ISSN 2079-0597, Russian Journal of Genetics: Applied Research, 2016, Vol. 6, No. 2, pp. 191–196. © Pleiades Publishing, Ltd., 2016.
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# The Cytogenetic Analysis of the Population of Ecologically Different Regions of North Ossetia

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Received May 15, 2014; in final form, September 30, 2014

**Abstract**—Blood samples of three groups of populations from different regions of North Ossetia situated at different distances from the source of pollution (a production plant with accidental releases) were used for cytogenetic analysis. Significant differences in the frequency of chromosome aberrations in leukocytes were found. The frequency was highest ($4.3 \pm 0.5$) in the population of the area situated at a distance of less than 3 km from the production plant. In the group of more distant areas (more than 5 km) this value was lower ($2.8 \pm 0.3$; $p \pm \leq \pm 0.003$). The frequency of chromosome aberrations in children from these groups was $4.1 \pm 0.7$ and $1.3 \pm 0.5$, respectively ($p \leq 0.04$). An increase in the percentage of chromosome-type aberrations in the population of North Ossetia may result not only from chemical but also from radiation mutagenesis. The application of Afobazole for the prevention of mutagenesis proved its protective properties ($p \leq 0.05$).

*Keywords*: chromosome aberrations, mutagenesis, environmental conditions, Afobazole

**DOI:** 10.1134/S2079059716020027

The effect of pollutants on human heredity, their ability to damage genetic mechanisms, reproductive system, and prenatal development, and to cause malignant conditions are among the most important current global problems (Bochkov, 2003; Bochkov and Durnev, 2011; Minina et al., 2009; Boffetta and Nyberg, 2003; Hagmar, 2004; Merlo et al., 2007; Norppa et al., 2006; Vodicka et al., 2010). The impact of pollutants is especially significant in the areas of the Republic of North Ossetia–Alania with a high level of anthropogenic load, in particular, in the industrial center of the republic (Vladikavkaz), where large areas are covered or affected by the transport system and plants of the nonferrous metal industry, metalworking, and mechanical engineering (Revich, 2007). According to a number of authors (Menchinskaya, 2004; Skupnevsky, 2006; Zengelidi, 2009) and the data of the North-Ossetian agrochemical laboratory and geological exploration, the level of soil pollution by eight heavy metals at a distance of 1 km from the plants of the nonferrous metal industry is extremely hazardous. The level of soil pollution in other areas of the city is highly hazardous. The soil concentration of heavy metals is extremely high even in some of the most distant suburban zones. A significant amount of the available data proves the genotoxic properties of heavy metal compounds (Bochkov and Chebotarev, 1989; Durnev and Seredenin, 1998; Chopikashvili et al., 1989; Chervona et al., 2012; Coelhoa et al., 2013; Salnikow and Zhitkovich, 2008). The unstable environmental situation and the global deterioration of the biosphere make the wide use of genetic monitoring indispensable. Genetic analysis is especially important for the estimation of disorders occurring before any morphological, population, or other changes can be seen (*Biological Control*..., 2010; Battershill et al., 2008; Sram et al., 2007). The purpose of this work was to determine the frequency of cells with chromosome aberrations in the leukocyte cultures of the population of ecologically different regions of North Ossetia after an accident at an iron and steel plant.

## MATERIALS AND METHODS

We analyzed the blood samples of 55 citizens of North Ossetia: 47 adults and 8 children (2 boys and 6 girls). The percentage of ages and sexes is presented in Table 1. The group of adults included 13 men (5 smokers) and 34 women (all nonsmokers). The age of adult subjects varied in the range from 18 to 75 years; the mean age was $34 \pm 3.2$ years. The age of the children varied from 8 to 17 years; the mean age was $14.6 \pm 1.0$ years. Moreover, one boy (15 years) and 6 adults (with the mean age of $31 \pm 3.7$ years) were examined twice: before and after the treatment with the use of Afobazole. The groups differed in the environmental conditions of their living areas. The main sources of pollution in Vladikavkaz are two large iron and steel plants: Elektrotsink (the second-largest factory in Russia) and Pobedit. From 1905 till 1990 the

**Table 1.** The age and sex distribution of the studied groups

| Groups | The number of subjects | | | Mean age ($X \pm S_X$) | | |
|---|---|---|---|---|---|---|
| | total number | men | women | total number | men | women |
| Group I | 24 | 5 | 19 | 31 ± 2.1 | 41 ± 5.4 | 28 ± 1.9 |
| Group II | 12 | 5 | 7 | 42 ± 5.4 | 36 ± 5.6 | 46 ± 8.4 |
| Group III | 11 | 3 | 8 | 34 ± 5.2 | 19 ± 1.0 | 40 ± 4.0 |
| Group IV | 4 | – | 4 | 16 ± 0.7 | – | 16 ± 0.7 |
| Group V | 4 | 2 | 2 | 14 ± 2.0 | 12 ± 3.5 | 16 ± 0.9 |
| Group VI | 7 | 2 | 5 | 31 ± 3.7 | 24 ± 8.5 | 34 ± 3.7 |
| Group VII | 7 | 2 | 5 | 31 ± 3.7 | 24 ± 8.5 | 34 ± 3.7 |

Elektrotsink factory processed complex ore concentrates produced by the Sadonsky lead-zinc combine (which is situated in the mountainous areas of North Ossetia) and produced zinc, lead, cadmium, sulfuric acid, copper, and a number of secret rare earth metals. Millions of tons of highly toxic waste has been accumulated during the period of operation of this plant. In October 2009, an accident occurred at the plant; it led to long-term accidental emissions. This study was performed after the accident. The atmospheric air of Vladikavkaz contains more than 100 different chemical compounds. The most hazardous and widespread of them include sulfur dioxide, nitrogen dioxide, carbon monooxide, hydrogen chloride, ammonia, and a number of heavy metals. Vladikavkaz has a technogenic dispersion halo of heavy metals; the formation of this halo is mostly influenced by the abnormal and extremely high concentrations of lead, cadmium, zinc, copper, silver, mercury, arsenic, tungsten, manganese, indium, bismuth, antimony, and others (Menchinskaya, 2004). The geographical location of Vladikavkaz, which is surrounded by mountains and weather with zero or low wind, led to a low intensity of pollutant dispersion in the air and to their accumulation in the lowest atmospheric layer, which has resulted in the pollution of other environments, including water bodies, bottom sediments, and soils. The participants were divided into five groups. Group I included 24 adults living at a distance of more than 5 km from nonferrous factories; this was the control group. Group II included 12 adults living at a distance of 3–5 km from the factories. Group III included 11 adults living at a distance of less than 3 km from the factories. Group IV included 4 children living at a distance of 10–15 km. Group V included 4 children living at a distance of less than 5 km. Two more groups (VI and VII) were formed by 14 subjects living at a distance of less than 5 km from the factories in order to investigate the effect of Afobazole on the rate of aberrant metaphases. Because of the unfavorable ecological situation of the region, some of the subjects received Afobazole, which is a selective anxiolytic (2-[2-(morpholino)-ethylthio]-5-ethoxybenzimidazole-hydrochloride). The medication and its dose were chosen based on the findings of some studies that proved its antimutagenic and antiteratogenic activity (Durnev et al., 2010; Shreder et al., 2008). We used Afobazole (Farmstandart) at a daily dose of 30 mg, 3 times per day during 14 days. Groups VI and VII included 7 subjects before and after receiving Afobazole, respectively; the blood samples were collected 24 h after the end of the therapy. The cytogenetic analysis was performed with the use of preparations of lymphocyte metaphase chromosomes cultivated in vitro according to the standard procedure (Bochkov, 1974). Whole blood (1 mL) was incubated by the macromethod in a complex medium consisting of 7.5 mL of RPMI 1640 medium (Sigma) and 1.5 of fetal serum (BioClot) in 15 mL tubes. In order to stimulate cell fission, 0.01 mg of phytohemagglutinin (PanEco) was added to each tube. The samples were fixed after 48 h of cultivation. Two hours before fixation, 50 μL of colchicine was added to the cultures; after the end of cultivation, the cells were treated with hypotonic solution of 0.55% KCl at a temperature of 37°C for 15 minutes. The fixation was carried out with the use of three portions of ethanol-acetate fixative (3 : 1) for 50 minutes. The obtained cell suspension was applied on cooled microscope slides, dried, coded, and stained by a 2% Giemsa stain solution. We analyzed 100 to 300 metaphase plates of each subject; we counted the percentage of cells with chromosome aberrations, the number of single fragments, chromatid exchanges, coupled fragments, and chromosome exchanges. Achromatic gaps were not included in the range of aberrations and were counted separately (Bochkov, 1974). The total number of analyzed metaphase plates is 15270. The data were processed with the use of Microsoft Office Excel 2007 and StatPlus 2009. The groups were compared by the method of the nonparametric Mann–Whitney U test.

**Table 2.** The frequency of chromosome aberrations in the blood cells of subjects

| Groups | The number of subjects | The number of analyzed metaphases | The number of metaphases with aberrations ($M \pm m$), % | The frequency of aberrations (%) | | | | Gaps (%) | Polyploidy (%) | The frequency of cells with more than one aberration (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | single fragments | chromatid exchanges | coupled fragments | chromosome exchanges | | | |
| Group I | 24 | 4000 | 2.8 ± 0.3 | 1.3 | 0.1 | 0.5 | 1.0 | 1.1 | 0.1 | 0.1 |
| Group II | 12 | 1610 | 3.4 ± 0.5 | 2.1 | 0.3 | 0.9 | 0.7 | 1.3 | 0.1 | 0.4 |
| Group III | 11 | 1940 | 4.3 ± 0.5* | 2.2* | 0.1 | 1.3* | 1.2 | 1.8 | 0.3 | 0.3 |
| Group IV | 4 | 530 | 1.3 ± 0.5 | 1.1 | 0.2 | 0.2 | 0.2 | 1.7 | – | – |
| Group V | 4 | 800 | 4.1 ± 0.7** | 2.4 | 0.4 | 1.0 | 1.0 | 1.8 | 0.1 | 0.6 |
| Group VI | 7 | 1500 | 4.0 ± 0.5 | 1.9 | 0.2 | 1.2 | 1.2 | – | – | 0.5 |
| Group VII | 7 | 1300 | 2.6 ± 0.4*** | 1.6 | – | 0.4 | 0.5 | 1.1 | – | 0.1 |

Comparison of groups by the Mann–Whitney test: *—with group I ($p < 0.001$ for % of metaphases with aberrations; $p \leq 0.04$ for single fragments; $p \leq 0.009$ for coupled fragments); **—groups IV and V ($p \leq 0.04$); ***—groups VI and VII ($p \leq 0.05$).

## RESULTS AND DISCUSSION

The mean frequencies of the aberrations and their different types in the lymphocyte cultures of subjects living in ecologically different regions of North Ossetia are presented in Table 2. The cytogenetic analysis of the blood samples of adults and children showed that there are significant differences in the percentage of cells with chromosome aberrations in subjects living near the factories. The frequency of aberrations in group III differed significantly from that in group I ($p \leq 0.003$). The most frequent type of aberrations in all the studied groups was single fragments; however, their percentage was lower than their predicted frequency in intact populations (Bochkov, 2001; Druzhinin, 2003; Sram et al., 2004). The increase in the frequency of chromosome aberrations was mainly due to double-hit rearrangements: coupled fragments and chromosome exchanges. As we can see in Table 2 and Fig. 1, the percentage of coupled fragments increased with a decrease in the distance from the factory and was 1.6 times higher in group III than in group I ($p \leq 0.009$).

It was found that the share of chromosome exchanges increased in all groups of adults (Fig. 1), which probably resulted from the dispersion of emissions from stationary sources of pollution along the direction of the rising wind to a distance of more than 5 km (Menchinskaya, 2004). Moreover, a number of crowded places (such as secondary and higher educa-

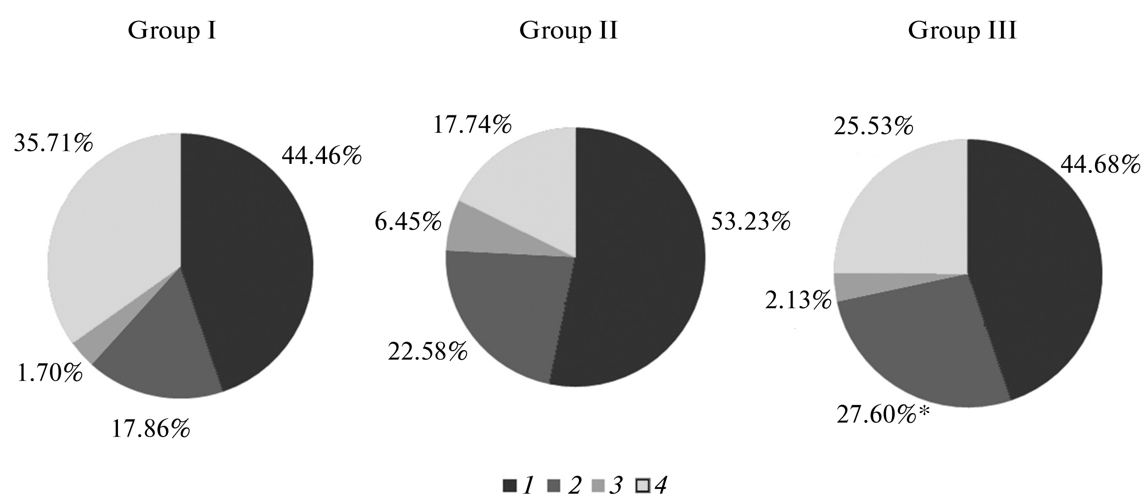


**Fig. 1.** The range of chromosome aberrations in the blood cells of subjects: *1*—single fragments; *2*—coupled fragments; *3*—chromatid exchanges; *4*—chromosome exchanges. Comparison with group I by the Mann–Whitney test: *—$p \leq 0.009$.

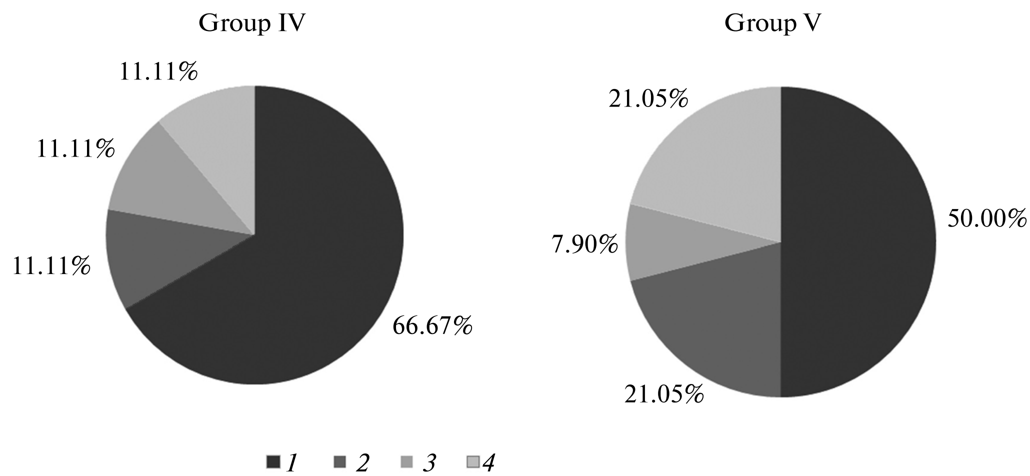


**Fig. 2.** The range of chromosome aberrations in the blood cells of children. *1*—single fragments; *2*—coupled fragments; *3*—chromatid exchanges; *4*—chromosome exchanges.

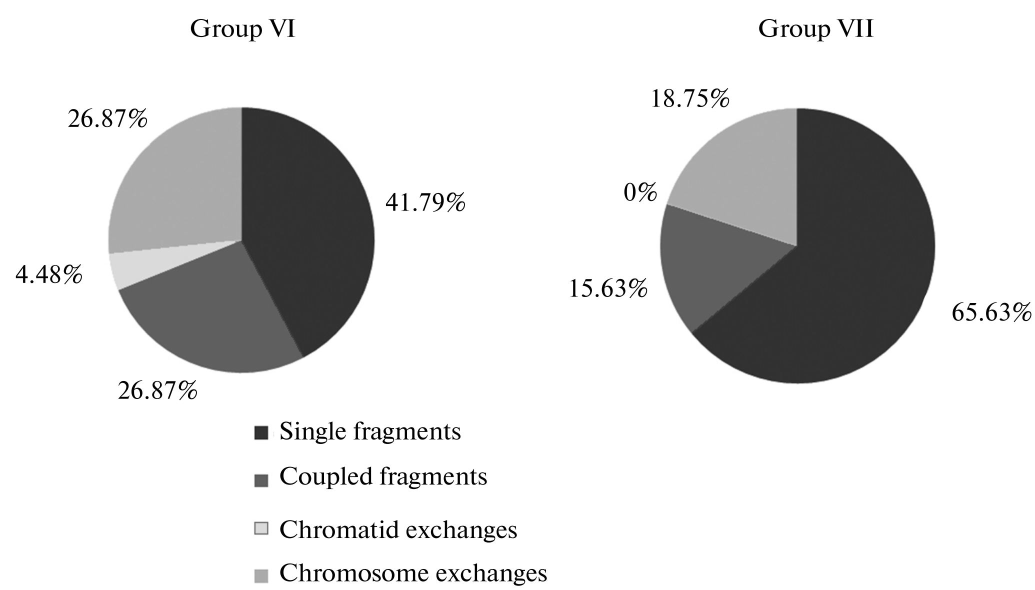


**Fig. 3.** The range of chromosome aberrations in the blood cells of subjects before and after receiving Afobazole.

tional institutions and hospitals) are situated in the city center, i.e., in close proximity to the factories. As Table 2 shows, the mean frequency of cells with chromosome aberrations differed significantly between the groups of children ($p \leq 0.04$). For example in group V the mean frequency of the aberrant metaphases was triple that in group IV; and the share of coupled fragments and chromosome exchanges was higher (Fig. 2). Moreover, cells with more than one aberration were found in the samples of children living near the factories, while in the group of children living at a distance of 10–15 km from the factories such cells were not recorded. The comparative analysis of samples of subjects before and after receiving Afobazole showed that there are significant differences in the frequencies of the aberrations between these groups ($4.0 \pm 0.5$ and $2.6 \pm 0.4$, respectively; $p \leq 0.05$). The percentage of coupled fragments and chromosome exchanges was lower after the course of antimutagenic treatment (by 11.24% and 8.12%, respectively) (Table 2, Fig. 3). The frequency of cells with more than one aberration also decreased after receiving Afobazole (from 0.5 to 0.1 per 100 metaphase plates). Our findings correspond with the data that the derivatives of 2-mercaptobenzimidazole can be considered as the most efficient antimutagenic compounds and are active against a wide range of mutagens (Durnev, 2008). Thus, the data obtained by the cytogenetic analysis of the adults and children of North Ossetia provide some evidence that the frequency of chromosome mutations varies in different regions, which may result from different levels of technogenic environmental pollution. The quantitative and qualitative analysis of chromosome aberrations shows that mutagenesis is caused by a complex of both chemical and physical factors. An increase in the percentage of double-hit aberrations, which are the markers of radiation pollution, may be caused by both natural and anthropogenic factors. The accumulation of radioactive wastes in the areas of factories situated in the city center also has some effect. Earlier radioactive wastes were accumulated in the Groznevsky repository. Since 1992, radioactive wastes were kept at the factories because of military operations in the Chechen Republic; the working sites were unequipped for this purpose, and the wastes were mishandled (Menchinnskaya, 2004). The mutagenicity of cadmium ions is partially due to its radioactive isotope which occurs in nature and provides β-rays (Ruposhev, 1976; Ruposhev, Garina, 1976). Some other factors, such as the radiation background in the mountain regions, can also have some effect. Thus, the cytogenetic analysis of blood samples shows that the frequency of cells with chromosome aberrations in the groups of the population living near the production plant with toxic accidental emissions is significantly higher than that in the groups of the population living at a distance of more than 5 km from the plant (4.3 ± 0.5% and 2.8 ± 0.3%, respectively; $p \leq 0.003$). The frequencies of the aberrations in the groups of children were 4.1 ± 0.7% and 1.3 ± 0.5%, respectively; $p \leq 0.04$). The frequency of chromosome aberrations in subjects receiving Afobazole after the course of treatment was significantly lower than before the course.

## REFERENCES


*Biologicheskii kontrol' okruzhayushchei sredy: Geneticheskii monitoring* (Biological Control Environment: Genetic Monitoring), Geras'kin, S.A. and Sarapul'tseva, E.I., Eds., Moscow: Akademiya, 2010.

Battershill, J.M., Burnett, K., and Bull, S., Factors affecting the incidence of genotoxicity biomarkers in peripheral blood lymphocytes: Impact on design of biomonitoring studies, *Mutagenesis*, 2008, vol. 23, no. 6, pp. 423–437.

Bochkov, N.P., Chebotarev, A.N., Katosova, L.D., and Platonova, V.I., The database for analysis of quantitative characteristics of chromosome aberration frequencies in the culture of human peripheral blood lymphocytes, *Russ. J. Genet.,* 2001, vol. 37, no. 4, pp. 440–447.

Bochkov, N.P., *Metod ucheta khromosomnykh povrezhdenii kak biologicheskii indikator vliyaniya faktorov vneshnei sredy na cheloveka* (Method of Accounting of Chromosomal Damage as a Biological Indicator of the Impact of Environmental Factors on Humans), Moscow: Nauka, 1974.

Bochkov, N.P. and Chebotarev, A.N., *Nasledstvennost' cheloveka i mutageny vneshnei sredy* (Human Heredity and Environmental Mutagens), Moscow: Meditsina, 1989.

Bochkov, N.P., Human ecological genetics, *Ekol. Genet.*, 2003, vol. 1, pp. 16–21.

Bochkov, N.P. and Durnev, A.D., The obvious and incredible about the concepts of mutation process in humans, *Gig. Sanit.*, 2011, vol. 5, pp. 9–10.

Boffetta, P. and Nyberg, F., Contribution of environmental factors to cancer risk, *Br. Med. Bull.,* 2003, vol. 68, pp. 71–94.

Chervona, Y., Arita, A., and Costa, M., Carcinogenic metals and the epigenome: Understanding the effect of nickel, arsenic, and chromium, *Metallomics*, 2012, vol. 4, no. 7, pp. 619–627.

Chopikashvili, L.V., Bobyleva, L.A., and Zolotoreva, G.N., Genotoxic effects of heavy metals and their salts in the experiment on *Drosophila* and mammals, *Tsytol. Genet.*, 1989, no. 3, pp. 35–38.

Coelho, P., Garciá-Lestón, J., Costa, S., et al., Genotoxic effect of exposure to metal(loid)s. A molecular epidemiology survey of populations living and working in Panasqueira mine area, Portugal, *Environ. Int.*, 2013, vol. 60, pp. 163–170.

Druzhinin, V.G., Quantitative characteristics of chromosome aberration frequency in the human population of a large western Siberian industrial region, *Russ. J. Genet.,* 2003, vol. 39, no. 10, pp. 1373–1380.

Durnev, A.D., Solomina, A.S., Zhanataev, A.K., et al., Effect of afobazole on genotoxic effects of tobacco smoke in the placenta and in the tissues of rat embryos, *Byull. Eksp. Biol. Med.*, 2010, no. 3, pp. 286–289.

Durnev, A.D. and Seredenin, S.B., *Mutageny - skrining i farmakologicheskaya profilaktika vozdeistvii* (Mutagens: Screening and Pharmacological Prevention Effects), Moscow: Meditsina, 1998.

Durnev, A.D., Methodological aspects of research involving the modification of chemical mutagenesis, *Byull. Eksp. Biol. Med.*, 2008, vol. 9, pp. 281–287.

Hagmar, L., Stromberg, U., and Bonassi, S., Impact of types of lymphocyte chromosomal aberrations on human cancer risk. Results from Nordic and Italian cohorts, *Cancer Res.*, 2004, vol. 64, pp. 2258–2263.

Menchinskaya, O.V., Ecological and geochemical aspects of technogenic pollution of metallurgical centers (on the example of Vladikavkaz), *Cand. Sci. (Geol.-Mineral.) Dissertation*, Moscow, 2004.

Merlo, D.F., Ceppi, M., Stagi, E., et al., Baseline chromosome aberrations in children, *Toxicol. Lett.*, 2007, vol. 30, pp. 60–67.

Minina, V.I., Druzhinin, V.G., Glushkov, A.N., et al., Quantitative characteristics of chromosomal aberration frequency in the residents of the regions with different levels of cancer incidences, *Russ. J. Genet.*, 2009, vol. 45, no. 2, pp. 211–217.

Norppa, H., Bonassi, S., Hansteen, I.L., et al., Chromosomal aberrations and SCEs as biomarkers of cancer risk, *Mutat. Res.*, 2006, vol. 600, pp. 37–45.

Revich, B.A., "*Goryachie tochki" khimicheskogo zagryazneniya okruzhayushhei sredy i zdorov'e naseleniya Rossii* ("Hot Spots" of the Chemical Pollution of the Environment and Public Health in Russia), Zakharov, V.M., Ed., Moscow: Akropol', 2007.

Ruposhev, A.R. and Garina, K.P., The mutagenic effect of cadmium salts, *Tsytol. Genet.*, 1976, vol. 10, no. 5, pp. 32–36.

Ruposhev, A.R., Cytogenetic effect of heavy metal ions on the seeds *Crepis capillaries* L., *Genetika*, 1976, vol. 12, no. 3, pp. 37–43.

Salnikow, K. and Zhitkovich, A., Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: Nickel, arsenic and chromium, *Chem. Res. Toxicol.,* 2008, vol. 21, no. 1, pp. 28–44.

Shreder, O.V., Smol'nikova, N.M., Durnev, A.D., and Seredenin, S.V., Effect of afobazole on teratogenic effects of cyclophosphamide in rats, *Byull. Eksp. Biol. Med.*, 2008, vol. 4, pp. 414–417.

Skupnevskii, S.V., *Abstract of Cand. Sci. (Biol.) Dissertation*, Vladikavkaz, 2006.

Sram, R.J., Rossner, P., and Smerhovsky, Z., Cytogenetic analysis and occupational health in the Czech Republic, *Mutat. Res.*, 2004, vol. 566, pp. 21–48.

Sram, R.J., Beskida, O., Binkova, B., and Chvatalova, I., Chromosomal aberrations in environmentally exposed population in relation to metabolic and DNA repair genes polymorphisms, *Mutat. Res.*, 2007, vol. 620, pp. 22–33.

Vodicka, P., Polivkova, Z., Sytarova, S., et al., Chromosomal damage in peripheral blood lymphocytes of newly diagnosed cancer patients and healthy controls, *Carcinogenesis*, 2010, vol. 31, no. 7, pp. 1238–1241.

Zengelidi, V.V., The impact of anthropogenic pollution on soils in Vladikavkaz, *Cand. Sci. (Biol.) Dissertation*, Vladikavkaz, 2009.

*Translated by Ya. Lavrenchuk*