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Evaluation of DNA-Damaging Potential of Bisphenol A and its Metabolites in Human Peripheral Blood in Vitro Study (Comet Assay and Micronucleus Test)

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ABSTRACT

Bisphenol A (BPA) is an industrial chemical used in the production of epoxy resins and produced worldwide at the highest volume. Polycarbonate compounds are commonly used in food packaging and manufacturing products. BPA is released from polycarbonates by exposure to UV light, ageing or heating and contact with either acidic or basic component. This research was performed to investigate in vitro genotoxic, cytotoxic and oxidative potentials of BPA in human blood lymphocyte cultures with and without metabolic activation using cytokinesis-block micronucleus test, Cytokinesis block proliferation Index and single cell gel electrophoresis analysis, respectively. Three doses of BPA (0.063µg/L, 0.128µg/L ve 0.32µg/L) were applied to lymphocyte cultures from 3 donors. There is a statistically significant difference between all the doses of BPA and negative control in both with and without metabolic activation cultures ($p<0.01$) for Micronucleus test, Cytokinesis block proliferation Index. Two parameters including genetic damage index and damaged cell percent were evaluated in single cell gel electrophoresis analysis. We found a significant difference between all the doses of BPA and negative control in both with and without metabolic activation cultures ($p<0.001$). Our results indicate that BPA is able to induce cytotoxic and genotoxic and oxidative damage in human blood lymphocytes cultures.

INTRODUCTION

Bisphenol-A (BPA) [2,2-bis (4-hydroxyphenyl)] is an industrial compound synthesized by condensation of two phenol groups and one acetone molecule at low pH. BPA is an important precursor chemical in the production of polycarbonates and synthetic resins. Bisphenol A (BPA) has received much attention due to its widespread human exposure (Vandenberg et al., 2009; Izzotti et al., 2009). One such estrogenic chemical is bisphenol A (BPA), is a monomer used in plastics manufacturing, including consumer products (e.g., food packaging materials, internal coatings of cans and drums, reinforced pipes, adhesives, flooring, and nail polish (Naik and Vijayalaxmi, 2009). Chemicals released into the environment can affect the endocrine functions of animals and humans, mimicking natural hormone action (Kavlock and Perreault, 1994). Such endocrine disruptors may represent a major toxicological and public health issue (Markey et al., 2003). Genotoxic and non-genotoxic effects of BPA have been reported (Naik and Vijayalaxmi, 2009; Hilliard et al., 1998; NTP report 1982; Schweikl et al., 1998; Honma et al., 1999).

The production of BPA within a year is almost 1.7 million kg. BPA can be dispersed in environment by various ways such as production, transport and application. This dispersion mostly has occurred by discharging to surface waters through waste waters without cleaning of wastes occurred during production or by leakage take placed in BPA storages (ECB 2003). In some studies, it is indicated that the level of BPA was 8-11 ng/ml in rivers which supplied of city water of Germany, Belgium and Holland (Staples et al., 1999). Humans are exposed to BPA via foods. BPA can be metabolized in living organisms. BPA metabolism in mammary is two pathways, glucuronidation and sulfation of BPA. BPA is glucuronidated by liver microsomes. Yokota et al. (1999) reported that the glucuronidation was mediated by *UDP-glucuronosyltransferase* (UGT) in the rat liver. The hepatic glucuronidation is slightly less in pregnancy than in nonpregnancy because of multidrug resistance-associated protein II and UGT decrease in pregnancy (Inoue et al., 2004). Moreover, the UGT levels in the human fetal liver are lower than those in the adult liver (Cappiello et al., 2000; Matsumoto et al., 2002; Strassburg et al., 2002). Matsumoto et al. (2002) indicated that the activity of UGT toward BPA and its protein and mRNA contents are not detected in the fetal rat liver. Sulfation of BPA by *sulfotransferases* in the liver is also included in the BPA metabolism pathway in mammary (Suiko et al., 2000; Nishiyama et al., 2002).

There are studies indicating the relationship between mutagenicity, genotoxicity or toxicity and BPA exposure in both in vivo and in vitro.

Today, S9 mix activation system has been applied in many *in vitro* cell cultures by many researchers (González Borroto et al., 2002; Marques et al., 2002; Eke and Çelik, 2008). Many mutagens require oxidative metabolism to reactive species before demonstrating mutagenicity.

Considering that, and the lack of data on the cytogenetic status induced by Bisphenol A, the aim of this study was to assess the genotoxic potential of bisphenol A and its metabolites in vitro in human peripheral blood lymphocytes with and without metabolic activation of S9.

MATERIALS AND METHODS

Donors and collection of blood samples

The study was carried out by using blood samples from three healthy, non-smoking male donors, aged 20, 21 and 23 years. Approximately, 10 ml of blood was collected, by venipuncture, into syringes containing sodium heparin as anticoagulant. Blood was taken the same day of the initiation of the experiment between 9.00 and 9.30 a.m. to minimize possible confounding effects of dietary factors.

Test chemicals

BPA (CAS No. 54–64–8) was obtained from Sigma (Figure 1). Mitomycin C (MMC, CAS No: 50-07-7, Kyowa). Methanol, acetic acid, potassium chloride, sodium chloride, trisodium citrate-2-hydrate, di-sodium hydrogen phosphate-2-hydrate, potassium dihydrogen phosphate and Giemsa dye were obtained from Merck (Darmstadt, Germany). Colchicine was purchased from Fluka (Buchs, Switzerland). Phytohaemagglutinin (PHA, M form) was obtained from Gibco BRL Life Technologies (Paisley, UK) and prepared according to supplier's instructions. Heparin was purchased from Braun (Melsungen, Germany. In the cultures without metabolic activation, the positive control was MMC at 2 µg/ml for treatments in cultures without S9. For cultures with metabolic activation (S9), cyclophosphamide (CP, CAS No. 6055-19-2, Sigma) was used as positive control at 1.4 µg/ml for testing MN. Hydrogen peroxide (cultures without S9) and Benzo-a-pyrene (with S9) were used as positive control for comet analysis,

Dose selection

The dose range was selected according to serum concentrations (0,063µg/L, 0,128 µg/L and 0,32 µg/L) of people exposed to bisphenol A.

Metabolic activation

S9 from (Cat No: 452591) ‘‘BD Biosciences” was used as metabolic activation system. The rats used to produce the S9 were not given arochlor, phenobarbital, or other chemicals.

The S9 mix freshly prepared, consisted of 1 ml of S9, 0.33 ml of 1 M KCl, 0.32 ml of 0.25 M MgCl2_6 H2O, 0.25 ml of 0.2 M glucose-6-phosphate, 1 ml of 0.04 M NADP, 2.10 ml of distilled water and 5 ml of phosphate buffer (pH 7.4).

Genotoxic evaluation

Lymphocyte cultures and Cytokinesis block Micronucleus Assay in peripheral blood lymphocytes

The study was carried out by using blood samples from three healthy non-smoking male donors. In three donors, results of clinical routine laboratory analyses were within normal range, and the absence of exposure to known genotoxicants was considered. Lymphocyte cultures were prepared according to the technique described by Scarpato et al. (1996) with slight modifications. Heparinized whole blood (0.8 mL) was added to 5 mL of culture medium RPMI 1640 (Sigma), supplemented to 20% with fetal calf serum (Sigma), with 0.2 mL phytohemagglutinin (Sigma), and with antibiotics (10,000 IU/mL penicillin and 10,000 IU/mL streptomycin). A final concentration of 6 µg/mL of cytochalasin B was added to cultures 44 h later to arrest cytokinesis. At 72 h of incubation, the cultures were harvested by centrifugation at 2000 rpm for 10 min. Then, to eliminate red cell and to keep the cytoplasm, the cell pellet was treated with a hypotonic solution (4–5 min 0.075 M KCl at 37°C). Cells were centrifuged, and Carnoy's fixative (methanol: acetic acid, 3:1, v/v) solution was freshly added. This fixation step was repeated five times. Next, cell pellets were resuspended in a small volume of fixative solution and dropped onto clean, cold slides. The slides were stained with 10% Giemsa dye solution.

In general, the same procedures were used for the assays conducted with and without metabolic activation. Nevertheless, in the case of cultures with metabolic activation, 24 h

after the initiation of cultures, 0.5 ml of the S9 mix were added together with the test agent, BPA (0,063µg/ml, 0,128 µg/ml, and 0,32 µg/ml). After an incubation period of 3 h at 37 ^{0}C, the test chemical and S9 mix were removed from the culture. Concurrent cultures, treated for 3 h without the activating system, were also set up. The pellet of lymphocytes was washed twice with 5 ml of RPMI 1640 medium and resuspended in complete medium and, after that, the cultures were incubated until the entire period of 72 h at 37 ^{0}C.

Lymphocyte cell collection for Comet Analysis

The experiments were performed on peripheral blood lymphocytes obtained from three healthy donors, peripheral blood mononuclear cells were isolated by Histopaque-1077 density gradient centrifugation, according to the manufacturer's instructions. Lymphocyte cultures were set up by adding 0.5 ml of lymphocyte suspension in 4.5 ml of RPMI 1640 medium supplemented with 20% fetal calf serum, 2mM l-glutamine, 10 mg/ml phytohemagglutinin, 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were incubated for 72 h at 37^{0}C.

Alkaline comet assay

Comet assay was performed with lymphocytes from two donors according to Singh et al. (1988). Firstly, lymphocytes from three donors were treated with BPA at three different concentration (0,063µg/L, 0,128 µg/L, and 0,32 µg/L). Briefly, 100 µl of cell suspension was mixed with 200 µl of 2% low melting temperature agarose at 37^{0}C and then placed on a slide pre-coated with the thin layer of 0.5% normal melting agarose. The cell suspension was immediately covered with a cover glass and the slides were kept at 4 ^{0}C for 5 min to allow solidification of the agarose. After removing the cover glass, the cells were lysed in a lysing solution (2.5M NaCl, 100mM EDTA, 10mM Tris, 1% Triton X-100, pH 10) for 1 h. After washing in re-distilled water the slides were placed in a horizontal gel electrophoresis chamber. The chamber was filled with cold electrophoretic buffer (1mM EDTA, 300mM NaOH, pH 13) and slides were kept at 4 ^{0}C for 40 min to allow the DNA to unwind. Electrophoresis was conducted at 20^{0}C using 25V and 185mA for 20 min. After electrophoresis, the slides were washed three times with neutralization buffer (0.4M Tris, pH 7.5). All preparative steps were conducted in dark to prevent additional DNA damage. The slides were stained with etidium bromide (0.1 mg/ml, 1:4) and analyzed with a fluorescence microscope (Olympus BX 51) equipped with a video camera CCD-4230.

Comet Slide scoring

Comet images were analyzed according to Collins et al. (1995). One hundred comet images were scored for each treatment by one scorer (S.Y.E.) visually under fluorescence microscopy (BX51 OLYMPUS). An intensity score from class 0 (undamaged) to class 4 (ultra high damage) [Sun et al., 2004] was assigned to each cell. The method of the observation was barred in a blind way during which the observer had no knowledge of the identity of the slide. Fifty cells per slide and two slides were examined per sample to evaluate DNA damage for each culture treated with/without S9. In comet assay, two different parameters were evaluated 1. Damaged cell percent (DCP), 2. Genetic damage index (GDI=AU). The arbitrary unit (AU=GDI) was used to express the extent of DNA damage and calculated using the following formula. The slides were blinded to the scorer. The cells were classified by eye in the five categories on the basis of the extent of DNA migration, undamaged (class 0), very little damage (class 1), moderate damage (class 2), high damage (class 3) ultra high damage (class 4).

$$AU = \sum_{i=0}^{4} i \times N_i$$

Ni = the number of scored cell in i level, i = the level of DNA damage (0, 1, 2, 3, 4).

DCP was expressed via following Formula;

DCP=class 2+ class 3+ class 4

RESULTS

Cytokinesis Block Micronucleus Assay and Comet Asay

Table 1 represents MN frequency in the binucleated cell with one/two MN, CBPI values of control and each dose groups and DCP, GDI values measured in comet assay performed in cultures with /without S9. All the dose of BPA caused the increase MN frequency and the decrease in CBPI values. There are significant differences between negative control group and BPA doses groups ($p<0.05$) in cultures and with/without S9 for MN frequency. Such values are much lower than those induced by the positive control, Mitomycin C and hydrogen peroxide or benzo-a-pyrene.

Table 1 shows DNA damage level (class 0-class 4). All the doses of BPA significantly induced the frequency of micronucleus and DNA damage in dose-dependent manner in human peripheral blood lymphocytes. Figure 2 and 3 represent micronucleus and comet views (level 0-4). There is a significant difference between both dose groups and negative control and among all dose groups ($p<0.05$).

DISCUSSION

The possible health effects of BPA have been a matter of concern because of the potential wide human exposure consequent to the widespread use of this chemical as plastic bottles, compact disks, dental sealant, food cans and bonding agents. We evaluated the effects in the base on genotoxicity of BPA using cytokinesis-block micronucleus test and single cell gel electrophoresis analysis (COMET) in human peripheral blood lymphocyte cultures with/without S9 metabolic activation system. We found that BPA induced micronucleus frequency in cytokinesis-block micronucleus test and increased DCP and GDI in comet analysis, besides decreased the CBPI value in peripheral blood lymphocyte cultures both with S9 and without S9.

It is seen that many environmental chemical substances induced DNA breaks in many investigations in such as in vitro and in via studies in many organisms and tissue cultures. In many studies, it is seen that Comet assay or single cell gel electrophoresis ascertains lesions such as DNA strand breaks and alkali-labile sites (Gajovik et al., 2013; Çelik et al., 2013; Eke and Çelik 2016). Due to BPA is one of the highest volume chemicals produced worldwide and has been used in the chemical industry for the production of products containing polycarbonate it is important to investigate the effects on the environment. BPA exposure can occur on different routes. BPA can be leached from plastic products and food- and- drink-packaging. Especially, in many studies, it is indicated that there are reports on the contamination of BPA from canned food (Goudson et al, 2002; Kang and Kondo, 2003). BPA can be metabolized by enzymes existing in plant and animals. In BPA metabolites, BPA glucuronide is characterized as a major metabolite of BPA via liver microsome pathway. Other metabolites such as BPA sulfate conjugate, BPA diglucuronide, were also identified. Jaeg et al (2004) reported that there are nine metabolites from the metabolism of BPA by mice liver microsomes and S9 fractions. Atkinson and Roy (1995 a,b) reported that BPA metabolite, bisphenol-*o*-quinone, could bind DNA in vivo and in vitro. Therefore, several

studies reported that the metabolic products of BPA can form the damages on DNA (Tsutsui et al., 1998; Tayama et al.,2008)

For BPA, there are also several published studies indicating its possible genotoxicity. Gajovik et al., (2013) reported that BPA induced DNA strand breaks in lung cells of mice exposed to BPA for 2 weeks at two different doses (5mg/kg and 10 mg/kg) using alkaline comet assay and evaluated the genotoxic effects of BPA in peripheral blood and bone marrow reticulocytes of mice using the micronucleus test. They found that there is a significant increase in the number of MN in peripheral blood reticulocytes after only 2-week' exposure to doses of 10 and 20 mg/kg BPA compared to the control group but no increase in MN frequency in bone marrow reticulocytes. They reported that both doses caused nearly twice as many micronuclei as in control and explained that the reason for a lower incidence of MN in bone marrow reticulocytes may be the dislocation of reticulocytes from bone marrow to peripheral blood to transform into mature erythrocytes. Iso et al. (2006) investigated the potential oxidative damage of PBA on the DNA in MCF-7 cell cultures using comet analysis and γH_2AX method and found that BPA increased comet tail length and DNA double-strand breaks. They showed that BPA caused DNA damage depending on MCF cells, although higher concentrations of BPA were needed. In another study performed in mouse lymphoma cells, it is found that BPA caused the genotoxicity but Lee et al. (2003) concluded this effect may be false positive due to cell death because $4x10^{-6}$-$4x10^{-4}$M BPA was cytotoxic. Hovewer, Iso et al.(2006) informed that the genotoxicity of 10^{-6} and 10^{-4}M concentration of BPA was depending on cytotoxicity. In our also study, BPA is both cytotoxic and genotoxic, we concluded that probably cytotoxicity and genotoxicity is mutually dependent. In the present study, All the concentrations of BPA decreased the CBPI value in peripheral blood lymphocyte cultures both with S9 and without S9. In many studies, CBPI is used as cell kinetic parameter for cytotoxicity. Suárez et al (2000) investigated the cytotoxic effects of Bisphenol A diglycidyl ether and its hydrolysis products in peripheral blood lymphocyte under culture conditions with/without S9. They found that these compounds decreased CBPI values and increased the micronucleus frequency in both culture conditions. In the present study, we also investigated the genotoxic and cytotoxic effects of BPA using cytokinesis-block micronucleus test. We measured the genotoxic effect by evaluating micronucleus frequency and found that BPA induced the micronucleus formation at three concentrations under culture conditions with/without S9 in peripheral blood lymphocytes *in vitro*. In biomonitoring studies, many researchers investigated the exposure

to BPA, for example, Battal et al.,(2014) analysed the total urinary BPA concentrations in 200 urine samples (children: 57; male: 74; female: 69) obtained from residents of a Mediterranean city, Mersin using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).They evaluated environmental exposure to free BPA and its major metabolite bisphenol A glucuronide (BPAG) in human urine and reported that determined amount of total BPA in the study performed by Battal et al.(2014) study is lower than the results of similar studies conducted in developed countries. Al-Saleh et al. (2017) evaluated the genotoxic effects of BPA and phthalate esters (PAEs) in TK6 human lymphoblast cell line treated samples from several wastewater plants. They found that BPA and some PAEs in the treated wastewater might have the potential to induce genetic damage in TK6 human lymphoblast cell line, despite their low levels. Kabuto et al. (2003) investigated the modifications in endogenous antioxidant capacity, including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, oxidative stress index, reduced glutathione (GSH), glutathione disulfide (GSSG), and thiobarbituric acid-reactive substance (TBARS) in the brain, liver, kidney, and testes of mice under bisphenol A (BPA), an endocrine disrupter, treated for 5 days. They reported that the injection of BPA induces overproduction of hydrogen peroxide in the mouse organs and therefore BPA may show the toxicity by increasing hydrogen peroxide. BPA acts both a prooxidant and an antioxidant because BPA has antioxidant activities structurally, its metabolites show prooxidant activity. Although BPA monoglucuronide is the major metabolite of BPA, it was indicated that BPA, in part, is metabolized to hydroxylated BPA via a bisphenol semiquinone by peroxidase (Knaak and Sulivan, 1966). It was shown that quinone and semiquinone metabolites of BPA are able to form DNA-BPA adducts (Atkinson and Roy, 1995a, b) via mutational changes in nuclear genome through inhibition of replication. Oxidative damage might be induced by free-radical generation via metabolic redox cycling between quinone and hydroquinone forms of BPA. The further studies are necessary to determine at molecular level the relationship between genotoxicity and cytotoxicity or whether BPA affects DNA repair system in eucaryotic organisms under in vitro or in vivo conditions.

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REFERENCES

1. Al-Saleh I., Elkhatib, R., Al-Rajoudi, T. Al-Qudaihi, G. (2017). Assessing the concentration of phthalate esters (PAEs) and bisphenol A (BPA) and the genotoxic potential of treated wastewater (final effluent) in Saudi Arabia. *The science of the Total Environment* 578, 440–451.
2. Atkinson, A., Roy, D., (1995a). In vitro conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite(s). *Biochemical and Biophysical Research Communication*. 210, 424–433.
3. Atkinson, A., Roy, D., (1995b). In vivo DNA adduct formation by bisphenol A. *Environmental and Molecular Mutagenesis* 26, 60–66.
4. Battal, D., Çok I., Unlusayin, I. & Aktaş A. & Tunctan, B. (2014) Determination of urinary levels of Bisphenol A in a Turkish population. *Environmental Monitoring and Assessment* 186, 8443–8452.
5. Cappiello, M., Giuliani, L., Rane, A., Pacifici, G.M. (2000) Uridine 5_-diphosphoglucuronic acid (UDPGLcUA) in the human fetal liver, kidney and placenta. *European Journal of Drug Metabolism and Pharmacokinetics* 25, 161–163.
6. Collins A, Dusinska M, Franklin M, et al. (1997) Comet assay in human biomonitoring studies: reliability, validation, and applications. *Environmental and Molecular Mutagenesis* 30:139–146.
7. Çelik A, Eke D, Ekinci SY, Yıldırım S. (2013) The protective role of curcumin on perfluorooctane sulfonate-induced genotoxicity: single cell gel electrophoresis and micronucleus test. *Food and Chemical Toxicology* 53, 249–255.
8. Eke, D., Çelik, A. (2008) Genotoxicity of thimerosal in cultured human lymphocytes with and without metabolic activation sister chromatid Exchange analysis proliferation index and mitotic index. *Toxicology in Vitro*, 22, 927–934.
9. ECB, "European Chemicals Bureau, (2003). European Union Risk Assessment Report 4,4'-ısopropylidenediphenol (Bisphenol-A). " Einecs No:201-245-8 Office for Official Publications of the European Communities,
10. Eke, D. and Çelik, A. (2016). Curcumin prevents perfluorooctane sulfonate-induced genotoxicity and oxidative DNA damage in rat peripheral blood. *Drug and Chemical Toxicology* 39(1): 97–103
11. Gajovik, A., Radzikowska, J., Dobrzyńska, M.M. (2013). Genotoxic effects of bisphenol A on somatic cells of female mice, alone and in combination with X-rays. *Mutation Research* 757, 120– 124.
12. González Borroto, J.I., Creus, A., Marcos, R., (2002). Genotoxic evaluation of the furylethylene derivative 1-(5-bromofur-2-yl)-2-nitroethene in cultured human lymphocytes. *Mutation Research* 519, 179–185.
13. Goodson, A., Robin, H., Summerfield, W., Cooper, I., (2004). Migration of bisphenol A from can coatings-effects of damages, storage conditions, and heating. *Food Additives and Contaminants* 21, 1015–1026.
14. Hilliard, C.A. Armstrong, M.J. Bradt, C.I. Hill, R.B. Greenwood, S.K. Galloway, S.M. (1998) Chromosome aberrations in vitro related to cytotoxicity of non mutagenic chemicals and metabolic poisons, *Environmental and Molecular Mutagenesis* 31, 316–326.
15. Honma, M. Hayashi, M. Shimada, H. Tanaka, N. Wakuri, S. Awogi, T. Yamamoto, K.I. Kodani, M.-U. Nishi, Y. Nakadate, M. Sofuni, T. (1999) Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the in vitro chromosomal aberration test, *Mutagenesis* 14, 5–22.
16. Inoue, H., Tusinata, A., Kudo, S., Ishii, T., Fukushima, Y., Iwano, H., Yokota, H., Kato, S. (2004) Bisphenol A glucuronidation and excretion in the liver of pregnant and nonpregnant female rats. *Drug Metabolism and Disposition* 33, 55-59.
17. Iso, T., Watanabe T. Iwamoto, T. Shimamoto A., Furuichi, Y. (2006) DNA Damage Caused by Bisphenol A and Estradiol through Estrogenic Activity, *Biological and Pharmaceutical Bulletin* **29**(2), 206—210
18. Izzotti, A. Kanitz, S. D'Agostini, F. Camoirano, A. De Flora, S.(2009). Formation of adducts by bisphenol A, an endocrine disruptor, in DNA in vitro and in the liver and mammary tissue of mice, *Mutation Research* 679, 28–32.
19. Jaeg, J.P., Perdu, E., Dolo, L., Debrauwer, L., Gravedi, J.P., Zalko, D., (2004). Characterization of new bisphenol metabolites produced by CD1 mice liver microsomes and S9 fractions. *Journal Agricultural Food Chemistry* 52, 4935–4942.
20. Kabuto H., Hasuike S., Minagawa N., Shishibori T. (2003).Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. *Environmental Research* **93**, 31—35.

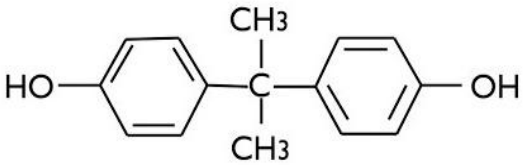
21. Kang, J.H., Kondo, F., (2003) Determination of bisphenol A in milk and dairy products by high-performance liquid chromatography with fluorescence detection. *Journal of Food Protection* 66, 1439–1443.
22. Kavlock R., Perreault S. (1994) Multiple chemical exposure and risks of adverse reproductive function and outcome, in R.S.H. Yang (Ed.), Toxicological of Chemical Mixtures: From Real Life Examples to Mechanisms of Toxicology Interactions, Academic Press, Orlando, pp. 245–297.
23. Knaak, J.B., Sullivan, L.J., (1966) Metabolism of bisphenol A in the rat. *Toxicology and Applied Pharmacology* 8, 175–184.
24. Lee M., Kwon, J. Chung, M-K. (2003) Enhanced prediction of potential rodent carcinogenicity by utilizing comet assay and apoptotic assay in combination. *Mutation Research* 541, 9–19.
25. Markey, C.M. Rubin, B.S. Soto, A.M. Sonnenschein, C. (2003) Endocrine disruptors: fromwingspread to environmental developmental biology, *The Journal of Steroid Biochemistry and Molecular* Biology 83, 235–244.
26. Marques, S., Oliveira, N.G., Chaveca, T., Rueff, J., (2002) Micronuclei and sister chromatid exchanges induced by capsaicin in human lymphocytes. *Mutation Research* 517, 39–46.
27. Matsumoto, J., Yokota, H., Yuasa, A., (2002). Developmental increase in rat hepatic microsomal UDP-glucuronosyltransferase activities toward xenoestrogens and decrease during pregnancy. *Environmental Health Perspectives* 110, 193–196.
28. Naik, P. Vijayalaxmi, K.K. (2009). Cytogenetic evaluation for genotoxicity of Bisphenol-A in bone marrow cells of Swiss albino mice, *Mutation Research* 676, 106–112.
29. Nishiyama, T., Ogura, K., Nakano, H., Kaku, T., Takahashi, E., Ohkubo, Y., Sekine, K., Hiratsuka, A., (2002). Sulfation of environmental estrogens by cytosolic human sulfotransferase. *Drug Metabolism and Pharmacokinetics* 17, 221–228.
30. NTP Report, TR-215 – Bisphenol A (CAS No 80-05-7) Carcinogenesis Bioassay of Bisphenol A (CAS No. 80-05-7) in F344 Rats and B6C3F1 Mice (Feed Study), National Toxicology Program. Technical Report Series, 1982.
31. Scarpato R, Migliore L, Angotzi G, et al. (1996). Cytogenetic monitoring of a group of Italian floriculturists. No evidence of DNA damage related to pesticide exposure. *Mutation Research* 319:215–222.
32. Schweikl, H. Schmalz, G. Rackebrandt, K. (1998) The mutagenic activity of unpolymerized resin monomers in Salmonella typhimurium and V79 cells, *Mutation Research* 415, 119–130.
33. Singh NP, McCoy MT, Tice RR, Schneider ELA. (1988) A simple technique for quantitation of low levels of DNA damage in individuals cells. *Experimental Cell Research* 175:184–191.
34. Staples, C.A., Willams, J.B., Blessing, R.L., Varineau, P.T.(1999) Measuring the biodegradability of nonylphenol ether carboxylates, octylphenol ether carboxylates, and nonylphenol. *Chemosphere*. 38: 2029-2039.
35. Strassburg, C.P., Strassburg, A., Kneip, S., Barut, A., Tukey, R.H., Rodeck, B., Manns, M.P., (2002) Developmental aspects of human hepatic drug glucuronidation in young children and adults. *Gut* 50, 259–265.
36. Suárez, S., Sueiro, R.A., Garrido J. (2000). Genotoxicity of the coating lacquer on food cans, bisphenol A diglycidyl ether (BADGE), its hydrolysis products and a chlorohydrin of BADGE. *Mutation Research* 470, 221–228.
37. Suiko, M., Sakakibara, Y., Liu, M.C., (2000). Sulfation of environmental estrogen-like chemicals by human cytosolic sulfotransferases. *Biochemical and Biophysical Research Communications*. 267, 80–84.
38. Sun LW, Qu MM, Li YQ, et al. (2004) Toxic effects of aminophenols on aquatic life using the zebrafish embryo test and the comet assay. *Bulletin Contamination Toxicology* 73:628–634.
39. Tayama, S., Nakagawa, Y., Tayama, K. (2008) Genotoxic effects of environmental estrogen-like compounds in CHO-K1 cells, *Mutation Research* 649, 114–125.
40. Yokota, H., Iwano, H., Endo, M., Kobayashi, T., Inoue, H., Ikushiro, S., Yuasa, A. (1999) Glucuronidation of the environmental estrogen bisphenol A by an isoform of glucuronosyltransferase, UGT2B1 in the rat liver. *Biochemical Journal* 340, 405-409.
41. Vandenberg, L.N., Maffini, M.V., Sonnenschein, C., Rubin, B.S., Soto, A.M., (2009) Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocrinology Review.* 30, 75–95.

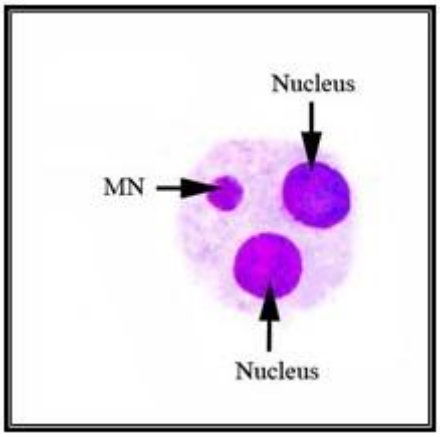
Table 1: Frequency of micronucleus and cytokinesis-block proliferation index values and DNA damage level (damaged cell percent and genetic damage index) in BPA-treated peripheral blood lymphocytes cultures

	1 MN mean±SD	2 MN mean±SD	CBPI mean±SD	DCP mean±SD	GDI mean±SD
S9-					
NK	3,00±1,00	0,33±0,58	2,54±0,01	12,67±1,00	54,67±5,77
0,063	5,33±0,58*	1,33±0,58*	2,35±0,03*	21,33±5,14*	76,67±14,22*
0,128	7,00±1,00**	4,00±1,00*	2,10±0,08**	27,00±2,65**	100,00±2,65**
0,320	7,67±0,58**	6,00±1,00**	1,80±0,05**	31,67±2,52**	120,33±6,81**
PK MMC(2µg/ml)	9,33±1,15***	7,33±0,58**	1,48±0,03***	90,67±4,16**	324,00±19,70***
S9+					
NK	3,67±0,58	1,67±0,58	2,54±0,02	8,00±1,00	40,67±3,21
0,063	5,33±0,58*	1,67±0,58	2,30±0,03*	23,00±1,00*	78,00±4,58*
0,128	6,67±0,58**	3,00±1,00*	2,08±0,04**	27,67±1,53**	102,67±4,16**
0,320	7,33±0,58**	5,00±1,00*	1,88±0,02**	37,67±1,53**	123,67±2,52**
PK MMC(2µg/ml)	9,67±0,58***	7,33±0,58***	1,48±0,03***	91,00±4,36***	315,00±4,58***

CBPI: cytokinesis block proliferation index;DCP; damaged cell percent;GDI: genetic damage index; MN: micronucleus;

NC: negative control; PC: positive control.




Nucleus
MN
Nucleus