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GENOTOXICITY AND POTENTIAL CHEMOSTERILANT EFFECTS OF ROSOL

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Rosol is an anticoagulant rodenticide which, according to the specification of its producer (VZ-Zemun), contains 0.5 g of warfarin-sodium in 100 mililiters of solution. Its cytogenetical effects on bone marrow mitotic cells and testicular meiotic cells of Mus musculus L. (1758) of the BALB/c strain were investigated in an in vivo experiment. Animals were intragastrically treated with Rosol at doses of 0,25 mg, 0,50 mg and 0,75 mg of warfarin-sodium/kg b. w. The applied doses of warfarin-sodium were chosen according to the results of Kastori (1993), who found 0,562 mg anticoagulant rodenticides could be detected in 1 kg of soil polluted by various pesticides.

The investigated doses of Rosol induced numerical (polyploidy and aneuploidy) and structural chromosomal changes (lesions, gaps, acentrics, Robertsonian fusions) of both cell types. The results obtained in the experiment point to genotoxic and mutagenic effects of Rosol or of its active component, warfarin-sodium. These effects are significant for rodent pest control as they might cause fertility failure of the treated rodents. The occurrence of numerical and structural chromosomal changes in testicular meiotic cells could lead to the production of genetically unbalanced gametes. If the unbalanced products mature and are capable of fertilization, unbalanced zygotes will be formed and may die in utero or give rise to congenitally abnormal offspring. It appears that the anticoagulant rodenticide Rosol causes antifertile effects and also acts as a chemosterilant.

Key words: Rosol, Warfarin-Sodium, Mice, Chromosomal Changes, Genotoxicity, Chemosterilant

INTRODUCTION

Nowdays the economic and epizootiological importance of mouse-like rodent control is well known. Methods for regulating the size of their populations include: preventive measures, mechanical-physical, biological, genetic and chemical methods. Chemical methods based on the application of acute, anticoagulant and chemosterilant rodenticides are the most frequently used (for rodent poest control). It appears that rodents become resistant to anticoagulant rodenticides which contain coumarin or its derivates. At the same time, all rodenticides are not toxic only for rodent pests, but also for domestic animals and humans. In addition to their anticoagulant effects, rodenticides based on coumarin and its derivates possess genotoxic, mutagenic and teratogenic effects (Baranov, 1971; Baranov, 1971; Baranov, and Dibon 1971; Capanna et al., 1971; Capanna et al., 1977; Gropp and tettanborn 1970; Haidari-Naser, 1980; Kataranovski, 1988; Kataranovski 1994; Meehan,1984; Ruvinski et al., 1986 a, b; Stanimirović, 1995; Stanimirović et al. 1995; Vučinić 1994, Marković 1996). The genotoxical and cytogenetical studies of the cited authors suggest that anticoagulant rodenticides, tested in mammalian somatic (mitotic) or geminative (meiotic) cells, induced numerical and structural changes in chromosomes. Therefore, the cytogenetical influence of Rosol on bone marrow mitotic cells and testicular meiotic cells of mice, was examined in order to determine the potential genotoxicity of Rosol or its active component at minimal doses which usually can be detected in the soil of ecological polluted regions (Kastori, 1993).

MATERIAL AND METHODS

The cytogenetical effects of the anticoagulant rodenticide Rosol on bone marrow mitotic cells and testicular meiotic cells of the BALB/c strain of mice were investigated in an in vivo experiment. In addition to the control group there were three experimental groups formed according to the applied doses of the examined substance. Warfarin-sodium (alpha-3 (α-acetonybenzyl) 4-hydroxycoumarin ($C_{19}H_{15}O_4Na$), at doses of 0,25 mg/kg, 0,50 mg/kg and 0,75 mg/kg b.w., was intragastrically injected into the experimental animals during the treatment period of 5 days. In each group there were 6 animals. For each animal from the control group or experimental groups 100 cells were cytogenetically examined. The applied warfarin-sodium doses were chosen according to the results of Kastori (1993), who established that 0,562 mg anticoagulant rodenticides could be detected in 1 kg of soil polluted by various pesticides. Mitotic chromosomes from bone marrow cells were prepared according to the method of Zimonjić et al. (1990). G-banding was done employing the methods of Seabright (1971), Yunis et al. (1978), and Durrillaix et al. (1981). Chromosomes and chromosomal bands were identified the basis of the criteria established by the Committee on Standardized Genetic Nomenclature for Mice (1972, 1979) and Cowell's photoatlas of mouse chromosomes (Cowell, 1984). Meiotic chromosome from testicular cells were prepared according to the method of Evans et al., (1964). The staining was done in Giemsa.

RESULTS AND DISCUSSION

Rosol is an anticoagulant rodenticide which contains warfarin-sodium. The results which we obtained during this investigation point to the inductive ability of Rosol or its active component to produce numerical (polyploidy and aneuploidy) and structural (lesions, gaps, breaks, acentrics, rings, Robetsonian fusions) chromosomal changes in bone marrow mitotic cells (Figures 3, 4, 4a) and testicular meiotic cells (Figures 5) at all the investigated doses. The results are shown in Tables 1 and 2, and also graphically represented in Figures 1 and 2.

Table 1. Cytogenetical changes in bone marrow mitotic cells of the control and experimental groups of mice

Types of cytogenetical change		Control and experimental groups of mice											
		Control group			0.25 mg/kg b. w.			0.50 mg/kg b. w.			0.75 mg/kg b. w.		
		X ± SD	&	%	X ± SD	&	%	X ± SD	&	%	X ± SD	&	%
Numerical changes	Aneupoloidy	4.50 ± 1.07	0.75		67.00 ± 3.31	11.17		111.25 ± 7.70	18.54		130 ± 40.16	21.69	
	Polyploidy	0.00 ± 0.00	0.00		10.50 ± 1.31	1.75		10.38 ± 1.77	1.73		18.00 ± 1.51	3.00	
Structural changes	Lesions	3.25 ± 0.71	0.54		5.50 ± 0.93	0.92		6.37 ± 1.06	1.06		33.00 ± 2.07	5.50	
	Breaks	1.38 ± 0.75	0.23		10.63 ± 1.06	1.77		6.50 ± 0.76	1.08		11.12 ± 1.64	1.83	
	Rings	0.00 ± 0.00	0.00		0.00 ± 0.00	0.00		6.63 ± 1.30	1.11		1.13 ± 0.83	0.19	
	Acentrics	0.00 ± 0.00	0.00		0.00 ± 0.00	0.00		13.25 ± 2.60	2.21		2.25 ± 1.67	0.38	
	Robertsonian translocation	0.00 ± 0.00	0.00		9.00 ± 1.60	1.50		38.38 ± 1.69	6.40		39.50 ± 1.43	6.58	
All cells with numerical and structural changes		8.80 ± 1.73	1.48		102.63 ± 4.10	17.11		190.38±15.53	31.73		237.63±7.44	39.61	

Table 2. Chromosomal changes of testicular meiotic cells

Types of cytogenetical change		Control and experimental groups											
		Control group			0.25 mg/kg b. w.			0.50 mg/kg b. w.			0.75 mg/kg b. w.		
		X ± SD	&	%	X ± SD	&	%	X ± SD	&	%	X ± SD	&	%
Numerical changes	Aneupoloidy	4.63 ± 1.69	0.77		62.38 ± 4.10	10.40		116.63±5.04	19.44		153.00±325	25.50	
	Polyploidy	0.00 ± 0.00	0.00		9.13 ± 1.25	1.52		11.38 ± 1.18	1.90		16.13 ± 2.42	2.69	
Structural changes	Lesions	3.63 ± 1.60	0.61		4.75 ± 1.04	0.79		5.62 ± 1.77	0.94		26.13 ± 2.79	4.36	
	Breaks	2.75 ± 1.39	0.46		8.38 ± 1.30	1.40		6.25 ± 0.71	1.04		12.88 ± 2.10	2.15	
	Acentrics	0.00 ± 0.00	0.00		0.00 ± 0.00	0.00		3.50 ± 1.31	0.58		6.50 ± 1.41	1.08	
	Robertsonian translocation	0.88 ± 0.99	0.15		8.50 ± 1.77	1.42		21.00 ± 2.51	3.50		52.63 ± 4.10	8.77	
All cells with numerical and structural changes		11.88 ± 2.23	1.98		93.13 ± 5.59	15.52		164.50 ± 6.85	27.42		247.25 ± 8.71	41.21	

At the same time, it has been established that at the investigated doses, warfarin-sodium decreased the proliferative activity of the bone marrow cells. This effect was manifested through a decrease in the mitotic index in the experimental groups of mice. The mean value of the mitotic index in the control group war 5.97±0.29. In the three experimental groups the mean valus for this parameter were. The mean value of the three experimental groups the mean values for this

parameter were 3.98± 0.48 (at the dose of 0.25 mg/kg b.), 3.18± 0.45 (at 0.50 mg/kg b.w.) and 1.94± 0.49 (at 0.75 mg/kg b.w.). In the three experimental groups the mean values for this parameter were 3.98±0.48 (at the dose of 0.25 mg/kgb.w.), 3.18±0.45 (0.50 mg/kg b.w.) and 1.94±0.49 (0.75 mg/kg).

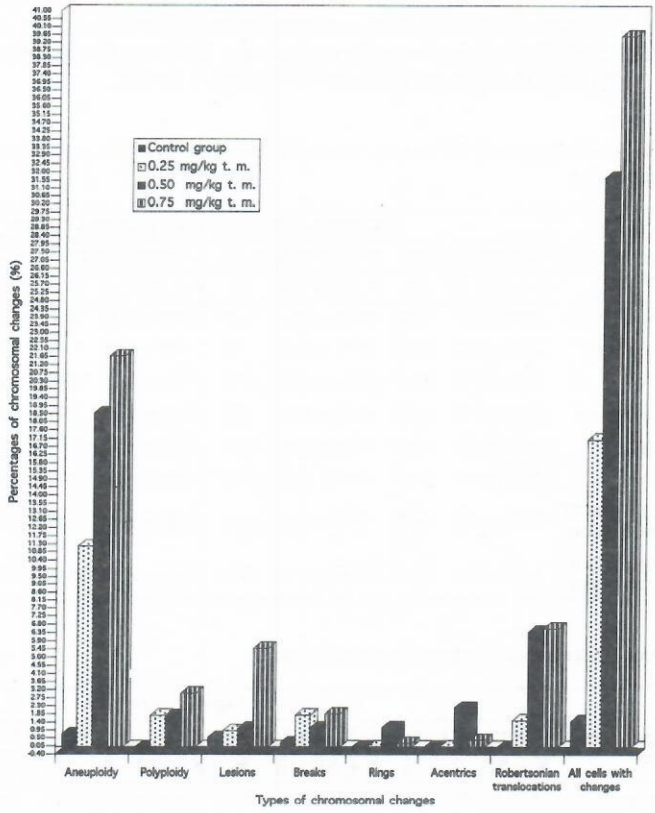

Figure 1. Chromosomal changes of bone marrow mitotic cells established in the control and experimental groups of mice, given in percentages

The percentage of mitotic and meiotic cells with numerical and structural chromosomal changes increased with increasing dose. However, the extent of the change was not proportional to the increase in dose for all types of chromosomal aberrations or more fully noticeable between the control group of animals and the highest dose (0,75 mg/kg b.w.). Thus, in this study we observed that the numbers of rings (1,11%) and acentrics (2,21%) in bone marrow mitotic cells were higher at the dose of 0.50 mg/kg b. w. than in those animals treated with warfarin-sodium at the dose of 0.75 mg/kg b. w. (rings-0.19%, acentrics-

0.38%). The reason for this could be selective effects of the highest dose of 0.75 mg/kg b.w., because of its higher toxicity for cells compared to the lower dose of 0.50 mg/kg b.w. Our consideration about this phenomenon is based on the investigations of Beutler (1985) and Marković (1996).

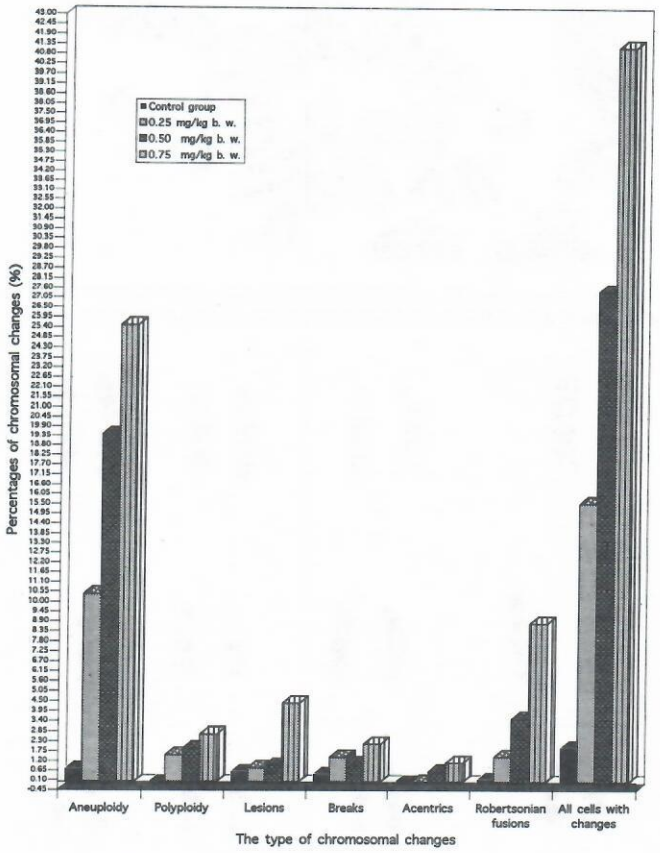

Figure 2. Chromosomal changes of testicular meiotic cells established in the control and experimental groups of animals, given in percentages

The presence of Rb 5/15 chromosome was observed in karyotypes of all the animals treated with increasing doses of the investigated substance. This Robertsonian chromosome (Rb 5/15) was observed in the karyotypes of natural mouse populations from regions with high concentrations of warfarin derivates in soil too (Stanimirović, 1995; Stanimirović et al. 1995).

The results obtained in the experiment have shown that Rosol could be considered as a genotoxic substance because this rodenticide induces changes at the mitotic and meiotic chromosome levels. Thus, the data presented here

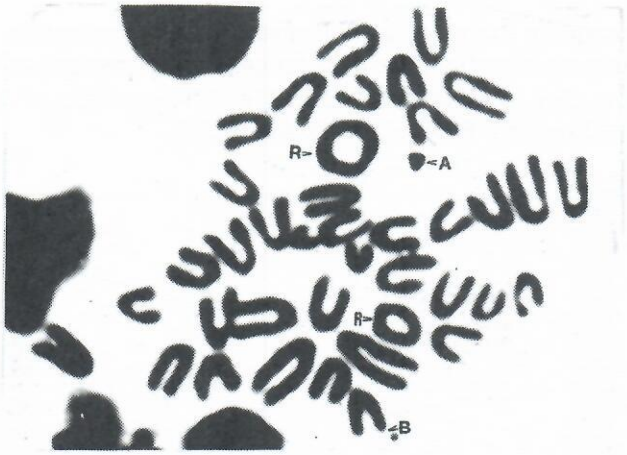

Figure 3. Two ring chromosomes (R), acentric (A) and breaks (B) in a bone marrow mitotic cell of a mouse treated with warfarin-sodium at the dose of 0,50 mg/kg b.w.

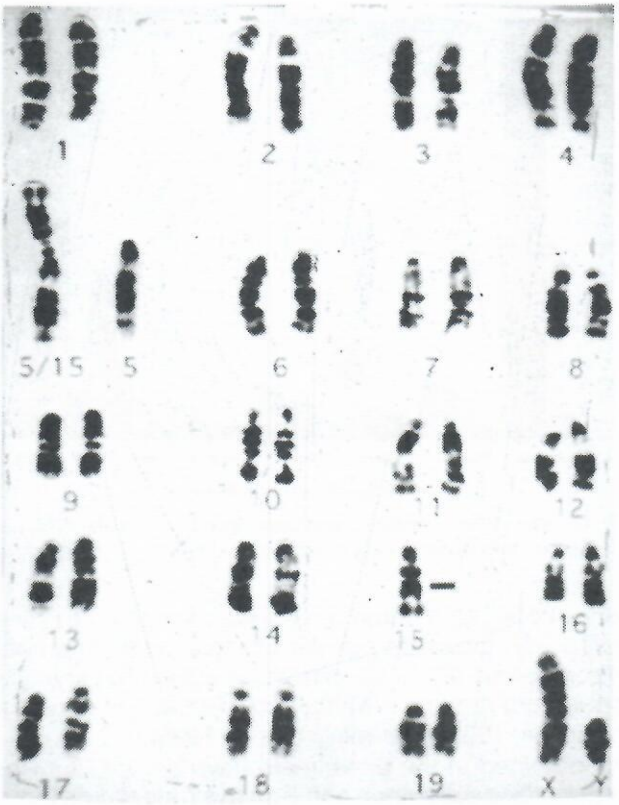

Figure 4. G-banded karyotype of a male mouse from the experimental group treated with warfarin-sodium at the dose of 0,75 mg/kg b. w. with one Rb. 5/15.

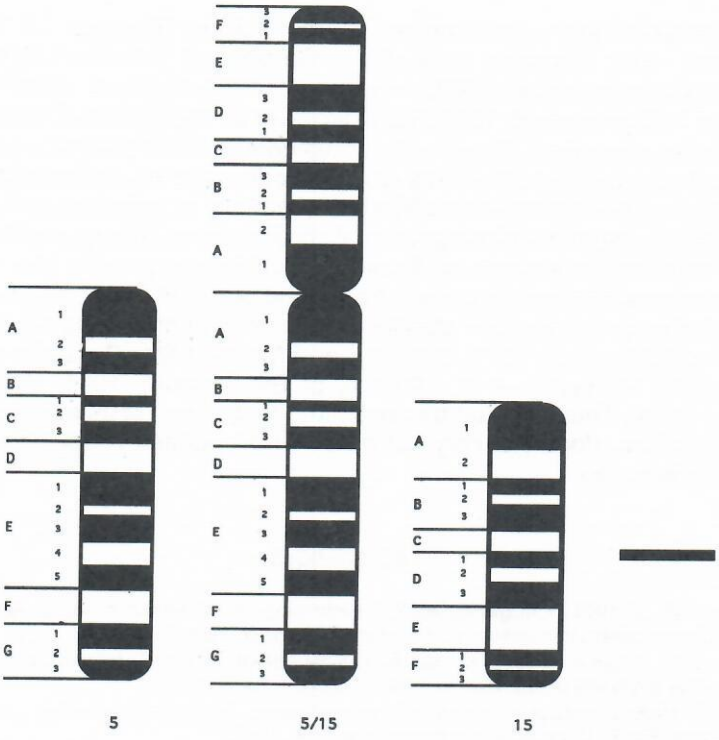

Figure 4a. Idiogram of 5., 15. and Rb.5/15 chromosome.

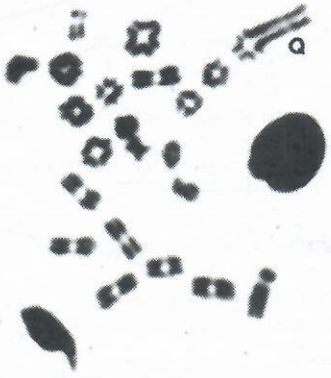

Figure 5. Late diakinesis/metaphase I of testicular meiotic cells with one quadrivalent (Q=quadrivalent).

support and extend the observations of other authors (Baranov, 1971; Baranov, and Diban 1971; Capanna et al. 1977; Gropp and Tellenbon 1970; Haidari-Naser,1980; Kataranovski, 1988; Kataranovski 1994; Meehan, 1984; Ruvinski, et al. 1986 a, b; Stanimirović, 1995; Stanimirović et al. 1995; Vučinić 1994, Marković 1996). At the same time, Rosol or its active component could be considered as a chemosterilant. Chemosterilants are chemicals used to render rodents of both sexes sterile. These chemicals disturb the maturity of gametes and in this way induce sterility. Numerical and structural chromosomal changes of the testicular meiotic cells may be a cause of disturbance in gametogenesis. The occurrence of chromosomal changes in gametes could be one of the factors that reduce the fertility of mice or induce their sterility (interference of crossingover, death of the embryos etc.(Baranov, 1971; Baranov, and Diban, 1971; Capanna et al. 1977; Gropp 1970; Ruvinskij, et al. 1986. a, b; Stanimirović, 1995; Vučinić 1994,; Marković 1996). Thus, it could be concluded that Rosol, or its active component warfarin-sodium, does not only act as an anticoagulant rodenticide, but as a chemosterilant, too.

REFERENCES

1. *Baranov V. S.* 1971. Citogenetičeskij i embriologičeskij analiz mišej s novoj markernoj Robertsonovskoj translokaciej., Biologija laboratornih životnih, 3, 27-28.
2. *Baranov V. S., Diban A. P.* 1971. Novaja markemaja Robertsonovskaja translokacija (centričeskoje slijanije autosom) u laboratoruih mišej, *Citologija, 13, 7,* 820.
3. *Beutler E.* 1985. Chemical toxicology of the erythrocyte. In: Toxicology of the Blood and Bone Marrow. Ed: R. D. Irons, *Raven Press, New York,* 39-50.
4. *Capanna E., Civotelli M. V., Cristaldi M.* 1977. Chromosomal rearrangement, reproductive isolation and speciation in mammals. The case of Mus musculus. *Boll. Zool., 44,* 213-246.
5. *Committee on Standardized Genetic Nomenclature for Mice* 1972. Standard karyotype of the mouse (Mus musculus). *Heredity, 63,* 69-72.
6. *Committee on Standardized Genetic Nomenclature for Mice:* 1979) New rules for nomenclature of genes, chromosome anomalies and inbred strains mouse. *Newsletter, 61* 4-11.
7. *Cowell J. K.* 1984. A photographic representation of the variability of the G-banded structure ofthe chromosomes of the mouse karyotype. Chromosoma, 89: 294-32-.
8. *Dutrillaux B., Vieges-Pequignot E.,* 1981. High resolution of R and G-banding on the same preparation. Human Genetics, 57., 53.
9. Evans E. P., Breckon, G., Ford, C. E. 1964. An airdrying method for meiotic preparations from mammalian testises. *Cytogenetics, 3*: 289-294.
10. Gropp, A., Tettenborn, U. 1970. Meiosis and Testicular Histology in XYY Males, *Morph. Asp. of Androl., 1,* 32-36.
11. *Haidari-Naser, M. A.,* 1980. Citogenetička dijagnostika efekata hemijskih zagađivača životne sredine na ćelijama čoveka i drugih sisara, doktorska disertacija, Veterinarski fakultet, Univerzitet u Beogradu. Doktorska disertacija, 1-120.
12. *Kataranovski, D.,* 1988. Analiza osnovnih parametara populacija vrste Ratus norvegikus, Berkenhout, 1769, u uslovima urbanih i suburbanih staništa. Prirodno-matematički fakultet, *Univerzitet u Beogradu. Doktorska disertacija,* 1-141.
13. *Kataranovski D.* 1994. The toxicity of rodenticides in rats following topicalapplication I LD50 and LD 100 values and changes of selected serum and urine biochemical parameters. *J. Serb. Chem. Soc. 59*: 787-795.
14. *Kastori R.,* (1993). Teški metali i pesticidi u zemljištu Vojvodine). *Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo, Novi Sad, Monografija* 1-314.

15. *Meehan, A. P.* 1984. Rats and mice, their biology and control. Rentokil Ltd., *East Grinstead, W. Sussex.* 383.
16. *Marković B.* 1996. Genotoksični efekat rodenticida "Rosol" kod laboratorijskog miša BALB/c soja. *Magistarska teza, Biološki fakultet Univerziteta u Beogradu.* 1-106.
17. Ruvinskij, A.O., Agnlnik S. I., Agnlnik A. I., Beljajev D. K., (1986. a). Segregacija hromosom u mišej heterozigotnih po Robertsonovskim translokacijam (Soobćenije 1), *Genetika, Tom 22, 9,* 2326-2332.
18. *Ruvinskij A. O., Agulnik S. I., Agulnik A. I., Beljajev D. K.,* (1986. b). Segregacija hromosom u mišej hetemzigotnih po Robertsonovskim translokacijam (Soobćenije 2), *Genetika, Tom 22, 10,* 25IO5-2511.
19. *Seabright M.* 1971. A rapid banding tehnique for human chromosomes. *Lancet 2,* 971-972.
20. *Stanimirović, Ž., Z.* 1995. Comparative analysis of natural and induced polymorphysm of chromosomes in the species Mus musculus, LInne 1758. *Doktorska disertacija, Biološki fakultet, Univerzitet u Beogradu,* 1-204.
21. *Stanimirović Z., Vučinić, Marijana, Soldatović, B., Vučićević, Marijana* 1995: A large acrocentric chromosome in the first pair of autosomes in natural populations of Mus musculus, Linne 1758. *Acta Veterinaria, 45/2-3,* 155-160., Beograd.
22. *Vučinić, M.* 1994. Uticaj kombinovane intragastrične aplikacije Bezalkorijum hlorida i Varfarin natrijuma na citogenetičke promene ćelija kostne srži i semenika laboratorijskih miševa. *Doktorska disertacija, Veterinarski fakultet, Univerzitet u Beogradu,* 1-69.
23. *Yunis J. J., Sawyer J. R., Bal D. W.,* 1978. The characterization of high resolution G-banded chromosomes of man. *Chromosoma, 67:* 293.
24. *Zimonjić, D. B., Savković, N., Anđelković, M.,* 1990. Genotoksični agensi - efekti, principi i metodologija detekcije, *Naučna knjiga, Beograd,* 1-425.

GENOTOKSIČNI I POTENCIJALNO HEMOSTERILANTNI EFEKAT ROSOLA

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SADRŽAJ

Citogenetički je ispitivan genotoksični i hemiosterilantni efekat rastućih doza (0,25mg/kg, 0,50 mg/kg i 0,75 mg/kg telesne mase) rodenticida "Rosol" (4-kumarinhidroksida) u in vivo eksperimentu na ćelijama kostne srži i semenim ćelijama laboratorijskog belog miša BALB/c soja vrste Mus musculus L. (1758). Utvrđeno je da ispitivane doze Warfarin-natrijuma, kao aktivne supstance rodenticida "Rosol" imaju sposobnost indukcije kako numeričkih (aneuploidije i poliploidije) tako i strukturnih (lezije, prekidi, acentrici, Robertsonove fuzije) aberacije hromozoma, što nedvosmisleno ukazuje na genotoksičnost ovog antikoagulantnog pesticida. Na osnovu dobijenih rezultata citogenetičkog ispitivanja ćelija kostne srži i semenih ćelija testisa eksperimentalnih životinja u sistemu in vivo, obzirom na poremećaje kariotipa (pre svega pojava Robertsonovih translokacija) i segregacije mejotičkih hromozoma u procesu spermatogeneze, kao i na osnovu teorijskih razmatranja posledica neregularnosti hromozomskih segregacija, zaključili smo da rodenticid Warfarin-natrijum osim antikoagulantnog može imati i hemisterilantno dejstvo, jer uslovljava poremećaje u spermatogenezi, uzrokujući smanjenu plodnosti nosilaca navedenih strukturnih aberacija (Robertsonovih translokacija), koje se manifestuju u pojavi defektnih spermatozoida, ili ako pak dođe do fertilizacije, u povećanom broju pobačaja, rađanju avitalnih potomaka ili preranom uginuću istih.