UDC 575:633.15
DOI: 10.2298/GENSR1130121J
Original scientific paper

CYTOGENETIC BIOMONITORING IN A SERBIAN POPULATION OCCUPATIONALLY EXPOSED TO A COMPLEX MIXTURE OF PESTICIDES

Dubravka JOVICIC [1], Jelena PAJIC [2], Boban RAKIC[2], Ljiljana RADIVOJEVIC [3], Milos PAJIC[4], Vaskrsije JANJIC[3], Aleksandar MILOVANOVIC[2]

[1]Singidunum University, Faculty of Applied Ecology (FUTURA), Belgrade, Serbia
[2]Serbian Institute of Occupational Health "Dr Dragomir Karajović", Belgrade, Serbia
[3]Institute of Pesticides and Environmental Protection, Belgrade, Serbia
[4]University of Belgrade, Faculty of Agriculture, Serbia

Jovicic D., J. Pajic, B. Rakic, Lj. Radivojevic, M. Pajic, V. Janic and A. Milovanovic (2013): *Cytogenetic biomonitoring in a Serbian population occupationally exposed to a complex mixture of pesticides.* Genetika, Vol 45, No. 1, 121-133.

The aim of this study was the analysis of chromosomal aberrations (CA) and premature centromeric division (PCD) in people exposed to pesticides at work. The research included 26 subjects occupationally exposed to pesticides, of average age 39.89 ± 8.66, and 32 control subjects of average age 40.57 ± 6.57. Mann-Whitney U tests showed statistically significant differences between the groups for mean values of all the examined variables. In addition, Spearman's (non-parametric) correlation test detected a positive linear correlation between CA and PCD. The presence of mostly chromatid and isochromatid breaks in the examinees indicates continuous exposure to pesticides. The absence of complex chromosomal rearrangements points to adequate protection of the subjects at their work places.

Key words: Occupational exposure, Human lymphocytes, chromosomal aberrations, pesticides

INTRODUCTION

Pesticides are extensively applied all over the world and in recent years their use has increased. At present there are more than 1000 chemicals classified as pesticides (WHO, 2004) and about 890 active ingredients in 20 700 pesticide products (TOMLIN, 2011).

Corresponding author: Jelena Pajic;Deligradska 29, 11 000 Beograd, tel: +381 11 34 00 976; fax: +381 11 26 43 675,e-mail: jejapajic@gmail.com;

The perfect pesticide should be toxic only to target organisms, be totally biodegradable to CO_2 and H_2O, and should not leave intermediate compounds in environment. Unfortunately, this is rarely the case and widespread use of pesticides in the contemporary agriculture is of increasing concern. The main problems in real system arising from the use of pesticides in agriculture are their toxicity to non-target organisms and environment (ROS *et al.,* 2006; RADIVOJEVIC *et al*., 2008; RADIVOJEVIC *et al.,* 2012).

Large amounts of these chemicals are released into the environment and being a potential hazard to human health. Toxicologic evidence of the carcinogenicity of several pesticides in animals, together with the fact that a large population of workers are exposed to such compounds, have attracted the attention of many studies (CARBONELL *et al.,* 1995; PADMAVATHI *et al*. 2000; GOMEZ-ARROYO *et al*., 2000; ZELJEZIC *et al*., 2002; UNDEGER *et al*., 2002; GROVER *et al*. 2003; HEUSER *et al.* 2007; JOVICIC *et al.,* 2012). With regard to genotoxicity, particular attention is focused on cytogenetic assays, because chromosome aberrations may be used as an early warning signal for cancer development. Data from the biomonitoring of human populations indicate that the increased frequency of chromosome aberrations is related to exposure to genotoxic agents, and may be employed to estimate cancer risk and genetic illness (WHO, 2004; HAGMAR *et al*. 2004). Biomonitoring studies using somatic cells have been extensively conducted to evaluate the possible genotoxic risk of a defined exposure and some indicators, such as chromosomal aberrations, have been shown to be relevant biomarkers for further cancer incidence (HAGMAR *et al*. 1998; IARC, 2007).

Using data from cytogenetic biomonitoring studies for risk assessment has many potential disadvantages, such as the difficulty of establishing consistent causal exposure-disease relationships, problems in obtaining reliable information on exposure levels in retrospective studies, overlapping contact with other chemicals and/or the problem of non-comparable populations among those exposed to different levels of carcinogens (HCN, 1994). Moreover, in the case of occupational contact with pesticides, there is great interindividual variability in the degree of exposure and it generally involves complex mixtures of many kinds of compounds.
Exposure to pesticides has been associated with increases in the incidence of non-Hodgkin´s lymphoma (HARDELL *et al*. 1999; ZHENG et al. 2001), pancreatic, stomach, liver and bladder cancer (SHUKLA *et al*. 2001; JI *et al*. 2001), Parkinson´s disease (GAUTHIER *et al.* 2001) and undesirable reproductive outcomes (ARBUCKLE *et al.* 2001) among others.

Many pesticides involved in carcinogenic risk, and classified as probable or possible carcinogens by International Agencies, are banned or their use is restricted in some countries; but, due to bioaccumulation and persistence in ecosystems, they are widespread environmental pollutants. Residues of pesticides have been detected in the food chain and in different biological media in humans.

In order to identify possible changes related to pesticide exposure, we have carried out a cross-sectional study in a group of occupationally exposed workers, where cytogenetic, biochemical and hematological parameters were analyzed simultaneously.

MATERIALS AND METHODS

The exposed group was composed of 26 individuals working in three different units of pesticide production (pesticide synthesis, emulsion concentrated production and powder and liquid pesticide production) and 32 controls. During the production process all subjects were simultaneously exposed to a complex mixture of pesticides (Table 1). No control individuals

were occupationally exposed to any particular chemical agent. Table 2 shows the characteristics of the studied groups regarding sex (male or female), age (in years), working experience (WE in years), duration of occupational exposure to pesticides (DOE in years), smoking habit (smoker or non – smoker), chromosomal aberrations (CAs) and premature centromeric division (PCD). The workers completed a specific questionnaire in which the type of working activity, duration of contact with pesticides, kinds of pesticides used, protective measures, etc., were recorded.

Table 1. Pesticides used in the pesticide factory

Herbicides		Insecticides/Acricides		Fungicides	
Active ingredient	Class	Active ingredient	Class	Active ingredient	Class
2,4 D	Phenoxycarboxylic acid	Abamectin	-	Copper oxychloride	Inorganic
Acetochlor	Chloracetamide	Acetamiprid	Neonicotinoid	Carbenadazim	Benzimidazole
Bentazone	Benzothiadiazinone	Bifenthrin	Pyrethroid	Chlorothalonil	Chloronitrile
Dicamba	Benzoic acid	Buprofezin	Thiadiazine	Difenoconazole	Triazole
Dichlobenil	Benzonitrile	Chlorpyrifos	Organophosphate	Fluazinam	Toluidine
Clethodim	Cyclohexanedione oxime	Cypermethrin	Pyrethroid	Iprodione	Dicarboximide
Clomazone	Isoxazolidinone	Fenitrothion	Organophosphate	Mancozeb	Dithiocarbamate
Clopyralid	Pyridinecarboxylic acid	Imidacloprid	Neonicotinoid	Pyrimethanil	Anilinopyrimidine
Fluroxypyr	Pyridinecarboxylic acid	Malathion	Organophosphate	Propamocarb hydrochloride	Carabamate fungicide
Glyphosate	Glycine derivative	Pyriproxyfen	Juvenile hormone mimic	Propineb	Dithiocarbamete
Linuron	Urea	Tebufenozide	Diacylhydrazine	Tebuconazole	Triazole
Nicosulfuron	Sulfonylurea				
Paraquat	Bipyridylium				
Pendimethalin	Dinitroaniline				
Quizalofop-P	Aryloxyphenoxypropionate				
Sulcotrione	Triketone				

Enzyme analysis

Levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholinesterase (SChE) were determined. These enzymes were analyzed spectrophotometrically using commercially available biochemical kits from Italy.

Chromosomal aberrations (CAs) and premature centromeric division (PCD)

CAs and PCD in lymphocytes were analyzed according to a standard protocol ((IAEA, 1986). Whole blood cultures were prepared using RPMI 1640 medium supplemented with 10% of fetal calf serum (Life technologies, http:// www.lifetechnology, com/). The lymphocytes were stimulated with phytohaemagglutinin (PHA, PAA) at 5 µg ml^{-1} for 48 h at 37°C. During the last 2 h of incubation colchicine (0.05µg mL^{-1}; Sigma – Aldrich, 3050 Spruce St., St. Louis, MO 63103) was added to the medium. The cells were exposed to hypotonic solution (20 min) by stepwise addition of 0.075 M KCL followed by fixation. Cells were spread on slides and dried over a flame. The slides were aged for the next 5-7 days. Giemsa stained slides were coded and

scored blind under a light microscope. Two hundred well-spread metaphases per subject were screened for PCD and chromosome damage. PCD was diagnosed when the separation between sister chromatids was equal to or more than the thickness of the chromatid (RUSHKOVSKY *et al.* 2003).

Table 2. General characteristics of the studied population

Variable	Exposed group	Mean	SD	Z -value	p-value
Age	control	40.57	6.57	-0.39	0.690
	exposed	39.89	8.66		
Working experience (WE)	control	16.23	6.25	-0.25	0.800
	exposed	16.12	9.6		
Duration of occupational exposure (DOE)	control	0.00	0.00	-6.97	0.000**
	exposed	11.56	7.42		
Apartate aminotrasferase (AST),	control	18.67	4.16	-2.46	0.014*
	exposed	22.19	5.80		
Alanine aminotransferase (ALT),	control	18.53	4.25	-3.65	0.000**
	exposed	27.58	12.49		
Serum cholinesterase (SChE).	control	6951.00	1651.55	-3.2	0.001**
	exposed	9878.92	3593.94		
Number aberrant cells	control	0.67	0.99	-5.64	0.000**
	exposed	4.35	2.35		
Acentric	control	0.33	0.55	-3.35	0.001**
	exposed	1.19	1.1		
Chromatid breaks	control	0.23	0.43	-6.12	0.000**
	exposed	2.27	1.04		
Chromosome breaks	control	0.13	0. 35	-4.44	0.000**
	exposed	1.27	1.15		
tPCD	control	0.23	0.50	-2.34	0.020*
	exposed	0.77	0.95		
PCD1-5	control	1.17	1.02	-3.19	0.001**
	exposed	2.88	2.2		
PCD5-10	control	0.40	1.31	-2.43	0.015*
	exposed	1.27	1.73		
Chromatid exchange	control	0.00	0.00	-3.01	0.002**
	exposed	0.27	0.45		

WE—working experience; DOE—duration of occupational exposure to pesticide tPCD—total number of chromosomes with PCD.

a ns $p < 0.05$; * $p < 0.01$; **

Statistical analysis

Frequencies of CAs: chromatid and chromosome breaks, acentrics, dicentrics and ring chromosomes, as well as PCD parameters, were evaluated for at least 200 metaphase cells. Three PCD parameters were included in the analysis: total number of chromosomes with PCD (tPCD) and frequencies of metaphase cells with 1-5 PCDs and 5-10 PCDs. In addition to descriptive statistics, appropriate non–parametric statistical methods were used (Mann–Whitney U-test, Pearson´s X^2 test and. Spearman rank test (non-parametric correlation test) on the whole sample (control-exposed) and the exposed group only. The software used for data analyses was STATISTICA (StatSoft, Tulsa,OK) and SPSS version10.0 (SPSS Inc., Chicago, IL).

RESULTS

Here we report the results of cytogenetic monitoring of 26 occupationally exposed workers and 32 matched controls using CA assays. The main objective was to show that exposure to pesticide mixtures in the manufacturing industry leads to an increase in cytogenetic damage.

Table 2. gives the main characteristics of the population studied. The Mann-Whitney U-test indicated statistically significant differences of average values of all examined variables in comparison to the control group. The numbers of acentric fragments ($p<0.01$), chromatid and isochromatid breaks ($p<0.01$), tPCD; ($p<0.05$), PCD1-5; ($p<0.01$) PCD 5-10 ($p<0.05$) and chromatid exchanges ($p<0.01$) differed between the groups (Figure 1). Moreover, the total number of aberrated cells in workers was significantly different from that in the control group of examinees ($p<0.01$). Table 2 also shows values for the analyzed enzymes (SChE, AST, ALT). According to earlier results (BHALLI *et al.* 2006; JOVICIC *et al.* 2012) serum cholinesterase (SChe) levels were lower in workers exposed to pesticides in comparison with the control group ($p<0.001$). Enzymes are very important biomarkers in biomonitoring studies. The values for ALT and AST were markedly higher than those for the control group of workers ($P<0.001$). Our results also show significantly higher concentrations of ALT and AST in exposed workers in comparison with the control group ($p<0.01$).

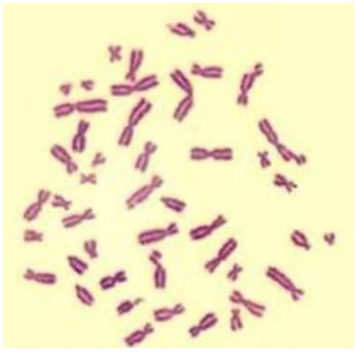

Fig 1. Metaphase with chromosome and chromatide breaks

The number of exposed years during employment was significantly greater than for the control group of examinees. Only age and total length of employment showed no significant difference in comparison to the control group (Table 2).

The frequency of PCD was expected to differ between the control and exposed individuals. We found that PCD frequency was significantly lower in the control than in the exposed group. JOVICIC *et al*. (2010) pointed to PCD as a marker of chromosome instability, so it was suggested that PCD is a suitable cytogenetic marker for workers professionally exposed to mutagenic agents. This could be explained partially by the observations of IKEUTCHI *et al*. (2004), who showed that PCD could be induced by an increasing hypotonic treatment (technical procedure). Other authors suggested that PCD might depend on the action of yet unknown environmental factors to which subjects from the control group were exposed (MAJOR *et al*. 1999).

Positive results for chromosomal aberration tests after occupational exposure to a mixture of pesticides were reported earlier (BOLOGNESI, 2003; ANTONUCCI *et al*. 2000; BOLOGNESI *et al.* 2009; EL-KHATIB *et al*. 2003). Similar results were obtained for a population monitored in Croatia, (CARBONELL *et al*. 1995; ZELJEZIC *et al*. 2002), i.e. the workers exposed were protected against pesticide fog but presented a frequency of CAs higher than controls. Statistically significant increases in the number of all types of chromatid and chromosome aberrations, sister chromatid exchanges frequency, micronucleus frequency and comet assay endpoint values observed in exposed subjects may indicate longterm occupational contact with a complex pesticide (GARAJ-VRHOVAC *et al*. 2001).

Table 3. Results of Pearson´s X^2 test for the variables examined

Variable	$\chi 2$	Degrees of freedom DF	p
Chromatid breaks	11.515	4	0.020 *
Chromosome breaks	10.099	3	0.018*

DF- degrees of freedom P<0.05

Table 4.The frequency of breaks and the number of aberrant cells in relation to smoking habit

Smoking	Variable	Number of breaks per 200 cells	Percent
Non–smokers	Chromatid breaks	0	54.1
		1-3	45.9
Smokers	Chromatid breaks	0	21.1
		1-4	78.9
Non–smokers	Chromosome breaks	0	73.0
		1-3	27.0
Smokers	Chromosome breaks	0	6.8
		1-3	63.2

Considering smoking habits, Pearson´s X^2 test showed that, regardless of whether workers are exposed or not, isochromatic and chromatic breaks occur more frequently among smokers. Thus, 79% of smokers had one or more chromatid breaks, whereas non-smokers had 46%. Moreover, 63% of smokers had one or more isochromatid breaks, while non-smokers had 27%. The results of Pearson´s X^2 test are shown in Table 3, while the frequency of the tested parameters is given in Table 4.

DISCUSSION

Cytogenetic damage in individuals occupationally exposed to pesticides has received the attention of investigators in several countries but no definitive conclusions have yet been made. Reviews on this matter (BOLOGNESI, 2003; BULL *et al.* 2006) suggest that most studies found increases in biomonitoring indices of genotoxicity in pesticide applications. Work environment, time of exposure and exposure conditions are described as factors affecting cytogenetic damage levels (KHUDER *et al.* 1997; BHALLI *et al.* 2006). Another factor complicating comparison of different studies performed to date is the large number and variety of chemicals generally used.

Similarly to our results, ANTONUCCI *et al.* (2000) observed that exposed and control subjects did not differ significantly in terms of smoking habits as observed with the statistical test used. BONNER *et al.* (2010) reported that workers who smoke and are exposed to pesticides exhibited a significant increase in total chromosomal aberrations compared to non-smokers who had no contact with pesticides. Although some studies showed higher levels of cytogenetic biomarkers in smokers than non–smokers (CARBONELL *et al*. 1990), BHALLI *et al*. (2006) reported that smoking has an additive effect on the frequency of BNMN (binucleated lymphocytes with micronucleus) and MNL (number of micronuclei in binucleated lymphocytes). Some other confounding factors like smoking habit and exposure time were also analyzed. Thus, smoking had an additive effect on the frequency of BNMN and MNL, which was reflected by more BNMN and MNL in lymphocytes of smokers than of non-smokers. On the other hand, micronuclei analyses conducted by Lucero (LUCERO *et al*. 2000) and PASTOR *et al*. (2002) showed that smoking habits did not cause any significant elevation in chromosomal aberrations.
In our exposed group, the length of exposure during employment only had an influence on PCD5-10 ($p<0.05$), while there was no statistical significance for the biochemical parameters SChe, ALT and AST. The results of Pearson´s X^2 test are shown in Table 5.

The lack of correlation between exposure time to pesticides and SCE frequencies might be related to the fact that the group less in contact with pesticides had similar SCE frequencies. This is in agreement with others (BOLOGNESI *et al.* 2003), who did not find any correlation between micronuclei frequency and exposure time to pesticides. Negative results for SCE were obtained in people exposed to insecticides, herbicides (SHAHAM *et al*., 2001) and a complex mixture of them (CARBONELL *et al*. 1990; GOMEZ-ARROYO *et al*. 1992). PASTOR *et al*. (2003) reported that no relation existed between exposure time and an increase in the frequency of micronuclei.

Besides that, the Spearman rank test indicated a positive linear correlation between CA and PCD. Spearman rank correlation showed that our group of examinees exhibited significant positive correlations with the number of aberrated cells as well as with the number of chromatid and isochromatid breaks, acentric fragments, frequency of premature centromeric division, and

tPCD, PCD1-5, PCD5-10 variables. For biochemical parameters, significant positive correlations occurred in ALT, SChE and ALT (Table 6).

Table 5. Influence of duration of exposure (DOE) on the genetic and biochemical parameters. Results Pearson´s X^2 test

Variables	$\chi 2$	DF	p-value
Number aberrant cells	11.41	9	0.25
Acentric	5.66	4	0.23
Chromosome breaks	4.99	3	0.17
Chromatid breaks	6.83	4	0.15
tPCD	2.01	3	0.57
PCD1-5	9.44	7	0.22
PCD5-10	12.39	4	**0.02***
Chromatid exchange	0.46	1	0.49
Aneuplody	4.34	5	0.50
Polyploidy	0.89	2	0.64
SChE	26.00	25	0.41
ALT	15.94	18	0.60
AST	17.96	17	0.39

DF - degrees of freedom,
Marginally significant P<0.05*

Table 6 .Spearman rank (nonparametric) correlations between variable pairs for the whole sample below diagonal and the exposed group above diagonal

	Number aberrant cells	Acentric	Chromatid breaks	Chromosome breaks	tPCD	PCD 1-5	PCD 5-10	Chromatid exchange	Aneuploidy	Polyploidy	ALT (U/L)	AST (U/L)	serum cholinesterase (SChE). (U/L)
Exposed group	0.761**	0.452**	0.825**	0.599**	0.316*	0.430**	0.327*	0.406**	0.361**	0.255	0.492**	0.331*	0.431**
Number aberrant cells		0.759**	0.911**	0.802**	0.632**	0.491**	0.416**	0.525**	0.521**	0.285*	0.573**	0.334*	0.414**
Acentric			0.568**	0.584**	0.569**	0.370**	0.356**	0.289*	0.548**	0.309*	0.407**	0.231	0.303*
Chromatid breaks				0.678**	0.411**	0.528**	0.408**	0.506**	0.454**	0.186	0.559**	0.319*	0.508**
Chromosome breaks					0.638**	0.492**	0.546**	0.604**	0.593**	0.226	0.557**	0.366**	0.204
tPCD						0.183	0.242	0.614**	0.437**	0.371**	0.332*	0.240	0.211
PCD 1-5							0.648**	0.354**	0.564**	-0.008	0.431**	0.367**	0.316*
PCD 5-10								0.424**	0.622**	0.007	0.401**	0.260	0.181
Chromatid exchange									0.533**	0.154	0.297*	0.204	0.254
Aneuploidy										0.121	0.396**	0.220	0.179
Polyploidy											0.025	0.036	-0.023
ALT (U/L)												0.616**	0.508**
AST (U/L)													0.434**

*p<005 **p<001 ALT: alanine aminotransferase AST: aspartate aminotrasferase (SChE). serum cholinesterase

Our results indicate that workers exposed to pesticides have an increased frequency of cytogenetic changes (chromatid and chromosome breaks) in comparison with the control group of examinees. We also noticed that it is necessary to biomonitor people professionally exposed to different groups of pesticides continually through regular analysis of biochemical and hematologic parameters. Moreover, constant improvement of cytogenetic methods will give a safer and fuller picture of potential changes in the karyotype (GROVER *et al.* 2003; PIPERAKIS *et al.* 2003; BOLOGNESI *et al*. 2004; COSTA *et al.* 2006; DA SILVA *et al.* 2008; REMOR *et al*. 2009). It was noted that employees working in pesticide production used adequate measures of protection, which was reflected in the presence of a relatively small number of drastic chromosome changes.

CONCLUSION

Therefore, according to data obtained in this study, occupational exposure to a complex mixture of pesticides was the main factor in the induction of CAs in the investigated population. These

observations indicate the need to increase the preventive measures habitually used by staff occupationally exposed to pesticides. Our results were obtained from a preliminary examination on a relatively small sample, so further studies including a larger number of subjects combined with investigations *in vitro* should be performed in order for the observed phenomena to be understood better. They also indicate the need for permanent biomonitoring of subjects occupationally exposed to various mixtures of pesticides, using a variety of cytogenetic methods.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Education and Science of the Republic of Serbia, through implementation of the project "Studies on plant pathogens, arthropods, weeds and pesticides with a view to developing the methods of biorational plant protection and safe food production" (TR31043).

Received December 21st, 2012
Accepted March 12th, 2012

REFERENCES

ANTONUCCI, G., I. COLUS (2000): Chromosomal aberrations analysis in a Brazilian population exposed to pesticides. Teratog Carcinog Mutagen *20:*265–272

ARBUCKLE, TE., L.LIN, L.S. MERY (2001): An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environ Health Perspect *109*:851–857

BHALLI, JA., QM. KHAN, MA..HAQ, AM KHALID, A.NASIM (2006): Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry. Mutagenesis *2*1:143–148

BOLOGNESI, C. (2003) Genotoxicity of pesticides: a review of human biomonitoring studies. Mutat Res *543:*251–272

BOLOGNESI, C., E.LANDINI, E.PERRONE, P. ROGGIERI (2004): Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centromeric probe. Mutat Res *557*:109–117

BOLOGNESI, C., G.CARRASQUILLA, S.VOLPI, KR.SOLOMON, EJ. MARSHALL (2009): Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate. J Toxicol Environ Health Part A *72*:986–997

BONNER, MR., BA. WILLIAMS, JA.RUSIECKI, A.BLAIR, LE.BEANE FREEMAN, JA.HOPPIN, M DOSEMECI., J. LUBIN, DP., SANDLER MC. ALAVANJA (2010) :Occupational exposure to terbufos and the incidence of cancer in the Agricultural Health Study. Cancer Causes Control *21*: 871–877.

BULL, S., K.FLETCHER, AR.BOOBIS, JM. BATTERSHILL (2006): Evidence for genotoxicity of pesticides in pesticide applicators: a review. Mutagenesis *21*:93–103

CARBONELL, E., M.PUIG, N.XAMENA, A.CREUS, R. MARCOS (1990): Sister chromatid exchanges in lymphocytes of agricultural workers exposed to pesticides. Mutagenesis *5*:403–405

CARBONELL, E., A.VALBUENA, N.XAMENA, A.CREUS, R. MARCOS (1995): Temporary variations in chromosomal aberrations in a group of agricultural workers exposed to pesticides. Mutat Res 344:127–134

COSTA, C., JP. TEIXEIRA, S. SILVA (2006): Cytogenetic and molecular biomonitoring of a Portuguese population exposed to pesticides. Mutagenesis *21*:343–350

DA SILVA, J.., CR. MORALES, D. VANINA, V. HEUSER, M. ANDRADE, FR. SILVA (2008): Evaluation of genetic damage in a Brazilian population occupationally exposed to pesticides and its correlation with polymorphisms in metabolizing genes. Mutagenesis *23*:415–422

EL-KHATIB, H, F. HAMMAM (2003): in: Human Monitoring for Genetic Effects, Cebulska-Wasilewska A, Au WW, Sram RJ (Eds.) NATO Science Series, Springer p. 142

GARAJ-VRHOVAC, V, D. ZELJEZIC (2001): Cytogenetic monitoring of Croatian population occupationally exposed to a complex mixture of pesticides. Toxicology *165*:153–162

GAUTHIER, E, I. FORTIER, F. COURCHESNE, P. PEPIN, J. MORTIMER, D.GAUVREAU (2001): Environmental pesticide exposure as a risk factor for Alzheimer's disease: a case-control study. Environ Res *86:*37–45

GOMEZ-ARROYO, S., Y DIAZ-SANCHEZ, M. MENESES-PEREZ, R. VILLALOBOS-PIETRINI, JL. RODRIGUEZ (2000): Cytogenetic biomonitoring in a Mexican floriculture worker group exposed to pesticides. Mutat Res *466*:117–124

GROVER, P., K. DANADEVI, M. MAHBOOB, RB. ROZATI, BS. BANU, MF. RAHMAN (2003): Evaluation of genetic damage in workers employed in pesticide production utilizing the Comet assay. Mutagenesis *18*:201–205

HAGMAR, L., S. BONASSI, U. STROMBERG, A. BROGGER, L. KNUDSEN, H. NORPPA, C. REUTERWALL (1998):ort from the European study group on cytogenetic Biomarkers and Health (ESCH). Cancer Res *64*:159–163

HAGMAR, L., U. STROMBERG, S. BONASSI, IL. HANSTEEN, L. KNUDSEN, C. LINDHOLM, H. NORPPA (2004): Impact of types of lymphocyte chromosomal aberrations on human cancer risk. Results from Nordic and Italian cohorts. Cancer Res *64:*2258–2263

HARDELL, L., M. ERIKSSON (1999): A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer *85*:1353–1360

HEALTH COUNCIL OF THE NETHERLANDS (HCN) (1994): Committee on the evaluation of the carcinogenicity of chemical substances. Regul Toxicol Pharmacol *19*:14 – 30

HEUSER, VD., B. ERDTMANN, K. KVITKO, P. ROHR, J. DA SILVA (2007): Evaluation of genetic damage Brazilian footwear–workers: biomarkers of exposure, effect and susceptibility. Toxicology *232*:235–247

IAEA, (1986: Biological dosymetry: chromosome aberration analysis for dose assessment, In. Technical Reports Series No. 260, International Atomic Energy Agency, Vienna, pp.59–63

IKEUTCHI, T., ZQ. YANG, K. WAKAMATSU, T. KAJI (2004): Induction of premature chromatid separation (PCS) in individuals with PCS trait and in normal controls. Am J Med Genet Part A *127:*128–132

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) (2007): Monographs on the Evaluation of Carcinogenic Risk to Human. World Health Organization, Lyon, France

JI, BT., DT. SILVERMAN, PA.STEWART (2001): Occupational exposure to pesticides and pancreatic cancer. Am J Ind Med *39*:92–99

JOVICIC, D., S. MILACIC, T. VUKOV, B. RAKIC, M. STEVANOVIC, DRAKULIC, R. RAKIC, N. BUKVIC (2010): Detection of premature segregation of centromeres in persons exposed D to ionizing radiation. Health Physics *98:*717–727.

JOVICIC, D., J. PAJIC, B. RAKIC, LJ. RADIVOJEVIC, M. PAJIC, S. GASIC, LJ. SANTRIC (2012):Cytogenetic effect in a Serbian population occupationally exposed to a complex mixture of pesticides. Abstracts of International MGPR Meeting 2012 and International Conference on Food and Health Safety: Moving Towards a Sustainable Agriculture, Belgrade, Serbia, 82

JOVICIC, D., J.PAJIC, B.RAKIC, LJ.RADIVOJEVIC, A.DJORDJEVIC, M. PAJIC, V.JANJIC (2012): Evaluation of cytogenetic changes in workers occupationally exposed to pesticides. In Proceedings of the International Congress "Ecology, health, work and sport", Banja Luka, 605-609

LUCERO, L., S. PASTOR, S. SUAREZ, R. DURBAN, C. GOMEZ, T. PARRON, A..CREUS, R. MARCOS (2000): Cytogenetic biomonitoring of Spanish greenhouse workers exposed to pesticides: micronuclei analysis in peripheral blood lymphocytes and buccal epithelial cells. Mutation Research 464: 255–262

MAJOR, J., MG JAKAB, A. TOMPA (1999): The frequency of induced premature centromere division in human populations occupationally exposed to genotoxic chemicals. Mutat Res *445*:241–249

PADMAVATHI, P., PA. PRABHAVATHI, PP. REDDY (2000): Frequencies of SCEs in peripheral blood lymphocytes pesticide workers. Bull Environ Contam Toxicol *64*:155–160

PASTOR, S., A,.CREUS N. XAMENA, C. SIFFEL, R. MARCOS (2002): Occupational exposure to pesticides and cytogenetic damage: results of a Hungarian population study using the micronucleus assay in lymphocytes and buccal cells. Environmental and Molecular Mutagenesis *40:* 101–109

PASTOR, S., A.CREUS, N T., PARRO´ A.CEBULSKA-WASILEWSKA, C.SIFFEL, S. PIPERAKIS, R. MARCOS (2003): Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers. Mutagenesis, *18*, 249–258

PIPERAKIS, SM, E.PETRAKOU, S.TSILIMIGAKI, M.SAGNOU, E.MONOGIUDIS, G.HANIOTAKIS, H.KARKASELI, E. SARIKAKI (2003): Biomonitoring with the comet assay of Greek greenhouse workers exposed to pesticides. Environ Mol Mutagen *41*:104–110

RADIVOJEVIC, LJ., S. GASIC, LJ. SANTRIC, R.STANKOVIC-KALEZIC (2008): The impact of atrazine on several biochemical properties of chernozem soil. Journal of Serbian Chemical Society 73: 951-959

RADIVOJEVIC, LJ., S. GASIC, LJ. SANTRIC, J. GAJIC UMILJENDIC, D. MARISAVLJEVIC (2012) :Short-time effects of the herbicide nicosulfuron on the biochemical activity of chernozem soil. Journal of Serbian Chemical Society 77: 845-854

REMOR, AP, AC TOTTI, DA MOREIRA, GP DUTRA, VD HEUSER, JM BOEIRA (2009): Occupational exposure of farm workers to pesticides: biochemical parameters and evaluation of genotoxicity. Environ Int 35:273–278

ROS, M., M. GOBERNA, JL MORENO, T. HERNANDEZ, C. GARCIA, H. INSAM, JA PASCUAL (2006): Molecular and physiological bacterial diversity of a semi-arid soil contaminated with different levels of formulated atrazine. Applied Soil Ecology *34*: 93-102

RUSHKOVSKY, SR, YV PETRENKO, VF BEZRUKOV (2003): Premature centromere division as a biomarker of genotoxic influences. In Abstract book of the sixth international symposium and exhibition on environmental contamination in Central and Eastern Europe and the Commonwealth of independent status, pp.1–4. Prague, Czech Republic

SHAHAM, J., Z. KAUFMAN, R.GURVICH, Z. LEVI (2001): Frequency of sister-chromatid exchange among greenhouse farmers exposed to pesticides. Mutatation Research *491*: 71–80

SHUKLA, VK, AN RASTOGI, TK ADUKIA, RB RAIZADA, DCS REDDY, S. SINGH (2001): Organochlorine pesticides in carcinoma of the gallbladder: a case-control study. Eur J Cancer Prev *10*:153–156

TOMLIN, CDS. (2011): The Pesticide Manual. Seventeenth Ed., British Crop Protection Council, Farnham, Surrey, UK.

UNDEGER, U., N. BASARAN (2002): Assessment of DNA damage in workers occupationally exposed to pesticide mixtures by the alkaline comet assay. Genotoxicity *76*:430–436

WORLD HEALTH ORGANIZATION (WHO) (2004): The WHO Recommended Classification of Pesticides and Guidelines to Classification. World Health Organization, Geneva

ZELJEZIC, D., V. GARAJ-VRHOVEC (2002) :Sister chromatid exchange and proliferative rate index in the longitudinal risk assessment of occupational exposure to pesticides. Chemosphere *46:*295–303

ZHENG, T., SH. ZAHM, KP. CANTOR, DD. WEISENBURGER, Y. ZHANG, A. BLAIR (2001): Agricultural exposure to carbamate pesticides and risk of non-Hodgkin lymphoma. J Occup Environ Med *43*:641–649

,

CITOGENETIČKA ISTRAŽIVANJA RADNIKA SRBIJE PROFESIONALNO IZLOŽENIH PESTICIDIMA

Dubravka JOVIČIĆ [1], Jelena PAJIĆ [2], Boban RAKIĆ [2], Ljiljana RADIVOJEVIĆ [3], Miloš PAJIĆ [2], Vaskrsije JANJIĆ [3], Aleksandar MILOVANOVIĆ [2]

[1]Univerzitet Singidunum, Fakultet za primenjenu ekologiju (FUTURA), Beograd, Srbija
[2]Institut za medicinu rada Srbije „Dr Dragomir Karajović“, Beograd, Srbija
[3]Institut za pesticide i zaštitu životne sredine, Beograd, Srbija
[4]Univerzitet u Beogradu, Poljoprivredni falkultet, Beograd, Srbija

Izvod

Cilj istraživanja bila je analiza hromozomskih aberacija (CA) i prevremene centromerne deobe (PCD) kod lica profesionalno izloženih pesticidima. Istraživanja su obuhvatala 26 ispitanika profesionalno izloženi pesticidima prosečne starosti 39.89± 8.66 i 32 ispitanika kontrolne grupe prsečne starosti 40.57± 6.57. Mann-Whitney U test pokazuje statistički značajnu razliku srednjih vrednosti parametara svih ispitivanih varijabli u odnosu na kontrolnu grupu. Osim toga, Spearmanova (neparametarska) korelacija je pokazala da postoji pozitivna linearna korelacija između CA i PCD. Prisustvo uglavnom hromatidnih i izohromatidnih prekida kod ispitanika ukazuju na kontinuirano izlaganje pesticidima. Odsustvo složenih hromozomskih rearanžmana ukazuje na dobru zaštitu ispitanika na njihovim radnim mestima.

Primljeno 21. XII 2012.
Odobreno 12. III.2012.