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# COMPARATIVE DATA OF THE CHROMOSOME FRAGILITY IN FIVE WILD SMALL MAMMAL SPECIES

T. Gerasimova, M. Topashka-Ancheva  
Institute of zoology, Bulgarian Academy of Sciences  
Correspondence to: Margarita Topashka-Ancheva  
E-mail: topashka.mn@lycos.com

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## ABSTRACT

*Small mammals are widely used as suitable indicators of environmental pollution and genotoxic damage.*

*The present investigation of the karyotype sensitivity to the influence of a strong mutagen was held under strictly controlled laboratory conditions. The data about the chromosome aberrations induced by treating of Mitomycin C (3.5 mg/kg) in bone marrow cells have been analyzed. The structural changes in the chromosomes including laboratory white mice ICR as well as five species small rodents – Clethrionomys glareolus (Schreber, 1780), Apodemus flavicollis (Melchior, 1834), Microtus levis (Miller, 1908), Mus spicilegus (Petényi, 1882) originating from Rila Natural Park, Vitosha Nature Park, Kresna Gorge and the vicinities of Pleven region were compared.*

*The analysis was performed according to the protocol described by Preston et al. (1987).*

*All treated groups of small mammals investigated showed significantly higher percentage of damaged cells in comparison with their own untreated controls ( $p \geq 0.99$ ).*

*Rodent species under present investigation have presented significantly lower sensitivity of their chromosome set when compared to the laboratory mice ICR ( $p \geq 0.99$ ). The lowest chromosome aberrations value (in %) was expressed within the specimens in Microtus arvalis ( $10.29 \pm 0.38$ ) while the highest sensitivity was shown in Mus spicilegus ( $21.5 \pm 3.03$ ). In the four other species investigated quite close values about the number of induced chromosome aberrations were expressed.*

*These findings have important implications for the use of zoomonitors as bioindicators in ecotoxicology and in application of genotoxicity findings in laboratory animal models to natural populations.*

**Keywords:** Kystendil, chromosome aberrations, ecotoxicology, Mitomycin C

## Introduction

The cytogenetic analysis of mouse-like rodent species has some cogent advantages. It is of common knowledge that chemical pollution (which may be considered as component of the general environmental pollution) leads to mutagenesis in somatic and germ cells. As a consequence, the relative frequencies of chromosomal aberrations (CA) increase. This fact is used in ecotoxicological investigations to indicate an existing pollution in natural populations (14), (12).

Small mammals are widely used as suitable indicators of environmental pollution and genotoxic damage. Rodent species are evolutionally closer to humans rather than bacteria, plants or insects and occupy a vast habitat. Due to this fact, it is possible to compare populations from the different, often very remote regions. The rodents are

characterised by large-size populations, an enhanced rate of reproduction and occupy a basic position in the food chain. Therefore they are pronounced to be a suitable monitor test systems (7). White laboratory mice are well known experimental animals, frequently used as controls in laboratory research. They are the most commonly used mammalian model organism, more common than rats.

De Souza Bueno et al. (9) showed that there is a difference in chromosome structure sensitivity of two species wild rodents—*Akodon montensis* and *Oryzomys nigripes* selected as bioindicators in relation to the cytogenetic end points analyzed. The analysis of the end points (the mitotic index, the frequency of cells with micronuclei in the bone marrow and peripheral blood, and the frequency of cells with CA in the bone marrow) that may reveal a difference in susceptibility to clastogenic agents between the investigated wild species (cells with breaks) showed that both rodent species under investigation are not equally sensitive in

respect of the clastogens present at impacted and control sites.

In previous investigations some authors (1), (3), (15) showed that chromosome structures of bone marrow cells in *A. flavicollis* are more sensitive to the effect of the environmental pollutants. Metcheva et al. (6), Topashka-Ancheva et al. (12) and data presented by Cristaldi et al. (2) showed that the chromosomes in bone marrow cells of *C. glareolus* are more susceptible to heavy metal loading or <sup>137</sup>Cs contamination. Recent investigations concerning the karyotype stability of *A. flavicollis* and ICR mice have showed three-fold higher karyotype sensitivity of the laboratory mice in comparison with the treated yellow-necked mice (13) with prevalence of breaks and fragments.

Kovalova & Glazko (5) have revealed the species-specific increase of cytogenetic anomaly frequency in bone marrow cells at a high level of radionuclide pollution (bank voles – metaphases with CA, common voles – leukocytes with micronuclei), and species-specific association between various types of cytogenetic anomalies.

Those results have led up the idea to examine the karyotype sensitivity of some small mammal species (typical for Bulgaria) towards the influence of some strong mutagens under strictly controlled laboratory conditions by comparison with the laboratory mice. For this purpose groups of five small mammal species were subjected to the action of the alkylating agent Mitomycin C at a dose identical for all five species - 3,5mg/kg body weight.

Mitomycin C was selected because of its proven genotoxic effect and high frequency of induced chromosome aberrations. This compound is a standard laboratory mutagen widely applied on different animal cells (4).

## Materials and methods

*Clethrionomys glareolus*, *Apodemus flavicollis*, *Microtus levis*, *Microtus arvalis*, *Mus spicilegus* originating from several conditionally unpolluted regions in Bulgaria were used for scoring of CA not earlier than 24 hours after capture. Ten male and female ICR strain laboratory mice, weighting 20g±1.5g were delivered by the animal breeding house of the Bulgarian Academy of Sciences, Sofia. All animals were kept under standard conditions – temperature 20°C, photoperiod 7am to 7pm, free access to standard animal food and water.

The animal experiments were conducted according to approved protocols, and in compliance with the requirements of the European Convention for Protection of Vertebrate Animals used for experimental and other Specific

Purposes and the current Bulgarian laws and regulations.

The cytogenetical analysis was performed according to the protocol described by Preston et al. (8). Mitomycin C (3.5 mg/kg body weight) was injected intraperitoneally (i.p.). 45 min prior bone marrow cell scoring, each animal was injected i.p. with 0.04% colchicine (1ml/100g body weight). Animals were euthanized by diethyl ether; bone marrow from both femurs was removed and flushed in 0.075 M potassium chloride, incubated at 37°C for 20 min, repelleted and fixed in cold 1:3 glacial acetic acid: methanol. Slides were prepared by flame-drying and stained in 5 % Giemsa solution (Sigma Diagnostic).

Up to 50 well-scattered metaphase plates were analyzed from each animal using light microscopy.

## Results and Discussion

The In vivo CA frequencies in bone marrow of the five small mammal species following single injection of 3.5 mg/kg body weight Mitomycin C are presented on **Table 1**.

The main types of aberrations – breaks, fragments, exchanges (centromer/centromeric fusions, telomere/telomeric fusions) and pericentric inversions were separately scored. It was found that Mitomycin C induced significantly high number of CA in the bone marrow cells of all treated species.

In all investigated species the treated groups showed reliably higher percentage of damaged cells in comparison with the untreated controls ( $p \geq 1.99$ ). The highest percentage of cells with CA was observed within the treated group of the laboratory mice ( $38.42 \pm 1.88$ ) followed by its relative species *Mus spicilegus* ( $21.5 \pm 3.03$ ). In the rest three groups the values are as follows: *Microtus levis* ( $14.11 \pm 1.96$ ), *Apodemus flavicollis* ( $11.01 \pm 0.81$ ) and *Clethrionomys glareolus* ( $10.38 \pm 1.57$ ). The lowest value appeared to be in *Microtus arvalis* ( $10.29 \pm 0.38$ ).

TABLE 1.

Number and frequency of chromosome aberrations found in *Apodemus flavicollis*, *Clethrionomys glareolus*, *Microtus levis*, *Microtus arvalis*, *Mus spicilegus* and ICR strain laboratory mice after experimental treatment with Mitomycin C (3.5mg/kg body weight /24 h)

Species	Number of metaphases scored		Type of chromosome aberrations					Percentage of cells with aberrations X ± SE %
			Breaks	Fragments	Rearrangements			
Substance					c/c	t/t	PInv	
<i>A. flavicollis</i>	10♂	500	20	33	2	0	1	12.20
Mit. C	8♀	411	13	11	7	4	2	9.52
								<b>11.01 ± 0.81</b>
<i>A. flavicollis</i>	5♂	232	2	2	0	0	0	1.72
untreated	6♀	266	7	2	0	0	0	3.38
								<b>2.66 ± 0.40</b>
ICR Mit. C	6♂	300	37	47	20	5	0	36.33
	4♀	200	29	35	18	3	0	42.5
								<b>38.42 ± 1.88</b>
ICR untreated	5♂	250	1	0	2	0	0	1.2
								<b>1.2 ± 0.49</b>
<i>M. arvalis</i> Mit. C	6♂	276	20	10	0	0	0	10.86
	7♀	350	20	10	0	0	0	10.86
								<b>10.29 ± 0.38</b>
<i>M. arvalis</i>	7♂	350	8	4	0	0	0	3.43
untreated								<b>3.43 ± 0.57</b>
<i>M. levis</i> Mit. C	6♂	292	20	9	0	1	7	14.11
								<b>14.11 ± 1.96</b>
<i>M. levis</i> untreated	2♂	100	3	0	0	0	0	3
	2♀	100	2	0	0	0	1	3
								<b>3.0 ± 0.57</b>
<i>Mus</i>	2♂	100	12	4	2	0	0	18
<i>spicilegus</i> Mit. C	2♀	100	13	12	0	0	0	25
								<b>21.5 ± 3.03</b>
<i>Mus spicilegus</i>	2♂	100	2	2	0	0	0	4
untreated	2♀	100	2	1	0	0	0	3
								<b>3.50 ± 0.5</b>
<i>C. glareolus</i> Mit.	2♂	75	6	1	0	0	1	11
C	2♀	90	3	5	1	0	0	9.75
								<b>10.38 ± 1.57</b>
<i>C. glareolus</i>	5♂	221	2	1	0	0	0	1.36
untreated	5♀	250	1	1	1	0	0	1.2
								<b>1.2 ± 0.42</b>

c/c – centromer/centromeric fusion; t/t – telomere/telomeric fusion; PInv - pericentric inversions

The high percentage of aberrant metaphases detected in treated *Mus spicilegus* group correlated with the data obtained by Topashka-Ancheva & Mecheva (14) for *Mus macedonicus* samples collected near the heavy metal polluted region of lead-zinc factory in the vicinities of Asenovgrad. All these results

refer to the conclusion that the chromosome structure of the species of genus *Mus* is more susceptible to the influence of clastogenic factors with various natures, the alkylating agent Mitomycin C in particular or heavy metal pollution.

The control groups of *Microtus arvalis*, *Apodemus*

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*flavicollis* and *Microtus levis* do not significantly differ in respect of the percentage of metaphase plates with CA. These results allowed an assumption that the reaction of the karyotype of these species to the surrounding ecological background is similar. The higher values observed in the control groups of *Microtus arvalis* ( $3.43 \pm 0.57$ ) could be due to the general pollution in the region from where the animals were collected (the vicinities of Gara Jana, Sofia).

Chromatid breaks and fragments as though chromosomal rearrangements (centromeric fusions, telomeric fusions, pericentric inversions and translocations) were separately scored in the cytogenetic analysis of the chromosome slides. Breaks and fragments significantly prevail over the chromosomal type aberrations (from 76.17% of all aberrations observed in laboratory mice, 85.55% in *A. flavicollis*, 88.22% in *C. glareolus*, 95.3% in *Mus spicilegus*, 96.67% in *M. levis*, up to 100 % in *M. arvalis*).

The prevalence of breaks and fragments in the treated groups of the five species results from the specific effect of the alkylating agent Mitomycin C. Jena and Bhunya (4) showed that Mitomycin C applied on bone marrow cells of *Gallus domesticus* provoked chromatid and isochromatid breaks, deletions and exchanges. The mechanism of action of Mitomycin C at the molecular level is due to the formation of DNA-DNA crosslinking adducts (11). It has been suggested that Mitomycin C may act as an alkylating agent (10). The types of CA observed depend on the chemical nature of the applied mutagen, and the number of the affected cells derives from the karyotype stability.

From the results of a previous experiment (13) we concluded that *A. flavicollis* exhibited higher karyotype stability in comparison with the laboratory mice. The data are in agreement to a certain extent with the results reported by Ieradi et al. (3) who showed low frequencies of micronuclei in the peripheral blood and bone marrow cells of *A. flavicollis* from an industrially polluted area in the Czech Republic. The results of the present experiment have supported to a great extent our previous data concerning the problem (12).

The differences in the chromosome damage frequencies found in the six investigated small mammal species could hardly be explained only by their phylogenetical distance. It is more likely that the selection pressure of natural environment over wild rodent species has resulted in strong chromosome repair mechanisms with a stable chromosome structure. In the

various strains of laboratory mice living in constant laboratory conditions such selection pressure is unlikely to be involved, which results in higher chromosome fragility.

It is concluded that data obtained from laboratory mice should be carefully extrapolated to other small mammal species living in nature. Future investigations on other species of small mammals will expand our knowledge of the relative sensitivities of their chromosomes and will improve the possibilities of using them as bioindicators of genotoxic damage.

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