

## Analysis and Detection of Bisphenol Contaminants in Aquatic Systems and Investigation of Their Molecular Biological Effects

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### Abstract

This study examined the concentration and molecular effects of bisphenol A (BPA), a prominent environmental pollutant, in various water sources. Recent research has reported rising BPA levels in aquatic ecosystems, primarily due to plastic pollution. This investigation focused on understanding the partial degradation of BPA under environmental conditions, specifically through exposure to light and oxygen.

The findings revealed significantly higher BPA concentrations in river samples and reverse osmosis (RO) water stations compared to control samples, indicating that these water sources face an elevated risk of contamination. To evaluate the molecular impact of BPA on living organisms, an *in vivo* comet assay was utilized. This sensitive technique assesses DNA strand breaks and repair mechanisms, providing valuable insights into genotoxic effects across different species, tissues, and types of DNA damage.

The results, analyzed using the single-cell gel electrophoresis index, showed a substantial increase in the comet index in laboratory animals exposed to BPA, particularly when measured by tail length. These findings highlight significant DNA damage associated with exposure to varying BPA concentrations. This study emphasizes the urgent need to address BPA pollution and its potential genotoxic effects on wildlife and, potentially, human health.

Keywords: Bisphenol, Concentration, Aquatic Systems, DNA damage, Molecular effects.

### Introduction

Bisphenol A (BPA) is primarily used to produce epoxy resins, polycarbonate plastics, and non-polymer additives for other plastics. Recent research revealed that BPA exposure negatively affects development and reproduction in both humans and animals, as demonstrated in various laboratory animal models and wildlife species (Wetherill *et al.*, 2007).

BPA, utilized in consumer items, is widely present in the environment and can enter the body through adsorption or ingestion (Kurosawa *et al.*, 2002). To evaluate the estrogenic effects of BPA, a luciferase test was conducted on three cell lines derived from different tissues using human ER $\alpha$  cDNA or ER $\beta$  cDNA. The findings revealed that BPA acts not only as an estrogen agonist via ER $\beta$  but also as an antagonist via ER $\alpha$  in certain cell types. Consequently, the specific tissue and the ER subtype may influence BPA activity.

Gould *et al.* (1998) reported that although BPA does not prevent the E2-induced increase in uterine weight, it can counteract the stimulatory effects of E2 on progesterone receptor levels and peroxidase activity. These findings suggest that BPA has a unique mode of action at ER $\alpha$  and is not just a weak estrogen mimic. The detection limits for BPA, bisphenol F (BPF), and bisphenol S (BPS) were 0.2, 0.2, and 0.1  $\mu\text{g/L}$ , respectively. Urinary levels of these bisphenols varied based on factors such as gender, household income, race/ethnicity, physical activity, smoking, and/or alcohol use. The study indicated that alternatives to BPA are nearly universally ingested by the American population. Further research is needed to understand the environmental, consumer, and lifestyle factors that influence BPF and BPS exposure, as their levels vary across different demographic groups.

(Mendy *et al.*, 2020) reported the presence of BPF, BPS, and BPF in 57.1%, 88.4%, and 94.8% of urine samples, respectively. Urinary BPF was positively associated with hay fever [odds ratio (OR): 1.66, 95% confidence interval (CI): 1.12–2.46] and current asthma (OR: 1.54, 95% CI: 1.16–2.04). In males, urinary BPS was linked to a higher likelihood of current asthma (OR: 1.64, 95% CI: 1.13–2.40). Additionally, in children aged 6–11 years, urinary BPA was associated with increased odds of asthma without hay fever (OR: 2.65, 95% CI: 1.05–6.68).

With advancements in electrochemical detection, the presence of BPA in water and human serum can now be accurately and efficiently assessed using coulometric arrays of four electrochemical sensors and high-performance electrochemical detection using liquid chromatography coupled with multiple electrodes (HPLC-ED). In Inoue *et al.*'s (2000) study, BPA was separated and detected using a reversed-phase column (CAPCELL PAK UG 120 C18) and a mobile phase consisting of 0.3% phosphoric acid and acetonitrile in a 60:40 ratio.

Prabhu *et al.* (2023) measured urine BPA levels in women with polycystic ovarian syndrome (PCOS) and healthy women using ELISA. The analysis identified metabolic trends in BPA levels among healthy women and those with PCOS. MetaboAnalyst 5.0 was employed for pathway and biomarker analysis. Compared to healthy women with low BPA levels, those with elevated BPA levels exhibited abnormal concentrations of certain steroids, sphingolipids, and other metabolites, indicating significant disruptions in steroid hormone biosynthesis, linoleic and linolenic acid metabolism, sphingolipid metabolism, and alternative metabolic pathways. These findings provide insight into metabolic markers in women with PCOS exposed to BPA, potentially informing precision medicine and treatment strategies.

(Asaad *et al.*, 20212) investigated the presence of bisphenols in pregnant women and their potential impact on miscarriage. Bisphenols pose environmental hazards and may have adverse effects on fetal development. This study discussed various bisphenol detection methods, including flow rate parameters and the chemical composition of CH<sub>3</sub>CN, distilled water, and phosphate buffer in a ratio of 66:33:7. The detector used was FLO (excitation = 360 nm, emission = 460 nm)

The current study contributes to the growing body of knowledge on the environmental and biological consequences of plastic pollution. It aimed to evaluate the molecular and genotoxic effects of BPA on living organisms by assessing the BPA contamination levels in various water sources, including river samples and reverse osmosis (RO) water stations. The study also examined the environmental degradation of BPA under specific conditions, such as exposure to light and oxygen, and understand its partial degradation in aquatic ecosystems. Furthermore, the extent of DNA damage caused by BPA exposure in laboratory animals was assessed by comet assay.

## Materials and Methods

This study measured BPA concentration in Samples obtained from local River Water

BPA extraction:

To extract BPA from water, 100 mL of river water was collected, and 5 mL of dichloromethane (DCM) was added to the sample. The mixture was then placed on an orbital shaker and agitated at 200 rpm for 24 h to ensure thorough mixing and extraction. The mixture was subsequently transferred to a separation funnel, where the organic phase containing the extracted BPA was collected. The organic phase was then dried under vacuum to remove the solvent.

The residue was re-suspended in 100  $\mu\text{L}$  of mobile phase, filtered through a 0.45  $\mu\text{m}$  pore filter to remove particulates, and stored at  $-20^{\circ}\text{C}$  until further analysis by high-performance liquid chromatography (HPLC).

HPLC separation and detection:

The separation and detection of BPA were performed according to the method described. A C18 column (Knaer), The mobile phase consisted of an isocratic mixture of water and acetonitrile (40:60 v/v) with a flow rate 1 mL/min. Detection was carried out at a wavelength of 210 nm. BPA was detected by matching the retention time and absorbance spectrum of the samples with those of known BPA standards. For quantification, a standard curve was prepared using BPA standards at known concentrations. A 10 ppm BPA standard was diluted to prepare a 5ppm solution using the following calculation:

$$5 \text{ ppm} = 0.005 \times 1000 \text{ ppm}$$
$$13.33 / 20 \times \text{required volume} / 20 \text{ mL of distilled water} = 0.005$$

The volume required for the second dilution (10 ppm) was calculated to be 0.15 mL, which was then diluted in 20 mL of distilled water to obtain the 5 ppm solution.

Assessment of molecular effects of BPA:

First, to assess the molecular effects of BPA, three different concentrations of BPA were injected into the thigh area of laboratory rats. Each rat was marked for identification and monitored carefully for one week. A fourth rat, which served as the control, was not injected with BPA. The injection volume for each concentration was 0.5 mL per rat. After one week, the rats were euthanized, and 5 mL of blood was collected directly from the heart for further analysis.

Comet assay:

The comet assay was performed to evaluate DNA damage in the blood samples, following the procedure described by Singh *et al.* (1988) and Steinert (1996). Briefly, cells were encapsulated in a mixture of low melting point agarose (LMA) and standard melting point agarose (NMA). The samples were then subjected to electrophoresis, and DNA damage was quantified by measuring the comet tail length and tail moment using single-cell gel electrophoresis. The comet index, a measure of DNA strand breaks, was calculated to assess the extent of genotoxicity induced by BPA exposure.

Statistical analysis:

The results were analyzed using SPSS 29 software. Statistical significance was determined using ANOVA to compare differences between the control and BPA-exposed groups.

## Results and Discussion

Bisphenols have been identified as emerging environmental contaminants due to their potential harmful effects on receptor-mediated pathways. Research suggests that BPA and BPS act through different mechanisms. BPA primarily alters genes involved in cellular metabolism, whereas BPS has minimal effects on gene expression and mainly impacts cell signaling (Cimmino *et al.*, 2020).

Analysis of bisphenol levels across all samples revealed the highest concentrations in river water and RO water stations compared to the control samples (Tables 1 and 2).

**Table 1. Bisphenol concentration ( $\mu\text{g/mL}$ ) in water samples.**

Sample	Area of peak	$\mu\text{g/mL}$	$\mu\text{g/mL}$ in sample	ng/L in samples	Sample Volume	Final Volume after extraction
1	86.111	4.3696715	0.004369672	4369.6715	100	0.1
2	148.884	7.916346	0.007916346	7916.346	100	0.1
3	217.746	11.807049	0.011807049	11807.049	100	0.1
4	249.642	13.609173	0.013609173	13609.173	100	0.1
5	215.517	11.6811105	0.011681111	11681.1105	100	0.1
6	770.32	43.02748	0.04302748	43027.48	100	0.1
7	2503.692	140.962998	0.140962998	140962.998	100	0.1
8	361.085	19.9057025	0.019905703	19905.7025	100	0.1
9	813.556	45.470314	0.045470314	45470.314	100	0.1
10	816.942	45.661623	0.045661623	45661.623	100	0.1
11	1452.093	81.5476545	0.081547655	81547.6545	100	0.1
12	177.225	9.5176125	0.009517613	9517.6125	100	0.1
13	45.248	2.060912	0.002060912	2060.912	100	0.1
14	211.916	11.477654	0.011477654	11477.654	100	0.1
15	126.645	6.6598425	0.006659843	6659.8425	100	0.1

Note: 15 samples, each with a volume of 100 mL, were analyzed to confirm the presence of bisphenols ( $\mu\text{g/mL}$ ).

BPA can be present in barreled drinking water due to contamination of the raw water, inadequate purification processes, and migration of BPA from the barrels (Hao, 2022). In 17% of surface water sources, the highest recorded concentration of BPA was 0.014  $\mu\text{g/L}$ . In all treated water samples, the BPA concentration was below 0.005  $\mu\text{g/L}$ .

Table 2. Bisphenol concentration (ng/L) in water samples

Sample	ng/L in samples	
	Mean	S.D
1	4369.7	49.2
2	7916.3	30.1
3	11807.0	15.6
4	13609.2	20.5
5	11681.1	17.8
6	43027.5	22.8
7	140962.9	25.6
8	19905.7	18.7
9	45470.3	12.4
10	45661.6	15.5
11	81547.7	19.3
12	9517.6	11.2
13	2060.9	9.2
14	11477.7	13.4
15	6659.8	12.5
<b>LSD (0.05)</b>		<b>200.57</b>

Note: 15 samples were analyzed to obtain the mean bisphenol content.

The concentrations of bisphenols in river water were higher compared to other samples; this occurred likely due to their accumulation in soils and pore waters (Vázquez-Tapia *et al.*, 2022).

Bisphenol concentrations in water samples vary significantly across studies and locations, reflecting their widespread presence and environmental implications. In the Dongjiang River basin, southern China, BPA was detected in surface water at a median concentration of 1.81 ng/L. Other bisphenol derivatives, such as BADGE, were also present but often below quantifiable levels (Yang *et al.*, 2024). A study employing a novel organic-diffusive gradients in thin film (o-DGT) passive sampler revealed freshwater bisphenol concentrations ranging from 9.2 to 323 ng/L, with BPA, BPS, and BPF consistently identified across multiple sites (Wang *et al.*, 2024). In northeastern Poland, wastewater samples showed significantly higher BPA levels, with concentrations reaching 400 ng/L in influent water and 100 ng/L in effluent, indicating substantial reduction during treatment (Kiejza *et al.*, 2022). In Andhra Pradesh, India, BPA levels in lake water measured 12 ng/L, along with the presence of other bisphenols such as BPF and BPE, reflecting elevated plastic pollution in the area (Katari *et al.*, 2022). Additionally, a study of the Jialing River in China recorded BPA concentrations ranging from 6.15 to 90.03 ng/L, highlighting its persistent contamination in surface waters (Xuerong *et al.*, 2022). These findings emphasize the variability of bisphenol concentrations across water bodies and the critical need for effective monitoring and mitigation strategies to protect aquatic ecosystems and human health.

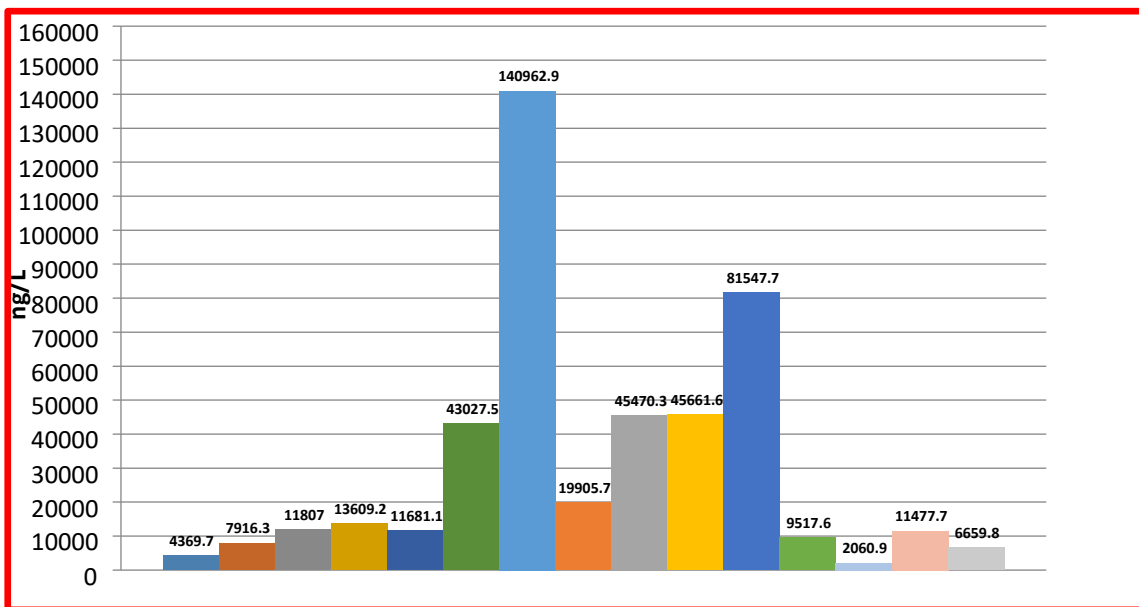


Figure 1. Relationship between bisphenol concentration (ng/L) (x-axis, 0–100) and peak area measurements (y-axis)

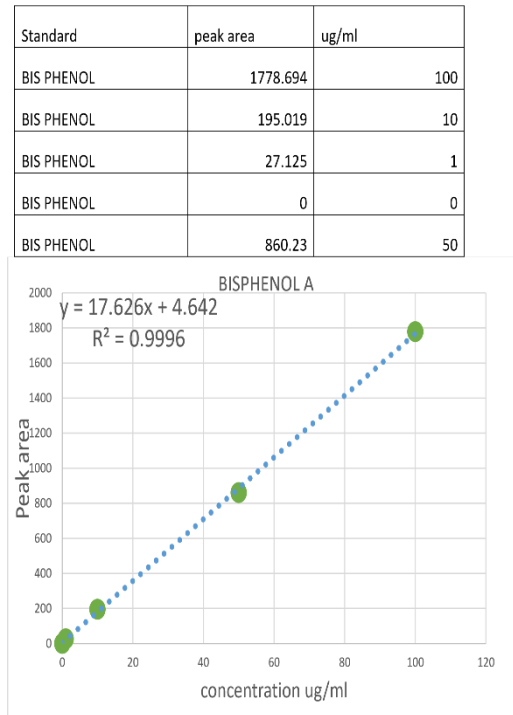


Figure 2. Quantitative analysis of bisphenol concentrations in samples using peak area measurements

(Li *et al.*, 2023) discovered that exposure to BPA reduces sperm quality and damages mouse testes through oxidative stress, iron accumulation, and mitochondrial damage. Furthermore, BPA was found to influence the expression of key proteins in testicular tissues, including ferritin heavy chain 1 (FTH1), cyclooxygenase 2 (COX2), glutathione peroxidase 4 (GPX4), and acyl-CoA synthetase 4 (ACSL4). These results align with the findings of the present study (Figure 2).

The comet assay, used to assess DNA damage, revealed that BPS induces genotoxic and cytotoxic effects in rats (Figure 3). Further investigations were conducted on the toxicity of BPS at the molecular level (Ali *et al.*, 2022).

Moreover, the migration of BPA into water was examined under varying conditions. Results showed that temperature plays a critical role in BPA migration. Although individual BPA concentrations in water containers were relatively low (ng/L), the daily intake could exceed these values significantly, given BPA's widespread use in consumer products. At a concentration of 0.6 mg/kg body weight, the overall exposure poses a potential health risk (Ginter-Kramarczyk *et al.*, 2020).

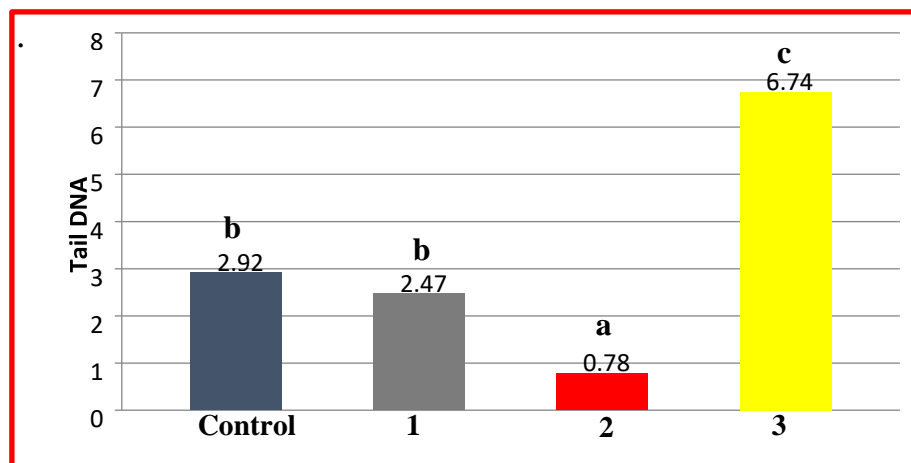


Figure 3. DNA damage in the rats after bisphenol injection according to comet assay.





Cytotoxicity studies revealed that BPA exposure did not affect cellular viability. However, genotoxicity research showed that BPA induced oxidative damage in the MRC-5 cell line and DNA damage in the Hep-2 cell line. Both cell lines exhibited an increase in micronuclei following BPA exposure (Ramos, 2019).

### Conclusions

The high concentrations of bisphenol detected in various water samples may result from raw water contamination and inadequate purification processes. Bisphenol compounds, particularly BPA, were successfully identified in multiple water sources, highlighting significant contamination, especially in industrial regions. Studies in laboratory animals demonstrated that bisphenol negatively affects various biological parameters, including DNA. Advanced analytical techniques confirmed its presence, whereas molecular studies revealed harmful effects on aquatic organisms, such as endocrine disruption. These findings underscore serious public health risks in terms of potential human exposure and ecological damage, emphasizing the urgent need for stricter regulations, further research on remediation strategies, and the development of safer alternatives.

**Author Contributions:** Noor Haider Al-Taei: Conceptualization, methodology, and primary writing of the draft manuscript; data analysis and interpretation.

Ekram Sabah Sahib Al Saidi: Conducting experimental procedures and analyses; contributing to the research design and data interpretation.

Fatema Ali Al Kafhage: Assisting in data collection, literature review, and manuscript refinement; providing critical feedback on the results.

Ayad M.J. Al-Mamoori: Contributing to developing analytical methods; involving in data validation, and authoring some manuscript sections.

Hayder Obayes Hashim: Supervising the project, providing expertise in molecular effects and environmental implications, and contributing to the overall editing and finalization of the manuscript.

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**Informed Consent Statement:** This research, titled “Detection of Bisphenol in Water and Study of Molecular Effects,” was conducted with informed consent obtained from all participants involved in data collection and sample analysis. Participants were thoroughly informed about the study’s purpose, methods, potential risks, and benefits. They were assured of their right to withdraw at any time without consequences.

All procedures involving human samples adhered to ethical guidelines set by the institutional research committee and complied with the 1964 Helsinki Declaration and its subsequent amendments. Participant confidentiality was strictly maintained, and data were reported in aggregate form to ensure anonymity.

By participating, individuals contributed to valuable research on the presence of bisphenol compounds in water and their molecular effects, thereby advancing public health knowledge and promoting environmental safety.

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**Conflicts of Interest:** The authors declare no conflicts of interest.



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