processes in planning, design, development, operation and management. Furthermore, we must appreciate the multiple objective nature of most complex systems and the fact that there is no single solution to most real-world problems, but there are choices and trade-offs [6]. In addition, there is also the temporal domain: we have to respond to past, present and future.

In risk assessment we are analyzing the three basic questions:

- What can go wrong?
- What is the likelihood that it would go wrong?
- What are the consequences?

In the next step risk management builds on this risk assessment process by asking another three basic questions:

- What can be done and what options are available?
- What are the trade-offs in terms of costs, benefits and risks?
- What are the impacts of current decisions on future management?

Of course a holistic risk management approach must also address all of the following four sources of failures:
CHAPTER 8. RISK-ADJUSTED MISSION VALUE

- Hardware
- Software
- Organization
- Personnel

These four elements are not necessarily independent of each other however. The process of design development is complicated. There are three different players with a different view on the issue of mission value or system value. Each one is important and they can in general not be compared directly. Figure 11.23 gives only a very rough impression of the complexity.

The issues to be regarded by the players might be:

- research results, easyness of experimenting, international reputation
- costs of the facility, social costs in case of an incident
- safety requirements for personnel, environment, animals, public
- risk aversion of managers
- probabilistic views of engineers
- value of successful operation

Only a trade-off between different solutions and different views can lead to a successful development regarding not only complete success or failures but missions with various degrees of success and degraded modes of failures. For this trade-off between mission risk and mission value a methodology will be developed in the following in order to include all opinions and world views in an appropriate manner.

We are regarding a mission which has to work in a safe environment. This may result in a successful outcome or in a complete failure. There are many ways to solve a problem and therefore there is more than one solution or alternative option. We describe the set of all possibilities - which have been negotiated among scientists, engineers, architects and managers - as $X = \{x, x_\phi\}$. A specific mission outcome is then $x = (x, x_\phi)$ with $x$ as successful outcome and $x_\phi$ as a mission failure. If the project team elects to implement mission $x$, then the successful mission is obtained with probability $s(x)$ or the failure outcome is obtained with probability $1 - s(x)$. More realistically there are various degrees of success and degraded modes of failures. The probabilities for all values of the whole set are a function of the:

- value judgements $v(x)$
- probabilities of success $s(x)$
- risk aversions $r(x)$

For the value judgements $v(x)$, the certainty equivalent must be defined for each successful outcome $x$. The certainty equivalent is the probability for the outcome for which the decision maker would be indifferent between choosing
the certainty equivalent and the gamble between the optimal solution and the least preferred option of the successful outcome.

The probability of success $s(x)$ is determined by standard probabilistic failure analysis.

The risk aversion $r(x)$ is defined by the certainty equivalent between the least-preferred outcome and the gamble for the optimal solution and the total failure of the mission.

Once these three sets of values are defined for all successful outcomes, their risk-adjusted mission value (RAMV) can be calculated easily according to standard decision theory and game theory as

$$\text{RAMV}(x) = s(x)\{(1 - r)u(x) + r\}$$

(8.1)

In this way a mission could be chosen with the highest RAMV by applying decision theoretical numerical calculation.
Chapter 9

Cost estimation and schedule

A rough cost and planning time estimation shall be made here.

Time is given in years prior to the expected landing of the Mars samples on earth. Cost is estimated in million Euro. It is obvious that the cost estimation can only be very preliminary. The following knowledge has been included here: the cost of existing recent facilities and the fact that all projects tend to become more expensive as the development is moving forward. This is a fact due to the growing demand on researchers and program managers, the increasing availability of more modern technology and the permanent underestimate of space usage.

Moveable laboratory equipment is not included in the price, as there is not enough information available about the necessary equipment. A clear research program must be designed first. This research program must be available prior to the beginning of the planning and must be the first key document for the planning team.

The time schedule is only achievable if several processes are running in parallel and the legal and communication process are done from the first step on in a very well planned manner. The planning team must be evaluated in a way that the relevant experts must not be changed during the whole process.

<table>
<thead>
<tr>
<th>Year</th>
<th>Action</th>
<th>Mio. EUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 9</td>
<td>Evaluation, forming and education of a planning team; study visits of existing facilities; site evaluation; functional project requirement description</td>
<td>0.5</td>
</tr>
<tr>
<td>- 8</td>
<td>Start of pre-project; developing of several options; risk communication concept; decision of construction site</td>
<td>3.5</td>
</tr>
</tbody>
</table>
- 7 Evaluation of the best option; 1\textsuperscript{st} design review; start of the main planning phase; start of risk analysis and public risk communication

- 6 2\textsuperscript{nd} design review; start of final design and engineering; official construction permission process including environmental impact study

- 5 Start building construction; defining acceptance criteria and tests

- 4 Construction

- 3 3\textsuperscript{rd} design review; technical commissioning; acceptance tests

- 2 Safety validation

- 1 4\textsuperscript{th} design review; test phase, education of staff; documentation; inauguration

0 Before launch of the Mars Sample Return Mission

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Before launch of the Mars Sample Return Mission</td>
</tr>
<tr>
<td>1</td>
<td>4\textsuperscript{th} design review; test phase, education of staff; documentation; inauguration</td>
</tr>
<tr>
<td>2</td>
<td>Safety validation</td>
</tr>
<tr>
<td>3</td>
<td>3\textsuperscript{rd} design review; technical commissioning; acceptance tests</td>
</tr>
<tr>
<td>4</td>
<td>Construction</td>
</tr>
<tr>
<td>5</td>
<td>Start building construction; defining acceptance criteria and tests</td>
</tr>
<tr>
<td>6</td>
<td>2\textsuperscript{nd} design review; start of final design and engineering; official construction permission process including environmental impact study</td>
</tr>
<tr>
<td>7</td>
<td>Evaluation of the best option; 1\textsuperscript{st} design review; start of the main planning phase; start of risk analysis and public risk communication</td>
</tr>
</tbody>
</table>

Table 9.1: Rough cost estimation in EUR (at the basis of 2004).

Operational cost and maintenance can not yet be calculated, too many factors and parameters are still open. This is only a feasibility study and only a functional description of the facility can provide the necessary values and costs. Choice of the site will also determine the running cost depending whether the building has to be extensively heated during winter or cooled in summer. Also cost on fuel, electricity and water can severely influence the operational cost. One thing, however, can be said at that time: cost depend mainly on labor cost. Therefore, as a team of around ten staff will be necessary to run the facility the labor cost would be around 2 Mio EUR. Assuming the energy cost to be another 1 to 2 Mio EUR it can be estimated that the operation of the facility will cost between 4 and 5 Mio EUR annually depending on the staffing.

The size and scope of the facility is not yet fixed. For this the Mars sample handling protocol must be finalized. In the following a function for the enlargement of the facility according to the need is developed in table 11.3. This would result in an additional cost of about 0.5 Mio EUR per 10\% of laboratory space just for the building, summing up at 2.5 Mio increase at a 50\% increase of laboratory space. Of course the real cost will increase stepwise as the technical installations do in general not allow for more than 10\% increase without adjustment of some of the parameters like air flow, heating and cooling and so on. It can only be estimated, that a 50\% enlargement of the building could be in the range of 5 to 10 Mio EUR.
Chapter 10

Conclusions

The purpose of computing is insight, not numbers.

Richard Hamming

Project planning, especially in the field of research, is full of uncertainties. We are sometimes unable to detect that the problem of an unsuccessful outcome of a project came from faulty processing of uncertain information. The purpose of this feasibility study was to help identify the important factors of the project and help to anticipate the unexpected. We would like to make clear that the problem is not solved forever. Many details may change but the basic problems and the methodology will stay more or less the same. It is a common behaviour to use or adapt methods and solutions which have been used in the past for similar problems. Therefore the proposed solution in this report is not completely new and the risks and uncertainties are quite well understood. This report tried to evaluate, order and structure the current knowledge so as to allow decisions with as complete understanding as possible of the current state-of-the-art of technology, its limitations and its implications.

As mentioned above, the study should not be regarded as a final draft design, but should get involved into a iterative exploration of alternative formulations and last but not least, should be the starting point for collecting and examining critiques from others, from experience of previous models and hence lead to the synthesis of the most useful elements for a new model. It should never be forgotten that goal development and choice are dependent behaviour in general. Human choice behavior is at least as much a process of discovering goals as of acting on them.

As shown in chapter 5, Risk-adjusted mission value, the criteria could be based on maximizing a multi-variate utility function as well as on a choice of doing the best job of reducing the risk that is possible with the current best available technology. It should not be missed, however, that „the best available” is often also economically determined. Therefore, in practice, this criteria is almost never applied as a pure strategy. Rather some „feasibility - affordability” criteria is added. There is also the remark that technology frequently changes. Especially in the life detection the assumptions must be adapted during the project to the detectable levels made possible by the rapid pace of development of modern measurement technology.

This could mean that we rarely know enough about the problem to solve it definitively. For that reason it might be a good idea to seek solutions that can be
adaptive over time, that can be modified and improved. Therefore the proposed solution tries not to be a fixed project but rather a proof that a solution can be found within the next planning phase which is adaptable over as long a period as possible until it has to be fixed in order to be realized. If we accept that the development of „insight and understanding“ should be the principal goal of the first planning phase, then a careful examination of the boundaries must be included. This feasibility study should therefore provide the following:

- Obtain an answer to the specifically formulated question whether it is possible to fulfil the task of the mission from the point of view of planetary protection.
- Provide insight and understanding that will be useful to ESA managers and national policy makers who are faced with decisions of building a facility.
- Provide arguments to support the chosen procedures and methods.
- Persuade others that things are under control and that ESA can be trusted.
- Advice and propose further work.

All of these requirements are fulfilled with this report:

1. According to the current technology and the state-of-the-art the safe working with Martian materials can be done in a facility.

2. The current technology has to be studied carefully during the whole planning phase and the project must be developed in a way that it can be adapted to new knowledge, new technologies and new materials and methods.

3. Calculations, models and materialization proposals are available which can be used in argumentations, peer reviews and future discussions.

4. Estimations on time schedule and cost are made which allow to set up the project at a reasonable time in order to minimize both the project and the mission risk.

5. Risk analysis of all kind must be performed in the near future and the results must be communicated in a proper and appropriate way to managers and the open public very soon.

6. Recommendations are made and a time planning is proposed.
Recommendations

1. A functional project definition must be started immediately. This includes several parallel branches:

(a) A group of scientists have to review and refine the NASA Draft Test Protocol. The requirements on instruments and methods must be defined.

(b) A containment expert must work out a functional technical description of the high containment facility resulting in a as accurate as possible definition of space needs, technical installations and cost estimation.

(c) A team must provide a thorough risk assessment for the whole transport including the way from Mars to Earth, the way from the landing site to the MSRF and the work inside the facility.

(d) A group should define research needs in view of decontamination and sterilization of known earthbound and unknown Mars-bound life forms.

2. The essential elements mentioned in chapter 4 must be elaborated more into detail taking into account future development and changes.

3. A study must treat the legal aspects in member states with a facility building capacity. According to the recent states with high containment experience and capability of a state-of-the-art standard this could include: Austria, France, Germany, Ireland, Netherlands, Sweden, Switzerland, United Kingdom.

4. Develop a risk communication program, by the ESA ethical working group and implement a risk communication program by means of internet and other technologies.
Bibliography


[12] Exobiology in the Solar System & The Search for Life on Mars; Report from the ESA Exobiology Team Study 1997-1998; ESA SP-1231
Chapter 11

Tables and pictures

<table>
<thead>
<tr>
<th>Component</th>
<th>Suit failure rate in 100 hours of lab work (total of 1'736 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fabric</td>
<td>0.27</td>
</tr>
<tr>
<td>gloves</td>
<td>0.36</td>
</tr>
<tr>
<td>air supply</td>
<td>0.05</td>
</tr>
<tr>
<td>fabric and gloves</td>
<td>0.63</td>
</tr>
<tr>
<td>fabric, gloves and air supply</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Table 11.1: Failure rates per 100 hours of suit use in the Australian Animal Health Laboratory in Geelong. The statistics is for laboratory work only; it’s much higher when work with large animals is involved.

<table>
<thead>
<tr>
<th>Typ of filter</th>
<th>IDG in %</th>
<th>LDG in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPA H 13</td>
<td>0.05</td>
<td>0.25</td>
</tr>
<tr>
<td>HEPA H 14</td>
<td>0.005</td>
<td>0.025</td>
</tr>
<tr>
<td>ULPA U15</td>
<td>0.0005</td>
<td>0.0025</td>
</tr>
<tr>
<td>ULPA U16</td>
<td>0.00005</td>
<td>0.00025</td>
</tr>
<tr>
<td>ULPA U17</td>
<td>0.000005</td>
<td>0.000025</td>
</tr>
</tbody>
</table>

Table 11.2: Definition of the HEPA and ULPA filter. HEPA = High Efficiency Particulate Air, ULPA = Ultra Low Penetration Air. IDG = integral penetration, this is the maximal accessible penetration of a new filter measured over the whole filter surface. LDG = local penetration.
### Chapter 11. Tables and Pictures

#### Table 11.3: Incremental cost model assuming a quadratic building with a extension of $a = 25\, m$, a building height of $h = 10\, m$ and a basement height of $h_{b} = 5\, m$

<table>
<thead>
<tr>
<th>Type</th>
<th>$a = b$</th>
<th>$\delta$</th>
<th>EUR/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>roof</td>
<td>$a$</td>
<td>$2\gamma a (1 + \gamma)$</td>
<td>700</td>
</tr>
<tr>
<td>ceiling</td>
<td>$a$</td>
<td>$2\gamma a (1 + \gamma)$</td>
<td>500</td>
</tr>
<tr>
<td>floor</td>
<td>$a$</td>
<td>$2\gamma a (1 + \gamma)$</td>
<td>400</td>
</tr>
<tr>
<td>floor basement</td>
<td>$a$</td>
<td>$2\gamma a (1 + \gamma)$</td>
<td>300</td>
</tr>
<tr>
<td>glass wall</td>
<td>$4ah$</td>
<td>$4a\gamma h$</td>
<td>600</td>
</tr>
<tr>
<td>outer wall</td>
<td>$4ah$</td>
<td>$4a\gamma h$</td>
<td>800</td>
</tr>
<tr>
<td>inner wall</td>
<td>$2ah$</td>
<td>$2a\gamma h$</td>
<td>500</td>
</tr>
<tr>
<td>wall basement</td>
<td>$2ah$</td>
<td>$4a\gamma h_{b}$</td>
<td>300</td>
</tr>
</tbody>
</table>

Table 11.3: Incremental cost model assuming a quadratic building with a extension of $a = 25\, m$, a building height of $h = 10\, m$ and a basement height of $h_{b} = 5\, m$

#### Table 11.4: Some characteristic parameters for a suit laboratory and a laboratory with biosafety cabinetts of class III (glove-box-lab)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Suit</th>
<th>BSC III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Construction</td>
<td>flexible, flammable</td>
<td>Impervious, resistant, expensive</td>
</tr>
<tr>
<td>Rigiity</td>
<td>non</td>
<td>rigid walls however open for gloves</td>
</tr>
<tr>
<td>Pressure</td>
<td>180 Pa</td>
<td>200 Pa</td>
</tr>
<tr>
<td>Air flow</td>
<td>140 - 260 l/min</td>
<td>3000 l/min</td>
</tr>
<tr>
<td>Gloves</td>
<td>various size and type</td>
<td>Thick, reduced texterity</td>
</tr>
<tr>
<td>Failure rate</td>
<td>about 1 failure per 200 h of</td>
<td>almost none</td>
</tr>
<tr>
<td></td>
<td>operation</td>
<td></td>
</tr>
<tr>
<td>Access/egress</td>
<td>not applicable</td>
<td>only by autoclave and dunk tank</td>
</tr>
<tr>
<td>Ergonomics</td>
<td>flexible working positions, heavy</td>
<td>fixed work positions</td>
</tr>
<tr>
<td></td>
<td>suit</td>
<td></td>
</tr>
<tr>
<td>Communication</td>
<td>simple</td>
<td>radio communication, noisy</td>
</tr>
<tr>
<td>Time demand</td>
<td>slow</td>
<td>rapid</td>
</tr>
<tr>
<td>Working alone</td>
<td>not allowed</td>
<td>possible</td>
</tr>
<tr>
<td>Maintenance</td>
<td>shut down period</td>
<td>use of second line</td>
</tr>
</tbody>
</table>

Table 11.4: Some characteristic parameters for a suit laboratory and a laboratory with biosafety cabinetts of class III (glove-box-lab)
Figure 11.1: The author in a Dover suit in the BSL-4 laboratory of the Canadian Health Science center, Winnipeg, Manitoba, CANADA.

Figure 11.2: BSL-4 laboratory of the University of Texas Medical Branch in Galveston TX.
Figure 11.3: Private laboratory of the company Rhone Merieux, P4 in Lyon, France. On the right of the picture, cages for nonhuman primates can be seen.

Figure 11.4: HEPA filter boxes on the upper floor of a laboratory.
Figure 11.5: In the diagram the airstream is depicted winding its way around a single fiber. The heavier particulates will either impact the fiber directly, or sometimes attach by close passage, due to static electrical attraction, or simply by physical attachment.

Figure 11.6: The diagram shows the effects of Brownian motion on particles approaching molecular dimensions. Viruses can be small enough to be dominated by Brownian motion as opposed to gravity or inertial forces.
From the figure it can be seen that the pressure difference depends from the air tightness of the room and the air volume difference between supply and exhaust air. The four curves stand for the following leakage areas (from top to bottom): 0.01, 0.005, 0.002, 0.001 m². A change of air flux from 70 to 60 m³/h results in a pressure difference in the top curve (Q4) of about 1 Pa, on the second curve (Q3) a difference of about 5 Pa. If the room is very airtight (the two curves at the bottom) a change of the difference of the supply and exhaust air results in a large pressure difference. This does not depend on the number of air changes per time unit (ACH) for a particular room.
Figure 11.8: In normal operation, the quality of the wall airtightness is not important. The dynamic containment is dominating as indicated in the first picture. However, if the dynamic containment gets lost by a complete power loss, the static elements become important. As a building without air conditioning tends to become under slight overpressure due to its temperature increase (heat production of a human being is about 70 W) the quality of the static containment becomes dominant as shown in the second picture.
Figure 11.9: A biosafety cabinet of class III, so-called glove box with three working places.
Figure 11.10: Three zones are important for the proper functioning of a shower. The two rooms $G_{\text{out}}$ and $G_{\text{in}}$ represent the change rooms outside and inside the containment respectively. They do not necessarily have to be closed rooms. The grey bands represent airtight doors, the blue arrows stand for the noncontaminated air and the red arrows for the potentially contaminated air. Grey arrows stand for passive air flow at leaky doors.
Figure 11.11: Airtight penetrations for pipes, tubes and cables can be achieved by commercial products.

Figure 11.12: Box-in-the-box with $V_1$, $V_2$ the volumes, $c_1$, $c_2$ the concentration of molecules under study and $q_1$, $q_2$ the fluxes into the boxes.
Packing and Labeling of Clinical Specimens

Figure 11.13: Packaging and labeling requirements for infectious substances and materials, as well as clinical specimens that have a low probability of containing an infectious substance. These regulations are derived from the Committee of Experts on the Transport of Dangerous Goods, United Nations Secretariat, and the Technical Instructions for the Transport of Dangerous Goods by air which is provided by the International Civil Aviation Organization (ICAO). A copy of the DGR may be obtained by calling 1-800-716-6326 or through the Internet at: www.iata.org or www.who.org
Figure 11.14: Biosafety levels according to the present scheme. The high containment level BSL-4 is extended according to the additional needs with respect of cleanliness. Starting with the BSL-4m (m = Mars) transport container having a defined atmosphere inside. After docking to the MSRF the atmosphere is analyzed and exchanged to a Mars-like atmosphere at normal terrestrial pressure and temperature BSL-4i (i = inert gas). After transfer of the sample into a biosafety cabinet of class III (glove box) the sample is held at clean room conditions BSL-4c (c = clean room). The same holds for the sample in a clean room outside the glove box. Final experimentation under animal room or normal biocontainment is done at conventional biosafety level 4 against the environment according to the classical animal biosafety conditions (BSL-4 or BSL-3ag as called in the USA).
Figure 11.15: The MSRF with its different biosafety levels starting from the transport container to the docking into the glove box and finally its release to the clean room environments and into the laboratory and animal facility before release to the environment.
Figure 11.16: A possible layout for a Mars Sample Receiving Facility. The design was made to concentrate all specific facilities like autoclaves, dunk tanks, air locks, chemical shower and body shower in one single point. This makes an up-scale to larger laboratory areas easier in the sense that the three rooms can be enlarged to the left and the upper part without changes in the technical part. Only air volume and sewage has to be adjusted.
Figure 11.17: *Pressure scheme of the MSRF*. The green surrounding area is at about -25 Pa with respect to the environment. The blue clean room area has to be positive against all attached areas, but the shower should be more negative than the safety corridor. Therefore the blue clean room could be at zero and the green shower at -50 Pa. The chemical shower in orange could also be at -50 Pa, the suit laboratory at -100 Pa and the animal room as the potentially highest contaminated room at -150 Pa.
Figure 11.18: The singularity problem of a three corner point can be solved in the shown way. „M” represents material air locks, „A” stands for autoclaves and the shower block are personnel showers.

Figure 11.19: An iterative process of risk analysis shall be used. Hazard and incident scenario should define the hazard and danger. The risk analysis gives some hints on the safety measures necessary and the remaining safety factor. Together with the implemented risk management in iterative steps it can be decided whether the safety measures and risk management are sufficient to make the risk acceptable.
Figure 11.20: The three input values of organisms, quantity and activity will lead to an estimation of the risk of the activity and therefore to a risk class and the necessary safety measures defined for risk class activity 1 to 4. For risk class activities with a potential for major incidents (class 3 and 4) the scenario leading to such an accident is also necessary in addition. If an unacceptable risk for men and environment results the input value, the safety measures and the risk management must be improved. This will in general also improve the safety factor for the time without major incident.
Figure 11.21: During a heavy storm with a wind velocity of 100 km/h a high wind load would result on the lee side while in the lee side of the building a considerable negative pressure would result. The values are approximations according to simple gas dynamic relations. Due to the nonairtight outer building structure and the many leaks in the connection to the upper floor, especially around the air ducts, the pressure distribution around the containment box would be according to the figure leading to a leakage of air from the inside to the outer corridor on the lee side of the building. If the lee side would be the side with the exhaust air duct, the containment would be in high overpressure compared to the outside. The airflow would hardly be able to react on that situation within a short enough time.
Figure 11.22: The meaning of redundancy can be demonstrated with the help of this simple figure. In case of a power loss there are some containment principles which are still functioning and therefore still guaranteeing the overall containment. From this it can be concluded that the UPS is absolutely essential and that the air locks cannot be used if negative pressure is to be guaranteed. The secondary barrier (walls, door seals etc.) will on the other hand define the time the negative pressure can be guaranteed.

Figure 11.23: Trading-off mission risk and mission value is a complex process between engineers, managers and scientists.