Bisphenol A Exposure Causes Prolactin Imbalance and alters Progesterone Functions in Rats
Chinenye E. Oguazu1*, Francis C. Ezeonu1, Enemali, M.O1, Kingsley I. Ubaoji1, Dike C. Charles1

1Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University, Awka - Nigeria

Abstract
The associations between Bisphenol A (BPA) exposure with prolactin and progesterone hormone levels is been considered globally. A three months study was conducted with female albino rats exposed to BPA. The blood samples were collected for assay of prolactin (PRL), and progesterone (PROG). A significant positive association between increased BPA concentration and higher PRL and PROG levels were observed. The results suggest that BPA exposure may lead to alterations in female reproductive hormone levels.

Keywords: BPA, prolactin, progesterone, exposure, chronic.

INTRODUCTION
Endocrine disrupting chemicals (EDCs) are a cluster of chemicals which can affect the endocrine system, including effects on hormone synthesis, secretion, or metabolism in the body. One such chemical, Bisphenol A (BPA), has brought about more and more concerns due to its wide spread exposure and potential harmful effects to human health (Burridge 2003, Vandenberg et al. 2007). BPA is a constituent of polycarbonate and epoxy resins, which may be used as the lacquer lining of food and beverage cans, and some dental sealants and composites. As a result of exposure from dietary and other sources, most people have BPA detected in their urine, despite their various lifestyles (Geens et al. 2012). BPA enters the body by the ingestion of contaminated food or beverages. It leaks from polycarbonate plastics, which are used to line food and drink containers such as bottles and cans. Further minor ways of penetrating into the body are through the skin e.g. contact with thermal receipts (Ehrlich et al. 2014, Liao and Kannan 2011) or inhalation e.g. cigarette smoke or dust (Braun et al. 2011, He et al. 2009).

Accumulating literature documents the alterations of circulating reproductive hormone concentrations following BPA exposures in animal models (Wetherill et al. 2007). Particularly, these effects can be observed at environmentally relevant low dose (Yang et al. 2011). In female rodents, effects of BPA prenatal exposure on the oocyte, developing reproductive tract, and timing of sexual maturation were observed (Calafat et al. 2008). Similarly, BPA has been linked to several endocrine disorders including precocious puberty, hormone dependent tumors such as breast and prostate cancer, and several metabolic disorders including obesity, diabetes, and polycystic ovary syndrome in human studies (Geens et al. 2012). BPA is capable of inducing toxic effect on non-reproductive vital organs; several studies have reported that absorption of BPA has caused extensive damage to the liver and kidney (Ezeonu et al. 2015, Oguazu et al. 2015). Human studies demonstrating BPA’s effects on circulating levels of reproductive hormones were limited and inconclusive, particularly for women (Nanjappa et al. 2012, Alonso-Magdalena et al. 2012, Vandenberg et al. 2009). A significant and positive relationship was reported between BPA exposure and circulating androgen concentrations (Steinmetz et al. 1997). An association between BPA and miscarriage has also been reported, indicating BPA’s possible disturbance to hormone homeostasis (Aldad, et al. 2011, Goloubkova et al. 2000).

In the present study, the associations between BPA level and concentrations of serum prolactin (PRL), and progesterone (PROG) was monitored.

MATERIALS AND METHODS
Non-pregnant female rats of age 5 weeks were acclimatized in the laboratory for seven days and randomly divided into eleven (11) groups experimental of 10 rats each and respectively administered 0.05, 0.1,
0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg of BPA/kgbw/day. The first group which served as control did not receive any treatment but distilled water instead. The graded doses of BPA were dissolved in distilled water and administered by oral gavage using intubation canular. Blood were obtained from the tail of the various groups by capillary action weekly, after BPA administration for thirteen (13) weeks. Blood samples were processed for clinical assay.

Animals were housed in aluminum wire-mesh cages in a well-ventilated animal house with a 12 h dark/light cycle and at room temperature and were provided commercial rat pellets (Vital feed from vital group of Company, Nigeria) and water ad libitum.

At the end of the experiments serum prolactine and progesterone were assayed using Chemwell 2910 Auotanalysier. All reagents were commercially obtained as already prepared kits. The kits for prolactine and progesterone were purchased from Egyptian company for Biotechnology (SAE) Cairo Egypt. Individual tests were carried out according to the kit specifications.

Differences between obtained values (mean ± SD) were carried out by one-way analysis of variance (ANOVA) using SPSS software version 20.0 followed by the Tukey-Kramer multiple comparison test. A P≤0.05 was taken as a criterion for a statistically significant difference.

RESULTS
Effect on prolactin

There is a significant increase in the prolactin level when compared with the control at p≤0.05. Groups 0.2mg/kg, 0.4mg/kg to 0.6mg/kg and 0.8mg/kg to 1mg/kg showed consistent rise in prolactin level over time (fig. 1a). Group 0.05mg/kg and 0.1mg/kg showed no statistical significant difference in the levels of prolactin week 7 to 9 period of exposure (fig.1b). Group 0.3mg/kg showed an initial increase at the onset of the experiment (week-1) and decreases through the weeks to as low as 0.06±0.008 at week 6 even below that of the control (fig.1a and 1b) then began to rise to its peak at 1mg/kg. An increase in prolactin was demonstrated throughout the weeks of administration (fig 1a, 1b and 1c), while the change in prolactin level for groups 0.8mg/kg and 1mg/kg tends to be constant throughout the duration of the study, other groups showed manifestingly steady increase in the course of time with group 6(0.5mg/kg) revealing the best time dependent incremental profile (see fig.1). Prolactin increases induced by group 1 and 2 are not statistically significant at week-7, 8 and 9.

From fig.1a, it was observed that after the administration of BPA at weeks-1 and 2, the effect shown by the entire dose group followed the same pattern. There is an increase from 0.05mg/kg to 0.1mg/kg; a decline at 0.2mg/kg group, a rise at 0.3mg/kg, a decline at 0.4mg/kg, an increase at 0.5mg/kg, another decrease at 0.6mg/kg, an increase through 0.7mg/kg to 0.8mg/kg, another decline at 0.9mg/kg and finally a rise at 1mg/kg. At week-3, it was observed that there was an increase at 0.05mg/kg to 0.1mg/kg, that decrease at 0.2mg/kg, then rises through 0.3mg/kg to 0.5mg/kg, and decline through 0.6mg/kg to 0.7mg/kg, rise at 0.8mg/kg, declined at 0.9mg/kg and rise to peak at 1mg/kg (fig.1a). At week-4, an increase was observed at 0.05mg/kg to 0.1mg/kg that decrease at 0.3mg/kg, rise through 0.4mg/kg to 0.5mg/kg, another drop through 0.6mg/kg to 0.7mg/kg that rise at 0.8mg/kg, drop again at 0.9mg/kg and finally peaks at 1mg/kg (fig. 1a).

Week-5 to 8, showed high prolactin level at 0.05mg/kg dosage group that decrease at 0.1mg/kg and spiked high at 0.2mg/kg. This was followed by a decline at 0.3mg/kg with its lowest value at week-6 (also see fig. 1b); then a spike increase of prolactin level was observed at 0.4mg/kg to 0.5mg/kg, which decrease through 0.6mg/kg to 0.7mg/kg and rise again at 0.8mg/kg, dropped at 0.9mg/kg and increase at 1mg/kg (fig 1a).

Week-9 to 13 (fig. 1c), showed a dose dependent increase in serum prolactin concentration after the administration of BPA from 0.05mg/kg to 0.2mg/kg group that declined at 0.3mg/kg and rise through 0.4mg/kg to 0.5mg/kg; it then decreased again through 0.6mg/kg to 0.7mg/kg and another dose dependent increase through 0.8mg/kg to 1mg/kg (fig. 5a).

The dose group 0.8mg/kg and 1mg/kg of BPA showed no time dependent effect (fig. 1a -1c). The group that received 0.5mg/kg body weight of BPA, showed a clear and pronounced time dependent effect (fig. 1b). Other dose groups showed time effect that is not time dependent; week-1 exhibited the maximum effect for the dose group 0.05mg/kg and 0.1mg/kg while week-6 showed the least effect for dose group 0.3mg/kg and 0.7mg/kg (fig.1b).
Fig-1a: Chart of serum prolactin concentration against durations of BPA exposure for week 1 to week 4

Fig-1b: Chart of serum prolactin concentration against durations of BPA exposure for week 5 to week 8

Fig-1c: Chart of serum prolactin concentration against durations of BPA exposure for week 9 to week 13
**Effect on progesterone**

There is a significant dose dependent increase in the progesterone level when compared with the control at \( p \leq 0.05 \). Only animals administered 1mg/kg (1000µg/kg) showed a consistent increase in progesterone levels (fig 2a). At group 0.4mg/kg, 0.5mg/kg, 0.8mg/kg and 0.9mg/kg, it was observed that there is fluctuation during the various weeks of administration (fig 2b). At other dose range there were marginal increases in progesterone levels which show some dose dependent relationship but bear no sensitivity to time (see fig 2b). Group 0.5mg/kg, 0.8mg/kg and 0.9mg/kg shows a non-significant decrease at week-7 and 8 (fig, 2c), week-1 and 2 (fig. 2a), and week-5, 6 and 7 respectively (fig 2b and 2c).

It was observed that at week-1 to 13, there was a dose dependent increase from 0.05mg/kg to 0.3mg/kg dose group (fig.2a – 2c). After which there was a decline in the progesterone levels by 0.4mg/kg dose group except at weeks-8 and 10, where its values (0.4mg/kg) is equal and higher than that of 0.3mg/kg group. It observed for weeks-1 to 3, that there was a rise at 0.5mg/kg, a decline at 0.6mg/kg, a rise at 0.7mg/kg, decline at 0.8mg/kg and finally a rise through 0.9mg/kg to 1mg/kg (fig.2a). At weeks-4 to 10, there was a decline through 0.4mg/kg to 0.5mg/kg, a rise through 0.6mg/kg to 0.7mg/kg, and decrease again through 0.8mg/kg to 0.9mg/kg, finally peaks at 1mg/kg, with group 0.9mg/kg showing it least (0.06±0.008) effect at week-6 (also see fig.2b and 2c). At week-11 to 13 (fig 2c), another dose dependent increase from 0.5mg/kg through to 1mg/kg was observed. Throughout the experiment the group that received 1mg/kg body weight of BPA had the maximum effect effect on progesterone, and the effect was time dependent (fig.2a -2c). The time of exposure does not have effect on the exposure to BPA for the dose group 0.05mg/kg to 0.3mg/kg, 0.6mg/kg and 0.7mg/kg (fig.2a – 2c).

![Fig-2a](image-url)

**Fig-2a; Chart of serum progesterone concentration against duration of BPA exposure for weeks 1-4**

![Fig-2b](image-url)

**Fig-2b: Chart of serum progesterone concentration against duration of BPA exposure for weeks 5-8.**
DISCUSSION

The present study examined the effect of BPA exposure on serum prolactin and progesterone hormones. The result obtained from the study revealed high prolactin and progesterone level. A significant positive association was found between BPA level and serum PRL and PROG concentration.

Progesterone is a hormone that helps to regulate the menstrual cycle, balance the effects of estrogen, proper fallopian tube function, vital for conception and maintaining pregnancy. Progesterone helps support a developing embryo and help sustain the developing baby. This finding is consistent with reported findings from in vitro and in vivo studies. In vitro studies showed that GH3/B6 pituitary cells, which express mER, respond to low level BPA exposure (in the picomolar to nanomolar range) by producing a calcium flux which leads to PRL release (Watson et al. 2007). BPA can also induce prolactin gene expression and cell proliferation in both primary anterior pituitary cells and GH3 cells (Steinmetz et al. 1997). In an animal study, injecting approximately 15 mg/(kg·day) of BPA into neonatal Fisher 344 rat pups resulted in an increase in serum PRL levels (Stoker et al. 1999). Similarly, treatment of variecomized Wistar rats with BPA at doses of 11–250 mg/kg per day induced hyperprolactinemia (Goloubkova et al. 2000). The observed association between BPA and increased PROG in the present study is also supported by the finding of an alteration of PR expression following BPA exposure in nonhuman primates (Aldad et al. 2011). In consequence, BPA deceptively triggered pregnancy-like effect in non-pregnant rats.

REFERENCES

Effects of xenoestrogen bisphenol A on uterine and pituitary weight, serum prolactin levels and immunoreactive prolactin cells in ovariectomized Wistar rats. *Archives of toxicology*, 74(2), 92-98.


