Eurasian Journal of Veterinary Sciences
https://eurasianjvetsci.org
pISSN:1309-6958/**eISSN:**2146-1953
Eurasian J Vet Sci 2026;42:e0477
DOI:10.63673/EurasianJVetSci.477

RESEARCH ARTICLE

The Effect of Bisphenol A on the Proportions of Alpha Naphthyl Acetate Esterase Positive Peripheral Blood Lymphocytes in Rats

Tugba Ozaydın[1,(*)], Emrah Sur[1], Mehmet Sait Atar[2], Ecem Arslan[3], Ali Caglayan Keles[3], Ilhami Celik[1]
[1]Selcuk University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Konya Türkiye
[2]Kastamonu University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Kastamonu, Türkiye
[3]Insitute of Health Science, Department of Histology and Embryology, Konya, Türkiye

Abstract

Bisphenol A (BPA) is one of the most important endocrine disrupting chemicals. Epoxy resins and polycarbonates are frequently manufactured using this compound. Enzyme histochemical techniques not only can be used to evaluate the general status of the immune system but also are used to determine the functional situation of the lymphocytes. Immunocompetent T-lymphocytes in certain animal species are characterized by the presence of ANAE (Alpha Naphthyl Acetate Esterase). The purpose of this study is to determine the effects of BPA on the proportions of ANAE positive peripheral blood lymphocytes (PBL) in rats. A total of 40 rats were used. Forty animals were randomly assigned to five experimental groups (n = 8 per group): control, vehicle-control, BPA-5, BPA-50, and BPA-500. The BPA-5, BPA-50 and BPA-500 groups were given 5, 50, and 500 µg/kg body weights/day, respectively. At the end of the 8 weeks, peripheral blood samples were obtained from the rats and blood smears were prepared for ANAE demonstration. On each smear, two-hundred lymphocytes were counted for the determining of the percentages of ANAE-positive lymphocyte percentages. The proportions of ANAE positivity were found as 43.37%, 36.50%, 32.62%, 31.37% and 30.75% respectively. It was determined that the differences of the proportions of ANAE positive PBL between the groups were statistically important ($p<0.01$). It was concluded that BPA had immunotoxic and/or immunosuppressive effect of PBL, and it might cause functional failure of lymphocytes affecting their enzymatic activity.

Keywords: ANAE, BPA, lymphocytes, rat.

(*) **Corresponding author:**
Tugba Ozaydın
ttelatar@selcuk.edu.tr

Received: 12.02.2025
Accepted: 07.05.2026
Published: 13.05.2026

How to cite this article?
Ozaydın T, Sur E, Atar MS, Arslan E et al 2026. The Effect of Bisphenol A on the Proportions of Alpha Naphthyl Acetate Esterase Positive Peripheral Blood Lymphocytes in Rats. Eurasian J Vet Sci, 42, e0477

INTRODUCTION

Bisphenol A (BPA) is a monomeric organic compound. It is produced in large amounts for use to produce epoxy resins and polycarbonate plastics all over the world. Epoxy resins and polycarbonate plastics are commonly used as protective coatings for metal products like beverage cans. In addition to food storage containers, infant bottles, electronic devices, thermal paper and medical devices are potential materials having risk about BPA. Besides, also some dental sealants or composites made of polymers could contain BPA (Maamar et al 2015, Kanam et al 2016). BPA can leach from mentioned materials under certain circumstances, especially pH and temperature and contaminate food, soil and air. Besides, it is claimed that the perinatal BPA exposure might cause development of metabolic and allergic disorders in adulthood (Nakajima et al 2012, Malaisé et al 2020). BPA exposure can occur via various pathways during every life stage, including in utero development. Consequently, considering environmental effects, BPA is of great concern for public health. BPA was identified as very high concern substance by European Chemical Agency (ECHA) in 2017 (Hormann et al 2014, Acconcia et al 2015, Kanam et al 2016, Abdelrazek et al 2019).

BPA is classified in endocrine disrupting chemicals (EDCs). Exposure of EDCs may develop hazardous problems threatening to life including formation of several hormone dependent neoplasms, especially in reproductive system organs. BPA has strong affinity to estrogen receptors and serves as a xenoestrogen (Lee et al 2013, Ahmed et al 2015). A reciprocal relationship exists between the immune and endocrine systems (Chryssikopoulos 1997). Estrogen has an important effect on immune cells. Karpuzoglu-Şahin et al (2001) demonstrated that the estrogen plays a role in modulating the immune system through the regulation of cytokines. Some studies have shown that estrogen enhances humoral immunity while suppressing cellular responses (Paavonén et al 1981, Yoshino et al 2004). As BPA specifically binds to the estrogen receptors, it mimics naturally hormones and the effects of BPA are similar to effects of the estrogens, so it alters the hormonal functions. Cytokines released by immune system cells interact hormones and this interaction plays a crucial for immune system functions. So, endocrine disrupting chemicals like BPA exposure may cause to detrimental effects on immune system cells because of its estrogenic effects (Chryssikopoulos 1997, Koike et al 2018). In addition to role of lactation promoting, prolactin, a peptide hormone, also plays a crucial role in modulating immune and inflammatory responses via different immune signaling pathways, especially react to self-antigen in autoimmunity. BPA has also strong effects on the prolactin secreting cells in pituitary gland. Therefore, BPA has also potential effects on emerging autoimmune diseases. Extensive studies have demonstrated that BPA exposure may act as a contributing factor to various immune system dysfunctions (Bauer et al 2012, Kharrazian 2014, Yılmaz and Öznurlu 2019, Çetin and Özaydın 2021).

The immunomodulatory effects and underlying mechanisms of BPA exhibit significant variations. These effects are modulated by a variety of factors, ranging from the experimental model and animal species to the dosage, timing, and route of BPA administration (Yılmaz and Öznurlu 2019). While Yoshino et al (2004) observed that prenatal BPA exposure significantly elevates immune responses—particularly Th1-mediated pathways—in adult mice, Palacios-Arreola et al (2017) found that exposure in early neonatal life (three-day-old females) predisposes them to breast cancer by impairing anti-tumoral immunity. Furthermore, evidence indicates that perinatal BPA exposure modulates the cellular immune response to food-derived antigens, thereby increasing the vulnerability of juveniles to intestinal parasitic infections (Ménard et al 2014).

To evaluate the functional ontogeny of the immune system, enzyme histochemical techniques have served as reliable tools. Alpha-naphthyl acetate esterase (ANAE) is widely utilized to identify for T lymphocytes, in various species including humans, mice, cattle, chickens and dogs (Mueller et al 1975, Knowles et al 1978, Sur and Çelik 2005).

The purpose of the present study is to evaluate the possible effects of BPA on the proportions of ANAE positive peripheral blood lymphocytes (PBL) in rats.

Material and Methods

Ethical approval

This study was performed after approval by the Ethical Committee of Selcuk University Chair of Experimental Medicine Research and Application Center (2015/51).

Animals and experimental design

In this study, forty male healthy rats were used. The groups were designed as control (untreated), vehicle-control (only given a mixture of ethanol-corn oil), BPA-5, BPA-50 and BPA-500 groups. Firstly, BPA was dissolved in ethanol, then corn oil added on this mixture. The BPA treatment groups received doses of 5, 50, and 500 µg/kg/d for eight weeks.

ANAE histochemistry

At the end of the study, peripheral blood samples were collected from the rats and blood smears were prepared. ANAE staining was carried on according to the method of İzgi et al (2023). For the preparing incubation solution, hexazotized pararosaniline and 20 mg of alpha naphthyl acetate (solved in acetone) were added on 80 ml of phosphate buffer (0.067 M, pH 5.0). Incubation solution pH was adjusted to 5.8. The slides were incubated in incubation solution at 37°C. At the end of the incubation, the smears were counter-stained with 1% methyl green.

Evaluation of histochemical staining

The blood smears were examined under a light microscope (Leica DM2500) and then were photographed by a digital camera (Leica DFC 320). Lymphocytes exhibiting one to three distinct red-brown granules were identified as ANAE-positive lymphocytes (Figure 1). ANAE positive peripheral blood proportions were determined by counting two-hundred lymphocytes on each stained smear.

Statistical analysis

The percentage of positive lymphocytes were subjected to the Angle (Arc Sinus) transformation method. Differences between groups were evaluated using one-way ANOVA with the Tukey's post-hoc test (SPSS Inc.; Chicago, IL, USA). $p<0.05$ was considered statistically significant.

Results

The ANAE-positive peripheral blood lymphocyte percentages of groups were given in Table 1. The dose-dependent decrease was observed in BPA-groups. The proportions of ANAE positivity were 43.37%, 36.50%, 32.62%, 31.37% and 30.75% respectively in groups control, vehicle-control, BPA-5, BPA-50 and BPA-500 groups. The differences of ANAE positive PBL between the groups were statistically important ($p<0.05$). The differences of ANAE positive PBL proportions among the groups were statistically important ($p<0.05$).

Table 1. ANAE-positive peripheral blood lymphocyte percentages of control and experimental groups.	
Groups	ANAE-positive peripheral blood lymphocyte percentages (%)
Control	43.37±6.04[a]
Vehicle-control	36.50±4.92[b]
BPA 5	32.62±2.97[bc]
BPA 50	31.37±4.13[c]
BPA 500	30.75±4.36[c]

[a-c:] Values within a column with no common superscripts are significantly different ($p<0.05$).

Discussion

BPA, one of the most hazardous endocrine disrupter compounds (EDCs), may disrupt not only the reproductive and endocrine system but also respiratory, circulatory, musculoskeletal and central nervous system by increasing oxidative stress (Nakamura et al 2006, Kandil and Sur 2018, Özaydın et al 2018b, Öznurlu et al 2021, Öznurlu et al 2022). BPA, used to produce food storage containers and water bottles, can be leached in very small amounts and contaminate the food and drinks they contain. Therefore, European Food Safety Authority (EFSA) regularly evaluates the food safety, and scientific advice to the risk managers, considering the results obtained from the latest studies for many years. Recently, EFSA re-assessed the public health risks associated with dietary exposure to BPA and, in April 2023, a report including scientific opinion regarding this topic was published. According to this report, the tolerable daily intake (TDI) for BPA lowered from 4 µg/kg bw per day to 0.2 ng/kg bw per day (EFSA CEP Panel 2023). However, studies performed in recent years have shown that long-term exposure to even low doses of BPA may have deleterious effects on various system (Nakamura et al 2006, Kandil and Sur 2018, Özaydın et al 2018b, Öznurlu et al 2021, Öznurlu et al 2022).

Immune system organs like thymus which produced immunocompetent T-lymphocytes are also close relationship to endocrine system and controlled by hormones (Goldman et al 2000, Çetin and Özaydın 2021). As T- and B-lymphocytes express estrogen receptors, the lymphocytes functions directly affected by estrogen (Nalbandian and Kovats 2005). So, having estrogen-like effects, due to binding to estrogen receptors, BPA may exert significant modulatory effects on the receptors of various immune system cells (Yoshino et al 2003).

The effects of BPA on immune system may be various such as proliferative, degenerative, necrotic and neoplastic responses, and it was reported that these effects might depend on many factors such as animal species, exposure route, dose of BPA, duration of BPA exposure and developmental stage of organs those of investigated (Ward et al 2006, Ménard et al 2014, Karnam et al 2016, Palacios-Arreola et al 2017). Drozdz et al (2011) demonstrated that BPA-contained dental composites caused decreasing the lymphocyte viability via directly interact with DNA

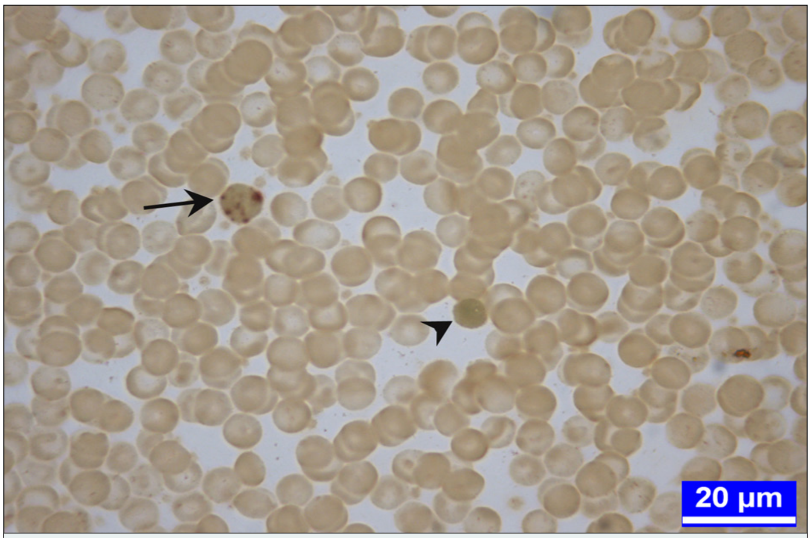

Figure 1. ANAE-positive (arrow) and negative (arrow head) peripheral blood lymphocytes are observed in blood smear prepared from a control animal. ANAE demonstration.

at the high concentrations, whereas Tiwari and Vanage (2017) reported that BPA trigger the oxidative stress in rat peripheral blood lymphocytes. Santovito et al (2018) observed increasing micronuclei frequency indicating that toxic effects of BPA in human cultured lymphocytes. Yin et al (2006) claimed that low dose of BPA induced macrophage proliferation while high doses inhibited this proliferation in Goldfish. Sugita-Konishi et al (2003) found that BPA caused a decline numbers of macrophage and T cells in mice spleen. In a study on rats performed by Özaydın et al (2018a) demonstrated that BPA causes the disturbing on immune system cytokines profiles, and distribution of CD4+ and CD8+ T lymphocytes in spleen and Peyer's patches of ileum. Similarly, Abdelrazek et al (2019) found that the spleen and thymus showed distinct lymphocytic depletion in BPA-administered rats.

Several studies investigating the embryotoxic effects of BPA on the immune system were performed. BPA exposure of pregnant animals may cause undesired changes in the number and function of cellular and humoral immune system cells in the offspring, including a decrease in T and B cells, and anti-inflammatory cytokines. This situation may lead to autoimmune and allergic disorders and immune deficiencies (Yoshino et al 2004, Palacios-Arreola et al 2017, Aydemir et al 2018, Koike et al 2018, Aydemir et al 2020). Ménard et al (2014) found that perinatal exposure to BPA at dose of 5 µg/kg of body weight/d significantly declined the proportions of Treg and T helper cells in the spleen and mesenteric lymph nodes of rats comparing to controls. Aydemir et al (2018) administered orally with BPA to adult pregnant female rats during pregnancy and they evaluated CD3, CD4, and CD8 thymocytes in thymus collected on day 21, 45 and 90 of offsprings. Investigators (Aydemir et al 2018) found that dramatically declined the mentioned cells on day 21 but not in adult animals. Same researchers also observed similar results in another study carried out lymph nodes and spleen. They determined a significant decrease in the immunopositive CD3 and CD20 in rats were born from BPA administered pregnant female rats and they concluded that BPA may affect organ function at cellular and molecular levels without it does not cause histologically distinct tissue damage (Aydemir et al 2020). Yılmaz and Öznurlu (2019) found that in ovo administered BPA impedes embryonic development of thymus. Çetin and Özaydın (2021) also observed that retarded development of bursa of Fabricius in in ovo BPA administered groups. Similarly, Yiğit et al (2013) found that the embryonic exposure to high dose BPA caused the distinct retardation of the bursa of Fabricius by decreasing the bursal index, the number and the diameter of the follicles in hens. All these results are important that BPA maternally transferred either placental or from eggs might be suppressed to humoral and cellular immune system in animals.

Enzyme histochemical techniques have important value not only to assess the development of immune system cells, especially lymphocytes, but also to differentiate them from each other. In peripheric blood smears, ANAE histochemistry is used to distinguish between T-lymphocytes and monocytes, dot and diffuse positivity, respectively, because of different reaction patterns. ANAE demonstration has widely been used and is accepted as a T-lymphocytes marker in certain mammals such as cattle (Kajikawa et al 1983), dog (Wulff et al 1981), mouse (Mueller et al 1975) and human (Basso et al 1980). This enzyme is not specific for some other species including rat T-lymphocytes. However, because the rates of enzyme positive lymphocytes have been considered to be a useful tool to obtain critical knowledge of health status of immune system, peripheral blood lymphocyte (PBL) counts and their enzymatic profiles were frequently evaluated in many experimental studies which determining the possible adverse or positive effects of some certain chemicals on immune system (İzci et al 2002, Sur and Çelik 2005, Çiftçi et al 2011, Sur et al 2011, Sur et al 2012, Yener et al 2013, Yener et al 2019). Çetin and Özaydın (2021) investigated the effects of BPA on embryonic development of bursa of Fabricius and acid phosphatase (ACP-ase) positive lymphocytes in peripheral blood, those of which accepted as B-lymphocytes, found that the decreasing ACP-ase positive lymphocytes percentage, by using enzyme histochemistry techniques. Similarly, Yılmaz and Öznurlu (2019) showed that BPA cause decreasing the ANAE positive peripheral blood lymphocyte rate in dose-depending manner when in ovo administered.

The data obtained from the present study showed lower ANAE-positive lymphocyte percentages in all BPA-groups in dose-dependent manner. A decrease in the proportion of the immune system cells may cause deleterious results, such as immunodeficiency. Consequently, these findings suggested that BPA may disrupt on immune system cells even at very low doses.

Conclusion

Developmental, metabolic, and reproductive processes are primarily orchestrated by the endocrine system through hormonal regulation. Endocrine disruptors modulate physiological processes through competitive binding or blockade of hormone receptors, which subsequently alters the intended hormonal signaling pathways. Many research highlights the bi-directional interactions between the endocrine and immune system. Cytokines and hormones serve as the fundamental signaling molecules facilitating this interaction. Therefore, EDCs—specifically BPA—may significantly alter immune homeostasis.

Declarations

Competing Interests

Authors declere that there are no conflicts of interest related to publication of this article.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement

Selcuk University Chair of Experimental Medicine Research and Application Center, Animal Experiments Ethics Committee. 17.03.2015, 2015/51 Number Ethics Committee Desicion.

Funding

This study was supported by the Scientific Research Projects Coordinatorship Unit, Selçuk University, (Project No: 15401073).

Acknowledgements

This study was presented as a poster presentation at the "7. International Molecular Biology and Biotechnology Congress and was pubished as a summary text in the congress book, 25-27 April 2018, Konya, TÜRKİYE".

Author Contributions

Motivation / Concept: TTO, ES, IC; Design: TTO, ES; Control/ Supervision: TTO, ES, MSA, EA, ACK; Data Collection and Processing: TTO, ES; Analysis and Interpretation: TTO, ES, MSA, EA, ACK, İC; Literature Review: TTO, ES, MSA, EA, ACK; Writing the Article: TTO, ES, MSA, EA, ACK; Critical Review: TTO, ES, MSA, EA, ACK

ORCID

TO: https://orcid.org/0000-0002-4552-5658
ES: https://orcid.org/0000-0002-3513-7424
MSA: https://orcid.org/0009-0007-2021-6289
EA: https://orcid.org/0009-0006-9238-564X
ACK: https://orcid.org/0000-0001-6587-7565
IC: https://orcid.org/0000-0002-3587-7609

References

Abdelrazek HMA, Eltamany DA, Soliman MTA, Greish SM, et al., 2019. Effect of lycopene on bisphenol A-induced immunological disturbances in albino male rats. Suez Canal Univ Med J, 22(1), 9-17. https://doi:10.21608/scumj.2019.44180

Acconcia F, Pallottini V, Marino M, 2015. Molecular mechanisms of action of BPA. Dose- Response, 13, 1-9. https://doi:10.1177/1559325815610582.

Ahmed WMS, Moselhy WA, Nabil TM, 2015. Bisphenol A toxicity in adult male rats: Hematological, biochemical and histopathological approach. Glob Vet, 14, 228-238. https://doi:10.5829/idosi.gv.2015.14.02.9332.

Aydemir I, Kum Ş, Tuğlu Mİ, 2018. Histological investigations on thymus of male rats prenatally exposed to bisphenol A. Chemosphere, 206, 1-8. https://doi: 10.1016/j.chemosphere.2018.04.145.

Aydemir I, Özbey C, Özkan O, Kum Ş, et al., 2020. Investigation of the effects of bisphenol-A exposure on lymphoid system in prenatal stage. Toxicol Ind Health, 36(7), 502-513. https://doi: 10.1177/0748233720941759.

Basso G, Cocito MG, Semenzato G, Pezzutto A, et al., 1980. Cytochemical study of thymocytes and T lymphocytes. Br J Haematol, 44, 577-582. https://doi: 10.1111/j.1365-2141. 1980. tb08712. x.

Bauer SM, Roy A, Emo J, Chapman TJ, et al., 2012. The effects of maternal exposure to bisphenol A on allergic lung inflammation into adulthood. Toxicol Sci, 130, 82-93. https://doi: 10.1093/toxsci/kfs227.

Çetin S, Özaydın T, 2021. The effects of bisphenol A given in ovo on bursa of Fabricius development and percentage of acid phosphatase positive lymphocyte in chicken. Environ Sci Pollut Res, 28, 41688-41697. https://doi: 10.1007/s11356-021-13640-z.

Chryssikopoulos A, 1997. The relationship between the immune and endocrine systems. Ann NY Acad Sci, 17, 83-93. https://doi: 10.1111/j.1749-6632. 1997.tb52132. x.

Çiftçi MK, Çelik İ, Tuzcu M, Sur E, et al., 2011. The evaluation of enzyme histochemical and histopathological findings in the diagnosis of Marek's Disease. Dicle Üniv Vet Fak Derg, 2(3), 50-57.

Drozdz K, Wysokinski D, Krupa R, Wozniak K, 2011. Bisphenol A-glycidyl methacrylate induces a broad spectrum of DNA damage in human lymphocytes. Arch Toxicol, 85, 1453–1461. https://doi: 10.1007/s00204-010-0593-x.

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2023. Scientific opinion on the re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal, 21(4), 6857. https://doi: 10.2903/j.efsa.2023.6857.

Goldman JM, Laws SC, Balchak SK, Cooper RL, et al., 2000. Endocrine-disrupting chemicals: prepubertal exposures and effects on sexual maturation and thyroid activity in the female rat. A focus on the EDSTAC recommendations. Crit Rev Toxicol, 30, 135-196. https://doi:10.1080/10408440091159185.

Hormann AM, vom Saal FS, Nagel SC, Stahlhut RW, et al., 2014. Holding thermal receipt paper and eating food after using hand sanitizer results in high serum bioactive and urine total levels of bisphenol A (BPA). PloS One, 9, e110509. https://doi.org/10.1371/journal.pone.0110509.

İzci C, Celik I, Alkan F, Oğurtan Z, et al., 2002. Topical administration of 2 % cyclosporine for treatment of keratoconjunctivitis sicca in dogs: Evaluation of clinical results, histopathology and local cellular immunity of the third eyelid gland. Am J Vet Res, 63, 688-694. https://doi: 10.2460/ajvr.2002.63.688.

İzgi M, Sur E, Öznurlu Y, Özaydın T, et al., 2023. Determination of embryotoxic effects of in ovo administered propofol on peripheral blood alpha naphthyl acetate esterase and acid phosphatase-positive lymphocytes. Turk J Anaesthesiol Reanim, 51(1):37-42. https://doi: 10.5152/TJAR.2023.22726.

Kajikawa O, Koyama H, Yashikawa T, Tsubaki S, et al., 1983. Use of alpha-naphthyl acetate esterase staining to identify T lymphocytes in cattle. Am J Vet Res, 44(8), 1549-1552.

Kandil B, Sur E, 2018. The light microscopic investigation of the effects of in-ovo administered bisphenol A (BPA) on the development of testes. Ankara Üniv Vet Fak Derg, 65, 273-281. https://doi.org/10.1501/Vetfak_0000002823.

Karnam SS, Ghosh RC, Mondal S, 2016. Effect of bisphenol - A on immune system of Wistar rats. Explor Anim Med Res, 6(1), 47-52.

Karpuzoglu-Şahin E, Hissong BD, Ansar AS, 2001. Interferon-γ levels are upregulated by 17-β-estradiol and diethylstilbestrol. J Reprod Immunol, 52, 113-27. https://doi: 10.1016/s0165-0378(01)00117-6.

Kharrazian D, 2014. The potential roles of bisphenol A (BPA) pathogenesis in autoimmunity. Autoimmune Dis, Article ID 743616. https://doi: 10.1155/2014/743616.

Knowles DM, Hoffman HT, Ferrarini M, Kunkel HG, 1978. The demonstration of acid α-naphthyl acetate esterase activity in human lymphocytes: Usefulness as a T-cell marker. Cell Immunol, 35, 112-123. https://doi: 10.1016/0008-8749(78)90131-4.

Koike E, Yanagisawa R, Win-Shwe TT, Takano H, 2018. Exposure to low-dose bisphenol A during the juvenile period of development disrupts the immune system and aggravates allergic airway inflammation in mice. Int J Immunopathol and Pharmacol, 32, 1–14. https://doi: 10.1177/2058738418774897.

Lee HR, Jeung EB, Cho MH, Kim TH, et al., 2013. Molecular mechanism(s) of endocrine-disrupting chemicals and their potent oestrogenicity in diverse cells and tissues that express oestrogen receptors. J Cell Mol Med, 17(1), 1-11. https://doi: 10.1111/j.1582-4934.2012.01649.x.

Maamar MB, Lesné L, Desdoits-Lethimonier C, Coiffec I, et al., 2015. An investigation of the endocrine-disruptive effects of bisphenol A in human and rat fetal testes. PloS ONE, 10, e0117226. https://doi.org/10.1371/journal.pone.0117226.

Malaisé Y, Lencina C, Cartier C, Olier M, et al., 2020. Perinatal oral exposure to low doses of bisphenol A, S or F impairs immune functions at intestinal and systemic levels in female offspring mice. Environ Health, 19, 93, https://doi.org/10.1186/s12940-020-00614-w.

Ménard S, Guzylack-Piriou L, Lencina C, Leveque M, et al., 2014. Perinatal exposure to a low dose of bisphenol A impaired systemic cellular immune response and predisposes young rats to intestinal parasitic infection. PloS ONE, 9, e112752. https://doi: 10.1371/journal.pone.0112752.

Mueller J, Brundel RG, Buerki H, Keller HU, et al., 1975. Nonspesific acid esterase activity: a criterion for differentiation of T and B lymphocytes in mouse lymph nodes, Eur J Immunol, 5, 270-274. https://doi: 10.1002/eji.1830050411.

Nakajima Y, Goldblum RM, Midoro-Horiuti T, 2012. Fetal exposure to bisphenol A as a risk factor for the development of childhood asthma: an animal model study. Environ Health, 11, 8. https://doi: 10.1186/1476-069X-11-8.

Nakamura K, Itoh K, Yaoi T, Fujiwara Y, et al., 2006. Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of Bisphenol A. J Neurosci Res, 84(6), 1197-1205. https://doi: 10.1002/jnr.21020.

Nalbandian G, Kovats S, 2005. Understanding sex biases in immunity. Immunol Res, 31, 91–106. https://doi.org/10.1385/IR:31:2:091.

Özaydın T, Öznurlu Y, Sur E, Çelik İ, et al., 2018a. The effects of bisphenol A on some plasma cytokine levels and distribution of CD8+ and CD4+ T lymphocytes in spleen, ileal Peyer's patch and bronchus associated lymphoid tissue in rats. Acta Histochem, 120 (8), 728–733. https://doi: 10.1016/j.acthis.2018.08.002.

Özaydın T, Öznurlu Y, Sur E, Celik İ, et al., 2018b. Effects of bisphenol A on antioxidant system and lipid profile in rats. Biotech Histochem, 93(4), 231-238. https://doi: 10.1080/10520295.2017.1420821.

Öznurlu Y, Özaydın T, Sur E, Özparlak H, 2021. The effects of in ovo administered bisphenol A on tibial growth plate histology in chicken. Birth Defects Res, 113, 1130–1139. https://doi: 10.1002/bdr2.1925.

Öznurlu Y, Özaydın T, Sur E, Kuşat T, 2022. The effects of bisphenol A injected into egg yolk on skeletal muscle development in chicken. Eurasian J Vet Sci, 38 (2), 90-100. https://doi: 10.15312/EurasianJVetSci.2022.369.

Paavonén T, Andersson LC, Adlercreutz H, 1981. Sex hormone regulation of in vitro immune response: Estradiol enhances human B cell maturation via inhibition of suppressor T cell in pokeweed mitogen-stimulated cultures. J Exp Med, 154, 1935-1945. https://doi: 10.1084/jem.154.6.1935.

Palacios-Arreola MI, Nava-Castro KE, Del Río-Araiza VH, Pérez-Sánchez NY, et al., 2017. A single neonatal administration of Bisphenol A induces higher tumour weight associated to changes in tumour microenvironment in the adulthood. Sci Rep, 7, 10573. https://doi: 10.1038/s41598-017-10135-1.

Santovito A, Cannarsa E, Schleicherova D, Cervella P, 2018. Clastogenic effects of bisphenol A on human cultured lymphocytes. Human Exp Toxicol, 37(1), 69–77. https://doi: 10.1177/0960327117693069.

Sugita-Konishi Y, Shimura S, Nishikawa T, Sunaga F, et al., 2003. Effect of Bisphenol A on non-specific immunodefenses against non-pathogenic Escherichia coli. Toxicol Lett, 136, 217-227. https://doi: 10.1016/s0378-4274(02)00388-0.

Sur E, Celik I, 2005. Effects of aflatoxin B1 on the development of chicken thymus and blood lymphocyte alpha-naphthyl acetate esterase activity. Flemish Vet J, 74, 432-439. https://doi: 10.21825/vdt.89102.

Sur E, Celik I, Oznurlu Y, Aydın MF, et al., 2011. Enzyme histochemical and serological investigations on the immune system from chickens treated in ovo with aflatoxin B1. Rev Med Vet, 162, 443-448.

Sur E, Dönmez HH, Boydak M, Ataman MB, 2012. Effects of glucomannan on the sacculus rotundus and peripheral blood lymphocytes in New Zealand rabbits during aflatoxicosis. The Sci World J, Article ID 632945. https://doi:10.1100/2012/632945.

Tiwari D, Vanage G, 2017. Bisphenol A induces oxidative stress in bone marrow cells, lymphocytes, and reproductive organs of Holtzman rats. Int J Toxicol, 36(2), 142-152. https://doi: 10.1177/1091581817691224.

Ward JM, Erexson CR, Lawrence J, Faucette L, et al., 2006. Immunohistochemical markers for the rodent immune system. Toxicol Pathol, 34, 616-630. https://doi: 10.1080/01926230600941340.

Yener Y, Sur E, Telatar T, Oznurlu Y, 2013. The effect of acrylamide on alpha-naphthyl acetate esterase enzyme in blood circulating lymphocytes and gut associated lymphoid tissues in rats. Exp Toxicol Pathol, 65, 143–146. https://doi: 10.1016/j.etp.2011.07.002.

Yener Y, Çelik İ, Sur E, Öznurlu Y, et al., 2019. Effects of long term oral acrylamide administration on alpha naphthyl acetate esterase and acid phosphatase activities in the peripheral blood lymphocytes of rats. Biotech Histochem, 94(5), 352-359. https://doi: 10.1080/10520295.2019.1571227.

Yılmaz D, Öznurlu Y, 2019. The determination of effects of in ovo administrated bisphenol a on the development of thymus and proportion of alpha-naphthyl acetate esterase enzyme lymphocyte by using histological and enzymehistochemical methods in chicken. Eurasian J Vet Sci, 35(3), 144–151. https://doi: 10.15312/EurasianJVetSci.2019.233.

Yiğit F, Aktaş A, Dağlıoğlu S, 2013. Effects of bisphenol A and diethylstilbestrol on the involution of bursa of Fabricius in the hens. J Fac Vet Med Istanbul Univ, 39(2), 168-174.

Yin D, Hu S, Gu Y, Wei L, et al., 2006. Immunotoxicity of bisphenol A to Carassius auratus lymphocytes and macrophages following in vitro exposure. J Environ Sci, 19, 232–237. https://doi: 10.1016/s1001-0742(07)60038-2.

Yoshino S, Yamaki K, Yanagisawa R, Takano H, et al., 2003. Effect of bisphenol A on antigen-specific antibody production, proliferative responses of lymphoid cells, and TH1 and TH2 immune responses in mice. Br J Pharmacol, 138, 1271-1276. https://doi: 10.1038/sj.bjp.0705166.

Yoshino S, Yamaki K, Li X, Sai T, et al., 2004. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses in mice. Immunol, 112, 489–495. https://doi: 10.1111/j.1365-2567.2004.01900. x.

Wulff JC, Sale GE, Deeg HJ, Storb R, 1981. Nonspesific acid esterase activity as a marker for canine T-lymphocytes. Exp Hematol, 9(8): 850-870.