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Genetic effects of phenylpyrazoles on C*itellus fulvus* from biotopes of South Kazakhstan

Abstract

It was investigated of genotoxical effects of fipronil, that is widely used in Kazakhstan, on background rodents from South Kazakhstan biotopes, where widely used phenylpyrazoles insecticides. The frequency of chromosomal and genomic mutations in bone marrow cells of *Citellus fulvus* from Arys and Shardara areas were significantly higher (P<0.01) than in rodents from Kazygurt area, that is furthest from the pesticide treatments. High level of chromatid aberrations are indicative for the presence of chemical mutagens. It was determined fipronil and it's degradation to fipronil-sulfone metabolite in *Citellus fulvus* from biotopes of South Kazakhstan, where used phenylpyrazoles pesticides.

Keywords: genotoxical effects, Citellus fulvus, pesticide treatments.

Introduction

Due to intensification of environmental pollution processes by ecologically hazardous factors of physical, chemical and biological nature, which impact all components of ecosystem, thorough investigation of potential toxic and genotoxic effects on living organisms including human acquires special importance. Majority of these factors are products of human activities [1-4]. Currently, according to CAS data about 50 000 000 organic and inorganic compounds were registered [5]. These data suggest that increase in number of chemical compounds, which are introduced into the environment by man, the probability of its pollution by mutagenic factors also increases. The mutagenic factors can penetrate into cells of living organisms and cause disruption of their genetical structures.

Number of published data suggest that doses of various pesticides, used in agriculture affect like mutagens, causing cytotoxic and negative genetic effects [6-10]. According to toxicologist's study presence of pesticides was determined in many food types, and the number of pesticide poisoning according to WHO data comprises 1% annually [World Health Organization, WHO]. Pesticides could persist in soil for a long of time. They migrate and pollute surface and underground water sources. As a result of pesticide influence the the following occurs: disruption of population content of agro- and bio-cenosis, as well as elimination of natural predators and pests, negative effects on on useful fauna, occurrence of pesticide resistant species populations, due to accumulation of trace concentrations of pesticides in agricultural crop types harvest quality may change, also it may cause negative influence on genetical material of all living organisms [11]. However pesticides application in various sectors of the human economical activity is inevitable, as it due to economic necessity.

Currently, in Kazakhstan, particularly in South Kazakhstan, phenylpyrazole insecticides (such as Adonis, Regent) are widely used against large number of grasshoppers [12-14]. The main active component of phenylpyrazole insecticides is fipronil. According to literature data fipronil can degrades in natural conditions and in mammalian organisms and forms toxic metabolites, which are characterized by high

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toxicity and stability in contrast to former chemical [12, 15].

Data by mutagenic effects of fipronil on mammals are quite controversial. Therefore conduction of analysis of genetic effects of insecticides, which are based on fipronil, on mammalian in natural conditions is very important. Therefore, the research subjects were the study of mutagenic effects of fipronil on background rodent populations (*Citellus fulvus)* from South Kazakhstan biotopes, where are used phenylpyrazoles insecticides.

Materials and methods

The material was *Citellus fulvus* from Shardara and Arys areas of South Kazakhstan, where widely used phenylpyrazoles insecticides. The control zones was Kazygurt area of South Kazakhstan, which is furthest from the pesticide treatments.

The cytological specimen preparations and cytogenetic analysis of bone marrow cells were performed using standard protocol [16]. Rodent chromosomes were stained with Giemsa stain. Slides were analyzed and photographed by light microscope Axioscope-40 (Zeiss). Fipronil and fipronil-sulfone were quantified by GC EC

Yuonglin instrument (South Korea), GC EC HP, GC 8000 (Series Carlo Erba). It was used modified protocol in gas chromatography analysis [17-19].

Statistical analysis for evaluation of all quantitative data standard Student's test were used. In order to obtain mean values of parameters were treated by standard methods of variational statistics [20]. Tables and graphic images of data were obtained using the Microsoft Excel Windows program.

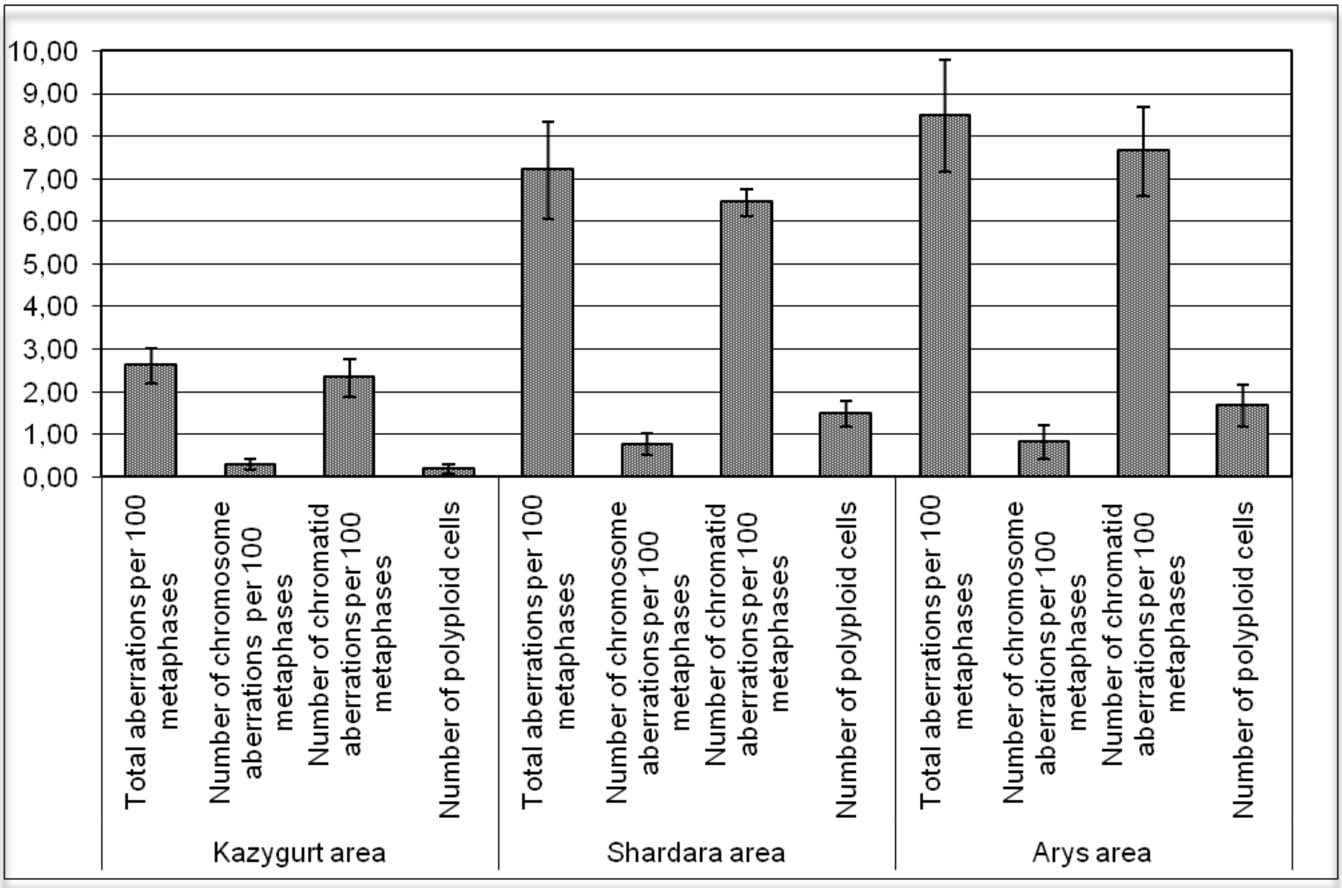

Figure 1 - The frequency of structural chromosomal and genome aberrations in bone marrow cells *Citellus fulvus*

Results and discussion

The results of cytogenetical analysis of bone marrow cells of *Citellus fulvus* from fipronil exposed biotopes demonstrated higher frequency of structural chromosomal aberrations (Figure 1). Figure 2 shows a normal karyotype of *Citellus fulvus*.

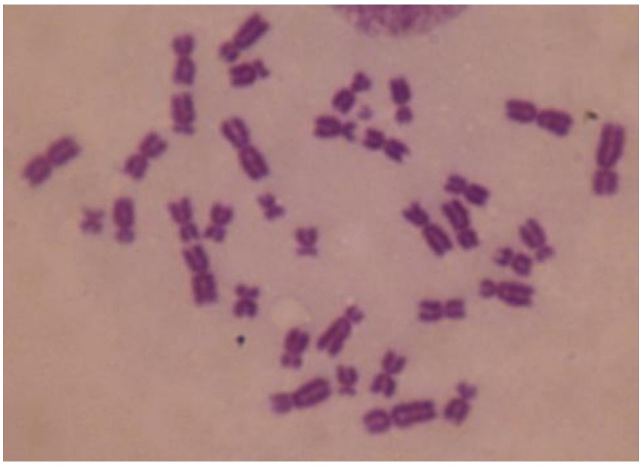

Figure 2 - Normal karyotype of *Citellus fulvus*, 2n=36, x 1000

The frequency of aberrant bone marrow cells in rodents from Arys and Shardara areas was high 2,5 ($P<0.05$) and 3 times ($P<0.01$), respectively, than the same rates in rodents from Kazygurt areas. Additionally, the level of polyploid cells increased significantly in those animals ($P<0.01$). Chromosomal aberrations were presented by pair chromosomal deletions and pair point deletions (figure 3); chromatid – cromatid deletions, chromatid point fragments and acenrtic fragments (figure 4). Polyploid cells were presented by tetraploid, hexaploid and octaploid cells (figure 5).

Thus, the cytogenetical study of bone marrow cells of rodents from fipronil exposed biotopes revealed significant increase of chromosomal aberrations frequency in comparison with the same rates in rodents from Kazygurt areas. Significant increase of structural mutations number resulted from chromatid aberrations. High level of chromatid aberrations in rodents from Arys and Sharadar areas indicate presence of chemical pollutants in that biotopes. The results demonstrate the increase of genetical burden in rodents from natural population, subjected to man-made chemical pollutants. The presence of high frequency of chromosome and genome aberration in *Citellus fulvus* indicatives of the presence of genotoxic factors in studied environment and can be considered as indication of general disorders in rodent organisms from polluted biotopes.

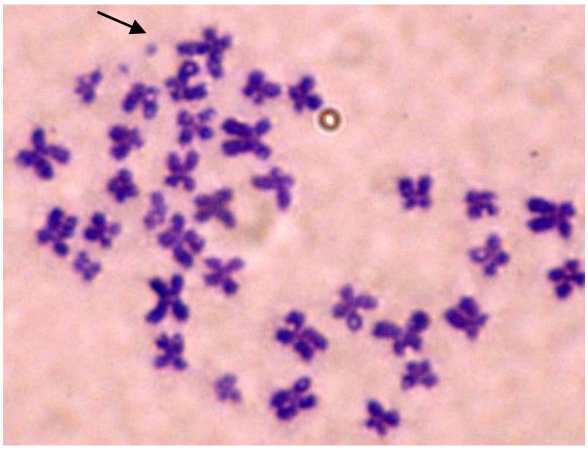

Figure 3 - Pair microfragments, x 1000

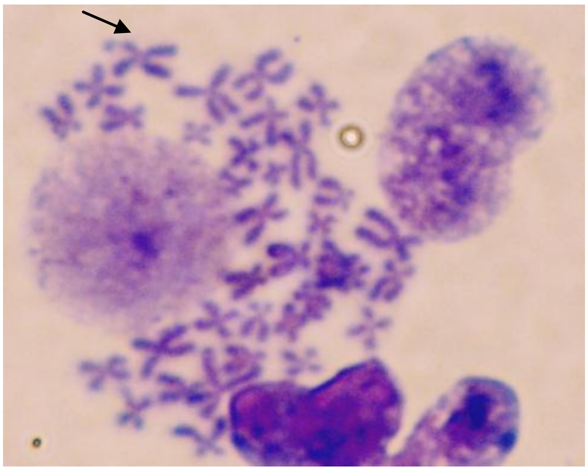

Figure 4 - Chromatide deletion, x 1000

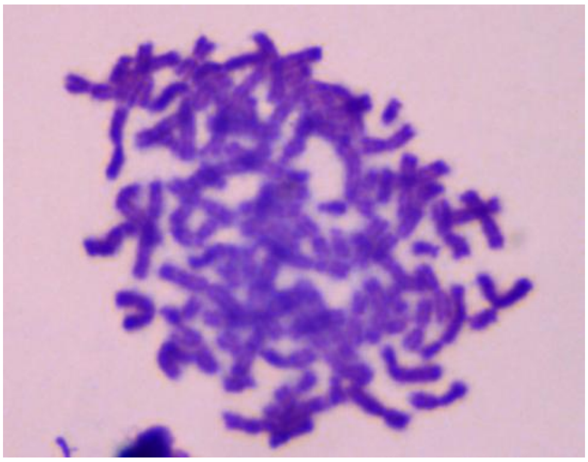

Figure 5 - Polyploid cell, 8n=36, x 1000

Above presented results testified the presence of chromosome and genome aberration in *Citellus fulvus,* Therefore, the research of the possibility of the presence xenobiotics in organism of *Citellus fulvus* from Arys and Saradara regions of South Kazakhstan with gas chromatography were performed.

It was determined fipronil and it's degradation to fipronil-sulfone metabolite in *Citellus fulvus* from biotopes of South Kazakhstan, where are widely used phenylpyrazoles pesticides. The metabolite content exceeded the fipronil content in rodent tissues, but the significant increase was observed in kidneys only (Figure 6). The investigation of fipronil and fipronil-sulfone content in background objects from fipronil exposed lands demonstrated their accumulation in rodent tissues.

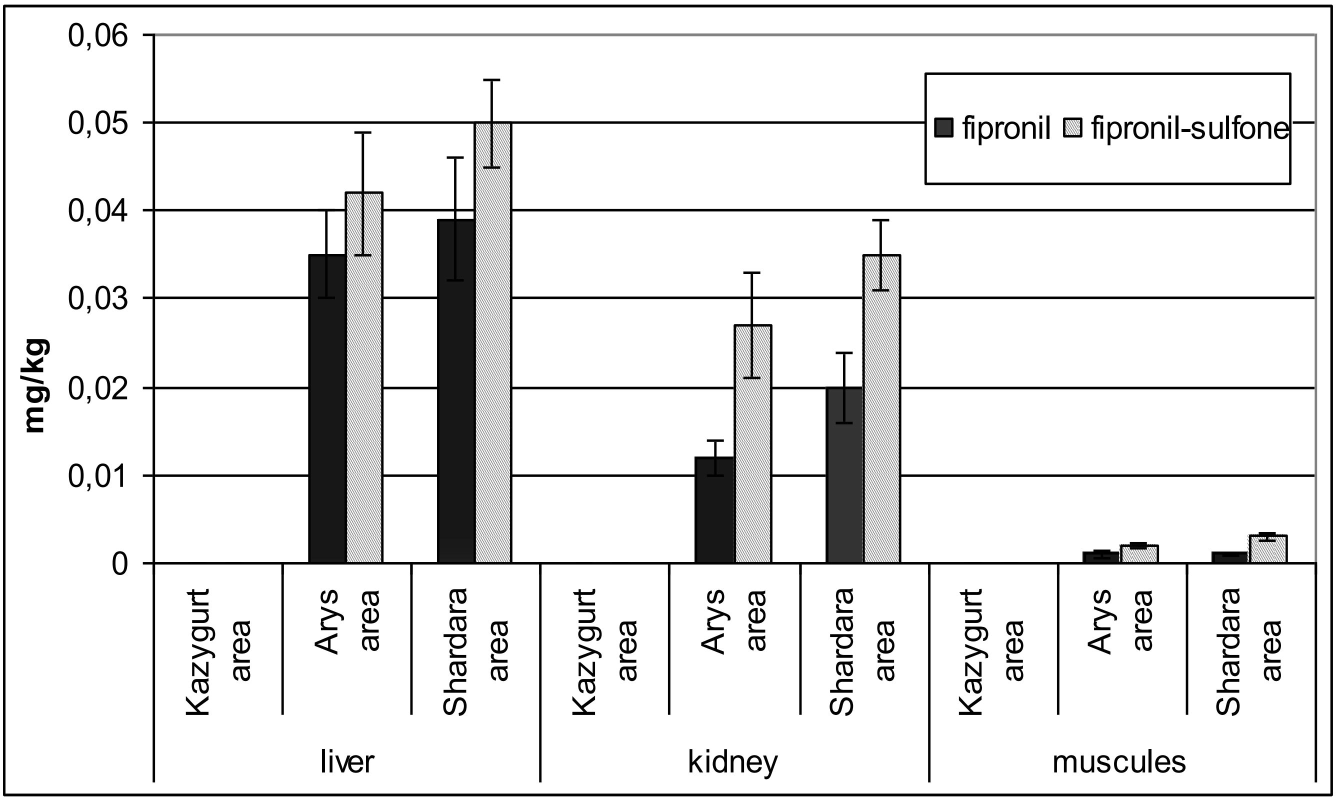

Figure 6 - Fipronil and fipronil-sulfone contents in *Citellus fulvus*

Thus, the frequency of chromosomal and genomic mutations in bone marrow cells of *Citellus fulvus* from Arys and Shardara areas were significantly higher (P<0.01) than in rodents from Kazygurt region. High level of chromatid aberrations in rodents from Arys and Sharadar areas indicate presence of chemical pollutants in that biotopes. The results demonstrate the increase of genetical burden in rodents from natural population, subjected to man-made chemical pollutants. The present structural changes in animal organs and high frequency of chromosome aberration in *Citellus fulvus* indicatives of the presence of genotoxic factors in studied environment and can be considered as indication of general disorders in rodent organisms from polluted biotopes [21, 22].

It was determined fipronil and it's degradation to fipronil-sulfone metabolite in *Citellus fulvus* from biotopes of South Kazakhstan, where are widely used phenylpyrazoles pesticides.

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References

1 Bostok K., Samner E. The chromosome of eukaryote celll. - Moskow: Piece. - 1981. – P.598.

2 Bersimbaev R.I. Chromosome aberrations in occupational uranium miners of North Kazakhstan // 8th International Conference on Enviromental Mutagens «Fundamental and Molecular mechanisms of Mutagenesis». – Shizuoka. - 2001. - P.178.

3 Bersimbaev R.I. The family analysis of the chromosome aberration frequencis in the population of the Semipalatinsk nuclear test site // 8th International Conference on Enviromental Mutagens «Fundamental and Molecular mechanisms of Mutagenesis». – Shizuoka. - 2001. - P.59.

4 Bigaliev A.B., Abilev S.K. Genetic and environment. - Karaganda: KSU. - 1989. – P.141.

5 CAS Registry Number and substance Counts, 2008 // http://www.cas.org/cgi-bin/regrepot.pl.

6 Garsia H.M. Effect of difenzogyat herbicide on cell division of wheat root tips // Physiol. Plant. - 1990. - Vol. 2. - P. 49-54.

7 Patil T.M., Shirashya V.S. Effect of methylparation and phosphamiden an mitotic chromosomal aberrations in some vegetable seeds // Environ. Biol. - 1991. - Vol. 3. - P. 249-254.

8 Holland N.T. et al. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo // Mutation Research (Genetic Toxicology and Environmental Mutagenesis). - 2002. - Vol. 521. - P. 165-178.

9 González M. et al. Effect of dithiocarbamate pesticide zineb and its commercial formulation, azzurro. IV. DNA damage and repair kinetics assessed by single cell gel electrophoresis (SCGE) assay on Chinese hamster ovary (CHO) cells // Mutation Research (Genetic Toxicology and Environmental Mutagenesis). - 2003. - Vol. 534. - P. 145-154.

10 Mahata J. et al. Chromosomal aberrations and sister chromatid exchanges in individuals exposed to arsenic through drinking water in West Bengal, India // Mutation Research (Genetic Toxicology and Environmental Mutagenesis). - 2003. - Vol. 534. - P. 133-143.

11 Sharma C.B.S.R. On the relative validity of the barley cytogenetic system as a monitor of pesticide mutagenecity // Environ. Mutagenes - 1982. - Vol. 4, № 3. - P. 329-335.

12 Insecticide factsheet. Fipronile // Journal of Pesticide Reform. - 2005. - Vol. 25, № 1. - P. 10-15.

13 Information bulletin RK. – Almaty: 2002. – P.159.

14 Sagitov A.O. et al. The prognostication of a volume of chemical treatment against Locusts in Kazakhstan // Protection and quarantine of plants. - 2002. - № 1. - P. 19-20.

15 Hainzl D., Cole L.M., Casida J.E. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct // Chem. Res. Toxicol. - 1998. - Vol. 11. - P. 1529-1535.

16 Grafodatsky A.S., Radgably S.I. Chromosomes of agricultural and laboratorial animals. - Novosibirsk: Science. - 1988. – P.127.

17 Vilchez J.L. et al. Determination of fipronil by solid-phase microextraction and gas chromatography-mass spectrometry // J. Chromatogr. A. - 2001. - Vol. 919. - P. 215-221.

18 The determination of fipronil and fipronil-sulfone in water, soil, potatoes, seeds by gas-liquid chromatography. Metodical protocols. MUK 4.1.1400-03: MSSD of RF 24.06.2003. – Moskow: 2003. – P.31.

19 Zhou P. et al. Dynamics of fipronil residue in vegetable-field ecosystem // Chemosphere. – 2004. - № 57. - P. 1691-1696.

20 Rokitsky N.A. Introduction in statistical genetics. – Minsk: Higher school. - 1978. – P.448.

21 Dmitriev Z.G. Cytogenetical unstability of three species of rodents in chemical plant of North Russia // Ecology. - 1997. - № 6. - P. 447-451.

22 Dubinin N.P. Common genetic – Moskow: Science. - 1986. – P.559.

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