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ORIGINAL ARTICLE

Cytogenetic methods and biomonitoring of occupational exposure to genotoxic factors

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Summary
The professional aim of this project was to map the detection of genotoxicants using cytogenetic methods. It summarizes the results of four studies carried out at three different workplaces in different industries. The main aim of the study was also to monitor types of exposure to genotoxicants and their impact on the human population: The exposure of blast furnace workers in the steel industry to genotoxic factors; the exposure of coke oven plant workers to genotoxic factors, and the exposure to genotoxic factors in workplaces in the chemical industry – in particular to heavy metals (Ni, Cd). Cytogenetic analysis and the micronucleus test are sensitive methods in the field of biomonitoring of human exposure in industry, and play an important role in the prevention of damage to human health.

Keywords: genotoxicants – micronucleus test – binucleated cells – furnace workers

INTRODUCTION

Extensive scientific development and the development of industry were characteristic of the 20th century; these two factors were often accompanied by the destruction of the environment as a result of their influence on organisms. There are a large number of harmful substances in the environment. Among the most dangerous are those able to react with and change genetic material, DNA (possibly RNA). These substances, which have mutagenic and carcinogenic effects, are called genotoxicants (Dobiáš and Vít 1995). The main source of pollution by chemical genotoxicants is the chemical industry, in which have been newly synthesised large numbers of chemical substances, the biological effects of which are mostly unknown. Organisms are increasingly exposed to chemical genotoxicants whose increased presence in the biosphere can substantially harm the population. An increased number of tumour diseases can occur as the consequence of mutation phenomena acting in the aetiology of the tumour creation process. These phenomena also have a share in the increased frequency of spontaneous

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miscarriages and genetically conditioned birth defects (Zudová and Samková 1980).

Another problem in the area of the assessment of genotoxical danger is the fact that complex mixtures are released into the environment as part of important industrial production processes, for example, the production of raw iron in blast furnaces or the production of coke in coke ovens. These mixtures can interact with the environment and influence the biosphere. It is evident that in most cases organisms are affected by complex mixtures containing genotoxicants. These elements can mutually influence each other and increase their harmful effects (Dobiáš and Vít 1995). There are a number of important studies focused on the assessment of the risk of the genotoxical potential of complex mixtures and the complex process of changing these harmful elements (Lewtas 1990).

MATERIALS AND METHODS

Three studies were carried out at the following locations affected by exposure to chemical genotoxicants: 1) a blast furnace – 14 observed persons (only males) with an average age of 39.27; 2) a coke oven – 10 observed males with an average age of 47.7, and 3) a plant where there was exposure to nickel and cadmium – 22 observed persons (8 females and 14 males) with an average group age of 37.73.

Table 1. **Blast furnace 2000,** micronucleus test

Number of preparations	Amount of binucleated cells	Amount of micronuclei	% MN
21	1000	2.00	0.20
22	1000	1.00	0.10
23	1000	1.00	0.10
26	1000	1.00	0.10
27	1000	2.00	0.20
28	1000	3.00	0.30
30	1000	1.00	0.10
31	1000	2.00	0.20
37	1000	0.00	0.00
38	1000	6.00	0.60
39	1000	4.00	0.40
40	1000	4.00	0.40
41	1000	1.00	0.10
42	unscored	-	-
43	1000	6.00	0.60
Average	-	2.43	0.24
S.D.		1.84	0.18

The following cytogenetic methods were used: cytogenetic analysis of human peripheral lymphocytes (CAPL) and the micronucleus test for the assessment of genotoxical potential. The frequencies of chromosomal aberrations and micronuclei (MN) present in the inspected cells, together with the proper assessment of biomarkers, constituted other expert objectives for the determination of the level of correlation among the observed cytogenetic parameters (Černá and Rössner 1988, Brusick 1994, Rössner 2000, Šmerhovský et al. 2001, Rössner et al. 2002, Adamus et al. 2004).

CAPL enables the detection of both structural and numerical chromosomal aberrations in human peripheral lymphocytes. It has been used as a brief and reliable test following exposure to genotoxic elements with a clastogenic effect. The expression of this effect is the increased frequency of structural chromosome aberrations in human peripheral lymphocytes (Černá and Rössner 1988, AHEM 20/1989, Albertini et al. 1996, AHEM

3/2003). The CAPL was performed as described by Hungerfod (1965) or Albertini et al. (2000).

The micronucleus test – modification with cytochalasin B- is a brief cytogenetic test carried out on mammal cells cultivated *in vitro*. The main point of the test is to monitor the frequency of MN in the cytoplasm of the exposed cells, which correlates with the extent of damage to the cytogenetic cell structure. MN is formed by the condensation of the fragmented nucleoprotein of chromosomal material. These micronuclei are the expression of the biological effect of genotoxic influence (Fenech and Morley 1985, AHEM 20/1989, IAEA 2001, AHEM 3/2003, Fenech et al. 1999, 2003). The micronucleus test was performed as described elsewhere (Fenech and Morley 1985, Albertini et al. 2000).

Table 2. **Cytogenetic analysis of lymphocytes and micronucleus test:** Coke oven 2003

Number of preparation	Amount of binucleated cells (MN/AB.C.)	Amount of micronuclei	% MN	% AB.C.
1	1000/200	17	1.70	2.00
2	1000/200	17	1.70	1.00
3	1000/200	13	1.30	0.00
4	1000/200	12	1.20	0.50
5	1000/200	40	4.00	3.00
6	1000/200	15	1.50	1.50
7	1000/200	18	1.80	1.00
8	1000/200	10	1.00	0.50
9	1000/200	20	2.00	0.00
10	1000/200	15	1.50	Unscored
Average	-	17.70	1.77	1.06
SD	-	7.95	0.80	0.93

AB.C = aberrant cells

The results of the CAPL and micronucleus tests were statistically adjusted using standard deviation calculated using Microsoft Office Excel 2003 and they complement the graphs shown later this study. The non-parametrical Mann-Whitney test at the significance level $2\alpha=0.05$ was used. A regressive and correlative analysis was used for the assessment of the relation between % AB.C. and % MN. Results are expressed as mean± S.D.

RESULTS AND DISCUSSION

The consequences of exposure to genotoxic factors in the workplace with iron casting processes (blast furnace)

Iron metallurgy is one of the most important industrial fields in our country. What is problematic is the stress which it causes in relation to environmental and workplace conditions. People who are employed in this field are exposed to a variety of risk factors (Kůsová et al. 2003).

14 persons were observed and assessed. 1000 double-nuclei cells were studied per each human subject. The average value of MN as a percentage was 0.24±0.18; other data are shown in Table 1.

The consequences of exposure to genotoxic factors in the workplace – coke production

Complex mixtures with a genotoxic effect constitute one of the most harmful chemical contamination elements, expecially polycyclic organic matter whose genotoxic impact is worse than polycyclic aromatic hydrocarbons (PAHs). When reacting with other pollutants, for example from local heating stations or car fume discharges, their biological effect increases (Daisey et al. 1980).

The mutagenic potential of these complex mixtures is conditioned especially by the content of carcinogenic PAHs whose biological effect shows additively. Among the most harmful structures is benzo(a)pyrene. The resulting genotoxic potential of the complex mixture is multiplied by the presence of other carcinogenic structures such as

PAHs (Dipple et al. 1976, Dobiáš 1998., Kůsová et al. 2000).

This study was dedicated to the application of the CAPL and the micronucleus test taking into account the risk of workers´ exposure to genotoxicants when operating the coke producing machinery. The 10 workers observed were assessed by both methods. The average value of % AB.C. 1.06±0.93 and MN in % 1.77±0.80 was calculated (Table 2.).

The consequences of exposure to genotoxic factors in a workplace in the chemical industry – exposure to heavy metal

The genotoxicity of certain heavy metals is a very important factor in the relationship of the human organism to the biosphere. The concentration of heavy metals in the emissions from local and industrial production sites is increasing. Contamination of this kind is permanent. The exposure of the environment to heavy metals represents one of the main risks for the genome of living systems (Dobiáš 1998).

Table 3. **Micronucleus test:** Plant with chemical exposure (Ni-Cd)

Number of preparation	Amount of binucleated cells (MN/AB.C.)	Amount of micronuclei	% MN	% AB.C.
4	1000/200	6	0.60	2.50
5	1000/200	1	0.10	2.50
6	1000/200	4	0.40	3.00
7	1000/200	3	0.30	3.50
8	1000/200	28	2.80	4.50
9	1000/200	13	1.30	3.00
10	1000/200	4	0.40	5.00
11	1000/200	2	0.20	3.50
12	1000/200	13	1.30	6.50
13	1000/200	20	2.00	3.50
14	1000/200	4	0.40	4.50
15	1000/200	7	0.70	3.50
16	1000/200	4	0.40	2.00
17	1000/200	6	0.60	2.00
18	1000/200	3	0.30	2.00
19	1000/200	21	2.10	3.50
20	1000/200	3	0.30	3.00
21	1000/200	12	1.20	3.50
22	1000/200	8	0.80	1.00
23	1000/200	15	1.50	3.50
24	1000/200	14	1.40	2.50
25	1000/200	3	0.30	2.50
Average	-	8.81	0.88	3.23
SD	-	7.09	0.71	1.16

The metals nickel, cadmium, chrome and the compounds of these elements are linked with genotoxic exposure. (IARC 1984, Cikrt et al. 1998, Buchancová et al. 2003). The mutagenity, carcinogenity and reproduction toxicity are the main reasons for monitoring the concentration of these elements not only in the working environment but also in the natural environment (Hunter et al. 1997). For this reason the last part of this study was devoted to the exposure to Ni-Cd in chemical industry workplaces. 22 individuals were tested by both methods. The following average values of cytogenetic biomarkers of exposure and effect were found: % aberrant cells 3.23±1.16 and micronuclei in % 0.88±0.71 (Table 3.).

Comparison of cytogenetic parameters

A comparison was made of the monitored cytogenetic parameters with the individuals exposed at the plants. The non-parameter Mann-Whitney test was used for the assessment of the statistical importance of the distribution of % AB.C. at the chemical plant with exposure to Ni – Cd, and the coke production site (Fig. 1). There was a statistically important difference between the plants when assessing the cytogenetic marker of % AB.C. ($p<0.05$). A value of 1.16 % AB.C. is nowadays considered the average value of spontaneous aberrations for the whole republic (AHEM 20/1989, Rössner 2000, Rössner et al. 2002, AHEM 3/2003). The monitored workers at the plant with chemical exposure with the average value % AB.C. 3.23±1.16 belong to the group with increased exposure to genotoxic structures.

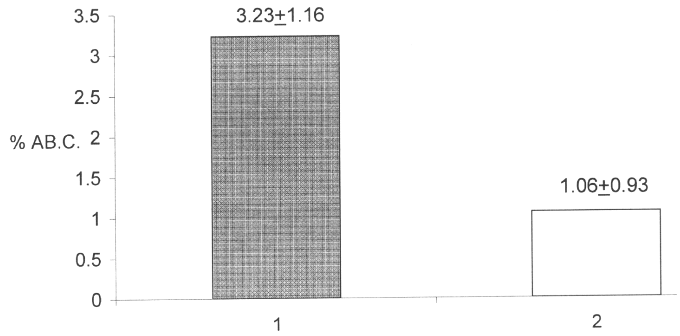

Fig. 1. **Comparison of relative counts of aberrant cells** (% AB.C.) between plant with chemical exposure (1) and coke oven plant (2).

The non-parameter Mann-Whitney test was used for the assessment of the statistical importance ($p<0.05$) of the distribution of % MN in the coke producing plant, the plant with chemical exposure (Ni-Cd) and blast furnace (Fig. 2). A statistically important difference was found between all the plants when assessing the cytogenetic marker % MN.

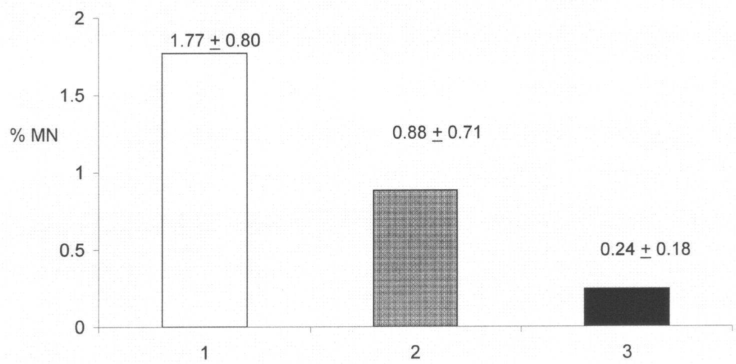

Fig.2. **Comparison of relative counts of micronuclei (**% MN**) values** between coke oven plant (1), plant with chemical exposure (2) and blast furnace (3).

Also, the data gained when assessing samples from the workers of the coke producing plant and the plant with chemical exposure to Ni-Cd, were also used for a mutual comparison of the output values of % AB.C. and % MN. First a regressive and correlation analysis of data from the coke processing plant there was carried out. No correlation was found between the cytogenetic markers of % AB.C. and MN in %. Subsequently the data which was gained from the coke producing plant and the plant with chemical exposure (Ni-Cd) were assessed using the same analysis. No correlation was found between % AB.C. and MN in %. On the basis of these results we can deduce that exposure to Ni-Cd causes the biological effect more sensitively measurable by the method of cytogenetic analysis whereas exposure to substances in the coke producing plant (for example PAHs and their derivates) cause, in the end, different changes, better detected by the means of the micronucleus test. Research with a larger number of individuals would be necessary.

The authors of this study do not now if a classification has been made of the researched groups according to the values of % MN found by means of a cytogenetic analysis. It is important to point out that that the method of the micronucleus test is not frequently used in the Czech Republic as a group exposure test and there are few workplaces which use it on a regular basis in our country.

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