Histopathological changes in the Kidney of mosquito fish, *Gambusia affinis* and guppy fish, *Poecilia reticulata* exposed to Bisphenol A

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ABSTRACT

Bisphenol A (BPA) is an industrial chemical, used to manufacture polycarbonate and numerous plastic articles and leach to salt and fresh water. Low levels of BPA have also been found to cause biological effects, and its mode of action appears to mimic that of the female hormone, estrogen. BPA therefore belongs to a group of chemicals termed “hormone disruptors” or “endocrine disruptors” that are able to disrupt the chemical messenger system in the body. The present study is aimed to assess the histological changes caused to the kidney tissues of both mosquito-fish (G*ambusia affinis*) and a guppy-fish (*Poecilia reticulata*) as a result of exposure to bisphenol A. These poeciliid fishes were exposed to 50µg/l of bisphenol A for 15 days (short term) and 30 days (long term). Histological examination of the kidney treated with Bisphenol A in both fish species showed a variety of histopathological effects; like inter tubular edema, shrinkage of glomeruli, glomerular distortion, vaculation of tubular cells, necrosis and atrophy of renal tubules, severe congestion and blood hemolysis, fluid stagnation in renal tubules and moderate aggregation of macrophages.

In conclusions, our study concluded that, the concentration 50µg/l of BPA has ability to induce harmful effect on the kidney tissue of fishes and these effects are time dependent.

Keywords: Kidney- Guppy fish-Mosquito-fish- BPA.

INTRODUCTION

The occurrence of endocrine-disrupting chemicals (EDCs) in aquatic environments has become a globally growing problem of concern in recent decade. EDCs consist of a wide range of natural and man-made chemicals, most of which are released to natural waters by anthropogenic activities and whose adverse effects on normal reproductive functions of aquatic species have widely been confirmed (Jobling *et al*., 1996; Routledge *et al*., 1998; Ohelmann, 2000; Irwin *et al*., 2001; Levy *et al*., 2004). For instance, widespread estrogenic chemical input has been regarded as an important factor related to the decline of fish abundance in freshwater systems (Jobling *et al*., 1998; Burkhardt-Holm *et al*., 2005; Kidd *et al*., 2007).

Bisphenol A (BPA) is an organic compound composed of two phenol rings connected by a methyl bridge, with two methyl functional groups attached to the bridge, the common name for 4,4-dihydroxy-2,2-diphenylpropane (International Union of Pure and Applied Chemistry [IUPAC] name). BPA is a white solid (available in crystals or flakes) with a mild phenolic odor under ambient conditions. Its melting point is 155°C, and its specific gravity is 1.060–1.195 g/cm3. BPA is generally considered to be a moderately hydrophobic compound (octanol–water partition coefficient [K_{ow}] of 103.4), with a slight polarity due to the two hydroxyl groups. It is soluble in acetic acid and very soluble in ethanol, benzene and diethyl ether (Lide, 2004, Kang *et al*., 2006 and Le *et al*., 2008).

Fish suffer regressive changes, mainly in organs directly exposed to the action of pollutants like gills, kidney (Sedrak, 1998). Kidney is the target organ playing a major role in excretion and elimination of the body waste metabolites and it is sensitive to action of toxic agents (Seldin and Giebisch, 1992).

Thus, the present study was planned to provide more information about the histopathological alternations induced in the kidney of the *Poecilia reticulata* and *Gambusia affinis* as a result of exposure to BPA in aquatic environment. In addition to assess the histological changes caused to the fish exposed to 50 µg/l BPA for 15 and 30 days.

MATERIALS AND METHODS

A total of 30 specimens of Mosquito-fish, *Gambusia affinis* and 30 specimens of guppy *Poecilia reticulata* were used in the present study. Fishes were obtained from EL-Sayeda Aisha market and transported to the laboratory in Zoology Department, Faculty of Science, Al-Azhar University; in a plastic package filled with oxygenated water. Then, Fishes were allowed to be adapted for two weeks to the laboratory conditions, in well-aerated dechlorinated tap water glass aquaria of 20-liter capacity. They were fed twice daily six days a week with commercial pelleted fish diet.

After fish acclimatization, fishes of each species divided into 3 groups in 3 glass aquaria (20-liter capacity) with stocking density of 10 fish/ aquarium. The first group was not exposed to BPA and served as control. The second group was exposed to 50µg/l for 15days (short term). The third group was exposed to 50µg/l for 30 days (long term).

Bisphenol A (>99% pure) was purchased from Aldrich Chemical (Milwaukee, WI, USA) and dissolved in dechlorinated tap water (50 mg/L). A BPA stock solution of 50µg/L was prepared by diluting the concentrated solution with dechlorinated tap water. To prepare 50µg/L we were take 1ml of (50 mg/L), and then dissolved in 1liter dechlorinated tap water.

Histological studies

To investigate the effect of BPA on kidney of both mosquito-fish (*Gambusia affinis*) and guppy (*Poecilia reticulata*), kidney of normal and treated fishes were fixed in alcoholic Bouin's solution for 24 hours. The specimens were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Transverse sections were cut at 4 to 6 microns and at least 10 slides from each region were prepared. Sections were stained with Harri's haematoxylin and subsequently counter stain with eosin. Finally, the slides were microscopically examined to identify the histopathological features, then photographed and described.

RESULTS

Histological observations of control kidney of *Gambusia affinis* revealed that kidney is mainly composed of renal tubules and renal corpuscles. The renal tubular are made from tall simple columnar epithelial cells, whereas the renal corpuscle is composed of glomerulus within Bowman's capsule which is formed of a double-walled epithelium and has a crescent–shaped lumen known as the capsular space. The renal tissues also have numerous blood supplies and hematopoietic tissue. The renal tubules were composed of proximal tubules, distal tubules and collecting duct. The proximal tubules were covered by tall columnar epithelial cells with basal nuclei, whereas distal tubules were lined with large, relatively clear columnar epithelial cells with central nuclei. The collecting duct was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei (Plate I, A&B).

After 15 days of exposure to 50µg/l BPA, the microscopic examination of kidney sections revealed that, tissue degeneration (edema), and congestion of blood vessels with blood hemolysis were observed. In addition, a moderate fluid stagnation (hyaline droplets) was obviously seen in lumen of renal tubules (Plate I, C&D). After 30 days of fish exposure to 50µg/l BPA, the microscopic examinations showed a moderate damage and of renal tubules represented by, separation or desquamation of the epithelial layer of renal tubules, degeneration and shrinkage of glomeruli, appearance of hemosiderin granules. Also, a remarkable increase of melano-macrophage aggregation was detected. Furthermore, severe congestion of blood vessels with blood hemolysis and blood clotting were observed inside blood vessels (Plate I, E& F).

Histological sections of control kidney of *Poecilia reticulata* revealed the similar structure like that of *G. affinis*, as shown in (plate II, A & B). After 15 days of exposure to 50µg/l BPA, the microscopic observation revealed that renal tubules were expanded; they were severely degenerated leading to presence of necrotic tubules. In addition, atrophy of renal tubules with pyknotic and karyorrhexis of nuclei was also observed. Degeneration and shrinkage of glomeruli were detected and blood hemolysis also can be seen in (Plate II, C&D).Whereas after 30 days of fish exposure to 50µg/l BPA, the result showed severe congestion of blood vessels with blood hemolysis was noticed, and distortion of some tubules with desquamation of the epithelial layer of renal tubules was recorded (Plate II, E&H).

DISCUSSIONS

Endocrine disrupters (EDs) reach living organisms through the air, soil, water and food, thus the major route transmission is the aquatic environment, were these substances bio accumulate through the food chain, fish ingestion is one of the main sources of human exposure to Endocrine disrupters (EDs) (Mita *et al*., 2011). BPA, one of the most abundant endocrine disruptors in the environment, is produced by the acid-catalyzed condensation of acetone with two phenols (kang *et al.,* 2006).

From an economic view point, fish form the basis of a large commercial fishery and aquaculture industry, as well as having wide spread recreational value, if EDCs in the aquatic environment are affecting reproductive success of fish, thus threatening population sustainability over time, commercial and sport fisheries could be impacted (Mills and Chichester, 2005).

The Environmental Protection Agency (EPA) set the BPA exposure limit at 50µg BPA/ kg body weigh/ day, based on the paper described by Morrissey *et al* (1987) and a study of National toxicology program (1982). This dose was based on the lowest observable adverse effect level (LOAEL), thus the EPA reference dose and FDA acceptable daily intake or ADI dose of 50µg/kg day this presumed "safe" dose is estimated by dividing the LOAEL by three 10-fold safety factors (IRIS, 1988).

The teleostean kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al*., 2003). Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman´s space (Takashima and Hibiya, 1995).

In the present study, the kidney of both *Gambusia affinis* and *Poecilia reticulata* exposed to 50µg/l BPA at different durations showed different signs of histopathological deformations varies in intensity according to the duration of exposure, represent as edema, atrophy of renal tubules, blood hemolysis, congestion of blood vessels, necrosis of renal tubular cells, shrinkage of glomeruli, and fluid stagnation in tubules. The presence of tubular degeneration, with the presence of necrosis in the renal tissue in the present study indicates that the kidney suffered damage after exposure to BPA. In this work, elevated time of exposure to BPA caused shrinkage glomeruli and blood hemolysis. This lesion may be return to cellular degeneration as well as increasing the amount of edematous fluid in the interstitial substance (Hadi and Alwan, 2012)

The initial stage in the degeneration process can progress to hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Hinton and Laurén, 1990; Takashima and Hibiya, 1995). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995).The atrophy of renal tubule interpreted according to Saenphet, *et al* (2009); they stated that, because of water reabsorption taking place in the distal tubules, relatively high concentrations of toxins may have an effect on renal cells.

Melanomacrophage centres, also known as macrophage aggregates, are distinctive groupings of pigment-containing cells located in the stroma of the haemopoietic tissue of the spleen and the kidney fishes (Agius and Roberts 2003). Several authors have suggested that the involvement of melano-macrophage centres in various disease processes and the changes brought about in them by chemical exposure (Long, *et al*. 1995; Couillard and Hodson 1996; Meinelt, *et al.* 1997) indicates that these centres can provide sensitive indicators of stressful conditions in the aquatic environment (Agius 1985; Wolke *et al*. 1985, 1995; Blazer *et al*. 1987; Pulsford, *et al.*1992; Spazier, *et al.* 1992).

Comparative studies on the incidence of melanomacrophage centres in the spleen, kidneys and liver of fish taken from waters polluted with toxic chemicals have, however, produced conflicting results (Pulsford *et al*. 1992). Some authors reported an increase (Kranz and Peters 1984; Wolke *et al*. 1985; Kranz and Gercken 1987), whilst others recorded the opposite (Kranz 1989). This latter worker observed an increase at low levels of pollution, which he ascribed to the capability of the cellular defense system to remove debris by increased phagocytic activity, with consequent increase in melanomacrophage centres.

The Haemosiderin is composed of ferric iron and protein and is derived from the catabolism of haemoglobin from effete erythrocytes and is therefore an intermediate metabolic product that occurs during recycling of components for erythropoiesis (Kranz, 1989).

From the present study, it can be concluded that, the concentration 50µg/l of BPA have ability to induce harmful effect on the kidney of fishes and the degree of damage varies in intensity according to the duration of exposure.

ACKNOWLEDGEMENT

We would like to record our deepest thanks to prof. Dr. Ahmad Mossad Azab professor of fish biology and ecology, Zoology Department, Faculty of Science, Al-Azhar University, Cairo for helping and supporting this study.

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LIST OF APPREVIATIONS

AT: Atrophy, BC: Bowman's capsule, BH: Blood hemolysis, C: Congestion, CT: Collecting duct, D: Degeneration, DT: Distal tubules, E: Edema, G: Glomerulus, HD: Hemosiderin droplets, HPT: Hematopoietic tissue, K: Karyorrhexis, M: Melno-macrophage, NT: Necrotic tubule, P: Pyknosis, PT: Proximal tubule, Se: Separation or desquamation, SC: Severe congestion. SG: Shrinkage glomeruli, ST: Shrinkage tubule, Sta: Stagnation, V: Vacuolation.

LEGEND OF PLATES

Plate I: Photomicrographs of transverse sections of Mosquitofish kidney of control and BPA-treated

(A): Photomicrographs of transverse sections of normal kidney of Mosquito fish showing normal Bowman's capsule, glomerulus, proximal and distal tubules. (H&E x100).

(B): Enlarged transverse section of normal kidney of Mosquito fish showing normal collecting duct, hematopoietic tissue, proximal and distal tubules. (H&E x400).

(C): Photomicrographs of transverse sections of kidney of Mosquito fish exposed to 50µg/l BPA for 2 weeks, showing abnormal renal tubules, congestion of blood vessels with hemolysis, and edema also detected. (H&E x100)

(D): Enlarged transverse section of transverse sections of kidney of Mosquito fish exposed to 50µg/l BPA for 2 weeks, showing, severe congestion, fluid stagnation in renal tubules and vaculation in renal tubular cells. (H&E x400)

(E): Photomicrographs of transverse sections of kidney of Mosquito fish exposed to 50µg/l BPA for 30 days, showing abnormal kidney structure, as separation or desquamation of the epithelial layer of renal tubules, degeneration and shrinkage of glomeruli, appearance of hemosiderin droplets. (H&E x100)

(F): Enlarged section of transverse sections of kidney of Mosquito fish exposed to 50µg/l BPA for 30 days, showing, severe congestion, Also, a remarkable increase of melano-macrophage aggregation was detected. Furthermore, severe congestion of blood vessels with blood hemolysis and blood clotting were observed inside blood vessels. (H&E x400)

Plate II: Photomicrographs of transverse sections of Guppy fish kidney of control and BPA-treated

(A): Photomicrographs of transverse sections of normal kidney of control Guppy fish, showing normal Bowman's capsule, glomerulus, collecting duct, proximal and distal tubules. (H&E x400).

(B): Enlarged transverse section of normal kidney of Guppy fish showing normal collecting duct, hematopoietic tissue, collecting duct, proximal and distal tubules. (H&E x400).

(C): Photomicrographs of transverse sections of kidney of Guppy fish exposed to 50µg/l BPA for 2 weeks, showing abnormal renal tubules, as shrinkage glomeruli, glomerular distortion, atrophy of renal tubules with multi necrotic tubular cells, and also blood hemolysis was recorded. (H&E x100).

(D): Enlarged transverse section in kidney of Guppy fish exposed to 50µg/l BPA for 2 weeks, showing abnormal kidney structure, atrophy of renal tubules with pyknotic nuclei and karyorrhexis of renal tubules cells. Notice vacuolation renal tubular cells. (H&E x400).

(E): Photomicrographs of transverse sections of kidney of Guppy fish exposed to 50µg/l BPA for 30 days, showing abnormal renal tubules, as distortion of some tubules with desquamation of the epithelial layer of renal tubules was recorded. (H&E x100).

(F): Enlarged transverse section in kidney of Guppy fish exposed to 50µg/l BPA for 30 weeks, showing abnormal kidney structure, severe congestion of blood vessels with blood hemolysis was noticed. (H&E x400).

Plate I

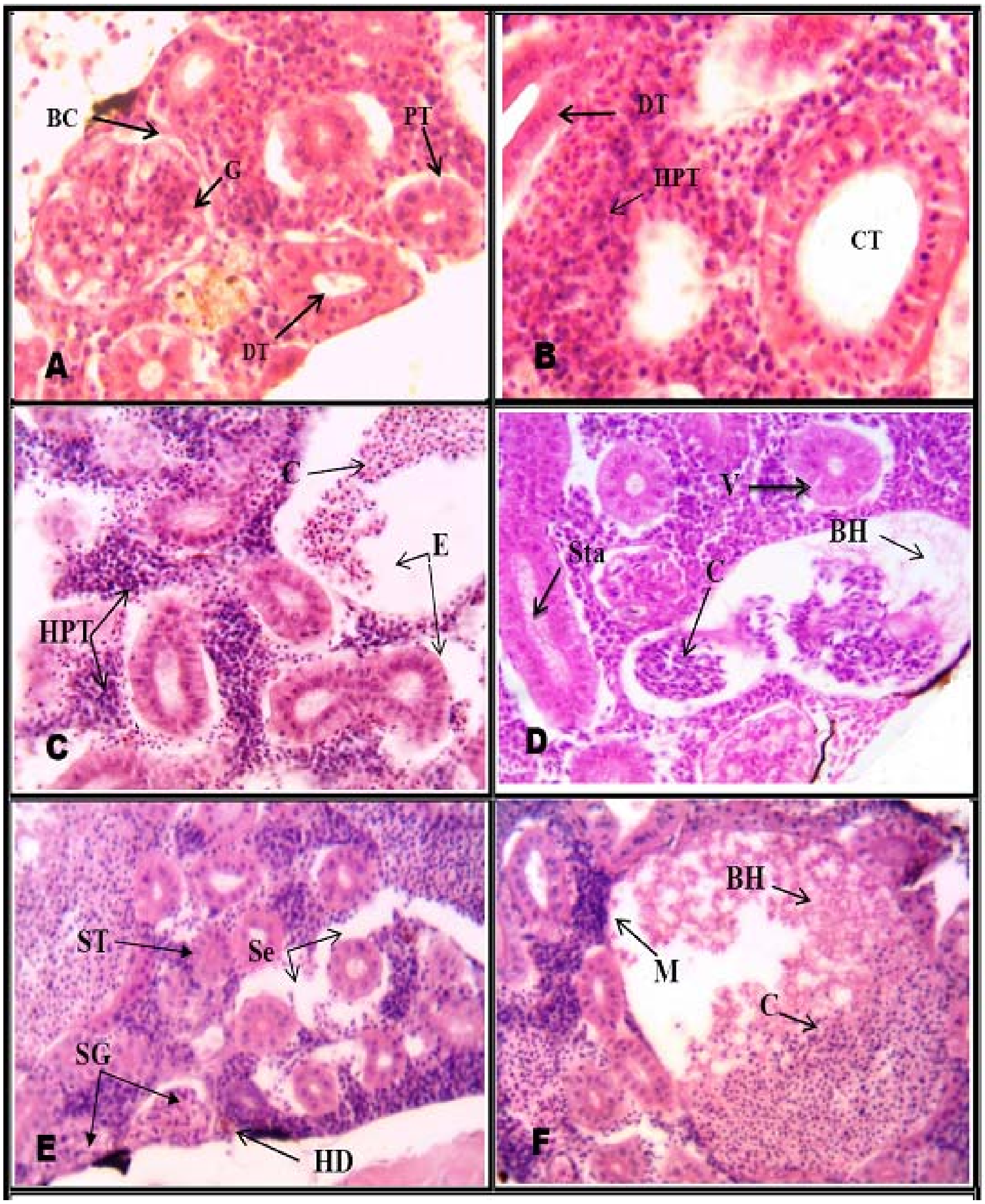
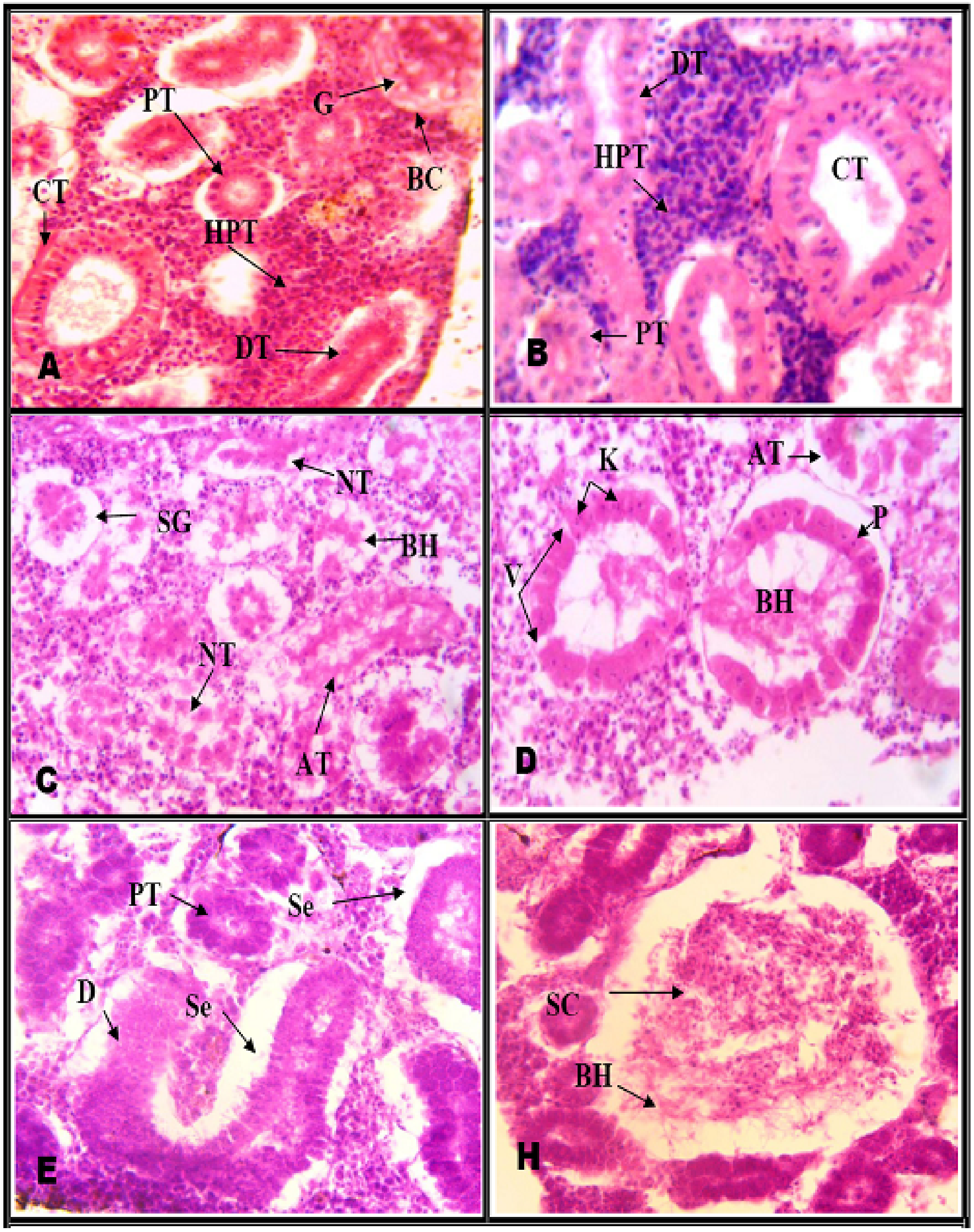


Plate II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ARABIC SUMMARY

التغيرات النسيجية المرضية في كلى أسماك أكل الناموس (جامبوزيا أفينيس) والجوبى (بوسيليا ريتيكيولاتا) المعرضة لمادة البيسفينول أ

فتحى الشاعر محمد فتحى - ليد على أبو شعير- سيد بكرى أحمد
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تهدف هذه الدراسة إلى تقييم الأضرار النسيجية التي لحقت بكلى الأسماك من نوعى الجامبوزيا أفينيس والبوسيليا ريتيكيولاتا بعد التعرض الى التركيز الآمن (٥٠ميكروجرام فى اللتر) من مادة البيسفينول أ BPA لمدة ١٥ يوماً و ٣٠ يوماً.

و قد أظهر الفحص الميكروسكوبى بعض التغيرات النسيجية المرضية التي حدثت في كلية كل من النوعين محل الدراسة عند تعرضها لجرعة ٥٠ ميكرو جرام فى اللتر ، ممثلة فى ظهورضمور وتأكل كامل للأنيبيات الكلوية مع نخر لبعض منها مع تكسير لانوية الانابيب الكلوية ، انكماش فى حجم الكبيبات وتشوه تام بها وفى الأنابيب الكلوية واحتقان شديد في الأوعية الدموية مع تحلل للدم بداخلها مع وجود ارتشاح للخلايا الأكولة. كما لوحظ أيضا تكون الفجوات فى الخلايا الطلائية المبطنة للأنيبيات الكلوية وإنفصالها عن الغشاء القاعدى لها.

وقد أظهرت الدراسة أن هذا التركيز (٥٠ميكروجرام فى اللتر) من مادة البيسفينول أ BPA ليس آمناً وأن له أضرار كبيرة على أنسجة الكلية، وأن هذه الأضرار تزداد شدتها مع زيادة مدة التعرض للمادة.