Chemical Mixtures in the Environment: Endocrine Disruption Properties of Phthalates and BPA

Tiffany Lee, T Nilawat, and Cesar Romano
Dept. of Environmental and Occupational Health, California State University, Northridge

Abstract

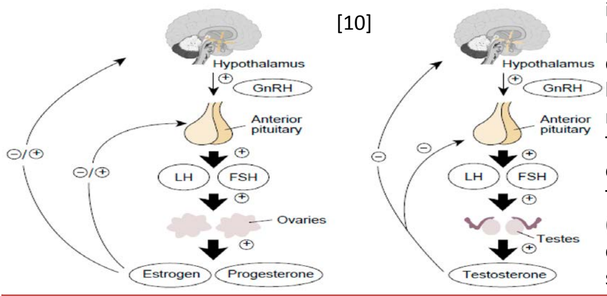
Many humans live in a modern industrial environment and are exposed daily to a myriad of chemicals. These chemicals are found in plastics, which are ubiquitous and are used for many purposes including for the wrapping and packaging of foods. Bisphenol-A (BPA) and phthalates are both endocrine disrupting chemicals commonly found in plastics. They affect gland functions and hormone production due to the similarity of the their chemical structures with estrogen. Regulatory agencies have not expressed clear concern with BPA or phthalate exposures but yet they have enacted limited regulations mainly to protect children. Studies show that both chemicals can cause alterations in thyroid hormone: synthesis, release, transport, and metabolism. Scientists have a limited understanding of the effects from singular chemical exposures and even less understanding from the effects of chemical mixture exposures. The goal of this poster is to understand the possible effects on the human body from exposure to a chemical mixture of BPA and phthalates. The findings enclosed suggest both BPA and phthalates affect similar endpoints.

Introduction

Humans are exposed to a plethora of anthropogenic, man-made, chemicals. These chemicals can be found in the from of plastics, which are ubiquitous and are used for many purposes including for the wrapping and transportation of foods. It is not uncommon that these chemicals leach into the food chain. This poster will focus on the effects of BPA and phthalates on the human body. Both are endocrine disrupting chemicals found in a variety of commercial products especially in plastics.

BPA is a synthetic organic compound. It makes plastic clear and tough and is commonly used in the production of polycarbonate plastics and epoxy resins, i.e. plastic bottles, baby bottles, printed circuit boards, and the lining of canned foods [1, 2, 3, 4]. Studies have indicated that BPA exhibits hormone like properties, that could possibly be linked to disrupted endocrine system function, obesity, cancer, heart disease, neurological effects, reproductive and sexual development divergence [5, 6].

Phthalates are plasticizers. Their function is to increase malleability, transparency, durability of a material. They are found in many consumer products, i.e. children's toys, biomedical supplements and equipment, cosmetics, and food packaging [4, 7, 8, 9]. Studies have indicated that phthalate exposure leads to endocrine system disruption and reproductive and sexual development divergence. There are numerous phthalate esters, the focus will be on the more common phthalates: Di(2-ethylhexyl) phthalate(DEHP), Dibutyl phthalate (DBP), Butyl benzyl phthalate (BBP), and Dimethyl phthalate (DMP).



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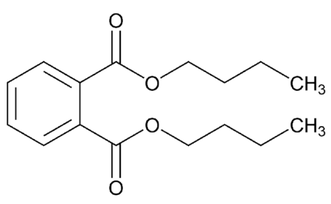
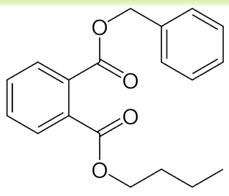
To understand the effects of BPA and phthalates on the human body it is important to discuss the function of the endocrine system. The system is made up of a collection of glands that secrete hormones into the circulatory system that target other organs or organ systems. These hormones regulate the body's various processes, i.e. sleep, metabolism, reproduction. These chemical processes are vital to the maintaining life. The glands that make up this system are the pineal, pituitary, pancreas, ovaries, testes, thyroid, parathyroid, hypothalamus, and adrenal glands. The subsystems of concern are the hypothalamus-pituitary-thyroid axis (HPT axis) and the hypothalamus-pituitary-gonad (HPG axis, the ovaries or testes are in place of the gonad). The table below describes the secreted hormone and their effects.

Initiating gland	Hormone	Target organ/gland	Effect
Hypothalamus	gonadotropin-releasing hormone (GnRH)	Anterior pituitary gland	stimulate the production of gonadotropin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH)
anterior pituitary	Follicle-stimulating hormone (FSH)	Gonads: Male - Testes:	Male - stimulates maturation of seminiferous tubules, spermatogenesis, production of androgen binding protein from Sertoli cells
		Female – Ovaries	Female - stimulates maturation of ovarian follicles in ovary
	Luteinizing hormone	Male - Testes	Male - stimulates testosterone synthesis from Leydig cells
		Female - Ovaries	Female - stimulates ovulation, formation of corpus luteum
Thyroid	Triiodothyronine (T3)		Stimulates body oxygen and energy consumption, stimulates RNA polymerase I and II, help regulate long bone growth
	Thyroxine (T4)		Stimulates body oxygen and energy consumption, stimulates RNA polymerase I and II, help regulate long bone growth
Testes	Testosterone		Anabolic growth of muscle mass and strength, increased bone density, maturation of sex organs, deepening of voice, growth of beard and axillary hair
	Estradiol		Prevent apoptosis of germ cells
	Inhibin		inhibit production of FSH
Ovaries	Progesterone		Prevent endometrial cancer by regulating effects of estrogen, promote healing by regulating collagen, increase core temperature during ovulation
	Androstenedione		substrate for estrogen
	Estradiol		promote formation of female secondary sex characteristics accelerate height growth, increase uterine growth,
	Inhibin	anterior pituitary	Inhibit production of FSH

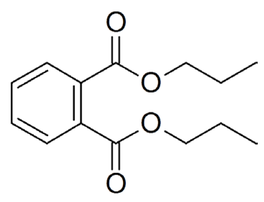
Chemical Structure



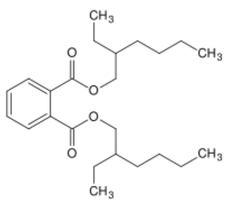
DBP – M.W. 278.34 g·mol^{-1}



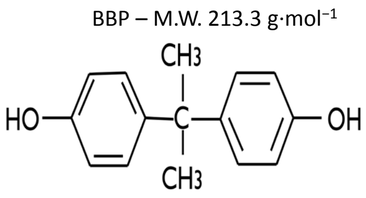
BBP – M.W. 213.3 g·mol^{-1}



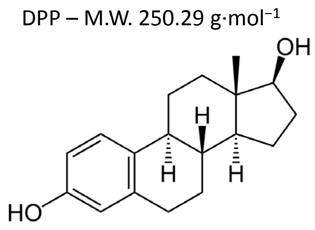
DPP – M.W. 250.29 g·mol^{-1}



DEHP – M.W. 390.57 g·mol^{-1}



BPA – M.W. 228.29 g·mol^{-1}



17-β estradiol – M.W. 272.38 g·mol^{-1}

Fate and Transport

BPA

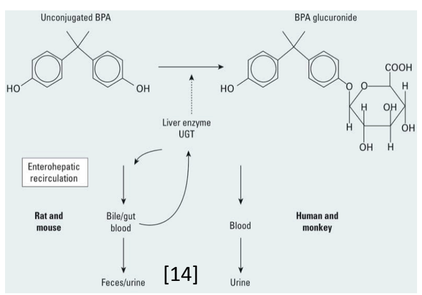
Nearly 75% of BPA used in the United States is attributed to polycarbonate plastic production. BPA was detected in the urine of 92% of participants of a 2003-2004 National Health and Nutrition Examination Study (NHANES) with a mean of 2.6 µg urinary BPA per liter[11]. Plastic can degrade and release BPA through normal use and/or due to high temperature and exposure to alkaline or acidic solutions [11]. Daily human intake is ~ 1 µg/kg/bw [3].

Phthalates

Phthalates are widely used as plasticizers in polyvinyl chloride (PVC) products. Plasticizers can account for up to 40% by weight of products [4]. Phthalates are not covalently bond with PVC, therefore they are free to migrate and are released into the environment by direct release, migration, evaporation, leaching and abrasion. Consequently, phthalates are able to transfer into food, drink, skin, and the environment; daily human intake is ~ 0.1-2 µg/kg/bw [7, 12].

- In 2008 the global production of both chemicals was approximately 5 million tons each[13].
- 50% of total phthalate consumption was for diethyl-hexyl phthalate (DEHP) [13].
- Exposure occurs through air, dust, water, food, and use of consumer and personal-care products. Thus, human exposure routes are ingestion, inhalation, and dermal absorption, while the primary exposure route is attributed to ingestion [1, 2].

Metabolism



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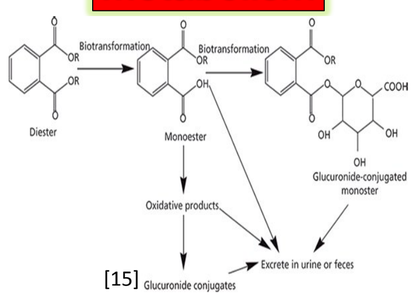

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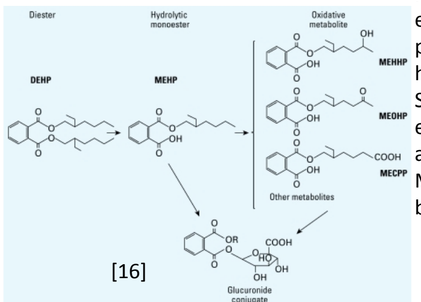

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BPA:

- 80-90% of BPA is rapidly biotransformed in the liver through glucuronidation to BPA-glucuronide (BPA-G). [17, 18, 19] BPA-G is a water-soluble metabolite and excreted in urine. Studies of BPA-G has indicated the glucuronide conjugated form is inactive [17, 18, 19].
- Unconjugated BPA possesses weak estrogenic activity [20].

Phthalates:

- Phthalates are rapidly hydrolyzed and metabolized into their corresponding monoester metabolites [9].
- Monoester metabolites are then conjugated with glucuronide or undergo further biotransformation [21].
- Phthalate metabolites are eliminated in urine or bile as free or glucuronidated conjugates [21].

DEHP:

- DEHP toxicity is associated with repeated or chronic exposure [21].
- Previously it was believed that DEHP is readily biotransformed into various metabolites that are excreted. However we now know DEHP can bioaccumulate in tissues and become mobilized or eliminated through perspiration [22, 23].
- Studies have found DEHP in sweat samples of participants with no measureable compound in the serum [22].

Regulations

Agency/Act [24, 25]	Action/Limit
FDA (July 17,2012)	BPA banned in infant feeding bottles and spill-proof cups
FDA (July 12, 2013)	BPA banned as coatings in packaging for infant Formula
FDA (October 19, 2011)	Allowable level for DEHP in bottled water 0.006 mg/l
Consumer Product Safety Improvement Act 2008	DBP, DEHP, and BBP banned in children's toys at concentrations higher than 0.1 %

Phthalates: The Food and Drug Administration (FDA) has reviewed safety and toxicity data for phthalates including infant toxicity. According to the FDA it is not clear what effect, if any, phthalates have on human health [24]. Despite FDA's lack of findings some regulations have been enacted.

BPA: The FDA determined that BPA is safe at the current levels occurring in food [25]. At the same time the FDA has headed to pressure form consumer advocates. The FDA recently granted two petitions requesting an amendment to their food additive regulations that will reduce BPA exposure to toddlers and infants [25].

BPA and Phthalate Mechanism of Action

Phthalates and the Thyroid:

- Cross-sectional study of urinary concentrations of DEHP metabolites with US adult urine samples found an inverse relationship with total T3, total T4, and thyroglobulin (Tg) (↓ total T3, ↓ T4, ↓ Tg) and positive relationship with TSH (↑ TSH) [26].
- Urine samples collected from 408 men were measured for MEHP, DEHP and their monoester metabolites and compared to levels of free T4, total T3, and TSH. The study found a significant inverse relationship between urinary DEHP metabolites and total T3, total T4, free T4, and Tg; and positive relationship with thyroid-stimulating hormone TSH (↓ total T3, ↓ total T4, ↓ free T4, ↓ Tg; ↑TSH). An adjusted regression coefficient for quintiles of oxidative DEHP metabolites displayed a monotonic dose-dependent decrease in total T4 [27].
- Metabolite Mono(3-carboxypropyl) phthalate (MCPP) was inversely related to serum levels of total and free T3 and total T4 (↓ total T3, ↓free T3, ↓ total T4) [28].
- DBP metabolites were found to be inversely related to serum levels of total and free T4 in Taiwanese study of 76 pregnant women (↓ total T4, ↓ free T4) [29].
- Dose dependent inverse association with DBP and T3 and T4 levels in rats (↓ T3, ↓ T4)[30].
- → The inverse relationship of phthalates and its metabolites in total and free thyroid hormones T3 and T4 coupled with its positive relationship with the thyroid stimulating hormone TSH suggests phthalates are associated with alterations in the thyroid hormone synthesis, release, transport, or metabolism as opposed to the hypothalamus or anterior pituitary gland.
- An in vitro study on effects of phthalates on one component of thyroid hormone biosynthesis was performed to examine the effects on the iodide uptake in rat thyroid cells mediated by the sodium/iodide symporter (NIS). Phthalate and metabolite exposure indicated significant enhancement of iodide uptake of thyroid follicular cells [31].
- A study to detect thyroid system-disrupting activity of DEHP, DBP, and MBP found the chemicals possessed anti-thyroid hormone activity which may suggest phthalates disrupt thyroid receptor to interfere with the function of the thyroid [32, 33].

Phthalates and Steroidogenesis:

- DEHP and DEP have been shown to up-regulate 17-β estradiol (E2) [34].
- DEHP, DEP, and DBP have been shown to down-regulate testosterone (T) production [34].

Phthalates and estrogen/androgen receptors:

- BBP is a weak Estrogen Receptor (ER) agonist [35].
- DBP, DHP, and DEHP each of which have alkyl chains between C3 and C6 in length, were found to induce ER α - mediated estrogenic activity. There is evidence that suggests the estrogenic activities of phthalates might be induced by binding to ER α [36].
- DHP and DEHP have been shown to be ER α antagonists. DBP and DHP have been shown to be Androgen Receptor antagonists [36].
- Studies have provided evidence that suggests phthalates are not AR agonists [36].
- Most phthalates, including DBP, and their mono metabolites do not bind to the AR and have little affinity to ER β, indicating that they are not direct AR and ER β antagonists. How ever DBP is found to have ER α affinity [37].
- Leydig cell hypoplasia (LCH) is characterized by an inability of the Leydig cells to produce testosterone and other androgen sex hormones.
- A primary target of phthalates are Leydig Cells (LC). LCs produce T and express ER α, ER β, and AR. Prenatal DBP exposure in rats induces LCH during adulthood [37].

BPA and the Thyroid :

- BPA analyzed in urine samples of 335 women during the second half of pregnancy. Thyroid hormones levels analyzed in blood samples of both mothers and newborns after birth presented an inverse relationship to total T4 and a positive relationship to TSH (↓ total T4; ↑TSH) [38].
- A study of urinary BPA concentrations of 167 men suggests an inverse relationship between BPA and thyroid-stimulating hormone (TSH) [39].
- In a study of BPA injected to Sprague Dawley rat liver cells, it was found that BPA inhibited T3 binding to the TR and suppressed its transcriptional activity T3 from endogenous TR [40].
- In vitro and in vivo zebrafish models show BPA effect regulation of gene expression involved in thyroid hormone synthesis. The study suggests a direct effect on thyroid follicular cell [41].
- BPA can impair thyroid hormone action by inhibiting T3 binding to the TR and by recruiting N-CoR on the promoter [34].

BPA and Steroidogenesis:

- BPA increases T levels in ovarian theca-interstitial (T-I) cells and is associated with mRNA expression of key enzymes involved in androgen production. BPA increases progesterone levels and suppresses E2 levels in granulosa cells. BPA may disrupt the normal expression of ovarian steroidogenic enzymes though an orphan nuclear receptor mechanism [42].

BPA and estrogen/androgen receptors

- BPA is an artificial estrogen. It has been used to:
  - Enhance the rapid growth of cattle and poultry [43]
  - As an estrogen replacement for women [43]
- BPA is similar to another well known potent estrogen diethylstilbestrol (DES) [43].
- Several studies have shown that BPA can activate ERs α and β [44].
- BPA can also bind to AR. Studies have shown anti-androgenic activity of BPA in cell systems through the formation of an AR/BPA complex that hinder endogenous androgens from regulating androgen dependent gene transcription [44].
- Recent studies have suggest BPA metabolites have greater affinity to ERs. When glucuronidation is unable to work efficiently as a detoxification pathway of BPA, metabolic activation to MBP may occur. MBP has attracted the attention of researchers since it has showed approximately 250 - 10,000 fold higher ER affinity than BPA in larvae and adult fish and 500 fold higher in rats [45]. The MBP length is closer to that of E2 compared to BPA.
- MBP is metabolized by recombination of the radical fragment of BPA, which is the one-electron oxidation product of carbon-phenyl bond cleavage, and its creation is dependent on both microsomal and cytosolic fractions [46].

Health Effects/Endpoints

Phthalates Specific:

- Despite their difference in molecular weight DEHP and DEP equally moderately toxic to cells[35].
- Environmental phthalate exposure can result in lower semen motility, increased percentage of semen with abnormal morphology, and lower semen concentrations. Thus, disruption in male fertility [47].
- Studies suggest that phthalates, particularly High Molecular Weight (HMW) phthalates, may be associated with allergic symptoms in adults and possibly children [48].
- Testicular injury, liver injury, liver cancer, anti-androgenic activity, and teratogenicity.

BPA Specific:

- Structural and neurochemical changes throughout the brain (i.e. Hyperactivity, Learning Deficits, Increased aggression, Increased likelihood of drug dependency) [43].
- Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting between 4% and 8% of reproductive aged women. It is characterized by chronic anovulation and hyperandrogenism. BPA concentrations are significantly higher in women with PCOS [42].
- Studies have shown links between BPA exposure and hormone- related cancers, including breast, prostate, and ovarian cancers and endometrial carcinoma [44].
- Abnormal sperm production in males and oocytes in females [43].
- Disruption of hormone production, fertility, and early sexual maturation in males and females[43].
- Immune Disorders and Increased growth rates [43].

Thyroid Related Illnesses Associated with Both Phthalates and BPA Exposure:

- Thyroid hormones are critical for growth and development of brain. Severe hypothyroidism is detrimental to neurodevelopment.
- Children born with normal thyroid function, but who experienced thyroid hormone insufficiency in the womb, display subtle cognitive impairments and abnormalities in brain imaging. Despite early detection and treatment, deficiencies also exist in children born with thyroid disorders [49].
- Adequate thyroid hormone supply is necessary throughout fetal and early infant life for proper development of the human hippocampus [49].
- Thyroid disruption has effect on waist circumference, insulin resistance, diabetes.
- BPA and phthalates may be related to the rising epidemics of obesity and Type 2 Diabetes [50].
- Type 2 Diabetes is closely associated with thyroid dysfunction. The most probable mechanism leading to Type 2 Diabetes in thyroid dysfunction could be attributed to factors contributing to insulin resistance. Hyper- and hypothyroidism have been associated with insulin resistance which has been reported to be the major cause of impaired glucose metabolism in Type 2 Diabetes [51].

Conclusion

We live in a plastic world. It is nearly impossible in most industrialized nations to avoid daily plastic use. Society's most beloved products including our foods are shrouded in plastic. Unfortunately, some of the constituents in plastics, like BPA and phthalates, leach into our environment and foods. Humans are suffering a chronic exposure to a mixture of these chemicals. The individual effects that BPA and phthalates have on humans are not well understood and there is even less data on their effects as a mixture. However, research studies have indicated that BPA and phthalates are endocrine disruptors. BPA acts like an artificial estrogen, acting to inhibit production of T3. Studies suggest phthalates are associated with alterations in the thyroid hormone: synthesis, release, transport, or metabolism. These endocrine disruptions have an impact on hormone production and ER and AR expression. The final result suggests both BPA and phthalates affect similar endpoints causing severe health effects which range from weight gain and neurochemical changes to diabetes and cancers. More research is needed to gain a better understanding of the joint effects of BPA and phthalates on human organisms. Plastics have been beneficial for the development of modern civilization but it is time to stop and analyze their deleterious effects on humans.

Citations

[1] Koch H, Calafat A. (2009). Human body burdens of chemicals used in plastic manufacture. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 2063-2078.
[2] Rudel R, Gray J, Engel C, Rawsthorne T, Dodson R, et al. (2011). Food packaging and bisphenol a and bis(2-ethyhexyl) phthalate exposure: Findings from a dietary intervention. Environmental Health Perspectives, 119(7), 914-920.
[3] Vandenberg L, Hauser R, Marcus M, Olea N, Welshons W. Human Exposure to Bisphenol A (BPA). Tufts University Sackler School of Gradute Biomedical Sciences. Living on Earth Webite https://loe.org/images/content/070803/Vandenberg%20Exposure%20Rep%20Tox%20resubmission.pdf
[4] ATSDR (2002) Agency for Toxic Substances and Disease Registry: Toxicological profile for di(2-ethylhexyl)phthalate(DEHP). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA. http://www.atsdr.cdc.gov/toxprofiles/tp9.pdf. Accessed June 13, 2014.
[5] National Toxicology Program. Bisphenol A (BPA). National Institute of Environmental Health Sciences Website. https://www.niehs.nih.gov/health/assets/docs_a_e/bisphenol_a_bpa_508.pdf. Published August 2010. Accessed December 4, 2015.
[6] Anderson SE, Meade BJ. Potential Health Effects Associated with Dermal Exposure to Occupational Chemicals. Environmental Health Insights. 2014;8(Suppl 1):51-62. doi:10.4137/EHI.S15258.
[7] Erythropel HI, Maric M, Nicell J, Leask R, Yargeau V. (2014). Leaching of the Plasticizer di(2-ethylhexyl)phthalate (DEHP) from Plastic Containers and the Question of Human Exposure. Applied Microbiology and Biotechnology. 98:24. 9967-9981.
[8] Cosmetics. Phthalates. U.S. Food and Drug Administration Website. http://www.fda.gov/Cosmetics/ProductsIngredients/Ingredients/ucm128850.htm. Updated September 19, 2014. Accessed December 4, 2015.
[9] Bahadar H, Maqbool F, Abdollahi M. 2014. Consumption of Phthalates Coated Pharmaceutical Tablets: An Unnoticed Threat. International Journal of Pharmacology, 10: 78-81.
[10] Thyroid System. Hypothalamic-Pituitary-Thyroid Axis. Wikipedia.
[11] McGovern V. 2009. Polycarbonate Plastics and Human BPA Exposure. 2009. Environmental Health Perspectives. 117(9). A406.
[12] Wittassek M, Koch HM, Angerer J, Brüning T. (2011), Assessing exposure to phthalates – The human biomonitoring approach. Molecular Nutrition Food Research. 55: 7–31. doi: 10.1002/mnfr.201000121
[13] Tran B, Teil M, Blanchard M, Alliot F, Chevreuil M. (2015). Fate of Phthalates and BPA in Agricultural and Non-agricultural Soils of the Paris Area (France). *Environmental Science and Pollution Research*, 22(14), 11118-11126.
[14] Enterohepatic Recirculation. National Institute of Health. http://ehp.niehs.nih.gov/wp-content/uploads/119/4/ehp.1002514.g001.png
[15] General Metabolic Pathways of Phthalates in Humans. Chromatography Online. http://www.chromatographyonline.com/analysis-four-phthalate-monoesters-human-urine-using-liquid-chromatography-tandem-mass-spectromet-0
[16] Calafat A, Needham L. DEHP Metabolites. Environmental Health Perspectives. http://ehp.niehs.nih.gov/090108/
[17] Olsen L, Lampa E, Birkholz D, Lind L, Lind P. (2012). Circulating Levels of Bisphenol A (BPA) and Phthalates in an Elderly Population in Sweden, Based on the Prospective Investigation of the Vasculature in Uppsala Seniors (Pivus). Ecotoxicology and Environmental Safety, 75(1), 242-248.
[18] Matthews B, Twomey K, Zacharewski TR. (2001). In Vitro and In Vivo Interactions of Bisphenol A and its Metabolite, Bisphenol A Glucuronide, with Estrogen Receptors α and β. *Chemical Research in Toxicology*. 14(2001), 149–157
[19] vom Saal F, Welshons W. (2006). Large Effects from Small Exposures. II. The Importance of Positive Controls in Low-Dose Research on Bisphenol A. Environmental Research, 100(1), 50-76.
[20] Yoshihara S, Mizutare T, Makishima M, Suzuki N, Fujimoto N, Igarashi K, Ohta S. (2003). Potent Estrogenic Metabolites of Bisphenol A and Bisphenol B Formed by Rat Liver S9 Fraction: Their Structures and Estrogenic Potency. Toxicological Sciences. (2004) 78 (1): 50-59.
[21] Mu D, Gao F, Fan Z, Shen H , Peng H, et al. (2015). Levels of Phthalate Metabolites in Urine of Pregnant Women and Risk of Clinical Pregnancy Loss. Environmental Science & Technology, 49(17), 10651-10657.
[22] Genuis S, Beesoon S, Lobo R, Birkholz D. 2012. "Human Elimination of Phthalate Compounds: Blood, Urine, and Sweat (BUS) Study," The Scientific World Journal, vol. 2012, Article ID 615068, 10 pages.
[23] Chiang HC, Kuo YT, Shen CC, Lin YH, Wang SL, Tsou TC. 2014. Mono(2-ethylhexyl)phthalate Accumulation Disturbs Energy Metabolism of Fat Cells. Molecular Toxicology Archives of Toxicology. 1-13.
[24] U.S. Food and Drug Administration (2015). Phthalates. *http://www.fda.gov/Cosmetics/ProductsIngredients /Ingredients/ucm128250.htm*, Accessed November 13, 2015.
[25] U.S. Food and Drug Administration (2015). Bisphenol A (BPA): Use in Food Contact Application.*http://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm064437.htm*. Accessed November 13, 2015.
[26] Meeker JD, Ferguson KK. 2011. Relationship between Urinary Phthalate and Bisphenol A Concentrations and Serum Thyroid Measures in U.S. Adults and Adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007–2008. *Environmental Health Perspectives*. 2011;119(10):1396-1402. doi:10.1289/ehp.1103582.
[27] Metabolites of Phthalates and Phthalate Alternatives. Laboratory Procedure Manual. Centers for Disease Control and Prevention Website. http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/PHTHTE_G_met.pdf Revised April, 2013. Accessed December 4, 2015.
[28] Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebaek NE, Hegedus L, Hilsted L, et al. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect. 2010;118:1458–1464.
[29] Huang PC, Kuo PL, Guo YL, Liao CC, Lee CC. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum Reprod. 2007;22(10):2715–2722.
[30] O'Connor JC, Frame SR, Ladics GS. Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. Toxicol Sci. 2002;69(1):92–108.
[31] Wenzel A, Franz C, Breous E, Loos U. Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. Mol Cell Endocrinol. 2005;244(1–2):63–71.
[32] Ghisari M, Bonefeld-Jorgensen EC. Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. Toxicol Lett. 2009;189(1):67–77.
[33] Shen O, Du G, Sun H, Wu W, Jiang Y, Song L, et al. Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. Toxicol Lett. 2009;191(1):9–14.
[34] Moriyama, K. , Tagami, T. , Akamizu, T. , Usui, T. , Saijo, M. , et al. (2002). Thyroid hormone action is disrupted by bisphenol a as an antagonist. *Journal of Clinical Endocrinology and Metabolism*, *87*(11), 5185-5190.
[35] Mankidy, R. , Wiseman, S. , Ma, H. , & Giesy, J. (2013). Biological impact of phthalates. *Toxicology Letters*, *217*(1), 50-58.
[36] Takeuchi, S. , Iida, M. , Kobayashi, S. , Jin, K. , Matsuda, T. , et al. (2005). Differential effects of phthalate esters on transcriptional activities via human estrogen receptors α and β, and androgen receptor. *Toxicology*, *216*(2), 223-233.
[37] Wakui, S. , Shirai, M. , Motohashi, M. , Mutou, T. , Oyama, N. , et al. (2014). Effects of in utero exposure to di(n-butyl) phthalate for estrogen receptors α, β, and androgen receptor of leydig cell on rats. *Toxicologic Pathology*, *42*(5), 877-887.
[38] University of california - berkeley; bpa linked to thyroid hormone changes in pregnant women, newborns. (2012). *OBGYN & Reproduction Week*, 898.
[39] Meeker J, Calafat A, Hauser R. (2010). Urinary Bisphenol A Concentrations in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic. Environmental Science & Technology, 44(4), 1458-1463.
[40] Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, et al. (2002). Thyroid hormone action is disrupted by bisphenol a as an antagonist. *Journal of Clinical Endocrinology and Metabolism*, *87*(11), 5185-5190.
[41] Gentilcore, D. , Porreca, I. , Rizzo, F. , Ganbaatar, E. , Carchia, E. , et al. (2013). Bisphenol a interferes with thyroid specific gene expression. *TOXICOLOGY*, *304*, 21-31.
[42] Zhou, W. , Liu, J. , Liu, J. , Liao, L. , & Han, S. (2008). Effect of bisphenol a on steroidogenesis-promoter gene expression and on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. *Molecular and Cellular Endocrinology*, *283*(1), 12-18.
[43] Erler, C. , & Novak, J. (2010). Bisphenol a exposure: Human risk and health policy. *Journal of Pediatric Nursing*, *25*(5), 400-407.
[44] Gao, H. , Yang, B. , Li, N. , Feng, L. , Shi, X. , et al. (2015). Bisphenol a and hormone-associated cancers: Current progress and perspectives. *MEDICINE*, *94*(1), e211-e217.
[45] Okuda, K. , Takiguchi, M. , & Yoshihara, S. (2010). In vivo estrogenic potential of 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, an active metabolite of bisphenol a, in uterus of ovariectomized rat. *Toxicology Letters*, *197*(1), 7-11.
[46] Okuda, K. , Fukuuchi, T. , Takiguchi, M. , & Yoshihara, S. (2011). Novel pathway of metabolic activation of bisphenol a-related compounds for estrogenic activity. *Drug Metabolism and Disposition*, *39*(9), 1696-1703.
[47] Duty, S. , Silva, M. , Barr, D. , Brock, J. , Ryan, L. , et al. (2003). Phthalate exposure and human semen parameters. *Epidemiology*, *14*(3), 269-277.
[48] Hoppin, J. , Jaramillo, R. , London, S. , Bertelsen, R. , Salo, P. , et al. (2013). Phthalate exposure and allergy in the us population: Results from nhanes 2005-2006. *Environmental Health Perspectives*, *121*(10), 1129-1134.
[49] Gilbert M, Rovet J, Chen Z, Koibuchi N. 2012. Developmental Thyroid Hormone Disruption: Prevalence, Environmental Contaminants and Neurodevelopmental Consequences. Neurotoxicology. 33(4) 842-852.
[50] Casals-Casas C, Desvergne B. 2011. Endocrine Disruptors: from Endocrine to Metabolic Disruption. Annual Review of Physiology. 73:135-62.
[51] Wang C. 2013. The Relationship Between Type 2 Diabetes Mellitus and Related Thyroid Diseases. Journal of Diabetes Research. 2013: 390534.