The “PHOENIX” Space Experiment: Study of Space Radiation Impact on Cells Genetic Apparatus on Board the International Space Station

M Yu Karganov\textsuperscript{1,2}, I B Alchinova\textsuperscript{1,2}, E N Yakovenko\textsuperscript{1,2}, V V Kushin\textsuperscript{3,4}, K O Inozemtsev\textsuperscript{3,4}, A Strádi\textsuperscript{5}, J Szabó\textsuperscript{5}, V A Shurshakov\textsuperscript{4} and R V Tolocheck\textsuperscript{4}

\textsuperscript{1} Research Institute for Space Medicine, Federal Biomedical Agency of Russia, Orekhoviy boulevard, 28, Moscow, 115682, Russian Federation
\textsuperscript{2} The Institute of General Pathology and Pathophysiology, Baltiyskaya str., 8, Moscow, 125315, Russian Federation
\textsuperscript{3} National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Kashirskoye shosse, 31, Moscow, 115409, Russian Federation
\textsuperscript{4} Russian Academy of Sciences, Institute of Biomedical Problems, Khoroshevskoye shosse, 76A, Moscow, 123007, Russian Federation
\textsuperscript{5} Hungarian Academy of Sciences, Centre for Energy Research (MTA EK), P. O. B. 49, H-1525, Budapest, Hungary

E-mail: mkarganov@mail.ru

Abstract. The preliminary results of the 1\textsuperscript{st} session of Russian “PHOENIX” long-term space experiment are presented. The survival of dried human lymphocytes and mouse bone marrow cells in 199 days space flight is studied. The degree of DNA fragmentation is analysed for samples flown in different ISS compartments. It is shown that biological data correlates with the results of space radiation dose measurements.

1. Introduction
It is commonly accepted that damage of genetics apparatus of cells in space flight conditions is primarily due to the impact of mixed space radiation. Indirect effects of radiations in water-equivalent medium account for 80–90 \% of the total damage in eukaryotes and prokaryotes. These effects are responsible for cell inactivation.

The Russian “PHOENIX” experiment is designed to study the effects of long-term space flight conditions on the genetic apparatus and viability of dried human lymphocytes and mouse bone marrow cells (BMC). The experiment is carried out onboard the Russian Segment of International Space Station (ISS).

2. Experimental Design, Instruments and Methods
Sealed vials with human lymphocytes and mouse BMC were packed in 12 “Bio-ecology” boxes arranged at 3 containers. Each box was equipped with the temperature sensor and passive space radiation dosimeter.
The containers were delivered to ISS in November 2014 and installed in Pirs, Poisk and Zvezda modules at panels №436, №103 and №103 respectively. Control vials with cells and travel background dosimeters were stored at the laboratory. Three boxes (one from each module) were descended to the Earth in 199 days exposure.

2.1. Methods for preparation and analysis of human lymphocytes and mouse BMC

Lymphocytes were isolated from the whole blood of healthy donors by centrifugation in a density gradient. Mouse BMC were isolated using the modified method [1]. The cell concentration was adjusted to $1 \times 10^6$ per 100 μl, this volume of the cell suspension was transferred into vials and lyophilized [2, 3].

2.1.1. DNA isolation. Lyophilized cells were rehydrated with 150 μl phosphate buffered saline and permeabilized in sucrose buffer. The samples were incubated for 10 min and then centrifuged at 3000 rpm for 15 min (Eppendorf 5702R centrifuge, Germany). The supernatant was decanted, the pellet (cell nuclei) was re-suspended in 400 μl buffer for proteinase K, and 20 μl 10 % SDS was added. After 5-min incubation, proteinase K (5 μl of stock solution with a concentration of 20 mg/ml) was added to final concentration of 250 μg/ml. The samples were incubated with proteinase K at 37° C overnight. Then, 400 μl buffered phenol was added and DNA was isolated routinely. The pellet was dried at room temperature until complete ethanol evaporation, and DNA was dissolved in TE buffer (overnight) [4]. The concentration and purity of isolated DNA were evaluated by the optical density of the obtained solution at 260 nm measured against TE buffer on the Nanodrop 1000 spectrophotometer (Thermo Scientific, USA). The samples were equalized by DNA content.

2.1.2. DNA electrophoresis in 1% agarose gel. Electrophoresis in 1% agarose gel was performed to identify and separate DNA fragments [4]. During separation in gel, the position of DNA was directly determined, as DNA bands in gel were stained with the fluorescent intercalating dye ethidium bromide in a low concentration. The mixture containing 1.0 μl 0.25 % bromphenol blue (BPB) in 50 % glycerin and 5.0 μl sample were applied into the slots of the gels. The current was set at 35 mA and dye migration front was observed. In 20 min, the current was switched off. The results were analyzed using transluminator (Biogene, Russia).

2.1.3. Evaluation of the morphofunctional parameters of cells and their components after rehydration. The cells from vials delivered from ISS and control samples remaining in the laboratory were simultaneously and uniformly rehydrated in sterile PBS. Then, aliquots of the cells suspensions were mixed with Hoechst 33258 fluorescent dye and examined under fluorescent microscope. Hoechst 33258 is the most widespread DNA-specific dye, which allows using it for nucleus contrasting in pro- and eukaryotic cells in both cultures and tissue samples as well as for detection of possible bacterial or mycoplasma contamination of the cell cultures.

2.2. Space Radiation Dosimetry

Passive dosimeters comprising CR-39™ “Tastrak” (TASL Ltd., Bristol, UK) solid state nuclear track detector (SSNTD) and LiF:Mg,Ti (93% 7Li and 7% 6Li “DTG-4” (ROSATOM, Russia) thermoluminescent detector (TLD) were applied in the experiment for the linear energy transfer (LET) spectra and dose measurements [5].

2.2.1. SSNTD treatment & track data analysis. “Tastrak” sheets were etched in 6 N NaOH solution at 70º C for 6 hours. The corresponding bulk etch rate ($V_b$) was 1.35±0.06 μm·h⁻¹ [6]. This value agrees well with result of [7]. After chemical etching the detector’s were manually scanned in 2-D and 3-D modes [6] using the Carl Zeiss® AxioScope.A1 microscope equipped with digital camera. Different geometrical parameters were measured and detector sensitivity $V = V_t / V_b$ (where $V_t$ is the track etch rate) was calculated for every individual track.
The tracks of long-range particles with incident angles close to 90° were evaluated via the so-called “classical” method. In this case diameters of the track opening and value of removed layer thickness are applied for the sensitivity V findings. The sensitivities V were converted to LET values using the calibration function [7]. The detector’s lower LET threshold turned out to be ~7.5 keV/μm (H₂O).

Oblique tracks were evaluated via the procedure firstly described in [8]. Track opening ellipse diameters and full projected length as well as the bottom sphere radius (for over-etched tracks) were applied for the sensitivity V findings. The calibration function [6] was used to obtain the corresponding LET values.

The long-range tracks were considered as primaries. Tracks observed in nuclear fragmentation events and single tracks of short-range particles (over-etched tracks) were attributed to the group of short-range secondaries.

The resulting total event LET spectra are the sum of primaries and secondaries. The track data processing, LET spectra calculations and dose rates evaluation were performed with the help of specially developed software based on MatLab®. The individual LET distributions of the secondaries and corresponding dose calculations were performed separately.

2.2.2. TLD treatment. Thermo-luminescent signal measurements were carried out using the HARSHAW-3500 TLD reader. Subsequent TLD data processing was performed with the help of “Windows Radiation Evaluation and Management System” software. The following parameters were used in the TLD treatment [9]: pre-heating at +50 °C; detector’s heating range +50…+300 °C; detector’s heating rate 4 °C / sec; full annealing time 63 sec; integration interval +150…+250 °C.

3. Results and Discussion

3.1. Cell viability & DNA damage

Cell viability was evaluated by trypan blue exclusion test (Figure 1). The morphology of human lymphocytes and mouse BMC was analyzed after Romanovsky–Giemsa staining (Figure 2). MTT assay showed the presence of mitochondrial activity of the cells after rehydration.

DNA were isolated from the cells and analyzed by electrophoresis followed by densitometry (see Section 2.1 and [10]). Four zones in Figure 3 reveal the most pronounced differences between the biological samples from different boxes. In the material from Poisk and Pirs modules density peaks were found corresponding to 1500-2000 bp values of the DNA marker (GeneRuler 1kb DNA Ladder, Thermo Scientific). The samples from Zvezda and Poisk modules were characterized by 15000 bp peak. In all cases, the peaks of samples from the Pirs module are shifted to the right relatively to the

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**Figure 1.** Hoechst 33258 staining (trypan blue exclusion test): mouse BMC (left), human lymphocytes (right). Upper left in each picture – control sample.

**Figure 2.** Romanovsky–Giemsa staining: mouse BMC (left), human lymphocytes (right). Upper left in each picture – control sample.
samples from the Zvezda and Poisk modules. This indicates the formation of smaller DNA fragments. Similar results were obtained for the human lymphocytes.

Double staining with fluorescent dyes ethidium bromide (necrosis) and acridine orange (visualizing live cells and specifically staining apoptotic cells) revealed cells in different physiological states. Standard methods for evaluation of the nucleus integrity revealed no apparent damage.

**Figure 3.** DNA from mouse BMC electrophoresis and densitometry data.

### 3.2. Space Radiation Data

#### 3.2.1. LET distribution

The LET spectra measured in different locations are presented in Figure 4. The contribution from primary and secondary high-LET space radiations are presented in Figure 5 separately.

**Figure 4.** Total event LET spectra measured in ISS compartments. **Figure 5.** The LET distribution of long-range primaries and short-range secondaries produced in nuclear fragmentations (Pirs module).

The relatively high flux of particles with LET < 100 keV/µm (H₂O) observed in the Pirs module (Figure 4) is supposedly due to the thinner shielding of compartment. The measured flux of secondaries exceeds the flux of primary component at LET > 100 keV/µm in all three modules of ISS. The measured flux of secondaries is 1–2 orders of magnitude higher than flux of long-range component at LET 100–600 keV/µm. Experimental data correlates reasonably with general conclusions of [11-14].
3.2.2. Doses. The dose rates measured by means of passive detectors are summarized in Table 1.

Contribution of nuclear fragments (D\textsubscript{c} and H\textsubscript{c}) into the total absorbed dose D\textsubscript{d} and dose equivalent H\textsubscript{d} varies in 2.0–5.6 % and 19.6–36.5 % respectively. This agrees well with the result [13].

The observed effect of DNA fragmentation depends on dose rate at the compartment. The smaller fragments of DNA were produced in the Pirs module due to the highest dose rates from all components of space radiation. Conversely, the fragments of bigger size were produced in Poisk and Zvezda modules due to relatively small dose rates.

Table 1. Space radiation doses measured in “Bio-ecology” boxes.

| ISS Compartments | TLD D\textsubscript{a}, μGy/day | SSNTD D\textsubscript{b}, μGy/day | Combined D\textsubscript{c}, μGy/day |
|-----------------|-------------------------------|---------------------------------|-------------------------------|
| Pirs            | 693±70                        | 492±40                          | 24±2                          |
| Poisk           | 492±40                        | 30±2\textsuperscript{c}         | 10±2                          |
| Zvezda          | 271±15                        | 271±15                          |
| SSNTD H\textsubscript{b}, μSv/day | 988±36\textsuperscript{c} | 399±28\textsuperscript{c} | 456±33\textsuperscript{c} |
| Poisk Q\textsubscript{b} | 8.6±0.4                      | 13.2±1.2                        | 11.8±1.1                      |
| Zvezda D\textsubscript{c}, μGy/day | 365±31                       | 171±25                          | 257±30                        |
| Combined H\textsubscript{c}, μSv/day | 729±70\textsuperscript{e} | 505±40\textsuperscript{e} | 287±15\textsuperscript{e} |
| Poisk Q\textsubscript{d} | 1602±79\textsuperscript{e}  | 874±49\textsuperscript{e}     | 705±36\textsuperscript{e}   |
| Zvezda          | 2.20±0.24                     | 1.73±0.17                       | 2.45±0.18                     |

\textsuperscript{a} Absorbed dose measured by TLD signal (non corrected).
\textsuperscript{b} SSNTD dose values at LET > 10 keV/\mu m (H\textsubscript{2}O) range.
\textsuperscript{c} Dose values, attributed to nuclear fragments at LET > 10 keV/\mu m (H\textsubscript{2}O) range.
\textsuperscript{d} Total dose values convolved from TLD and SSNTD data by method [15]. The Q(LET) function proposed in ICRP pub. No. 60 was applied in the calculations.
\textsuperscript{e} Sum of primaries and secondaries.

4. Conclusion

The preliminary results of the “PHOENIX” space experiment shows a good survival of human lymphocytes and mouse bone marrow cells nuclei after 199 days space flight, when the doses and dose equivalents varied in the range 57–145 mGy and 140–319 mSv respectively. The degree of DNA fragmentation depends on the dose rate directly. The smallest DNA fragments were detected in the samples exposed at the Pirs module with the maximum dose rates.

The further efforts will be focused on the analysis of data from the next sessions of the “PHOENIX” experiment.

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