Chromosomal instability in rodents caused by pollution from Baikonur cosmodrome

Saule Kolumbayeva · Dinara Begimbetova · Tamara Shalakhmetova · Timur Saliev · Anna Lovinskaya · Benazir Zhunusbekova

Accepted: 7 June 2014/Published online: 3 July 2014 © Springer Science+Business Media New York 2014

Abstract An assessment of the health status of ecosystems exposed to man-made pollution is a vital issue for many countries. Particularly it concerns the consequences of contamination caused by the activity of the space industry. Each rocket launch is accompanied by the introduction of parts of the rocket propellant into the environment. This study aims to scrutinize the effect of the components of rocket fuel on the induction of lipid peroxidation and chromosomal aberrations on rodents inhabiting the area exposed to pollution from Baikonur cosmodrome. The results showed the increase of the level of lipid hydroperoxide and malondialdehyde in the livers of *Citellus pygmaeus* Pallas and *Mus musculus* L., which indicates an augmentation of free radical activity and DNA damage. The cytogenetic analysis of bone marrow cells

S. Kolumbayeva \cdot T. Shalakhmetova \cdot A. Lovinskaya \cdot

B. Zhunusbekova

Department of Biology and Biotechnology, Al-Farabi Kazakh National University, 71 av. Al-Farabi, Almaty 050040, Kazakhstan e-mail: saule.kolumbayeva@kaznu.kz

T. Shalakhmetova e-mail: tamara.shalakhmetova@kaznu.kz

A. Lovinskaya e-mail: ankalav@mail.ru

B. Zhunusbekova e-mail: benazir_zhunusbe@mail.ru

D. Begimbetova \cdot T. Saliev (\boxtimes)

Department of Regenerative Medicine and Artificial Organs, PE "Center for Life Sciences", JSC Nazarbayev University, Unit 9, 53 Kabanbay batyr Ave, Astana 010000, Kazakhstan e-mail: timur.saliev@nu.edu.kz

D. Begimbetova e-mail: dinara.begimbetova@nu.edu.kz revealed that the frequency of chromosomal aberrations was a few times higher in the rodents from contaminated territory. The signs of oxidative stress and high level of chromosomal aberrations indicate the environmental impact of the cosmodrome, and its possible toxic and mutagenic effects on ecosystems.

Keywords Rocket · Cosmodrome · Contamination · Rodents · Lipid peroxidation · Chromosomal aberrations · DNA damage

Abbreviations

UDMH	Unsymmetrical dimethyl hydrazine
NDMA	Nitrosodimethylamine
ROS	Reactive oxygen species
LHP	Lipid hydroperoxide
MDA	Malondialdehyde
NO	Nitric oxide
PLO	Product of lipid oxidation
SH-group	Sulfhydryl group

Introduction

An evaluation of the genetic consequences of environmental pollutants is one of the most pressing issues of the present time. Almost all environmental pollutants are potentially dangerous factors that can have a negative, multi-level impact on an organism. The polluting agents can induce qualitative and quantitative disturbances in ecosystems thus affecting the viability and adaptation ability of natural populations (Cotman and Zagorc-Koncan 2005; Medina et al. 2007; Yadav and Jha 2013).

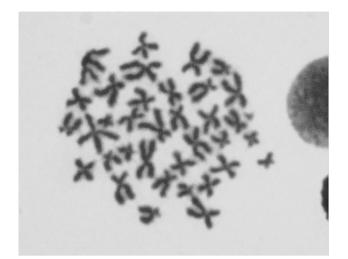


Fig. 1 Karyotype of bone marrow cells of *Citellus pygmaeus*, 2n = 36, Gimsa, $\times 1,000$

One of the major contributors to environmental pollution is the space industry, which has been rapidly growing in recent decades (Erickson et al. 1989; Hansard et al. 2011; Gamper-Rabindran and Finger 2013; Swart 2013). The operation of rocket and space systems may affect the environment through generation of noise, vibration, and contamination by highly toxic rocket fuel as a result of the fall of parts of launched rockets (Yarmishko et al. 1999; Chugunov et al. 2000; Byr'ka et al. 2010). The main cause of soil contamination by fuel components is aerogenic dissemination and ground spills at the places where rocket parts fall to earth. It has been revealed that the fall of the first rocket stage results in the introduction of propellant (from 0.3 up to 2 tons) into the environment. The places, where residual rocket parts fall, usually have an ellipsoidal shape with an area of several hundred thousand square kilometers. Other sources of environmental contamination by rocket fuel are emergency rocket launches, spills during refueling operations, and rocket transportation (Romanov and Romanova 2003).

Since the late fifties, some territories of Kazakhstan and Russia have been experiencing the impact of rocket and space activities, including Baikonur, Plesetsk and others (Villain 1996; Lipanov 2003; Shatalov 2003). The results of field studies conducted at these territories indicated the presence of a component of rocket fuel 'unsymmetrical dimethyl hydrazine' (1,1-Dimethylhydrazine; UDMH) and its oxidation products, including *N*-nitrosodimethylamine (NDMA). NDMA has been found out in the soil, water and plants in the concentrations exceeding maximum permissible dose (Kaplan and Kaplan 1985; Ismagliov et al. 2004; Yang and Liu 2006).

For many years the Ulytau District of Karaganda region has been the landing site for the first stages of rockets. As a

 Table 1
 Distribution of 1,1-DMH at the point of fall of the part of rocket «Proton»

Depth of sample (cm)	C, mg/kg (field data, expedition 09.06.2003)	Soil moisture (%)	C, mg/kg (field data, expedition 14.08.2005)	C, mg/kg (calculated data)
0	122.00	12	52.00	94.1
8	112.00	14	48.80	84.5
20	2.50	14	4.52	9.8
40	0.53	14	0.87	0.18
50	0.22	14	0.53	0.18
68	0.16	14	0.01	0.18

result, the area has been contaminated by the components of rocket propellant, including heptyl and its derivatives such as UDMH (Batyrbekova et al. 2008). It must be noted that in the environment UDMH can either remain stable or it can be oxidized to nitrosodimethylamine (NDMA), which is a strong carcinogen by itself (Liteplo and Meek 2001). As a consequence, the health status of the domestic population has been affected.

The monitoring of the polluted areas near cosmodrome "Baikonur" conducted few years ago showed the regularity of distribution of highly toxic unsymmetrical dimethylhydrazine and its derivative products in the soils of Central Kazakhstan (Fig. 1).

The highest level of the soil pollution by propellants and their derivatives in Central Kazakhstan has been observed at the point of landing of the rocket's vehicle and fuel tank as a result of fuel spillage. It has been also revealed that the levels of 1,1-DMH and its derivatives reduced proportionally with the distance from the point of rocket fall. The high contamination by 1,1-DMH has been observed in the topsoil (0–40 cm height), which exceeds the maximal permissible level more than 1,000 times.

Another dangerous factor is the feasibility of leaking and migrating components of rocket fuel and its derivatives from polluted areas to uncontaminated soils. The speed and progress of spreading of toxic agents depends on many factors, including conditions of natural soil barriers. The transformation of chemical components of rocket fuel occurs most intensively in the first 2 years after exposing to the nature. Then the process gets stabilized, including the levels of 1,1-DMH. Even in the regions, which did not experience the space-rocket activity for long period (over 20 years), the level of contamination of soil by 1,1-DMH still remains high (0.41–6.8 mg/kg) (Table 1).

Moreover, the pollution at space-rocket test sites has been enhanced by specific local climatic conditions. From geographical point of view, the regions adjacent to the Baikonur cosmodrome belong to the desert and semi-desert area types, and they are characterized by low annual rainfall and frequent winds. These areas are characterized by poor biodiversity and low biological productivity, which makes them extremely sensitive and vulnerable to anthropogenic pressure. Therefore, the study of the functional state of natural ecosystems exposed to human activities is extremely important (Ghaznavi 1999; Ellis 2000; Medina et al. 2007; Sun and Tian 2012). Such studies require a biological model in order to characterize the contamination effect caused by human's technological activity. In our case, we have chosen the rodents from polluted areas as a model of bio-indication. Among all the species of mammals, rodents have a special interest for biologists and environmentalists. This is due to large number of rodents found in nature, their broad representation of the different food chains of ecosystems, and the opportunity to extrapolate the results of eco-toxicological analysis of this group to human beings (Schroder et al. 2003; Sanchez-Chardi et al. 2009; Gaschak et al. 2011).

The purpose of this study was to investigate the chromosomal instability in rodents (*C. pygmaeus*; *M. musculus*) as a result of the pollution from Baikonur cosmodrome.

Materials and methods

The results of Russian and Kazakh expeditionary fieldworks conducted at the places of fall of space rocket residuals have indicated the presence of UDMH and its oxidative metabolite *N*-nitrosodimethylamine (NDMA) in the soil, water and plants in higher concentrations comparing to the maximum permissible level (Kasimov et al. 1996; Kenessov 2010). As it was aforementioned, for many years the Ulytau District of Karaganda Region has been used as the landing site for the first stages of rockets, leading to contamination of the territory by the components of propellant (Batyrbekova et al 2008). The presence of these pollutants might have toxic and genotoxic impact on living organisms, including the humans, since all substances circulate in the environment and can be transmitted through the food chain.

As it was noted above, the rodents are convenient objects for the environmental monitoring (Schroder et al. 2003). In Kazakhstan widespread rodent species are little ground squirrels and house mice (*C. pygmaeus* and *M. musculus*). These rodents live in large areas, including polluted areas.

The objects of the study were representatives of the common rodents *C. pygmaeus* and *M. musculus*, trapped in Ulytau District of Karaganda Region (an area affected by pollution from Baikonur cosmodrome), and the rodents captured in the area of the river Sarisu in Kyzyl-Orda region (zone of conventional control). The trapping sites were the peripheries of small settlements and farms near contaminated areas of Ulytau district and conventional

control zone. All trapped animals were males. The rodents were humanely killed via deep anesthesia by using Nembutal sodium solution (pentobarbital sodium). The excised livers were weighed and placed into a chilled 0.05 M Tris–HCl buffer (pH 7) with 0.1 M KCl and 0.9 mM EDTA. Then the organs were triturated with a Potter homogenizer in a 0.05 M Tris–HCl buffer. After this, the extracts were centrifuged for 10 min at $1,000 \times g$ to obtain a 10 % homogenate. The contents of primary and secondary products of lipid hydroperoxide (LHP) and malondialdehyde (MDA) were determined.

For determination of LHP, the diene structures of LHPs were isolated from 10 % liver homogenate by using a mixture of heptane and isopropyl alcohol (ratio of 1:1). Then a solution of HCl (pH 2) was added. The heptane layer was collected and the absorbency was measured at a wavelength of 233 nm on spectrophotometer SF-46 (Milaform, Russia). The contents of the LHPs were calculated taking into account that the molar extinction coefficient is 2.2×10^{-5} cm/M $\times 1$ per 1 g of liver (wet weight) and expressed as mMol/mg.

The MDA content was determined by reaction with thiobarbituric acid (Schmedes and Holmer 1989). 1 ml of 17 % trichloroacetic acid was added to 2 ml of 10 % liver homogenate, and then it was agitated for 10 min. Then 1 ml of 0.8 % thiobarbituric acid was added to the solution, after this it was heated in a steam bath for 10 min, cooled down and centrifuged at $4,000 \times g$ for 10 min in a centrifuge. The color intensity of the produced trimethine complex was detected by using a spectrophotometer SF-46 (Milaform, Russia) at a wavelength of 532 nm. The amount of MDA was calculated using the molar extinction coefficient equal to 1.56×10^{-5} /cm/M on 1 g liver wet weight and expressed as mMol/mg.

Cytological samples for metaphase analysis were prepared by exploiting standard methods (Preston et al. 1987). Prior to sacrificing, each rodent was weighed. The colchicine solution was injected intraperitoneally into the animals (0.04 % of 1 ml per 100 g of body weight). After 1.5–2 h the animals were sacrificed, and then the femurs were excised from the body. The epiphyses were cut. The bone marrow was washed using a syringe with a hypotonic solution of potassium chloride (0.56 %), and placed in a preheated (up to 37 °C) graduated centrifuge tube of 10 ml.

The washed bone marrow was homogenized. Tubes with the cell suspension were placed into water bath at a temperature of 37 °C for 8–12 min. Then the cell suspension was subjected to hypotonic treatment, and centrifuged for 5 min at 1,000 rpm/min. The supernatant was removed with a pipette, and the precipitate was fixed with methanol and glacial acetic acid (3:1), and then placed in the refrigerator for 20 min. The fixative was changed 2–3 times followed by an intermediate re-suspension and centrifuging. For the final fixation the 1.5-2 ml of cooled fixative was added to the suspension.

The fixed cells were carefully re-suspended in a fixative, and 5–6 drops of the suspension was applied with a Pasteur pipette on a chilled wet slide from a height of 5–6 cm. The glass was passed through the flame of a spirit lamp to burn off the fixative. After this the glass was dried in a stream of warm air. The samples were labeled and left before the final drying for 2–3 days, then stained by using Giemsa azure-eosin dye (Merch, Germany).

Cytogenetic analysis was performed by using the metaphase method, where the overall frequency and spectrum of chromosomal aberrations were determined. Metaphase plates were analyzed and photographed under a light microscope Axioskop-40 (Zeiss, Germany). The metaphase plates were accepted for further analysis if they met the following criteria's:

- The metaphase plate should have a roughly round shape and intact chromosomes;
- All chromosomes must be well distributed and uniformly stained; their density should not differ by more than two times;
- All the elements of the plate have to be uniquely identified prior to the count;
- The plates with presence of random chromosomes were excluded from analysis;
- The plates with many overlapping chromosomes, particularly longitudinal, were excluded from analysis due to the possibility of exchange aberrations;
- Only cross-imposition of the long arm was allowed, whilst the longitudinal imposition of big and middle chromosomes was not accepted.

It must be noted that the loss of chromosomes in the metaphase plate is technically inevitable. In this regard, the analysis was allowed only for cells with a chromosome number not less than 40 and not more than 44, i.e. 42 ± 2 chromosomes.

Statistical analysis of the quantitative studies was carried out according to the standard procedure using Student's t test. To obtain an average of the value, statistical indicators were treated by exploiting the conventional methods of variation statistics.

Results and discussion

We have previously demonstrated in in-vivo studies that the toxicity of 1,1-DMH and NDMA contributes to an increase of lipid peroxidation and inhibition of antioxidant enzymes (Mahmoud et al. 2011, 2012). This oxidative stress has been mainly induced by NDMA, which conforms to other reports (Parks et al. 2001; De Felice et al. 2007). Apart from that, we have observed an enhancement of the mutation process in laboratory animals (Mahmoud et al. 2011, 2012; Carlsen et al. 2009).

The accumulation of MDA in the liver of animals from the contaminated areas occurred as a result of reduced activity of antioxidant enzymes, including catalase. It is known that the metabolism of 1,1-DMH and NDMA can lead to the formation of nitric oxide (NO), which binds to the active site of the enzyme therefore reducing its catalytic activity (Brunelli et al. 2001; Sigfrid et al. 2003). As a result of the fall of catalase activity, peroxide has been accumulated in the liver, which enhanced the process of lipid peroxidation.

Table 2 illustrates the results of the determination of biochemical products of lipid peroxidation. The content of LHP (primary product LHP) in the liver of *C. pygmaeus* and *M. musculus* was 1.7 and 1.9 times (p < 0.01) higher in comparison to the animals from the control group. The content of malondialdehyde (secondary PLO) in the liver of both species from the contaminated habitats were 1.6-fold (p < 0.01) higher in comparison to the animals from the animals from the control zone.

The concentrations of 1,1-DMH and NDMA in soil and plants were elevated in the native habitat areas of the rodents (*C. pygmaeus* and *M. musculus*). Such amounts of those agents can cause toxic and genotoxic effects in mammals. In this regard, we have investigated products of lipid peroxidation in liver and levels of chromosomal aberrations of *C. pygmaeus* and *M. musculus*, inhabiting the territories contaminated by rockets propellants.

These results indicate the amplification of free radical processes, which can be caused by the presence of toxic environmental factors, in particular by the components of rocket propellants (1,1-DMH and NDMA).

The results of cytogenetic analysis of bone marrow cells of rodents collected in the area of Ulytau showed increased frequency of metaphases with structural and genomic mutations compared to the rodents from the area of conditional control (Table 3).

The frequency of aberrant and polyploid cells, and the number of chromosome aberrations per 100 metaphases were 2.26, 0.74 % and 2.64 in animals from the control area, while the same indicators of the rodents from the polluted region increased up to 3.2 (p < 0.001), 3.3 (p < 0.05) and 3.5-fold (p < 0.001) respectively. Besides the aneuploid metaphases, the polyploid chromosomes were detected as well. The range of chromosomal aberrations was represented by structural rearrangements of chromosomal and chromatid type, including chromosomal pair terminal fragments. Figures 1 and 2 represent normal karyotype of male *C. pygmaeus* (diploid cell with 36 chromosomes) and chromosomal aberrations respectively. Amid chromosomal aberrations the single concentric rings

Place of rodent trapping	Name of species	Amount of analyzed cells	Frequency of aberrant cells, $(M \pm m)$ (%)	Number of chromosoal aberrations on 100 metaphases			Frequency of polyploid
				Total aberrations	Chromosome type	Chromatid type	cells, (M ± m) (%)
Area of conditional control (near the river Sarisu of Kyzylorda region)	<i>Citellus pygmaeus</i> Pallas	1,050	2.26 ± 0.32	2.64 ± 0.32	0.64 ± 0.21	2.30 ± 0.25	0.74 ± 0.38
	Mus musculus L.	1,120	2.62 ± 0.36	3.00 ± 0.30	0.60 ± 0.17	2.40 ± 0.32	1.00 ± 0.39
Ulytau district of Karaganda region (zone exposed to pollution from Baikonur spaceport)	Citellus pygmaeus Pallas	1,250	7.28 ± 0.83**	9.66 ± 0.78**	1.90 ± 0.44*	7.76 ± 0.71**	2.45 ± 0.45*
	Mus musculus L.	1,310	5.81 ± 0.33**	7.98 ± 0.47**	2.13 ± 0.46*	5.85 ± 0.36**	2.54 ± 0.24*

Table 2 Levels of LHP products and MDA in the liver of *Citellus pygmaeus* and *Mus musculus* inhabiting areas exposed to pollution from the Baikonur cosmodrome

* P < 0.05, ** P < 0.001 in comparison with values of control group

Table 3 Frequency spectrum and structural abnormalities of chromosomes of bone marrow cells of *Citellus pygmaeus* and *Mus musculus* from the habitats of Ulytau District of Karaganda Region

Place of rodent trapping	Name of species	Level of LHP (mMol/mg)	Level of MDA (mMol/mg)
Area of conditional control (near the river Sarisu of Kyzylorda region)	Citellus pygmaeus Mus	2.11 ± 0.19 1.74 ± 0.19	2.69 ± 0.30 2.29 ± 0.29
	musculus L	1.74 ± 0.19	2.27 ± 0.27
Ulytau district of Karaganda region	Citellus pygmaeus	3.50 ± 0.35**	4.39 ± 0.33**
(zone exposed to pollution from Baikonur Spaceport)	Mus musculus L	3.22 ± 0.30**	3.72 ± 0.35*

* p < 0.05, ** p < 0.01 comparing to control

were observed along with paired terminal deletions. The aberrations of the chromatid type were presented by single terminal deletions, point fragments, and acentric rings. The significant increase of chromosomal rearrangements (3.0 times, p < 0.05) and chromatid (3.4 fold, p < 0.001) types was detected. In the spectrum of structural chromosome aberrations the chromatid-type aberrations prevailed (p < 0.001).

Cytogenetic analysis of bone marrow cells of the house mouse (M. musculus from the territory Ulytau District of Karaganda Region) showed elevated levels of cells with chromosome aberrations in comparison with the animals from 'clean' areas (Table 3). Figure 3 demonstrates normal



Fig. 2 Chromosomal pair terminal fragments of bone marrow cells of *Citellus pygmaeus*. Gimsa, $\times 1,000$

karyotype of male *M. musculus* (diploid cell with 40 acrocentric chromosomes).

Thus, the frequency of aberrant and polyploid cells, and the number of chromosomal aberrations in 100 metaphases of *M. musculus* from the control zone were respectively 2.62, 1.00 and 3.00 %; whereas the same parameters of the mouse from contaminated areas were 2.6 (p < 0.001), 2.5 (p < 0.05) and 2.7-fold more (p < 0.001), respectively. Apart from polyploid metaphases (Fig. 4), the cells with the aneuploid set of chromosome were detected. The range of chromosomal aberrations was represented by all types with dominance of violations of chromatid type, including

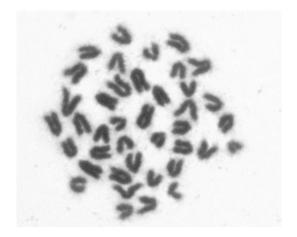


Fig. 3 Karyotype of bone marrow cells of *Mus musculus*, 2n = 40, Gimsa, $\times 1,000$

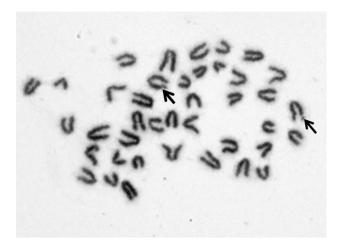


Fig. 5 Chromatid single terminal fragment and chromatid single terminal deletion of bone marrow cells of *Mus musculus* (*black arrows*). Gimsa, $\times 1,000$



Fig. 4 Polyploid bone marrow cells of *Mus musculus*. Gimsa, $\times 1,000$

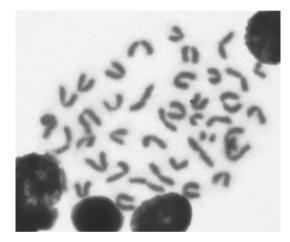


Fig. 6 Chromosomal pair terminal deletions of bone marrow cells of *Mus musculus*. Gimsa, $\times 1,000$

chromatid single terminal fragmentation, chromatid single terminal deletion, and chromosomal pair terminal deletions (Figs. 5, 6). A significant increase in both chromosomal rearrangements (3.6-fold, p < 0.05) and chromatid (1.8 fold, p < 0.001) types was detected in *M. musculus* from the contaminated region. The level of polyploid metaphases in animals from areas affected by Baikonur cosmodrome was significantly higher compared to the control group. Among the samples with polyploid metaphase the cells with mainly tetraploid chromosome were detected.

As a result of cytogenetic studies the fact of chromosomal instability in representatives of *C. pygmaeus* and *M. musculus* species (from areas exposed to the rocket propellants) has been established. It indicates about the presence of genotoxic factors in their natural habitat. The detected high level of chromosomal abnormalities of the chromatid type indicates a significant chemical contamination of the environment. The results showed increasing mutation load in rodent populations from natural ecosystems affected by anthropogenic pressure. An acceleration of the frequency of mutations increases the number of individuals with congenital defects that, ultimately, constitutes a threat for the existence of the general population (Ayas et al. 2000; Beauchamp et al. 2007; Malaquias et al. 2009).

A significant increase of lipid peroxidation products in the rodents liver from contaminated habitats compared to the control area (p < 0.05, p < 0.01), indicates the presence of xenobiotics in the environment. The frequency of structural and genomic mutations in bone marrow cells of the small ground squirrels and the house mice from Ulytau District of Karaganda Region was significantly higher (p < 0.05, p < 0.01) compared to the animals from the control area.

The genotoxic effect of UDMH and its oxidation product NDMA has been already demonstrated by authors of this article in experiments on laboratory animals. The spectrum and high level of chromosomal aberrations in animal models were similar to the genomic and structural abnormalities found in rodents from areas contaminated by heptyl and its derivatives, and to the results of control group too.

It must be noted that many harmful environmental factors can affect the genetic apparatus of the cell, resulting in damage of the genome, which may take the form of chromosomal aberrations (Seo 2012). The level of aberrations may indicate the degree of contamination of the environment; and it can play the role of a biomarker for mutagenic factors (Vargas 2003). One of the most promising approaches for the detection of mutagenic factors in the natural environment is the cytogenetic method. It has been demonstrated that even in optimal conditions for an organism, the various genetic aberrations can spontaneously arise (Yamaguchi 1975; Woodruff and Thompson 1982; Lebedeva et al. 1993; Sal'nikova 2012). When exposed to adverse environmental factors, the frequency of genetic defects may increase as a result of damage to the immune and DNA repairing systems, which can lead to a breach of cytogenetic homeostasis. As a result, there are individuals in a local biosphere with an increased frequency of chromosomal abnormalities. The study of such abnormalities allows an evaluation of the degree of environmental contamination by toxicants.

It has been shown that different pollutants and toxicants can induce lipid peroxidation associated with the activity of free radicals and the production of reactive oxygen species (ROS) in mammals (Goodman and Hochstein 1977; Dickens et al. 1988; Wardle 1990; Ferrari 2000). In turn, ROS may trigger the degradation of membranes, a reduction of activity of enzymes (such as enzymes of cytochrome P450 system) and hormones that can lead to DNA damage, impairment of cell cycles and, ultimately, initiate cell death (Takeuchi et al. 1996; Bennett 2001; Ogawa et al. 2003; Qi 2010). Previously some studies have already demonstrated that free radicals can damage DNA via single-and double-strand breaks, the formation of DNA-protein cross-links, and oxidative modification of nucleotide bases (Cole et al. 1979; Devi et al. 2000; Albino et al. 2006; Bryant 2012; Schipler and Iliakis 2013).

The most common type of DNA damage is single-strand break that, unlike double-strand breaks, can be almost completely repaired. This DNA damage can be triggered by products of lipid peroxidation. The amplification of lipid

peroxidation is an irreversible oxidation of SH-groups of proteins, which can consequently lead to a violation of their functions and the entire chromatin in whole. Moreover, the activation of lipid peroxidation processes may induce the formation of cross-linking protein and protein covalently bonded polymers. The secondary products of lipid peroxidation such as MDA have crosslinking properties (Marnett 1999). It is well-known that the lipid free radicals (lipid peroxyl radicals) have an ability to form covalently linked protein polymers (Kikugawa et al. 1989), including DNA cross-linking, and other variations of crosslink covalent bonds between different amino acid residues of proteins, DNA bases and lipids (Noll et al. 2006). Such changes may result in a breach of transcription and translation, the occurrence of mutations, and the impairment of functional activity of proteins.

Currently the products of lipid peroxidation of chromatin are considered as one of the leading mechanisms of damaging of the nuclear genetic apparatus (Nair and Bartsch 2003; Almeida 2006; Negi et al. 2012; Winczura et al. 2012; Yardim-Akaydin et al. 2013).

Based on our findings we hypothesize that NDMAinduced lipid peroxidation of chromatin can lead to DNA damage. Moreover, UDMH and its oxidation products may adversely affect the cellular repair system, which in turn could contribute to formation of the stable mutations.

Conclusions

The presented study provides the information about the effect of pollution caused by rocket activity on native species found in the wild. The obtained data demonstrated the role of derivatives of rocket fuel in the induction of free radical cascades and the developing of lipid peroxidation. It consequently leads to DNA damage thus affecting the health of the inhabitants of polluted territories. The analysis of such processes can help to extend the understanding of toxic and mutagenic effects of 1,1-DMH and NDMA on living organisms.

During these studies we have demonstrated that chromosome instabilities could play a role of bio-indicators of the level of contamination of the environment by products of space-rocket activities. Based on these findings, one can estimate the genetic risk of this type of eco-toxicants. The gained knowledge about the morphogenetic and cytogenetic changes induced by components of rocket propellant and its derivatives will provide a foundation for developing recommendations regarding ecological regulations for local authorities.

To summarize, the biodiversity conservation of wildlife, as well as improving the health of the population can be addressed through the development and implementation of environmental and health measures on the basis of knowledge about the sensitivity of organisms to the action of harmful environmental factors.

Acknowledgments The authors would like to thank Paul Bartlett for helping to write this article. The experimental work was supported by the Committee of Science Department of Education and Science of the Republic of Kazakhstan through the grants: 'Induced mutagenesis in mammals and measures for decreasing its risk by using biologically active substances of natural origin' (Grant number 0106RK00279, period of grant 2006–2008) and 'Study of genotoxic effects of environmental pollutants on mammals' (Grant number 0112RK00580, period of grant 2012–2014).

Conflict of interest The authors disclose no conflict of interest.

References

- Albino AP, Huang X et al (2006) Induction of DNA double-strand breaks in A549 and normal human pulmonary epithelial cells by cigarette smoke is mediated by free radicals. Int J Oncol 28(6):1491–1505
- Almeida AM (2006) Mitochondrial DNA damage associated with lipid peroxidation of the mitochondrial membrane induced by Fe2+ -citrate. An Acad Bras Cienc 78(3):505–514
- Ayas S, Poblete P et al (2000) Porphyria cutanea tarda: frequency of mutations in the hemochromatosis and uroporphyrinogen decarboxylase gene in patients from germany and its implications for public health services. J Investig Dermatol 115(3):585
- Batyrbekova S, Kenessov B et al (2008) Environmental monitoring of territories of "Baikonur" cosmodrome polluted with highly toxic rocket fuel components. Abstracts of Papers of the American Chemical Society, vol 236
- Beauchamp NJ, Taybert J et al (2007) High frequency of missense mutations in glycogen storage disease type VI. J Inherit Metab Dis 30(5):722–734
- Bennett MR (2001) Reactive oxygen species and death—oxidative DNA damage in atherosclerosis. Circ Res 88(7):648–650
- Brunelli L, Yermilov V et al (2001) Modulation of catalase peroxidatic and catalatic activity by nitric oxide. Free Radical Biol Med 30(7):709–714
- Bryant HE (2012) DNA double-strand break damage and repair assessed by pulsed-field gel electrophoresis. Methods Mol Biol 920:315–321
- Byr'ka AA, Bogolitsyn KG et al (2010) Application of analytical methods for estimating contamination of atmospheric air during launch of carrier rockets of different classes from the Plesetsk Cosmodrome. Inorg Mater 46(15):1627–1631
- Carlsen L, Kenessov BN et al (2009) Assessment of the mutagenic effect of 1,1-dimethyl hydrazine. Environ Toxicol Phar 28(3): 448-452
- Chugunov VA, Martovetskaya II et al (2000) Microbiological degradation of asymmetric dimethylhydrazine, a toxic rocket fuel ingredient. Appl Biochem Microbiol 36(6):544–549
- Cole A, Meyn RE et al (1979) Relative contribution of unrepaired single-strand and double strand DNA breaks to biological damage. Biophys J 25(2):A155–A155
- Cotman M, Zagorc-Koncan J (2005) Risk assessment approaches for ecosystem responses to a point source of pollution on receiving waters. River Basin Manag III 83:315–324
- De Felice FG, Ferreira ST et al (2007) Soluble AE A beta oligomers induce tau hyperphosphorylation and NMDA receptor-mediated oxidative stress. J Neurochem 102:19

- Devi GS, Prasad MH et al (2000) Free radicals antioxidant enzymes and lipid peroxidation in different types of leukemias. Clin Chim Acta 293(1–2):53–62
- Dickens BF, Kramer JH et al (1988) Free-radicals, calcium, and membrane lipid-peroxidation. Faseb J 2(4):A834–A834
- Ellis JB (2000) Risk assessment approaches for ecosystem responses to transient pollution events in urban receiving waters. Chemosphere 41(1–2):85–91
- Erickson ED, Johnson JH et al (1989) Pollution problems from ordnance. 3. Study of the organic contaminants released to the environment during the disposal of rocket motors by burning. J Hazard Mater 21(2):161–176
- Ferrari CKB (2000) Free radicals, lipid peroxidation and antioxidants in apoptosis: implications in cancer, cardiovascular and neurological diseases. Biologia 55(6):581–590
- Gamper-Rabindran S, Finger SR (2013) Does industry self-regulation reduce pollution? Responsible care in the chemical industry. J Regul Econ 43(1):1–30
- Gaschak SP, Maklyuk YA et al (2011) Radiation ecology issues associated with murine rodents and shrews in the chernobyl exclusion zone. Health Phys 101(4):416–430
- Ghaznavi DN (1999) "Effect of agricultural, municipal and industrial pollution on the Indus ecosystem." Indus river: biodiversity, resources, Humankind: pp 42–46
- Goodman J, Hochstein P (1977) Generation of free-radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. Biochem Biophys Res Commun 77(2):797–803
- Hansard R, Maher BA et al (2011) Biomagnetic monitoring of industry-derived particulate pollution. Environ Pollut 159(6):1673–1681
- Ismagliov ZR, Kerzentsev MA et al (2004) Oxidation of unsymmetrical dimethyl hydrazine over oxide and noble metal catalysts. Solution of environmental problems of production, storage and disposal of highly toxic rocket fuels. In: Defense industries: science and technology related to security: impact of conventional munitions on environment and population. NATO Science Series IV-Earth and Environmental Sciences, vol 44. Springer, Netherlands, pp 293–313
- Kaplan DL, Kaplan AM (1985) Biodegradation of N-Nitrosodimethylamine in aqueous and soil systems. Appl Environ Microbiol 50(4):1077–1086
- Kasimov NS, Grebenyuk VB et al (1996) The behavior of rocket-fuel components in soil, water, and plants. Eurasian Soil Sci 28(1):79–95
- Kenessov B, Koziel J et al (2010) Screening of transformation products of 1,1-dimethylhydrazine in soils affected by hydrazine-based rocket fuel spills using solid phase microextraction coupled to gas chromatography-mass spectrometry. Abstracts of Papers of the American Chemical Society, vol 239
- Kikugawa K, Kato T et al (1989) Fluorescent and cross-linked proteins formed by free-radical and aldehyde species generated during lipid oxidation. Lipofuscin Ceroid Pigment 266:345–357
- Lebedeva LI, Skorova SV et al (1993) Possible mechanisms of chromosomal-aberrations. 6. Mitotic delay as a protective process—origin of spontaneous chromosome breaks. Genetika 29(11):1826–1831
- Lipanov AM (2003) Historical survey of solid-propellant rocket development in Russia. J Propul Power 19(6):1067–1088
- Liteplo RG, Meek ME (2001) N-nitrosodimethylamine: hazard characterization and exposure-response analysis. J Environ Sci Health Part C-Environ Carcinog Ecotoxicol Rev 19(1):281– 304
- Mahmoud KE, Shalahmetova T et al (2011) Combined effect of vanadium and nickel on lipid peroxidation and selected parameters of antioxidant system in liver and kidney of male rat. Afr J Biotechnol 10(79):18319–18325

- Mahmoud KE, Shalahmetova TM et al (2012) Antioxidant biological effects of crude oil and its inorganic component (vanadium) in liver and kidney of rats. J Animal Vet Adv 11(5):124–131
- Malaquias AC, Brasil AS et al (2009) The G allele at KRAS IVS4 + 92 is associated with higher frequency of congenital heart disease and severity of short stature in Noonan syndrome patients with PTPN11 mutations. Horm Res 72:462
- Marnett LJ (1999) Lipid peroxidation—DNA damage by malondialdehyde. Mutat Res Fund Mol Mech Mutagen 424(1–2):83–95
- Medina MH, Correa JA et al (2007) Micro-evolution due to pollution: possible consequences for ecosystem responses to toxic stress. Chemosphere 67(11):2105–2114
- Nair J, Bartsch H (2003) Oxidative stress and lipid peroxidationinduced DNA damage in human metal storage diseases. Naunyn-Schmiedebergs Arch Pharmacol 367:R3–R3
- Negi R, Pande D et al (2012) In vivo Oxidative DNA damage and lipid peroxidation as a biomarker of oxidative stress in preterm low-birthweight infants. J Trop Pediatr 58(4):326–328
- Noll DM, Mason TM et al (2006) Formation and repair of interstrand cross-links in DNA. Chem Rev 106(2):277–301
- Ogawa Y, Kobayashi T et al (2003) Radiation-induced reactive oxygen species formation prior to oxidative DNA damage in human peripheral T cells. Int J Mol Med 11(2):149–152
- Parks JK, Smith TS et al (2001) Neurotoxic A beta peptides increase oxidative stress in vivo through NMDA-receptor and nitricoxide-synthase mechanisms, and inhibit complex IV activity and induce a mitochondrial permeability transition in vitro. J Neurochem 76(4):1050–1056
- Preston RJ, Dean BJ et al (1987) Mammalian in vivo cytogenetic assays—analysis of chromosome-aberrations in bone-marrow cells. Mutat Res 189(2):157–165
- Qi XY (2010) Reactive oxygen species scavenging activities and inhibition on DNA oxidative damage of dimeric compounds from the oxidation of (-)-epigallocatechin-3-O-gallate. Fitoterapia 81(3):205–209
- Romanov VI, Romanova RL (2003) Risk factors of negative impact on objects of the environment upon accidents during launches of rocket-space hardware. Cosm Res 41(5):494–501
- Sal'nikova L, Chumachenko A et al. (2012) "Association study of the frequencies of spontaneous and induced chromosome aberrations in human lymphocytes." Radiobiol Environ Secur, pp 187–198
- Sanchez-Chardi A, Penarroja-Matutano C et al (2009) Bioaccumulation of metals and effects of a landfill in small mammals Part III: structural alterations. Environ Res 109(8):960–967
- Schipler A, Iliakis G (2013) DNA double-strand-break complexity levels and their possible contributions to the probability for error-prone processing and repair pathway choice. Nucleic Acids Res 41(16):7589–7605
- Schmedes A, Holmer G (1989) A new thiobarbituric acid (Tba) method for determining free malondialdehyde (Mda) and hydroperoxides selectively as a measure of lipid-peroxidation. J Am Oil Chem Soc 66(6):813–817

- Schroder JL, Basta NT et al (2003) Ecotoxicological risks associated with land treatment of petrochemical wastes. I. Residual soil contamination and bioaccumulation by cotton rats (*Sigmodon hispidus*). J Toxicol Environ Health Part A 66(4):305–325
- Seo YR (2012) Genomic approach for understanding the mutagenic and carcinogenic mechanisms of heavy metal nickel in terms of gene–environment interaction. Int J Mol Med 30:S65–S65
- Shatalov DV (2003) The history of the beginning of the Russian Plesetsk cosmodrome. Hist Rocket Astronaut 25:137–144
- Sigfrid LA, Cunningham JM et al (2003) Cytokines and nitric oxide inhibit the enzyme activity of catalase but not its protein or mRNA expression in insulin-producing cells. J Mol Endocrinol 31(3):509–518
- Sun HM and Tian WJ (2012) "A review of impacts of oil pollution on coastal wetland ecosystem." Progress in environmental science and engineering (Iceesd 2011), Pts 1–5 356–360: 2551–2554
- Swart J (2013) Intra-industry trade and heterogeneity in pollution emission. J Int Trade Econ Dev 22(1):116–139
- Takeuchi T, Nakajima M et al (1996) Relationship between the intracellular reactive oxygen species and the induction of oxidative DNA damage in human neutrophil-like cells. Carcinogenesis 17(8):1543–1548
- Vargas VMF (2003) Mutagenic activity as a parameter to assess ambient air quality for protection of the environment and human health. Mutat Res Rev Mutat Res 544(2–3):313–319
- Villain J (1996) A brief history of Baikonur. Acta Astronaut 38(2):131–138
- Wardle EN (1990) Free-radicals and lipid-peroxidation in relation to pathophysiological mechanisms. Saudi Med J 11(3):181–186
- Winczura A, Zdzalik D et al (2012) Damage of DNA and proteins by major lipid peroxidation products in genome stability. Free Radical Res 46(4):442–459
- Woodruff RC, Thompson JN (1982) Genetic-factors that affect rates of spontaneous mutation and chromosome-aberrations in *Dro*sophila melanogaster. Cytogenet Cell Genet 33(1–2):152–159
- Yadav VB, Jha SK (2013) Status of trace and toxic elements pollution in creek ecosystem using TXRF method. J Radioanal Nucl Chem 295(3):1759–1762
- Yamaguchi O (1975) Spontaneous occurrence of chromosomeaberrations in *Drosophila melanogaster*. Jpn J Genet 49(5): 332–333
- Yang R, Liu JH, et al (2006) "Toxic mechanism and protection of unsymmetrical dimethyl hydrazine (UDMH)." Proceedings of the 3rd International academic conference on environmental and occupational medicine, pp 132–134
- Yardim-Akaydin S, Caliskan-Can E et al (2013) Lipid peroxidation and DNA damage in apnea patients with or without metabolic syndrome. Sleep Biol Rhythms 11(2):116–124
- Yarmishko VT, Yarmishko MA et al (1999) Response of plants to highly toxic components of liquid-propellant rocket fuel. Russ J Ecol 30(6):435–439