MARS 96 PLANETARY PROTECTION PROGRAM AND IMPLEMENTATIONS FOR MARS ENVIRONMENT PRESERVATION

A. DEBUS - CNES Toulouse, France
J. RUNAVOT - CNES Toulouse, France
G. ROGOVSKY - NPO LAVOCHKINE Moscow, Russia
V. BOGOMOLOV - NPO LAVOCHKINE Moscow, Russia
N. KHAMIDULLINA - NPO LAVOCHKINE Moscow, Russia
V. TROFIMOV - LEKBIOTECH RESEARCH AND DEVELOPMENT CENTER Moscow, Russia

ABSTRACT

In the frame of extraterrestrial exploration missions and since the beginning of the Solar System exploration, it is required, **according to the article IX of the OUTER SPACE TREATY** (London / Washington January 27., 1967) to preserve planets and Earth from biological contamination. Consequently, COSPAR (Committee of Space Research) has established Planetary Protection recommendations in order to protect other worlds environment from biological contamination by terrestrial microorganisms and to protect Earth environment from back contamination. According to these recommendations, projects involved in planetary exploration programs include in their development plans specifications for biological cleanliness.
After the last planetary protection program applied for the Viking missions two decades ago, the Mars 96 mission has taken in account the last updated Cospar recommendations and a biological decontamination program has been implemented commonly by Russia and France. This program include sterilizations, biocleanings and landers integration in sterile environment in order to ensure Mars environment protection.

HISTORIC OVERVIEW FOR MARS PLANETARY PROTECTION

Cospar recommendations for Mars Exploration

Before the upcoming Mars missions planned at the beginning of the last decade of this century (Mars 96, Pathfinder, Mars Global Surveyor, Planet B), Cospar recommendations were the following:

Orbiters: According to the COSPAR Policy[1], Mars orbiter is a category III mission. The main goals is to limit the impact probability and to control the bioload. The requirements were:
- documentation related to contamination control and organic inventory,
- implementing procedures such as trajectory biaising, cleanroom integration and bioload reduction if necessary.

Landers: According to the COSPAR Policy[1], Mars landers are in category IV. The main goals is to limit the probability of nonnominal impact and to limit the bioload. The requirements were:
- detailed documentation related to microbial reduction plan and organic inventory
- implementing procedures such as cleanroom assembly, bioload reduction, bioshield
- monitoring of bioload via bioassays

Evolution of recommendations and application for Mars missions

Since the first Martian missions, the allowed probability of contamination of Mars by terrestrial microorganisms has been set by COSPAR and specified to 10^{-3} for all probes entering the Mars atmosphere[1]. Each mission had to remain within a determined probability of contamination (10^{-4} for each Viking lander for example) and the sum of all probabilities related to each planned mission was not permitted to exceed 10^{-3}. The permissable number of microorganisms for each vehicle before launch was determined by a probabilistic approach, based on the probability of survival of the terrestrial bioburden during the different events of the mission (launch, travel, UV exposure, planet atmosphere, ...etc). The most important factor in this calculation was Pg, the probability of growth and

proliferation of terrestrial microorganisms under environment conditions of Mars. As knowledge of Mars increased, estimated Pg decreased and it appeared, after the Viking mission, that Pg was very low. The evolution is:

- 1963 : $P_g = 1$
- 1964 : $P_g = 10^{-3}$
- 1967 : $P_g = 10^{-4}$
- 1971 : $P_g = 10^{-6}$, probability taken into account for the Viking landers
- 1978 : $P_g < 10^{-10}$ ($< 10^{-7}$ for the polar caps and 10^{-8} for sub-surface deeper than 6 cm), probabilities estimated after the Viking missions.

Consequently, the decontamination level of a martian lander is the result of the biolooad reduction induced by different events of the mission, of the probability of survive on Mars and of the active bioload reduction performed by the project teams. The sterilization specification is consequently dependant of Pg and the decreasing of Pg in function of Mars environment knowledge induces evolution of recommendations and specifications. The consequences for missions were, for the first missions, to perform hard sterilization, for Viking to realize soft sterilization (except for life detection experiments) and for Mars 96 bioload reduction with selective sterilizations.

The Viking Planetary Protection Program

The program was built at the beginning of the 70th and one single method was specified: dry heat sterilization. All components were chosen in function of their compatibility with high temperature and the entire process has been validated and qualified. The principal events were the following:

1- Determination of the initial bioload of each Viking lander: estimated to 10^9 microorganisms

2- Integration at equipments level in cleanroom with biocleaning procedures

Dry heat sterilization (120°C - 54h) to 10^{-6} level of life detection experiments only

Estimated bioload after this step: 3.10^5 microorganisms (300 spores / m^2)

3- Landers integration in cleanroom with biocleaning procedures, integration of the bioshield

Final global sterilization step (111.7 °C - 40h) at lander level

Final estimated bioload: 100 microorganisms for each lander

With a probability of growth and proliferation of 10^{-6} (avalaible value for the Viking mission), the probability of Mars contamination for each Viking lander was about 10^{-4} (one microorganism for 10 000 landers).

COSPAR NEW RECOMMENDATIONS FOR MARS ENVIRONMENT PRESERVATION

Space Studies Board proposal

Because of the Mars exploration missions planned during this decade and taking into account new informations on Mars from the Viking mission, NASA's Planetary Protection Office requested that the Space Studies Board, represented by the concerned Scientific Community, make recommendations for this upcoming Mars missions[6,9]. It appeared, after the last Mars mission (Viking), that the probability of Mars contamination was very low and consequently, this fact was discussed in order to propose for COSPAR approval new recommendations. The conclusion was that the biodecontamination constrains decreased, but active bioload reduction stay necessary.

COSPAR new recommendations

After several meetings, held in 1991 and in 1992, the Space Studies Board proposed the following recommendations[5,8]:

The category IV is divided into two sub-categories:

Category IVa: Spacecrafts (including orbiters) without exobiological experiments as Mars 96 must be subject to at least Viking presterilization procedures, but such spacecrafts don't need sterilization.

Category IVb: Landers and vehicles carrying instrumentation for in-situ investigations of extant Mars life should be subject to at least Viking level sterilization procedures.

MARS 96 MISSION PLANETARY PROTECTION PROGRAM

Mission overview

MARS 96 mission was an international scientific mission to Mars lead by Russia, with significant participation of France and other countries such as

Germany, Finland and USA. It included an orbiter, two small scientific stations and two penetrators deployed on the Martian surface. The launch was planned on november 16., but unfortunatelly, technical problems induced the lost of the spacecraft just after launch. Nevertheless, the planetary protection program has been entirely realized, because as it will be described lower in this paper, all actions were done before the final spacecraft integration. This program, its definition, specifications, plans and implementions is the result of remarquable common work between russian and french teams.

MARS 96 specifications

For category IV missions without life detection experiments as MARS 96, requirements contain:
- cleanroom assembly: the acceptable class is 100 000 (FS 209) or better.
- bioburden reduction: the maximal acceptable level of decontamination is 300 spores per square meter and 3.10^5 spores per vehicle (values delivered from VIKING data). Only the external and internal exposed surfaces are concerned. It must be noticed that MARS 96 landers are smaller than the Viking landers and consequently, the representative maximal level (more stringent case) is 300 spores per square meter and this last value is define as the Mars 96 specification. The specification is given in function of a spore level, because spores are the most resistant of all microorganisms. Consequently, performing a method able to reach a level of 300 spores per square meter (from an initial level of about 10^4 spores/m^2 to 10^6 microorganisms/m^2) will kill all other microorganisms.
- selective bioburden reduction. Large surfaces and subsystems capable of disseminating terrestrial life on the planet (parachutes, balloons, wheels, guiderope external structures, ...) must be sterilized.
- the limit on probability of accidental impact by hardware other than lander, probe or orbiter must be less than or equal to 10^{-5}.
- recontamination prevention.

Remarks:
- the required recontamination level concerns each category IV orbiter, probe, vehicle or landing system. A landing system is defined as all subsystems included in a single landing event.
- for orbiters of category III as MARS 96, trajectory biaising and orbit lifetime computations are required. But Mars 96 orbiter did not need any implementation of sterilization procedures because the probability of spacecraft crash did not exceed 10^{-5} and because it's orbit was in accordance with quarantine requirements (orbit lifetime with 0.9999 confidence for the first 20 years and 0.95 confidence during the next 20 years).
- in case of a lander crash, the affected area must be limited and localized.
- processes and techniques used for establishing burden levels must be the same as those used for Viking or other improved assay methods [7]. In each case, the project teams have to demonstrate equivalence of techniques other than those used for Viking.

Mars 96 Planetary Protection Plan

The general integration philosophy of landers is described on table 1.

Table 1: implementations for Mars 96 landers during different step of integrations

	INTEGRATION PHASES	IMPLEMENTATIONS
1a	Landers equipments and sub-systems	Sterilization or biocleaning + ultraviolet rays exposure Sterile packaging Microbiological control
1b	Experiments	Sterilization or biocleaning + ultraviolet rays exposure Sterile packaging including sterile test interfaces Microbiological control
2	Landers integration in NPO Lavochkine Moscow	Sterile class 100 cleanrooms Sterile procedures for equipement and operators (clothes, tools, ...) Surface biocleaning / UV exposure Microbiological control Sterile packaging
3	Recontamination prevention	Bioshields

MARS 96 PLANETARY PROTECTION IMPLEMENTATIONS

Landers integration

According to the Planetary Protection Plan described on table 1, implementations can be divided into different levels:

Instrument and Subsystem level: Equipment, instruments, and subsystems have been sterilized to 10^{-3} level (1 microorganism for 1000 items) or better, or biocleaned to a level of less than 300 spores per square meter. The applied methods are described on table 2. All biodecontamination or sterilization were subject to the following scheme:

1/ After a first compatibility study, sterilization methods are chosen in function of each equipement

2/ For each method, procedures have been used in order to reach the required decontamination level (verification of microbiological efficiency). For all methods, procedures have been validated.

3/ For each method, the compatibility with the concerned sub-systems or instruments have been validated in order to verify that the effect of sterilization on equipments was acceptable. Validation tests have been performed on critical materials and for the french and some of the russian payload, the hydrogen peroxide gas plasma has been qualified at instrument level[2].

4/ The decontamination level was controlled on every sub-system or instrument using the following procedure:

* for sterilized items:
  - initial bioburden assessments
  - packaging
  - sterilization through the bags (as usual) with microbiological efficiency indicators (sheets of specific materials loaded with a definited number of reference spores (typically a few millions of the most resistant spores)
  - analysis of microbiological efficiency indicators and determination of the sterility levels

* for biocleaned / UV exposure equipments
  - initial bioburden assessments
  - final bioburden assessments
  - sterile packaging
  - determination of decontamination levels

Packaging: Because of testing on different sites, equipments must be packaged in a minimum of two bags in order to preserve the cleanliness conditions. For experiments a special packaging system was used in order to conduct acceptance tests without impacting the biological cleanliness. A third antidust bag was required for final protection during transportation.

Landers level: The integration site was a cleanroom in class 100 000 in which the small stations and penetrators integration was conducted in NPO Lavochkine (Moscow) under two 100-class laminar flow installations. They were required to control surface recontamination and to ensure a surface bioburden level of less than 300 spores per square meter. The contamination level was controlled by the means of bioburden assessments, periodic surface cleaning, and by use of sterile procedures for operators (clothes, handling), equipment, and the working environment. A bioshield finally covered each probe in order to prevent recontamination during the integration steps.

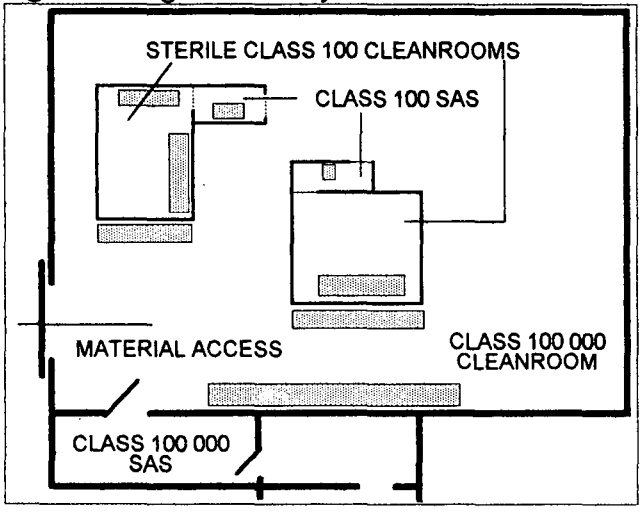
Table 2: Used decontamination methods for Mars 96 landers sub-systems and experiments

DECONTAMINATION METHODS	CONCERNED ITEMS
Gamma rays sterilization	Landers parachute and airbags systems Some of small station and penetrators sub-systems
Dry heat sterilization	Finnish meterological sensor of the small stations
Hydrogen peroxide gas plasma sterilization	Small stations french payload Penetrators ARS abd BSS experiment units Some of penetrators sub-systems
Biocleaning (alcohol or sporicide cleaning completed with ultraviolet rays exposure) performed in sterile class 100 cleanroom	Small stations finnish experiment Small stations US experiment Small stations russian experiments Some of small stations and penetrators subsystems and housekeepings

* *Class 100,000 Cleanroom:* All integration operations were done in a class 100,000 cleanroom (see Figure 1). This facility has been constructed to have the 100,000 features, particularly: positive pressure, dust filters, access by SAS (small autonomous stations), class 100,000 clothes, and particular cleanliness control (cleaning procedures). In this large cleanroom, two class 100 laminar flow facilities have been installed. Since the class 100 cleanroom has transparent walls, most of the control instruments and operators necessary for control during integration may stay outside the class 100 cleanrooms thus avoiding possible recontamination.

* *Class 100 Laminar Flow Cleanrooms:* The integration of the 2 small stations and the 2 penetrators were done in two class 100 cleanrooms (see Figure 1) as defined by FS 209B.

Figure 1: Integration site layout in NPO Lavochkine



Positive pressure was induced by a laminar flow blowing from top to bottom, which avoids particles (dust and microorganisms) settling. The size of the integration area under the laminar flow was about 10 m^2 for a height equal to 3.5 meters, allowing the integration of two landers at the same time. The access to the class 100 cleanrooms was through a common SAS joining each cleanroom across a corridor. An integration official was in charge of the cleanroom utilization and of the application of procedures. Access to this area, for operators and equipment, was done by means of the SAS where operators change their 100,000 class clothes for the sterile class 100 clothes, according to stringent changing procedures. The transparent walls of these cleanrooms lended a contribution to cleanliness because all ground support equipment remain outside while their screens or numeric information displays were visible from inside.

* *Cleanroom Cleanliness Control and Maintenance*. The integration area was kept clean by means of a daily cleaning with alcohol and sporide of all working surfaces (tables, chairs, etc.) except probe surfaces and other equipment (tools, control instruments, electrical cables, etc.) as well as a weekly cleaning with special disinfecting agents for the cleanroom structures.

* *Material Access Conditions*. Equipments were moved from the storage rooms into the 100,000 class cleanroom according to technological plans. All equipment and probe instruments have been decontaminated, as defined by the specifications. The external bag was removed for acceptance tests and the internal bag was first cleaned in the SAS and then opened in the class 100 cleanroom. All surfaces of other control instruments, tools, etc. have been cleaned with alcohol and/or sporicide wipes.

* *Sterile Clothes*. Class 100,000 clothes were typically suits designed for this level of cleanliness. For the class 100 laminar flow cleanroom some specific sterilized clothes were worn in the class 100 SAS. At each sterile cleanroom access, operators have to wear decontaminated overboots, overalls, hood, mask, and gloves. Except for the gloves which were thrown away, all other clothes were decontaminated after each change. Taking into account that the mission specification did not impose absolute sterility, the clothes were not sterilized, but were washed and completely decontaminated using chlorine water solutions. Typically one wash in ten was followed with actual sterilization. In this case, the clothes were washed and dried before packaging into bags. The bags used depend on the sterilization method. The two methods used were:

- steam sterilization (20 min at 120 °C)
- gamma sterilization (25 kGy).

* *Microbiological Recontamination Control:* Contamination of the lander surfaces were monitored by means of periodic microbiological assessments performed by the Lekbiotech Research and Development Center of Moscow. Bioassays were made according to an assessment plan and the

results of these analyses determined the method of surface biocleaning. Assessments were perfomed usually twice a week on landers surfaces and cleanrooms, and more often when it was required by integration processing. The bioassays method was similar to the Viking one and levels were given as an average of 6 to 8 assessments. In function of the determined levels, corrective actions were defined (biocleanling, time of ultraviolet exposure) in order to keep the following specifications:

- class 100 000 cleanroom: less than 2000 micro-organisms/m^2
- class 100 sterile cleanroom: less than 250 micro-organisms/m^2
- lander surfaces: less than 250 microorganisms/m^2

* *Surface Cleaning:* External surfaces were cleaned by the following methods:

- Ethyl alcohol was used daily to remove spores. Since alcohol is not a sporicide the alcohol flasks were typically changed every 5 cleanings to guard against contamination of the ethanol by spores.
- Ultraviolet exposure was used as a sporicide in order to complete biocleaning in case of excessive recontamination. Surface exposures occurred overnight and were stopped two hours before starting work in order to flush from the room the ozone generated by the process. The results of the analyses and estimates of the total contamination produced by all the integration factors (including microorganisms settling on the surfaces of the installation coming from the air, microorganisms produced by the personnel during their work, microorganisms coming with the supplied units and elements, and microorganisms introduced by the technological process itself) at NPO Lavochkine allowed the calculation of the necessary minimal power levels of UV radiation for different cleanliness classes. The results of this study enabled the choice of the characteristics, the number of the UV lamps and the exposure durations. In anycase, protocols were defined in order to reach on probes surfaces a level of 100 spores / m^2 just after exposition.

* *Packaging and Transport of Landers:* To carry out transport operations, the descent modules were packaged into a sterile envelope and placed on the transport devices within a sealed shield with a respiratory valve. These devices were placed in a special container, including cooling and dust / spores filtration subsystem, during transportation to the launch pad in Baikonour.

* *Bioshield Description:* After their reintegration, the landers were placed under a metallic cover. Gas exchange between both sides of the bioshield occured through a valve equipped with biological filters.

* *Results:* Before integration, all subsystems and experiments presented a contamination level of less than 300 spores / m^2. During the integration, the surfaces recontamination was limited and controlled by the above described means. Nevertheless, the level sometimes increased above the specification and correction were brought, as required, by surfaces biocleaning and ultraviolet exposure. The final values were the following:

Small station flight model 1:	270 spores / m^2
Small station flight model 2	210 spores / m^2
Penetrator flight model 1:	240 spores / m^2
Penetrator flight model 2	270 spores / m^2

Documentation

Documents related to planetary protection was included in the project documentation. The principal types of documents were specifications, plans and procedures. Detailed documents are listed in the summary on table 3.

Added to the procedures, it is important to note that inventories of materials, and particularly of organic materials were done through the material lists of landers.

Studies

The principal goals of studies were to validate sterilization methods and procedures and to establish protocols. Detailed studies are described in the summary on table 3.

Manpower

Except the subcontractors the planetary protection team includes 5 peoples. Their tasks were the following:

- NPO Lavochkine planetary protection responsible: it was in charge, commonly with the french responsible, to define specifications, procedures and to follow the work of its subcontractors. During landers integration, its task was to ensure the decontamination specification.

Table 3: MARS 96 Planetary Protection russian / french cooperation scheme

TASKS	RUSSIA	FRANCE
IMPLEMENTATIONS		
- Class 100 000 cleanroom	X	
- Class 100 cleanroom for small station integration		X
- Class 100 cleanroom for penetrators integration	X	
- Cleanrooms sas	X	
- Class 100 sterile clothes		X
- Usual equipment for cleanrooms (carpets, bags, sterility indicators.....)	X	X
- Ultraviolet rays sterilization device	X	
- Hydrogen peroxide gas plasma sterilizator		X
- Gamma rays implementations for validation tests	X	X
- Gamma rays implementation for flight models equipments sterilization	X	
- Cleaning agents and sporicides	X	X
- Microbiological assessments	X	X
DOCUMENTATION		
- Mars 96 quarantine program	X	
- Sterilization / biodecontamination specifications	X	X
- Class 100 cleanroom biocleaning procedures		X
- Class 100 clothes biocleaning and sterilization procedures		X
- Cleanroom access procedures for operators and equipment		X
- Class 100 sterile clothes wearing procedures		X
- Payload bioburden assessments procedures	X	X
- Landers bioburden assessments procedures	X	
- Surfaces cleaning procedures	X	X
- Ultraviolet irradiation protocols	X	
- Microbiological assessment plan	X	
- Landers sterile integration plan	X	X
- Decontamination certificates	X	X
- Hydrogen peroxide gas plasma sterilization procedure		X
STUDIES		
- Equipments and materials compatibility with sterilization methods	X	X
- Hydrogen peroxide plasma gas sterilization cycle biological validation		X
- Materials validation testing with hydrogen peroxide gas plasma sterilization process	X	X
- Materials validation testing with gamma rays sterilization	X	X
- Ultraviolet surface dose exposure times	X	
- Specific alcohol / formaldehyde sporicide validation	X	X

- CNES planetary protection responsible: it was in charge, commonly with the russian responsible, to define specifications, procedures and to follow the work of its subcontractors. It was supported by CNES laboratories in order to performed validation tests.

- NPO Lavochkine cleanroom responsible: it was in charge of the particle and biological cleanliness of all integration implementations. Its task was to control if all procedures were correctly applied.

- Two NPO Lavochkine representative in charge of studies

Associated subcontractors

Microbiology and sterilzation are not specific spatial tasks and consequenly, subcontractors participated actively to this programs and it is important to note the remarquable implication and

interest that all of them brought in this project. The subcontractors were:

- Lekbiotech Research and Development Center of Moscow: It was in charge of active sterilization and of microbiological assessments done in NPO Lavochkine.

- Paris V Pharmacy University: It was in charge of bioassays performed in France (french and a part of russian payload) and support CNES for procedures writing (sterilization, cleanroom cleanliness control, sterile clothes biocleaning).

- Johnson and Johnson Medical of Chatenay Malabry (France): it performed and followed the sterilizations by hydrogen peroxide plasma gas.

- Caric Mediris of Orsay (France): it performed sterilization by gamma rays for validition tests, with the help of its belgian unit. Most of them were done for the validation of the martian aerostat. This part of the project was unfortunatelly cancelled in 1994.

PARTICULAR REQUIREMENTS AND CONSTRAINS FOR MARS EXPLORATION MISSIONS

The most of scientific programs need classic integration procedures according to requirements for dust cleanliness, compatibility with space environment and with mission specifications. Planetary exploration programs, and particularly martian programs for which planetary protection is required need unusual tasks. They must be taken into account early in the mission program in order to planned all works, personnals and funding. The specific tasks are:

- to include planetary protection in mission development plan
- to build specifications including biological decontamination requirements
- to build specific procedures for biological cleanliness at different levels
- to make studies in order to validate methods and procedures
- to specify all materials choice taking into account the effect of decontamination methods
- to follow the tasks of subcontractors. Subcontract are indispensable to support project teams for specific and unusual tasks as sterilization and bioburden assessments.
- to realize integration in unusual environment. In sterile cleanrooms, operators have to deal with clean and sterile procedures for wearing, handling and working. They must at every time control their work and control themselves, bodies entirely covered except eyes. Working in such conditions during a few hours is difficult and teams rotations must be organized in order to limit the duration of teams work to four consecutive hours maximum.

Taking in charge these tasks need additionnal personnal and funding. The total cost of planetary protection tasks for the entire mission has not be calculated, but on the french side, the CNES participation may be estimated, including personnals, at about 1.5 MF (300 000 USD).

BENEFITS FOR FUTURE MARS EXPLORATION MISSIONS

At this time, american MGS 1 and Pathfinder has been subject to planetary protection requirements. For future missions, all works will be usefull for future Mars exploration missions, because new sterilization methods for space application have been validated. Working in sterile conditions, with stringent procedure will also be usefull, and particularly for the next sample return missions for which hard specifications will probably be built.

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