# Chromosomal Aberrations in Blood Lymphocytes of the Residents of 30-km Chornobyl NPP Exclusion Zone

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#### Abstract

A comparative cytogenetic examination of 33 self-settlers in the 30 km ChNPP Exclusion Zone and 31 residents in villages of Yahotyn district, Kyiv region was carried out in 1998-99. The levels of soil contamination of their residential areas with <sup>137</sup>Cs, <sup>90</sup>Sr and <sup>238,239+240</sup>Pu were 74–477 kBq/m<sup>2</sup>, 33–288 kBq/m<sup>2</sup> and 1.5-10.0 kBq/m<sup>2</sup>, respectively for the former, and 1.9–5.8 kBq/m<sup>2</sup>, 0.6–2.8 kBq/m<sup>2</sup> and 0.01-0.05 kBq/m<sup>2</sup>, respectively for the latter. Using various data about the radiation situation in the Exclusion Zone, the effective doses on whole-body of the self-settlers were estimated to be 30–333 mSv for the whole residing period after the accident. The mean frequencies of aberrant cells and chromosomal aberrations for the Zone self-settlers were significantly higher than those for the residents in Yahotyn district, while the values of the latter group were found to be above the spontaneous levels reported in literatures. The individual variability in the distribution of the same indices was significantly larger for the self-settlers than for the Yahotyn residents. The compared groups also differ in the distribution of aberrations in cells. A repeated examination of 20 Zone self-settlers was conducted in 2001. A significant decrease in chromosome type aberration frequency was found at the expense of fragments frequency decrease. However, the total frequency of chromosomal aberrations didn't differ in 1998-99 and in 2001.

#### **INTRODUCTION**

After the Chornobyl accident on  $26^{th}$  of April 1986, the territory within a 30-km radius from ChNPP was separated as Exclusion Zone. The people residing in this area were evacuated during 4-7<sup>th</sup> of May. However, right from the first days and weeks after the evacuation some people have been returning to the Zone villages without permission. In addition, nearly 100 people were not evacuated at all. They are called "self-settlers". The number of them was about 500 people in 1986 in the Ukrainian part of the Exclusion Zone territory, 1200 - in 1988, 660 - in the end 1998 and 477 - in 2001. Up to 80 % of them are old age pensioners. They lead relatively isolated life, farm and consume mostly food products grown in Zone, which determines their permanent external and internal irradiation.

It is a well-known fact that one of the most significant effects of radiation is destabilization of genetic structures of somatic cells leading to an increase in the risk of somatic diseases, first of all, of cancers. In order to investigate the efficiency of long-term permanent suprabackground radiation in the conditions of Exclusion Zone we have been conducting periodically cytogenetic examinations of self-settler groups from 1995. The first examination was carried out using micronucleus test on cultures of peripheral blood lymphocytes. A significant increase was revealed in the micronuclei frequency in cells of the Zone self-settlers of the age up to 55, compared to that of Kyiv residents of the same age [1,2]. These findings pointed out an expediency of further investigations. In this work we present the results of chromosomal aberration frequency in blood lymphocytes of ChNPP Exclusion Zone residents investigated in the end of 1998 – beginning of 1999, and in 2001.

# SUBJECTS AND METHODS OF STUDY

# The subjects of examination

In 1998-1999 we examined 33 people residing in the villages in Exclusion Zone, located northwestward (Stari Shepelychi, Rudnya Illinetska, Lubyanka) and southeastward (Opachychi, Kupovate, Horodyshche, Paryshiv) of ChNPP. These people returned to their homes in Exclusion Zone mostly in June 1986 - April 1987 after the evacuation. For comparison we have simultaneously examined 31 people (control group) from the inhabitants of the villages in Yahotyn district, Kyiv region. The climatic and socioeconomic conditions as well as health care of both examined groups were analogous. Table 1 represents the data on the radionuclide contamination in soil of residing areas of the people observed. The group of comparison was formed to match age, sex and smoking status of the ChNPP Exclusion Zone self-settlers (Table 2). None of the persons of the control group had had any conscious contact with radiation factor except for routine medical radiological diagnostic procedures and had not been exposed to chemotherapy. In 2001 20 people among the 33 Zone self-settlers observed in 1998-1999 were repeatedly examined. At the moment of obtaining of blood samples no one from those examined had any acute illness or exacerbation of chronic diseases. The participation in examination was voluntary. A special questionnaire was offered to the examined persons and all data obtained were entered into a database.

# The cytogenetic protoco

Venous blood samples were obtained in field conditions, either in the places of residence of the

| Region                           | Inhabited locality       | <sup>137</sup> Cs | <sup>90</sup> Sr      | <sup>238.239+240</sup> Pu |
|----------------------------------|--------------------------|-------------------|-----------------------|---------------------------|
|                                  | Rudnya Illinetska        | 129.5±25.9        | 70.3±22.2             | 1.5                       |
|                                  | Lubyanka                 | 407.0±101.7       | 144.3±31.7            | 5.9                       |
|                                  | Stari Shepelychi         | 477.3±118.9       | 77.3±118.9 288.6±85.1 |                           |
| ChNPP Exclusion<br>Zone          | Paryshiv                 | 115.4±22.0        | 54.0±11.3             | 2.2                       |
|                                  | Opachychi                | 214.6±59.2        | 133.2±25.9            | 5.6                       |
|                                  | Kupovate                 | 107.0±22.2        | 44.4±14.8             | 3.0                       |
|                                  | Horodyshche              | 74.0±14.2         | 33.3±6.3              | 2.6                       |
|                                  | Sulymivka, Chernyakhivka | 1.9 – 2.9         | 0.64                  | 0.01                      |
| Yahotyn district,<br>Kyiv region | Lozovyi Yar, Chervone    | 2.6               | 0.96                  | 0.04                      |
|                                  | Panfily                  | 5.0               | 0.8                   | 0.04 - 0.1                |
|                                  | Kapustyntsi              | 5.0               | 0.8                   | 0.02 - 0.04               |
|                                  | Yahotyn                  | 2.9 - 5.8         | 0.9 - 2.8             | 0.03 - 0.05               |

Table 1. Density of radionuclide contamination in soil in ChNPP Exclusion Zone and villages in Yahotyn district, Kyiv region, kBq/m<sup>2</sup>.

#### Table 2. Characteristics of examined groups.

| Group                               | Range of effe<br>dose | ctive equivalent<br>s, mSv | Number | Age<br>(range,      | Sex |    | Smoking status  |              |
|-------------------------------------|-----------------------|----------------------------|--------|---------------------|-----|----|-----------------|--------------|
| Group                               | Whole body            | body Red bone<br>marrow    |        | mean),<br>in years  | F   | М  | Non-<br>smokers | Smok-<br>ers |
| ChNPP<br>Exclusion<br>Zone          | 30 - 333              | 13 - 630                   | 33     | 24 - 68<br>53.9±1.7 | 17  | 16 | 28              | 5            |
| Yahotyn<br>district,<br>Kyiv region | -                     | -                          | 31     | 23 – 72<br>51.5±2.2 | 18  | 13 | 26              | 5            |

examined people or in rural outpatient clinics. All samples were drawn into vacutainers containing heparin as antocoagulant. Samples were transported within eight hours in Kyiv to Laboratory of Radiobiology of Institute for Nuclear Research, National Academy of Science of Ukraine.

Lymphocytes culture and cytogenetic slide preparation were conducted according to [3]. 0.25 ml of whole-blood have been cultivated in 3 ml of RPMI-1640 medium supplemented with 15% fetal bovine serum with 1.5 % phytohaemagglutinin-P (Sigma, USA) for 48 h at 37°C in darkness. Colchicine in final concentration of 7.5mg/ml was added to the culture 3 hours prior to fixation. The slides were prepared following a standard technique, using for hypotonia - 75mM solution of KCl and for fixation – ethanol-acetic acid (3:1), for chromosome staining - Giemsa stain (2 % solution). All slides were encoded. The slides were studied with oil immersion using x900 magnification. On the average 380 metaphases meeting the required standards from every person were studied. Analysis was conducted by means of visual partial karyotype determining. We counted all chromatid type aberrations including chromatid fragments and chromatid deletions), interstitial deletions, dicentric chromosomes (*dic.*) and centric rings (*c.r.*), abnormal monocentrics, which could emerge as the result of terminal and interstitial deletions, reciprocal translocations and insertions. The blank gaps were not taken into account.

#### **Doses estimation**

Individual effective equivalent irradiation doses (both external and internal on whole body and red bone marrow) of the Zone self-settlers were determined by calculational methods. Dose reconstructing was based on the data of the mean density of soil contamination of residential areas with radionuclides of <sup>137</sup>Cs, <sup>90</sup>Sr, <sup>238,239,240</sup>Pu and <sup>241</sup>Am together with the time and duration of personal residence in Zone in the post-accident period. Also the data were used from representative measurements of exposure dose rate in houses and homesteads; <sup>137</sup>Cs and <sup>90</sup>Sr content in nutritional products of local origin; individual <sup>137</sup>Cs content in the bodies of examined people measured by a whole body counter.

#### Statistical Analysis

Characteristic distribution of chromosomal aberrations among the examined persons was determined by means of  $\chi^2$  test. Significance of the differences between the groups was estimated with  $\chi^2$ -test, Wilcoxon-Mann-Whitney U-test, Students t-criterion and Fisher test. Age-relation was estimated by means of the Spearman ranks correlation coefficient [4,5].

#### **RESULTS AND DISCUSSION**

#### Cytogenetic examination in 1998-1999

# Frequency of aberrant cells and chromosomal aberrations:

Distribution of the examined persons by the number of chromosomal aberrations per 100 analyzed lymphocytes is shown in Fig. 1. The obtained distributions fit the normal type in both of the Zone self-settlers and the village residents of Yahotyn district: for the Yahotyn district group  $-\chi^2 < \chi^2_{0.01}$  and for the Zone group  $-\chi^2 < \chi^2_{0.05}$ . As is seen from the bar chart (Fig. 1), one third of the Zone self-settlers showed the frequency of chromosomal aberrations exceeding the maximum value of this parameter in the comparison group. The comparison of individual distributions by the number of aberrant cells and chromosomal aberrations per 100 cells showed a significant difference between two groups ( $\chi^2 > \chi^2_{0.01}$ ). However, the comparison utilizing the U-criterion revealed that the distribution of persons by the chromosomal aberrations frequency statistically significantly differs in two groups only when the results of persons younger than 60 years old were compared (p < 0.001). The inter-individual variation (dispersion) and the mean group frequency of aberrant cells and chromosomal aberrations in peripheral blood lymphocytes were significantly higher for the Zone self-settlers than for the Yahotyn district inhabitants (Table 3). V.Yu. Nugis [6] observed that the increase of individual variation in frequencies of



Fig. 1. Distribution of examined persons by the number of chromosomal aberrations (CA) per 100 cells.

chromosomal damages in the population signals chronic exposure to genotoxic factors. It is noteworthy that higher levels of aberrant cells ( $3.20\pm0.15$  %) (mean±SEM, here and below) and chromosomal aberrations ( $3.51\pm0.17$  %) than those usually reported for the "clean" regions were found in the inhabitants of the Yahotyn district. According to a generalized data on the spontaneous level of mutagenesis for the year 2000 reported by our Russian colleagues, the frequencies of aberrant cells and chromosomal aberrations in blood lymphocytes are  $2.13\pm0.08$  % and  $2.21\pm0.14$  %, respectively [7]. The data of the same authors also indicates that the mean frequency of chromosomal aberrations in the early 70-th was  $1.19\pm0.06$  % [8]. A generalized cytogenetic monitoring of healthy population of Ukraine up to 1986 testifies that before the Chornobyl accident the frequency of aberrant cells and chromosomal aberrations in peripheral blood lymphocytes of the Ukrainian population were  $1.43\pm0.16$  % and  $1.47\pm0.19$  %, respectively [9]. The above allows us to assume that the registered parameters of mutagenesis in Yahotyn district are caused not only by the age of the examined persons (65% of them were 50+ according to Zone self-settlers group age structure), but also by general deterioration of the environment during the period after 1986.

Two persons from the Exclusion Zone and one Yahotyn district resident had one multiaberrant cell each (we referred cells having more then 7 aberrations to multiaberrant).

# Age dependency of chromosomal aberrations:

Age dependency of chromosomal aberrations frequency in blood lymphocytes was analyzed by the method of the Spearman ranks correlation coefficients. A strong age dependency was observed in the group of Yahotyn district: the Spearman ranks correlation coefficient was 0.71 (p < 0.0005), while it was

 Table 3. Frequency of aberrant cells and chromosomal aberrations per 100 lymphocytes from ChNPP Exclusion Zone and Yahotyn district residents.

 (individual range: mean +SD)

| (individual range, inean ±5D).   |                         |                                |                           |                           |                     |  |  |  |  |
|----------------------------------|-------------------------|--------------------------------|---------------------------|---------------------------|---------------------|--|--|--|--|
| Group                            | Number<br>of<br>persons | Number of<br>analyzed<br>cells | Aberrant cells            | Chromosome<br>aberrations | Multiaberrant cells |  |  |  |  |
| ChNPP Exclusion<br>Zone          | 33                      | 11789                          | 1.92 - 10.20<br>5.02±1.95 | 2.24 - 10.80<br>5.32±2.10 | 0.017±0.066         |  |  |  |  |
| Yahotyn district,<br>Kiev region | 31                      | 12273                          | 1.82 - 5.50<br>3.20±0.84  | 1.82 - 6.00<br>3.51±0.97  | 0.009±0.25          |  |  |  |  |

|                             |                |                |               | (intervie       | uur runge,            | IVI-OLIVI                                       | )                   |               |                   |               |  |  |
|-----------------------------|----------------|----------------|---------------|-----------------|-----------------------|---|---------------------|---------------|-------------------|---------------|--|--|
|                             | Chromatid type |                |               | Chromosome type |                       |   |                     |               |                   |               |  |  |
|                             |                |                |               | _               | Inter-                | dicentrics + centric rings                      |                     |               | abnormal          |               |  |  |
| Group                       | breaks         | excha-<br>nges | total         | frag-<br>ments  | sticial.<br>deletions | with<br>fragment                                | without<br>fragment | total         | monocen-<br>trics | total         |  |  |
| ChNPP                       | 0.6-5.6        | 0-1.0          | 1.0-6.6       | 0.3-4.9         | 0-0.5                 | 0-1.0   | 0- 0.9              | 0-1.3         | 0 - 1.4           | 0.3-5.6       |  |  |
| Exclusion<br>Zone           | 3.01<br>±0.24  | 0.13<br>±0.04  | 3.14<br>±0.24 | 1.59<br>±0.20   | 0.02<br>±0.02         | 0.22<br>±0.05                                   | $0.10 \\ \pm 0.03$  | 0.33<br>±0.06 | 0.23<br>±0.05     | 2.16<br>±0.24 |  |  |
| Yahotyn                     | 1.0-4.0        | 0 - 3.0        | 1.0-4.0       | 0 - 2.2         | 0 - 0.5               | 0 - 0.5   | 0 - 0.25            | 0 - 0.5       | 0 - 0.8           | 0.3-2.5       |  |  |
| district,<br>Kyiv<br>region | 2.31<br>±0.12  | 0.02<br>±0.01  | 2.33<br>±0.12 | 0.89<br>±0.12   | 0.04<br>±0.02         | $\begin{array}{c} 0.08 \\ \pm 0.03 \end{array}$ | $0.05 \\ \pm 0.02$  | 0.13<br>±0.03 | 0.12<br>±0.03     | 1.18<br>±0.13 |  |  |

 Table 4. Frequency of various types of chromosomal aberrations per 100 cells in the Exclusion

 Zone self-settlers and the residents in Yahotyn district.

 (individual range\_M+SEM)

-0.23 (p> 0.05) for the Zone group, i.e. in the latter group even some tendency to an inverse relationship with age is observed. It might be caused by the influence of additional factors in ChNPP Exclusion Zone, in particular, by the influence of radiation factor and perhaps by lower sensitivity of the elderly people (60+) to it. The similar results were obtained in our previous studies in which micronucleus test was utilized [2].

## Ratio of chromosomal aberration types:

Table 4 shows the results of the analysis of frequency of various types of chromosomal aberrations in peripheral blood lymphocytes. Correlation between chromatid type and chromosome type aberrations in both groups corresponds to the ratio reported in the literature for spontaneous mutagenesis (2:1 - 1.5:1) [8]. The proportion of chromosome type aberrations in self-settlers' lymphocytes does not significantly differ from that of the control group;  $39.2\pm2.8$  % and  $33.6\pm2.9$  %, respectively (t=1.4, p=0.16).

The induction of chromosome type aberrations is known to be characteristic for radiation exposure. Their mean group frequency in lymphocytes of the self-settlers was increased 1.8-fold compared with that of the Yahotyn residents, but at the same time there was a 1.3- fold increase in the frequency of chromatid type aberrations.

#### Chromosome type aberrations:

Exchange rearrangements are the most specific markers of radiation exposure. Unstable chromosome exchanges (*dic.* and *c.r.*) were detected in 60 % of the Zone self-settlers and in 45 % of the Yahotyn district inhabitants. In the Zone self-settlers' cells unstable exchanges occurred on the average 2.5 times more often than in the control group of Yahotyn district. In both groups the majority of unstable exchanges were accompanied by fragments. Routine staining of stable chromosome exchanges (abnormal monocentrics) also revealed a significant increase of mean group frequency in the Zone self-settlers (1.9-fold) with one third of the examined self-settlers and 16 % of Yahotyn district residents simultaneously revealing both stable, and unstable aberrations.

Reported spontaneous levels of unstable chromosome exchanges vary within the range of 0.03 % and 0.3 % [7, 10-12]. According to [11,13] the mean population frequency of the total *dic.* and *c.r.* is taken as 0.13 % – 0.16 %. Up to 1986 these damages were registered in the rural population of Ukraine with the frequency of 0.05 per 100 metaphases [9]. In our research we have revealed *dic.* and *c.r.* in the control area residents with the frequency of 0.13±0.03 %, i.e. 2.6 times exceeding the pre-Chornobyl accident level for agricultural regions of Ukraine, but comparable with the mean population one.

The frequency of paired fragments per 100 metaphases was  $1.59 \pm 0.20$  in lymphocytes of the Zone self-settlers versus  $0.89\pm0.12$  in lymphocytes of the control group, while the mean pre-Chornobyl accident

level for the rural population of Ukraine was 0.47 [9]. Reported spontaneous levels of paired fragments are 0.25 - 0.65 per 100 cells [7, 10, 14]. It should be noted that in our research the increase of frequency of paired fragments in the Zone self-settlers is caused to a great extent by the excess of the number of isochromatid deletions. In addition in the course of analysis attention was paid to the fact that in cells of the Zone self-settlers some paired fragments were shown to be much longer than those usually observed.

#### Chromatid type aberrations:

As seen in Table 4, a significant increase of chromatid type aberrations per 100 metaphases of  $3.14\pm0.24$  was registered in the Zone self-settlers compared with the value of  $2.33\pm0.12$  in Yahotyn district residents. The pre-accident level for the rural population and the currently reported spontaneous levels of this indices were 1.05 [9] and 0.6 -1.5 [7, 10, 14], respectively.

Thus, in the conditions of chronic irradiation the frequency both of chromosome type (at the account of single- and two-hit damages) and chromatid type aberrations increased in lymphocytes of the Zone self-settlers. The elevated frequency of single-hit acentrics in the presence of two-hit *dic* and *c.r.* is characteristic for continuous exposure to low dose rates of low-LET radiation [15]. Increase of the acentrics frequency was revealed in cells of persons having been exposed to occupational irradiation for 20 years [10].

It is difficult to interpret the causes of the increase of chromatid type aberrations, which is characteristic for non-radiation mutagenesis, in the Zone self-settlers. Probably, the influence of chemical mutagenes in places of the Zone self-settlers' residence is not worse than in the Yahotyn district villages. Since the time of the accident no pesticides or other fertilizers have been utilized in the Exclusion Zone, while traces of the pre-accident soil contamination are still found. As for the salts of heavy metals we determined the elevated content of zinc and cadmium (in comparison with standard index) in crops grown in the self-settlers' homesteads. The levels of air contamination in the villages by nitrogen and carbon oxides, ozone and heavy metals aerosols do not exceed maximum allowable concentration [16]. It is unlikely that the self-settlers use more medications in comparison with the Yahotyn district residents.

The following assumptions were made in the literature concerning the increase of chromatid type damages due to prolonged irradiation: a) reparation of chromosome damages in conditions of low dose rate irradiation results in partial transition of chromosome type aberrations to the class of chromatid type aberrations [17]; b) the low doses of radiation cause instability of the chromosome system, which increases sensitivity to other forms of mutagenes, such as viruses, chemical factors [18]. Data are available, according to which radionuclides in low doses affect cells more likely as chemical mutagenes, toxins than



Fig. 2. Distribution of aberrant cells by the number of aberrations per cell in lymphocytes of the groups of ChNPP Exclusion Zone self-settlers (■) and the residents of Yahotyn district (□). % of the total number of examined cells.

-Left: total chromosome type aberrations

-Center: total chromosome type exchanges

-Right: unstable chromosome type exchanges

#### as radiation sources [19].

# Distribution of aberrations in cells:

The important information for finding out the type, dose and uniformity of mutagenic exposure is the distribution of aberrations in cells. In both groups of the Zone self-settlers and the Yahotyn district residents we have found persons having cells with two and three chromosome type aberrations. Some persons had even two or three such cells (two self-settlers and four persons from Yahotyn district ).

In such cells of the Yahotyn district residents aberrations are represented mainly by acentrics, which might be caused by the past viral infection [20] without clinical manifestations. Among the Zone self-settlers' cells with several chromosome type aberrations, cells prevail with 2-3 chromosome exchanges. Six self-settlers had such cells, and in five of them cells with 2 unstable exchanges were revealed, which is characteristic for exposure to radiation factor. One cell with two unstable exchanges with accompanying fragments was also found in one of Yahotyn district residents (27 years old), which probably testifies to the fact of some unaccounted irradiation in the region considered "radiation clean".

The comparison of the distributions of aberrant cells by the number of aberrations per cell is shown in Fig.2 for all chromosome type aberrations, all chromosome type exchanges and unstable chromosome type exchanges. A significant inter-group differences was revealed by  $\chi^2$  criterion ( $\chi^2 > \chi^2_{0.01}$ ) between the Yahotyn district residents and the Zone self-settlers.

#### Cytogenetic examination in 2001

# Time trend of chromosome aberration:

In order to investigate the dynamics of cytogenetic effects in the Zone self-settlers, 20 people from 33 already examined in 1998-99 were repeatedly analyzed in 2001. As it is seen in Table 5 this analysis has not revealed any changes in the mean group frequency not only of quantitative aberrations (metaphases with 47 chromosomes and tetraploids) but also of the total qualitative chromosome damages; the frequencies of aberrant cells and the number of aberrations per aberrant cell.

 Table 5. Dynamics of cytogenetic effects in the peripheral blood lymphocytes of the ChNPP Exclusion

 Zone residents. (Number of examined persons - 20)

| Vear of          | Number                  | Frequer                             | ncy per 100 cel | lls (individua        | Number of        | Number of                 |                                     |                          |
|------------------|-------------------------|-------------------------------------|-----------------|-----------------------|------------------|---------------------------|-------------------------------------|--------------------------|
| examina-<br>tion | of<br>analyzed<br>cells | Metaphases<br>with 47<br>chromosome | Tetraploids     | Aberrant<br>cells     | "Rogue"<br>cells | Chromosome<br>aberrations | aberrations<br>per aberrant<br>cell | with<br>"rogue"<br>cells |
| 1998-99          | 6978                    | 0.73±0.14                           | 0.48±0.10       | 1.9–10.2<br>5.44±0.52 | 0.14             | 2.2–10.8<br>5.79±0.55     | 1.06                                | 7                        |
| 2001             | 7923                    | 0.75±0.10                           | 0.39±0.07       | 2.2–9.5<br>4.99±0.41  | 0.09             | 2.2–10.4<br>5.41±0.49     | 1.08                                | 4                        |

# Table 6. Dynamics of frequency of different type chromosomal aberrations in the blood lymphocytes of the ChNPP Exclusion Zone residents. (Number of examined persons - 20)

|                  | Frequency per 100 cells (individual range, M±SEM) |         |         |                 |             |                            |                     |        |                          |         |  |  |
|------------------|---|---------|---------|-----------------|-------------|----------------------------|---------------------|--------|--------------------------|---------|--|--|
| Year of          | Chromatid type                                    |         |         | Chromosome type |             |                            |                     |        |                          |         |  |  |
| examina-<br>tion |   | <b></b> |         |                 | incratitial | dicentrics + centric rings |                     | abnor- |                          |         |  |  |
|                  | breaks  | changes | total   | ments           | deletions   | with<br>fragment           | without<br>fragment | total  | mai<br>mono-<br>centrics | total   |  |  |
| 1008             | 1.0-5.6   | 0-1.0   | 1.0-6.6 | 0.3-4.9         | 0-0.5       | 0-0.9                      | 0-0.5               | 0-1.3  | 0-1.4                    | 0.3-5.9 |  |  |
| 1998-            | 3.0   | 0.16    | 3.17    | 1.77            | 0.025       | 0.29                       | 0.10                | 0.39   | 0.32                     | 2.58    |  |  |
| 1999             | ±0.33   | ±0.07   | ±0.33   | ±0.30           | ±0.025      | ±0.081                     | ±0.04               | ±0.09  | ±0.08                    | ±0.35   |  |  |
|                  | 1.2-8.1   | 0-0.9   | 1.2-9.0 | 0-2.0           | 0-0.4       | 0-0.8                      | 0-1.0               | 0-1.4  | 0-0.9                    | 0.2-2.8 |  |  |
| 2001             | 3.53  | 0.29    | 3.82    | 0.79*           | 0.07        | 0.27                       | 0.18                | 0.45   | 0.25                     | 1.63*   |  |  |
|                  | $\pm 0.40$  | ±0.07   | ±0.45   | ±0.14           | ±0.03       | ±0.06                      | ±0.06               | ±0.09  | ±0.06                    | ±0.16   |  |  |

\* P<0.05

Studying the dynamics of different types of chromosome damage showed a significant decrease in the mean group frequency of chromosome type aberrations at the expense of a decrease in the yield of fragments, i. e. single-hit lesions. No alteration was found in the mean group frequencies of two-hit chromosome exchanges induction (Table 6).

#### "Rogue" cells:

We also analyzed the dynamics of "rogue" cell incidence, using the conventional definition of "rogue" cell proposed by M. A. Pilinskaya [21]. It has been proposed to refer to the cell as "rogue", when containing more than one lesion with at least one of them being chromosome type exchange aberration. Table 7 shows an aberration spectrum seen in "rogue" cells. "Rouge" cells of the Zone residents contained mostly 2-3 chromosome exchanges. Some self-settlers were indicated to have several "rogue" cells. There were also "rogue" cells similar to those described by Awa and Neel [22] that contain policentrics and "double minutes". It is important to note that two individuals (encoded #15 and #29) were shown to have "rogue" cells in 1998 as well as in 2001. Individuals encoded #1 and #2 belonged to the same family. One of them exhibited "rogue" cells in 1998 and the other in 2001. In the inhabitants of the control Yahotyn district "rogue" lymphocytes mostly contained one chromosome exchange per cell.

The mean frequency of "rogue" cell incidence among analyzed lymphocytes in the first and second examinations of groups involving 20 individuals were 0.14 and 0.09 per 100 cells, respectively (Table 6). The frequency of these cells among lymphocytes analyzed in 1998-99 from the whole group of self-settlers consisting of 33 individuals was 0.10 per 100 cells. Therefore, this value was generally similar to

| G                                | <sup>238,239,240</sup> Pu, |           | Year of examination               |  |  |  |  |
|----------------------------------|----------------------------|-----------|-----------------------------------|--|--|--|--|
| Group                            | $kBq/m^2$                  | Coded No. | 1998 – 1999 (n=33)                | 2001 (n=20)  |  |  |  |
|                                  |                            | 1         | <u>2(dic+ace):</u><br>tr, dic+ace |  |  |  |  |
|                                  | 1 - 2                      | 2         |                                   | tr, del, ace;<br>2tr, cr, dic+ace, 2min;<br>tric, dic+ace, min |  |  |  |
|                                  | 1 – 2                      | 8         | dic+ace, tr, del                  |  |  |  |  |
|                                  | 1-4                        | 19*       | dic, del                          |  |  |  |  |
| ChNPP<br>Exclusion<br>Zone       | 2-4                        | 29        | tr, 2(dic+ace)                    | tetrac, min, 2ace;<br>2tr in tetraploid;                       |  |  |  |
|                                  |                            | 15        | tr, ace                           | dic+ace, tr  |  |  |  |
|                                  |                            | 12        | dic, del                          |  |  |  |  |
|                                  | 2 - 10<br>4 - 10           | 6         | 2(dic+ace)                        |  |  |  |  |
|                                  |                            | 21        | dic+ace, del                      |  |  |  |  |
|                                  |                            | 32        |                                   | 2dic, dic+ace  |  |  |  |
|                                  |                            | 30*       | 2dic, del, 10ace;<br>2dic         |  |  |  |  |
|                                  | 10 - 40                    | 18        | 2(dic+ace)                        |  |  |  |  |
|                                  |                            | 1y        | dic+ace, 2ace                     |  |  |  |  |
| Yahotyn                          |                            | 11y       | dic+ace, del                      |  |  |  |  |
| district,<br>Kyiv region<br>n=31 |                            | 12y       | dic, ace                          |  |  |  |  |
|                                  | 0.02 - 0.1                 | 18y       | dic+ace, ace                      |  |  |  |  |
|                                  |                            | 20y       | cr+ace, dic+ace                   |  |  |  |  |
|                                  |                            | 26y       | dic, 2del                         |  |  |  |  |
|                                  |                            | 27y       | tr, ace                           |  |  |  |  |

 Table 7. Aberration spectrum in "rogue" peripheral blood lymphocytes of Exclusion Zone self-settlers and residents of Yahotyn district, Kyiv region.

\* - individuals examinated in 1998-99 only.

the values of both investigation periods for 22 self-settlers, and 1.5-fold higher than that for Yahotyn district residents' lymphocytes (0.06 per 100 cells).

Cells containing multiple chromosome exchanges had been also found in residents of Belarus and Russia [23, 24]. According to [21] no "rogue" cells had been found in course of the examination of rural population of Ukraine before the Chornobyl accident.

According to the reported data, cells with two unstable exchanges are observed in acute general irradiation starting from a dose of 100 cGy onward [25]. Effective whole body doses of irradiation for the self-settlers due to external and internal irradiation, reconstructed by us till the beginning of 1999, did not exceed 33 cSv and on red bone marrow - 63 cSv. Probably, emerging of cells with multiple chromosomal aberrations under low dose radiation exposure could be connected with the presence of hypersensitive lymphocyte subpopulation [26]. In the case of the Zone self-settlers we intend most probably to assume that the incidence in blood of extremely "rouge" cells is due to non-uniform internal irradiation owing to getting into the body of  $\alpha$ - emitting transuranium elements. It is likely that <sup>238,239,240</sup>Pu and <sup>241</sup>Am get into the body first of all with aerosols and probably with vegetative nutrition products. <sup>241</sup>Am content is accumulating on the Zone territory following  $\beta$ -decay of <sup>241</sup>Pu. During the post-accident years its amount increased from 0.003 to 0.04 PBq [27]. At the period of our investigations the level of soil contamination by <sup>241</sup>Am in the Zone appeared to coincide with the contamination level by all Pu-isotopes. Taking this situation into account, researches connected with transuranium elements are very important.

#### CONCLUSION

The results of our examination of ChNPP Exclusion zone self-settlers testify to the destabilizing effect of continuous low dose rate irradiation on genetic structures of somatic cells in conditions of living in the areas contaminated with radionuclides and to the expediency of cytogenetic monitoring of the population. The data obtained from studying of cytogenetic effects dynamics show that 12-15 years after the accident the level of chromosome damages in ChNPP Exclusion Zone self-settlers' lymphocytes comes to a plateau. The comparison of the results of cytogenetic examination of Yahotyn district inhabitants with the reported data on spontaneous levels of chromosomal damage allows us to assume the activation of mutagenesis in this region.

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