Chapter 1

Bisphenol A: Understanding Its Health Effects from the Studies Performed on Model Organisms

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Abstract

Bisphenol A [4,4′-(propane-2,2-diyl)diphenol] (abbreviated as BPA) is a synthetic xenoestrogenic chemical and endocrine disruptor. It is a most common plasticizer that is used widely to produce epoxy resin and polycarbonate plastics, enters the living system through food and water contamination and generates health hazards. Researches are being conducted to explore the adversity that BPA exerts in living body, and for this reason, model organisms are of scientific choice. Rodents, zebrafish, *Drosophila*, nematodes, crustaceans and echinoderms are being used for monitoring the effect of BPA on their life history traits, nervous system, endocrine system, reproductive systems, behaviour, etc., which could help us to anticipate what kind of challenges BPA is putting in human life. This systematic review is focused on the latest research trend on BPA toxicity on different model organisms.

Keywords: bisphenol A, rodent, zebrafish, *Drosophila melanogaster*, invertebrates, reproductive system, life history traits, developmental defects, gene expression

1. Introduction

Bisphenol A [4,4′-(propane-2,2-diyl)diphenol] (abbreviated as BPA) is a synthetic xenoestrogenic chemical and endocrine disruptor [1–3] widely used in dentistry, food packaging and as lacquers to coat food cans, bottle-tops and water pipes since the 1960s. It is a most common plasticizer that is used widely to produce epoxy resin and polycarbonate plastics. It was first synthesized by Dianin in 1891 and was investigated for potential commercial use in the 1930s during a search for synthetic estrogens. BPA enters the living system inconspicuously through various routes, particularly through food and water contamination, and creates multitude of imperilments at cellular, molecular and genetic level. The EC_{50} and LC_{50} values of BPA range

from 1.0 to 10 mg/L (Environment Canada 2008), and BPA is declared as 'moderately toxic' and 'toxic' to aquatic biota by the European Commission and the United States Environmental Protection Agency (US EPA), respectively [4], Commission of the European Communities 1996]. Moreover, environmentally relevant concentrations (12 mg/L or lower) of BPA were also found to be harmful as far as wildlife is concerned [5]. BPA exerts its effect through direct binding to estrogen receptor (ER) in a wide range of species that includes invertebrates, fish, amphibians, reptiles, birds and mammals [6]. BPA binds both ERα and ERβ receptors, with approximately 10-fold higher affinity to ERβ [7].

The toxicokinetics of BPA exposure reveal that after oral administration in human, BPA is metabolized rapidly in the intestine and liver. BPA is not completely metabolized via Phase I reactions, but it is rapidly conjugated with glucuronic acid (Phase II metabolism) to produce non-active BPA-glucuronide in the gut wall and liver. Little amount of BPA also reacts with sulphate to form BPA-sulphate compound. The formation of BPA conjugates with other chemical moieties is a detoxification process [8, 9]. The BPA conjugates formed in the liver reach the kidney through blood circulation and then excreted in the urine with terminal half-lives of less than 6 hours [10, 11]. According to a declaration made in 2010 by U.S. Food and Drug Administration, exposure to BPA is alarming because of possible health hazards it exerts on brain, behaviour and prostate gland of foetuses, infants and children. The European Food Safety Authority (EFSA) reviewed new scientific information on BPA in the years 2008, 2009, 2010, 2011 and 2015, concluding on each occasion the known level of exposure to BPA to be hazardous. In February 2016, France announced that it intends to propose BPA as a REACH Regulation candidate substance of very high concern (SVHC).

Owing to difficulty in doing research on human subjects, researchers prefer to use model organisms to test the toxic effect of xenobiotic agents in living system. This approach is also popular in the research on BPA as the agent is ubiquitously present in our 'plastic wrapped world' and no perfect control subject could be obtained in natural environment. Several model organisms from different taxa are in use for studying the effects of BPA on their life history, morphological traits, reproductive functioning, neural functioning and behaviour. The outcome of these studies helps to anticipate the probable adversity that BPA inflicts in human body. Keeping all these factors in mind, a critical review on latest research works is presented here to understand the deleterious effects of BPA exposure on different vertebrate and invertebrate model organisms that could facilitate the understanding of human health hazards due to exposure to this xenoestrogen and endocrine disruptor BPA.

2. Vertebrate model animals

2.1. Studies on rodents

Owing to close taxonomic proximity, rodents, including rat, mice and hamster, resemble most with of humans among all other commonly used vertebrate models, and many symptoms of human conditions can be replicated in mice and rats. For that reason, rodents occupy the most preferred model animal in biomedical research, and keeping pace with this global trend, BPA researchers also rely on rodents to unravel the BPA effects on mammals.

2.1.1. Effects on reproductive system

Almost all xenobiotic agents have been tested for their toxicity in rodents to anticipate the probable effects on human body owing to taxonomic closeness of rodents and human as primate. There is extensive evidence that BPA imperils development of reproductive system in male rats and mice, although there appear to be species, strain and dose differences in the sensitivity of specific outcomes to BPA [3]. There are numerous studies of the effects of low doses of BPA on the development of the female and male reproductive organs in rats and mice. Findings include chromosomal abnormalities in oocytes in females [12, 13] and long-term effects on accessory reproductive organs that are not observed until mid-life, such as uterine fibroids and para-ovarian cysts [14]. In Newbold's study [14], outbred female CD-1 mice were treated on days 1–5 with subcutaneous injections of BPA (10, 100 or 1000 μg/kg/day). At 18 months of age, ovaries and reproductive tract tissues exhibited significant increase in cystic ovaries and cystic endometrial hyperplasia in the BPA-treated group. Progressive proliferative lesion of the oviduct and cystic mesonephric (Wolffian) duct remnants was also seen in BPA-treated groups [15].

The effect of BPA on male reproductive organs and function includes decrease in testosterone secretion [16] and sperm production [17, 18]. Impacts on other reproductive structures include reduction in the size of the epididymis at a dose of 2 ng/g and enlargement of the size of prostate ducts in the male foetuses when pregnant females were exposed to a dose of 10 μg/kg BPA/day [19, 20]. These findings are consistent with effects of low doses of positive control chemicals, such as diethylstilbestrol (DES) and ethinyl estradiol. Moreover, the testicular function impairment includes germ cell sloughing, disruption of the blood-testis-barrier and germ cell apoptosis [21, 22].

Impairment in testicular function is also evident in other studies [23, 24, 25]. The effects of BPA resemble more or less the estrogenic effects on the testes [18, 26, 27] with reduction in daily sperm production [28], deformed acrosomal vesicles, acrosomal caps, acrosomes and nuclei of the spermatids. Tohei et al. [29] reported that plasma concentration of testosterone was decreased, and LH was increased in rats after administration of BPA. Testicular content of inhibin was decreased. The testicular response to human chorionic gonadotropin (hCG) for progesterone and testosterone release was also decreased in BPA-treated rats. These results suggest that BPA directly inhibits testicular functions by disrupting the pathway of negative feedback regulation.

Studies have revealed that BPA exposure also affects the female systems, and it is found to be associated with a number of anomalies like polycystic ovarian syndrome [30], endometriosis [31] and anovulation. Studies have also been conducted to evaluate effects of BPA on development of mammary gland. *In utero* exposure to 25 and 250 μg BPA/kg body weight showed changes in the mammary glands of CD1 mice, including a significant increase in the percentage of gland ducts, terminal ducts, terminal end buds and alveolar buds at 6 months of age [32]. Perinatal exposure to 25 and 250 ng BPA/kg body weight showed increased area of terminal end buds relative to the gland ductal area [33]. Studies in both rats and mice have shown that BPA induces change in mammary gland morphology that may predispose animals to develop cancer [34, 35]. **Table 1** shows the summary of results from experiments on reproductive system of laboratory rodents.

Affected area	Model; time and route of exposure	Effect	Citation
Ovary	Mice; developmental, pellet implantation	Disruption of early oogenesis	Susiarjo et al. [12]
Ovaries and reproductive tract tissues	CD-1 mice; developmental, injection	Increase in cystic ovaries and cystic endometrial hyperplasia	Newbold et al. [14]
Mammary gland	Mice, Rats; developmental, injection, minipump	Enhanced growth and differentiation	Markey et al. [32]; Munoz-de-Toro et al. [33]; Soto et al. [13]; Durando et al. [34]; Murray et al. [35]
Testes	Mice, rats ; developmental, adult, oral, injection	Decreased testosterone secretion and sperm production ; deformed sperm with reduced motility	Akingbemi et al. [16]; Aikawa et al. [17]; Toyama et al. [18]; Al-Hiyasat et al. [23]; Chitra et al. [24]; Sakaue et al. [26]
Seminiferous tubules	C57BL/6 mice; adult, oral	Disrupted	Takao et al. [25]
Blood	Rats, adult, oral	↓ Plasma testosterone and ↑ LH	Tohei et al. [29]
Prostate gland	CF-1 mice, CD-1 mice; developmental, oral	↑ weight, ↑ prostate duct volume	Thayer et al. [27]; Timms et al. [20]
Epididymis	CF-1 mice; developmental, oral	↓ size	vom Saal et al. [19]

Table 1. Summary table of the various effects of BPA exposure on reproductive system of laboratory rodents.

2.1.2. Effects on nervous system

BPA has both indirect and direct effects on the nervous system. Since gonadal hormones in conjunction with other neurotrophins regulate cell death, neuronal migration, neurogenesis and neurotransmitter plasticity [36], BPA, in disrupting sex hormone functions, can affect brain development. Estrogen plays a major role in development and differentiation of certain parts of male and female brains. Male and female brains are exposed to different amounts of estrogen during development, and this appears to shape some regions of the brain differently. One of these regions is the hypothalamus, which controls a variety of basic functions including hunger, mood and sex drive. Due to its estrogenic and antiandrogenic activities, BPA can interfere with the dimorphic development of the neuronal networks of male and female brain regulating [37] the activation of hypothalamic estrogen or androgen receptors, testosterone-activating enzymes and hippocampal aromatase expression [38].

As BPA disrupts thyroid function, it can also affect the development of the nervous system because thyroid hormones regulate prenatal and neonatal development of the brain [39]. Juvenile hypothyroidism due to BPA exposure leads to diminutive dentritic growth in hippocampal neurons of rat brain, resulting in cognitive defects including impaired memory, defective perception and attention problems [40]. In a prenatal study [41] of brain development in mice treated with BPA in a dose 20 μg/kg, body revealed decrease in growth in the ventricular

zone of the BPA-treated offspring, whereas in the cortical plate, growth was increased. In addition, the expression of thyroid Receptor gene TRα (and other genes) was significantly upregulated in the cortical area of the BPA-treated group. BPA induces cortical plate growth via upregulation of the thyroid pathway. In doing so, BPA might have disrupted normal neocortical development by accelerating neuronal differentiation and migration. BPA exposure may also interfere with the development and expression of normal sex differences in cognitive function, via inhibition of estrogen-dependent hippocampal synapse formation in female rat [42] and testosterone-induced hippocampal synapse formation in male mice [43].

In addition, BPA may directly cause neurodegeneration. BPA enhances hydroxyl radical formation in the rat brain [44], and it is induced by 1-methyl-4-phenylpyridinium ion (MPP+) [45]. This leads to neurodegeneration of the *substantia nigra* and produces acute Parkinsons like symptoms. In this study, 10 μM BPA was infused into the rat striatum to generate OH radical, and *in vivo* micro-dialysis technique was used for evaluating toxic effects on nervous tissues. In another study [46], BPA was shown to increase intracellular reactive oxygen species at a concentration of 1, 10, 25 and 50 μmol/L and induce apoptosis at a concentration of 100 μmol/L in mesencephalic neuronal cell culture. Besides, BPA has a significant impact on the dopaminergic system and hippocampal-associated cognitive functions. **Table 2** represents the various observations on the nervous system of laboratory rodents exposed to BPA.

2.1.3. Effects on chromosomes

Recently, researches have unravelled the fact that maternal exposure to a very low dose (20 ng/g body weight) of BPA disrupts alignment of chromosomes during meiosis in the embryonic oocyte during formation of the primary follicles. This abnormality was also observed in mice that were housed in polycarbonate cages and that were provided water in polycarbonate bottles that had been damaged by exposure to a harsh detergent during washing [47]. This finding suggests that exposure to BPA during the time that meiosis resumes in the mid-cycle surge by luteinizing hormone (LH) can result in an increase in foetal aneuploidy and subsequent spontaneous abortion in humans [47]. The effect of BPA on aneuploidy has also been examined in cell culture [48–51]. In the study by Tsutsui et al. [48, 49], treatment of Syrian hamster

Affected area	Model; time and route of exposure	Effect	Citation
Brain	Mice; developmental, injection	↓ growth of ventricular zone, ↑ cortical plate growth	Nakamura et al. [41]
Hypothalamus	Mice, rats; developmental, injection	Affect sex differences in brain development	Negri-Cesi [38]
Hippocampus	Sprague-Dawley rats; adult, injection	Inhibits synapse formation at CA1 area	MacLusky et al. [42]; Leranth et al. [43]
Striatum	Rat; adult, infusion	Neurodegeneration of *substantia nigra*	Obata and Kubota [44]

Table 2. Summary table of the various effects of BPA exposure on nervous system of laboratory rodents.

embryo cells with BPA (100 μM) for 48 hours resulted in statistically significant increases in the percentage of aneuploid metaphases with chromosome losses. Reports are also available that revealed delay in the meiotic cell cycle, possibly by a mechanism that degrades centrosomal proteins and thus perturbs the spindle microtubule organization and chromosome segregation in mouse oocyte during meiosis. When cultured cells were exposed to BPA during the transition from meiosis-I to meiosis-II, a delay in meiosis-I had been observed. This transition phase usually lasts for 8–10 hours in mice, but for BPA-exposed culture, 53% of cells remained in meiosis-I. Insignificant counts of cells were found in anaphase [52].

2.1.4. Effects on behaviour

With inevitable effects of BPA on nervous system, behavioural patterns of rodents are reported to be affected by BPA exposure. An increase in defensive aggression was reported in the offspring of male Sprague-Dawley rat whose mother was offered oral BPA dose (40 μg/kg/day) throughout gestation [53]. In addition, increased aggressiveness (using a composite score of aggression) in male CD-1 mouse offspring was evident as a result of oral administration of low dose of BPA (2 and 20 ng/g of body weight) to pregnant females on gestation days 11–17 [54, 55].

A series of studies demonstrated that prenatal and neonatal exposure to BPA upregulates activities of the dopamine system and induced hyperactivity among the experimental rat [56]. Support to this primary report came from the study [57] that revealed prenatal and neonatal exposure of mice to BPA caused upregulation of dopamine D1 receptors, produced hyperlocomotion and increased rewarding responses induced by methamphetamine. Narita et al. [58] demonstrated that exposure of mice to BPA during either organogenesis or lactation, but not implantation and parturition, significantly enhanced the morphine-induced hyperactivity and rewarding effects. In a rat model, Ishido et al. [59] demonstrated that neonatal exposure to BPA (87 nmol/10 μl/rat) caused significant hyperactivity at 4–5 weeks of age, and significantly decreased gene expression of dopamine transporter at 8 weeks.

Negishi et al. [60] demonstrated that BPA impaired both passive and active avoidance learning among offspring of Fisher 344 rats that were fed a low dose of BPA (0.1 mg/kg/day orally) during pregnancy and lactation. There are also evidences of depressed maternal behaviour in female exposed [61, 62]. There are also reports by Dessi-Fulgheri et al. [63] about decrease in play behaviour of juvenile Sprague-Dawley rats due to exposure of BPA. Authors observed a masculinization of female behaviour in two behavioural categories, that is, play with females and sociosexual exploration, an effect probably mediated by the estrogenic activity of BPA in the central nervous system.

Foetal/neonatal exposure to low doses of BPA causes sex differences in brain structure, chemistry and behaviour. BPA interferes with the normal processes of sexual differentiation, with brain changes in both male and female rat and mice [61, 64]. Evidence of anatomical alterations in brain sexual differentiation was evident in male and female offspring born to mother exposed to 25 or 250 ng BPA/kg body weight per day [65]. In Fujimoto's experiment, prenatal exposure to BPA affected male rats and abolished sex differences in rearing behaviour in the open-field test and struggling behaviour in the forced swimming test. **Table 3** shows the summary of the experimental results on the behavioural aspects of laboratory rodents.

Event	Model; time and route of exposure	Effect	Citation
Defensive aggression in male	Sprague-Dawley rat, CD-1 mice; developmental, oral	Increased	Farabollini et al. [53]; Kawai et al. [54]
Hyperactivity, hyperlocomotion and rewarding response	Mice, rats; adult, developmental, oral, injection	Increased	Mizuo et al. [56]; Suzuki et al. [57]; Narita et al. [58]; Ishido et al. [59]
Passive and active avoidance learning	Fisher 344 rats; developmental, oral	Impaired	Negishi et al. [60]
Maternal behaviour in females	CD-1 mice, rats; adult, developmental, oral	Decreased	Palanza et al. [61]; Della Seta et al. [62]
Play behaviour in juveniles	Sprague-Dawley rats; developmental, oral	Decreased	Farabollini et al. [63]
Sex differences in behaviour	CD-1 mice, rats; developmental, oral	Lost	Fujimoto et al. [64]; Palanza et al. [61]; Rubin et al. [65]

Table 3. Summary table of the various effects of BPA exposure on behaviour of laboratory rodents.

2.1.5. Other miscellaneous effects

There are evidences on effects of BPA on subsequent activity of enzymes in tissues and thus metabolic processes [66–69]. Study showed very low dose (10 μg/kg) of BPA stimulates insulin production and secretion, which is then followed by insulin resistance at a dose of 100 μg/kg in mice [70]. In the study by Sakurai et al. [71], a high dose of BPA has been revealed to stimulate an increase in the glucose transporter and glucose uptake into adipocytes in cell culture. Study showed that perinatal exposure to a low dose of BPA increased adipogenesis in female rats at weaning [72].

BPA appears to possess complex immuno-modulating effects. It may stimulate or suppress the immune system. It may also alter immune response pathways. There is extensive evidence that BPA modulates both T helper 1 and T helper 2 cytokine production and alters antibody production [73–75]. Yamashita et al. [76] used immune cells from BALB/c mice and demonstrated that BPA induces innate immune response by increasing cytokine synthesis, including tumour necrosis factor (TNF) and IL-1 in macrophages, and stimulates both T and B cells in adaptive response pathway. Using IL-2 and IFN-γ as markers for Th1 response and IL-4 for Th2 response, the authors found that BPA stimulated Th1 cells to produce IFN-γ and Th2 cells to express IL-4. The authors inferred that BPA does not selectively activate the Th1 or Th2 path. BPA also enhances Th1 or Th2 response *in vivo*, depending on the doses [74, 77]. In addition, prenatal exposure to BPA was shown to augment both Th1 and Th2 responses in adulthood [74]. BPA has been reported to modulate immune function at doses between 2.5 and 30 μg/kg/day [70, 73].

2.2. Studies on zebrafish

Zebrafish (*Danio rerio*) as vertebrate model system is popular for studying developmental events. The reasons for choosing zebrafish in developmental biology research include its easy

maintenance and rearing, prolific fecundity, transparent embryo, absence of placenta that eases the study of morphological characters and even teratogenic effects on anatomy due to experimental exposure to xenotoxicants. Researchers have taken this opportunity to facilitate their understanding in the effects of BPA on vertebrate model. Summary of the results of experiments on zebrafish model is given in **Table 4**.

2.2.1. Effects on development and reproduction

Laboratory studies showed that BPA causes developmental and reproductive effects in zebrafish. There are evidences of delayed hatching, altered axial curvature and tail malformation in zebrafish embryos following exposure of fertilized eggs to BPA [78]. In a study by William et al. [79], BPA altered early dorso-ventral patterning, segmentation and brain development in zebrafish embryos at a concentration of 50 μM within 24 hours of exposure.

Effects on development and reproduction	Endpoint	Life stage and route of exposure	Effect	Citation
	Hatching, axial curvature, tail morphology	Fertilized eggs, directly in a plate	Delayed hatching, altered axial curvature, tail malformation	Hua and Lin [78]
	Early dorso-ventral patterning, segmentation and brain development	Embryo, directly in a plate	Altered	William et al. [79]
	Fertilization and egg production	Breeding adult, in aquarium	Reduced rate of fertilization, increased egg production	Laing et al. [83]
	Testes	Adult, in aquarium	Degenerated, increased number of sustentacular cells, decreased percentage of germ cells	Lora et al. [81]
	Ovary	Adult, in aquarium	Deteriorated ovarian tissues, increased number of atretic follicles, distorted and less developed oocytes	Yon and Akbulut [82]
	Transcription of genes involved in reproductive function	Adult,	Altered	Laing et al. [83]
	Oocyte maturation	Adult, in aquarium	Disrupted	Fitzgerald et al. [84]
Effects on nervous system and behaviour	Hypothalamus	Embryo, directly in culture plate	Increased neurogenesis and hyperactivity	Kinch et al. [89]
	Larval hyperactivity, Adult learning behaviour	Embryo, directly in culture plate	Increased activity, learning deficit	Saili et al. [90]
Effects on chromosomes	Oocyte maturation	Adult, in aquarium	Disrupted by chromatin modification	Santangeli et al. [85]

Table 4. Summary table of the various effects of BPA exposure on zebrafish (*Danio rerio*).

Perturbations in expression of cytochrome P450 aromatase activity have also been observed in zebrafish. Estrogen synthesized in the brain by the action of P450 aromatase is known to have organizing effects on the developing central nervous system. In fish, estrogen increases the predominant brain isoform (P450aromB), implying that xenoestrogens like BPA could act as neurodevelopmental toxicants by altering the expression of P450aromB [80].

Lora et al. [81] found several alterations in the zebrafish testes including a pronounced degeneration of all cellular components, an increase in the percentage of the Sertoli cells and a marked decrease in the percentage of germ cells due to exposure of BPA. Histological studies also showed severe deterioration of ovarian tissue such as disintegration of vesicular structures of mature oocytes, irregularities at cytoplasm, reduction in the number of primary and developing oocytes, deformation at the ooplasm and structure of the mature oocytes and irregularities at nucleolus. The number of the atretic oocytes increased due to BPA exposure. Structurally distorted and less developed oocytes were also observed [82]. A study by Laing et al. [83] documented significant increase in egg production, together with a reduced rate of fertilization in zebrafish exposed to BPA, associated with considerable alterations in the transcription of genes involved in reproductive function and epigenetic processes in both liver (vtg1, esr2b, hdac3, mbd2, mecp2 and dnmt1) and gonad tissue (esr2a, cyp19a1a and amh). Their study demonstrated how BPA disrupts reproductive processes in zebrafish. BPA can also disrupt zebrafish oocyte maturation by a novel nongenomic estrogenic mechanism [84]. BPA exerts this nongenomic estrogenic action on zebrafish oocytes directly through binding to the membrane estrogen receptor Gper and activating a Gper-dependent Egfr/Mapk3/1 pathway. BPA activates this pathway by increasing phosphorylation of Mapk3/1and cAMP concentrations in zebrafish oocytes. Activation of this pathway prevents the resumption of meiotic maturation in fish oocytes [83]. Study showed that BPA downregulated oocyte maturation-promoting signals through changes in the chromatin structure mediated by histone modifications in zebrafish [85].

2.2.2. Effects on nervous system and behaviour

Zebrafish has been used extensively to elucidate basic mechanisms underlying behavioural toxicology [86]. Zebrafish was also employed as a model for identifying sex-specific effects on social interactions induced by developmental BPA exposure [87, 88]. A study by Kinch et al. [89] revealed that treatment of embryonic zebrafish with very low-dose BPA (0.0068 μM, 1000-fold lower than the accepted human daily exposure) resulted in 180% increase in neurogenesis within the hypothalamus. Fish embryos exposed to BPA exhibit hyperactivity with ontogenetic growth possibly due to the accelerated neural growth. The authors also found that these effects are probably not due to an effect on estrogen receptors (or estrogen-like receptors) but may be due to its deleterious effects on the synthesis of key enzyme in steroid hormone synthesis, Aromatase B. This study also demonstrated that developmental BPA exposure led to larval hyperactivity or learning deficits in adult zebrafish [90]. There are evidences for temperature-specific impairment of swimming performance, disturbances in muscle activity and gene expression in zebrafish due to exposure of BPA [91]. This result suggests that BPA toxicity is compounded with the effects of climate change.

2.2.3. Other miscellaneous effects

BPA can alter sex ratio of zebrafish by inducing feminization of the fry [92]. Zebrafish embryos exposed to BPA also showed signs of feminized brains [86]. Kinch et al. [93] investigated morphological changes to developing zebrafish caused by exposure to BPA including changes in body length, pericardia (heart) and the head. Na et al. [94] observed a significant damage in the liver of zebrafish after 96 hours of exposure to BPA. This result further confirmed that liver was the target organ of BPA.

3. Invertebrate model animals

3.1. Study on *Drosophila melanogaster*

Drosophila melanogaster remains as one of the popular organism in studying the effects of BPA on eukaryotic biological system. The study on *Drosophila* includes change in gene expression profile, change in behaviour and nervous system, alteration in juvenile growth and development, history traits and fecundity and metabolism.

3.1.1. Effects on life history traits and developmental event

In comparison to other studies on effects of BPA on biological aspects in *Drosophila melanogaster*, adequate references are available on the researches on *Drosophila* life history traits. The effects of BPA on growth and development in *Drosophila* were observed, which demonstrated a statistically significant increase in larval growth for the low-dose treatment group (0.1 mg/L), but not in the high-dose treatment group (10 mg/L). BPA exposure caused an increase in body size in treated flies at 48, 72 and 96 hours following egg laying (AEL), suggesting a non-monotonic dose response. The increase in growth rate found for all treatment groups was associated with a statistically significant increase in food intake observed at 72-hour AEL. Furthermore, it was observed that the increased growth rate was coupled with an earlier onset of pupariation and metamorphosis, resulting from increased activity of insulin/insulin growth factor signalling (IIS) in *Drosophila*. Thus, this suggests that BPA exerts its effects through disruption of endocrine signalling in *Drosophila* since the timing of the onset of pupariation in *Drosophila* is controlled through the complex interaction of the IIS and the ecdysone signalling pathways. All these observations suggest that the effect is probably due to disruption of insulin-like signalling in cellular system [95].

Another study on life history traits of *Drosophila* [96] obtained some contradiction to the above-mentioned observation. The author reported a delay in both the mean pupation and the mean maturation times in treated group. In that experiment, larvae of *D. melanogaster* were exposed to three different concentrations: 0.1, 1 and 10 mg/L BPA. In the 0.1 and 1 mg/L exposed groups, the mean offspring numbers were significantly less than that of the control groups, indicating that mean fecundity was significantly decreased. Thus, administration of BPA in both food and through body wall absorption resulted in altered fecundity [96]. Mean decrease in fecundity as compared to control in *Drosophila* exposed to BPA is also evident

in the work of Atli et al. [96]. William et al. [97] have reported that BPA exposure causes inhibition of lipolysis during starvation, leading to significantly increased lipid content after 24 hours of fasting. Furthermore, it also suppresses the expression of insulin-like peptide in *Drosophila,* indicating that BPA may inhibit lipid recruitment during starvation in *Drosophila*.

3.1.2. Effects on behaviour and nervous system

BPA causes [98] behavioural modifications in *Drosophila melanogaster,* which, in turn, suggests intuitively the role of environmental risk factors for the behavioural impairments like autism and attention deficit hyperactivity disorder (ADHD) in human. The study revealed disturbance in the locomotion patterns of BPA-exposed *Drosophila* that may relate to the decision-making and the motivational state of the animal. Furthermore, an increase in repetitive behaviour and disturbance in grooming behaviour and abnormal social interaction of *Drosophila* following BPA exposure were seen.

A recent study conducted by Streifel [99] shows that administration of BPA in the prenatal environment had significant impacts on some aspects of *Drosophila* behaviour, which includes increased time spent in seeking behaviour, increased numbers of peristaltic contractions, increased linear as well as angular movement, decrease in turn angle value as well as potentially significant impacts on motor nerve morphology. These findings suggest implication of BPA as ubiquitous neurotoxin that acts upon the delicate process of neurodevelopment.

3.1.3. Effects on global gene expression profile

Alteration in gene expression profile in *Drosophila* has been studied by Branco et al. [100]. The authors reported that the effects due to BPA on genome-wide gene expression of *D. melanogaster* can be enhanced by the ingestion of high dietary sugar. The authors have found that acute and chronic exposure to BPA causes gross downfall in transcription of testis-specific genes and overexpression of ribosome-associated genes across tissues. In addition, it causes alteration of transposable elements that are specific to the ribosomal DNA loci, suggesting that nucleolar stress might implicate in BPA toxicity. This observation suggests that BPA and dietary sugar might functionally interact, with consequences to regulatory programmes in both reproductive and somatic tissues [100].

3.2. Study on other invertebrate model

As compared to vertebrates, the number of research works regarding BPA exposure on invertebrates is minimum. Invertebrates are frequently used as bioindicators for endocrine-disrupting chemicals. Research suggests that some invertebrates appear to be quite sensitive to BPA, and effects have been documented even at environmentally relevant concentrations [101].

3.2.1. Effects on life history traits and developmental events

A study conducted by Lemos et al. [102] revealed that low BPA concentrations disrupt the endocrine function of terrestrial arthropod *Porcellio scaber* by causing a sex-ratio shift. In this

study, endocrine system-related chronic effects were identified at a lower dose of BPA than the concentration having acute toxic effects on isopods, indicating impairment of molting, incomplete ecdysis.

The effects of various concentrations of BPA on the development of two sea urchin species *Hemicentrotus pulcherrimus* and *Strongylocentrotus nudus* were examined [103]. This study suggested that the sensitivity of sea urchin embryos and juveniles to endocrine disrupter chemicals changes during the stages of development. The development in the first 12 hours following fertilization up to the morphogenesis of embryo was found to be most sensitive. Even higher concentrations of BPA exposure (>300 mg/L) resulted in developmental arrest and mortality in the sea urchin *Paracentrotus lividus* [104].

Studies on lepidopteran corn stalk borer *Sesamia nonagrioides* revealed that BPA induces various developmental disorders through interfering effect in ecdysteroidal pathway [105] and over expression of heat-shock proteins [106]. Study on freshwater insect *Chironomus riparius* showed that adult emergence times were significantly delayed on moderate BPA exposure [107]. Marcial et al. (2003) and Watts et al. [108, 109] found that the marine copepod *Tigriopus japonicus* showed developmental inhibition at a very low concentration of BPA (0.1 mg/L). However, it is unclear if these effects have any long-term impacts in adult life. Experimental exposure to higher concentration of (11.4 mg/L) BPA for 1 hour caused premature larval metamorphosis in the marine polychaete worm *Capitella capitata* [110].

A study conducted on *Hydra vulgaris* by Pascoe et al. [111] pointed that the structure and physiology of polyps were adversely affected at concentrations greater than 42 μg/L BPA. Also, inhibition of regeneration ability was recorded above 460 μg/L BPA concentration. The results indicate that signalling processes necessary for the control and regulation of cell movement and differentiation during normal development, regeneration and sexual reproduction in *H. vulgaris* are not disrupted by BPA at low environmentally relevant concentrations.

3.2.2. Effects on reproductive system and fecundity

As far as published literatures are concerned, several studies have been conducted to unravel the adverse effects of BPA on reproductive systems and reproductive functioning in various invertebrate animals. In the study of Manshilha et al. [112], an increased fecundity (neonates per female), in comparison with the negative control group (100.3 ± 1.6%), was observed when daphnids were cultured and allowed to breed in the polycarbonate (PC) containers (145.1 ± 4.3%–264.7 ± 3.8%) for single and multiple generations. A strong dose-dependent ecotoxicological effect was evident, and it was suggested that BPA leached from plastic materials acts as functional estrogen *in vivo* at very low concentrations. In contrast, neonate production by daphnids cultured in polypropylene and non-PC bottles was slightly but not significantly enhanced (92.5 ± 2.0 to 118.8 ± 1.8%). Multigenerational tests also demonstrated magnification of the adverse effects, not only on fecundity but also on mortality of the species. Reproductive impairment in Daphnia due to exposure to BPA is also evident in the study by Tišler et al. [113].

Andersen et al. [6] found an increase in egg production in copepod *Acartiatonsa* exposed to 20 μg BPA/L. Moreover, inhibition in normal development at BPA concentrations above

environmentally relevant levels (100 mg/L) was also evident. At extremely high exposures (16,000–80,000 mg/L), abnormal growth and inhibition of gemule germination was found in freshwater sponges *Heteromyenia sp.* and *Eunapius fragilis* [114]. A study conducted by Oehlmann et al. [115] on freshwater snail *Marisa cornuarietis* and of the marine prosobranch *Nucella lapillus* revealed that BPA affects the reproductive system and has a negative impact on snails even at nominal concentration, that is, 1 μg/L. Affected *Marisa* females were designated as 'superfemales' and were characterized by the presence of additional female organs, hyperplasia of the accessory pallial sex glands, malformations of the pallial oviduct causing increased female mortality and a strong stimulation of oocyte and spawning mass production. In these follow-up studies, Oehlmann et al. [116] tried to bridge several gaps in knowledge by conducting additional experiments. Here, the authors confirm the previous results and additionally conclude that the occurrence of superfemales is associated with adverse effects on reproduction and survival, even at sub-micrograms per litre concentrations of BPA (NOEC, 7.9 ng/L; EC10, 13.9 ng/L). However, if snails are exposed to BPA under conditions that maximize the reproductive output, particularly during the spawning season or at elevated temperatures, the induction of superfemales is at least partially masked. The superfemale induction is probably mediated by binding of BPA with estrogen receptor, because the response can completely be reversed by coexposure to potent estrogen inhibitors. Furthermore, the extreme BPA sensitivity of *M. cornuarietis* and other prosobranch snails probably due to higher affinity of the compound for the estrogen receptor in this species was compared. Overall, the results suggest that BPA imposes a potential hazard for prosobranch population in the field even at environmentally relevant concentrations. Experimentally determined EC_{50} values of BPA for different invertebrate model organisms have been given in **Table 5**.

3.2.3. Effects on gene expression profile

Change in expression pattern of genes and alteration in RNA expression pattern due to BPA exposure are also within the scientific interest. Planelló et al. [117] studied the effects of BPA on the expression of some selected genes, including housekeeping, stress-induced and hormone-related genes in *C. riparius* larvae. They found that exposure to BPA at a concentration of 3 mg/L for 12–24-hour exposure did not influence the levels of ribosomal RNA or those

Species	EC_{50} (mg/L)	NOEC (mg/L)	Reference
Waterflea Daphnia magna	10.2	4.1	Alexander et al. [4]
Mysid Mysidopsis bahia	1.1	0.51	Surprenant [123]
Chironomid Chironomus tentans	2.7	1.4	Mihaich et al. [124]
Copepod Tigriopus japonicus	4.32	3.5	Marcial et al. [108]
Snail Marisa cornuarietis	>4.03 (LC_{50})	1.32	Mihaich et al. [124]
Snail Marisa cornuarietis	2.24 (LC_{50})	1.18	Mihaich et al. [124]

Table 5. Summarized presentation showing experimentally determined effective concentration (EC_{50}) and no effect concentration (NOEC) of BPA on different invertebrate animals [103].

of mRNAs for both L11 or L13 ribosomal proteins which were selected as representative of housekeeping genes involved in ribosome biogenesis. Nonetheless, BPA treatment induced the transcription of the HSP70 gene. Interestingly, BPA causes significant increase in transcript of the ecdysone receptor (EcR), suggesting that BPA can selectively affect the expression of the ecdysone receptor gene suggesting a direct interaction with the insect endocrine system.

Significant level of DNA strand break has been detected in snail *Potamopyrgus antipodarum* under exposure to BPA [118]. DNA-damaging effect of BPA on aquatic insect *C. riparius* has also been reported by Martinez-Paz et al. [119].

4. Conclusion

Bisphenol-A (BPA), found ubiquitously in our environment, has received a tremendous amount of attention from research scientists, government panels and the popular press. Extensive investigational work has been and is still being carried out in various fields like: (1) mechanisms of BPA action; (2) levels of human exposure; (3) routes of human exposure; (4) pharmacokinetic models of BPA metabolism; (5) effects of BPA on exposed animals and (6) links between BPA and cancer. BPA interferes with hormone signalling via two mechanisms: altering the availability of ovarian hormones and altering binding and activity of the hormone at the receptor level [120–122].

Besides understanding the probable human health hazards, study of BPA effect on model organisms facilitates our concern to the issues like biodiversity loss, environmental degradation and overall imbalance in ecological functioning. Today's world is extremely dependent on plastics, and this dependency inevitably brings the challenges of BPA exposure to the environment. Invertebrate and vertebrate fauna from terrestrial and aquatic ecosystems get affected equally, and the situation is going worse every day. Tantalizingly, the role of BPA in biodiversity loss is not being analysed when the issue comes on the table for discussion. So, mass awareness is to be build up among the people that include students, scholar, academician, conservationist, wildlife activist, NGOs working with environmental issues, policy-makers and politicians across the nation. It is hard to make BPA free world, but the extent of its adverse effect could be mitigated by our concern and consciousness.

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