

## GENETIC INDICATORS OF THE DIOXIN EXPOSURE TO ECOSYSTEMS AND HUMAN HEALTH

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### Introduction

The published results from the studies on dioxins' genotoxicity on genetical models in vitro, on mammals and in epidemiological observations on humans are inconsistent. At the same time it is impossible to eliminate direct or indirect effect of dioxins, which results in inducing genetic instability. The analysis of the mechanism of dioxin' toxicity indicates its capability to significantly modify the effect of other factors, which are acting on the live organisms exposed to dioxins.

### Methods and Materials

The evaluation was carried out in the region of Chapaevsk (Samara oblast), a center of chemical and military-chemical industry. The air in the city contains over 100 chemical pollutants, some of them are hazardous substances class 1 or 2, such as dioxins, furans, hydrogen chloride, etc. The integrated assessment of the state of mammalian populations was performed using small mammals (bank vole – *Clethrionomus glareolus* and pygmy wood mouse – *Apodemus uralensis*) and the following methods: classic cytogenetic for estimation of the chromosomal aberrations and micronuclei in somatic cells, immunological and physiological investigations. Medico-genetic characteristics of Chapaevsk population included the estimation of congenital morphogenetic options (CMO) (about 400 children, 87 attributes), congenital malformations (CM) (retrospective studies for 15 years – 14299 deliveries, 19 forms – 168 cases of CMO), chromosomal aberrations in lymphocytes of peripheral blood and micronuclei in epithelial cells of mucous membrane of mouth (from 45 women: 15 women were workers of chemical Plant, 16 were from 1-3 km from the Plant, and 14 from more distant district of 5-8 km from the Plant), oxidant status (spontaneous peroxide hemolysis, chemoluminescence of plasma and saliva).

### Results and Discussion

The frequency of chromosomal aberrations in the voles and in the pygmy wood mice from industrial territories was significantly higher than in the mammals from recreational zone (9.04% and 9.63% versus 2.85%). The most aberrations were the single fragment, but sometimes centric ring chromosomes were observed. The level of the micronuclei in the same regions was 0.2% and 1.8% compare to 0.08% in condition control. General assessment of the immune status has shown a secondary immunodeficiency among mammals from ecologically unfavorable territories. In addition, mammals from more heavily polluted regions demonstrated statistically significant inhibition of growth processes and short-term stress effect, either hormonal or chemical.

We have also studied medico-genetic parameters in women in Chapaevsk with high dioxin exposure. Congenital morphogenetic options - number of attributes per 1 child (4.5 in boys and 4.4 in girls), where two times higher than in less polluted regions. Congenital malformations frequency per 1000 newborns was 11.75 (compare to 20.8 in EUROCAT), however the number of congenital hydrocephalus, agenesis and dysgenesis of kidneys was higher.

There was no statistically significant differences in the frequency of epithelial cells with micronuclei in scraping of the mucous cheek ( $0.33^{0/00} - 0.56^{0/00}$ ) for the examined groups of women. However, statistically significant differences in other indexes of genotoxic and cytotoxic effects were observed. Also, the highest level of pathologic forms of the nucleus was identified in one group.

The results of cytogenetic studies unambiguously demonstrate the existence of the spatial gradient within the town territory – from the plant to the distant district (Table 1). The comparison of levels of chromosomal aberrations in lymphocytes of workers of the plant (Zavod), as well as of inhabitants of Titovka settlement, and those of the “control” district “Nagorniy” has shown that all average parameters were arranged in decreasing order as follows: “Zavod” > “Titovka” > “Nagorniy”. The difference among groups was not statistically significant for the following parameters: fraction of cells with chromosomal aberrations, number of single fragments, paired fragments, exchanges of chromatide type and total number of aberrations per 100 cells. Statistically significant difference was revealed between groups “Zavod” – “Nagorniy” for the following characteristics: the ratio of aberrations of chromatide and chromosomal types (with the increase of the fraction of aberrations of chromosomal type in the group “Zavod”), the number of exchanges of chromosomal type per 100 cells and the portion of cells with exchanges of chromosomal type. All these parameters for the group “Titovka” were close to those of the group “Zavod”, but they demonstrated no significant difference with those of the control group “Nagorniy”.

Table 1.

Results of analysis of chromosomal aberrations in lymphocytes of Chapaevsk inhabitants.

	Workers of the Plant (Zavod)	1-3 km from the Plant (Titovka)	5-8 km from the Plant (Nagorniy)
	n=15	n=16	n=14
Number of cells	4500	4800	4015
Number of cells with aberrations, % ± m	5.84 ± 0.48	5.35 ± 0,43	4.91 ± 0.40
Total of aberrations per 100 cells	6.22 ± 0.54	5.71 ± 0.54	5.11 ± 0.41
Cells with aberrations, %			
1.0 – 2.9	1	0	0
3.0 – 4.9	2	7	8
5.0 – 6.9	9	7	5
7.0 – 8.9	2	1	0
9.0 – 10.9	1	1	1
Chromosomal exchanges per cell			
0	4485	4789	4011
1	12	10	3
2	2	0	1
3	1	0	0
4	0	1	0
Total of 1 - 4	15	11	4

The increase in the number of exchanges of chromosomal type in lymphocytes of humans is a distinct biological indicator of a radiation effect or some other factors related to a group of mutagens of the radiomimetic mechanism of action.

According to the published data, the number of exchanges of chromosomal type for healthy donors varies from 0.010 to 0.071 per 100 metaphases, and the number of dicentric chromosomes – from 0.010 to 0.068 per 100 metaphases. If in the group "Nagorniy" the above parameters are close to the spontaneous values (0.125 and 0.075 per 100 cells, respectively), then these values for other examined groups are essentially higher. They are respectively 0.422 and 0.200 for the group of workers of the plant (Zavod) and 0.292 and 0.146 per 100 cells for the group of Titovka inhabitants. The values mentioned are comparable to the levels of the same parameters in lymphocytes of inhabitants of the polluted districts after the accident at Chernobyl Nuclear Power Station and the levels of other districts with radioactive pollution. The important fact is that the examined subjects in the groups "Zavod" and "Titovka" exhibit the presence of lymphocytes with 3 and 4 exchanges of chromosomal type, as well as of dicentric chromosomes with paired fragments and acentric rings, and this is an evidence of the continuing effect of mutagenic factors.

According to the results of the combination of the three used methods, the stability of cells and biological liquids to a damage by active radicals of oxygen for the examined women from the group "Zavod" differs significantly from the respective parameters of the town residents. So, the female workers of the plant demonstrate the increase of intensity of the processes of peroxidized oxidation in the blood plasma together with the adaptive stabilization of the erythrocytes membrane. The chemoluminescence of the saliva, on the contrary, decreases, and it is possible that this is related to a local depression of the phagocytes section of immunity (specific peroxidase which determines principally the intensity of a quick flash of the saliva chemoluminescence induced by the hydrogen peroxide). The respective differences of parameters of the conditionally control group "Nagorniy" from those of the group of inhabitants "Titovka" display statistical significance of about 0.10 – 0.20 and may be evaluated as the tendency analogous to the differences in the group "Zavod".

The results of the psychological testing demonstrated the great psychological discomfort, as shown by the parameters of the high state anxiety and degree of overfatigue for the female workers of the chemical plant in comparison with female inhabitants of the town.

The tendency toward decrease of chromosomal aberrations for total number per 100 cells among the groups 1–2–3 was analogous to that for the portion of cells with chromosomal aberrations and the difference between these three groups was expressed more clearly. The number of exchanges of chromatide type per 100 cells in group 1 was two times higher than those in two other groups, although the differences in this parameter were not statistically significant. The more essential decrease in the number of exchanges of chromosomal type per 100 cells was noted: from 0.47 in group 1 to 0.29 in group 2 and to 0.12 in group 3. Differences in this parameter between groups 1 and 3 were statistically significant ( $P < 0.05$ ). The predominance of "fresh" exchanges of chromosomal type (dicentrics with paired fragments, acentric rings) was observed in all the groups in comparison with other types of exchanges<sup>1, 2, 3</sup>. This gave indications for a possibility of the formation of "fresh" exchanges in a previous, in respect to the time of the investigations, period. The correlation analysis for the obtained cytogenetic data and levels of dioxins in blood was conducted for 14 donors randomly distributed between the three examined groups. No correlation for the mentioned characteristics was found.

The increase in the number of exchanges of chromosomal type in lymphocytes of humans is a distinct biological indicator of a radiation effect or some other (chemicals, for example) factors related to a group of mutagens of the radiomimetic mechanism of action.

These data coincide with results of inspections of the workers exposed to dioxins, which didn't reveal a connection between concentration of TCDD in blood and levels of chromosomal aberrations and SCE in lymphocytes<sup>4,5</sup>. The reason for the observed cytogenetic changes is not clear. However in a study<sup>4</sup> inspecting workers engaged in cable manufacturing, where basic toxic agents were dioxins, furans and heavy metals, there was also statistically significant increase in the amount of acentric fragments and dicentric chromosomes in lymphocytes of workers in comparison with control group.

Apart from it, the load by the most minor doses of a cloudy can call events for types of additive or synergy in the case of instability of a genome. Moreover, the modifying effects of other environmental factors can be realized in padding genetically events. Molecular mechanisms of interactions lie in operation of an AhR-receptor, change of routes of signal transduction of growth factors, straight line of a gene activation of cytochrome P-450 (CYP1A1 etc.), antiestrogenic activity of dioxins, etc. Basically they were determined also by a polymorphism and variability of the answer of an organism to dioxins action.

It is necessary to take into account, that there is always a category of people, for which the potential danger of genetic damages due to variety of reasons is high. It could be due to increased individual sensitivity of genome to mutagens action by virtue of genetic polymorphism of biochemical systems (insufficiency of ferment work of systems of a reparation DNA or xenobiotics detoxication), disturbances of immune or hormonal system, disbalance of oxidant and psychological status. The estimation of individual sensitivity to the toxicants, particularities of genetic polymorphism are very useful for our understanding of real danger, real risk of human' contacts with persistent toxic substances<sup>6</sup>.

Comparison of the results from studies of mammals and humans let conclude that the most part of changes in genetic, oxidative, immune systems was similar, the type of danger in organisms had the same tendency and target organs. The assessments performed by analyzing mammals and humans revealed serious consequences of the impact of Chapaevsk city environment on the health.

### References

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