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DOI: 10.18413/2658-6533-2020-6-3-0-2

Aplastic anemia, clinical implications and DNA damage in workers with occupational exposure to aromatic hydrocarbons in Rio de Janeiro

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Abstract
Background: Brazilian gas station workers (GWS) are daily exposed to petroleum-derived hydrocarbons which are harmful. **The aim of the study:** The aim of this study was to evaluate the clinical complaints and the genotoxic effects in GWS. **Materials and methods:** Overall, 88 workers were recruited versus 127 controls. To test the influence of the time of exposure, we have divided the workers into two groups arbitrarily in: Group 1 (G1), with the short time of exposure, ≤ 3 years; and Group 2 (G2), with the longer time of exposure, > 3 years. **Results:** The most relevant complaints were headache (32%) and fatigue (20%), lipothymia (11%), and less commonly sleeplessness and crustiness (both 5%), drowsiness (4%), irritability (3%) and pruritus (1%). 12% of them reported having alcoholic histories. The workers presented concerning alterations found in the blood. Polycythemia (5/88), leukocytosis (10/88) and anemia (19/88) were the most frequent. Hepatic enzymatic damage showed an increase in LDH, bilirubin and AST. Anemia was not associated with the higher LDH rate. Micronucleus (MN) and comet tests were determined in erythrocytes and leukocytes, respectively. The MN test was significant for the total workers group (P = 0.034). As for the class of the comet tails, they were significantly higher for G1 (P = 0.001) and for the total of workers (P = 0.001), for the G2 the significance was borderline (P = 0.05). **Conclusion:** There were important clinical and laboratorial complaints. Genotoxicity assays indicated DNA damage and they can be useful to prevent serious diseases in this group.
Keywords: comet assay; gas station workers; genotoxicity; hepatic enzymes; micronucleus test

For citation: Melo K, Santiago F, de Lucena SBG, et al. Aplastic anemia, clinical implications and DNA damage in workers with occupational exposure to aromatic hydrocarbons in Rio de Janeiro. Research Results in Biomedicine. 2020;6(3):308-317. DOI: 10.18413/2658-6533-2020-6-3-0-2

Introduction. Gas station workers (GSW) are chronically exposed to aromatic compounds such as benzene, toluene, ethylbenzene, and xylenes, which are found in diesel and in gasoline. A dispersal plume is produced by the permeability of the gases burning fuel during the fluid injection in the cars at gas station. Benzene poisoning is a group of signs, symptoms, and complications occurring by acute or chronic exposure to aromatic hydrocarbons by inhalation and skin contact [1]. Acute effects of benzene include mucocutaneous irritation, or even pulmonary edema, and some central nervous system symptoms (narcosis, headache, dizziness, drowsiness, nausea, tremors, and seizures). Chronic effects, among the most common, are blood alterations, such as hypoplasia, myelodysplasia, or severe aplastic aplasia [2, 3]. The diagnosis is made by the medical history, including regular blood counting and biochemical biomarker exams [1-3].

Aplastic anemia is hazardous and with high mortality without an adequate treatment. It is determined by loss and insufficiency of hematopoietic stem cells in front of body's necessity, resulting in bone marrow failure syndrome. The signs include: pancytopenia, hypocellular bone marrow [4, 5].

The genotoxicity of chemical-induced DNA damage can be measured. One efficient method is the single-cell gel electrophoresis assay (comet assay) reflecting the DNA repair capacity. The resulting DNA fragments are like a comet image on the electrophoretic gel. The comet assay has excellent sensitivity and accuracy and it is an important and inexpensive method [6-8]. Another method indicated is the micronucleus test (MN) counting [9, 10]. A MN shows the color of pyknotic nucleus on May-Grünwald Giemsa stain and generates a positive Feulgen reaction for DNA. They are round, no larger than 0.5μm in diameter and, generally, only one is present in an erythrocyte. In pathologic situations, they appear to represent chromosomes that have separated from the mitotic spindle during abnormal mitosis and contain a high proportion of centromere material along with heterochromatin [11].

The aim of the study. The present study aims to estimate clinical effects due to exposure to benzene in the human body, mainly in GSW of the city of Rio de Janeiro and to discuss the current Brazilian law concerning benzene poisoning. The methods included micronuclei counting and comet assay. The clinical exams and questionnaires were applied simultaneously.

Material and Methods. Study population: **t**he workers included in the exposed group were selected from four gas stations in the northern part and midtown of the city of Rio de Janeiro. Workers were of any age 18 or older, with the minimal time of exposure of 6 months. In addition to the exposed group, two subjects that were treated in the Hematology Service at Pedro Ernesto University Hospital (HUPE) while this study was going on were invited to participate in this study because of their matching occupational history and symptoms. The control group was formed by individuals who had never worked with aromatic hydrocarbons or in a laboratory. They were selected among the administrative staff from our University and from a hospital in Rio de Janeiro, and among teachers from an elementary school nearby. The recruitment occurred between 2016 and 2018. The mean age of the workers group was 38 years and for the control group – 42 years.

Clinical and the other sociodemographic questionnaires were applied to the individuals involved in this study. The clinical evaluations made by Medical Doctors samples were collected for the regular exams and genotoxicity tests (comet and MN). This study was approved by the research ethics committees of HUPE (CAAE 34310014.9.0000.5259), and National Cancer Institute José Alencar Gomes da Silva National Cancer Institute (121/09).

To test the influence of the time of exposure, we divided the workers into two groups arbitrarily: Group 1 (G1), with the short time of exposure, ≤ 3 years; and Group 2 (G2), with the longer time of exposure, > 3

years. In all statistical tests used, a level of significance of 5% ($P < 0.05$) was considered.

Blood exams. Samples of 15 mL of blood were collected to perform blood count tests with quantitative and qualitative analysis of the three blood series (erythrocytes, leukocytes, and platelets) and reticulocyte count in EDTA tubes, in addition to AST, ALT, GGT, total bilirubin, and fractions, and LDH, HSV, CRP and ANF were tested. For conducting the comet and MN tests, 5mL of blood was collected in tubes with heparin. The non-parametric Mann-Whitney test was used to evaluate the differences between the quantitative clinical variables between the exposed group and control group, since the normality assumption was not verified using the Shapiro-Wilk test. The data of the research were treated in the statistical program IBM SPSS, version 20.

Comet assay. After collection, the blood was stored at room temperature until the Comet assay that was in three hours. We followed the protocol for preparation of comets of leukocytes on slides and visualized by light microscopy (100x magnification) [7]. One hundred comets per individual were analyzed using the image analysis system and were classified as 0, 1, 2, 3 and 4. The Genetic Damage Indicator (IDG) was calculated as seen in previous publications [10, 12, 14]. Comet tail classification was performed according to double-blind visual criteria of the first and then confirmed by a second examiner. In case of a disagreement, the two observers discussed it to reach a conclusion. A non-parametric Wilcoxon test was used for the evaluation of differences between the frequency of shorter comets [IDG 0, 1, 2] and the frequency of longer comets [IDG 3, 4], since the data did not follow Gaussian distribution. The data of the research were treated in the statistical program PASW, version 18.

Micronucleus test. The blood was smeared on microscope slides. The smears were air dried, stained by May Grünwald-Giemsa. Two slides were prepared for each subject and 1000 erythrocytes per slide were examined to determine the MN frequency [15]. The Mann-Whitney non-parametric test was used to evaluate the differences between micronuclei number in 1,000 erythrocytes between the exposed group and control group, since the normality assumption was not verified using the Shapiro-Wilk test. The data of the research were treated in the statistical program IBM SPSS, version 20.

Results and discussion. Table 1 shows the number of participants/gender and the laboratory tests of this study. The median age of the exposed group was 38 years old, and 42.5 for the control group. Clinical complaints were reported by the GSW. The most relevant were headache (32%) and fatigue (20%), lipothymia (11%), and less commonly sleeplessness and crustiness (both 5%), drowsiness (4%), irritability (3%), and pruritus (1%).

The enzymatic hepatic damage evaluation is shown in Table 2. The exposed group presented a significantly higher level for bilirubin (mean 0.69, $P<0.0002$), AST (mean 27.97, $P<0.05$) and LDH (301.3, $P<0.05$).

Table 1

Number and gender of participants

Parameter	Exposed	Controls
Comet test	57 / M=24 F=33	127 / M=69 F=58
Micronucleus test	90 / M=43 F=47	124 / M=64 F=60
Blood counting	88/ M=43 F=45	119 / M=56 F=63
γGT	88 / M=43 F=45	116 / M=56 F=60
ALT	88 / M=43 F=45	119 / M=56 F=63
LDH	88 / M=43 F=45	119 / M=56 F=63
AST	88 / M=43 F=45	124 / M=64 F=60
Bilirubin	88 / M=43 F=45	119 / M=56 F=63

Note: F = Female, M = Male. Inconsistent numbers due failure/absence of blood test reagents or collection.

Table 2

Biochemical variables in the exposed groups x the control´s

Variable	Group	Descriptive Measures						*P*-value
		N	Mean	SD	1Q	Median	3Q	
Bilirubin	Exposed	88	0.69	0.46	0.32	0.52	0.98	0.0002
Bilirubin	Control	119	0.44	0.26	0.27	0.37	0.52	
ɣGT	Exposed	88	39.48	34.07	21.00	31.00	43.00	0.0709
ɣGT	Control	116	38.81	46.55	17.00	23.50	43.50	
AST	Exposed	88	27.97	31.86	18.25	22.00	29.00	0.0431
AST	Control	124	22.85	12.73	17.00	20.50	25.00	
ALT	Exposed	88	25.85	18.1	14	20	31	0.5168
ALT	Control	119	23.62	17.27	13	19	28	
LDH	Exposed	88	301.3	104.6	228	283.5	358.5	<0.001
LDH	Control	119	202.56	52.23	165	200	232	

Note: ɣGT = Glutamyl transferase, AST = Aspartate transaminase, ALT = Alanine transaminase, LDH = Lactate Dehydrogenase. The probabilities of significance (*P* value) refer to the Mann-Whitney test.

In regard to the blood count results, leukocytosis (10/88), leukopenia (4/88), neutropenia (6/88), and lymphocytosis (13/88) were detected. Normochromic and normocytic anemia (not associated with higher LDH, see Table 3) and polycythemia were the alterations found in the red blood cell section, the latter was seen in 5/88 workers (one blood collection alone). Because of the hard conditions to perform the propaedeutic adequately in a gas station room, it was not possible to make differential diagnosis of polycythemia because the exams for dehydration, chronic obstructive pulmonary, and predisposition to clonal changes could not be assessed.

Table 3

Assessment of LDH concentration and the presence or absence of anemia

Variable	Anemia	Descriptive measures						*P*-value
		N	Mean	SD	Q1	Median	Q3	
LDH (U/L) – G1	No	41	339.59	110.584	267.50	340.00	370.00	0.131
	Yes	7	276.29	82.476	210.00	241.00	352.00	
LDH (U/L) – G2	No	27	276.85	99.410	204.00	238.00	371.00	0.391
	Yes	12	231.58	59.888	193.50	246.50	286.50	
LDH (U/L) – G1 + G2	No	68	314.68	109.964	231.50	290.50	370.25	0.014
	Yes	19	248.05	70.357	204.00	241.00	294.00	
LDH (U/L) - controls	No	109	202.38	51.427	165.00	200.00	231.00	0.481
	Yes	10	219.60	65.337	165.75	207.00	263.00	

Note: G1 = workers with ≤ 3 years of exposure, G2 = workers with > 3 years of exposure. The probabilities of significance (*P*-value) refer to the Mann-Whitney test.

The two patients that were being treated in our hospital had pancytopenia, biopsy showing bone marrow hypoplasia, with normal karyotype and no vitamins deficiency, that a diagnosis of severe aplastic anemia, in all instances, could be attributed to chronic exposure to aromatic hydrocarbons. One patient was a male petrochemical worker with fifteen years of exposure. He was treated at Pedro Ernesto University Hospital for sepsis, and cellulitis of the left leg, leading to the amputation of this leg and later resolution of the problems with amplified antibiotics and bone marrow recovery. This man retired due to disability and he was followed up by physicians. The other patient was a 30-year-old male gas station worker with five years of exposure, with pancyt-

openia and hemiplegia of inferior members; he died of sepsis after three months.

As for the class of the comet tails, they were significantly higher for G1 (P = 0.001) and for the total workers (P = 0.001), indicating early DNA damage. For G2 the significance was borderline (P = 0.05), see Table 4. Examples of comets (0 - 4) can be seen in Fig. 1 (A - E), respectively.

Table 4

Distribution of frequency of comets [IDG 0, 1, 2] and comets [IDG 3, 4] in the exposed group

Comets	Group	Descriptive Measures						*P*-value
		N	Mean	D.P	1Q	Median	3Q	
0, 1, 2	G1	44	12.91	9.80	7.00	9.50	14.75	<0.001
3, 4		44	72.16	39.50	57.00	61.00	72.75	
0, 1, 2	G2	13	45.62	23.54	23.00	45.00	61.50	0.050
3, 4		13	87.00	65.95	51.00	63.00	98.50	
0, 1, 2	Total Exposed	57	20.37	19.60	9.00	11.00	24.00	<0.001
3, 4		57	75.54	46.58	55.00	61.00	77.00	

Note: G1 = ≤ 3 years of exposure, and G2 = > 3 years of exposure. The probabilities of significance (*P*-value) refer to the Wilcoxon test. IDG = Genetic Damage Indicator.

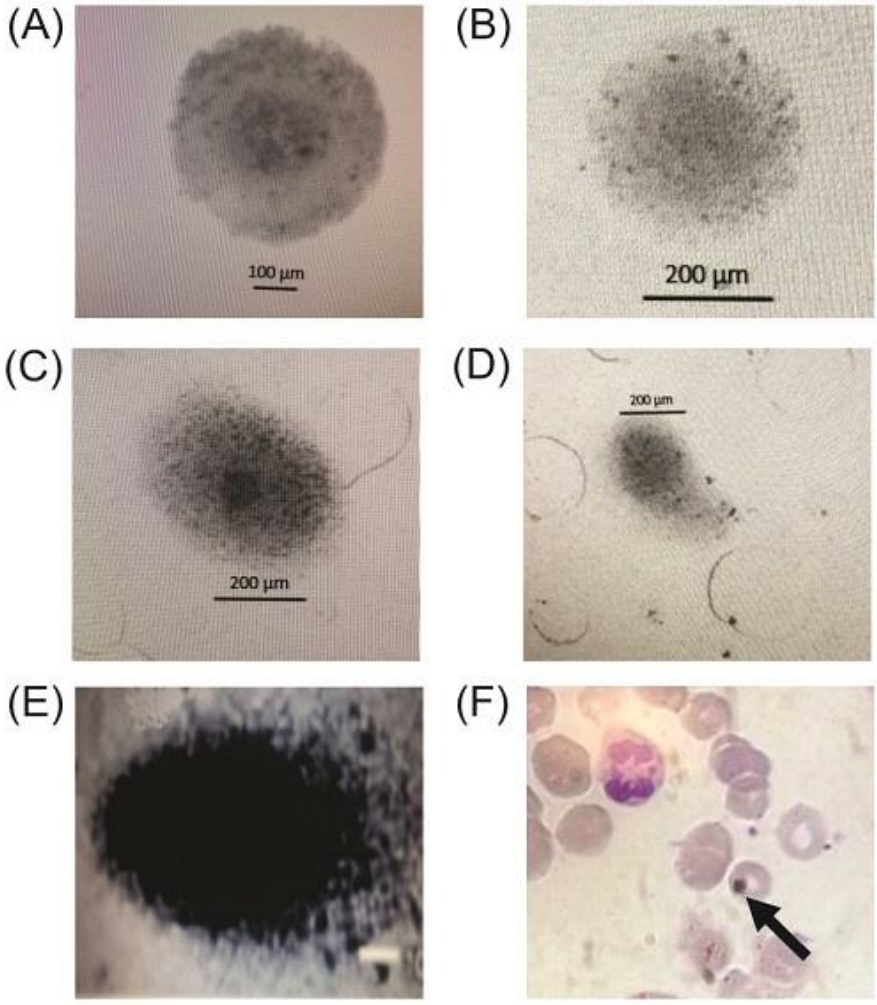

Fig 1. Comets and MN
(A) Comet class 0; (B) Comet class 1; (C) Comet class 2; (D) Comet class 3; (E) Comet class 4; (F) Micronucleus (arrow), May Grünwald-Giemsa stain

The MN test result was not significant when the groups G1 (workers ≤ 3 years of exposure) and G2 (workers with > 3 years of exposure) were compared to the controls;

however, the result was significant for the total workers group (P = 0.034), see Table 5. Example of MN can be seen in Fig. 1F.

Table 5

Evaluation of MN number in 1,000 erythrocytes in the exposed group x the control´s

Group	Descriptive Measures						*P*-value
	N	Mean	SD	1Q	Median	3Q	
G1	47	12.30	5.00	8.00	12.00	16.00	0.069
Control	123	10.44	5.82	6.00	11.00	16.00	
G2	43	11.79	3.81	9.00	11.00	14.00	0.141
Control	124	10.38	5.83	5.25	11.00	15.75	
Total Exposed	90	12.06	4.46	9.00	11.50	16.00	0.034
Control	124	10.38	5.83	5.25	11.00	15.75	

Note: MN = Micronucleus, G1 = workers with ≤ 3 years of exposition, and G2 = workers with > 3 years of exposition. The probabilities of significance (*P*-value) refer to the Mann-Whitney test.

Alterations in the blood of the workers were observed, including anemia, polycythemia, and leukocytosis; the latter two are not commonly described in current literature. These alterations in the blood are not described as chronic benzene poisoning alone. On the other hand, in spite of the difficult conditions for performing the propaedeutic adequately in a gas station room, it is not possible to think of another diagnosis, for example, cyanocobalamin vitamin or folic acid deficiencies. Increased levels of hemoglobin and hematocrit could indicate dehydration, chronic obstructive pulmonary and clonal changes predisposition could not be assessed in the gas station workers. It is worth noting the hot weather in Rio de Janeiro. Some experimental studies have demonstrated the association between anemia and benzene exposure [3, 16].

Experimental and clinical studies reinforce that aromatic hydrocarbons can cause liver damage [16-19]. Biochemical markers of enzymatic hepatic damage (bilirubin, AST, and LDH) were increased. Metabolic, infectious and immunologic injuries, such as overweight and steatohepatitis, primary biliary cirrhosis, alcohol drinking, viral infection, hepatocellular, carcinoma, and untoward drug side effects also cause the same biochemical markers to rise [15, 17, 18]. Therefore, interpreting these results is difficult without any complementary exam, especially because 12% (11/88) of GSW reported that they had alcoholic histories, but only two had increased levels of LDH. Neither a higher LDH level nor alcoholism was associated with anemia (Table 3), indicating that the anemia was not due to hemolytic compounds [20, 21] an inflammatory response might explain this result. As it is unclear whether the aromatic hydrocarbons exposure causes the liver damage, we suggest that a series of studies, excluding the confounding factors, be conducted in the future.

Micronuclei and comet techniques are not new and have been applied in other studies for the purpose of comprehensive biological monitoring [22-24]. The use of procedures to monitor the group of workers is very important in pointing out DNA damage. In order to test the influence of the time of exposure on the increasing formation of micronucleus and comet assay, we divided the workers into two groups: G1, ≤ 3 years of exposure; and G2, > 3 years. As for MN analysis, it was not possible to differentiate between G1 and G2 (Table 5), and only the total number of workers was significant (P = 0.034). However, with regard to the class of the comet tails, they were statistically longer in G1 (P = 0.001, Table 4) in compari-son to the control group; G2, was borderline (P = 0.005) probably because the N was small and the total was clearly differentiated (P = 0.001). In our work group population, the comet assay seemed to be more sensitive than MN test. Using the micronuclei and single-cell gel electrophoretic techniques many au-thors discussed the DNA damage findings on benzene exposure effects, excluding those

caused by smoking; some of them also tested other substances [25-31].

According to the clinical questionnaires, it was observed that none of the exposed workers had occupational diseases according to the Brazilian Ministry of Labor and Employment criteria. In spite of that, the workers presented a wide range of clinical complaints. Other papers have reported the same symptoms in GSW [1, 32]. Our study is especially important because it includes a high number of females; most studies reported data only from male workers.

We asked the GSW presenting symptoms why they did not seek health care. The excuse was that they did not want to use their days off for doctors´ visits and exams. On the other hand, the employers did not release them from a shift to be able to perform X-rays and abdominal ultrasound tests, available only in the hospital. We emphasize that although the workers were not considered ill, the results of the DNA damage tests (comet and micronuclei assays) indicated that damage was occurring, and in the long run, chronic diseases, even cancer, could rise consequently [31, 33]. The fact that two inpatients were in a prolonged-stay unit of intensive care at HUPE and had a history of exposure to aromatic hydrocarbons over five years, and finally were diagnosed as severe aplastic anemia, indicated a threat to their health. We think that one biological biomarker may be insufficient for surveillance, so other molecular and cytogenetic assays are necessary as well as additional periodic clinical examinations. The workers should be more frequently examined, considering their ongoing genome risks. It is up to the authorities to seek solutions to the problem of improper health care of these workers.

Conclusion. The most relevant complaints were headache, fatigue, and lipothymia. Alterations in the blood of the workers were observed including polycythemia, anemia, higher LDH and leukocytosis, indicating health problems. The genotoxicity assays suggested DNA damage and the comet test seemed to be more sensitive than the micronucleus test for surveillance. We hope that the results can guide the competent authorities to take the correct measures that enable to perform more effective surveillance of populations occupationally exposed to benzene, in particular, gas stations workers, in order to prevent cancer and other diseases associated with benzene and other compounds exposure; as well as improvement of routine health care, and surveillance of genome damage.

Financial support

No financial support has been provided for this work.

Conflict of interests

The authors have no conflict of interest to declare.

References

1. Bahadar H, Mostafalou S, Abdollahi M. Current understandings and perspectives on non-cancer health effects of benzene: a global concern. Toxicology and Applied Pharmacology. 2014;276(2):83-94. DOI: https://doi.org/10.1016/j.taap.2014.02.012

2. White MC, Infante PF, Walker B Jr. Occupational exposure to benzene: a review of carcinogenic and related health effects following the U.S. Supreme Court decision. American Journal of Industrial Medicine. 1980;1(2):233-243. DOI: https://doi.org/10.1002/ajim.4700010214

3. Linet MS, Yin SN, Gilbert ES, et al. A retrospective cohort study of cause-specific mortality and incidence of hematopoietic malignancies in Chinese benzene-exposed workers. International Journal of Cancer. 2015;137(9):2184-2197. DOI: https://doi.org/10.1002/ijc.29591

4. Atta EH, Lima CBL, Dias DSP, et al. Predictors of early mortality after rabbit antithymocyte globulin as first-line treatment in severe aplastic anemia. Annals of Hematology. 2017;96(11):1907-1914. DOI: 10.1007/s00277-017-3086-7

5. Clé DV, Atta EH, Dias DSP, et al. Rabbit antithymocyte globulin dose does not affect response or survival as first-line therapy for acquired aplastic anemia: a multicenter retrospective study. Annals of Hematology. 2018;97(11):2039-2046. DOI: 10.1007/s00277-018-3416-4

6. Ostling O, Johanson KJ. Microelectrophoretic study of radiation-induced DNA damages in individuals mamalian cells. Biochemical and Biophysical Research Communications. 1984;123:291-298. DOI: https://doi.org/10.1016/0006-291X(84)90411-X

7. Møller P**.** The comet assay: ready for 30 more years. Mutagenesis. 2018;33(1):1-7. DOI: https://doi.org/10.1093/mutage/gex046

8. Zhang L, Eastmond DA, Smith MT. The nature of chromosomal aberrations detected in human exposed to benzene. Critical Reviews in Toxicology. 2002;32(1):1-42. DOI: https://doi.org/10.1080/20024091064165

9. Lovreglio P, Maffei F, Carrieri M, et al. Evaluation of chromossome aberration and micronucleous frequencies in blood lymphocytes of workers exposed to low concentrations of benzene. Mutation Research - Genetic Toxicology and Environmental Mutagenesis. 2014;770:55-60. DOI: https://doi.org/10.1016/j.mrgentox.2014.04.022

10.Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Molecular Biotechnology. 2004;26(3):249-261. DOI: https://doi.org/10.1385/MB:26:3:249

11.Mohandas N. Structure and composition of the erythrocyte. In: Kaushansky K, Lichtman MA, Prchal JT, et al. Williams Hematology. New York: Mcgraw Hill; 2016:467-468.

12.Singh NP, McCoy MT Tice RR, et al. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research. 1988;175(1):184-191. DOI: https://doi.org/10.1016/0014-4827(88)90265-0

13.Bastos V, Duarte IF, Santos C, et al. Genotoxicity of citrate-coated silver nanoparticles to human keratinocytes assessed by the comet assay and cytokinnesis blocked micronucleus assay. Environmental Science and Pollution Research. 2017;24:5039-5048. DOI: https://doi.org/10.1007/s11356-016-8240-6

14.Collins AR, Ai-guo M, Duthie SJ. The kinetics of repair of oxidative DNA damage (strand breaks and oxidised pyrimidines) in human cells. Mutation Research – DNA Repair. 1995;336(1):69-77. DOI: https://doi.org/10.1016/0921-8777(94)00043-6

15.Mrdjanović J, Šolajić S, Dimitrijević S, et al. Assessement of micronuclei and sister chromatid exchange frequency in the petroleum industry workers in province Vojvadina, Republic of Serbia. Food and Chemical Toxicology. 2014;69:63-88. DOI: https://doi.org/10.1016/j.fct.2014.03.041

16.Martínez-Rodríguez JL, Gutiérrez-Hernández R, Reyes-Estrada CA, et al. Quantitative measurement of oxidative damage in erythrocytes as indicator in benzene intoxications. Toxicology Mechanisms and Methods. 2018;28(6):450-460. DOI: https://doi.org/10.1080/15376516.2018.1455786

17.Deng Q, Liu J, Li Q, et al. Interaction of occupational manganese exposure and alcohol drinking aggravates the increase of liver enzyme concentrations from a cross-section study in China. Environmental Health. 2013;12:30. DOI: https://doi.org/10.1186/1476-069X-12-30

18.Dere E, Ari F. Effect of Benzene on liver functions in rats (Rattus norvegicus). Environmental Monitoring and Assessment. 2009;154(1-4):23-27. DOI: https://doi.org/10.1007/s10661-008-0374-7

19.Malaguarnera G, Cataudella E, Giordano M, et al. Toxic hepatitis in occupational exposure to solvents. World Journal of Gastroenterology. 2012;18(22):2756-2766. DOI: https://doi.org/10.3748/wjg.v18.i22.2756

20.Moro AM, Brucker N, Charão MF, et al. Biomonitoring of gasoline station attendants exposed to benzene: effects of gender. Mutation Research - Genetic Toxicology and Environmental Mutagenesis. 2017;813:1-9. DOI: https://doi.org/10.1016/j.mrgentox.2016.11.002

21.Ekpenyong CE, Asuquo AE. Recent advances in occupational and environmental health hazards of workers exposed to gasoline compounds. International Journal of Occupational Medicine and Environmental Health. 2017;30(1):1-26. DOI: https://doi.org/10.13075/ijomeh.1896.00800

22.Rekhadevi PV, Rahman MF, Mahboob M, et al. Determination of genetic damage and urinary metabolites in fuel filling station attendants. Environmental and Molecular Mutagenesis. 2011;52(4):310-318. DOI: https://doi.org/10.1002/em.20622

23.Carere A, Antoccia A, Crebelli R, et al. Genetic effects of petroleum fuels: cytogenetic monitoring of gasoline station attendants. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis. 1995;332(1-2):17-26. DOI: https://doi.org/10.1016/0027-5107(95)00081-9

24.Pitarque M, Carbonell E, Lapeña N, et al. No increase in micronuclei frequency in cultured blood lymphocytes from a group of filling station attendants. Mutation Research. 1996;367(1):161-167. DOI: https://doi.org/10.1016/0165-1218(95)00091-7

25.Roma-Torres J, Teixeira JP, Silva S et al. Evaluation of genotoxicity in a group of workers from a petroleum refinery aromatics plant.

Mutation Research - Genetic Toxicology and Environmental Mutagenesis. 2006;604(1-2):19-27. DOI: https://doi.org/10.1016/j.mrgentox.2005.12.005

26.Andreoli C, Leopardi P, Crebelli R. Detection of DNA damage in human lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites. Mutation Research. 1997;377:95-104. DOI: https://doi.org/10.1016/j.cccn.2004.04.010

27.Maluf SW. Monitoring DNA damage following radiation exposure using cytokinesis-block micronucleus method and alkaline single cell gel electrophoresis. Clinica Chimica Acta. 2004;347(1-2):15-24. DOI: https://doi.org/10.1016/j.cccn.2004.04.010

28.Sul D, Lee E, Lee MY, et al. DNA damage in lymphocytes of benzene exposed workers correlates with trans,trans-muconic acids and breath benzene levels. Mutation Research – Genetic Toxicology and Environmental Mutagenesis. 2005;582(1-2):61-70. DOI: https://doi.org/10.1016/j.mrgentox.2004.12.011

29.Megyesi J, Biró A, Wigmond L, et al. Use of comet assay for the risk assessment of oil- and chemical-industry workers. Orvosi Hetilap. 2014;155(47):1872-1875. DOI: 1 https://doi.org/0.1556/OH.2014.30041

30.Naidoo RN, Makwela MH, Chuturgoon A, et al. Petrol exposure and DNA integrity of peripheral lymphocytes. International Archives of Occupational and Environmental Health. 2016;89(5):785-792. DOI: https://doi.org/10.1007/s00420-016-1116-8

31.Pandey AK, Bajpayee M, Parmar D, et al. Multipronged evaluation of genotoxicity in Indian petrol-pump workers. Environmental and Molecular Mutagenesis. 2008;49(9):695-707. DOI: https://doi.org/10.1002/em.20419

32.Santiago F, Alves G, Otero UB, et al. Monitoring of Gas Station Attendants Exposure to Benzene, Toluene, Xylene (BTX) Using Three-Color Chromosome Painting. Molecular Cytogenetics. 2014;7(1):15. DOI: https://doi.org/10.1186/1755-8166-7-15

33.Aksoy M. Malignancies due to occupational exposure to benzene. American Journal of Industrial Medicine. 1985;7(5-6):395-402. DOI: https://doi.org/10.1002/ajim.4700070506

Received 5 June 2020
Revised 20 July 2020
Accepted 25 July 2020

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