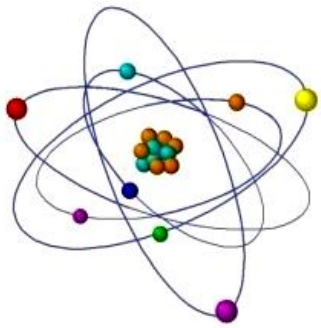
GONADAL DYSGENESIS AS A MARKER OF GENOMIC INSTABILITY IN POPULATIONS OF *DROSOPHILA MELANOGASTER* FROM DIFFERENT OF RADIATION FACTOR IMPACT REGIONS OF UKRAINE



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ABSTRACT: *To investigate the differences of the gonadal dysgenesis frequency as an indicator of the activation of mobile elements through natural populations of Drosophila melanogaster, selected from Ukrainian regions with different radiation impact. Follow-up study of the dynamics of this indicator under chronic exposure in laboratory conditions for 10 generations. The study was conducted in two stages. The first one included trapping of insects in regions with different radiation load with subsequent assessment of both the time of maturation and the index of the gonadal dysgenesis through the first (F1) generation, obtained in laboratory conditions. At the second stage, the dynamics of this indicator was investigated for the F1- descendants of each ten consequent generations, which were developed both with and without additional gamma-exposure. Differences of the gonadal dysgenesis frequency as an indicator of the activation of mobile elements were revealed in F1 - descendants of natural populations of Drosophila melanogaster, selected from regions of different radiation impact. Under conditions of additional low rate chronic irradiation in laboratory conditions for 10 generations, significant differences in changes in the level and dynamics of this indicator were established depending on the accumulated dose of Drosophila populations from the city of Netishyn (Khmelnytskyi NPP) and Magarach city. There were no signs of adaptation.*

The discrepancy between the real and expected biological effects has reflected the difference in the intensity of the radiation background, which was traditionally determined by the gamma - emitters and did not take into account the wide range of other genotoxic elements from nuclear power emissions. A complex, non-monotonic type of frequency dynamics of gonadal dysgenesis could be determined by the interaction of radiation damage, protection and recovery.

Key words: Drosophila melanogaster; radiation, gonadal dysgenesis, genomic instability

INTRODUCTION

Genetic and radiobiological studies of the last decades have shown that the most important effect of radiation exposure with low intensity is the emergence of genomic instability and its transmission through generations. Forms of manifestation and distant consequences of genomic instability are equally diverse (structural reorganization of the genome, activation of mobile elements, developmental violations, oncological diseases) as well as difficult to predict [1,2,3,4,5,6,7]. This phenomenon is investigated on various experimental models and has already been identified by some key features: the possibility of transgenerational transmission, the non-clonality of genome disorders, that is the instability of the reaction, which is determined by many components.

Scientific developments have established significance and threat of the induced genomic instability, but with hygienic (anthropocentric) normalization, this phenomenon is not taken into account due to its insufficient research [8]. Environmental regulation, which only forms its own principles, does not consider this phenomenon at all. No less difficult to predict the effects of radiation exposure is to take

into account radioadaptation - a phenomenon that undoubtedly extends to a set of processes that lead to genomic instability. The study of the morbidity structure of the population in areas of high radiation background leads to the conclusion that low-intensity radiation exposure is dangerous only as a "new" environmental factor and has no serious consequences for human health as a constitutive component of the environment for many generations [9]. The limit between the "new" and the constitutive has not yet been established, but this phenomenon indicates the importance of radioadaptive processes and the need to develop approaches to study the speed of their formation and results.

Before the effects of genomic instability were understood as a radiobiological phenomenon, they were discovered and studied by geneticists. The mobile elements were found in maize in the late 40s as the part of the genome, which was able to cleave and re-integrate again into the genome. Then it was indicated that almost all organisms, including humans, have had this jumping part of the genome [10]. It is now well established that up to 80% of spontaneous mutations are associated with the activation of mobile elements [11]. Also, it is indicated, that various stress effects, including radiation, lead to the activation of mobile elements [10].

Drosophila melanogaster is the most genetically investigated object. This species is radioresistant (LD_{50} =1228 Gy for adult males and 1250 Gy for adult females) and synanthropic, it constantly accompanies human. As in the other prokaryotic and eukaryotic organisms, the Drosophila genome contains mobile elements of various types, the activation of which is effected with the environmental factors, including radiation exposure [11, 12]. This leads to genomic instability and increasing yield of mutations. The activation of mobile elements from P-, hobo- and I- families causes an increase in the frequency of gonadal dysgenesis, i.e. atrophy of one or both gonads of *Drosophila melanogaster*.

The study is devoted to the investigation of the level and dynamics of the gonadal dysgenesis frequency as a marker of mobile elements' activation through natural populations of Drosophila from Ukrainian regions with different radiation impact with follow-up study of the dynamics of the index under low-rate chronic exposure in laboratory for 10 generations.

MATERIAL AND METHOD

The research was conducted on the natural Drosophila populations caught in the settlements of Ukraine with different radiation exposure. The insects from Netishyn were caught in 2014. The populations from Magarach, Pyriatyn and Lubny were received in 2014 from the collection of Taras Shevchenko Kyiv National University (Kyiv, Ukraine). Data on the radiation situation in Netishyn were obtained from the official website of Khmelnytskyi NPP. At the time of selection, the dose rate was 0,10 μGy/h. Accumulated dose through generations was approximately 0,02 Gy. Magarach, Pyriatyn, Lubny are settlements that are not included into the permanent radiological control zone due to the lack of enterprises related to radionuclide emission. According to official data, these regions like the entire Khmelnytskyi region belong to the "green" zone, for which the radiation background is ≤ 10 μR/h.

At catching used attraction of individuals with a fruit smell, with the subsequent placement into the test tubes with a nutrient medium. The study was conducted in two stages. The first one included the assessment of both the time of maturation and the index of the gonadal dysgenesis (GD) through the first (F1) generation, obtained in laboratory conditions. At the second stage, the dynamics of this index was investigated for the F1- descendants of each ten consequent generations, which were developed under laboratory conditions both with and without additional gamma-exposure with tree variants of the dose rate.

Exposure conditions. The source of prolonged exposure was the vessel with a solution of $^{137}CsCl$ located in the center of the tripod, with concentric slits for fixing test tubes with flies. The report presents the results obtained under exposure with different dose rate $1{,}2 \cdot 10^{-8}$, $0{,}3 \cdot 10^{-8}$ and $0{,}12 \cdot 10^{-8}$

Gy/sec. The flies were in glass vessels of 50 ml, the volume of the nutrient medium was 10 ml. Chronic exposure was conducted for 10 generations under room temperature + 21- + 23 ^{0} C.

Assessment of the frequency of gonadal dysgenesis**.** Gonadal atrophy was assessed in 50 individuals of each sex obtained from exposed parents. Gonadal dysgenesis was considered 0 if both gonads were complete morphologically, as 1 (GD (1)) if one gonad was underdeveloped or absent, and 2 (GD (2)) if both gonads were reduced or absent. From 4 tubes were selected both 50 males and females and evaluated the frequency of gonadal dysgenesis for each population.

The percentage of gonadal dysgenesis was calculated by the formula:

$$\%GD = ½\%GD(1) + \%GD(2)$$

Significance of differences between variants of the experiment was evaluated by parametric Student's t-test at 0,05 significance level.

RESULTS AND DISCUSSION

Estimation of the level of gonadal dysgenesis in F1-generation of insects immediately after moving to laboratory conditions indicated a significant difference in the index between populations from different radiation zones of Ukraine. Both males and females from Netishyn population demonstrated a 10 - 12-fold increase in the level of dysgenesis compared to the same rate in the populations from "clean" regions (Fig. 1).

The maturation period estimation (age of the first clutch) of the first generation of insects after the moving to laboratory conditions showed a significant maturation slowdown through the individuals of the Netishyn population (Fig. 2). Thus, the first stage of the study shows a significant difference in such important vital indexes as the maturation time and the yield of gonadal dysgenesis through populations from both "clean" regions and under constant low-intensity radiation exposure.

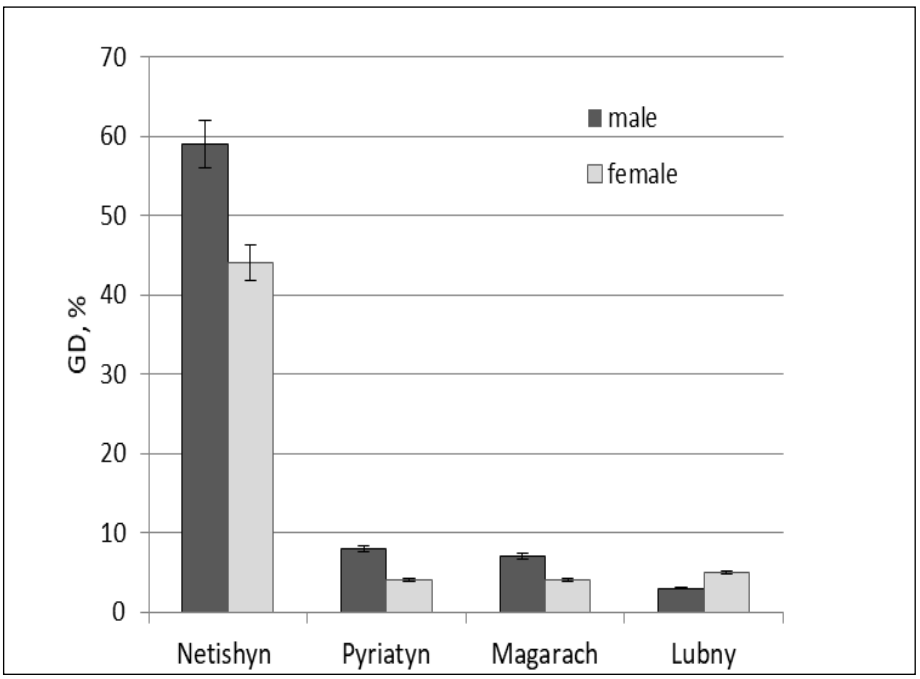

Fig. 1. Initial gonadal dysgenesis (GD) yield through F_1-generation of populations from different regions of Ukraine.

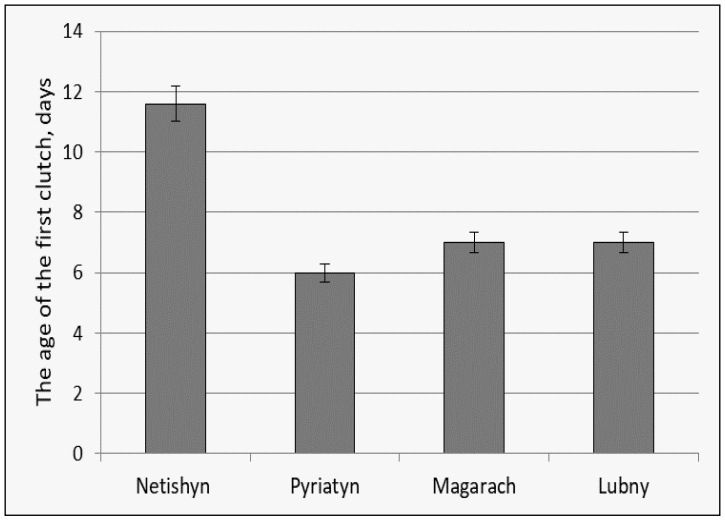

Fig. 2. The age of the first clutch of the F_1- generation when moving individuals from different natural populations to laboratory conditions.

The next study stage concerned the investigation of the GD dynamics through generations within populations from both areas with different radiation exposure without and with additional chronic gamma-exposure. Due to the technical limitations of the experiment, as well as the same level of both gonadal dysgenesis and maturation (Fig. 1, Fig. 2) within three populations from "pure" areas, in the second series of the experiment only Netishyn population and the population from Magarach were used. According to the data (Fig. 3) there is a big difference between the dynamic characteristics of GD frequency in generations without additional exposure. The Netishyn population demonstrated a clearly expressed oscillatory curve, while this index for the Magarach population had a relatively monotonous one. It is known that oscillatory dynamics is typical for systems with negative feedback and is an integral, final part of the homeostatic curve [13]. It shows a kind of over-regulation in the work of recovery processes. This phenomenon is well studied for technical and living systems under a single stress exposure. It is expected that the typical appearance of the homeostatic curve, which has one distinct pessimum and a series of fading oscillations on the ascending branch of the curve, will be significantly modified under constant stress exposure.

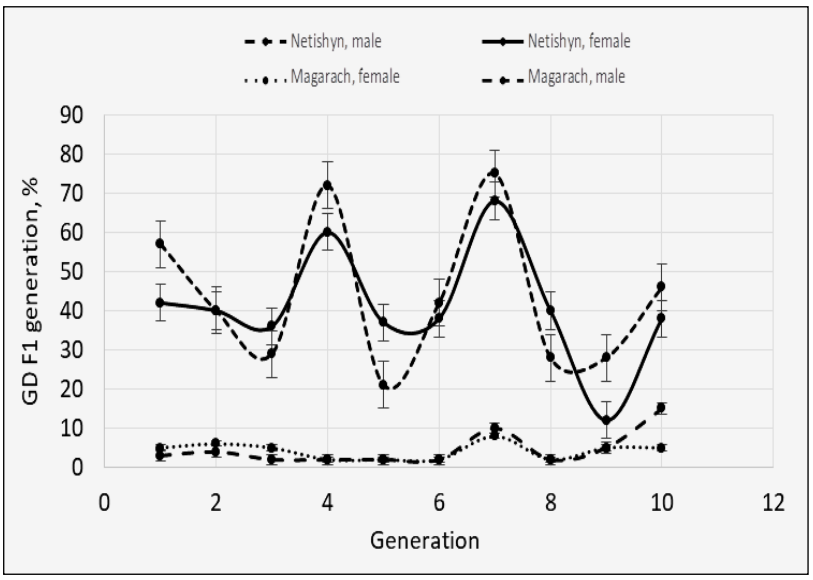

Fig. 3. Dynamics of gonadal dysgenesis (GD) yield without additional chronic exposure.

Similar oscillatory kinetics has been observed with another experimental model, the hybrid P - M system of the gonadal dysgenesis (the hybridization of Drosophila pure lines *Canton-S* without P-element and *radius incompletus (ri)* which includes the mobile element). Regular alternation of decrease and increase in the GD yield has been observed without additional exposure, so we can assume that the oscillatory dependence of the index is the result of chronic stress exposure of any nature. Additional chronic exposure of hybrid insects indicated changes in the oscillatory kinetics. There appeared a certain trend to both decreasing and increasing of the GD yield depended on the dose of additional exposure (Kravets et al. 2009).

Additional chronic exposure with the dose rate $0{,}12 \cdot 10^{-8}$ and $3 \cdot 10^{-8}$ Gy/sec led to the opposite reactions within both populations (Fig. 4, Fig. 5). The exposure of the first six generations of the Netishyn population indicated decreasing GD yield while maintaining the oscillation kinetics. Then, from the seventh generation, there was an increasing of the index followed by a decreasing with damped fluctuations. Increasing exposure of dose rate expanded a negative effect for the Magarach population. In Netishyn population a general dynamics of changes in both direction and GD yield was the same with slightly reduced increasing of the indexes for the seventh exposed generation (Fig. 5).

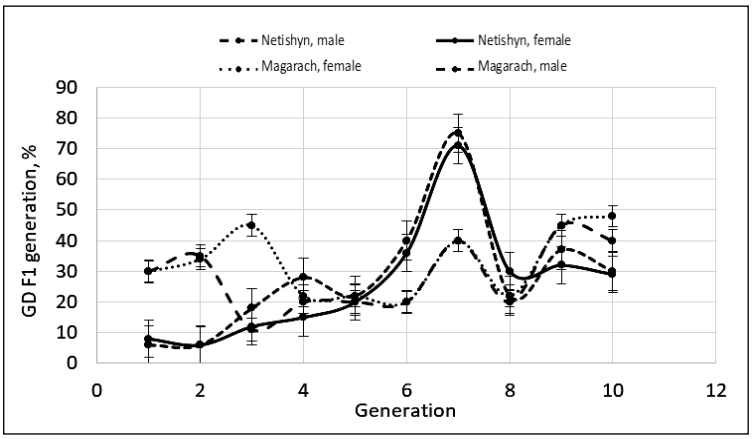

Fig. 4. Dynamics of gonadal dysgenesis (GD) yield under additional chronic exposure with dose rate $0{,}12 \cdot 10^{-8}$ Gy/sec.

Dynamic curve of GD yield for the Magarach population lost its monotony and for the fist – third generations exposed with the lowest dose rate has demonstrated a sharp increase of the index up to 30% for female and 45% for male.

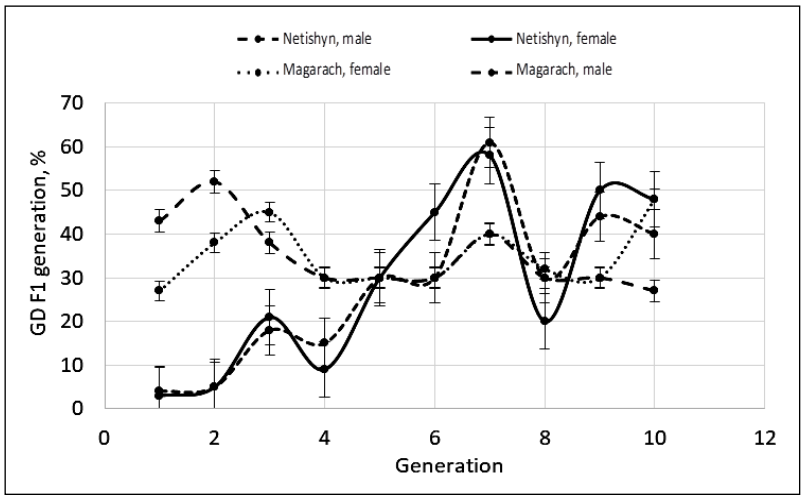

Fig. 5. Dynamics of gonadal dysgenesis (GD) yield under additional chronic exposure with dose rate $0{,}3 \cdot 10^{-8}$ Gy/sec.

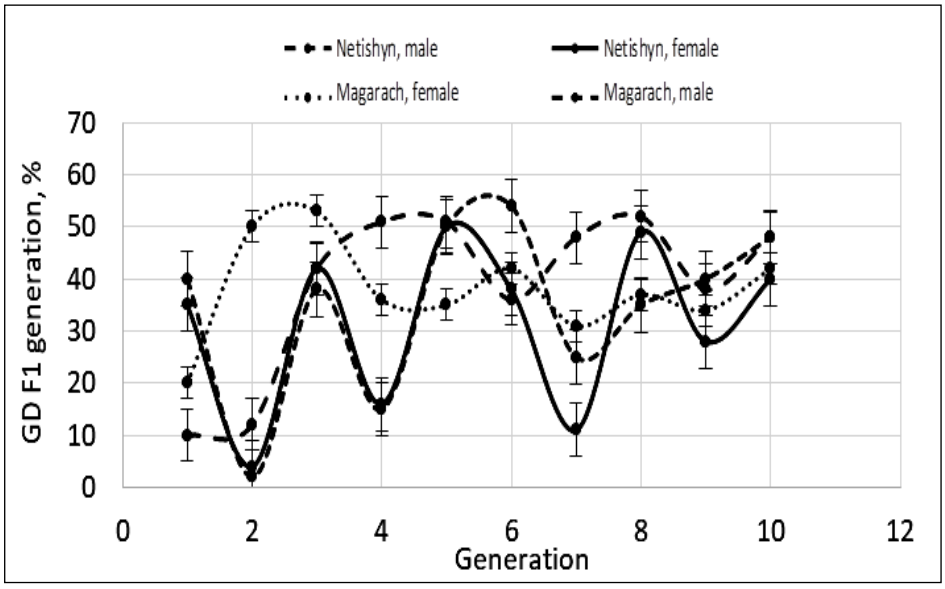

Fig. 6. Dynamics of gonadal dysgenesis (GD) yield under additional chronic exposure with dose rate $1{,}2\cdot10^{-8}$ Gy/sec.

Under increasing exposure dose rate up to $1{,}2\cdot10^{-8}$ Gy/sec the decrease compared to the control variant of the Netishyn population was disappeared, and dynamic curves of the GD yield became similar for the insects of both populations. The GD yield for the Netishyn population on average ranged from 2 up to 55%. For the Magarach one, the index was from 10 up to 53%.

The general dynamic type of dependence has become similar to the control one for the Netishyn population without additional exposure. Note that the study of small dose effects on different experimental models with their single or prolonged exposure has showed that dose curves have a complex non-monotonic nature, which differs significantly from the curves in the field of "large" doses [14,15,16].

The explanation of this phenomenon should take into account all currently known processes related to both the development of damage and the protective mechanisms of living things. Thus, a generalized explanation by Burlakova (1999) for single exposure dose curves in the field of small doses is based on the information about a complex system of damage development, induction and recovery processes. Within the system, each component has its own dose curve and turning-on "threshold". “Overlaying” of these dose curves causes complex nonmonotonic structure of the general curve "dose - effect" [14].

This idea has been developed by Kolomiytseva (2003) for the times of prolonged exposure when the effects have cumulative character and their appearance in time corresponds to the accumulative dose.

Interpretation of the results presented in this report should also include up-to-date information on the regulation of the mobile elements' activity. It is known that the activation of mobile elements induces a number of DNA protective mechanisms. The most important role in regulation of the activity of genome mobile elements is played by epigenetic mechanisms regulating gene expression. Inactivation of mobile elements is done by both switching the methylation of their sequences into *de novo* mode and activity of short non-coding RNA together with Argonaut protein complexes, which is a key component of the RNA interference [17]. A powerful defense mechanism is the reparative processes' activation, like a reaction to radiation damage [18]. Radiobiological study of the interaction of these two main mechanisms of mobile elements' activity regulation is just beginning. Right now, it allows us to approach the interpretation and preliminary estimates of the time of adaptive response

formation under exposure with certain doses and its absence under other one.

The most difficult to interpret the results is to explain the fact that according to the official data, all insect populations have originated from the regions belonging to the "green zone". One of the assumptions in the interpretation of this contradiction is related to the difference in the time of radionuclides' entry into the environment and their chemical composition, which determine both the natural radiation background in settlements of Poltavschina and Magarach and the technogenically changed in Netishyn. The generally accepted definition of radiation background intensity by gamma emitters [19] ignores a wide range of other radionuclides associated with NPP emissions and characterized with high genotoxic properties [20].

Exposure under ultra-low doses of technogenically-modified radiation background for tens of generations, as observed within Netishyn population, apparently has not become a constitutive factor forming a new stable pattern of genomic instability in generations which allows maintaining population density at the required level.

The effects of additional exposure with increased intensity (for example dose rate 0,12*10-8 Gy/sec) could occur with different stages. There are decreasing yield of genomic instability or its increasing up to the initial level with subsequent adaptive effect.

Thus, the data obtained on a simple experimental model show a complex interaction between the development of damage processes, on the one hand, and both protective and repair processes, on the other.

They also indicate the extreme complexity of interpreting the results of epidemiological studies and the uncertainty in predicting the effects of chronic exposure. The data show a significant discrepancy between the actual and expected radiation biological effects. Drosophila is an extremely radioresistant species with a high regenerative potential [21] and studying genomic instability on species with different radiosensitivity could provide an additional important information on the development of radiation safety standards for humans and biota.

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REFERENCES

[1]. Pelevina II, Gotlib VY, Kudriashova OV, Serebrianyi GG. 1996. Genome instability after exposure to low doses of radiation (in the 10-kilometer zone of the Chernobyl accident and in laboratory conditions). Radiat. Biol. Radioecology. 36 (4): 546-560.

[2]. Suskov II, Kuzmina NS. 2003. Polygenic realization of mutagenic effects in the body of people exposed to radiation in low doses. Radiat. Biol. Radioecology. 43 (2): 150.

[3]. Kuzmina NS, Suskov II. 2002. Expression of genomic instability in lymphocytes of children living under conditions of prolonged exposure to radiation factors. Radiat. Biol. Radioecology. 42: 735.

[4]. Dancause KN, Yevtushok L, Lapchenko S, Shumlyansky I, Shevchenko G, Wertelecki W, Garruto RM. 2020. Chronic radiation exposure in the Rivne-Polissia region of Ukraine: Implications for birth defects. American Journal of Human Biology. 22 (5): 667-674.

[5]. De Toledo SM, Buonanno M, Harris A, Azzam EI. 2017. Genomic instability induced in distant progeny of bystander cells depends on the connexins expressed in the irradiated cells. Int. J. Radiat. Biol. 93 (10): 1182-1194.

[6] Fang L, Li J, Li W, Mao X, Ma Y, Hou D, Zhu W, Jia X, Qiao J. 2019. Assessment of Genomic Instability in Medical Workers Exposed to Chronic Low-Dose X-Rays in Northern China. Dose Response. 17 (4): 1559.
[7]. Siama Z , Zosang-Zuali M , Vanlalruati A, Jagetia G C, Pau KS, Kumar NS. 2019. Chronic low dose exposure of hospital workers to ionizing radiation leads to increased micronuclei frequency and reduced antioxidants in their peripheral blood lymphocytes. Int. J. Radiat. Biol. 95(6):697-709.
[8]. [ICRP] International Commission on Radiological Protection publication 103. 2007. The 2007 recommendations of the ICRP. Published for the ICRP by ELSEVIER. 37:2-4.
[9]. Dobrzyński L, Fornalski K W, Feinendegen LE. 2015. Cancer Mortality Among People Living in Areas with Various Levels of Natural Background Radiation. Dose Response. 13 (3): 2391.
[10]. Golubovsky MD. 2000. Century of Genetics: Evolution of Ideas and Concepts. St. Petersburg: Borey Art.
[11]. Zainullin VG. 1996. Mutability of natural populations and laboratory lines of *Drosophila* under conditions of chronic irradiation in low doses of low intensity. Radiat. Biol. Radioecology. 36 (4):561-566.
[12]. Kozeretskaya IA, Protsenko AV, Afanasieva ES, Rushkovskyi SR, Chuba AI, Miusse TA, Meller AP. 2008. Mutation processes in natural populations of *Drosophila* and *Hirindo rustica* from the radioactively contaminated territory of Ukraine. Cytology and Genetics. 42 (4): 63-68.
[13]. Novoseltsev VN. 1978. Control Theory and Biosystems: Analysis of Protection Properties. Moskva: Nauka.
[14]. Burlakova EB. 1999. Features of the biological effect of small doses of radiation. Radiat. Biol. Radioecology. 39:26.
[15]. Kolomiytseva IK. 2003. Non-monotonicity of the dose-effect relationship in the region of low doses of ionizing radiation. Radiat. Biol. Radioecology. 43:179.
[16]. Kravets AP, Gatilova GD, Grodzinsky DM. 2008. Dynamics of the release of cytogenetic anomalies in the seedling meristem under chronic seed irradiation. Radiat. Biol. Radioecology. 48 (3): 208-219.
[17]. Law JA, Jacobsen SE. 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nature Reviews Genetics. 11: 204-220.
[18]. Yushkova E, Zainullin V. 2016. Interaction between gene repair and mobile elements-induced activity systems after low-dose irradiation. International Journal of Radiation Biology. 92 (9): 485-492.
[19]. Moiseev AA, Ivanov VI. 1990. Dosimetry and Radiation Hygiene Handbook. Moskow: Energoatomizdat.
[20]. Bazhenov AV, Buldakov LA, Vasilenko IY. 1990. Harmful chemicals. Radioactive substances. Leningrad: Chemistry.
[21]. Paithankar G J , Deeksha K , Patil R K. 2017. Gamma radiation tolerance in different life stages of the fruit fly Drosophila melanogaster. Int J Radiat Biol. 93 (4):440-448.