

HOW SAFE ARE OUR BABIES? AN IN-SIGHT ON EFFECT OF BISPHENOL A (BPA) ON DEVELOPMENT

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ABSTRACT

Globally anthropogenic materials have replaced natural materials. These substances which were industrially useful have proved to be highly deleterious in recent decade. One of these compound is Bisphenol A (BPA), which is used in almost all food cans and containers. This paper focus on little known aspects of BPA which is an endocrine disruptor on oogenesis, gene implants, embryo development, mammary glands, prostate, testis urethra. Exposure to even small concentrations of BPA have shown severe impact on different stages of embryo development. The behaviour modifications as well as carcinogenic effects are also discussed based on the studies on various animal models.

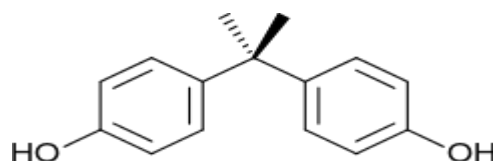
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INTRODUCTION

Anthropogenic compounds have displaced the natural compounds in our day to day life. The microorganisms have shown magnificent capacity to adapt to new and changing environment.^{1,2} Humans and higher organisms are still struggling to find solution to these toxic compounds.³ The natural compounds are not only environmental friendly but also provides long and healthy life.^{4,5}

The contemporaneous world man has moved from natural products to synthetic products which come with a prize to pay. WHO reported about the global trend, wherein babies are seldom breast fed by their lactating mothers. It is justified due to global shift from home makers to the working women. Social and work pressure on mothers makes it difficult for them to breast feed their newborns. The consequence of this results in bottle feeding. The question thus may arise “what’s wrong in bottle feeding?” other than depriving the child of essential antibodies present in colostrum.

A Galaxy of compounds has been reported all over the world to be present in plastic bottles but the least studied compound is Bisphenol A. Our intensive literature search shows that there are very few published work available on effect of Bisphenol A on biotic system especially in developmental process. This paper is destined to reveal all the information on various facets of Bisphenol A and understand the metabolic mechanisms in the body during development.



Chemical structure of Bisphenol A

Bisphenol A which is commonly denoted as BPA is an organic compound which was first synthesized in 1891 by Aleksander Dianin, a Russian chemist. BPA has a chemical nomenclature of 2, 2 – bis (4-hydroxyphenyl) propane. The detrimental effects arise as BPA starts to

leach from the bottle and enters into the diet of the baby. Although the effect of BPA in this case would be postnatal, more serious effects are seen in pre-natal exposure.⁶

Post natal effects of BPA are manifested as change in neuro-behaviour,^{7,8} cognitive skills and social behaviour,^{9,10} underdeveloped reproductive system,¹¹⁻¹³ and deformed mammary glands.¹⁴⁻¹⁸

The effects of BPA are agonistic to action of estrogens. It alters developmental processes by blocking or inducing different cell signalling in estrogen responsive and sensitive tissues even at a very low dose (exposure). The compound is capable of binding to the estrogen receptor α and β and induces estrogenic activity.¹⁹ The significance of BPA was realized during the mid-1930s with the discovery of its ability to act as an agonist to estrogen in ovary ectomized (estrogen deficient) rats. The phenomena was discovery by two biochemists Edward Charles Dodds and William Lawson but failed to explain the mechanism hence tried to solve this mystery through their endocrinology background. Their discovery was of much importance, due to the fact that isolation of naturally occurring hormones was very difficult at that point of time; hormone which could agonise the estrogen can be easily duplicated hence it served as alternate source to natural hormones. This opened a new arena of extensive research on BPA which revealed severe developmental effects on the foetus.

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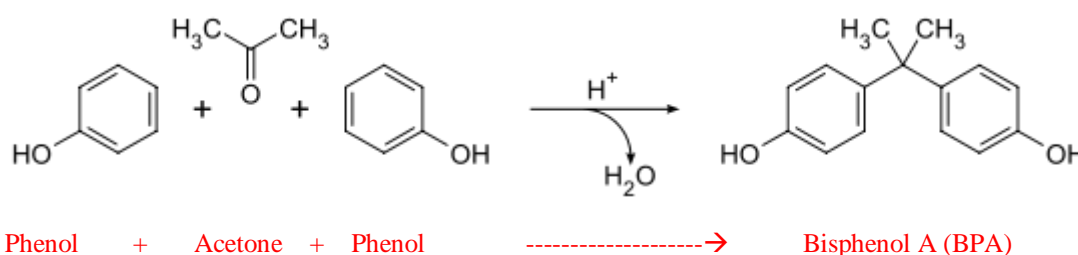
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In early 1950s BPA's industrial uses were realized. It was found to provide a strong backbone for plastics and epoxy resins and was used as plastic coatings in food packaging industry²⁰. It provided transparency, heat resistance, high resilience, and low weight to certain polycarbonates. Epoxy resins containing BPA act as good sealants that are corrosion resistant and prevent contamination⁶. It is used in production of baby bottles,²¹ dental prostheses,²² PVC stretch films and in even papers.²³

Leaching of BPA from food cans and containers

Any metallic food container requires certain level of rust and corrosion resistance in order to be used as food containers. This is achieved by coating them with epoxy resins along the inner side. These resins are synthesized by condensation reaction between BPA and epichlorhydrin to create Bisphenol A diglycidyl ether (BADGE)²⁴.



Synthesis of Bisphenol A

The complete polymerization results in leaching of BPA from the epoxy resin into the food stored in it. The Table No 1 shows the leaching levels of BPA from food cans and epoxy resins. Several studies have been conducted on the conditions that facilitate migration of BPA from the coating of cans.²³ The studies were aimed at

determining the influence of heating time and temperature, storage time and temperature, and various other factors on the level of BPA migration and have estimated leaching to be in the range of 4–23 g of BPA per can.²⁵

Table 1: shows the leaching levels of BPA from cans and containers coated with epoxy resins.²³

Detection method	Sample	Leaching levels	Reference
HPLC	Cans containing 20 types of food products	4-23 µg/can	25
GC-FID	Three epoxy resin	0.32-89.79 ng/ml	26
HPLC/FD	Cans with epoxy resin linings	7-31 ng/ml	26
GC-MS	Cans with epoxy resin linings	100% detection rates	27

Studies revealed a greater impact of heating temperature than heating time on BPA migration²⁶. Presence of Vegetable oil and sodium chloride solutions were also found to significantly increase BPA leaching. Heat catapults the dissolution of DPA in the packed food in BPA coated food containers.²⁷ Preservation of canned foods by heating at 100° C leads to an increased concentration of BPA estimated to be 1.7–55.4 times the normal concentration.²³

Effect on early Oogenesis

BPA exposure was proven to induce changes in Meiotic Chromosome behaviour. Ovary of mouse foetus when exposed to BPA at the time of development showed increased synaptic defects and number of recombination events between homologous chromosomes in cell cycle's meiotic prophase. Studies on rhesus monkeys showed a highly significant increase in mean MLH1 (MutL Homolog1) values per cell in oocytes from exposed compared with placebo treated foetuses.^[29] An increased meiotic aneuploidy in oocytes was also observed in the mouse colony when they were

accidentally exposed to BPA from the animal cages and plastic water bottles used to feed them.³⁰

Effect on gene imprinting

Mendelian inheritance supports that all inheritance is based on DNA nucleotide sequence. In nature both alleles of one gene are not equally expressed but shows differential expression of genes depending on parental inheritance. The imprints or epigenetic instructions are laid down in the parental germ cells. The embryo developing in the mother receives the signals from fetal growth genes which manifests different character in the offspring rather than dictated by genetic makeup.

Exposure to BPA in the case of pregnant, viable, yellow agouti mice lead to altered agouti expression and decreased DNA methylation at 9 CpG islands within the intracisternal-A particle retro-transposon locus in the offspring.³¹ Higher dose of BPA exposure in F1 hybrid mice generated by reciprocal mating of C57BL/6 and B6(CAST7), resulted in disruption of parental specific, monoallelic expression of the Snrpn, Igf2 and Kcnq1ot1 genes in a tissue-specific manner.

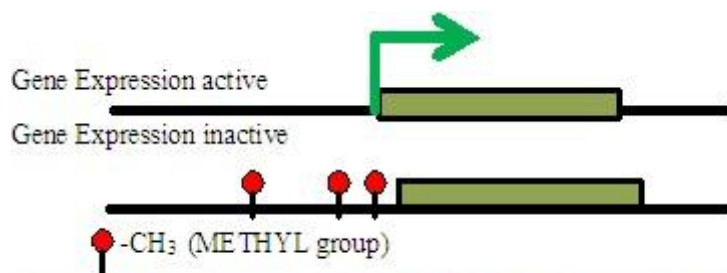


Fig. 1 shows the Loss of Imprinting (LOI) due to methylation of genes.

Higher dose of BPA exposure resulted in significantly more placentas exhibiting biallelic expression of the paternally expressed *Snrpn* gene. Loss of imprinting (LOI) as shown in Fig.1 occurred in 13/28 placentas from higher dose BPA exposed mice compared to 0/23 in controls.³²

Effect on Embryo



In experimental test mice exposed to 100 mg/kg/day BPA on a pre-implantation basis, it was seen that the exposure regimen inhibited implantation of the embryo. Further it was noted that the 100 mg/kg/day BPA exposure regimen delayed embryo transport in the reproductive tract, and also delayed early embryo development.³³ A decrease in the number of embryos was observed after administering BPA at 10mg/kg dose in experimental mice.³³



Xenopus embryo exposed to high concentration of BPA (2.5×10^{-5} M) resulted in morphological abnormalities including scoliosis and malformation of the head region. Most of the embryos did not survive the treatment and those which did were with malformations.^{34,35}

Studies on zebrafish embryos showed that BPA exposure at 5 μ M during the developmental window of 72 to 96 HPF resulted in 25 fold overexpression of *AroB* which indicated strong developmental effects on brain at the molecular, cellular and functional levels.³⁶

Effect on the mammary gland

Development of the mammary gland and the organization of its tissues are altered by exposure to BPA as shown in several animal experiments.^{14,15,16}

The perinatal BPA exposure slowed the ductal invasion of stroma¹⁴. The study further revealed the appearance of the mammary glands of the non-pregnant mice to

closely resemble that of their pregnant counterparts.¹³ Higher estradiol sensitivity in BPA exposed test animals were witnessed and a higher rate of neoplastic development were also reported in mice.¹⁵

Effect on Testis, prostate and urethra

A decrease in testis weight, of mice at 8 and 12 weeks of age when treated with BPA at an early developmental stage as compared to the control group⁹. Further it was noted that a larger effect on testis weight was seen in the low dose group than compared to the higher dose group.⁹ The wet weight of the testis in the 5 and 10 μ g/ml – treated mice was found to be significantly lower than the control group.²⁰

Low dose exposure of BPA in fetal mice results in an increased number of dorsal, lateral and ventral ducts in the prostate compared to that of control.³⁷ The volume of the paired coagulating glands were also increased^[33]. Malformations in the urethra around the neck of the bladder was also reported following exposure to low dose BPA in test mice.³⁷

Effect on Behaviour

Mice showed a temporary high aggression score after BPA treatment as they reached their sexual maturity around 8 weeks of age following exposure during fetal development.⁹ Another study reported an increased change in the maternal behaviour of mice treated with low dose of BPA. The study further characterizes the exposure pattern responsible for inducing the altered behaviour. It was reported that either exposure as a foetus or during late pregnancy, not including both, was capable of inducing maternal behavioural changes such as lower levels of nursing and higher levels of other behaviours including resting and self-grooming.¹⁰

Carcinogenic Effects

Studies conducted on rats exposed to 250 μ g of BPA prenatally, which is a significantly high dose, resulted in changes in the number of epithelial structures. An increase in the number of terminal end buds (TEB) and terminal ducts (TD) was observed in the high dose group.³⁸ Prenatal exposure to BPA has also been shown to induce preneoplastic lesions in the mammary gland. The study further points out that BPA exposed rats could be more sensitive to estrogen predisposing them to higher proliferation and reduced apoptosis leading to malignancies.³⁹ Low dose BPA exposure effects, in the human context, was reported in 2009 to be responsible for inducing proliferation of the human seminoma cells by activating the PKA and PKG signalling pathways.¹¹

It was further hypothesized that low dose BPA exposure during foetal development, contributed to “testicular germ cell carcinogenesis”.⁴⁰

Even in low dosage of BPA in neonatal exposure, induced a series of abnormalities which were carcinogenic in nature.¹¹ Progressive proliferative lesions in the oviduct was seen in all the BPA treated mice. An increased cystic endometrial hyperplasia was also observed in all of the BPA treated groups. Further a 25 % increase in the incidence of neoplastic lesion associated with stromal cell sarcomas was also noticed in the BPA in the group of 100 mice.¹¹

Further Studies

BPA is an industrially useful compound and thin film coating can be given to control its leaching. Studies on the effect of Biofilm formation, cellular changes at the time of adsorption of microbes, biological remediation of the BPA can be undertaken to minimize the extent of

hazards of endocrine disruptor in environment. The microbes in the environment are known to form Biofilm on any moist surface and are capable to uptake, metabolize and utilize most of the xenobiotic compound as carbon source to generate energy. Our laboratory is dedicated in R & D to understand Biofilm formation and cellular modification in bacteria.

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