

ORIGINAL RESEARCH ARTICLE

Estrogenic Effects of Bisphenol-A and Octylphenol on Reproductive Health of Male Albino mice

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Received 09 Nov 2012; Revised 08 Feb 2013; Accepted 20 Feb 2013

ABSTRACT

The present study was carried out to evaluate the estrogenic effects of Bisphenol-A and Octylphenol in reproductive health of male albino mice. The albino mice of equal weights are taken into five groups, group I mice were left untreated, group II receives vehicle control, group III received Octylphenol and group IV received Bisphenol-A both with the amount of 80mg/day/kg body weight and group V received 17- β estradiol in amount of 120 μ g / kg / day .These compounds were administered subcutaneously in a volume of 100 μ l for 7 consecutive days. Significant decrease in the weight of Testis, Epididymes and Sperm count were observed in the mice treated with the two industrial chemicals and the effects were similar with that of 17- β Estradiol. The results showed estrogenic property of Bisphenol-A and Octylphenol.

Key words: Bisphenol-A, Octylphenol, Estradiol, Sperm, epididymes, testis.

INTRODUCTION

Our environment is a sink of large numbers of chemicals. Many of these can disturb different physiological systems of animals as well as human body. Many of these chemicals have teratogenic effect while some have adverse effect on reproductive system, endocrine & immune system of the adult body. There are thousands of sources of these chemicals of which are industrial source, mining areas, many cosmetics, detergents, plastics etc. Even now-a-days each house becomes a store house of millions of chemicals.

Among these chemicals some act adversely on the endocrine system of the body, because they act as hormone mimic. Some of them can block the action or synthesis of natural hormones produced by endocrine glands, these chemicals are called Endocrine disruptors as they disturb or disrupt the normal functioning of the body. They are the exogenous chemicals those interfere with the normal function.

Numerous natural and artificial chemicals might act as endocrine disruptors causing the regression of male reproductive abilities. One of such chemicals, 2,2-bis (4-hydroxyphenyl) propane (Bisphenol-A; BPA), is a monomer of polycarbonate plastics used in a coating on food

and drink packages and also in dental sealants. Similarly composites and sealants used in dentistry release BPA monomers in to saliva (Olea *et al.*, 1995).

A growing concern is that BPA melts at high temperatures achieved by boiling and microwaves and thus, can easily be consumed. Some studies have shown that BPA has xenoestrogenic and anti-androgenic effects on “*in-vitro*” systems. However, result concerning its xenoestrogenic effects on “*in-vivo*” mammalian bodies have been unclear.

The estrogenic compounds that occurred at concentrations in effluents nonylphenol and Octylphenol. These are break down product of surfactants which has been used in detergent & household products and spermicides. Vom Saals (2000) asserts that this chemical is not fixed in plastic forever.

These studies also implicate that BPA is causing the increase in prostate enlargement seen in men. In US, 65% of men at the age of retirement have enlarged prostate glands and 40000 men die of prostate cancer each year.

Numbers of works have been done in this field to evaluate possible effects of hormone mimics or

estrogen mimics on the health of human and wildlife and other animals.

But in India Research is still in infancy. Seeing the increase use of chemicals having endocrine disrupting effects potency of these chemicals and their impact on health must be evaluated. The present study tried to evaluate the possible Estrogenic effects of two environmental chemicals Bisphenol-A and Octylphenol The non-steroidal estrogenic compound Bisphenol-A (BPA) is a monomer used in the manufacture of polycarbonate plastics and resins. Bisphenol-A may be ingested by human as it repeatedly leaches from the lining of tin cans into food from dental sealants into saliva and from polycarbonate bottles into their content, because Bisphenol-A is weakly estrogenic approximately 10,000 fold less potent than 17 β -estradiol (Milligan *et al.*, 1998). The ester bonds in these Bisphenol-A based polymers are subjected to hydrolysis. Leaching of BPA has led to widespread human exposure. Many Evidences of decreasing quality of semen during the past 50 years have been found (Carlsen.E. *et al.*, 1992).

The rate of leaching of BPA from food and beverages containers leading to widespread human exposure. Median BPA level in human blood and tissue including in fetal blood is higher than the level that cause adverse effects on mice. BPA advanced the onset of puberty (Howdshell *et al.*, 1999) and altered the mammary gland development in mice (Markey, *et al.*, 2001). Adverse effects of BPA on human and animal reproduction are suspected, because it is now well established that BPA is an estrogenic compound (Soto and Sonnenschein *et al.*,)

It has been claimed that BPA acts the same manner as female hormone. BPA is present in reusable food contact plastic and migrate to food (Biles, J. E, Mc Neal, *et al.*, 1997). Bisphenol-A (BPA) has been reported to have some xenoestrogenic effects on the reproductive system of male animals. The body growth showed no significant difference between BPA-administered and control birds when treated with BPA. However, the weight of the combs and testes were lower in the BPA-treated birds. Histologically, testes of the control birds were well matured; the seminiferous tubuli were filled with sperm. In contrast, the testes of most of the BPA-treated birds showed an immature appearance with smaller seminiferous tubule and limited spermatogenesis. These findings suggest that the

xenoestrogenic property of BPA might disturb the growth of the comb and testes of male chickens by a possible endocrine disrupting mechanism (Furuya, M., 2003).

BPA produces identical effects to those produced by estradiol on rat uterus and vagina. Because BPA is having estrogenic activity and estrogens have receptor on uterus and vagina and other reproductive organs. Vagina was particularly sensitive to the chemical (Steinmetz R. *et al.*, 1998). The xenoestrogen BPA induces growth differentiation and C-fos gene expression in the female reproductive tract in vagina, uterus etc. (Steinmetz, R. *et al.*, 1998). In male BPA has an effect on the size of the reproductive organs of male and daily sperm production and behaviour. These may be malformation of the male offspring following maternal exposure to estrogenic chemicals (Gupta Ms. *et al.*, 2000).

Injection (sc) of Bisphenol -A (50 μ g/animal, about 15-20 mg/kg/day) for the first 5 days after birth resulted in a decrease in the percentage of moving sperm, and and increase in the incidence of mal formed sperm, in the epididymis of mice at 10 weeks of age, although no marked changes were found in the testicular histology between BPA-treated and vehicle-treated control mice.(Aikawa *et al.*, 2004).

The *in-vitro* and *in- vivo* studies have proved that octylphenol and Bisphenol-A both have estrogenic activity. In recent years , the estrogenic activity of environmental estrogen responses has clearly demonstrated in a number of *in vitro* systems. A number of these *in vitro* estrogenic responses have been adopted as assay system to measure estrogenic compounds(ex-Ishikawa assay, Yeast assay, MCF-7). It is reported that BPA, OP have estrogenic activity (White *et al.*, 1994) although their affinity for estrogen receptor is atleast 10,000 times lower that of estradiol. They are estrogenic when they are in high concentration. In an acute mammalian assay using ovarioectomized mice Milligan *et al* reported that bisphenol A is about 10,000 fold less potent than Estradiol and octylphenol and nonylphenol are about 100,000 fold less potent than estrdiol. But in another study it was reported that BPA was only 100-500 fold less active than estradiol. In Yeast screen containing human androgen receptor shows that Bisphenol A act as anti androgen. (Sohani and Sumpter, 1998).

Former studies shows that injection of 4-tert-octylphenol at the dose level 80mg/kg body

weight to 2 months old male rats 3 times weekly for 25-28 days resulted in marked decrease in the weight of testis, epididymes and accessory sex organs. (Boockfor and Blake)

MATERIALS AND METHODS

Chemicals:

All materials, chemicals, laboratory equipments necessary to complete the experiment were supplied by the department of Zoology, Gauhati University. Bisphenol-A and Octylphenol were a generous gift from Prof Stuart Richard Milligan of King's College, London.

Experimental animal:

The animal that used in the experiment was Albino mice of strain H3R-He from the animal house of Department of Zoology, Gauhati University. Experiment was conducted using healthy adult Albino male mice of approximately equal weight (25g). Besides animals, all lab equipments centrifuge, colorimeter, water bath, microtome all other equipments test tubes, haemocytometer all were supplied by the department.

Animal procedures:

In-vivo technique was applied to detect the estrogenicity of BPA and octylphenol.

The two test chemicals BPA and OP were administered to male albino mice and estrogenic effect were observed in reproductive organs and also in liver.

Preparation of the test chemicals:

The two test chemicals were prepared in ethanol and diluted with Olive Oil, containing 10% alcohol for required dose of subcutaneous injection. Bisphenol A was prepared for injecting 80mg/day/kg of body weight and octylphenol was prepared for injecting 80mg / day / kg of body weight for subcutaneous injection to albino mice.

Preparation of 17 β estradiol:

17- β estradiol is prepared in ethanol. A stock solution of 1mM is prepared and diluted with Olive Oil. The estradiol was prepared for injection of 120 μ g / kg / day.

Administration of the test chemicals:

The compounds are administered by subcutaneous injection in the thigh region (Kalita, 1998) in a volume of .1ml (100 μ l) at 24 hrs of interval for 7 consecutive days.

Design of the Experiments:

Before the experiment the albino mice were taken and are grouped into 5 groups.

Group I and II are control (untreated) and vehicle control (ethanol mixed with olive oil 1:10 ratio). Group III are receiving Octylphenol, Group IV

received Bisphenol A while, Group V received estradiol (E_2) or positive control. The test compounds are administered subcutaneously at 24 hrs interval for 7 consecutive days. At 24 hrs of 7th or last treatment mice were weighted and sacrificed by cervical dislocation.

After injection and cervical dislocation mice were dissected and first of all testes and epididymes along with vas deferens were taken out carefully, trimmed of fat and observed for any seen abnormalities then took the weight in Sartorius to get accurate result. To observe the effect of the chemicals on sperm count, had collected the epididymal fluid with Gilson pipette of 1 μ l from the head of the epididymis and diluted with .5ml of distilled water and sperms were counted by taking 1 μ l of diluted fluid in haemocytometer chamber (both upper & lower) and counted in each square.

RESULTS

Experiment 1:

The effect of BPA, OP and Estradiol on adult male mice – effect on the weight of testes.

We have taken the averages value of right and left testis and values are plotted in mean \pm SEM (n=6).

Table 1: The effect of BPA, OP and Estradiol on weight of testis in adult male mice

S. No	Experimental groups	Weight of Testes (mg) mean \pm SEM
1	Group-I (Control or untreated)	82.8 \pm .71
2	Group-II (Vehicle or Olive Oil control)	88 \pm 1.36
3	Group-III (Octylphenol treated)	74 \pm 1.27
4	Group-IV (Bisphenol-A treated)	54.7 \pm 1.34
5.	Group-V (Estradiol treated)	46 \pm 1.7

Results in the (Table 1) showed that testes weights were reduced significantly in all the mice treated with E_2 , BPA, OP relative to the controls. In the animals treated with E_2 testes weight were reduced markedly than BPA treated mice and OP treated mice.

Experiment 2: The Effect of BPA, OP and Estradiol in the weight of epididymes.

Table 2: The Effect of BPA, OP and Estradiol in the weight of epididymes, values are plotted in mean \pm SEM (n=6).

S. No	Experimental Groups	Weight of Epididymes (mg) mean \pm SEM
1	Group-I (Control or untreated) (n=6)	29.4 \pm .742
2	Group-II (Vehicle or Olive Oil control) (n=6)	28.03 \pm .462
3	Group-III (Octylphenol treated)	24.5 \pm .275
4	Group-IV (Bisphenol-A treated)	19.45 \pm .59
5	Group-V (Estradiol treated or positive control)	18.31 \pm .72

Results in the (Table 2) showed that epididymes weight was reduced significantly in all the mice

treated with E₂, BPA, OP relative to the controls. In the animals treated with E₂ epididymes weight were reduced markedly than BPA treated mice and OP treated mice.

Experiment-3 Effects of BPA, OP and Estradiol on sperm quantity and count.

Sperm count was done using haemocytometer and the average counting of upper and lower chamber has been taken. 1 μ l of epididymal fluid was diluted to .5ml or 500 μ l distilled water.

Dilution factor = 500. And from this diluted fluid 1 μ l is taken in the haemocytometer chambers.

Table 3.1: The effects of BPA, OP and Estradiol in the sperm count

S. No	Experimental Animal	Average value of both chambers
1	Group-I (-)Control	75
2	Group-II (Vehicle control)	72
3	Group-III (OP treated)	58
4	Group-IV (BPA treated)	60
5	Group-V (Estradiol treated (+) control)	36

Table 3.2: Total sperm in 1 μ l fluid (epididymal)

S. No	Experimental Group	Sperm in 1 μ l x dilution factor DF = 500 μ l
1	Group-I Control (-)	75 x 500 = 37500
2	Group-II (Vehicle control)	72 x 500 = 36000
3	Group-III (Octylphenol)	58 x 500 = 29000
4	Group-IV (BPA)	60 x 500 = 30000
5	Group-V (Estradiol)	36 x 500 = 18000

Results in the (Table 3.1 & 3.2) showed that sperm number in epididymal fluid were reduced significantly in all the mice treated with E₂, BPA, OP relative to the controls. In the animals treated with E₂, sperm numbers in epididymal fluid were reduced markedly than BPA treated mice and OP treated mice.

The results of the present study showed reduction in testis and epididymis weight after administration of 120 μ g/kg bw of E₂, 80mg/kg of bw of BPA and 80mg/kg of bw of OP. Reduction of weight of testis and epididymis were more prominent in Estradiol treated animals. The present results were in agreement with the findings of other workers. The present results shown a marked decrease in the weight of testis of albino male mice treated with Estradiol, Bisphenol-A and Octylphenol but the effect of Estradiol was much stronger than the effects of BPA and OP. Steinmetz *et al* postulated that the potency of bisphenol-A was 1000-5000 fold less than Estradiol *in vitro*. It is reported that BPA, OP have estrogenic activity (White *et al.*, 1994) although their affinity for estrogen receptor is atleast 10,000 times lower that of estradiol

DISCUSSION

Effects of BPA and OP on male albino mice:

Exposure to different environmental chemicals had far greater impact on the male reproductive system. They are associated with different reproductive abnormalities like feminization, reduced fertility, impaired hormone secretion in wildlife (Colborn *et al.*, 1993). BPA and estrogenic chemicals have large effect on the size of reproductive organs, daily sperm production and behaviour (Vam Saal *et al*). Since it is not possible to evaluate all these effects at a time, The present study was designed to observe the effects of BPA and OP on the following reproductive characteristics – the weight of testes and epididymes, sperm count Histological changes in liver.

The results of the present study showed reduction in testis and epididymis weight after administration of 120 μ g/kg bw of E₂, 80mg/kg of bw of BPA and 80mg/kg of bw of OP. Reduction of weight of testis and epididymis were more prominent in Estradiol treated animals. The present results were in agreement with the findings of other workers. The present results shown a marked decrease in the weight of testis of albino male mice treated with Estradiol, Bisphenol-A and Octylphenol but the effect of Estradiol was much stronger than the effects of BPA and OP. Steinmetz *et al* postulated that the potency of bisphenol-A was 1000-5000 fold less than Estradiol *in vitro*. It is reported that BPA, OP have estrogenic activity (White *et al.*, 1994) although their affinity for estrogen receptor is atleast 10,000 times lower that of estradiol. The present study had taken one concentration for each chemical, therefore could not evaluate dose-effect relationship. Boockfor and Blake (1997) reported that injection of 4 tert-octylphenol at the dose level 80mg/kg bw to 2 months old male rats 3 times weekly for 25-28days resulted in marked decrease in the weight of testis, epididymis and accessory sex organs. Sharpe *et al* (1995) found reduction in rat testicular weight and sperm production rate with relatively high exposure levels of environmental estrogenic compound i.e. OP (10-1000 μ g/l). Fisher *et al* (1999) reported that the neonatal exposure to high levels of BPA and OP caused deleterious changes in testis weight and other end points. They hypothesized that prolonged exposure to exogenous estrogen during both fetal and postnatal life could reduce sertoli cell number and thus reduced sperm output in adult life.

In the present studies, found a marked decrease of sperm number in Estrodiol treated male mice. Sperm number in BPA and OP treated mice also showed reduction but the effect is lower than estrodiol or E₂ treated mice. Exposure to these chemical resulted in significant reduction in testis size and a corresponding decrease in daily sperm production. Blake C.A, Boockfor F.R. reported that chronic administration of the environmental pollutant 4-tert-octylphenol to adult male rats interferes with the secretion of luteinizing hormone, follicle stimulating hormone, and testosterone.

BPA also act as a anti androgen blocking the action of dihydrotestosterone in Yeast Screen containing androgen receptor (Sohani & Sumpter 1998). Inhibition of testicular steroidogenesis by the xenoestrogen BPA is associated with reduced pituitary lutenizing hormone secretion and decreased steroidogenic enzyme gene expression in rat leydig cells. Testosterone production increase rapidly under the stimulus of anterior pituitary gonadotropin hormones at the onset of puberty. Decrease in LH also decreases the synthesis of testosterone by the testis. The androgens, principally testosterone and DHT are involved in sexual differentiation, spermatogenesis and development of secondary sexual organs, anabolic metabolism and gene regulation and male pattern behavior (Wilson, J.D *et al*, 1983).

Testosterone is essential for spermatogenesis, therefore decrease in LH leads to decrease in spermatogenesis or sperm production.(Guyton,). As from previous reports it was cleared that Estradiol exerts a local negative feedback effects on the multiplication and differentiated of leydig cells and on the capacity of differentiated leydig cells to synthesize testosterone (Guyton & Hall, 1996) and Estradiol may also play a role in negatively regulating the synthesis and release of gonadotropins (LH and FSH). Due to this reason, In adult testes estradiol appears to be an important local regulator of leydig cell number and function . Because of these physiological roles of estradiol, exposure to exogenous estrogen like Bisphenol-A and Octylphenol may have the potential to reduce the number and function of both sertoli cells and leydig cells and any other functions (musculinization and spermatogenesis) dependent on these cells (Sharpe *et al.*, 1994). Now from above study it is proved that BPA, OP are responsible for the

reduction of weight of testis, epididymis and are also responsible for reduction of sperm count. Experiments involving exposure of rats to various xenoestrogen during both fetal and postnatal life could reduce sertoli cell number and thus reduced sperm output in adult life. Exposure involving exposure of rats to various xenoestrogens during the period of sertoli cell multiplication showed that in adult life such exposure resulted in small (8-12%) but highly significant reduction in testis size and a corresponding decrease in daily sperm production (Sharpe *et. Al* 1995). Sharpe *et al* (1995) hypothesized that changes in testis weight are very useful to gauge the overall effects of treatment that correlate with daily sperm production. Such findings suggested that there was the theoretical possibility might have contributed to the decline in sperm counts in men as investigated by many workers (Bostofe *et al* 1983; DEPA, 1995). The epididymis act to store and mature the sperms (Cooper, 1996). In the present study it was confirmed reduction in testis weight and spermatogenesis was responsible to the reduction of the weight of epididymes. . This study has focused on the potential effects of Bisphenol-A and Octylphenol (OP) on the reproductive health of male albino mice. Environmental estrogens influence many aspects of mammalian reproduction and many other physiological, behavioral and anatomical functions. From the animal experimentation and studies on human, it has been established that endocrine disruptors may pose problem to the health of human and animals. Though a number of studies have been done with these chemicals by different scientist, but still there are confusion regarding the exposure of man and animals to these chemicals and the risk of exposure. It is now a very essential need to assess the contamination level of these chemicals in our environment and their effects on different organs and physiological process of our body keeping this in mind, In the present studies the estrogenic effects of BPA and OP on reproductive health were investigated *in vivo* using male albino mice.

CONCLUSION

Both BPA and OP act like estradiol but their potency is lower than that of endogenous estrogen, 17 β estrodiol. Both the chemicals have reduced the weight of testes and epididymes. Exposure to BPA and OP resulted in decrease in sperm number in epididymal fluid.

ACKNOWLEDGEMENT

Authors are grateful to the Department of Zoology, Gauhati University for providing laboratory facilities and supplying albino mice for the study.

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