



BIOREMEDIATION SCIENCE AND TECHNOLOGY RESEARCH

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MINI REVIEW

Toxicity Effect of Bisphenol-A in Several Animal Studies: A Mini Review

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HISTORY

Received: 27 September 2013
 Received in revised form: 21 November 2013
 Accepted: 18 December 2013

KEYWORD

bisphenol-A
 pollution
 plastic
 estrogenic activity
 toxicity

Abstract

The main highlight of Bisphenol-A (BPA) is the exhibition of the estrogenic properties, which is found to be a threat to human beings. This mini review summarizes available data in the literature (1997–2011) on toxicity effect of BPA in several animal studies. There are needs of more validated studies to be carried out to determine the toxicity effects of BPA on human beings.

INTRODUCTION

BisphenolA (BPA) is a type of chemical inducing the estrogenic endocrine interrupting effect on human beings, which is found related to the production of plastic products [1]. It is a monomer, consists of empirical formula of $C_{15}H_{16}O_2$ and chemical formula of 4,4-isopropylidenediphenol, which undergoes the polymerization process to produce polycarbonate. BPA is a highly-produced chemical in worldwide, where over 6 billion pounds are being produced every year, and over 100 tons have been released into atmosphere [2]. The main highlight of BPA is the exhibition of the estrogenic properties, which is found to be a threat to human beings. The functional activities of estrogen receive high concerns of all users, since it is affecting reproduction, cellular development, health and even worsening the risk of carcinogenesis [3].

The polycarbonate products that produced by the polymerization process of BPA monomers are used in the manufacturing of plastics and epoxy resins that can be used as linings of metal cans and lacquer coatings of canned foods. Besides, BPA are widely used in plastic manufacturing industry, such as polyvinyl chloride (PVC), toys, water pipes, soda bottles, baby bottles and mineral water bottles [4]. In the medical equipment industry, BPA is used in the manufacturing of impact-resistant safety equipment, dental sealants and composites. In addition, pvc is used in the manufacturing of medical products such as bags that containing intravenous fluids, feeding tubes,

parenteral nutrition tubes, respiratory masks, endotracheal tubes and umbilical catheters. Meanwhile, the epoxy-based coatings are widely used in different fields of applications. For example, finishings for product and marine, the manufacturing of decorative floor, coatings of tank, can and drum and floor varnishes [5].

The exposure of human population towards BPA became a concern, since it is proven that the exposure is threatening the normal reproductive development of laboratory animals, as well as human beings [6]. There were series of past researches carried out to determine the toxicity effect of BPA. Vomsalet *et al.*, in the year of 1997, carried out a research to determine the increment of prostate weight in male offspring at 6 months of lives. BPA was administered to pregnant mice, where the prostate weight of male offspring were examined at the 6th months of lives. The studied proved that BPA effects in prostate enlargement of male mice significantly. However, there are controversies debating the causal effect of BPA on the reproductive organs development in animal studies [7]. Moreover, ho *et al.*, [8], contributed their research in the mean of relationship between the BPA exposures with the prostate disease. They found the related developmental exposure of mice subjects to low and mild concentrations of BPA increases the sensitivity and susceptibility of hormonal carcinogenesis,

Tylet *et al.*, [7] in the year of 2008, conducted a cohort study to determine the production of offspring systemic toxicity through altering the fertility and pregnancy of 280 virgin female and male Cd-1 (Swiss) mice using different concentrations of BPA. The

growth and development of offspring were monitored over two generations of mice, begin with the lowest dose of the parental generation with their systemic toxicity produced ($\mu\text{g}/\text{kg}$ body weight/day), up to the maximum level of toxicity produced (high milligram/kg/day doses). Several organ tissues were examined using different kinds of chemicals to check for the status.

Table 1. Series of examinations conducted by Tylet *et al.* [7].

Organ Tissues Subjected For Examination	Chemical Used	Targeted Purpose
vaginal smear was taken from each female, subjected for microscope check	Toluidine blue	to determine the stage of estrus at demise
testis tissues was taken from each male	periodic acid schiff & hematoxylin	to identify treatment-related effects
ovary tissues was sampled from each female	hematoxylin & eosin	to identify the presence or absence of small, growing and antral follicles & corpora lutea compared with control ovaries
epididymis from each male was frozen and tissues were sampled from each	instrument used: automated sperm analysis system	to check on sperm motility
posterior pair of mammary glands in adult female	bnf (stick to the file card to keep the preparation flat)	to check on the enlargement & morphology of mammary gland

Tylet *et al.*, [7] found that the adult systemic effects only begins with 300ppm of BPA from parental generation. At this level, only centrilobular hepatocyte hypertrophy was traced. Meanwhile, up to 3500ppm of BPA content in body fluids of parental generation of mice, signs such as declined body weight, declined testes weights and undescended testes only in weanlings were shown. However, there was no any effect and no monotonic dose-response curve shown for lower dose of BPA (0.018 – 30ppm). From the study conducted, the no observable effect level (NOEL) was 30ppm of BPA in body fluids. Hence, they concluded BPA was not a selective reproductive or developmental toxicant in mice.

In the year of 2008, Moral *et al.* [9] also conducted a research to study the prenatal exposure to the BPA on the mammary gland morphology during puberty stage, and gene expression signature for different stages of development of the tissues, using 8-week-old female Sprague-Dawley CD rats (*Rattus norvegicus*). This study was meant to determine the toxicity effect of BPA on altering the risks of breast cancer, in high exposure towards female populations, since breast cancer is a highly estrogen-dependent malignancy [10]. Female offspring of these mice were transferred, and weaned at the 21st day and killed in the estrous phase. Mammary glands' tissues were fixed and examined under microscope. The genomic profiles were determined using the microarray analysis. Meanwhile, gene expression in mammary glands were found expressed in the mammary gland differently, and validated by using real-time PCR method. It was found that high dose of BPA was altering the undifferentiated epithelial structures of breast tissues, where it also established the highest indications on gene expression by 100 days. In relation with the changing gene expression, it could be resulting in altering the normal development of the mammary gland. Moral *et al.* concluded high prenatal exposure to BPA is increasing the

susceptibility of the mammary gland towards gene alterations, increasing the risk of breast cancer malignancy.

Jenkins *et al.*, [11] in the year of 2009, conducted a research with similar purpose, using neonatal or prepubertal rats exposed to BPA via lactation. Femae Sprague Dawley CD rats were used as subjects of the study, and surrogated artificially. The collection of mammary glands were carried out at the 21st day and 50th day of age respectively by using ketaminexylazine as anesthesia respectively. Two sets of mammary glands were frozen in liquid nitrogen for immunoblotting, where the other sets were fixed in formalin and later in paraffin, for morphology examination. The tumorigenesis study was carried out as well, using 30mg of dimethylbenzanthracene (DMBA)/kg body weight, under exposure of different concentrations of BPA. From the study carried out, there was an increase dose effect in the combination of DMBA treatment with lactation exposure to BPA on the mammary tumor multiplicity and reduced tumor latency compared with controls. Therefore, they concluded that the carcinogenesis of mammary glands in a DMBA-induced model of mice increases with the maternal exposure to BPA during lactation.

Studies on effects of BPA on male reproductive health was again, highlighted by Li *et al.*, [12] in the year of 2009. Male Kunming (China) mice were used as subjects in the study to investigate the main function of apoptosis in the development of male reproductive organs, relating to the toxicity of BPA. Studies of Li *et al.*, [12] and Moral *et al.*, [9] were both using oral gavage of BPA as the induction method, where the control group of mice were fed on the same amount of sesame oil and corn oil respectively. Meanwhile, up to the necropsy of the study, Li *et al.* [12] froze one of the testes of each male for Western blotting purpose. Meanwhile, the other one from each male was subjected for weighing and fixing in Bouin's solution for 18 hours. The tissue blocks were then subjected to deparaffinization, rehydration, and stained with hematoxylin and eosin to examine the changes of testes morphology. From the study, they found the significant underdevelopment testes and obvious disruption of spermatogenesis at the level of 480 and 960 mg/kg/day of BPA. Besides, there were many apoptotic cells found in testes for these levels of BPA exposure. Comparing with control group, these cells are found in significantly higher amount existence in mice testes. Therefore, Li *et al.*, [12] concluded that for those who are highly exposed to BPA, concern of reproductive health of human beings should be given high attention.

Carwile *et al.*, [6], studied the relationship between the frequency of exposures of polycarbonate beverage containers and the BPA concentration in urine, using non-randomized intervention of college students. 77 Harvard college students were recruited as subjects. A 7-days period was fixed as the washout period for them, where they were required to consume the cold beverages using stainless steel bottles. Urine from each participant was sampled at 1700 hours and 2000 hours on the 1st and 2nd day, and 1600 hours and 1900 hours on the 3rd day. After the washout phase, participants were required to drink from polycarbonate plastic bottles. Urine samples were collected at the same time interval with that of the washout phase. Total urinary concentrations of BPA was determined and evaluated using high-performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS). From the study conducted, they found the one whole week of drinking from polycarbonate plastic bottles

shown the significant increase of BPA concentration in urine. This was proved by the larger amount of creatinine ($\mu\text{g/g}$) contained in urine samples for the week of drinking from polycarbonate plastic bottles. They concluded that the frequent consumption of cold beverages from polycarbonate containers was more prone to BPA exposure, besides exposing to other possible BPA sources.

Studying on urine and serum profiles as a result of BPA exposure, Teeguarden *et al.*, [13] conducted a research in the year of 2011, to find out the causal-effect relationship of internal exposure of adult humans to BPA and relationship between serum and urinary pharmacokinetics of BPA. 20 volunteer healthy adults, aged between 18 to 55 years old were chosen as subjects. All of them were fasted overnight, with access only to BPA-free water. Every subject was required to ingest 100% of meals prescribed for them, comprising of standard grocery food items for breakfast, lunch and dinner. Blood and urine samples were collected after 24 hours of ingestion. In the study, the content of BPA in urine samples was found to be higher than the 95th percentile of the United States adult populations. Meanwhile, BPA concentration that could be screened in blood serum samples were found 42 times lower than that of urine samples' concentrations. Since these standard grocery food items were classified as high prone food items towards BPA, more than 80% of the serum samples shown non-detectable BPA level, and found to be less than limit of detection (LOD = 1.3nM).

The development of reproductive organs became a quest for BPA exposure. Meanwhile, study was carried out to examine the effect of BPA exposure on the alterations of early adipogenesis, and to investigate the effect of BPA on adipose tissue development. Sommet *et al.*, [14] carried out a study to evaluate the impact of adipogenesis of perinatal exposure. Virgin male and female Sprague-Dawley rats were used as subjects for the study, where the induction of BPA into subjects were through feeding water. Subjects were fed with drinking water consisting 1mg/L of BPA, starting from day 6 of gestation until the end of lactation. Body weight gain and food and water intake during pregnancy and after gestation of maternal and offspring mice were accessed and measured to evaluate the exposure of BPA at different stages of pregnancy, to see the effect of it on maternal physiology and even after gestation.

Meanwhile, body weight increment was only found in female mice, where the parametrial white adipose tissue weight of increased after weaning. Besides, the gene expression for a few lipogenic genes which contribute to the adiposity gains such as acetyl-CoA carboxylase, stearoyl-CoA carboxylase, fatty acid synthase, and lipoprotein lipase also shown increment in weaning. In a conclusion, the study contributed a series of data which available to prove the perinatal exposure to a low concentration of BPA increases the risk of adipogenesis in females at weaning stage. This was due to the high chance of early determination of adult body weight, which might lead to changes that highly depended on the sex and nutritional status.

The exposure of BPA is evoking a great deal of controversies lately, especially its effect on the growth and development of human being. From the series of studies and researches carried out, there were arguments about the animal studies that have been carried out. For instance, low dose of BPA is known to be susceptible as a weak environmental estrogen, where the method

used to determine the concentration (e.g. microassay), induction of specific indigenous genes, and increment in cell number. This might alter the evaluation of relative potencies when comparing hormones that are prone to inactivation in living animals with those which are not. Therefore, such cohort studies might not be representative enough to measure a true potency of a hormone in biological system (e.g. fetal and adult organism). Another point of argument on the cohort studies using animals is the presence of other possible estrogens in the studies. It is unavoidable that subjects are prone to other sources of estrogens, occurring naturally in surrounding, such as detergents, feed, plastics and other food sources. This may alter the sensitivity and accuracy of the study conducted.

Vandenberg *et al.*, [2], in their review paper, mentioned about another point which is worth to be a point of discussion. Most of the cohort animal studies were involved in oral prescription of BPA as induction of BPA exposure, where series of examinations were carried out to determine the gene alterations and significant body changes. Meanwhile, the concentrations that are used for orally gavaging prescriptions are not representative for human exposure, through dietary intakes. Vandenberg *et al.* summarizes possible sources of human exposure to BPA in their review paper. BPA was found in air samples, floor dust, playing area soil, liquid and solid foods prone to children. These sources of BPA exposure are not even reaching the levels of concentrations used as low-dose BPA exposure's concentrations in cohort studies. Hence, the true and significant symptoms and signs of low-dose BPA exposure to human beings are still waiting to be verified and validated.

In a nutshell, there are needs of more validated studies to be carried out to determine the toxicity effects of BPA on human beings. Similarity of pharmacokinetics mechanisms of human beings towards reactions of BPA are strictly pursued, since there are differences between the normal used mice subjects and human beings, in the contexts of the extents of exposures, as well as the indication of the minimal toxicity effect works on human beings.

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