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Toxicity Studies of Bisphenol-A Induced Sprague Dawley Rats

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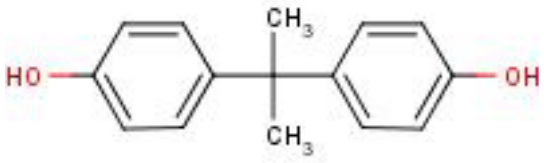
ABSTRACT

Bisphenol A is a monomer usually found in commonly used consumer plastic goods. Although much attention in recent years has been placed on BPA`s impact as an endocrine disruptor. The Rodent studies on toxicity of BPA have been debated in recent years that the values of oral lethal dose of Bisphenol A were extremely different in the literature. The Present study was an attempt to determined the oral LD_{50} of Bisphenol A in the Sprague dawley rats and to measured the effect of the high doses of Bisphenol A compound on different organs of the rat. Eight groups of male Sprague Dawley rats were used (n = 48). The treated groups (G-I to G-VIII) received eight different doses of Bisphenol A (G-I=2250 mg/kg, G-II=2500 mg/kg, G-III=2750 mg/kg, G-IV=3000 mg/kg, G-V=3250 mg/kg, G-VI=3500 mg/kg, G-VII=3750 mg/kg, G-VIII=4000 mg/kg) by orally through drinking water. All rats were examined twice daily for mortality and impairment during the 2-week experiment. The bodyweight (BW) of the rats as well as the tissues viz brain, liver and kidneys were measured in all groups of BPA treated animals. The results indicated that they loss in their weights. Behavioral changes were also noticed and discussed in detail of the text. Dissection is realized for each died rats and the results revealed that 50% of rats kill was observed in groups of G-III, G-IV, and G-V. Finally the LD_{50} of Bisphenol A in the SD rats was noticed as 3228 mg/Kg body weight.

INTRODUCTION

Bisphenol A (2, 2-bis (4-hydroxyphenol) propane), a chemical compound found in plastic products, is being used increasingly in industrial manufacturing materials. Numerous reports state that BPA production was (2,214,000) metric tons worldwide per year in 2003 and (3,200,000) tons in 2005 (Calafat *et al.*, 2005). The industrialized process of BPA involves the combination and condensation of one part of acetone with two parts of phenol (in the presence of a catalyst and promoter) under conditions of high temperatures and low pH, followed by purification of the product.



Bisphenol A – 2, 2,-bis (4-hydroxyphenyl) propane

Bisphenol A (BPA), a chemical widely used in the manufacture of polycarbonate plastics and epoxy-phenolic resins. Polycarbonate plastics are used in manufacture of food and drink containers (e.g., milk, water and infant bottles) and epoxy resins are used as an interior protective lining for food and beverage cans (Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch). Although recent works point out to adverse effects of BPA on adult brain, there is still limited toxicogenomic information on BPA-induced neurotoxicity during adult life (Inagaki *et al.*, 2012; Eilam-Stock, *et al*., 2012). Due to its major applications in the production of plastic food or beverage containers and the coating of food cans, people of different ages are inevitably exposed to BPA in daily life. The human population is widely exposed to low levels of BPA, primarily by way of the diet by migration from food and beverage containers. (Vandenberg *et al*., 2007).

The omnipresent and extensive use of BPA containing products results in a high human exposure worldwide (Vandenberg *et al.*, 2010). It is thought that human exposure mainly occurs through diet as polymers containing BPA can be hydrolyzed under high temperature and acidic or basic conditions leading to leaching into food and drink containers (Welshons *et al.*, 2006). However, Research Article ISSN 2250-0480 Vol 2/Issue 2/Apr-Jun 2012L-20 Life Science Zoology is a recent evidence, it also indicates that exposure may occur through

dermal contact with thermal papers used widely in cash register receipts (Biedermann *et al.*, 2010). A number of effects of BPA in animals have been broadly investigated and target organs identified in repeat-dose animal studies include intestine, liver and kidney. However, the effects of most concern have been those related to the hormonal activity of BPA and potentially related effects on physical, neurological and behavioural development. (International Food Safety Authorities Network (INFOSAN) No. 5/2009 - Bisphenol A).

Bisphenol A is metabolized more quickly following oral exposure compared to non-oral exposures such as inhalation because of "first pass effects" (Tyl *et al*., 2002). To date, there is an argument about the toxicity of BPA. Although FDA has labeled BPA as a safe agent (FDA, 2008), newly budding data has stirred discussion on urgency of more studies to make human health risk assessment of BPA exposure (Hugo *et al.*, 2008). The extraordinary interest of researchers on the health risks posed by BPA stands mainly on its accepted endocrine (estrogenic) disrupting activity, on apparent low-dose effects and non-monotonic dose response relationships and on its developmental toxicity. LD_{50} is a standard measurement of acute toxicity that is stated in milligrams (mg) of pesticide per kilogram (kg) of body weight. LD_{50} represents the individual dose required to kill 50 percent of a population of test animals (e.g., rats, fish, mice, cockroaches)

The LD_{50} of Bisphenol A values found in the literature are extremely different. According to NTP (1982), it was 4100 mg/kg (BW). Morrissey *et al*., (1987) and Goodman *et al*., (2006) reported it was 3300-4240 mg/kg (BW) Chapin *et al*., (2008) and Michelle Twaroski (WHO, 2010) reported it was 3,250 mg/kg (BW). The aim of the present study is to determine oral LD_{50} of Bisphenol A on *Rattus norvegicus* for analysis of differentially expressed proteins under BPA toxicity and also probiotic treatment.

MATERIAL AND METHODS

Chemicals:

Bisphenol A (BPA) (CAS # 80-05-7) of analytical reagent grade (Purity>90%) was procured from SRL, Mumbai, India. All other chemicals used in this study were of the highest purity available and purchased from BROS Scientific Company, Tirupati, India.

Feed, water, and housing:

The pellet diet was used in this toxicological research and throughout an experiment in the same way, BPA was delivered in drinking water and the water exists free of chlorine or other

reactive ions and also made free of BPA and other estrogens; reverse osmosis and carbon filtration was often necessary to achieve this requirement BPA. Polypropylene cages have been used successfully in these studies (Howdeshell *et al*., 2003).

Analysis of LD $_{50}$:

Adult male (n=48) (body weight 190 ± 10 g) Sprague Dawley rats were purchased from Sri Venkateswara traders, Bangalore, India. The rats were fed on Pellet diet (Godrej agrovet ltd, Mumbai, India) and water ad libitum, maintained under standard laboratory conditions (temperature, 22–25°C; light: dark cycle, 12:12 hr). The rats were acclimatized for one week before experimentation. Rats were randomly divided into eight (8) groups consisting of six animals in each group, with different concentration dissolved in distilled water for 14 days. The treated groups (G-I to G-VIII) received eight different doses of Bisphenol A (G-I=2250 mg/kg, G-II=2500 mg/kg, G-III=2750 mg/kg, G-IV=3000 mg/kg, G-V=3250 mg/kg, G-VI=3500 mg/kg, G-VII=3750 mg/kg, G-VIII=4000 mg/kg) by orally through drinking water. All rats were examined twice daily for mortality and impairment during the 2-week experiment. The bodyweight (BW) of the rats as well as the tissues viz brain, liver and kidneys were measured in all groups of BPA treated animals. The body weight of the animals was recorded on the day of initiation of the treatment and also on the day of sacrificing. Behavioral changes were noticed and recorded at BPA treated animals.

Statistical analysis:

The statistical methods used varied by endpoint and were described in supplemental materials. The dose range of primary interest for the present study was between 2250 to 4000 µg BPA/kg BW/day and a major purpose of the study was to provide information for a subsequent chronic study. The LD_{50} of Bisphenol A was calculated by probit analysis (Finney, 1952). The data was presented as mean ± S.D.

RESULTS AND DISCUSSION

Clinical signs:

All the bisphenol treated animals showed treatment related clinical signs such as decreased locomotor activity, sedimentation, soft stool, diarrhea and urination were observed. Irregular movements, biting of teeth, aggressive nature, unrest and frequent urination were noticed in increased dosage concentration of Bisphenol A treated rats. (Cichna-Markl, 2012)

Analysis of LD $_{50}$:

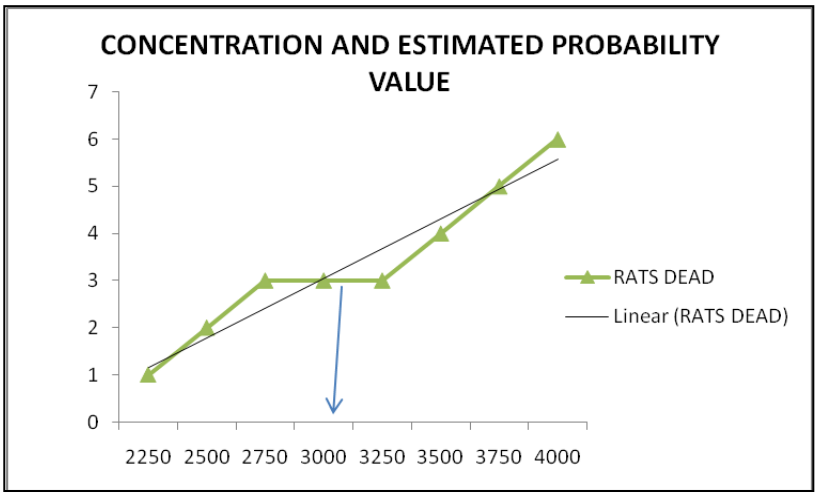
Table 1: Derivation of LD_{50} of Bisphenol-A to rat by probit analysis. Computations for the fitting of a regression equation by arithmetical method.

S.No	BPA Conc (mg/kg)	Log Con (X)	No.of Rats (R)	Dead (r)	% kill (p)	Emarical probit	Expected Co-efficient(Y)	Weight Co-efficient(W)	nw	Working probit (y)	nwx	nwy
1	2250	3.35218	6	1	16.66667	1.534	0.651890	0.325945	1.955671	1.934	1.955671	3.782269
2	2500	3.39794	6	2	33.33333	2.223	0.899685	0.449843	2.699055	2.723	5.398111	7.349528
3	2750	3.43933	6	3	50	2.531	1.185302	0.592651	3.555907	2.931	10.66772	10.42236
4	3000	3.47712	6	3	50	2.995	1.001669	0.500835	3.005008	2.995	12.02003	9
5	3250	3.51188	6	3	50	4.011	0.747943	0.373972	2.243829	4.611	11.21915	10.34630
6	3500	3.54406	6	4	66.66667	5.513	0.725558	0.362779	2.176673	6.513	13.06004	14.17667
7	3750	3.57403	6	5	83.33333	4.995	1.001001	0.500501	3.003003	5.123	21.02102	15.38438
8	4000	3.60206	6	6	100	5.563	1.078555	0.539277	3.235664	5.863	25.88531	18.97070

$\bar{x} = 3.62$ Y bar = 4.1

LD 50 = $\bar{x} + \frac{3.6 - Y\ bar}{b} = 3.62 + \frac{3.6-4.1}{4.5} = \log_{10} 3.5089$ or 3227.668 mg/kg body wt.

Figure 1: LD_{50} of Bisphenol A showing in the graph



Estimated Probability value

Concentration

The present results (**Table-1 & Figure: 1**) indicated that the dose dependent concentration of Bisphenol A showed the significant and variable changes on Sprague dawley rats. The maximum toxicity was observed in the dosage of Group –VIII (4000 mg/kg) and half of the animals were died at the dosage of Group III (2750 mg/kg), Group IV (3000 mg/kg) & Group V (3250 mg/kg).These results were undergo for computations for the fitting of a regression equation by arithmetical method (**Table-1**). The Median Lethal Dose (LD_{50}) of Bisphenol A was derivated as 3228 mg/kg body weight.

Animal and tissue weights:

All animals in the control group as well as BPA-treated groups, showed similar progressive decrease in animal and tissue weights throughout the two week experimental period of study (Table 2 & 3).

Animal weight:

The obtained results showed that the effect of Bisphenol A in the body weights of rats at different concentrations were presented in **Table-2**. The animal weight was decreased under the dose dependent concentrations of Bisphenol A.Values were represented as mean ± S.D. of 6 individuals.

Table 2: Effect of Bisphenol A in the animal weights at different concentrations

BPA Concentration (mg/Kg)	Animal Weight (initial)	Animal Weight (final)
2250	190±1.2	185±1.1
2500	195±1.5	180±1.5
2750	205±1.9	200±1.9
3000	194±1.9	180±1.3
3250	198±1.5	185±1.9
3500	200±1.8	180±2.4
3750	205±1.6	190±1.5
4000	202±1.2	185±1.6

Tissue weight:

Effects of Bisphenol A on tissue weight ratio at different concentrations were also represented in **Table-3**. The weight of the Hypothalamus, Liver, Kidney tissues showed that the variations in almost all dosage levels which itself indicating the toxicity of Bisphenol A causing irregularities in all metabolic activities. Values were represented as mean ± S.D. of 6 individuals.

Table 3: Effect of BPA on Tissue weight ratio at different concentrations

BPA Concentration(mg/kg)	Hypothalamus	Liver	Kidney
2250	0.924±0.03	2.52±0.25	1.38±0.05
2500	0.917±0.11	2.46±0.25	1.37±0.05
2750	0.876±0.03	2.42±0.28	1.35±0.42
3000	0.854±0.03	2.40±0.21	1.28±0.05
3250	0.812±0.02	2.37±0.21	1.26±0.06
3500	0.789±0.01	2.29±0.27	1.18±0.08
3750	0.762±0.07	2.21±0.24	1.08±0.08
4000	0.756±0.04	2.19±0.24	1.12±0.09

CONCLUSION

The oral administration of BPA was used in this study because the primary route of environmental exposure was through by dietary intake (contaminated food and water).The experiment was mainly focused to determine an LD_{50} of Bisphenol A whose data were old, rare, and divergent in the literature (NTP feed study,1982). The LD_{50} of Bisphenol A values found in the literature were extremely different. According to NTP (1982) it was 4100 mg/kg (BW); Morrissey *et al*., (1987) and Goodman *et al*., (2006) reported it was 3300-4240 mg/kg (BW) Chapin *et al*. (2008) and Michelle Twaroski (WHO, 2010) reported it was 3,250 mg/kg (BW). However most recent LD_{50} value published in some Material Safety Data Sheet of Societies producing Bisphenol A as Sigma Aldrich was >2000mg/kg of Bisphenol A by oral route. In the present study, the higher dose used (Group –VIII (4000 mg/kg)) was the maximum dosage concentrations in which death of 100% of the rats were observed and the median Lethal Dose (LD 50) of BPA was 3228 mg/Kg. Probit analysis was used to access

the median Lethal Dose (LD 50) of BPA whose obtained value is nearer to the 3250 mg/Kg by Chapin *et al.*, (2008).

Rats were administered orally with low and high doses of Bisphenol A and the weights of animals were decreased .The effects of BPA were observed on various tissue parameters of hypothalamus, liver and kidney organs. The body weights of rats were monitored throughout the experiments and the effect of toxic compounds of the general health status of the animals were recorded. Depression in body weight or reduction in the body weight gain may reflect a variety of responses, including internal and external environmental factors such as rejection of food and water, treatment induced anorexia or systematic toxicity.

The current findings showed that the body weights were decreased under the dose dependent concentrations of Bisphenol A intoxicated rats. A study at 2500 mg/Kg and above respectively decreased mean body weights in males by Til (1973).Takashi (2001) reported that a significant decrease in a body weight gain was observed at the dosage of 466 mg/Kg body weight when compared with control. In Another study of Yamasaki *et al.,* (2002a) and Yamasaki *et al* (2002 b) reported that sub acute toxicity of BPA for 28 day reveals that body weight was decreased in males at a dosage of 600 mg/Kg body weight. Stump *et al.*,(2010) showed that statistical significant reductions in mean body weights were observed at 2250 mg/Kg given through the diet. Accordingly results showed in present study makes clear understanding that decrease in a relative mean body weight was observed with respect to the concentration of the Bisphenol A treated to the rats. It was a basic study of our research. Further studies we carried out for proteomic study of Biphenol A toxicity on sprague dawley rats.

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