

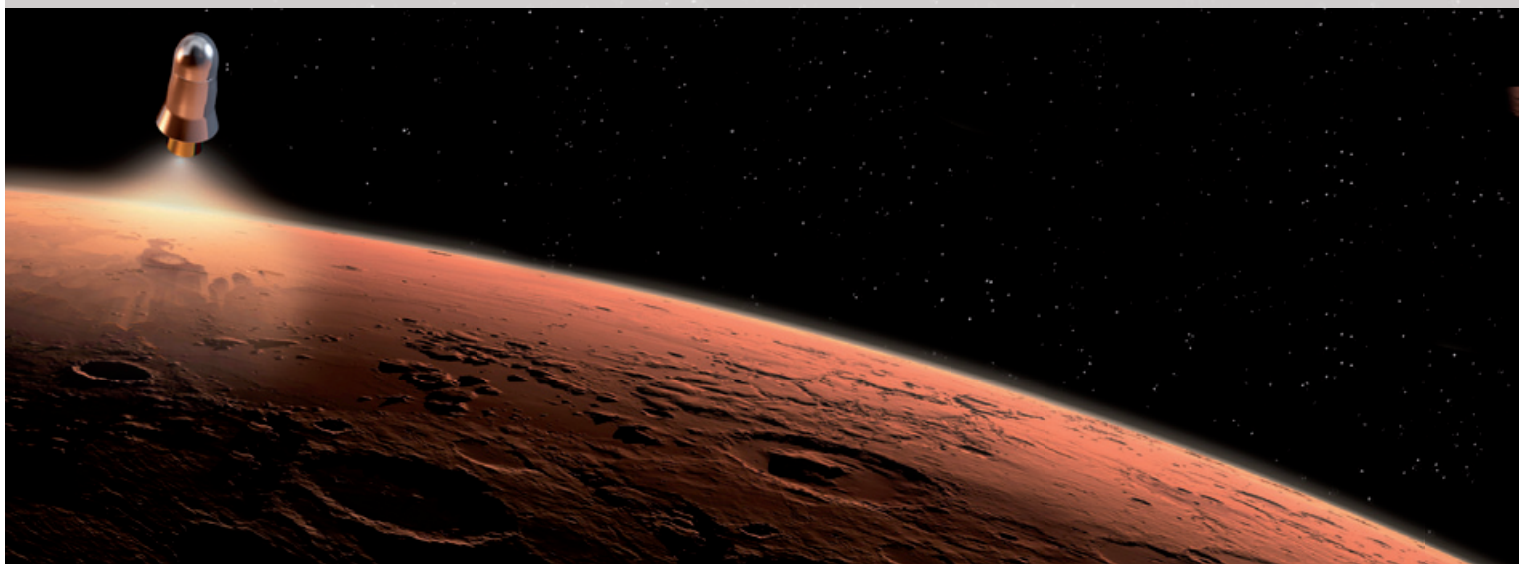


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Mars Sample Return backward contamination – Strategic advice and requirements

Report from the ESF-ESSC Study Group on MSR Planetary Protection Requirements.



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Foreword: Mission Statement



As planetary protection regulations have a significant impact on mission design, engineering and overall cost, it is critical that the guidelines are implemented with proper justification and are re-evaluated on a regular basis.

In June 2011, the European Space Agency asked the European Science Foundation (ESF) in coordination with its European Space Sciences Committee (ESSC) to perform a study regarding planetary protection regulations for a Mars Sample Return (MSR) mission. Specifically, ESF was asked to perform a study on the level of assurance of preventing an unintended release of Martian particles into the Earth's biosphere in the frame of an MSR mission. ESF commissioned a study group of 12 high-level, international and multidisciplinary experts (see Annex 1 for Study Group composition) to evaluate the current requirements, and to provide new insights and recommendations where applicable. The Study Group was formed following a call for nominations addressed to several research organisations in Europe and beyond as well as to the ESF standing committees on Life, Earth and Environmental Sciences (LESC), Medical Research (EMRC), Physical and Engineering Sciences (PESC) as well as Social Sciences (SCSS) and Humanities (SCH).

The mandate of the Study Group was to: **“Recommend the level of assurance for the exclusion of an unintended release of a potential Mars life form into the Earth’s biosphere for a Mars Sample Return mission”.**

The starting point of this activity was the requirement used since the late 1990s specifying that: *‘the probability that a single unsterilised particle of 0.2 micron diameter or greater is released into the Earth environment shall be less than 10⁻⁶’.*

The value for the maximum particle size was derived from the NRC-SSB 1999 report ‘Size Limits of Very Small Microorganisms: Proceedings of a Workshop’, which declared that $0.25 \pm 0.05 \mu\text{m}$ was the lower size limit for life as we know it (NRC, 1999). However, the past decade has shown enormous advances in microbiology, and microbes in the 0.10–0.15 μm range have been discovered in various environments. Therefore, the value for the maximum particle size that could be released into the Earth’s biosphere is revisited and re-evaluated in this report. Also, the current level of assurance of preventing the release of a Mars particle is reconsidered.

To complete its mandate, the Study Group met on three occasions between June and November 2011 and commissioned the organisation of a workshop dedicated to risk perception held in January 2012. The outcome and recommendations from the risk perception workshop (see Annex 2 for details) were used as direct inputs in the formulation of the advice contained in this report.

1. Mars Sample Return Mission and planetary protection – background



1.1 Planetary protection regulatory framework

In 1967, the United Nation’s Outer Space Treaty defined the grounds for planetary protection, stating that:

“parties to the Treaty shall pursue studies of Outer Space, including the Moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extra-terrestrial matter and, where necessary, shall adopt appropriate measures for this purpose” (United Nations, 1967).

Currently, over 100 countries are party to the treaty, and the Committee on Space Research (COSPAR) maintains and propagates this planetary protection

policy, while also providing guidelines to spacefaring nations.

Planetary protection considers two types of contamination: forward and backward contamination. Forward contamination refers to the introduction of Earth organisms to other celestial bodies, whereas backward contamination refers to the release of extra-terrestrial material into the Earth’s biosphere.

Planetary protection regulations are further adapted for specific missions, depending on the targeted body and its significance to the origin of life and/or chemical evolution, and the type of mission (i.e. lander, flyby, or sample return mission). COSPAR has identified five categories of space mission depending on the target body, its potential interest for the study of chemical evolution and/or origin of life and the type of mission (e.g. direct contact, Earth return) with suggested planetary

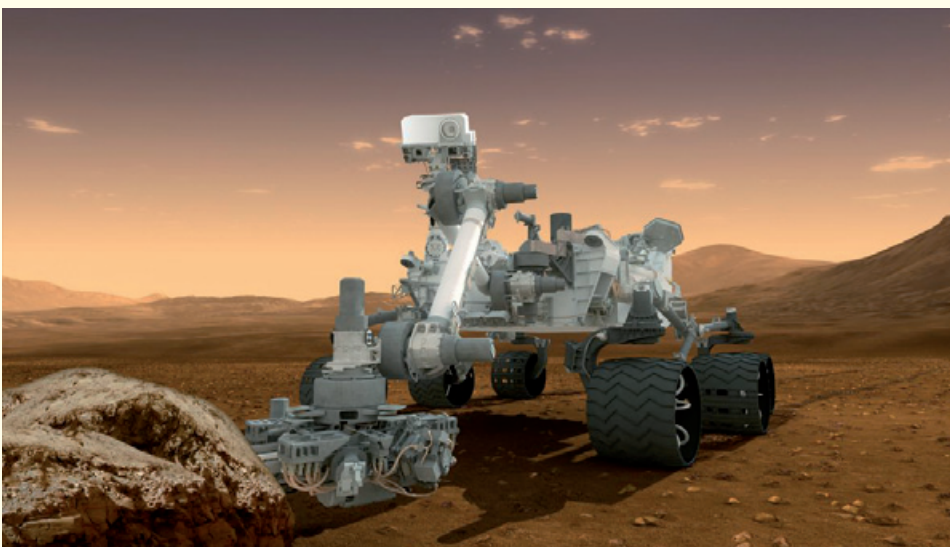


Figure 1. NASA’s Mars Science Laboratory Curiosity rover will investigate Mars’ past or present ability to sustain microbial life.
Credit: NASA/JPL-Caltech

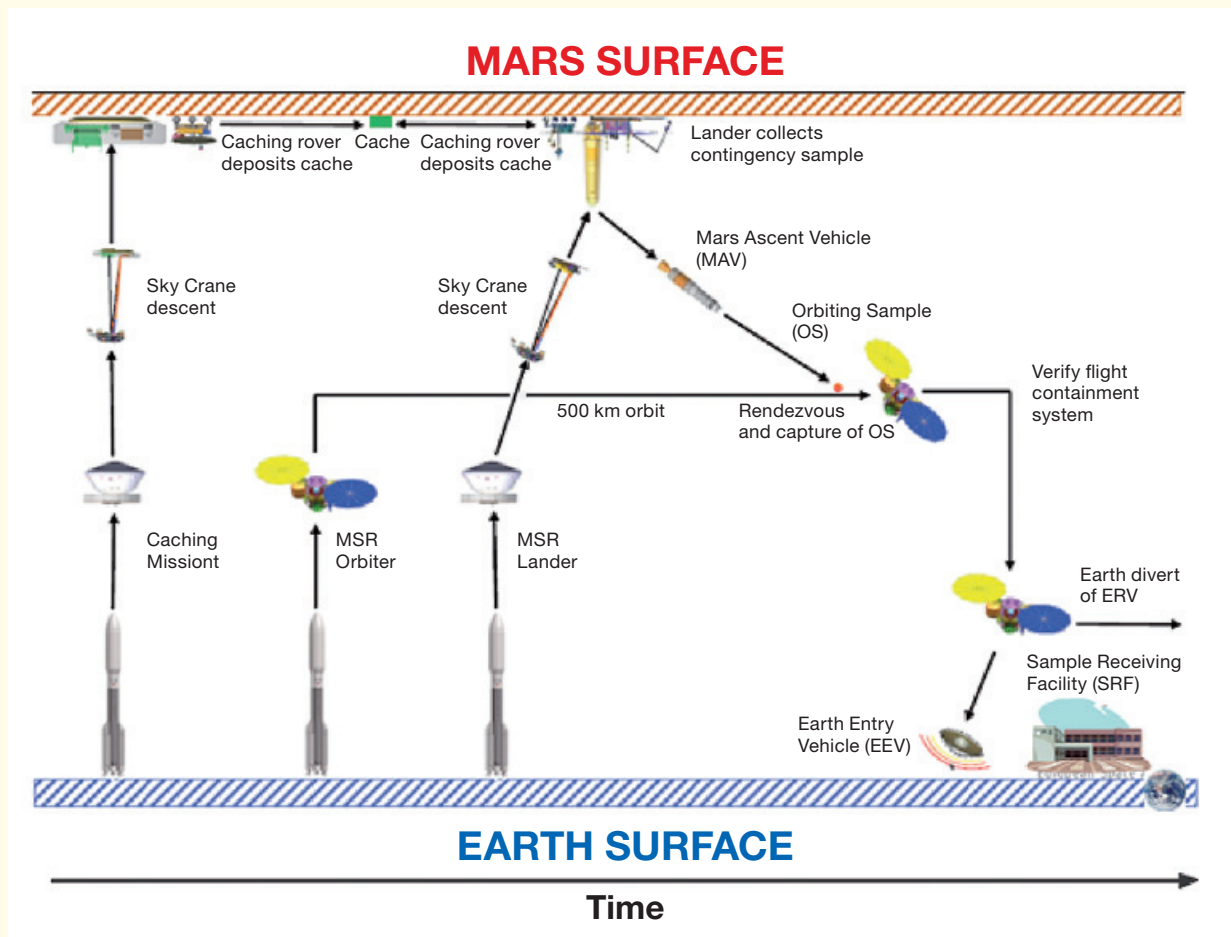


Figure 2: An example of a possible Mars Sample Return mission architecture

protection requirements for each, ranging from Category I (no requirements) to Category IV (more restrictive), and Category V (Earth return missions – the most requirements) (COSPAR, 2002–2011).

1.2 Mars Sample Return Mission concept

Figure 2 depicts the mission architecture of a possible Mars Sample Return (MSR) mission. The mission may include three launches from Earth (one for the caching mission, one for the MSR orbiter/Earth Entry Vehicle and one for both the fetch rover and Mars Ascend Vehicle) and one launch from the Mars surface (Mars Ascend Vehicle). Planetary protection regulations will address both forward and backward contamination during this mission; the activity of the ESF-ESSC Study Group and this report focus on the latter.

COSPAR defined specific category III/IV/V requirements for Mars missions; category IV in particular is divided into three subcategories:

- Category IVa. Lander systems not carrying instruments for the investigations of extant Martian life,

- Category IVb. For lander systems designed to investigate extant Martian life,
- Category IVc. For missions which investigate Martian special regions.

An MSR mission is regarded as a Category V mission with restricted Earth return, this category having the highest planetary protection requirements. When considering a lander system designed to investigate extant Mars life, the outbound portion of the mission must meet Category IVb forward contamination requirements to avoid not only contamination but also false positive indications for on-going and future life-detection experiments. The main concern, however, lies in the potential backward contamination of the Earth’s biosphere by Mars material through the Earth Entry Vehicle and the sample it contains. COSPAR recommends strict requirements (Category V), including:

- *Unless the samples to be returned from Mars are subjected to an accepted and approved sterilisation process, the canister(s) holding the samples returned from Mars shall be closed, with an appropriate verification process, and the samples shall remain contained during all mission phases through trans-*

port to a receiving facility where it (they) can be opened under containment.

- The mission and the spacecraft design must provide a method to “break the chain of contact” with Mars. No uncontained hardware that contacted Mars, directly or indirectly, shall be returned to Earth. Isolation of such hardware from the Mars environment shall be provided during sample container loading into the containment system, launch from Mars, and any inflight transfer operations required by the mission.
- Reviews and approval of the continuation of the flight mission shall be required at three stages: 1) prior to launch from Earth; 2) prior to leaving Mars for return to Earth; and 3) prior to commitment to Earth re-entry.
- For unsterilised samples returned to Earth, a programme of life detection and biohazard testing, or a proven sterilisation process, shall be undertaken as an absolute precondition for the controlled distribution of any portion of the sample.

1.3 Sterilisation: concept, methods and limitations

Sterility is defined as the state of being free from viable (micro-)organisms (adapted from ISO/TS 11139: 2006). Sterilisation is a term referring to any process that eliminates or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present in air or on a surface, contained in a fluid, or inside porous materials such as certain rocks. In recent years the term has evolved to also include the disabling or destruction of infectious proteins such as prions.

Sterilisation procedures are developed for life as we know it with a water-mediated carbon chemistry. Tests to confirm the efficiency of sterilisation processes are performed routinely as cultivation assays. However, it has been known for many years that only a very small portion of all microorganisms from a whole microbial community present in a certain environment can be grown in the lab. The term “the great plate count anomaly” was used for the first time by Staley and Konopka (1985), but the phenomenon had already been observed by other scientists. It describes the difference in orders of magnitude between the numbers of cells from natural environments that form colonies on agar media and the numbers countable by microscopic examination. Thus culturability is a parameter which can indicate viability, but lack of growth on or in media does not indicate the absence of cells or cell death.

There are different reasons why many microorganisms do not grow under laboratory conditions:

- i. they are dead,
- ii. they need environmental conditions which have not yet been reproduced in laboratories, e.g. extremely long incubation times, necessity of specific chemical compounds or physical factors, need for other organisms,
- iii. the organisms can be cultivated but have transiently entered a VBNC (viable but not cultivable) state as a response to stress (antibiotics, toxic metals, UV light, biocides, starvation, osmotic stress, etc.).

Therefore the statement of something being sterile and the application of methods for sterilisation are based on growth experiments which are conducted under defined conditions with respect to nutrients, temperature, gas composition and pH. However, for certain scientific questions it is necessary to determine whether microorganisms, e.g. in an environmental sample, are viable and metabolically active, even if they cannot be cultured. Different molecular-based methods can be applied for the investigation of different biological endpoints (Rochelle *et al.*, 2011). Examples are the application of fluorescent dyes for the investigation of membrane integrity, membrane potential, and protein synthesis, the *in vitro* amplification of nucleic acids to detect and quantify ribosomal and messenger RNA, or the measurement of enzymatic activities to demonstrate respiration.

Sterilisation processes can be divided into physical, chemical or mechanical methods (see Box 1). Each of these methods has advantages and limitations which have to be considered before choosing a method for a specific purpose.

For planetary protection ESA and NASA currently have only one approved method of spacecraft sterilisation – the dry heat microbial reduction (DHMR) process. This technique was used on the Viking Mars landers, which were built and launched in the 1970s. However, advanced materials, electronics, and other heat-sensitive equipment being used on spacecraft today could be damaged by such high-temperature treatment. Therefore, both space agencies are developing and standardising alternative sterilisation methods for application on spacecraft components and systems.

For an MSR mission other sterilisation techniques may have to be applied depending on the actual assumptions about putative Mars life forms. If we expect life as we know it (Chapter 3.1) with a water-mediated carbon-based biochemistry many of the sterilisation techniques mentioned above

Box 1. Sterilisation processes**Physical methods****Heat sterilisation**

Heat sterilisation is the most widely used and reliable method of sterilisation. It is a bulk sterilisation method. It can only be applied to thermostable materials. The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water.

- **Steam sterilisation**
Humidity can damage sensitive materials.
- **Dry heat sterilisation**
Higher temperatures are necessary than for steam sterilisation.

Radiation sterilisation

- **Ionising radiation**
Ionising radiation is routinely used for the sterilisation of medical devices. It is a bulk sterilisation method. Ionising radiation induces damage in DNA and in other cellular components. The penetration depth depends on the type and energy of the radiation (X-rays, γ radiation, β radiation). It can only be applied to radiation-resistant materials.
- **UV radiation**
UVC radiation is germicidal due to the induction of DNA damage. It is only effective on surfaces, which makes the dosimetry and the application on three dimensional objects difficult.

Chemical methods

- **Chemical vapour sterilisation**
Chemically reactive gases such as formaldehyde and ethylene oxide possess biocidal activity by alkylation reactions with cellular components such as proteins and nucleic acids. Hydrogen peroxide induces oxidative damage. These gases are potentially mutagenic and carcinogenic and/or toxic and corrosive. They are only effective on surfaces.
- **Gas plasma sterilisation**
Cold atmospheric gas plasma inactivates microorganisms by complex chemical reactions induced by excited atoms and molecules, radicals and ions. These reactions take place at moderate temperatures. The efficiency depends on the type and energy of the plasma source, the gas or gas mixture and the exposure time. Gas plasmas are only effective on surfaces.
- **Sterilisation with liquid chemicals**
Chemicals such as peracetic acid or hydrogen peroxide solutions are used for sterilising medical devices. They disrupt bonds in proteins and enzymes and may also interfere with cell membrane transportation through the rupture of cell walls and may oxidise essential enzymes and impair vital biochemical pathways. The disadvantage of this method of sterilisation is that the devices must be immersible in an aqueous solution.

Mechanical methods

- **Filtration sterilisation**
Filtration does not destroy but removes the microorganisms. It is used for both the clarification and sterilisation of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles. The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material.

could be utilised. If we expect other forms of life, e.g. based on a solvent other than water or based on an element other than carbon for scaffolding (NRC, 2007a), then it may be difficult not only to detect extraterrestrial life forms, but also to ensure sterilisation.

1.4 Summary of advice from past committees

It is crucial to recognise that significant efforts have gone into developing the current policies for planetary protection, and considerable research has been performed regarding future sample return missions. In order not to re-invent the wheel, Table 1 presents key reports regarding planetary protection for an MSR mission. The reader is recommended to refer to the included documents for further discussion on issues not presented or discussed thoroughly in this report.

Due to recently re-ignited interest in an MSR mission, the National Research Council Space Studies Board (NRC-SSB) was commissioned by NASA to re-evaluate recommendations produced in the 1997 report ‘Mars Sample Return: Issues and Recommendations’ (NRC, 1997). The key recommendations from the 2009 re-evaluation include (NRC, 2009):

- “Samples returned from Mars by spacecraft should be contained and treated as though potentially hazardous until proven otherwise”
- “No uncontained Mars materials, including spacecraft surfaces that have been exposed to the Mars environment should be returned to Earth unless sterilised”

Table 1. Important background documents regarding a Mars Sample Return mission

Category	Report
Background Policies	<ul style="list-style-type: none"> • United Nations, Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, Including the Moon and Other Celestial Bodies, 1967 (United Nations, 1967). • COSPAR, COSPAR Planetary Protection Policy, 20 October 2002, as amended March 24, 2005, July 20, 2008, and March 24, 2011 (COSPAR, 2002–2011).
Mars Sample Return	<ul style="list-style-type: none"> • National Research Council, Mars Sample Return: Issues and Recommendations, 1997 (NRC, 1997). • National Research Council, An Astrobiology Strategy for the Exploration of Mars, 2007 (NRC, 2007b). • iMARS, Preliminary Planning for an International Mars Sample Return Mission, 2008 (iMARS, 2008). • National Research Council, Assessment of Planetary Protection Requirements for Mars Sample Return Missions, 2009 (NRC, 2009).

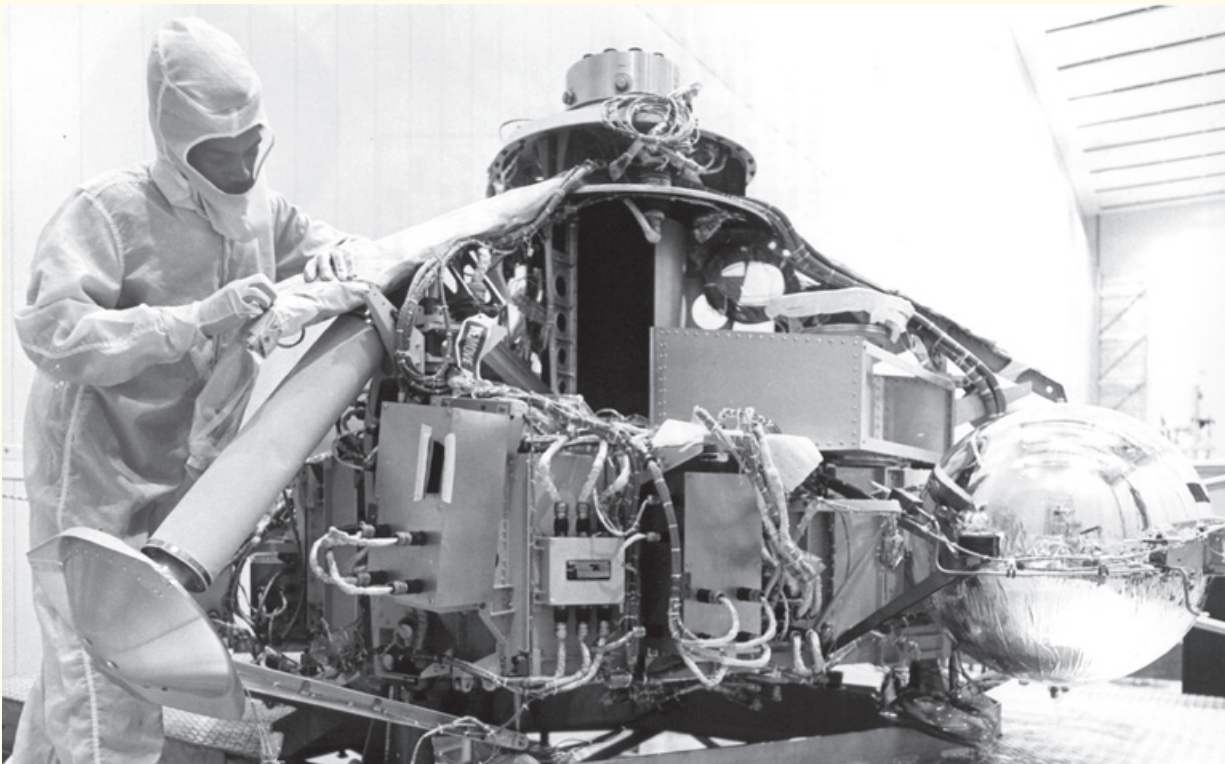


Figure 3. The Viking I spacecraft in a clean room. Credit: NASA

2. From remote exploration to returning samples



2.1 New missions for new knowledge

For any space mission, an analysis must be performed on the benefits and risks involved to justify the investment made. A sample return mission, however, requires extra attention to elucidate the vast benefits not only for science and technology, but also the general public. The benefits of Mars exploration and of a Mars sample return are vast; a few examples of overarching benefits include (but are not restricted to):

- Public engagement and excitement in science and space exploration
- Improving the picture of a ‘larger world’
- Exploration and discovery as part of the destiny of mankind
- The possibility of discovering extra-terrestrial life
- Gathering knowledge to pave the way for potential future human exploration
- The history of science shows that discovery has always led to future discoveries

The past fifteen years have shown an enormous growth of interest in Mars, the most Earth-like planet in our solar system, and in the search for

environments amenable for extant or extinct life. Since 1997, there have been four successful Mars orbiters (Mars Global Surveyor, Mars Odyssey, Mars Express and Mars Reconnaissance Orbiter), two successful landers (Mars Pathfinder and Phoenix), and four rovers (Sojourner, Spirit, Opportunity, and Curiosity – scheduled to land in August 2012). Combining data collected by the numerous orbital and landed spacecraft and with data from laboratory studies of over 50 Mars meteorites, a picture can be painted of a rocky planet with a scant atmosphere, past evidence for abundant water, and the possibility of life.

A Mars sample return has been deemed the highest priority in Mars exploration, as it would promise dramatic advances in the understanding of Mars as a whole (McCoy, Corrigan and Herd, 2011). Several reports from international space agencies and research councils have declared the importance of an MSR mission, and conveyed its necessity in answering fundamental, high-priority scientific questions (e.g. ESA, 2006; ISECG, 2007; iMARS, 2008). Through the study of a sample, researchers could make great progress in understanding the history of Mars, its volatiles and climate, its geological

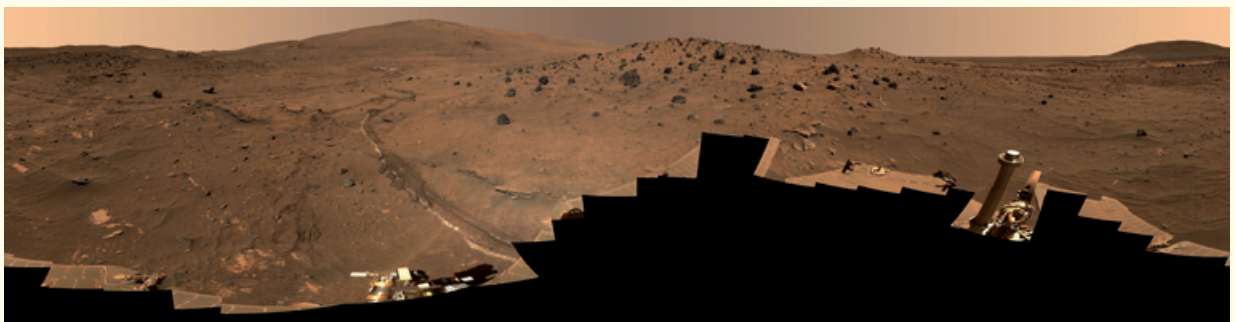


Figure 4. An image taken by the Spirit Rover of the Mars surface. Credit: NASA

and geophysical history, and gain new insights into astrobiology. A Mars sample return has also been deemed an essential precursor to any human exploration missions to Mars (NRC, 2007b).

Although some questions may be answered through *in situ* studies carried out by robotics on the Mars surface, returning a sample to Earth is desirable for several reasons (NRC, 2007b):

- Many experiments and their sample preparations will be too complex for an *in situ* robotic mission
- Returning a sample allows for flexibility in dealing with the unknown and unexpected discoveries via new protocols, experiments and measurements
- There are major limitations with regard to size and weight of instrumentation that can be flown
- There is a significant communication delay to Mars, which impedes the ability to deal with emergencies
- There is a much greater diversity in available instruments and an almost unlimited range of analytical techniques that can be applied on Earth
- The ability to repeat experiments in multiple laboratories and confirm key results is available on Earth
- Participation of entire analytical community is possible
- There is the potential to propagate organisms if they are discovered

In addition to the above points, returning a Mars sample will bring enormous public excitement and engagement to space-related activities, along with pride and prestige to this accomplishment of mankind. For a full discussion on the pros and cons of a Mars sample return vs. *in situ* analysis, the reader is directed towards the National Research Council's report, 'An Astrobiology Strategy for the Exploration of Mars', pp. 73–77 (NRC, 2007b).

2.2 The importance of not compromising the sample and the Mars surface

From an astrobiological point of view, protecting Mars from forward contamination by Earth life is extremely important to ensure that future life science experiments on the planet are not compromised. Contamination would affect the validity of all research done on the sample, as it could potentially be difficult to distinguish between Mars and Earth organisms. All sample acquisition and handling systems must undergo significant bioload reduction prior to launch to minimise the possibility of Earth organisms entering the sample. COSPAR

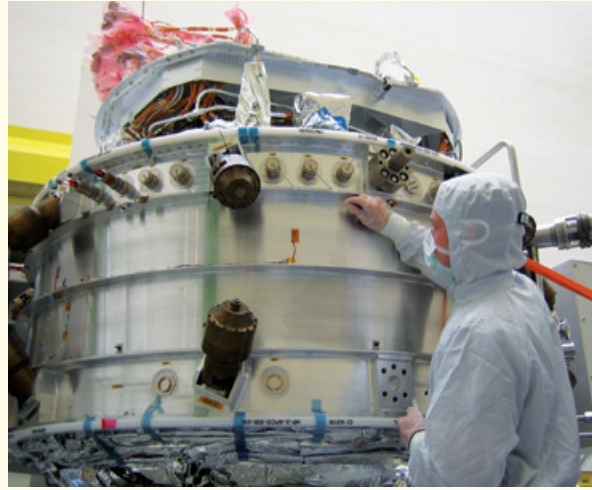


Figure 5. Microbial sampling of a spacecraft for bioburden determination for planetary protection purposes. Credit: DLR

has implemented forward contamination guidelines, stating that for category IVb (required for the outbound leg) (COSPAR, 2002–2011):

- The entire landed system is restricted to a surface bioburden level of ≤ 30 spores, or to levels of bioburden reduction driven by the nature and sensitivity of the particular life-detection experiments

OR

- The subsystems which are involved in the acquisition, delivery, and analysis of samples used for life detection must be sterilised to these levels, and a method of preventing recontamination of the sterilised subsystems and the contamination of the material to be analysed is in place.

Forward contamination of the Mars environment is not within the scope of this report, and the reader is encouraged to refer to the National Research Council's report Preventing the Forward Contamination of Mars (NRC, 2006). One may also review past space missions with strict planetary protection guidelines to understand lessons learned and see where improvements can be made.

2.3 The challenge raised by a returned sample

It should be clear that the introduction of a possible organism from Mars, or a population of Mars organisms, would be very difficult to accomplish even if it were being done on purpose. The Mars environment (cold and dry) is very different from most environments on Earth (largely warm and wet). Free oxygen in the Earth's atmosphere may be an even greater hazard for Mars organisms: it has

the ability to strip electron from (organic) molecules and is therefore poisonous for any organism that has not developed the ability to produce antioxidants (the Great Oxygenation Event around 2.4 billion years ago wiped out most of the early Earth's anaerobic organisms). Earth does have cold and dry environments, some of which are anoxic, but there is only a limited chance that Mars organisms would find their way to those places. Adding the presence of predatory and competitive Earth organisms, the chances for survival for an alien microbe (and its potential hazard) becomes even lower. The challenges of contaminating the Earth are daunting for an invading Mars microbe, and certainly the probability of success for such an invasion is much less than one.

It is highly unlikely that any Mars organisms, if they exist, would be obligate parasites of Earth organisms. It is quite certain that no humans or other macro-organisms have been in regular contact between Earth and Mars, and only a limited number of Earth microbes have made the trip since the beginning of Mars exploration by robotic spacecraft in the early 1970s. Even in the face of potential natural interchange of materials from Earth to Mars (e.g. Mileikowsky *et al.*, 2000) there are severe limitations on any recent contact between the two planetary biospheres, if, indeed, there proves to be one on Mars at all.

With an eye to this sparse potential for contact, while still acknowledging its possibility, and given the inherent differences between the available niches on Earth compared to those that are possibly inhabitable on Mars, the US National Research Council's Space Studies Board concluded in 1997 that the "*contamination of Earth by putative Mars microorganisms is unlikely to pose a risk of significant ecological impact or other significant harmful effects. The risk is not zero, however*" (NRC, 1997). Even now, with an expanded understanding of the potential for more frequent interchanges than was appreciated in 1997, the Space Studies Board concluded in 2009 that "*the potential for large-scale pathogenic effects arising from the release of small quantities of pristine Mars samples is still regarded as being very low.*" The report also noted that "*extreme environments on Earth have not yet yielded any examples of life forms that are pathogenic to humans*" (NRC, 2009).

This is not to say that these exercises in logic can provide any guarantee of safety. Indeed, the implications of Mileikowsky *et al.* (2000) are that it is possible that the natural interchange of materials between Mars and Earth, perpetuated as a result of large impact events across the history of the solar

system, could have also involved the infrequent exchange of live microorganisms from time to time. This could have resulted in either colonisation of one planet by life from the other, and the potential for biospheric exchange that may have had evolutionary consequences. Joshua Lederberg, himself a pioneer in the consideration of the consequences of an interplanetary exchange of organisms, noted the limitations of mankind's ability to deal with the problem of a sample returned from Mars and its possible consequences for Earth life (Lederberg, 1999):

"Whether a microorganism from Mars exists and could attack us is more conjectural. If so, it might be a zoonosis to beat all others.

On the one hand, how could microbes from Mars be pathogenic for hosts on Earth when so many subtle adaptations are needed for any new organisms to come into a host and cause disease? On the other hand, microorganisms make little besides proteins and carbohydrates, and the human or other mammalian immune systems typically respond to peptides or carbohydrates produced by invading pathogens.

Thus, although the hypothetical parasite from Mars is not adapted to live in a host from Earth, our immune systems are not equipped to cope with totally alien parasites: a conceptual impasse" (Lederberg, 1999).

With those thoughts in mind, it may seem that the risk posed by returning a dangerous biological entity (e.g. a virus-type, microorganism, etc.) is quite low. Nevertheless, it still cannot be guaranteed to be impossible. It is believed that if such a biological entity exists, humans would be able to kill it (by the sundering of covalent bonds in a rigorous sterilisation process).

2.4 Considering backward contamination through particle size

When dealing with the issue of containment of a Mars sample, it is important to focus on what it is about the sample that must be contained to achieve the desired result (e.g. safety of the Earth, non-contamination of the sample, engineering feasibility, and so on). It does not advance the case for a "safe sample return" by specifying an unachievable goal or an irrelevant one, nor does the imposition of multiple monitoring systems necessarily result in a more reliable containment process. Monitoring systems, particularly critical sensors, themselves, are often less reliable than the process that they are monitoring (Wu, 2005) and during an Earth-entry by a

returning spacecraft from Mars, there will be very little, if any, time to sort out such failures from the malfunctioning of the containment system itself.

In the context of a potential joint MSR mission with CNES and NASA, to deal with the possibility of a sample from Mars carrying a Mars microbe, it was originally decided by the NASA Planetary Protection Officer (Rummel, 1999) to focus on the containment of a particle of a certain size as a way of defining the requirement for project implementation. This was couched as a draft requirement, subject to further discussion prior to defining the final requirements. However, subsequent discussions and project work argued that organism size, or the dust particle or rock on (or in) which an organism could be lodged, was an appropriate way to characterise a physical entity that might be a biological hazard, and was amenable to engineering solutions that could be verified remotely and be long-lasting.

Alternative containment options, such as the establishment of a gas-tight or hermetic seal, posed much larger problems in terms of engineering and monitoring complexity, and exacerbated the problem posed by the possible failure of monitoring sensors during the mission – especially at critical points during the return of the sample to Earth.

The ESF-ESSC Study Group concurs with the approach adopted since 1999 and confirms that containment of particles larger than a given size is an appropriate constraint to be considered when designing the mission.

With the (draft) determination that a particle was the right entity to contain, the original letter (Rummel, 1999) used a particle size that was used in standard microbiological laboratory practice as the then-accepted minimum size of an organism to be filtered from air or a liquid in order for that air or liquid to be specified as “sterile.” In a Space Studies Board workshop published the same year, it was concluded (as a consensus) that “*given the uncertainties inherent in this estimate [of the required protein-making machinery], the panel agreed that 250 ± 50 nm constitutes a reasonable lower size limit for life as we know it*” (NRC, 1999). Thus, at the time, and until the publication of this report, the original $0,2 \mu\text{m}$ draft requirement was considered to be appropriate for the state of knowledge at that time.

The ESF-ESSC Study Group highlights that considering the knowledge that has been produced over the past years, the $0,2 \mu\text{m}$ value is no longer valid. New developments in microbiology should be taken into consideration when determining the specification for a future Mars Sample Return Mission.

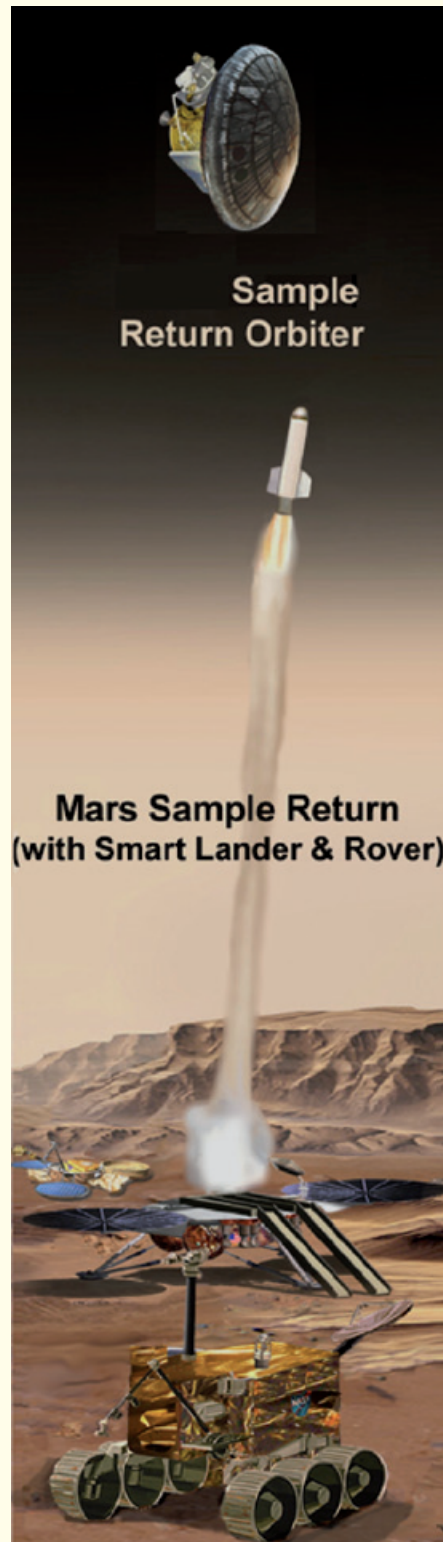


Figure 6. Whatever is done to contain a Mars sample in the Mars environment will have to be done as part of the rover/lander that collects the sample, the payload container that is loaded on the Mars Ascent Vehicle, or the orbiter that will collect the sample and return it to the orbit of Earth. Credit: CNES/JPL

3.

Life as we know it and size limits



3.1 Life as we know it

So far, there is only one example of life, i.e. life on Earth. Despite impressive developments in our understanding of biological processes at the cellular and molecular level and new approaches in the emerging field of synthetic biology, where biological components and systems are designed and constructed that do not already exist in the natural world, we still lack a generally agreed-upon definition of life (Tsokolov, 2009; Tirard *et al.*, 2010). Instead several characteristics can be listed for describing living organisms. These include organisation in the form of cells as basic units of life, the ability to regulate the internal cellular environment to maintain a constant state, the transformation of energy by converting chemicals and energy into cellular components and decomposing organic matter, the capability to grow and reproduce, the ability to respond to external stimuli and to adapt to environmental changes. However, non-living matter can also exhibit some of these features.

There are three prerequisites for life as we know it:

- (i) **Water:** Life on Earth requires water which has to be available at least temporarily in a liquid state. This limits the temperature range for extraterrestrial environments to be defined as habitable. Water serves as a selective solvent necessary for diffusion processes, as a reaction partner in metabolic reactions, as a heat conductor and as a stabiliser for complex biochemical molecules.
- (ii) **Carbon and other key elements:** All organisms are composed of chemical compounds made from carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur and several other trace elements. In particular it is the capability of carbon to form four covalent bonds to other atoms

that enables the formation of a huge number of complex organic molecules with stable carbon-carbon bonds.

- (iii) **Energy:** Life, which can also be described as a self-sustained chemical system capable of undergoing Darwinian evolution, needs an energy source for metabolic processes. Most organisms on Earth depend directly or indirectly on the radiation energy of the sun either by performing photosynthesis or by using organic compounds produced by photosynthesising organisms. However, some groups of organisms can gain chemical energy by using different electron donors, e.g. H₂, Fe(II) or S⁰, and electron acceptors, e.g. O₂, Fe(III) or S⁰.

3.2 Approaching the issue of minimum size limit for life

The dimension of cells, the basic units of life, is generally expressed as the diameter or volume of coccoid cells, or length, diameter and volume of rod-shaped cells. Small cells are also categorised by genome size although there is no clear correlation between genome size and cell size (see below: ‘smallest cells observed and their characteristics’).

Virus particles are small infectious agents that can replicate only inside living cells. Similar to cells, virus dimensions are also measured as capsid size or length for head-tail bacteriophages and rod-shaped and filamentous viruses, and as genome size. Bacteria range in size from 700 to 750 μm for the largest, *Epulopiscium fishelsoni* (isolated from surgenfish gut; Angert *et al.*, 1993) and *Thiomargarita namibiensis* (isolated from marine reduced sediments; Schulz *et al.*, 1999), to approxi-

mately 0.1–0.2 μm for the small forms of human pathogenic *Mycoplasma* species (Robertson *et al.*, 1975). Most of the ultrasmall “free-living” microorganisms are between 0.2–0.4 μm , although there are reports of “free-living” bacteria that can pass through a 0.1 μm filter (Miteva and Brenchley, 2005; Wang 2007). Starved bacterial cells from marine environments are also known to miniaturise to diameters of less than 0.4 μm (Velimirov, 2001). A common starvation response in soils is spore or cyst formation. Bacterial spores are 0.8–1.2 μm in length and bacterial cysts are generally greater than 1 μm in diameter.

Theoretical considerations

What is the theoretically smallest possible size of a free-living microorganism? This question was addressed in a National Research Council workshop Report (NRC, 1999) in response to the report by McKay *et al.* (1996) suggesting that 50 nm (0.05 μm) particles observed on the Mars meteorite ALH 84001 could be fossil bacteria. It was determined from calculations of molecule size and structure that DNA, due to its folding characteristics and the necessity for a minimal number of genes, controls cell size. A 50 nm diameter cell, 75% of which is occupied by proteins (average MW of 30 kDa or a diameter of about 4 nm per protein) and ribosomes (diameter of 20 nm) could contain only eight genes (8 kb DNA).

The NRC workshop report concluded that $0.25 \pm 0.05 \mu\text{m}$ was the lower size limit for life as we know it – the minimal size of a cell that would contain the minimal material (e.g. number of genes, proteins) to be free living as an autotroph. Since the report was published it is clear that there are smaller cells seen in different samples (see below).

Cells in the environment may have less than the minimal number of genes for growth in the free-living state but grow because of associations with other cells in more of a mutualistic association that supply key nutrients. If the ‘minimum’ cell contained 250 genes (250 kb DNA) and the cell was 50% DNA, the diameter would be 110 nm. The cell needs water and if it is assumed that it contains 50% water, then the cell size would be 136 nm. A cell growing on CO_2 as the source of carbon would require 750 genes and if the DNA occupies 50% of the cell volume, the cell would be 156 nm in diameter. It appears that a coccoid cell with the minimum number of genes to be free living in an environment other than a living host would have a minimum cell diameter of approximately 0.15–0.2 μm . A rod-shaped cell could have a width of less than 0.1 μm with a variable length but greater than 0.2 μm . It is possible

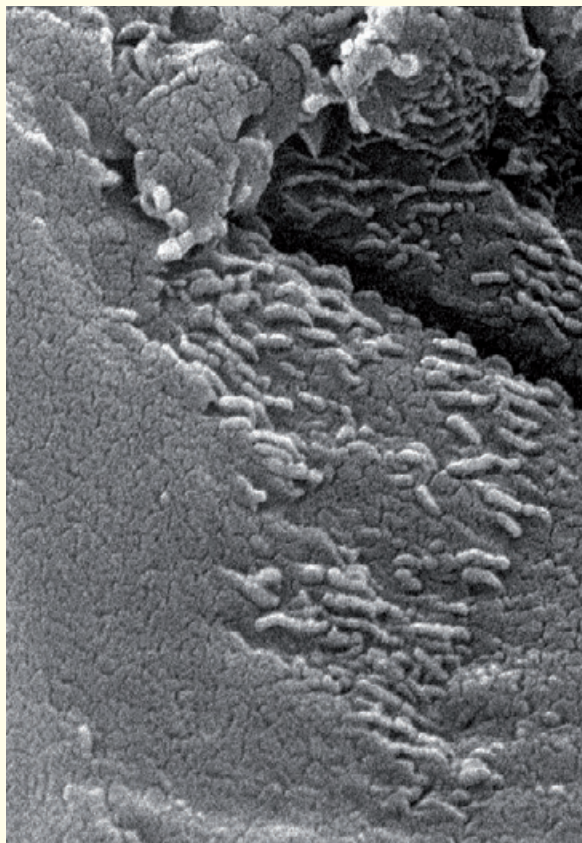


Figure 7. Scanning Electron Microscope images like this one of Mars meteorite ALH84001 have been interpreted as evidence of past life on Mars. Credit: NASA Johnson Space Center

that smaller cells exist that have greatly reduced genomes but have an obligatory requirement to co-exist with other organisms as the source of required genes or gene products.

3.3 Characteristics of the smallest cells

There are different categories of minimally sized cells: free-living growing cell, free-living dormant cell, endo- and exo-symbionts, parasites, and syntrophic cell communities. The smallest cells are bacterial endosymbionts and bacterial parasites that have greatly reduced genome sizes (Feldhaar and Gross, 2009). As parasites, these cells have co-evolved with their host and have lost genes that are furnished by the host. However, not all parasites have eukaryotic animal or plant hosts and one of the smallest is the archaeon *Nanoarchaeum equitans* (490 kbp, about 550 genes; Huber *et al.*, 2002, see Figure 9), that is a parasite of the hyperthermophilic archaeon *Ignicoccus* species. *N. equitans* has an extremely compact genome and virtually no noncoding DNA and is 0.4 μm in diameter (Huber *et al.*, 2002; Küper *et al.*, 2010). As mentioned, an

important fact to keep in mind is that there are cells with small genomes that do not necessarily have the smallest cell dimensions. For example, the insect endosymbiont *Candidatus Carsonella ruddii* has the smallest bacterial genome (160 kbp) with many genes of reduced length and many overlapping genes (Thao, 2000; Feldhaar and Gross, 2009). The *C. ruddii* cells are elongated tubes that appear to exceed 0,5 μm in length, although electron micrographs show wide variations in cell length (Nakabachi *et al.*, 2006). Another insect symbiont, *Candidatus Sulcia muelleri*, has a genome size of 245 kbp, but can elongate to more than 30 μm (Moran *et al.*, 2005). This elongated size is likely due to the ability of this organism to have from 200 to 900 genome copies per cell, making it an ideal candidate for single cell genomics (Woyke *et al.*, 2010). For comparison, mitochondria have approximately 1.6 kbp and are 0.5 to 1 μm , whereas chloroplasts have a similar genome size but can vary in cell size from 2 to 10 μm .

The smallest free-living cells

Ultrasmall free-living microorganisms have been isolated from marine waters, soils, oil slimes, ice cores and acidic mine wastes. First described in seawater by Torrella and Morita (1981), ‘ultramicrobacteria’ have cell volumes less than 0.1 μm^3 and are generally less than 0,5 μm in diameter. This report expanded the idea that most small cells in oligotrophic marine environments were small because they were starved, to the possibility that actively growing cells could also be ultrasmall. Initial attempts to isolate bacteria from oligotrophic marine waters using *in situ* levels of dissolved organic material yielded a diversity of small microbes that formed microcolonies on agar plates (e.g. Schut *et al.*, 1993). A similar approach was used to isolate one of the most abundant microorganisms in the marine environment, originally referred to as SAR 11, based on its detection by molecular methods in water from the Sargasso Sea (Giovannoni *et al.*, 2005). The isolate, ‘Pelagibacter ubique’, grows in the dilute organic content of seawater and requires reduced sulfur, and at 1,350 genes, has the smallest genome of any free-living bacteria yet discovered (there are many examples of cells with ≤ 1600 genes). ‘P. ubique’ is a rod shaped cell varying in length from 0.37 to 0,84 μm and with an average cell diameter of 0.12–0.2 μm (Giovannoni *et al.*, 2002). ‘P. ubique’ has no introns, inteins or transposons and a very short intergenic spacer region (ITS) but still retains the metabolic capability of other alphaproteobacteria and is only capable of slow growth. Ultrasmall bacteria have also been isolated from freshwater environments but are not phyloge-

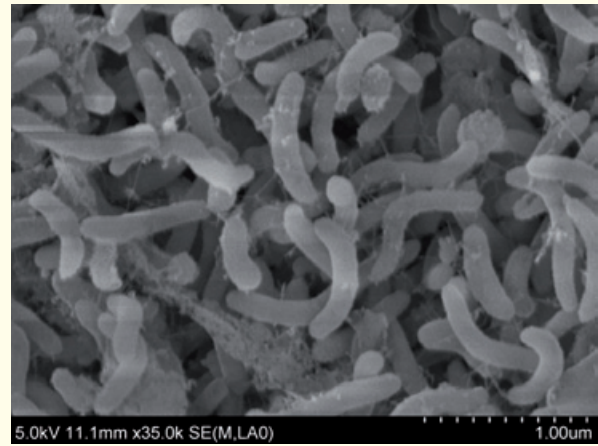


Figure 8. The bacteria “*Pelagibacter ubique*”.

Credit: Center for Microbial Oceanography: Research and Education

netically related to marine bacteria. For example, Hahn *et al.* (2003) isolated nine ultrasmall bacteria of the class *Actinobacter* from freshwater lakes and a pond in Europe and Asia. All were isolated from filtrates after passing through a 0,2 μm filter. The cell volumes were less than 0.1 μm^3 with lengths less than 0,5 μm . The small sizes were maintained even when cultured in media with high levels of organic material.

There are a number of reports of ultrasmall bacteria from soils. Isolates described include an alphaproteobacteria related to *Kaistia* species (Duda *et al.*, 2007; Panikov, 2005). These cells are heterotrophic and aerobic and display two cell sizes during their growth cycle, cells, 0.4–0,8 μm in diameter, and ultrasmall cells approximately 0.2–0,3 μm in diameter. It was also demonstrated that these free-living ultrasmall bacteria can also be parasitic to cyanobacteria and heterotrophic bacteria in addition to being free living on organic compounds (Suzina *et al.*, 2008). Two anaerobic, fermentative, ultras-small bacteria were isolated from anoxic rice paddy soil that were members of the *Verrucomicrobiales* lineage of bacteria (Janssen *et al.*, 1997). The mean diameter of these isolates was 0.35–0,5 μm with a cell volume of 0.03–0.04 μm^3 . It is interesting that the ultrasmall size was stable even with increases in the organic substrate concentration of the growth medium. Similarly, more than 250 bacterial colony forming units were isolated per ml of melted 120,000-year-old Greenland glacier ice core after the sample was filtered through 0.2–0,4 μm filters (Miteva and Brenchley 2005). Some colony forming units of bacteria were even isolated after prefiltration of the melted ice core through a 0,1 μm filter. Even after cultivation, some of the cells were less than 0,5 μm in diameter. The isolates included different proteobacteria and both high- and low-GC Gram

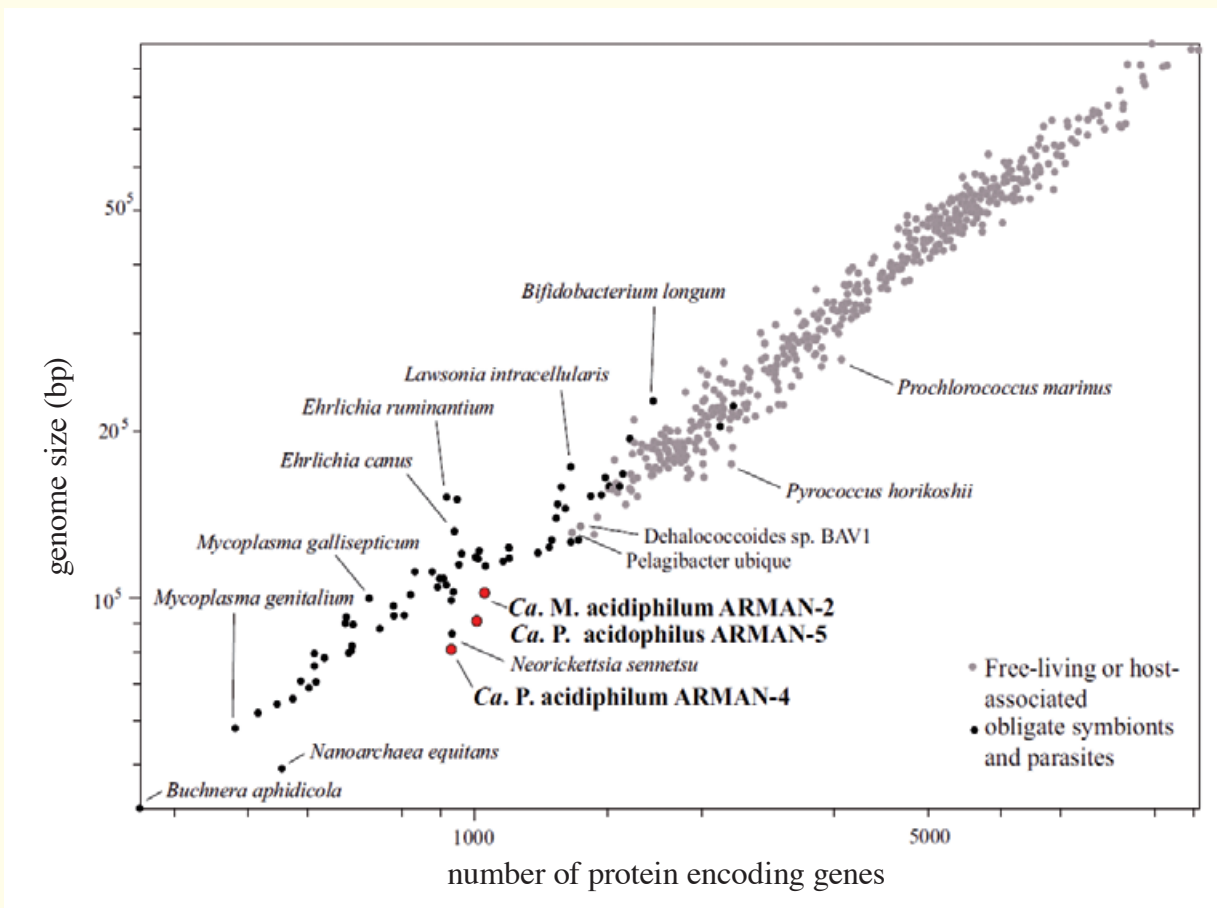


Figure 9. Plot of archaeal and bacterial genomes (from the National Center for Biotechnology Information Database) sizes versus the number of proteins encoding genes per genome (Baker *et al.*, 2010). Ca: Candidatus; M: Micrarchaeum; P: Parvarchaeum.

positive bacteria (actinobacteria and Firmicutes, respectively). Wang *et al.* (2007) discuss bacteria that passed through a 0,1 μm filter and were subsequently able to grow on natural assimilable organic carbon with specific growth rates of up to 0.47 h^{-1} .

The Archaea, the third domain of life, have many unique characteristics including the ability to grow in the most extreme Earth environments, novel metabolisms, and an evolutionary history that places them on the early Earth (Jarrell *et al.*, 2011). Many archaea that grow in extreme environments and particularly those that grow at hyperthermophilic temperatures ($>80^\circ\text{C}$) have small cell sizes and small genomes. As a general rule, the cell size and volumes of many genera of hyperthermophilic archaea can vary by as much as four orders of magnitude. The smallest cell sizes of hyperthermophilic archaea are rods of *Thermofilum* at 0.15–0,17 μm in diameter and between 1 and 100 μm in length, the 0,3 μm diameter spheres that protrude from rod-shaped cells of *Pyrobaculum* and *Thermoproteus*, and the 0.2–0,3 μm diameter flat disks (0.08–0,1 μm wide) in *Pyrodictium* and *Thermodiscus* species (NRC, 1999). An ultra-small archaeon has been imaged from the biofilms found in acid mine drainage referred to

as ARMAN organisms (archaeal Richmond Mine acidophilic nanoorganisms; Comolli *et al.*, 2009). The cells were approximately 0,3 μm in diameter with cell volumes of 0.009–0.04 μm^3 and only ~ 92 ribosomes. A metagenomic and proteomic analysis of three lineages of ARMAN organisms showed genome sizes from 800 to 999 kb and approximately 1000 protein coding genes (Baker *et al.*, 2010; see Figure 9 for genome size versus number of genes). These ultra-small Euryarchaea have a high number of genes with similar sequences found in both bacteria and Crenarchaea indicating that ARMAN branch early in evolutionary history (Baker *et al.*, 2010).

3.4 Viruses

Viruses are infective agents that consist of either RNA or DNA inserted into a protein coat that may or may not be surrounded by a lipid membrane. Viruses that infect bacteria are called bacteriophages and can either cause lysis of the host cell or enter into a relatively stable lysogenic state where the viral genome is incorporated into the host genome

and replicates with the host genome. As with bacterial and archaeal parasites, viruses require a host cell for replication and for synthesis of viral biochemical products. Unlike microbial parasites and endosymbionts, there is no evidence that viruses descended from ‘free-living’ cells. The origin of viruses and their early evolution and their possible role in the origin and early development of life is not known (Forterre, 2005; Forterre and Prangishvili, 2009).

Since viruses are presumed to be associated with organisms from all domains of life, it follows that if there were Earth-like life forms on Mars, they would also likely have viruses (most likely bacteriophages). Thus, the detection of viruses or virus-like particles (unusual morphologies) on a Mars sample would most likely indicate that cellular life was also present. However, there are many gaps in our understanding of viruses of most organisms, since the emphasis has been on human and other animal pathogenic viruses, and viruses that target medically important bacteria. For example, very little is known about archaeal viruses and particularly those that infect hyperthermophilic species – those that have been identified had morphologies that had not previously been observed (Prangishvili *et al.*, 2006a,b). A DNA virus that infects the acidophilic, hyperthermophilic *Sulfolobus* species has a gene sequences that shows a relationship to viruses from all three domains of life (Prangishvili *et al.*, 2006b).

Only relatively recently has it been realised how abundant and diverse viruses are in most environments. In the ocean, for example, their numbers exceed those of all bacteria (prokaryotes) by an order of magnitude (Suttle 2005; Rohwer and Thurber, 2009). Moreover, genome sequences of viruses and host bacterial species show the ubiquity of laterally transmitted genes (Paul, 2008; Sullivan *et al.*, 2005, 2009). These include viral immunity systems in bacteria and archaea, host metabolic genes in the viral genome that aid viral reproduction by keeping the host metabolically active during infection, and entire viral genomes (Anderson *et al.*, 2011; Krupovic *et al.*, 2011). There is a strain of *Escherichia coli*, for example, that has 18 whole viral genomes inserted in its chromosome and many bacteria have ‘pathogenicity islands’ and ‘genomic islands’ that include genes transmitted from viruses.

The detection of viruses in a Mars sample could be difficult because of size and morphology, such as small filamentous viruses. Retroviruses, such as Rous sarcoma virus, have the smallest genome among the RNA viruses at 3.5 kb and a particle diameter of 80 nm. The hepadnaviruses, such as hepatitis B, have the smallest DNA genome at 3.2 kb and a particle diameter of 42 nm. The parvoviruses

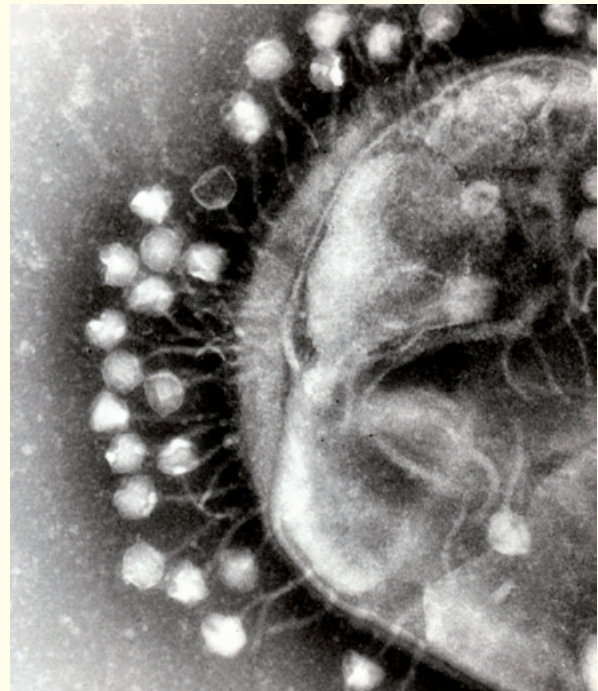


Figure 10. Electron micrograph of Bacteriophages.
Credit: Graham Colm

have a particle size of 18–26 nm with a 5 kb genome. The *Escherichia coli* bacteriophage ϕ -X174 has the smallest genome of any phage thus far described at 4 kb. The DNA bacteriophages have a size range from 50 to >200 nm. The smallest virus observed, the single-stranded DNA porcine circovirus type 2, has a particle size of 17 nm (Faure *et al.*, 2009). The mimivirus, that infects protists, is 400 nm in diameter with the largest known viral genome at 1.2 Mb. It is interesting that even the ultra-small acidophilic ARMAN archaeon was observed to have attached viruses (Comolli *et al.*, 2009).

However, as stated above viruses are not able to reproduce by themselves but need a host organism. For potential consequences on the Earth’s biosphere either these putative virus-type Mars entities have to be able to use a terrestrial cell as host, which would require a very specific and sophisticated adaptation to these cell types, or the putative Martian host has to be present in the same Martian sample and has to be alive and metabolically active to enable the replication of that entity.

3.5 Gene transfer agents (GTAs)

In addition to bacteriophages that can be both lytic and genetic-transfer agents, there have been reports of viral-like transducing particles known as gene transfer agents (GTAs). These bacteriophage-like particles were first reported in the purple non-sul-

fur alphaproteobacterium *Rhodobacter capsulatus* (Imhoff, 1984) and have since been found in the genome of most species in the order Rhodobacterales as well as specific strains of archaea and other gram-negative and gram-positive bacteria (Biers *et al.*, 2008; Lang and Beatty, 2000; Leung *et al.*, 2010; Matson *et al.*, 2005).

GTAs resemble small bacteriophages, ranging in size from 30 to 80 nm with 4.4 to 13.6 kb DNA (Lang and Beatty, 2007). A universal characteristic of GTAs is that they randomly incorporate segments of the host genome into the viral capsid where they can transfer this to different hosts, including phylogenetically unrelated bacteria and archaea, without resulting in lysis of the host cell. In this manner, it is believed that it is possible for GTAs to incorporate any of the host genes during replication (Lang and Beatty, 2007).

While the origin of GTAs is not known, it has been suggested that they are defective phages (Lang and Beatty, 2000; Stanton, 2007; Matson *et al.*, 2005), thus implying that GTAs have lost their parasitic nature and have instead been usurped by the host for the purposes of gene exchange. GTAs have also been hypothesised to be involved in the incorporation of the mitochondria (believed to be an alpha-proteobacterium) into the proto-eukaryotic host (Richards and Archibald, 2010), thus implying an ancient origin for GTAs and their possible important role in the early evolution of prokaryotes and eukaryotes.

While many questions remain about the origin of GTAs, their prevalence in different species of bacteria and archaea, and their host range includ-

ing cross-domain infection, there is evidence that a large portion of marine viromes consist of GTAs. Surprisingly, it is now estimated that GTA transduction rates are more than a million times higher than previously reported for viral transduction rates in marine environments (McDaniel *et al.*, 2010). Clearly, GTAs are a major source of genetic diversity in marine bacteria.

3.6 From new knowledge to new requirements

There is considerable evidence that bacteria and archaea from a variety of environments can pass through 0,22 μm filters. Many of these are free-living organisms in marine, freshwater, soil and ice environments. Also, many archaea living in extreme environments have greatly reduced genome sizes and ultrasmall cell dimensions. Other archaea have been observed from extreme acid environments using microscopic and molecular methods that are morphologically ultra-small with significantly reduced genomes, and appear to be very deeply rooted in the phylogenetic tree of life (Baker *et al.*, 2010). While these archaea, like most microorganisms from a diverse range of different environments, have not been cultured, they clearly point to the fact that it is difficult to make generalisations about the smallest cells, their phylogeny and physiology. Cells approaching the theoretical minimal size limit could exist, particularly if they live in association with other cells as a syntrophic biofilm, in a starved state, or survive desiccation.

If an acceptable minimal size limit for living organisms does exist, then various methods could be employed to assess a sample for the presence or absence of putative organisms or to guarantee that such a particle would not escape from a sample container. **The geochemical and physical context of the sample will be critical information for constraining the potential physiological groups of microbes that could exist in a Mars sample, given that a life form on Mars will likely use the same energy sources as Earth life and could develop some of the same characteristics as Earth life in similar settings.** For example, microorganisms on Earth that live on rocks, including deep basaltic and desert rocks, deep sediment cores, and brine pockets in ice, are likely to have a lifestyle that involves attachment to the solid strata and the ability to form biofilms. Microbes attached to solid substrates, and particularly those that form biofilms, are difficult to observe and enumerate, and, in many cases, difficult to differentiate from non-life forms. Good

Table 2. Smallest organisms

Organisms	Size
Microorganisms	
Smallest bacteria: <i>Mycoplasma</i> species	0.1–0,2 μm
A theoretical coccoid cell with the minimum number of genes to be free living	0.15–0,2 μm
A theoretical rod-shaped cell with the minimum number of genes to be free living	Width: <0,1 μm Length: >0,2 μm
Rods of “Pelagibacter ubique” archaea	0.12–0,2 μm in diameter 0.37–0,84 μm in length
Rods of <i>Thermofilum</i> bacteria	0.15–0,17 μm in diameter 1–100 μm in length
Viruses	
Smallest virus observed: single-stranded DNA porcine circovirus type 2	0,017 μm
Gene Transfer Agents	
General GTAs	0.03–0,08 μm

examples of these difficulties were the numerous reports of “nanobacteria”, 100 nm sized particles first observed in association with specific minerals and hypothesised to cause the formation of these minerals, and later identified in human blood. They have recently been reassessed and determined to be mineral deposits, not organisms (Martel and Young, 2008; Raoult *et al.*, 2008).

Since it is unlikely that samples from Mars will include liquid water (melted ice) or air samples with viable microorganisms, the biggest concern lies in the possibility of attached microorganisms and/or virus-type entities on drill cores and rocks. If this were the case, then there would be a considerably lower chance for microbes to escape from the sample container. However, once the samples are in the lab for analysis, then there is a real possibility that a particle of any size that is carbon-based could pose a danger and should remain quarantined until proven to be safe.

The ESF-ESSC Study Group concurs with the conclusions from the NRC reports (1997, 2009) that large-scale effects arising from the intentional return of Mars materials to Earth are primarily those associated with replicating biological entities. However, bearing in mind new knowledge produced over the past years, the Study Group considers that, if there were Earth-like life forms on Mars, virus-type and GTA-type entities’ ability to interact with Earth organisms cannot be ruled out. Based on this, and following the recommendation expressed during the risk perception workshop it oversaw (see Annex 2), **the ESF-ESSC Study Group recommends that not only self-replicating free-living biological entities are considered as potentially having consequences for the Earth’s biosphere but also virus-type and gene transfer agent-type entities.**

The Study Group also concurs with another conclusion from the NRC reports (1997, 2009) that the potential for large-scale effects on the Earth’s biosphere by a returned Mars life form appears to be low, but is not demonstrably zero. It adds that if this risk appears to be low for free-living self-replicating organisms, considering their specificities and replication requirements, **the potential risk posed by virus-type and gene transfer agent-type entities can be considered to be far lower and almost negligible, but still cannot be demonstrated to be zero.**

As a consequence, the ESF-ESSC Study Group recommends that:

- **The release of any particle smaller than 0,01 μm diameter should be considered as acceptable.**



Figure 11. A Mars sample simulant (MSS) is a 10 g sample, intended to simulate the variety of material that could be returned from Mars in terms of shape, hardness and other properties affecting the handling of a sample. Credit: Dr. B. Hofmann, University of Berne

Unsterilised particles smaller than 0,01 μm would be unlikely to contain any organisms, whether free-living self-replicating (the smallest free-living self-replicating microorganisms observed are in the range of 0.12–0,2 μm , i.e. more than one order of magnitude larger), GTA-type (the smallest GTA observed is 0,03 μm , i.e. three times larger) or virus-type (the smallest GTA observed is 0,017 μm , i.e. almost twice as large).

This level should be considered as the bottom line basic requirement when designing the mission systems and operation.

- **The release of particles larger than 0,01 μm but smaller than 0,05 μm can be considered as tolerable if it can be demonstrated that such a range is the best achievable at reasonable cost.** In case the requirement of not releasing a particle larger than 0,01 μm cannot be met, the release of a single unsterilised particle of up to 0.05 μm can be considered as a potentially tolerable systems-level adjustment to achieve the required overall level of assurance (as presented in Chapter 4.5).

In such a case, and because the particle could theoretically contain a virus-type or GTA-type organism, the actual maximum particle size potentially released would have to be independently reviewed by interdisciplinary groups of international experts to determine:

i) whether this size value is the best reasonably achievable at reasonable costs,

IF YES:

ii) taking into consideration the latest scientific developments in the fields of astrobiology, microbiology, virology and any other relevant discipline, whether the release of such a particle can be considered tolerable.

• **Any release of a single unsterilised particle larger than 0,05 μm is not acceptable.**

A dimension of 0,05 μm is less than half of the smallest diameter of any free-living self-replicating microorganism observed (“Pelagibacter ubique” with a diameter of 0.12–0,2 μm but a length of 0.37–0,84 μm). This size is also half of the diameter of the smallest (non-free-living) microorganisms observed. The ESF-ESSC Study group considers that a particle smaller than 0,05 μm would be unlikely to contain a free-living microorganism, but that larger particles may bear such an organism. As self-replicating free-living organisms are likely to be the main concern following a release event, the study group considers that the release of a particle larger than 0,05 μm is not acceptable under any circumstance.

3.7 Perspectives for the future

The recommendation put forward above represents a drastic decrease of the size requirement (from 0,2 μm to 0,01 μm). Besides new knowledge gained in microbiology, the main driver behind this is the consideration given to Mars virus-type and GTA-type entities as potentially impacting the Earth’s biosphere.

Within free-living microorganisms’ machinery, molecules have to be of a certain size to code for particular protein products and that functionality represents a fundamental minimum size threshold.

Based on our current knowledge and techniques (especially genomics), one can assume that if the expected minimum size for viruses, GTAs or free-living microorganisms decreases in the future, and this is indeed possible, it will be at a slower pace than over the past 15 years.

However, no one can disregard the possibility that future discoveries of new agents, entities and mechanisms may shatter our current understanding on minimum size for biological entities. As a consequence, **it is recommended that the size requirement as presented above is reviewed and reconsidered on a regular basis.**

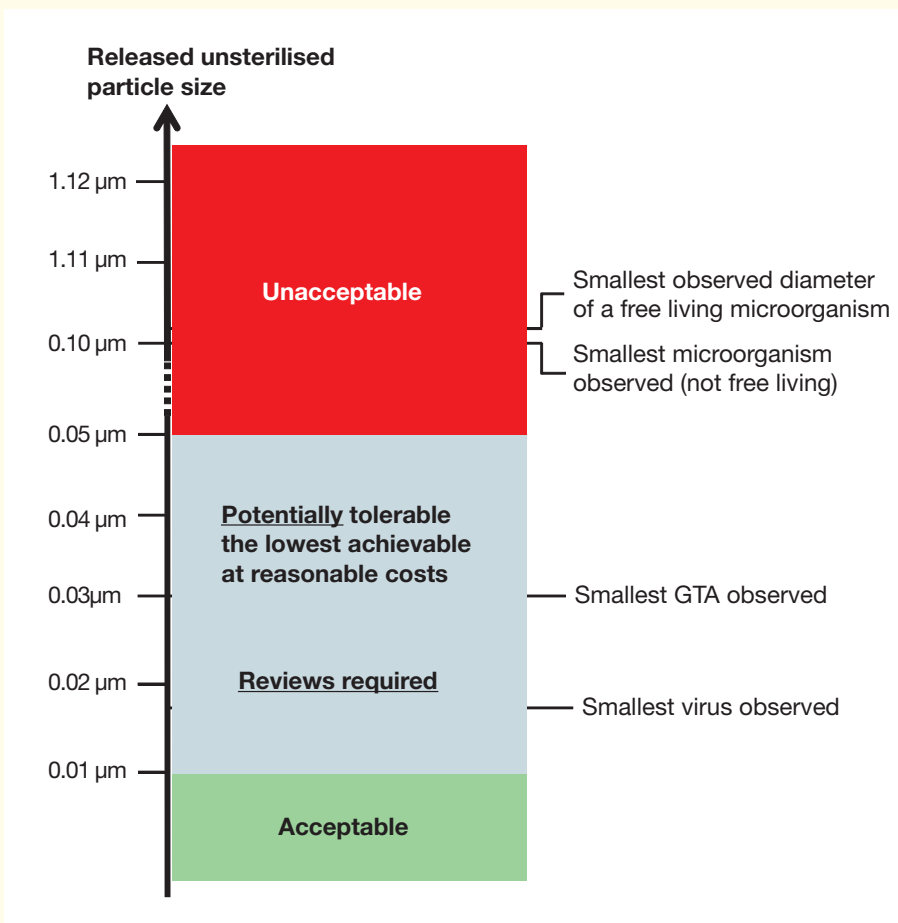


Figure 12. Representation of acceptable, tolerable and unacceptable size range for unsterilised particle released

4. Defining the adequate level of assurance for a non-release



4.1 From risk to level of assurance

It is crucial to have a common understanding of the terms used in the frame of this MSR study. In everyday language, the term ‘risk’ is often loosely used to describe different concepts, being sometimes qualitative (referring to a hazard or damage) or quantitative (referring to probability). To be workable in the current context, the definition of risk requires more accuracy. In 1981, Kaplan and Garrick approached risk as being dependent on three components (Kaplan and Garrick, 1981):

- A (set of) scenario(s) (*i.e. what can go wrong?*)
- A (set of) probability(ies) (*i.e. how likely is it that it will happen?*)
- The consequences resulting from the scenario(s) (*i.e. if it does happen, what are the consequences?*)

The current guideline in the frame of an MSR mission states that “*the probability that a single unsterilised particle of 0.2 micron diameter or greater is released into the Earth environment shall be less than 10^{-6}* ”. It is important to note that this requirement only considers two of the three risk elements as defined above:

- A scenario: the release of an unsterilised particle larger than a given size
- A probability: less than one in a million (10^{-6})

Without dealing with potential consequences, it identifies the event and sets an upper limit for the p variable. In the present document, the probability of not releasing an unsterilised particle variable will be labelled ‘level of assurance’.

It is crucial to consider that for an MSR mission and in the context of the advice provided by this report, **the required level of assurance for not**

releasing an unsterilised particle into the biosphere is not the same as the level of assurance for not contaminating the Earth with a Mars organism (see Chapter 5). The introduction of an unsterilised particle into the Earth’s biosphere could be caused by a breach in containment but also by Mars particles attached to outside surfaces of the spacecraft. The overall level of assurance (covering both scenarios) can be specified because it is theoretically and practically calculable, based on design data and known uncertainties in the environments encountered during the mission. It has to be specified as an acceptably low number because of the theoretical and practical challenges in calculating further probability that an unknown organism from Mars might be present and survive on Earth, especially if not knowing a priori about the organism or the environment in which it may be found.

Focused on the review of the current requirement, the current report does not intend to define an acceptable level of risk, but rather it aims at defining an appropriate level of assurance for preventing the release of an unsterilised particle larger than a given size.

4.2 Approaching the unknown and considering consequences

Known knowns

The third component of Kaplan and Garrick’s definition of risk very much depends on the understanding of how the event, or its main constituent, will interact with its environment. In some cases, consequences are well known and understood and this allows for risk/benefit analyses and informed decisions to be made on the acceptability of a risk



Figure 13. The bacteria *Legionella pneumophila* is an example of a Risk Group 2 pathogen

and/or actions to be taken to reduce this risk. This has been the case with the United Nation Stockholm Convention on Persistent Organic Pollutants that abolishes or strongly constrains the production and use of organic compounds recognised as causing adverse effects on humans and the ecosystem (United Nations, 2001).

Another relevant example is how pathogenic agents are handled depending on their classification. In this instance, the World Health Organisation (WHO) sets a general framework, identifying four groups of pathogens (WHO, 2004). For each group of organism or agent, recommendations are given on adequate laboratory biosafety levels, practices and equipment.

- **Risk Group 1 (no or low individual and community risk):** A microorganism that is unlikely to cause human or animal disease.
- **Risk Group 2 (moderate individual risk, low community risk):** A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
- **Risk Group 3 (high individual risk, low community risk):** A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
- **Risk Group 4 (high individual and community risk):** A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

It is clear that understanding goes with knowledge and that research on mechanisms linking an event to its consequences is required to perform risk/benefit analyses and apply adequate decisions. It is interesting to note that while the negative impact of the insecticide DDT on human health and the ecosystem is acknowledged by the Stockholm Convention, exception (with restriction) is made on its use as a disease vector control agent for malaria. This exception is driven by the fact that, in some cases, the benefits of using DDT are considered to overcome its negative effects.

Known unknowns

Due to the complexity or novelty of some issues, having a sound (or at least adequate) level of knowledge and understanding of what drives potentially negative consequences is sometimes not possible.

This is the case, for example, when considering the impact of electromagnetic fields (EMF) on human health. The total number of mobile phone subscriptions has increased from 962 million in 2001 to 5.4 billion in 2010 (ITU, 2012). While the benefits of mobile phones are well acknowledged (e.g. providing communication services to populations in developing countries without having to build tight and costly cable-based networks), detailed and structured information on the long-term impact of EMF on health have not had time to develop.

In 2011, WHO's International Agency for Research on Cancer (IARC) classified the EMF produced by mobile phones as possibly carcinogenic to humans. While research and assessment are still on-going at the national and international levels, IARC recommends pragmatic measures to reduce exposure such as the use of hands-free devices or texting (WHO-IARC, 2011). Similarly, several national health agencies recommend some limitations in the use of mobile phones for the population in general and children in particular.

The EMF case shows an example of a lack of knowledge about potentially negative effects, but in this case, regulators and scientists know what they do not know. The major unknown here is the potential increased risk for glioma, a type of brain cancer, associated with the use of mobile phones. This clarifies what type of research has to be conducted and the kind of studies that have to be performed.

Unknown unknowns

So far, no evidence of extinct or extant life on Mars has been found, and there is no known 'Mars biology'. Any assumption made on potential Mars organisms can only be speculated on by combining our knowledge of life on Earth (especially extre-



Figure 14. Researchers working in a Biosafety level 4 Laboratory. Credit: Inserm, P. Latron

mophile biology in analogue ecosystems) with our knowledge and understanding of Mars geology and environmental conditions. This lack of knowledge, or uncertainty, prevents definitive conclusions from being reached on major factors that would allow for a real assessment of the risk of contamination posed by an MSR mission, including:

- Whether life exists on Mars or not
- If there are living organisms on Mars, it is not possible to define the probability of a sample (with a given size and mass) actually containing organisms
- If there are living organisms in the sample, it is not possible to definitively assess if (and how) a Mars organism can interact with the Earth's biosphere.

On the latter point, there is consensus among the scientific community (and among the ESF-ESSC Study Group, as presented above) that the release of a Mars organism into the Earth's biosphere is unlikely to have a significant ecological impact or other significant effects. **However, it is important to note that with such a level of uncertainty, it is not possible to estimate a probability that the sample could be harmful or harmless in the classical frequency definition of probability** (i.e. as the limit of a frequency of a collection of experiments). However it is possible to establish the risk as low, as a consensus of the beliefs of the experts in the field as represented by their experience.

Unless future Mars landers and/or rovers discover living organisms on Mars and gather significant information before a Mars sample is returned, knowledge about Mars biology (if any) will have a very steep development curve with an MSR: the sample will land overnight and the scientific investigations will have no or only limited preliminary steps. This differs significantly from, for example, the incremental development of synthetic biology that becomes increasingly complex, building upon past experience and experiments.

While, based on assumptions, some aspects of the release of unsterilised Mars material can be framed in some way, with such a level of uncertainty, unknown (and therefore unexpected) consequences driven by unknown mechanisms are conceivable and by definition are hardly manageable and predictable. In this context, confinement of the sample appears to be the best prevention method. This principle is also applied when an unknown pathogen with a high case fatality rate is isolated: it is assimilated to Risk Group 4 and contained in laboratories with the highest level of confinement until further knowledge about the pathogen allows it to be downgraded to a lower risk group. **Following the same principle, a priori assignment of a Mars sample to Risk Group 4 appears to be the best measure.**

4.3 The Precautionary Principle in the context of MSR

As emphasised above, a clear distinction has to be made between the assurance level of preventing a release and the potential risks resulting from such a release. The risks cannot be definitely evaluated or demonstrated to be low. In the document *Electromagnetic Fields and Public Health – Cautionary Policies* (WHO, 2000), the World Health Organisation defines the Precautionary Principle as “a risk management policy applied in circumstances with a high degree of scientific uncertainty, reflecting the need to take action for a potentially serious risk without awaiting the results of scientific research”.

The UNESCO World Commission on the Ethics of Scientific Knowledge and Technology (COMEST) asserts that the Precautionary Principle should apply when the following conditions are met (UNESCO-COMEST, 2005):

- there exist considerable scientific uncertainties;
- there exist scenarios (or models) of possible harm that are scientifically reasonable (that is based on some scientifically plausible reasoning);
- uncertainties cannot be reduced in the short term without at the same time increasing ignorance of other relevant factors by higher levels of abstraction and idealisation;
- the potential harm is sufficiently serious or even irreversible for present or future generations or otherwise morally unacceptable;
- there is a need to act now, since effective counteraction later will be made significantly more difficult or costly at any later time.

The understanding and application of the Precautionary Principle differs widely depending on the topic considered or the country or region applying it. Stewart (2002) reduced the Precautionary Principle to four basic versions, from the least to the most constraining:

- *Non-preclusion Precautionary Principle*: Scientific uncertainty should not automatically preclude regulation of activities that pose a potential risk of significant harm.
- *Margin of Safety Precautionary Principle*: Regulatory controls should incorporate a margin of safety; activities should be limited below the level at which no adverse effect has been observed or predicted.
- *Best Available Technology Precautionary Principle*: Activities that present an uncertain potential for significant harm should be subject to best technology available requirements to minimise the risk of harm unless the proponent of the activity shows

that they present no appreciable risk of harm.

- *Prohibitory Precautionary Principle*: Activities that present an uncertain potential for significant harm should be prohibited unless the proponent of the activity shows that it presents no appreciable risk of harm.

The *Non-preclusion* Precautionary Principle approach cannot apply in the context of the return of a sample from Mars. Not only can the potential negative impact on the biosphere not be discarded but this programme will draw major attention from the public. It can be expected that the issue of the potential risk posed by the mission will also be raised by influential individuals or groups and neither the public nor policy makers will accept that the programme is implemented without control, monitoring or a regulatory framework.

As mentioned earlier, the consequence of a release cannot be estimated and no previous observations will be available. As a consequence, the *Margin of Safety* Precautionary Principle approach is not justifiable or applicable.

It is not possible to demonstrate that the return of a Mars sample presents no appreciable risk of harm. Therefore, if applied, the *Prohibitory* Precautionary Principle approach would simply lead to the cancellation of the MSR mission.

Based on Stewart’s structure, the only model relevant to apply the Precautionary Principle would be the *Best Available Technology* Precautionary Principle. This approach relates strongly to the Best Available Technique optimisation concept used in some pollutant emission regulations. Justification for the use of this concept and further details are provided in Chapter 4.4 below.

The definition of Precautionary Principle and the associated conditions presented above align perfectly with the potential risks posed by a Mars sample and the **ESF-ESSC Study Group recommends that the Best Available Technology Precautionary Principle is applied when considering the potential release of unsterilised Mars particles.**

4.4 Emission optimisation strategies

The Concept of As Low As Reasonably Achievable (ALARA)

Over the past decades, radiological protection strategies have been based on the concept of optimisation between reducing the doses and the costs associated with these reductions. In its 1990 Recommendations of the International Commission on Radiological

Protection (ICRP, 1991), ICRP describe the optimisation of protection for practice as:

“In relation to any particular source within a practice, the magnitude of individual doses, the number of people exposed, and the likelihood of incurring exposures where these are not certain to be received should all be kept as low as reasonably achievable, economic and social factors being taken into account. This procedure should be constrained by restrictions on the doses to individuals (dose constraints), or the risk to individuals in the case of potential exposures (risk constraints), so as to limit the inequity likely to result from the inherent economic and social judgements.”

This description of the optimisation of protection, introducing the term ALARA, focuses on individual doses and refers to risks assessed using the dose/risk relationship recommended by ICRP. ALARA has proved to be an effective tool for managing human risks after low dose exposures taking into account individual doses, the number of exposed individuals and the likelihood that an exposure situation will occur (NEA-OECD, 2003).

In the context of radiological protection, while the ALARA approach does not mention a minimum level of exposure (it has to be ALARA), optimisation is constrained by an upper limit stating that the dose limit for members of the public is set at 1 mSv per year from all contributing artificial radiation sources (NEA-OECD, 2003).

The ALARA approach is focused on protecting individuals and is based on a well-known dose-effect relationship. In principle, ALARA could be applied to the protection of the environment by adding the environmental detriment aspects to the human health dimension but there are many uncertainties on how radiation impacts on the environment and therefore the full breadth of environmental consequences cannot be estimated.

The concept of Best Available Technique (BAT)

The BAT concept is an optimisation approach aimed at limiting the release of pollutants into the environment. In particular, it is specified in the *OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic* (1992) and is also at the core of the European Union *Integrated Pollution Prevention and Control (IPPC) Directive* (2008).

The IPPC directive defines Best Available Technique as (EU, 2008): *the most effective and advanced stage in the development of activities and their methods of operation which indicate the practical suitability of particular techniques for pro-*

viding in principle the basis for emission limit values designed to prevent and, where that is not practicable, generally to reduce emissions and the impact on the environment as a whole.

- (a) *‘techniques’ shall include both the technology used and the way in which the installation is designed, built, maintained, operated and decommissioned;*
- (b) *‘available techniques’ means those developed on a scale which allows implementation in the relevant industrial sector, under economically and technically viable conditions, taking into consideration the costs and advantages, whether or not the techniques are used or produced inside the Member State in question, as long as they are reasonably accessible to the operator;*
- (c) *‘best’ means most effective in achieving a high general level of protection of the environment as a whole.*

The focus of BAT is more on methods that will eliminate or reduce the input of hazardous waste into the environment rather than determining its assimilative capacities (Moberg *et al.*, 2004).

ALARA and BAT comparison

It has been noted that there are obvious similarities between ALARA and BAT. Both strategies consider science, technology and economics to optimise protection of man and the environment. They both consider that arbitration has to be made on level of emission and cost associated with reaching or improving that level. However, BAT has a more far-ranging application under conditions where detriment (either biological, societal or economical) is difficult to assess (Moberg *et al.*, 2004), whereas ALARA covers situations where all components of risk (including consequences) are known.

ALARA is impact-oriented and focuses on human health through well-defined dose-effect relationships. By considering the release at its source, BAT allows the integration of consequences that are beyond those borne only by individuals and also to limit the impact on the environment as a whole, including non-human species. Another difference lies in the fact that ALARA considers all sources potentially affecting individuals, while BAT focuses on a single source of release (NEA-OECD, 2003).

Nevertheless, both approaches tend towards the same objectives and it can be assumed that applying BAT to all sources of release would allow ALARA to be achieved for the environment and not only for humans.

It appears clear that BAT is a suitable

approach to be considered in the frame of a Mars Sample Return mission and adapted to the specificities of the systems and operations involved. This would allow mobilising the use of the best technologies and operational concepts in order to minimise the probability of an unintended release and the magnitude of this release.

However, while BAT only implies that available techniques (at a reasonable cost) are used, it seems important to set a limit to define and recommend adequate requirements for the release probability and magnitude. Should these requirements not be achievable with available technology, new technologies would have to be developed to meet them.

4.5 Quantitative risk levels used by regulators

4.5.1 The use of 'one in a million'

Many hazardous substances are present in daily life; in the environment, in houses, in water and food, etc. This is also the case for exposure to potentially harmful radiation when going through airport security, during medical examination or even sun tanning. Regulatory authorities have to perform risk/benefit analyses prior to authorising and framing the use of various substances or procedures. These decisions have to be based on levels of risk considered acceptable or tolerable.

When investigating what is considered to be an acceptable – or tolerable – level of risk, one often comes across the figure 'one in a million' or 10^{-6} . This value originates from the concept of *de minimus* risk contained in a 1973 notice in the US Federal register, where *de minimus* risk is considered to be 'essentially zero' and therefore below which no further regulatory action is required (Kelly, 1991). Following this, and apparently without wide expert consultation on the relevance of this value and its application, 'one in a million' was set by FDA as the being 'maximum lifetime risk that is essentially zero' (Kelly, 1991).

From there, the concept of 'one in a million' spread beyond the United States as being a standard when defining thresholds above which adverse effects are not considered tolerable. It is primarily applied when considering the risk of an individual to adverse health effects due to exposure to chemicals, toxic waste or radiation.

Several examples of the use of 'one in a million' can be found in various regulations, legislations and institutional guidelines from around the world.

The values from these examples only provide risk

levels at the individual level, i.e. defining the acceptability for negative consequences borne by only one individual. While all pointing towards the same level, in some cases (e.g. ECHA and EPA) higher levels of risk can also be considered as acceptable.

It is important to note that while the figure used (10^{-6}) is the same in most of the cases above, its significance and overall value varies considerably. It varies on the seriousness of the consequences, from the risk of developing a cancer (e.g. US EPA Superfund of ECHA guidelines), to the risk of death (e.g. Australian EPA guidance or UK HSE). Interestingly, the DALY approach allows integration of the full scope of seriousness.

More importantly, the use of the 10^{-6} value varies depending on the timeframe it covers: it can be either a lifetime risk (especially in the US) or an annual risk (as in the UK or Australia). Therefore, the HSE guidance of an individual risk of death of 10^{-6} per annum is hardly comparable to the FDA regulation of 10^{-6} lifetime risk of cancer.

While it is almost impossible to find a justification for it, it appears that the 10^{-6} value has been accepted and is now considered by regulators as being the 'gold standard' to be met to demonstrate excellence in risk management (Kelly, 1991).

4.5.2 Approaching events with unknown consequences

The values given in Table 3 were based on the ability to clearly define and quantify negative consequences for an individual exposed to a specific hazard (in most cases, the risk of developing a cancer). However, as already stated, in the case of the MSR mission, potential consequences of a release cannot be determined.

All over the world, populations face hazards that are not attributable to human intervention; these include for instance: natural hazards (earthquakes,



Figure 15. Tsunami Hazard Zone sign. Credit: NOAA

Table 3. Use of ‘one in a million’ in various regulatory frameworks

Organisation	Scope	Risk	Limit of acceptability
US Environmental Protection Agency (EPA)	‘Superfund’ programme to clean up uncontrolled hazardous waste sites.	Lifetime cancer risk to an individual exposed to a cleaned-up site	10 ⁻⁴ to 10 ⁻⁶
US Food and Drug Administration (FDA)	Food produced from animals exposed to carcinogenic compounds	Lifetime cancer risk to an individual eating food from animal exposed to carcinogenic compounds	10 ⁻⁶
European Chemical Agency (ECHA)	Exposure to chemicals	Lifetime cancer risk levels to an individual exposed to chemicals	10 ⁻⁵ to 10 ⁻⁶
EU Air Quality Directive	Exposure to carcinogenic compounds	Lifetime cancer risk levels to an individual exposed to carcinogenic compounds	10 ⁻⁶
EU Drinking Water Directive	Exposure to carcinogenic compounds	Lifetime cancer risk levels to an individual exposed to carcinogenic compounds	10 ⁻⁶
World Health Organisation (WHO)	Drinking Water Quality	Annual individual DALY (disability-adjusted life year) – provides a way to approach overall disease burden on people by compiling various effects (degree of illness to death) resulting from a single source	10 ⁻⁶
UK Health and Safety Executive (HSE)	General guidance for setting a limit between unacceptable and tolerable risk	Annual individual risk of dying	10 ⁻⁶
Australia Environment Protection Authority	Offsite Individual Risk from Hazardous Industrial Plant	Annual individual risk of dying as a result of an industrial accident	10 ⁻⁶
International Standards Organisation (ISO)	Reliability for structures (buildings, bridges...)	Annual individual risk of dying as a result of the collapse of a structure	10 ⁻⁶

tsunamis, floods and volcanic eruptions), asteroid impact or the spread of diseases. While mitigation strategies can be implemented, these events cannot be controlled and are often hardly predictable. However, probabilities can be associated to their occurrence, for example:

- The Calaveras Fault in the East Bay, and the San Gregorio Fault along the San Francisco Peninsula coast, have probabilities of 7% and 6%, respectively, of producing a magnitude 6.7 or greater earthquake in the next 30 years (USGS, 2008)
- Average interval between Tunguska-class asteroid impacts for total Earth: 300 years, for populated area: 3,000 years, for urban areas: 100,000 years (Morrison, 1992)
- The average span between global influenza pandemics is 27 years (Stafford, 2005)

By definition, events attributable to human activity are potentially avoidable (at least by not performing these activities). **The general public’s level of acceptance to these man-induced events is much lower than for natural events; as a consequence, regulatory authorities tend to impose assurance levels that are much tighter than the probability of occurrence of natural events.**

Some example of regulatory guidelines approaching this kind of event whose consequences cannot be accurately defined can be found: the sterility assurance level in the pharmaceutical and medical

industry, the reliability of aircraft and the reliability of nuclear facilities. **As for the MSR release case, these examples should not be considered as probabilities that certain consequences will happen but rather that events will happen that would allow consequences to materialise.**

Sterility Assurance Level (SAL)

Sterilisation (see Chapter 1.3 for the various methods) is key in medicine, surgery and drug production. The process aims to destroy all microorganisms on the surface of an object or in a substance (e.g. a liquid) in order to avoid transmission of disease associated with the use of that item. While it is hardly possible to demonstrate that an item is totally sterile, or to achieve total sterilisation, some indicators have been devised to quantify the efficiency level of sterilisation processes and define the required level depending on the use of sterilised items.

Sterility assurance level (SAL) refers to the probability of a viable microorganism being present on an object after sterilisation. SAL is normally expressed a 10⁻ⁿ; a SAL of 10⁻⁶ means that there is one chance out of one million that a single viable microorganism is present on an item that has undergone sterilisation process. The US Center for Disease Control (CDC) Guideline for Disinfection and Sterilisation in Healthcare Facilities (2008) states that “a SAL of 10⁻⁶ generally is accepted as appropriate

for items intended to contact compromised tissue (i.e. tissue that has lost the integrity of the natural body barriers)”. It further states that “the choice of a 10^{-6} SAL was strictly arbitrary and not associated with any adverse outcomes (e.g. patient infections)”. This level actually follows the US Pharmacopeial convention’s guideline for sterilisation (USP, 2011): “The process must result in a biologically verified lethality sufficient to achieve a probability of obtaining a non-sterile unit that is less than one in a million”.

A SAL level of 10^{-6} for most critical medical and surgical items and injectable drugs is a standard that is also applied in Europe by the European Pharmacopeia (2011) and the Council of Europe’s guide to safety and quality assurance for organs, tissues and cells (2004).

At the international level, the Pharmaceutical Inspection Convention and the Pharmaceutical Inspection Co-operation scheme (PIC/S) expressed the following recommendation (PIC/S, 2007): “Although “sterility” is an absolute term, the assurance that any given item is sterile is a probability function, commonly expressed as a negative power to the base ten. The minimum acceptable Sterility Assurance Level (SAL) for terminally sterilised drugs is generally based on the probability of a non-sterile unit of 10^{-6} ”.

The SAL concept is applied for the most critical pharmaceutical and medical products without discriminating between pathogenic and harmless organisms. A SAL recommended level of ‘one in a million’ is similar to the individual tolerable risks levels presented in Table 3, with the main difference being that it frames the sterilisation process without knowing the impact that an unsterilised unit would have on an individual or the population.

Civil Aviation

Airworthiness certification processes in Europe and the United States require that some specific safety targets are met in order to consider a commercial transport aircraft to be airworthy. In its airworthiness regulation for large transport aircraft, the European Aviation Safety Agency (EASA) identifies five levels of severity of system failure condition (EASA, 2006):

- No effect on operational capabilities or safety
- Minor failure condition: slight reduction in functional capabilities or safety margins
- Major failure condition: significant reduction in functional capabilities or safety margins
- Hazardous failure condition: large reduction in functional capabilities or safety margins
- Catastrophic failure condition: normally with hull loss

Hazardous failure conditions are expected to imply

serious or fatal injury to a small number of passengers or cabin crew while catastrophic failure conditions are expected to result in multiple fatalities.

Probability targets have been set for each failure condition. For the condition of catastrophic failure, EASA states:

“In assessing the acceptability of a design it was recognised that rational probability values would have to be established. Historical evidence indicated that the probability of a serious accident due to operational and airframe-related causes was approximately one per million hours of flight. Furthermore, about 10 per cent of the total were attributed to Failure Conditions caused by the aeroplane’s systems. It seems reasonable that serious accidents caused by systems should not be allowed a higher probability than this in new aeroplane designs. It is reasonable to expect that the probability of a serious accident from all such Failure Conditions be not greater than one per ten million flight hours or 1×10^{-7} per flight hour for a newly designed aeroplane. The difficulty with this is that it is not possible to say whether the target has been met until all the systems on the aeroplane are collectively analysed numerically. For this reason it was assumed, arbitrarily, that there are about one hundred potential Failure Conditions in an aeroplane, which could be Catastrophic. The target allowable Average Probability per Flight Hour of 1×10^{-7} was thus apportioned equally among these Failure Conditions, resulting in an allocation of not greater than 1×10^{-9} to each. The upper limit for the Average Probability per Flight Hour for Catastrophic Failure Conditions would be 1×10^{-9} , which establishes an approximate probability value for the term “Extremely Improbable”.

In the United States, the FAA uses the same values (FAA, 1988), with the same approach (Azevedo, 2008).

Going upstream this approach, it appears clear that the regulatory safety targets for large aircraft are set in order to meet an overall probability of 10^{-6} catastrophic event per flight hour (including from non-system operational causes) and that this probability is based on historical data. The consequences of such a catastrophic event cannot be precisely defined but would result in fatalities (both for passengers and on the ground) ranging from a couple to several hundreds.

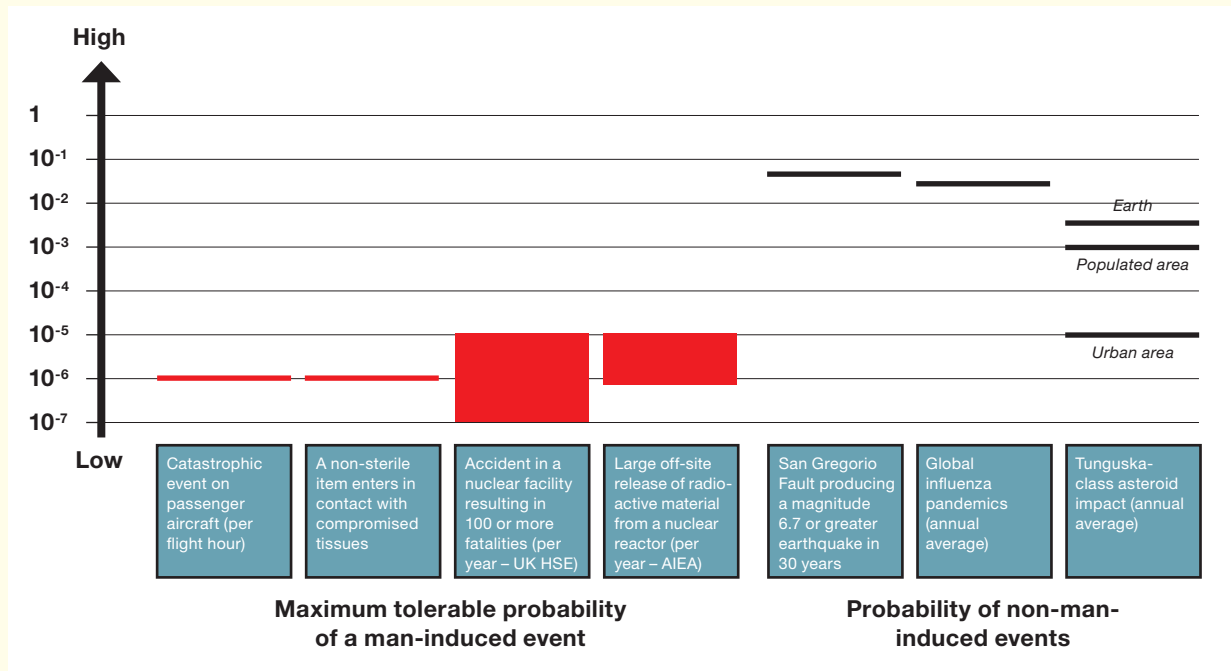


Figure 16. Maximum tolerable probabilities for some man-induced events and probabilities of some non-man-induced events

Nuclear Safety

The two worst events considered by operators and regulators of nuclear power plants are core damage and large release events. Core damage refers to an event leading the nuclear fuel becoming spoiled, possibly resulting in core meltdown. Large release events occur when, following core damage, the reactor containment is breached and radioactivity is released into the environment.

Following the recommendation from its International Nuclear Safety Group (INSAG), the International Atomic Energy Agency (IAEA) adopted the following safety targets (IAEA, 2001):

For Core Damage Frequency:

- 10⁻⁴ per reactor-year for existing plants,
- 10⁻⁵ per reactor-year for future plants.

For large radioactive release:

- 10⁻⁵ per reactor-year for existing plants,
- 10⁻⁶ per reactor-year for future plants.

This document further defines *large off-site release of radioactive material*: as being “A large release of radioactive material, which would have severe implications for society and would require the offsite emergency arrangements to be implemented.” It states “such off-site release can be specified in a number of ways including the following:

- As absolute quantities (in Bq) of the most significant nuclides released,
- As a fraction of the inventory of the core,
- As a specified dose to the most exposed person off the site,
- As a release giving “unacceptable consequences.”

Finally, IAEA mentions that although there is no consensus on what constitutes a large off-site release, numerical criteria similar to large radioactive release targets have been specified in a number of countries.

In the United Kingdom, the UK Health and Safety Executive (UK HSE) derives a number of Safety Assessment Principles (SAPs) in the area of nuclear facilities. Those SAPs have been benchmarked against the IAEA Safety Standards. When considering an accident in a nuclear facility, UK HSE specifies that (UK-HSE, 2006):

Target 9: *The targets for the total risk of 100 or more fatalities, either immediate or eventual, from on-site accidents that result in exposure to ionising radiation, are:*

- Basic Safety Level (BSL): 1x10⁻⁵ per year
- Basic Safety Objective (BSO): 1x10⁻⁷ per year

UK HSE defines BSL as the higher limit for tolerable risk (any larger risk is considered unacceptable) and BSO as the limit below which risk can be considered as broadly acceptable. In between these two values is the ‘tolerable region’ for which the As low As Reasonably Practicable (ALARP) principle has to be demonstrated. For the purposes of radiation protection legislation, ALARP and ALARA can be regarded as essentially the same in terms of requirements (SNIFFER, 2005).

The values discussed above are gathered in Figure 16.

4.6 Updating the appropriate level of assurance

Along with the draft determination to contain a particle of a certain size, the original categorisation letter for the MSR mission (Rummel, 1999) specified a level of assurance for containment of $<10^{-6}$ that a particle would be released into the Earth's biosphere. The intention of specifying that level of assurance was both to begin at a level that has had widespread public and governmental acceptance, and that would be stringent enough to drive out mission design and cost issues to help understand the risk–benefit calculation associated with the decision to bring a sample back from Mars.

From the review of the current guidelines and regulations applied worldwide and in line with the positions adopted at the international level and the recommendations expressed during the risk perception workshop it oversaw, the ESF-ESSC Study Group considers that the current assurance level (lower than one in a million) for the release of a potentially hazardous unsterilised Mars particle is appropriate and should be kept.

It has to be highlighted that the level of assurance is not equivalent to an acceptable risk, but

rather the level of assurance only provides the maximum probability of the (unknown) potential risk. This value has to be understood as representing a reduction factor to the (undetermined) risk posed by the potentially hazardous nature of the sample.

The case of the NASA-ESA Ulysses mission provides a good illustration of such a reduction factor. The Ulysses spacecraft, launched in October 1990 by the Space Shuttle Discovery, was a scientific probe aimed at studying the sun and the solar system. Ulysses was powered by a radioisotope thermoelectric generator (RTG) fuelled by Plutonium 238. The potential release of this highly radioactive material following an accident or an uncontrolled re-entry raised some concerns among the general public, space agencies and governments. A survey of the risk was performed by the Interagency Nuclear Safety Review Panel (INSRP). This review concluded (Sholtis *et al.*, 1991) that the highest calculated added individual risk associated with the Ulysses mission increased lifetime cancer risk to no more than 0.00015% (1.5×10^{-6}). However, to be real, this risk required fuel to be accidentally released in the environment. If one considers that the likelihood of an accidental release that results in fatal cancer was less than 1 in 100,000, the actual added

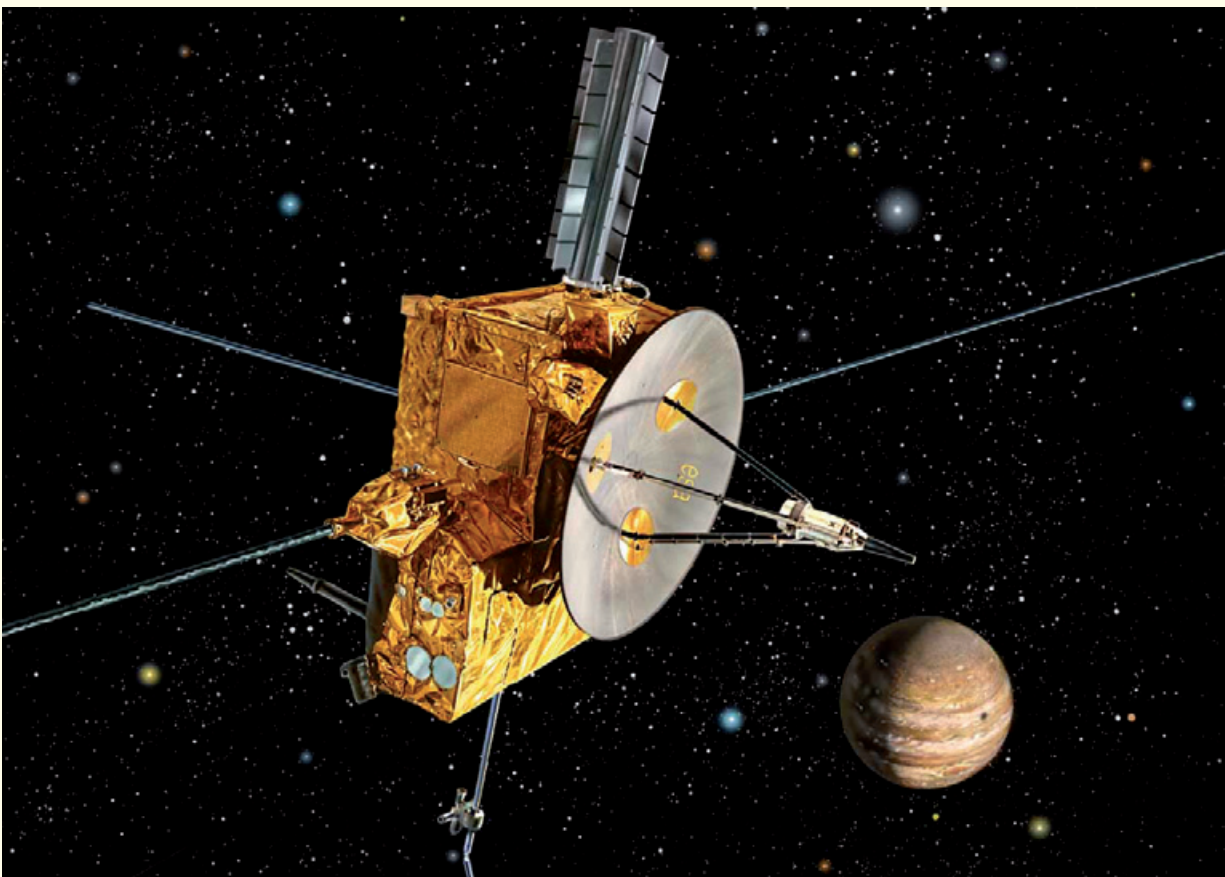


Figure 17. An artist's impression of the Ulysses spacecraft. Credit: NASA/ESA

risk of fatal cancer associated with the Ulysses mission was smaller than 0.00015% by five orders of magnitude and turned out to be 1.5×10^{-11} .

4.7 Potential verification methods

Verification of sterility of the surfaces of spacecraft elements that come into contact with the Earth's biosphere, either upon return from the Mars surface or due to re-contact at some later date, is difficult. Sensory indications are often not reliable enough to be consistent with the 10^{-6} requirement. Because of this, only indirect methods of verification might be used. These include exclusion of potential particles from the spacecraft surfaces in the first place, sterilisation of the surfaces at some point prior to re-entry into the Earth's biosphere by direct or indirect means such as re-entry heating, and ensuring that surfaces are not contaminated after sterilisation via leakage.

Initial sealing of the Mars sample can be assured to a high level of reliability via the use of a proven container concept along with a sealing concept that has been shown to be reliable to first order with a sensory back up system, utilising outgassing for example. Although sensory systems are limited, as previously mentioned, the combined low risk of failing to create a seal in the first place and the additional conditional probability of detection using leak detection sensors, should be able to provide the required level of assurance that the sample is encased within the magazine consistent with the risk of release requirement.

However, it is possible that the sample magazine could be penetrated by a micrometeoroid during transit from Mars, thereby causing exterior contamination and release upon entry. While sensory systems that detect leakage might be limited in risk protection, potential sensory systems that would detect any penetration of the Earth Return Vehicle to a high level of reliability should be feasible.

Upon return to Earth, the sample would still have to be transported from the landing site to the curation facility. While the Study Group was not tasked with considering human factors, it has to be highlighted that the use of human handling in this process and the transport itself entails the risk of human error and the potential for accidental release. For this reason, care must be taken to minimise human interaction with the sample and to provide adequate protection via transport containment to guard against an accident during transport to the curation facility.

5.

From release to risk: a framework to approach the consequences



5.1 The sequence of events leading to environmental consequences

Returned samples from Mars may contain two potential hazards: viable biological entities (including virus-types), and/or a highly toxic chemical(s). In the case of a toxic chemical, the risk would be confined to a very limited area, whereas in principle, a viable life form could pose a much wider risk.

For a significant environmental risk to arise from a viable life form, the following sequence of events is necessary (see also Figure 19):

1. Release due to loss of containment or ineffective spacecraft surface sterilisation
2. Survival in an environment very different from Mars
3. Replication, either in the external environment or following intake by Earth organism(s)
4. Dispersal/transfer
- 5a. Pathogenicity of the replicating species
AND/OR
- 5b. Displacement or outcompeting of terrestrial species, disturbance/breakdown of terrestrial ecosystems

At some point in this event chain, one or more Earth organisms must be exposed. This could be at any stage from 2 to 5 above. Thus, the location of release is an important consideration. In principle, the greatest impact (if any) would be if the location of exposure was a breeding site for the key species, and the least impact would be in a desert-like environment.

Release due to failure of containment or of the spacecraft surface sterilisation

For there to be a risk, as opposed to a hazard, there must exist an event that would initiate an exposure of the environment to the components of the Mars sample. In principle, there are four main ways for an environmental exposure to be initiated from the accidental/deliberate release a Mars sample into the Earth's biosphere:

- A break-up of the container during atmospheric entry (due to a design fault or sabotage),
- An unsuccessful full sterilisation of the Earth Entry Capsule, potentially having Mars particles attached to its outside surfaces,
- Damage to the vehicle due to heavy impact with the Earth,
- Escape of material during transport or from the laboratory.

In the first and (possibly) second cases, there is potential for contamination over a quite wide area (especially if the capsule breaks up at high altitude). However, the sample will be small (the quantity of unsterilised particles even smaller) and therefore deposition per unit area will be very low.

In the two latter cases, the release would be a point source. Based on failure of containment of pathogenic material in the past, it is reasonable to assume that the most likely cause of a release would be due to human error or a deliberate human act following the introduction of the material into the laboratory.



Figure 18. Acidophilic microorganisms thrive in the acidic waters of Rio Tinto, Spain. Credit: F. Perez, CAB

Survival in an environment very different from Mars

Due great differences in the environmental conditions of Mars and Earth, it is very likely that any life form existing on Mars would have great difficulty surviving under Earth conditions. However, the possibility must be considered that a Mars life form that is not adversely affected by Earth's environment could be present in a returned sample. It is evident that biological organisms can survive in extreme physical and chemical conditions on Earth (extremophiles), and there is a considerable body of information on how they have adapted to survive under these conditions.

Replication either in the external environment or following intake by Earth organism(s)

Depending on its ability to cope with a new set of physico-chemical environmental conditions, a surviving Mars organism could undergo replication in the external environment or following uptake by an Earth species.

Survival and ability to replicate would allow the Mars organism to colonise the Earth's biosphere. These organisms would potentially disturb the functioning and equilibrium of the ecosystem they settle in, by, for example, competing for resources or representing new resources, and thereby have an indirect consequence on Earth species.

Dispersal/transfer

In the case of environmental pathogens on Earth, dispersal/transfer may occur via physical forces, e.g. wind and water, or via biological organisms. Dispersal via wind and water inevitably involves considerable dilution of the organism concentration and therefore the most effective mode is generally by transfer through close contact between organisms.

Pathogenicity of the replicating species

The potential pathogenicity of a potential living, replicating and dispersed Mars organism is unknown. If one wants to approach the issue using Earth-based examples, Table 4 lists a few incidents that have taken place and have some parallels with scenarios set out above. **It is very important to note that these examples have not been chosen due to any potential of such organisms being present in the Mars sample, but rather to reflect potential scenarios.**

The prospect of pathogenicity arising within Earth species is anticipated to be greatest if the Mars life form has a similar biochemistry to that of life on Earth. If pathogenicity does occur, it will be experienced in Earth organisms deemed vulnerable or susceptible species. This raises the question of what is the potential for the Mars life form to come in contact with the vulnerable species as a consequence of the chain of events.

Table 4. Examples of major environmental impacts from a local release of an Earth pathogen

Agent	Likely causal agent	Nature of incident
Economic		
BSE	Prion	From 1989, thousands of cattle affected, millions of cattle sacrificed
Foot and mouth disease	Aphtho virus	2001, UK: claimed waste food from a restaurant was responsible ultimately for a few thousand animals with the disease and many thousands sacrificed
Potato blight	<i>Phytophthora infestans</i>	1845, Ireland: 1.5 million humans died due to starvation, a further 1.5 million emigrated
Ecological		
Dutch elm disease	Ophiostoma	10 million elm trees lost in the UK alone
Myxomatosis	Myxoma virus	1952: release on a single estate in France resulted in the deaths of 90% of the wild rabbits in two years

Table 5. Criteria for minor and major environmental accidents

Category	Least likely to have a significant environmental impact	Most likely to have a significant environmental impact
Identification	Early detection	Very delayed detection
Quantity of life form over time	Very slow or no replication, therefore only very small quantities available	Efficient replication, leading to ever increasing amounts over time
Transfer	Limited or no spread from original contamination site	Effective spread by one or more vectors
Pathogenic potency of the life form/ effects	Few species affected, minor and reversible effects	Several species or a particularly vulnerable species affected, delayed, serious effects
Impacts <i>Note: It is very difficult to define which organisms are and will be economically important – it is even more difficult define which species are not ecologically important.</i>	Affected species are not economically or ecologically important	Affected species are economically and/ or ecologically important

5.2 Estimate of the overall risk

A clear distinction needs to be made between an adverse event (i.e. a hazard) and one that causes an impact sufficient enough to overwhelm the response capacity of a local community or region using only its own resources (Jha, 2010). Overall, in regard to environmental risks as opposed to direct human risks, it is possible to identify the most and least likely events that could lead to a major environmental impact. These are outlined in Table 5.

There is a no adequate basis to identify which scenarios are most likely to occur. Based on the understanding of pathogenic organisms on Earth, it might be anticipated that the scenarios least likely to have a significant impact on the biosphere are the most realistic ones, but the justification for this conclusion is weakly based.

Inevitably, there are great uncertainties within each step of the event chain (see Figure 19) and to

represent a significant risk, some conditions will have to be met. However, as discussed in Chapter 2.3 and stated in Chapter 3.6, the overall risk posed by returning a dangerous biological entity from Mars is quite low, not even considering the reduction factor of one in a million recommended in Chapter 4.5.

5.3 Direct consequences for human health

Specific cases of environmental consequences are those borne by the population. Human pathogens must be able to grow and replicate at 37°C in order to colonise and cause pathogenesis. Mars, on the other hand, is a cold planet that seldom experiences temperatures greater than 20°C. Human pathogens have co-evolved to avoid many of the body’s defence mechanisms, or, in some cases, to hijack them, caus-

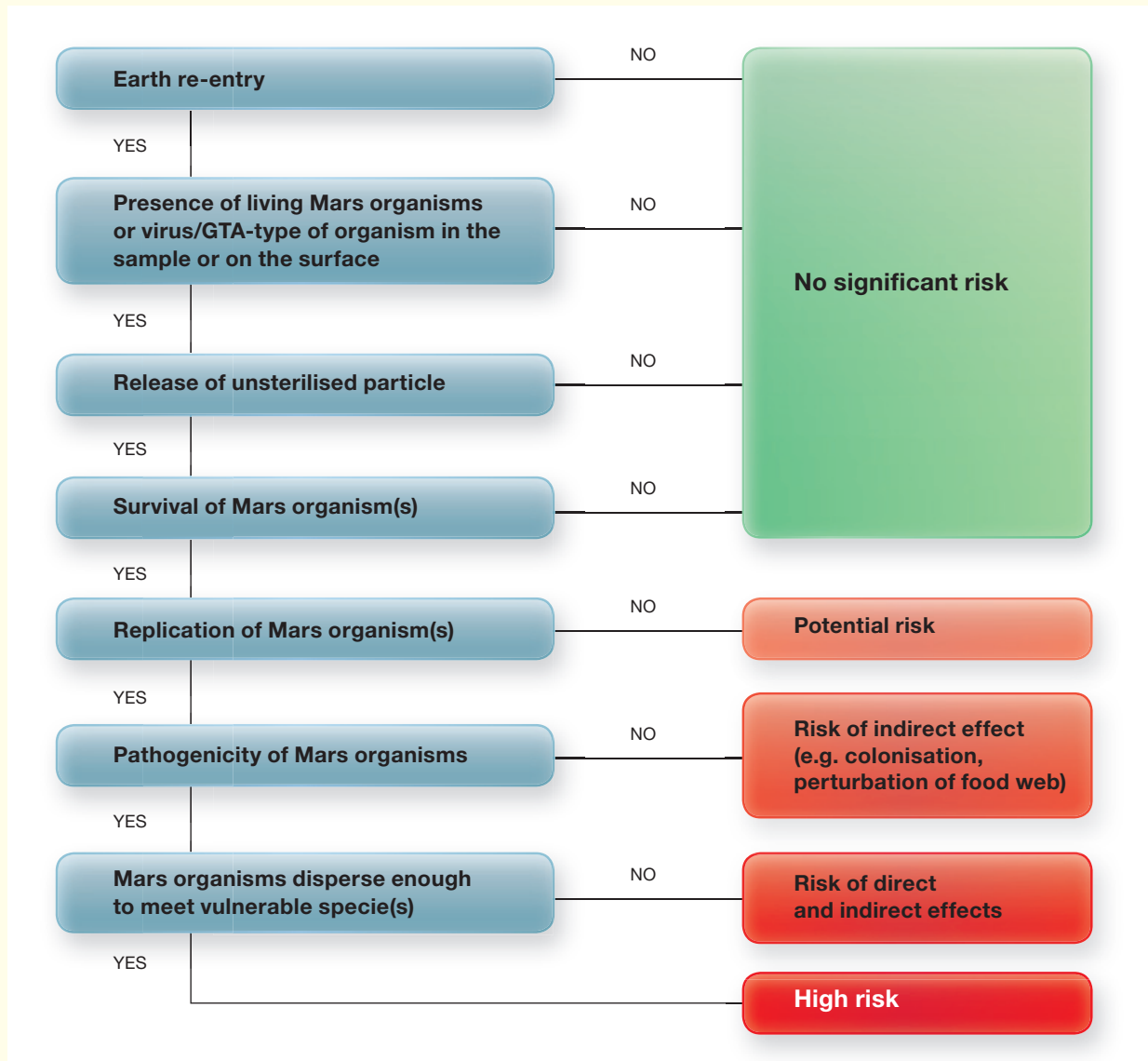


Figure 19. Flowchart representation of the event chain necessary for substantial environmental consequences

ing disease and death. Most human pathogens also have another animal or protozoan reservoir, which is not available on Mars. It is very rare for environmental microorganisms without another animal host to cause disease, and such diseases have high infectious doses and are unable to be transmitted from person-to-person. These factors make it exceedingly unlikely that any microorganism capable of causing human disease could originate from Mars.

Modelling a release and its consequences

There will be two factors in any risk assessment of an MSR mission: the likelihood that there will be a release of sample during the return and/or handling of the sample, and the consequence of such a release. While the risk of a release can be calculated semi-quantitatively from engineering data or by using performance data from high containment microbiology laboratories, it is difficult to predict

consequences in an accurate fashion. In order for a consequence value to be reached, a number of assumptions must be made about the potential presence of life in the sample and on its potential worst case consequence.

In recent years, great progress has been made in mathematical modelling of epidemics of infectious diseases. These models have been developed to measure the effectiveness of interventions used to reduce the spread of infection and to measure the cost benefit. The two areas of greatest interest have been the release of bioterrorism agents and the spread of an emerging disease. To populate the models, a series of assumptions need to be made about the disease and how it spreads. These assumptions are built on evidence obtained from the scientific literature and from epidemiological investigations. These assumptions are then expressed in numerical terms, e.g. a case fatality rate of 50%, and as statistical functions. These models are normally developed

Table 6. Consequences of secondary transmission following undetected/unreported initial infection as modelled in the frame of NIH-BRP (2012)

		Percentage of simulations in which a consequence occurred			
		SARS-associated coronavirus	1918 H1N1 Virus	Ebola Virus	Yersinia pestis
	Number of Simulation Performed	500,000	100,000	100,000	100,000
Consequence					
Number of public infections	1 or more	38%	62%	62%	57%
	10 or more	21%	40%	18%	17%
	100 or more	8.8%	28%	0.03%	<0.001%
	1,000 or more	0.2%	4%	N/A	N/A
	10,000 or more	<0.0002%	<0.001%	N/A	N/A
Number of public fatalities	1 or more	24%	36%	56%	52%
	10 or more	9.1%	5.6%	12%	3.5%
	100 or more	0.3%	0.02%	<0.001%	<0.001%
	1,000 or more	<0.0002%	<0.001%	N/A	N/A

not only to give an idea of the number of possible casualties, but to assess the efficacy of various interventions. A list of assumptions commonly used in models is given below:

- Susceptibility of population
- Incubation period
- Transmissibility from person to person/number of secondary cases per infected individual
- Time window of transmission
- Case fatality rate
- Hospitalisation of patients – hospitals can increase transmission and amplify outbreaks
- Transmission routes (aerosol, ingestion, etc.)
- Survival/reproduction in the environment
- Can it infect animals/plants
- Duration of the symptomatic phase

Due to our lack of knowledge on potential Mars pathogens, it is impossible to answer any of the points listed above and therefore to model the consequence of the potential release of a Mars organism.

If one wants to approach the issue using known transmissible highly pathogenic organisms as a benchmark, the draft Risk Assessment report for the Boston University National Emerging Infectious Diseases Laboratories (NIH-BRP, 2012) provides up-to-date estimates concerning the consequences of an undetected/unreported initial infection leading to secondary transmissions. Table 6 provides these estimates for some Risk Group 3 and 4 pathogens:

- The SARS-associated coronavirus
- The 1918 H1N1 Virus – responsible of the Spanish flu pandemic of 1918

- The Ebola virus
- The *Yersinia pestis* bacterium – responsible for the bubonic plague

The figures presented in Table 6 give the estimated chances of some defined consequences (number of public infections, number of public fatalities) based on the number of simulations in which consequences occurred (out of several tens of thousands of simulations performed).

It is very important to note that these pathogenic agents have not been chosen due to any potential of such organisms being present in the Mars sample, but rather to reflect potential worst case scenarios. It is also very important to note that the results given in Table 6 are specific to the demographic and societal characteristics of the Boston urban area and they incorporate the positive effect of instituting mitigating procedures such as vaccines, drugs, and isolation.

These results suggest that, under the base case assumptions used in the model, a laboratory worker or any individual infected with SARS-CoV who enters the public would have about a 38% chance of transmitting infection to at least one contact. There is an estimated 8.8% chance that an outbreak would grow to 100 total cases, and a 0.2% chance of 1,000 total cases. The estimated 10% case fatality for SARS-CoV leads to an estimate of a less than a 10% chance of 10 or more total fatalities occurring.

When considering these figures, it is key to keep in mind that they reflect the consequences of an initial infection, they do not consider the probability that one individual actually gets infected following

exposure. This probability will strongly depend on the quantity of pathogen one is exposed to and the method of dispersal (e.g. if the pathogen has been aerosolised or not).

5.4 Being prepared

As it is not possible to definitively exclude a release scenario having a consequence on the biosphere, it is crucial to work on the definition and development of potential scenarios, addressing the question “What might happen in case of an unintended release?”. These scenarios should be scientifically sound with uncertainties well defined, acknowledged and regularly updated.

While it is a clear prerequisite, containment at the highest level is not sufficient alone. Even if significant know-how and operational experience in handling and managing highly pathogenic organisms has been gained over the past decades, coping with a breach in containment potentially leading to the release of unsterilised material from the sample is another issue. One of the key strategies in reacting to the release of a biological agent is rapidly and effectively detecting any consequences of such a release and responding effectively. In this context, experience gained in public health domains and emergency response preparation is highly relevant.

From a crisis management position, if the effects of the release are unusual and of rapid onset, it is most likely that the causative agent will be identified fairly quickly and actions can be taken to try to limit the spread. However, if the onset is slow and the effects are not unusual, significant spread may occur before the nature of the threat is realised.

If the Mars life form has an unknown, fundamentally different biochemistry to life forms on Earth but nonetheless is able to cause adverse consequences in the Earth’s biosphere, this must be considered to be an extreme worst case scenario. If this were to be the case, a major rethink of the applicability of current strategies for dealing with pathogens would be required. Some of the questions that could arise include:

- Can it be assumed that the consequences are limited to one or a few species?
- Are currently available biocides effective?
- How can the presence of the life form(s) be detected?

Building capacity to respond to a release of Mars material is of utmost importance and should benefit from available experience in the fields of public health and emergency response. In addition to current prevention strategies, it is recommended that potential release scenarios (including undetected release) are clearly defined and investigated, and that response strategies are developed from these.

The relevance and significance of the scenarios developed as well as of their associated monitoring and response strategies will be key elements in optimising the efficiency of mitigation, containment or limitation of potential consequences. Therefore, the appropriate level of means and expertise will be required in their development and they will have to be reviewed, reassessed and updated on a regular basis.

It is critical that such strategies are designed to be implemented as soon as possible and at the local level and that they encompass:

- observation of pre-defined indicators
- rapid detection of anomalies
- effective warning procedures
- analysis, resistance and mitigation procedures

6.

Perceived risk: differences between the general public and experts



Research on *perceived risk* started in the late 1960s, with Starr's (1969) work on risk and voluntariness. Lowrance's (1976) work on determinants of the acceptability of risk was also influential. His work helped to explain why some technologies were less acceptable than others. Both Starr's and Lowrance's work followed the increasing use of probabilistic tools to conduct safety analyses in the context of new technologies such as space exploration programmes (see for example Kolluru, 1995) and nuclear power facilities (see for example Royal Society, 1983).

Slovic and colleagues (1979) were the first to show that the way people perceive risk differs from probabilistic assessments, but that their perception of risk is both predictable and quantifiable. As argued by Fischhoff *et al.* (1978) biases in perceived risk can be related to one of the most general judgmental heuristics: *cognitive availability*. People who use this heuristic judgement perceive an event as likely or common if instances of it are relatively easy to imagine or recall. Thus, overestimated risks (e.g. nuclear accidents, air crashes) tend to be dramatic and sensational whereas underestimated risks concern less spectacular events that usually claim one or a few victims at a time, and are also common in non-fatal forms. Not surprisingly, overestimated risks also often receive disproportionate attention from the news media.

Experts versus non-experts

Experts' judgments of risk often differ systematically from those of non-experts; their perceptions tend to reflect the complete range, from high to low risk, inherent in statistical measures. Lay people's perceptions of risk, however, tend to be compressed

into a smaller range, and are not as highly correlated with annual mortality statistics. When asked about perceived risk, it seems as though experts see the task primarily as one of judging statistics, whereas lay people's judgments are influenced by a variety of other factors. It needs to be stressed, however, that these effects are generally restricted to absolute estimates of risk and do not affect the ordering of risks. Risk estimates of lay people are generally quite adequate at rank order level. People's performance in these tasks is about as good as one would expect, given their limited knowledge about, and experience with, many of the hazards presented to them. The factors discussed below, however, impact risk perception by the general public.

Risk acceptability

What are the possible causes of the limited *acceptability* of some technological hazards? This can be related to specific characteristics of these hazards. Fischhoff *et al.* (1978) assessed "risk profiles" of various hazards, and showed that less acceptable hazards such as nuclear power scored close to the extreme high-risk end on characteristics such as *involuntary, unknown to those exposed or to science, uncontrollable, unfamiliar, potentially catastrophic, severe and dreaded*. Overall these ratings could be explained by two higher order factors. The first factor was primarily determined by the characteristics *unknown to those exposed* and *unknown to science*, and to a lesser extent by *newness, involuntariness* and *delay of impact*. The second factor was primarily defined by *severity of consequences, dread*, and *catastrophic potential*. Controllability contributed to both factors. Thus unknown, new risks with cata-

strophic potential tend to be seen as less acceptable.

Overall, results of these early studies on people’s perception of technological risks helped to understand public reactions and predict future acceptance and rejection of specific technologies. The main conclusion was that people’s fears of some technologies are determined by their concerns about issues such as the controllability of potentially catastrophic consequences. A similar point can be made about more recent technological developments in areas such as biotechnology and human genetics.

The role of affect and intuition

Loewenstein (2001) was one of the researchers who noted that the vast majority of theories of choice under risk or uncertainty are “cognitive and consequentialist”. These rational choice models typically assume that people analytically assess both the desirability and the likelihood of possible outcomes to arrive at a calculated decision. Affect (a person’s good or bad, positive or negative feelings) and emotions (e.g. anger, fear) were typically ignored in these models. Loewenstein’s work is part of broader empirical and theoretical developments distinguishing “two parallel, interacting modes of information processing: a rational system and an emotionally driven experiential system” (Epstein, 1994). The rational processing system is analytic, logical, and deliberative and encodes reality in abstract symbols, words and numbers. In contrast, the experiential system is holistic, affective and intuitive. The latter system encodes reality in concrete images, metaphors linked in associative networks. Among other findings, this research has identified an ‘affect heuristic’ – an orienting mechanism that allows people to navigate quickly and efficiently through a complex, uncertain and sometimes dangerous world, by drawing on positive and negative feelings associated with particular risks (Finucane *et al.*, 2000).

Affective images and risk

Research on the role of affect investigates the relationship between affect, imagery and perceived risk. *Affect* refers to a person’s good or bad, positive or negative feelings about specific objects, ideas or images. *Imagery* refers to all forms of mental representation or cognitive content including perceptual and symbolic representations. The study of affective images in risk perception attempts to identify images that carry a strongly positive or negative emotional ‘charge’ (Slovic *et al.*, 1998). For example, many of the images the American public associated with ‘nuclear waste repository’ (images such as death, cancer, and the mushroom cloud) evoked strong feelings of dread, which resulted in the view that a proposed nuclear waste repository was an extremely dangerous risk (Slovic *et al.*, 1991).

Social context

The acceptability of risk is also related to societal decision-making processes. An extensive body of research shows that transparency of the decision-making process helps to make risk more acceptable. Risk–benefit distributions are also important. Local acceptability tends to be low when the local community is confronted with a risk, while the benefits are for the country as a whole. Numerous examples of this so-called Not-In-My-Backyard (*NIMBY*) effect illustrate the importance of equity-related issues (e.g. Van der Horst, 2007; Dear, 1992). Trust has also been researched extensively, and a variety of studies point at the importance of this factor in public reactions to risks associated with new technological developments (Siegrist *et al.*, 2000; Slovic, 1999). Perceived equity, trust and transparency of the decision-making process all affect public reactions; i.e. both the perception and the acceptability of the risks involved. These public reactions should affect risk communication and risk mitigation. Public reactions or the anticipation of the nature of



Figure 20. Risk assessment, risk management and the public.

these reactions should also affect the nature of risk assessment. A more apprehensive public warrants more extensive risk assessment and a more elaborate risk communication programme. Figure 20 summarises the various interdependent processes.

Perception of risk in the context of MSR

The concept of hazardous macro- or micro-organisms from space causing death and destruction to humanity has been a topic for popular literature and films for the past 100 years, from *War of the Worlds* to the *Andromeda Strain* and beyond. The theory of panspermia was proposed by Svante Arrhenius (Arrhenius, 1908). Later Hoyle and Wickramasinghe postulated that a range of pathogenic micro-organisms such as SARS may emerge from space to infect humanity, keeping the concept of extraterrestrial pathogens in the public eye. However, the majority of scientists do not agree with this idea. The effects of such theories are to most definitely increase popular perception of the possible risks associated with a sample return. In actuality, a Mars sample return contains many of the risk features defined by Bennett *et al.* (2010) as being more worrying (and less acceptable) to the public. These factors include:

1. To be involuntary rather than voluntary
2. As inescapable by taking personal precautions
3. To arise from an unfamiliar or novel source
4. To result from man-made, rather than natural sources
5. To threaten a form of death arousing particular dread
6. To be poorly understood by science
7. As subject to contradictory statements from responsible sources

The literature summarised in the previous paragraphs results in a number of concrete suggestions for risk management, risk mitigation and risk communication. If we start with the social context described in the final paragraphs of this section, transparency of the decision-making process about how to deal with the possible risks and equity issues are likely to be less prominent than in the case of siting new facilities that are associated with risk by the general public. NASA and ESA do not suffer from a structural lack of trust as seems to apply to the nuclear industry and its regulating agencies (in some countries at least). It is important to nourish this situation.

Given the fact that possible consequences of an unintended release of a potential Mars life form into the terrestrial biosphere are unknown it is difficult to predict public reaction to possible

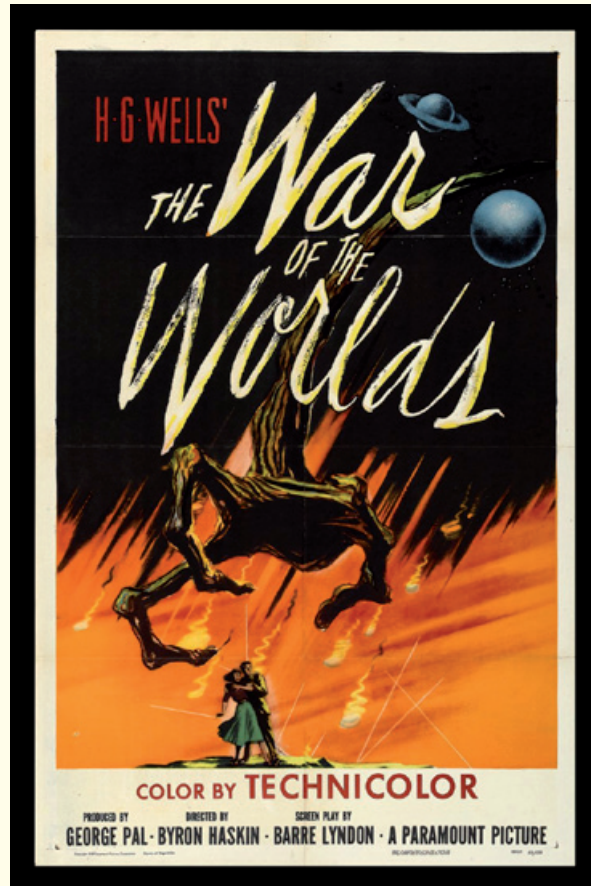


Figure 21. Poster of the 1953 movie War of the Worlds

risks. Images that the general public associates with Mars life forms are less clear-cut and probably less valenced than those people tend to associate with technological hazards such as nuclear energy and toxic waste. This is reassuring, but it would be advisable to monitor these images and also their relation to the risks people associate with the possible escape of Mars life forms. Given these uncertainties it seems best to adopt a cautious approach when considering the possible consequences of an unintended release.

7. Regulatory and legal aspects of a Mars Sample Return Mission



The current legal framework surrounding a sample return mission states that there is an obligation to prevent contamination or adverse changes to the environment of the Earth, with concomitant responsibility and liability. In Outer Space and as far as space activities are concerned, general international law and space law, including the UN treaties and resolutions, apply.

7.1 Obligation to prevent pollution/contamination of Outer Space and the Earth

In international environmental law, some principles have been elaborated by the way of declarations (Declaration of the United Nations Conference on the Human Environment, Stockholm, 1972; Rio Declaration on Environment and Development, 1992), later turned into legal principles which have been recognised by the International Court of Justice (ICJ).

In its 1996 advisory opinion *Legality of the threat or use of nuclear weapons* (ICJ, 1996) ICJ states: “*The existence of the general obligation of States to ensure that activities within their jurisdiction and control respect the environment of other States or of areas beyond national control is now part of the corpus of international law relating to the environment*”. In 1997 the court stressed “*the great significance that it attaches to respect for the environment, not only for States but also for the whole of mankind*” (ICJ, 1997).

In space law some provisions deal with environmental issues resulting from space activities. Article 1 of the Outer Space Treaty (United Nations, 1967) provides that “*exploration and use of Outer Space, including the Moon and other celestial bodies,*

shall be carried out for the benefit and in the interests of all countries, irrespective of their degree of economic or scientific development, and shall be the province of all mankind.” They shall be free for exploration and use by all States. A special reference is made to “*freedom of scientific investigation in Outer Space, including the Moon and other celestial bodies, and States shall facilitate and encourage international co-operation in such investigation.*”

The most relevant provision may be found in Article IX of the Outer Space Treaty: “States Parties to the Treaty **shall** pursue studies of Outer Space, including the Moon and other celestial bodies, and conduct exploration of them **so as to avoid their harmful contamination** and also **adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter** and, where necessary, **shall adopt appropriate measures** for this purpose.”

This provision sets a precise obligation on States taking part in an MSR mission.

The Moon agreement enters into more detail; it is in force but has not been ratified by any spacefaring nation (United Nations, 1979). In its Article 5.3 a reference is made to an obligation for States Parties to promptly inform the UN “*Secretary General as well as the public and the international scientific community, of any phenomena they discover in Outer Space, including the Moon, which could endanger human life or health, as well as of any indication of organic life.*”

7.2 Responsibility and liability of States

According to Article VI of the Outer Space Treaty, every activity in Outer Space is under the responsibility of a State and must be authorised and continuously supervised by the State for which this activity is a “national activity”. The State should make sure the activity is conducted in accordance with international law including the Outer Space treaties. For a space sample return the relevant provision is especially the obligation contained in Article IX of the Outer Space Treaty. If this obligation is not fulfilled, the State which violates Article IX would be responsible according to international law. In case of any damage there may be an obligation to indemnify the victim.

Under the Liability Convention (United Nations, 1971), the launching State is liable for “damages caused by the space object”. If a sample has detrimental consequences on Earth it may be considered that the State having launched the spacecraft is liable under this convention (absolute liability without any ceiling either in amount or in time; Liability Convention Article 1 – loss of life, personal injury

or impairment; or loss of or damage to property of States or of persons, natural or juridical, or property of international intergovernmental organisations).

A distinction must be made between damage which may be considered as “caused by a space object”, such as a contamination event taking place at the re-entry of the capsule, and, for example, the case of a leak from an Earth-based laboratory. In the latter, the point is much more uncertain; the damage would perhaps not be considered as “caused by the space object”.

If the State is not liable under the Liability Convention because the damage is not directly “caused by a space object”, as required by the Liability Convention, it would nevertheless still be in violation of international law (Article IX of the Outer Space Treaty). The difference is that the settlement of dispute mechanism and the nature of the responsibility/liability is not the same: absolute liability and the possibility to have the constitution of a claim commission under the Liability Convention, versus responsibility for an illegal act under general international law and the usual settlement of dispute mechanism for violation of Article IX of the Outer Space Treaty.

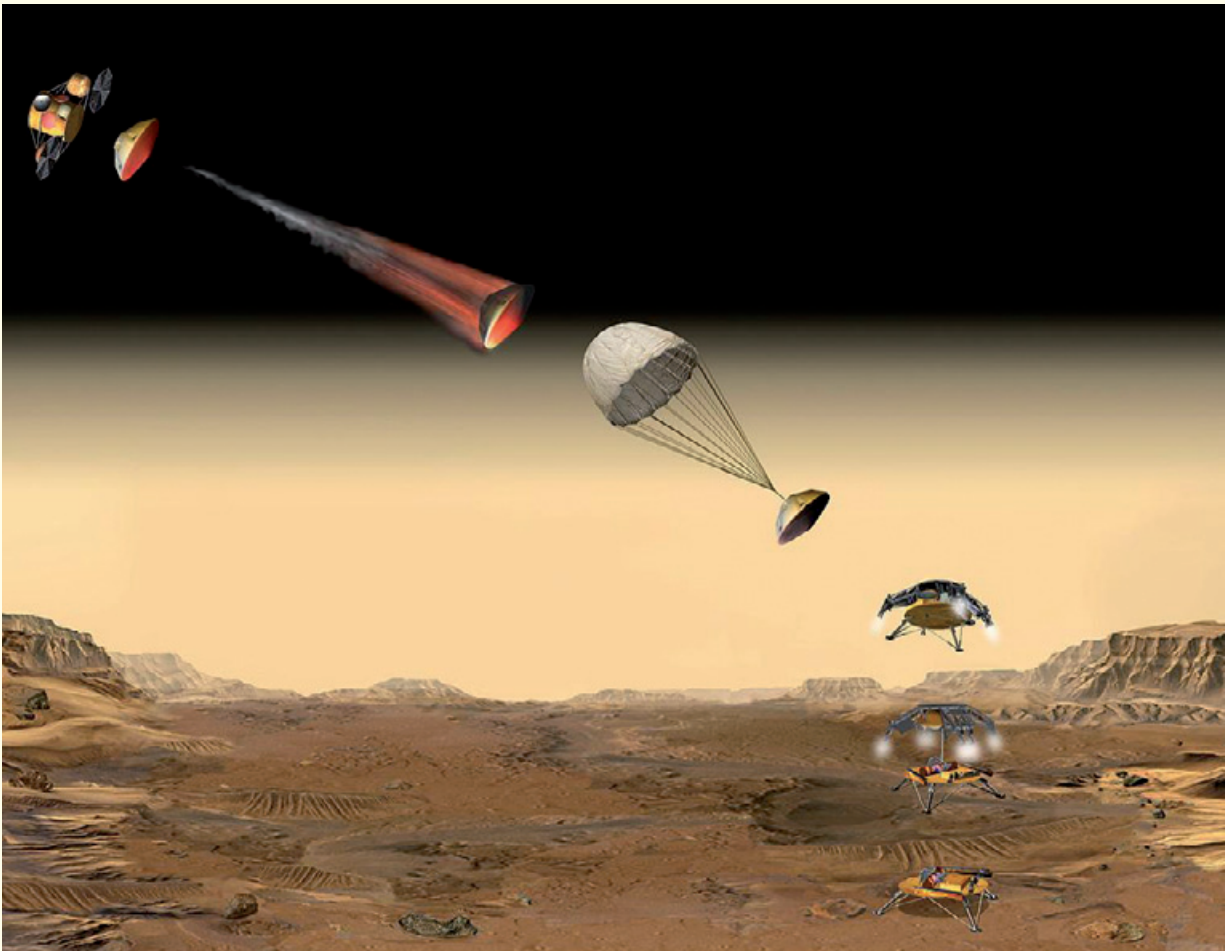


Figure 22. This artist's conception of Mars Sample Return mission shows the entry, decent and landing sequence onto Mars. Credit: NASA/JPL

7.3 The necessity/utility to give some legal value to measures preventing damage

Various levels of legal obligation may be accepted by States in order to implement their obligations to “adopt appropriate measures” for the purpose of preventing contamination.

Article IX of the Outer Space Treaty creates an obligation to adopt “appropriate measures” but does not indicate either what they would be or what kind of rules should be used. They could be:

- Prevention measures
- Notification to other States (all States or the States involved)
 - Of the activity
 - Of possible hazard
 - Of any problems
- Cooperation between States to prevent damage or in case of damage

The legal instrument to use:

- International compulsory regulation (treaty or agreement for instance drafted by UN COPUOS and analogous with the IMO Ballast Water Convention)
- International compulsory regulation accepted by an agreement between States involved
- International code of conduct accepted but without legal compulsory value (see: EADC code of conduct and its COPUOS–STSC version)
- Implementation of these texts within a domestic legal order
 - As a compulsory rule
 - As a code of conduct

In any case, the States involved must be conscious of their obligation to adopt these measures and of the consequences of damage on the States’ possible responsibility and liability.

A cooperation mechanism may be set in place along the lines of Antarctic cooperation among Consultative Parties under the Antarctic Treaty and the Madrid Protocol. These discussions are not open to every State; only those States involved in the activity are deemed “Consultative Parties”.

8.

Study Group findings and recommendations



8.1 Mars exploration and sample return

1. The past fifteen years have shown an enormous growth of interest in Mars, the most Earth-like planet in our solar system, and in the search for environments amenable for extant or extinct life. A Mars Sample Return has been deemed the highest priority in Mars exploration, as it would promise dramatic advances in the understanding of Mars as a whole. Such a mission requires extra attention to facilitate the vast benefits it will produce not only for science and technology, but also the general public. The history of science shows that discovery has always led to future discoveries and in this context the perspectives offered by Mars Sample Return Mission are tremendous.
2. Through the study of a sample, researchers could make great progress in understanding the history of Mars, its volatiles and climate, and its geological and geophysical history, and gain new astrobiological insights. A Mars Sample Return has also been deemed an essential precursor to any human exploration of Mars. Although some questions may be answered by *in-situ* studies done by robotics on the Mars surface, returning a sample to Earth is desirable for several reasons including (but not restricted to): the ability to perform complex experiments and analyses on the sample, greater flexibility in dealing with the unknown and potentially unexpected discoveries and the ability to repeat experiments and confirm key results.

8.2 Uncertainties, Precautionary Principle and optimisation

3. So far, no evidence of extinct or extant life on Mars has been found, and there is no known 'Mars biology'. Any assumption made about potential Mars organisms can only be speculated upon by combining knowledge of life on Earth (especially extremophile biology in analogous ecosystems) with knowledge and understanding of Mars' geology and environmental conditions. This lack of knowledge, or uncertainty, prevents definitive conclusions from being reached on major factors that would allow for a real assessment of the risk of contamination posed by an MSR mission. Therefore, with such a level of uncertainty, it is not possible to definitely estimate a probability that the sample could be harmful or harmless (in the classical frequency definition of probability) nor the nature and magnitude of the consequences of a release.
4. The concept of *Best Available Technology Precautionary Principle* instructs that activities that present an uncertain potential for significant harm should be subject to best technology available requirements to minimise the risk of harm unless the proponent of the activity shows that the activity presents no appreciable risk of harm. This concept aligns well with the conditions posed by an MSR mission.
5. With regard to optimisation strategies, it appears clear that the concept of Best Available Technique (BAT) used in the field of pollutant emission control is a suitable approach in the frame of an MSR mission. Adapted to the spe-

cificities of the systems and operations involved in an MSR mission, such a concept would allow for mobilising the use of the best technologies and operational concepts (at a reasonable cost) in order to minimise not only the probability of an unintended release but also the magnitude of such a release.

However, while BAT only implies that available techniques (at a reasonable cost) are used, it seems important set a limit to define and recommend adequate requirements for the release probability and magnitude. Should these requirements not be achievable with available technology, new technologies would have to be developed to meet them.

RECOMMENDATION 1:

Considering the many uncertainties and unknowns about putative Mars biological entities and the potential consequences of releasing such entities into the Earth's biosphere, as well as about public perception of risk in the frame of an MSR mission, the ESF-ESSC Study Group recommends that the Best Available Technology Precautionary Principle is applied when considering the potential release of unsterilised Mars particles.

RECOMMENDATION 2:

In accordance with past advice, the ESF-ESSC Study Group recommends that a Mars sample should be applied to Risk Group 4 (as defined by the World Health Organisation) a priori.

RECOMMENDATION 3:

The ESF-ESSC Study Group recommends that the Best Available Technique (BAT) optimisation concept is used as a benchmark and adapted to the specificities of an MSR mission in order to guarantee that the probability of an unintended release and also the magnitude of this release is minimised.

BAT only implies that available techniques (at a reasonable cost) are used, yet it seems important to set a limit to define and recommend adequate requirements for the release probability and magnitude. Should these requirements not be achievable with available technology, new technologies would have to be developed to meet them.

8.3 On particle size

6. Overall, the ESF-ESSC Study Group concurs with the approach adopted since 1999 confirming that containment of particles larger than a given size is an appropriate constraint to be considered when designing an MSR mission.

7. The original particle size limit (0,2 μm) was based on the estimated minimal size for a free-living autotroph cell (250 ± 50 nm). An extensive literature review on recent developments and findings in the field of microbiology invalidated this value. In particular:

- The rod shaped archea *P. ubique* has a cell diameter of 0.12–0.20 μm (length between 0.37 and 0,84 μm)
- The bacterium *Thermofilum* has a diameter of 0.15–0,17 μm (length between 1 and 100 μm)
- Some colony-forming units of bacteria were isolated after prefiltration of melted ice core through a 0,1 μm filter

Furthermore, it is believed that theoretically a coccoid cell with the minimum number of genes to be free living in an environment other than a living host would have a minimum cell diameter of approximately 0.15–0,2 μm . A rod-shaped cell could have a width less than 0,1 μm with a variable length but greater than 0,2 μm .

8. Viruses are presumed to be associated with organisms from all domains of life on Earth, so it follows that if there were Earth-like life forms on Mars, they would also be likely to have viruses. Furthermore, gene transfer agents (GTAs) are able to randomly incorporate segments of the host genome into a viral capsid which can be transferred to different hosts, including phylogenetically unrelated bacteria and archaea, without resulting in lysis of the host cell. In this manner, it is believed to be possible for GTAs to incorporate any of the host genes during replication and it is now estimated that GTA transduction rates are more than a million times higher than previously reported for viral transduction rates in a marine environment.

The smallest observed virus, the single-stranded-DNA porcine circovirus type 2, has a size of 0,17 μm ; GTAs are in the range of 0.03 to 0,08 μm .

RECOMMENDATION 4:

The ESF-ESSC Study Group concurs with the conclusions from NRC reports (1997, 2009) that large-scale effects arising from the intentional return of Mars materials to Earth are primarily those associated with replicating biological entities. However, bearing in mind new knowledge produced in recent years, the Study Group considers that, if there were Earth-like life forms on Mars, virus-type and GTA-type entities' ability to interact with Earth organisms cannot be ruled out. Based on this, the ESF-ESSC Study Group recommends that not only self-replicating free-living biological entities are considered as potentially having consequences on the Earth's biosphere but also virus-type and GTA-type entities.

9. The Study Group also concurs with another conclusion from the NRC reports (1997, 2009) that the potential for large-scale effects on the Earth's biosphere by a returned Mars life form appears to be low, but is not demonstrably zero. It adds that if this risk appears to be low for free-living self-replicating organisms, considering their specificities and replication requirements, the potential risk posed by virus-type and GTA-type entities can be considered to be far lower and almost negligible, but still cannot be demonstrated to be zero.

8.4 Public perception

10. Given the fact that possible consequences of an unintended release of a potential Mars life form into the terrestrial biosphere are unknown it is difficult to predict public reactions to possible risks. Images that the general public associates with Mars life forms are less clear-cut and probably less valenced than those people tend to associate with technological hazards such as nuclear energy and toxic waste. This is reassuring, but it would be advisable to monitor these images and also their relation to the risks people associate with the possible escape of Mars life forms.

RECOMMENDATION 5:

The Study Group considers transparent communication about accountability, benefits, risks and uncertainties relating to an MSR mission to be crucial throughout the whole process. It is recommended that tools to effectively interact with individual groups of stakeholders are developed.

8.5 On the required level of assurance

11. The required level of assurance for not releasing an unsterilised particle into the biosphere is not the same as the level of assurance for not contaminating the Earth with a Mars organism. This level of assurance only provides the maximum probability of the (unknown) potential risk, and this value has to be understood as representing a reduction factor to the (undetermined) risk posed by the potentially hazardous nature of the sample. Therefore, the required level of assurance for not releasing an unsterilised particle is not equivalent to an acceptable risk.
12. From the review of the current guidelines and regulations applied worldwide and in line with the positions adopted at the international level, it appears that the current assurance level (lower than one in a million) for the release of a potentially hazardous unsterilised Mars particle is appropriate and in line with international standards for excellence in management of risk with consequences that are hard to estimate.

RECOMMENDATION 6:

Based on standards established and adopted at the national and international levels, the ESF-ESSC Study Group recommends that the probability of release of a potentially hazardous Mars particle shall be less than one in a million.

8.6 Implication for design

Based on recommendations 1 to 5:

RECOMMENDATION 7:

The probability that a single unsterilised particle of 0,01 μm diameter or greater is released into the Earth’s environment shall be less than 10^{-6} .

If the size requirement cannot be met without decreasing the overall level of assurance for the non-release of such a particle, the release of a single unsterilised particle of up to 0.05 μm can be considered as a potentially tolerable systems-level adjustment, assuming that it has been demonstrated that this size is the lowest achievable at a reasonable cost.

In such a case, the actual maximum particle size potentially released (as planned from design) would have to be independently reviewed by interdisciplinary groups of international experts to determine:

- whether this size value is the best reasonably achievable at a reasonable cost,

And, if yes:

- taking into consideration the latest scientific developments in the fields of astrobiology, microbiology, virology and any other relevant discipline, whether the release of such a particle can be considered as tolerable.

The release of a single unsterilised particle larger than 0,05 μm is not acceptable under any circumstance.

13. The recommendation put forward above represents a drastic decrease of the size requirement. The main driver behind this is the consideration given to Mars virus-type and GTA-type entities as potentially impacting the Earth’s biosphere. Based on our current knowledge and techniques (especially genomics), one can expect that if the expected minimum size for viruses, GTAs or free-living microorganisms decreases in the future, and this is indeed possible, it will be at a slower pace than over the past 15 years.

However, no-one can discard the possibility that future discoveries of new agents, entities and mechanisms may shatter our current understanding on minimum size for biological entities.

RECOMMENDATION 8:

Considering that (i) scientific knowledge as well as risk perception can evolve at a rapid pace over the time, and (ii) from design to curation, an MSR mission will last more than a decade, the ESF-ESSC Study Group recommends that values on level of assurance and maximum size of released particle are re-evaluated on a regular basis.

8.7 Accompanying measures

14. As it is not possible to definitively exclude release scenarios having consequences on the biosphere, it is crucial to work on the definition and development of potential scenarios, addressing the question: “What might happen in case of an unintended release?”. The relevance and significance of the scenarios developed as well as of their associated monitoring and response strategies will be key elements in optimising the efficiency of the mitigation, containment or limitation of potential consequences.

RECOMMENDATION 9:

Building capacity to respond to a release of Mars material is of utmost importance and should draw upon available experience in the fields of public health and emergency response. In addition to current prevention strategies, it is recommended that potential release scenarios (including undetected release) are clearly defined and investigated, and that response strategies are developed from these. It is critical that such strategies are designed to be implemented as soon as possible and at the local level and that they encompass:

- observation of pre-defined indicators
- rapid detection of anomalies
- effective warning procedures
- analysis, resistance and mitigation procedures

Scenarios and response strategies should be reassessed and updated on a regular basis.

RECOMMENDATION 10:

Considering the global nature of the issue, consequences resulting from an unintended release could be borne by a larger set of countries than those involved in the programme. It is recommended that mechanisms dedicated to ethical and social issues of the risks and benefits raised by an MSR are set up at the international level and are open to representatives of all countries.

15. In general, the geochemical and physical context of the Mars sample to be returned will be critical information for constraining the potential physiological groups of microbes that could exist in the sample, given that a life form on Mars will likely use the same energy sources as Earth life and could develop some of the same characteristics as Earth life in similar settings.

RECOMMENDATION 11:

Information on the geochemical and physical context of the Mars sample to be returned and having access to this information will be key elements to define and refine scenarios and assumptions about potential Mars biological entity(ies) returned to Earth. Such information should be gathered and made available to the relevant stakeholders as soon as possible in the process.

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Annexes

Annex 1: **ESF-ESSC Study Group composition**

First name	Surname	Organisation	Town	Country
Walter	Ammann	Global Risk Forum	Davos	Switzerland
John	Barros	University of Washington	Washington	USA
Allan	Bennett	Health Protection Agency – Microbiology Services	Salisbury	UK
Jim	Bridges	University of Surrey	Guildford	UK
Joseph	Fragola	Valador Inc.	Herndon	USA
Armel	Kerrest	University of Western Brittany	Vannes	France
Hervé	Raoul	Laboratoire P4 Inserm Jean Mérieux	Lyon	France
Petra	Rettberg	DLR	Köln	Germany
John	Rummel	COSPAR Panel on Planetary Protection (PPP) Liaison – East Carolina University	Greenville	USA
Mika	Salminen	National Institute for Health and Welfare	Helsinki	Finland
Erko	Stackebrandt	Former DSMZ director	Paris	France
Jean-Pierre	Swings	European Space Sciences Committee, Liège University	Liège	Belgium

ESF Support Staff

Nicolas Walter, Senior Science Officer
 Karina Marshall-Bowman, Junior Science Officer
 Johanne Martinez-Schmitt, Administrator

Annex 2: Risk perception workshop – participation, consensus statements and recommendations



European Space Sciences Committee
ESF-ESSC Study Group on Mars
Sample Return Requirements

Risk Perception Workshop

9–10 January 2012, Berlin, Germany

Meeting Consensus Statement and Recommendations

List of participants

- Walter Ammann – Global Risk Forum, Switzerland
- Anne Cambon-Thomsen – INSERM-U558, Faculté de Médecine Toulouse, France
- Thomas Epprecht – Independent Risk Consultant, Switzerland
- Joseph Fragola – Valador Inc., USA
- Peter Mani – Techrisk GMBH, Switzerland
- Piet Sellke – University of Stuttgart, Germany
- Joop Van der Pligt – University of Amsterdam, The Netherlands
- Stefan Wagener – National Microbiology Lab, Public Health Agency of Canada, Canada
- Laurie Zoloth – Northwestern University, USA
- Gerhard Kminek – European Space Agency, The Netherlands
- Nicolas Walter – ESF, France

Consensus Statement

- There is no ‘Mars biology’ known to us, therefore, there are no experts on Mars biology, and expert consensus is hard to reach and harder to justify.
- However, significant knowledge has been gained on side issues such as handling pathogenic agents, biosafety operations and strategies to respond to the release of hazardous material.
- The recommended constraint on the unsterilised particle size should be reconsidered taking into account not only the size of self-replicating organisms but also virus-type organisms.
- Unlike for synthetic biology and nanotechnologies, knowledge development about Mars biology (if any) will not be incremental – the sample will arrive in one go.
- Prevention is not enough to deal with the unknown; preparedness and fast reaction is the required approach (e.g. response procedures in civil security).

- Risk can never be demonstrated to be zero. In this respect, zero risk to release a potential Mars organism in the Earth’s biosphere cannot be presented as a valid option. Even if no samples are returned from Mars, a meteorite from the planet could still be a potential vector.
- A transparent approach (including communication) is crucial to gain trust:
- The ‘*trust me I am a scientist*’ approach cannot be considered valid when considering potential release of Mars organisms. The uncertainties have to be clearly listed and explained;
- Structured and targeted communication throughout the whole process is crucial to gain trust.
- Mars research may yield beneficial outcomes that range from useful to tremendous; not undertaking the MSR mission may result in lost opportunities.
- Cultural differences in risk perception and acceptability are real.
- Reversibility of the effect is an important characteristic to consider.
- Risk is an element that does not stand alone. It has to be considered together with risk management and risk communication approaches and strategies, as well as potential benefits.

RECOMMENDATION 1

A strong argument based on our best current knowledge of biology and the Mars environment has been made that the risks resulting from the introduction of a potential Mars life form are very low. Over the past years, this argument has reached consensus among the scientific community. However, this risk cannot be demonstrated to be zero.

Hence, it is recommended to adopt a conservative approach:

- the current assurance level (lower than one in a million) for the release of an unsterilised Mars particle larger than a given size in at least one dimension is considered appropriate.

This recommendation should apply to all phases of the mission.

RECOMMENDATION 2

Building capacity to respond to a release event is of utmost importance. In addition to current pre-

Annex 2: **Risk perception workshop – participation, consensus statements and recommendations**

vention strategies, it is recommended that potential release scenarios are clearly defined and response strategies are developed. It is critical that such strategies are implemented as soon as possible and at the local level and encompass:

- observation of pre-defined indicators
- rapid detection of anomalies
- effective warning procedures
- analysis, resistance and mitigation procedures

Experience gained in the in public health domains and preparation for an emergency response would be highly relevant.

RECOMMENDATION 3

Potential risks from an MSR are characterised by their complexity, uncertainty and ambiguity, as defined by the International Risk Governance Committee's risk governance framework. As a consequence, civil society, the key stakeholders, the scientific community and relevant agencies' staff should be involved in the process of risk governance as soon as possible.

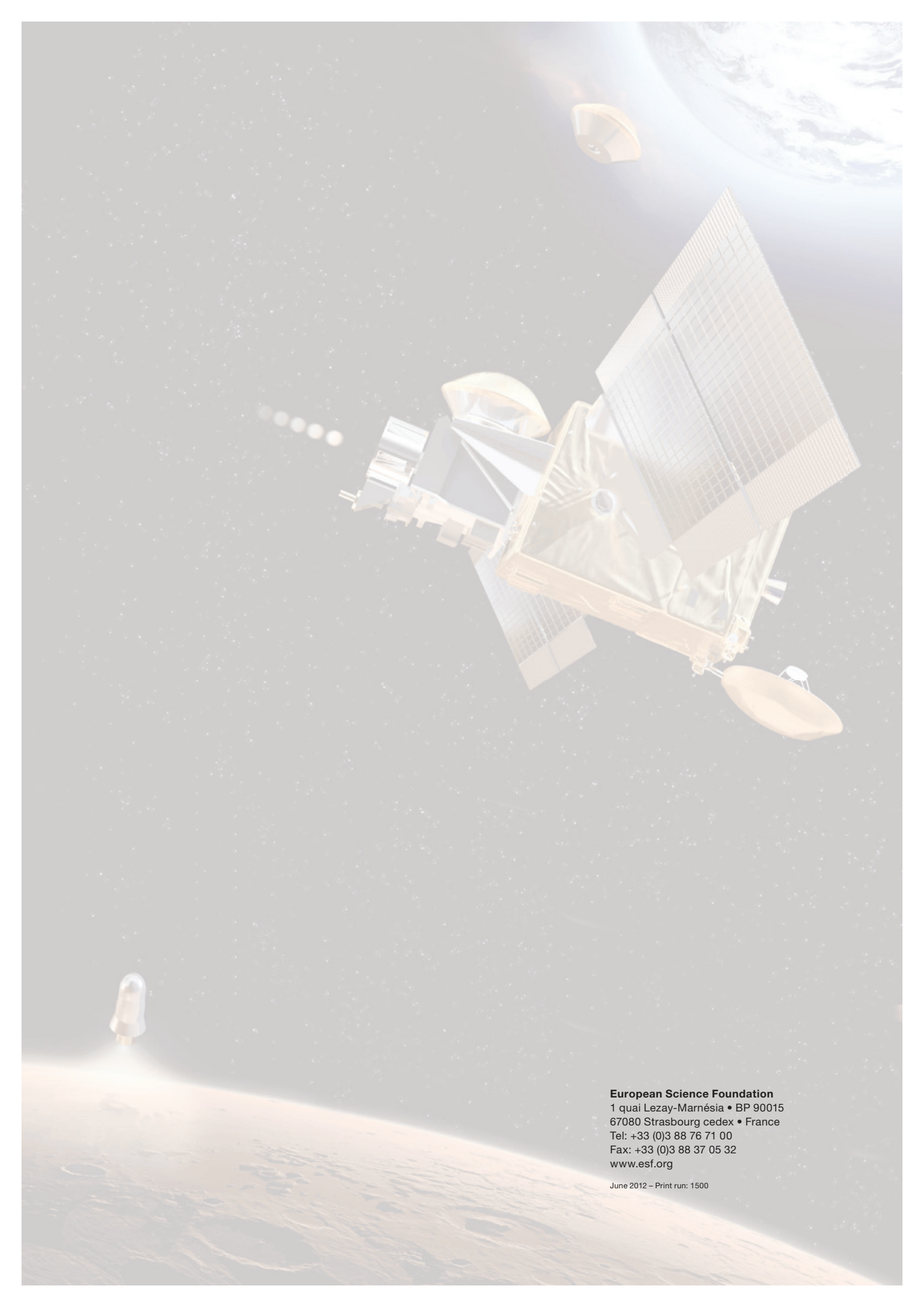
In this context, transparent communication covering the accountability, the benefits, the risks and the uncertainties related to an MSR is crucial throughout the whole process. Tools to effectively interact with individual groups should be developed (e.g. a risk map).

RECOMMENDATION 4

Potential negative consequences resulting from an unintended release could be borne by a larger set of countries than those involved in the programme. It is recommended that mechanisms and fora dedicated to ethical and social issues of the risks and benefits raised by an MSR are set up at the international level and are open to representatives of all countries.

RECOMMENDATION 5

Given that both knowledge and risk perception evolve over time and that, from design to curation, the MSR mission will take more than one decade, it is recommended that values on the level of assurance and maximum size of a released particle are determined and re-evaluated on a regular basis, together with release scenarios and response strategies.



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