JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

EVALUATION OF DOSE-DEPENDENT ACTIVITY OF BISPHENOL F ON VIABILITY PARAMETERS AND STEROIDOGENESIS IN H295R CELLS

Nikola Knížatová[1], Hana Greifová[1], Katarína Tokárová[1], Tomáš Jambor[1], Norbert Lukáč[1]

Address(es):
[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

*Corresponding author: nikola.knizatova@gmail.com

https://doi.org/10.15414/jmbfs.4765

ARTICLE INFO

Received 6. 10. 2020
Revised 16. 4. 2021
Accepted 12. 5. 2021
Published 1. 10. 2021

Short communication

OPEN ACCESS

ABSTRACT

Increasing concern over bisphenol A (BPA) as an endocrine-disrupting chemical and recent imposition of restriction on the use of BPA paved the way for entry of its analogues in the market. Bisphenol F (BPF) is one of the major analogues of commercial value. Thus, its increasing production and application make it vulnerable to human exposure. The aim of our *in vitro* study was to assess the potential effect of BPF on H295R cells mitochondrial activity, metabolic activity, membrane integrity, lysosomal function, and testosterone synthesis. Adrenocortical carcinoma cells were cultivated during 24 h in the presence of BPF (0.1, 0.5, 1, 10, 25, 50, 75, 100, 300, 500 μM). Exposure doses of BPF caused a significant decrease of mitochondrial activity starting from 1 μM, we observed a slight increase in mitochondrial activity at the lowest concentration (0.1 μM). Metabolic activity decreased with increasing dose of BPF - from 10 to 500 μM. A significant increase in metabolic activity was observed after cultivation with 0.1 μM BPF and a slight increase was observed after cultivation with 0.5 μM BPF. We observed a slight increase in lysosomal function and membrane integrity after cultivation with 0.1 and 1 μM, although higher exposure doses (25 - 500 μM) caused significant decrease in membrane integrity and lysosomal function. Lowest exposure dose of BPF (0.1 μM) caused a significant increase in testosterone synthesis, on the other hand, higher exposure doses (50 - 500 μM) caused significant decrease of testosterone production. The obtained results confirmed that BPF at higher concentrations caused cytotoxicity and possibly have endocrine-disrupting potential.

Keywords: BPF, H295R, membrane integrity, metabolic activity, mitochondrial activity, lysosomal activity, testosterone

INTRODUCTION

In recent years, there is increasing evidence of possible negative effects of bisphenol A (BPA) used in plastics, receipts, food packaging, and other products to human health due to its actions as an endocrine disrupting chemical (EDC) **(Rochester, 2013; Rochester and Bolden, 2015)**. Scientists, regulators, and the general public have raised concerns about the use of BPA, which has prompted industry to seek alternative chemicals such as bisphenol F (BPF). **(Vandenberg *et al.*, 2010; Rochester and Bolden, 2015)**. BPF is industrially used to make epoxy resins and coatings, especially for systems needing increased thickness and durability, such as tank and pipe linings, industrial floors, road and bridge deck toppings, structural adhesives, grouts, coatings, and electrical varnishes **(Fiege *et al.* 2000)**. BPF epoxy resins are also used for several consumer products such as lacquers, varnishes, liners, adhesives, plastics, water pipes, dental sealants, and food packaging **(Office of Environmental Health Hazard Assessment 2012)**. **Cabaton *et al.* (2006)** discovered that after administration of single dose of BPF to pregnant and nonpregnant rats, BPF was absorbed and metabolized, with at least six metabolites identified, (4,4- dihydroxybenzophenone (DHB) and hydroxylated-BPF [BPF-OH]) were main metabolites. BPF residues were measured in the placenta, amniotic fluid and the fetuses. According to **Cabaton *et al.* (2006) and Cabaton *et al.* (2009)**, BPF and its metabolites are excreted primarily in the urine (43-54% of administered dose) and to a lesser extent in the feces (15-20%). Ideally, substitutes used to replace a chemical of concern should be inert, or at least far less toxic than the original chemical, but BPF is structural analogue to BPA, thus its effects in physiological systems may be similar. BPA has been identifies as endocrine disruptor based on *in vitro* and animal laboratory studies **(Wetherill *et al.* 2007, Richter *et al.* 2007; Vandenberg 2014, Rochester and Bolden, 2015)**. Humans are exposed to bisphenol analogues via the same pathways that have been demonstrated for BPA, including oral, dermal, hand to-mouth transfer, as well as other mechanisms **(Chen *et al.*, 2016)**. According to the literature, the intake of dietary BPF in the form of contaminated food and water is the main source of exposure. Mainly, exposure to BPA analogues comes from microwaving food in plastic containers made from these materials, from using plastic bowls and cups that are worn out and may be leaching monomers, or even from tap water in areas where bisphenols were used to coat the inside of water pipes **(Wu *et al.*, 2017, Liao *et al.*, 2012, Thoene *et al.*, 2020)**. Exposures to levels of BPA found in environment have been associated with adverse health outcomes in children and adults in more than 75 human studies **(Rochester 2013, Rochester and Bolden 2015)**. According to review of **Rochester and Bolden (2015)**, BPF showed estrogenic, androgenic, and thyroidogenic activities. BPF increased the weight of the testes **(Higashihara *et al.* 2007)**, Cowper's gland weight **(Yamasaki *et al.* 2003)**, increased thyroid weight and altered thyroid hormone concentrations and also caused changes to hematological parameters and enzyme expression **(Higashihara *et al.* 2007)**. BPF also showed other *in vitro* effects such as cytotoxicity, cellular dysfunction, DNA damage, and chromosomal aberrations **(Audebert *et al.* 2011; Cabaton *et al.* 2009; Lee *et al.* 2013; Nakagawa and Tayama 2000; Pisapia *et al.* 2012)**, and decreased adiponectin production and secretion *in vitro* **(Kidani *et al.* 2010)**. The H295R cell line is a cell of human adrenal carcinoma that has intact steroidogenic pathways and is able to secrete all steroid steroidogenesis intermediates. Due to the ability to quantify the modification in gene transcription, enzyme activity and hormone output at the same time, this cell line has been widely used as a cell model to test the chemical disruption of the steroidogenesis pathway **(Feng et al., 2016; Rotroff et al., 2013)**. The aim of our *in vitro* study was to evaluate the potential impact of BPF on mitochondrial activity, metabolic activity, membrane integrity, lysosomal function and synthesis of testosterone by H295R cells.

MATERIAL AND METHODS

Cell culture and treatment

The NCI-H295R cells were obtained from the American Type Culture Collections (ATCC CRL-2128; ATCC, Manassas, VA, USA). The cells were cultured in a Good Laboratory Practice (GLP) certified laboratory (National Institute of Chemical Safety, Budapest, Hungary; OGYI/45151-4/2012) according to

previously established and validated protocols. After the initiation of the H295R culture from the original ATCC batch, the cells were cultured throughout four passages, split and frozen down in liquid nitrogen. The cells used in the scheduled experiments were cultured for a minimum of two additional passages to achieve an optimal hormone production using new H295R batches from frozen stocks. The H295R cells were grown in 25 cm^2 plastic tissue culture flasks (TPP, Trasadingen, Switzerland) in Dulbecco's Modified Eagle's Medium/Nutrient F-12 Ham 1:1 mixture (Sigma, St. Louis, MO, USA) supplemented with 1.2 g/L $NaHCO_3$ (Molar Chemicals Halasztelek, Hungary), 12.5 mL/L of BD Nu-Serum (BD Bioscience, Bath, UK) and 5 mL/L of ITSC Premix (BD Bioscience) in a CO_2 incubator at 37°C with a 5% CO_2 atmosphere. The culture medium was changed 3 times/week, and after obtaining an acceptable cell density, it was removed from the culture flasks. The H295R cells were detached from the bottom of the culture flasks with 0.25% trypsin-EDTA for 3 min (Sigma, St. Louis, MO, USA). The cells were subsequently centrifuged (10 min., 125 x g) and re-suspended in fresh cell culture medium. The cell number was counted using a Burker chamber and adjusted to required cell concentration. The cell suspension was plated into sterile 96-well cell culture plates ($6*10^4$ cells/100 µL/well) for cytotoxicity and hormone measurements. The cells were incubated for 24 h in a CO_2 incubator at 37°C under a humidified atmosphere of 95% air and 5% CO_2. To explore the effect of bisphenols, cells were cultured for 24 h in medium containing specific concentrations of BPF (0.1, 0.5, 1, 10, 25, 50, 75, 100, 300, 500 µM) dissolved in dimethyl sulfoxide (Sigma, St. Louis, MO, USA). Control samples were samples without any treatment and negative control were samples treated with dimethyl sulfoxide (Sigma, St. Louis, MO, USA). The specific concentration range of bisphenols was selected according to the results of our pilot range-finding experiments.

Mitochondrial activity assay

In vitro mitochondrial activity of H295R cells exposed to bisphenol F, was determined using the MTT ((3-4,5-dimetyltiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, which measures the reduction of a yellow tetrazolium salt to insoluble blue formazan in mitochondria of viable cells **(Mosmann, 1983)**. H295R cells were exposed to different concentrations of BPF. After 24 h of treatment, cells were incubated with MTT tetrazolium salt (Sigma-Aldrich, St. Louis, USA) in CO_2 incubator at 37°C under a humidified atmosphere of 95% air and 5% CO_2 for 1 h. The supernatants were removed and formed formazan crystals were dissolved by adding isopropanol (p.a. CentralChem, Bratislava, Slovak Republic). Dissolved formazan was measured by an ELISA reader (Multiscan FC, ThermoFisher Scientific, Vantaa, Finland) at 570 nm against 620 nm wavelengths. Cells from three different experiments were analyzed for each treatment. All data were expressed in percentage of control, which was set to 100% **(Jambor *et al.*, 2018; Greifová *et al.*, 2020)**.

Triple assay

For cell viability, three cellular activities were monitored with three indicator dyes: metabolic activity with alamarBlue (ThermoFisher Scientific, USA), plasma membrane integrity with 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM; Sigma-Aldrich, USA), and lysosomal activity with neutral red (NR; Sigma-Aldrich, USA). **Schirmer *et al.*, (1997)** first identified the use of these 3 dyes in 96-well plates to provide an overview of the cytotoxicity/cytoprotectivity of treatments for cells. With minor modifications, this protocol was followed in this review. With this approach, three cell viability parameters are calculated on the same collection of cells without interference at the same stud&. In the first step, after 24 h of treatment, a solution of almarBlue and CFDA-AM in MEM medium (minimum critical medium eagle; Sigma-Aldrich, St. Louis, USA) was applied to cells seeded in the 96-well plate. The cells were incubated for one hour followed by measurement at individual wavelengths. The cells were then washed with PBS and neutral red dye in MEM medium was added to the cells for 1 h. After incubation cells were washed twice with PBS (phosphate- buffered saline; Sigma-Aldrich, St. Louis, USA) and exposed for another 30 minutes to the lysis buffer followed by measurement at a specific wavelength. The multiple endpoint assay is based on measurements, determined here using a Glomax Multi + Combined Spectro-Fluoro Luminometer (Promega Corp., USA) at respective excitation/emission wavelengths of 525/580-640 nm for alamarBlue, 490/510-570 nm for CFDA-AM and 525/660-720 nm for NR. Cells from three different experiments were analyzed for each treatment. All data were expressed in percentage of control, which was set to 100%.

Assessment of testosterone production

The culture medium was collected, centrifuged (300x g, 4°C) and stored at -20°C until hormone measurement. The testosterone concentration in the samples was assessed by the ELISA method (enzyme-linked immunosorbent assay) using commercial kit (Testosterone, Dialab, Neudorf, Austria) according to the instructional manual. The absorbance was measured at 450 nm by the ELISA reader (Multiscan FC, ThermoFisher Scientific, Vantaa, Finland) at 450 nm **(Greifová *et al.*, 2020)**.

Statistical analysis

Obtained data were statistically analyzed using the GraphPad Prism 8 (GraphPad Software Incorporated, San Diego, California, USA). Descriptive statistical parameters (minimum, maximum, standard error, etc.) were evaluated at first. One-way analysis of variance (ANOVA) with Dunnett's posttest was used for statistical evaluations. The level of significance was set at ***($P < 0.001$), **($P < 0.01$) and *($P < 0.05$). Each experiment was performed three times independently with cells from different passages (5-10) and expressed in percentage of the control groups, which was set to 100%. Results were presented as means (± SEM).

RESULTS AND DISCUSSION

Mitochondrial activity

We observed a slightly increased mitochondrial activity after cultivation with 0.1 µM of BPF *($P > 0.05$)* and a slightly decreased mitochondrial activity after cultivation with 0.5 µM of BPF *($P > 0.05$)*, although administration of higher concentrations (1 - 500 µM) caused significantly decreased *($P < 0.01$)* mitochondrial activity when compared to the control group (Tab. 1).

Metabolic activity

Significant increase *($P < 0.05$)* of metabolic activity was observed in experimental group treated with 0.1 µM and a slight increase *($P > 0.05$)* was observed in experimental group treated with 0.5 µM of BPF. On the contrary, doses higher than 10 µM of BPF caused inhibition of metabolic activity (Tab. 1).

Table 1 - Effects of bisphenol F on viability parameters and steroidogenesis in H295R cells

	Mitochondrial activity (%)	Metabolic activity (%)	Lysosomal activity (%)	Membrane integrity (%)	Testosterone production (%)
C	100.00 ± 0.41	100.00 ± 1.04	100.00 ± 0.73	100.00 ± 1.20	100.00 ± 1.12
NC	99.17 ± 0.32	100.10 ± 2.13	100.40 ± 0.98	100.40 ± 2.63	104.00 ± 0.36
0.1 µM	101.70 ± 0.76	111.50 ± 3.89*	104.20 ± 0.99	111.60 ± 3.48	111.80 ± 1.79***
0.5 µM	96.92 ± 0.65	101.70 ± 1.61	105.50 ± 1.51	101.80 ± 2.01	105.60 ± 0.37
1 µM	93.64 ± 0.46***	94.05 ± 2.19	105.40 ± 1.42	95.03 ± 3.15	102.80 ± 0.22
10 µM	93.64 ± 0.46***	84.33 ± 4.31**	96.86 ± 2.60	92.37 ± 3.23	105.20 ± 3.17
25 µM	94.61 ± 0.83***	83.01 ± 2.23***	92.04 ± 2.61**	84.73 ± 4.52***	100.80 ± 0.77
50 µM	94.53 ± 0.42***	77.72 ± 2.80***	92.99 ± 1.08*	84.96 ± 1.52***	87.98 ± 1.29***
75 µM	93.57 ± 0.65***	71.60 ± 3.59***	92.14 ± 1.04**	76.50 ± 2.18***	72.01 ± 0.98***
100 µM	92.38 ± 2.01***	72.80 ± 1.52***	87.49 ± 1.21***	73.52 ± 0.66***	65.25 ± 2.22***
300 µM	86.04 ± 0.44***	87.32 ± 2.67***	81.63 ± 0.34***	60.89 ± 1.11***	63.33 ± 1.20***
500 µM	73.58 ± 0.51***	43.87 ± 2.87***	74.72 ± 1.39***	56.07 ± 0.83***	56.22 ± 0.80***

Legend: C, NC – control groups. The level of significance was set at *** (*P<0.001*), ** (*P<0.01*) and * (*P<0.05*) between control and experimental groups.

Lysosomal activity

Lysosomal activity measurement in H295R cells treated with BPF for 24 h showed significant changes *($P < 0.01$)* only in experimental groups supplemented with the

higher doses of BPF (25 - 500 μM), which resulted in the decline of values (Tab. 1).

Membrane integrity

Cell membrane integrity measurement in H295R cells treated with bisphenol F for 24 h showed significant changes (*P < 0.001*) only in experimental groups treated with the higher doses of BPF (25 - 500 μM), which resulted in the decline of values. Cell samples incubated with 0.1 and 0.5 μM of BPF exhibited slightly improved (*P > 0.05*) level membrane integrity compared with the control group (Tab. 1).

Testosterone production

Assessment of the testosterone production using ELISA revealed that BPF treatment led to significant increase *(P < 0.001)* in samples cultivated with 0.1 μM. Inversely, significant decrease *(P < 0.001)* of testosterone production was detected after BPF administration at concentrations 50, 75, 100, 300, and 500 μM (Tab. 1).

Concern over the endocrine-disruptive effects of BPA has resulted in hundreds of laboratory studies. However, a proper human hazard assessment of analogues such as BPF that are believed to have a less harmful toxicity profile is lacking. Although relatively few studies have examined the hormonal actions of BPF (especially *in vivo*), the *in vitro* literature indicates that BPF has actions and potencies similar to those of BPA and supports the biological plausibility of its hormonal activity *in vivo*, which is not surprising because BPF is a structural analogue of BPA and thus mechanisms of action would be expected to be similar (**Rochester and Bolden, 2015)**. Nowadays, many studies prove the endocrine disrupting potential of BPA analogues, BPF also showed other *in vitro* effects such as cytotoxicity, cellular dysfunction, DNA damage, and chromosomal aberrations **(Audebert *et al.*, 2011, Cabaton *et al.*, 2009, Lee *et al.*, 2013, Pisapia *et al.*, 2012)**. According to our results, BPF at concentrations 25 - 500 μM significantly decreased cell viability, metabolic activity, lysosomal activity and membrane integrity. On the other hand, low concentrations (0.1 and 0.5 μM) of BPF increased all of these parameters. Several *in vitro* studies also showed cytotoxic potential of BPF, which is in agreement with our results **(Lee *et al.*, 2013, Fic *et al.*, 2013, Satoh *et al.*, 2004)**. According **to Russo *et al.* (2018)** bisphenols IC_{50} values confirming their poor acute toxicity. As compared to BPA, bisphenol F was found as the less toxic congener. Their results are partly consistent with the scale of phospholipid affinity showing that toxicity increases at increasing membrane affinity. Therefore, phospholipophilicity determination can be assumed as a useful preliminary tool to select less toxic congeners to surrogate BPA in industrial applications. According to our results, cell metabolic activity, membrane integrity and testosterone production appears to be the most sensitive parameters to the activity of bisphenol F, followed by lysosomal activity membrane integrity and the least sensitive parameter was mitochondrial activity. **Cabaton *et al.* (2009)** showed that BPF was effective on HepG2 cell DNA fragmentation at noncytotoxic concentrations. An *in vivo* study in gestating Wistar rats revealed that BPF decreased the 5α-reductase expression and dopamine (DA)−serotonin (5-HT) systems in the prefrontal cortex of juvenile female rats at postnatal day 21 **(Cabaton *et al.*, 2009; Castro *et al.*, 2015; Chen *et al.*, 2016)**. **Ullah *et al.*, (2018)** suggested that BPF had toxic effect on the testes and spermatogenesis (testosterone production was reduced), and also observed that BPF induced oxidative stress in the reproductive tissues of male rats, which is in agreement with our results because significant decrease *(P < 0.001)* of testosterone production was detected after BPF administration at concentrations 50, 75, 100, 300, and 500 μM. Decreased (*P > 0.05*) testosterone levels with 50 and 75 μM BPF in H295R cells were also observed in a study by **Feng *et al.*, (2016)**. While the mechanism of action of testesterone decrease was not clear, previous work indicated decreased StAR, CYP11A1 and HSD3B2 expression **(Peretz *et al.*, 2012; Ziv-Gal *et al.*, 2013; Feng *et al.*, 2016)**. Additional research is urgently needed to fill in knowledge gaps and deepen toxicity evaluations, given that the production and applications of bisphenol analogues are on the rise and that many of them have already been present in environmental compartments, foods, and humans.

CONCLUSION

The ban on BPA resulted in its replacement by its analogues, such as BPF. The manufacturing and application of this analogue in the future, is expected to increase. Consequently, we have investigated the potential impact of BPF on mitochondrial activity, metabolic activity, membrane integrity, lysosomal function and synthesis of testosterone by H295R cells. The results of the cytotoxicity evaluation of BPF indicated that a significant level of cytotoxicity was observed at the following tested concentration: 25, 50, 75, 100, 300, and 500 μM. However, its low concentrations led to the improvement of viability parameters (mitochondrial activity, metabolic activity, membrane integrity, and lysosomal activity), as well as testosterone production, which indicates the biphasic, hormetic response of BPF in biological systems.

Acknowledgments: The present work was developed with the support of the Res. Centre AgroBioTech built under the project Building Research Centre, AgroBioTech ITMS 26220220180 and the APVV-15-0543, APVV-19-0243, APVV-18-0312, VEGA 1/0038/19, VEGA 1/0163/18.

REFERENCES

Audebert, M., Dolo, L., Perdu, E., Cravedi, J. P. & Zalko, D. (2011). Use of the γH2AX assay for assessing the genotoxicity of bisphenol A and bisphenol F in human cell lines. *Arch Toxicol*., 85, 1463–1473. **https://doi.org/10.1007/s00204-011-0721-2**

Cabaton, N., Dumont, C., Severin, I., Perdu, E., Zalko, D., Cherkaoui-Malki, M. & Chagnon, M. CH. (2009). Genotoxic and endocrine activities of bis(hydroxyphenyl)methane (bisphenol F) and its derivatives in the HepG2 cell line. *Toxicology*, 255, 15–24. **https://doi.org/10.1016/j.tox.2008.09.024**

Cabaton, N., Chagnon, M. C., Lhuguenot, J. C., Cravedi, J. P. & Zalko, D. (2006). Disposition and metabolic profiling of bisphenol F in pregnant and nonpregnant rats. *J Agric Food Chem.*, 54, 10307–10314. **https://doi.org/10.1021/jf062250q**

Castro, B., Sanchez, P., Torres, J. M. & Ortega, E. (2015). Bisphenol A, bisphenol F and bisphenol S affect differently 5α-reductase expression and dopamine-serotonin systems in the prefrontal cortex of juvenile female rats. *Environ. Res.*, 142, 281−287. **https://doi.org/10.1016/j.envres.2015.07.001**

Feng, Y., Jiao, Z., Shi, J., Li, M., Guo, Q. & Shao, B. (2016). Effects of bisphenol analogues on steroidogenic gene expression and hormone synthesis in H295R cells. *Chemosphere*, 147, 9–19. **https://doi.org/10.1016/j.chemosphere.2015.12.081**

Fic, A., Žegura, B., Sollner Dolenc, M., Filipic, M. & Peterlin Masic, L. (2013). Mutagenicity and DNA damage of bisphenol A and its structural analogues in HepG2 cells. *Arh Hig Rada Toksikol.*, 64, 3–14. **https://doi.org/10.2478/10004-1254-64-2013-2319**

Fiege, H., Voges, H. W., Hamamoto, T., Umemura, S., Iwata, T., Miki, H., Yasuhiro, F. Dorothea, H. B. & Paulus, G. W. (2000). Phenol derivatives. *Ullmann's Encyclopedia of Industrial Chemistry*. Winheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 643–647. **https://doi.org/10.1002/14356007.a19_313**

Greifová, H., Jambor, T., Tokárová, K., Speváková, I., Knížatová, N., & Lukáč, N. (2020). Resveratrol attenuates hydrogen peroxide-induced oxidative stress in TM3 Leydig cells in vitro. *Journal of Environmental Science and Health, Part A*, 1–11. **https://doi.org/10.1080/10934529.2020.1717899**

Higashihara, N., Shiraishi, K., Miyata, K., Oshima, Y., Minobe, Y. & Yamasaki, K. (2007). Subacute oral toxicity study of bisphenol F based on the draft protocol for the "Enhanced OECD Test Guideline no. 407.". *Arch Toxicol*., 81, 825–832. **https://doi.org/10.1007/s00204-007-0223-4**

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.-L., Wu, Y. & Widelka, M. (2016). Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity—A Review. *Environmental Science & Technology*, 50(11), 5438–5453. **https://doi.org/10.1021/acs.est.5b05387**

Jambor, T., Greifova, H., Kovacik, A., Kovacikova, E., Tvrda, E., Forgacs, Z., Massanyi, P. & Lukac, N. (2018). Parallel effect of 4-octylphenol and cyclic adenosine monophosphate (cAMP) alters steroidogenesis, cell viability and ROS production in mice Leydig cells. *Chemosphere*, 199, 747-754. **https://doi.org/10.1016/j.chemosphere.2018.02.013**

Kidani, T., Kamei, S., Miyawaki, J., Aizawa, J., Sakayama, K. & Masuno H. (2010). Bisphenol A downregulates Akt signaling and inhibits adiponectin production and secretion in 3T3-L1 adipocytes. *J Atheroscler Thromb.*, 17, 834–843. **https://doi.org/10.5551/jat.4051**

Lee, S., Liu, X., Takeda, S. & Choi, K. (2013). Genotoxic potentials and related mechanisms of bisphenol A and other bisphenol compounds: a comparison study employing chicken DT40 cells. *Chemosphere*, 93, 434–440. **https://doi.org/10.1016/j.chemosphere.2013.05.029**

Liao, C., Liu, F., Alomirah, H., Loi, V. D., Mohd, M. A., Moon H. B., Nakata, H. & Kannan, K. (2012). Bisphenol S in urine from the United States and seven Asian countries: Occurrence and human exposures. *Environ. Sci. Technol.*, 46, 6860–6866. **https://doi.org/10.1021/ es301334j**

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.*, *65*(1-2), 55-63. **https://doi.org/10.1016/0022-1759(83)90303-4**

Nakagawa, Y. & Tayama, S. (2000). Metabolism and cytotoxicity of bisphenol A and other bisphenols in isolated rat hepatocytes. *Arch Toxicol.*, 74, 99–105. **https://doi.org/10.1007/s002040050659**

Office of Environmental Health Hazard Assessment. Biomonitoring California: p,p'-Bisphenols and Diglycidyl Ethers of p,p'-Bisphenols. (2012). Available: **http://www.oehha.ca.gov/multimedia/biomon/pdf/041113Bisphenols_priority.pdf**

Peretz, J., Craig, Z.R. & Flaws, J.A. (2012). Bisphenol A inhibits follicle growth and induces atresia in cultured mouse antral follicles independently of the genomic estrogenic pathway. *Biol. Reprod.*, 87, 63e63. **https://doi.org/10.1095/biolreprod.112.101899**

Pisapia, L., Del Pozzo, G., Barba, P., Caputo, L., Mita, L., Viggiano, E., Russo, G. L., Nicolucci, C., Rossi, S., Bencivenga, U., Mita, D. G. & Diano N. (2012). Effects of some endocrine disruptors on cell cycle progression and murine dendritic cell differentiation. *Gen Comp Endocrinol*, 178, 54–63. **https://doi.org/10.1016/j.ygcen.2012.04.005**

Richter, C. A., Birnbaum, L. S., Farabollini, F., Newbold, R. R., Rubin, B. S. & Talsness, C. E. (2007). In vivo effects of bisphenol A in laboratory rodent studies. *Reprod Toxicol*., 24, 199–224. **https://doi.org/10.1016/j.reprotox.2007.06.004**

Rochester J. R. & Bolden A. L. (2015). Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes. *Environ Health Perspect*, 123, 643–650. **https://doi.org/10.1289/ehp.1408989**

Rochester J. R. (2013). Bisphenol A and human health: a review of the literature. *Reprod Toxicol*., 42, 132–155. **https://doi.org/10.1016/j.reprotox.2013.08.008**

Rotroff, M. D., Dix, J. D., Houck, A. K., Knudsen, B. T., Martin, T. M., McLaurin, W. K., Reif, M. D., Crofton, M. K., Singh, V. A., Xia, M., Huanf, R. & Judson S. R. (2013). Using in Vitro High Throughput Screening Assays to Identify Potential Endocrine-Disrupting Chemicals. *Environmental Health Perspectives*, 121(1), 7–14. **https://doi.org/10.1289/ehp.1205065**

Russo, G., Capuozzo, A., Barbato, F., Irace, C., Santamaria, R. & Grumetto, L. (2018). Cytotoxicity of seven bisphenol analogues compared to bisphenol A and relationships with membrane affinity data. *Chemosphere*, 201, 432-440. **https://doi.org/10.1016/j. chemosphere.2018.03.014**

Satoh, K., Ohyama, K., Aoki, N., Iida, M. & Nagai, F. (2004). Study on anti-androgenic effects of bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and their derivatives using cells stably transfected with human androgen receptor, AR-EcoScreen. *Food Chem Toxicol.*, 42, 983–993. **https://doi.org/10.1016/j.fct.2004.02.011**

Schirmer, K.; Chan, A. G. J.; Greenberg, B. M.; Dixon, D. G. & Bols, N. C. (1997). Methodology for demonstrating and measuring the phototcytotoxicity of floranthene to fish cells in culture. *Toxicol. In. Vitro*, 11(1-2), 107-119. **https://doi.org/10.1016/s0887-2333(97)00002-7**

Thoene, M., Dyika, E., Gonkowski, S. & Wojtkiewicz, J. (2020). Bisphenol S in Food Causes Hormonal and Obesogenic Effects Comparable to or Worse than Bisphenol A: A Literature Review. *Nutrients*, 12(2), 532. **https://doi.org/10.3390/nu12020532**

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Shaheen, G., Rehman, H. & Butt, M. A. (2018). Bisphenol A and its analogs bisphenol B, bisphenol F, and bisphenol S: Comparative in vitro and in vivo studies on the sperms and testicular tissues of rats. *Chemosphere*, 209, 508–516. **https://doi.org/10.1016/j.chemosphere.2018.06.089**

Vandenberg L. N. (2014). Non-monotonic dose responses in studies of endocrine disrupting chemicals: bisphenol A as a case study. *Dose Response*, 12, 259–276. **https://doi.org/10.2203/dose-response.13-020.Vandenberg**

Vandenberg, L. N., Chahoud, I., Heindel, J. J., Padmanabhan, V., Paumgartten, F. J. & Schoenfelder, G. (2010). Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environ Health Perspect*, 118, 1055–1070. **https://doi.org/10.1289/ehp.0901716**

Wetherill, Y. B., Akingbemi, B. T., Kanno, J., McLachlan, J. A., Nadal, A. & Sonnenschein, C. (2007). In vitro molecular mechanisms of bisphenol A action. *Reprod Toxicol*., 24, 178–198. **https://doi.org/10.1016/j.reprotox.2007.05.010**

Wu L. H., Zhang X. M., Wang F., Gao C. J., Chen D., Palumbo J. R., Guo Y. & Zeng E. Y. (2017). Occurrence of bisphenol S in the environment and implications for human exposure: A short review. *Sci. Total Environ*., 615, 87–98. **https://doi.org/10.1016/j.scitotenv.2017. 09.194**

Yamasaki, K., Takeyoshi, M., Sawaki, M., Imatanaka, N., Shinoda, K. & Takatsuki, M. (2003). Immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals. *Toxicology*, 183, 93–115. **https://doi.org/10.1016/s0300-483x(02)00445-6**

Ziv-Gal, A., Craig, Z. R., Wang, W., & Flaws, J. A. (2013). Bisphenol A inhibits cultured mouse ovarian follicle growth partially via the aryl hydrocarbon receptor signaling pathway. *Reproductive Toxicology*, 42, 58–67. **https://doi.org/10.1016/j.reprotox.2013.07.022**