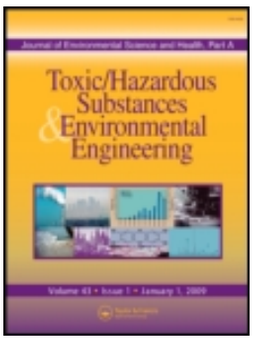

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Efficacy of UV-C photolysis of bisphenol A on transcriptome alterations of genes in zebrafish embryos

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ABSTRACT

The purpose of this study was to investigate the efficacy of UV-C direct photolysis of bisphenol A (BPA) as a remediation method of BPA contamination. We used zebrafish embryos as a model organism to test the toxicity and residual biological activity by measuring cytochrome P4501A1 (CYP1A), aromatase B (Aro B) and heat shock proteins (HSP-70) transcript levels. The mRNA levels of CYP1A gene increased about two fold while exposure of zebrafish embryos at 72 hpf resulted in significant induction ($P = 0.048$) of Aro B at 100 μg/L of BPA. Exposure of zebrafish embryos at 72 hpf to increasing concentrations of BPA resulted in significant induction ($P = 0.0031$) of HSP-70 transcript level. UV treatment of BPA resulted in a significant reduction in toxicity by reducing mortality of zebrafish embryos. The results suggest that UV-C direct photolysis may be an effective method for remediation of BPA contamination. Further studies will be necessary for better understanding of the identity and relative activity of the UV degradation by-products.

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KEYWORDS
Bisphenol A; endocrine disruption; zebrafish embryos; QPCR; biomarker genes; UV photolysis

Introduction

Production of bisphenol A (BPA) worldwide exceeds 3 million metric tons per year. BPA is used extensively in both industrial and domestic consumer products made from polycarbonate plastics and epoxy resins.[1] Recent studies have linked BPA to adverse health effects in infants, young children, and adults. While debate is going on relative safe levels for endocrine disruptive chemicals (EDCs), it is generally recognized that significant efforts will be needed to reduce environmental levels of BPA worldwide. It should be noted that instruments can be used to accurately detect concentrations of chemicals in the environment,[2] but biological assay can conclusively determine the toxic effects of contaminants and no observed adverse effect level (NOAEL). BPA can enter body through contaminated water, treated wastewater, food and physical handling of plastic coated surfaces. Significant attention has been given to BPA contamination due to its high level of production and widespread use in production of polycarbonate derived products.[3] BPA's solubility in water is much greater than its half maximal effective concentration (EC50) and can cause acute toxicity in aquatic organisms in the range of 1–15 mg/mL for freshwater and marine species.[4] Like other xenobiotics, it is documented to alter expression of various genes and physiological processes. Several studies have focused on the responses to chemical stressors at the molecular level in aquatic species and there is a great demand for cost effective approaches to evaluate its toxicity and develop effective remediation measures.[5,6] In the present study, we used zebrafish (*Danio rerio*) embryos as a model organism to identify effective biomarkers for BPA. Zebrafish embryos are widely used in the area of environmental toxicology, and considered as a suitable model organism to assess developmental abnormalities in vertebrates, including mammals.[7,8] Use of zebrafish also meets the OECD guideline for testing lethal effects of chemicals on embryonic stages of fish and considered a possible alternative test organism for acute toxicity in juvenile and adult fish.[9]

Gene expression has been used as a viable approach in many studies as validated biomarker to assess the effects of exposure to EDCs, including the investigation of photolysis and other advanced oxidation processes used for degradation of chemicals to remediate their toxic effects.[10] Owing to rising concern regarding the presence of BPA and other EDCs, the treatment of these chemicals is being extensively tested, and advanced oxidation processes (AOPs) are considered to be an effective remediation method to degrade organic pollutants. Various AOPs have been evaluated for degradation of BPA in aqueous solution. Among these, direct UV photolysis and UV+H_2O_2 provide relatively simple and economical methods. To determine the efficacy of the treatment process, changes in biological activity of BPA have been evaluated by a number of investigators in the past in addition to analytical methods.[11,12]

Studies revealed that BPA targets multi-receptor pathways in a complex mechanism within the endocrine system.

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Here, we focus on transcriptome alterations of genes involved in controlling various physiological parameters to assess the activity of BPA. The genes investigated include cytochrome P450 aromatase (Aro B), as a target for estrogen-like activity of BPA. Disruption of Aro B can adversely affect sexual hormones and impair function of steroidogenic enzymes, responsible for conversion of androgens to estrogens.[13–16] BPA was shown to influence brain specific expression of aromatase and this induction appears to be mediated through estrogen-mediated pathways as well as androgen receptors.[17,18] Most vertebrates tested contain detoxification mechanisms as protection against chemical contaminants that includes target genes family of cytochrome P450, such as cytochrome P4501A1 (CYP1A1).

In the present study, we used gene expression as a quantitative biological assay to assess biological activity of BPA. The expression of heat shock protein 70 (HSP-70) was measured, since its variation can lead to adverse health effects in vertebrates.[19] The expression of both inducible heat shock genes and constitutive forms has been found to be affected in response to physical and chemical stress.[20] We also measured CYP1A mRNA as potential biomarker for BPA in zebrafish embryos. CYP1A is phase I biotransformation enzyme, involved in the metabolism of chemicals by increasing their hydrophilicity through hydrolysis, reduction and oxidation.[21] The main objective of the present study was to use zebrafish embryos as a suitable model organism to test the hypothesis that exposure to BPA alters expression of genes coding for proteins involved in estrogen mediated pathway as well as stress response. We also aimed to identify potential biomarkers for testing the efficacy of UV based advanced oxidation processes to convert BPA into inactive by-products in an effort to develop an effective remediation procedure to address serious challenges resulting from contamination with BPA and other ubiquitous organic environmental chemicals.

Materials and methods

The animal care committee at the University of Calgary approved all protocols involving use of zebrafish embryos and adults. Embryos were obtained from the wild type adult zebrafish purchased from the Aquatic Imports, Calgary, Canada. Fish were kept in the laboratory in a dechlorinated RO water recirculating system with automatic pH and salt dosing unit (aquatic system water) at 14:10 h light and dark. pH solution contained 16 g/L $NaHCO_3$ (VWR International, Canada) and conductivity was maintained by 25 g/L sea salt solution (VWR International, Canada). Salinity and conductivity of the medium ranged between 0.5–1 g/L and 300–1,500 μS, respectively.

Zebrafish larvae were fed brine shrimp (*Artemia nauplii*, INVE, Salt Lake City, UT, USA), and adult fish were maintained on commercial diet twice daily (Zeigler). Seventy embryos per replicate (five replicates for each treatment) were transferred to sterile plates with aquatic system water (4 mL). At 5 h post fertilization (hpf), embryos were spiked with 1 mL of reverse osmosis (RO) water containing different concentrations of BPA along with the vehicle control group. Mortality was estimated and the system water with BPA solutions was renewed daily. After 72 hpf, the embryos were snap frozen for further analysis. Throughout the treatment period, embryos were kept in an incubator at 28 ± 0.5°C.

UV treatment of BPA

BPA (Sigma Aldrich, St. Louis, MO, USA) was used to make 35 ppm stock solution in Milli-Q water by sonication. Based on earlier studies on direct UV photolysis, the aqueous solution was irradiated with UV-C radiation using low pressure mercury lamp ($\lambda = 254$ nm) for 90 min in a Rayonet photo-reactor.[22]

HPLC analysis

BPA degradation and the intermediates generated during the degradation process were analyzed by HPLC, using a Varian Prostar 210 HPLC instrument (Palo Alto, CA, USA) equipped with a 325 nm liquid chromatography (LC) UV-visible detector. A Zorbax SB C-18 (4.6 150 mm, 5 μm, Agilent, Wilmington, DE, USA) column was used to separate the mother compound from its by-products. HPLC analysis showed the disappearance of BPA along with formation of photoproducts.

BPA exposure to zebrafish embryos

Stock solution with 35 ppm concentration of BPA was diluted to 50, 100, 150, 200 and 250 μg/L for exposure to zebrafish embryos. Similarly, UV treated BPA of 35 ppm stock was also diluted to the similar concentrations and used for exposure to zebrafish embryos to measure the mRNA levels of target genes.

RNA extraction and reverse transcription

Total RNA was extracted using TRIZOL reagent (Invitrogen, Canada) according to the manufacturer's protocol. Total RNA was quantified and the quality determined using Nanodrop (Thermo Scientific, Waltham, MA, USA) spectrophotometer at 260 and 280 nm. Reverse transcription of 4 μg of total RNA was carried out in a total volume of 18 μL according to manufacturer's protocol. An oligo d (T) anchor and M-MLV reverse transcriptase (Invitrogen, Burlington, ON, Canada) were used.

Quantitative real-time polymerase chain reaction (QPCR)

QPCR was done to determine the relative abundance of mRNA extracted from zebrafish embryos/larvae. All primers were optimized via gradient PCRs and a melt curve was performed for every QPCR plate. The efficiencies of reactions were determined by performing the QPCR on serial dilutions of cDNA. The efficiencies for all genes were between 90–105%, which is considered in the optimal range for QPCR analysis. PCR amplification was conducted using BIORAD I cycler Multicolor Real Time PCR Detection System (Bio-Rad, Hercules, CA, USA) and iQ TM SYBR Green Supermix (Bio-Rad), according to the manufacturer's instructions. Each reaction well contained 12.5 μL SYBR Green PCR Master Mix (Qiagen, Mississauga, Canada), 1 μL of diluted cDNA, 0.25 μM dNTPs, 0.25 μM of each

Table 1. QPCR primers used for the analysis of zebrafish genes.

Primer Name	Sequence	Reference	Annealing temp (°C)
β-Actin	F-CGCGCAGGAGATGGGAACC R- CAACGGAAACGCTCATTGC	Keegan et al.[23]	59.7
CYP1A	F-CATATCGTAGTATCCGTGGCTAAC R- CCCGAACTCATCGCTCATATT	Voelker et al.[24]	55
Aro B	F-AAGAGTTACTAATAAAGATCCACCGGTAT R- TCCACAAGCTTTCCCATTTCA	Sawyer et al.[25]	58
HSP-70	F- GCACAAGAAGGACATCAGTCAGA R-CGATGCCCTCGTACAGAGAGT	Kreiling et al.[26]	54

primer and ultrapure distilled water (Invitrogen, Buvlington, ON, Canada) in a total volume of 25 μL. QPCR included initial denaturation at 95°C for 3 min, 40 cycles of denaturation at 95°C for 10 s, and annealing at the gene specific temperature for 20 s. All forward and reverse primers along with their optimized annealing temperatures are shown in Table 1.[23–26] Each experimental sample was run in triplicates to ensure consistency. Relative quantification approach was employed for determination of mRNA by QPCR. It is based on the expression levels of a target gene versus a housekeeping gene. Zebrafish β-actin gene was used as internal control and to calculate the threshold cycle (Ct). The calibrated ΔCt value ($\Delta\Delta$Ct) for each sample and β-actin was calculated as [$\Delta\Delta$Ct = 2^−(ΔCt sample−ΔCt internal control)].[27]

Statistical analysis

Statistical analysis was performed using one-way ANOVA and two-way ANOVA as indicated to determine the difference among means of mRNA transcript levels for various BPA dosages and effect of UV photolysis, respectively. When ANOVA showed the presence of significant difference (P less than 0.05), post hoc multiple comparison of the mean was performed using the Tukey's test (Prism statistical software, Graph Pad Software, Inc., La Jolla, CA, USA).

Results

Time-related effects of BPA

In the first study, BPA dose of 100 μg/L was chosen as a sublethal concentration to determine time-related effects of BPA (48 and 72 hpf) in terms of transcript levels for zebrafish embryos. QPCR was carried out to determine transcript abundance for CYP1A, Aro B and HSP-70 genes as described above. The mRNA level of each target gene was expressed after normalizing to the expression of β-actin as a housekeeping gene, with respect to its respective control.

As shown in Figure 1, BPA exposure did not significantly affect transcript levels after 48 hpf. However, at 72 hpf, exposure to BPA significantly altered Aro B and HSP-70 transcript levels, compared to the control (Fig. 1). Exposure to BPA did not significantly alter GPX mRNA level, either at 48 or at 72 hpf (Fig. 1). The overall results from the initial experiment indicate that exposure of BPA in zebrafish embryos at 72 hpf may provide a suitable model system to test the efficacy of UV-C direct photolysis treatment on biological activity.

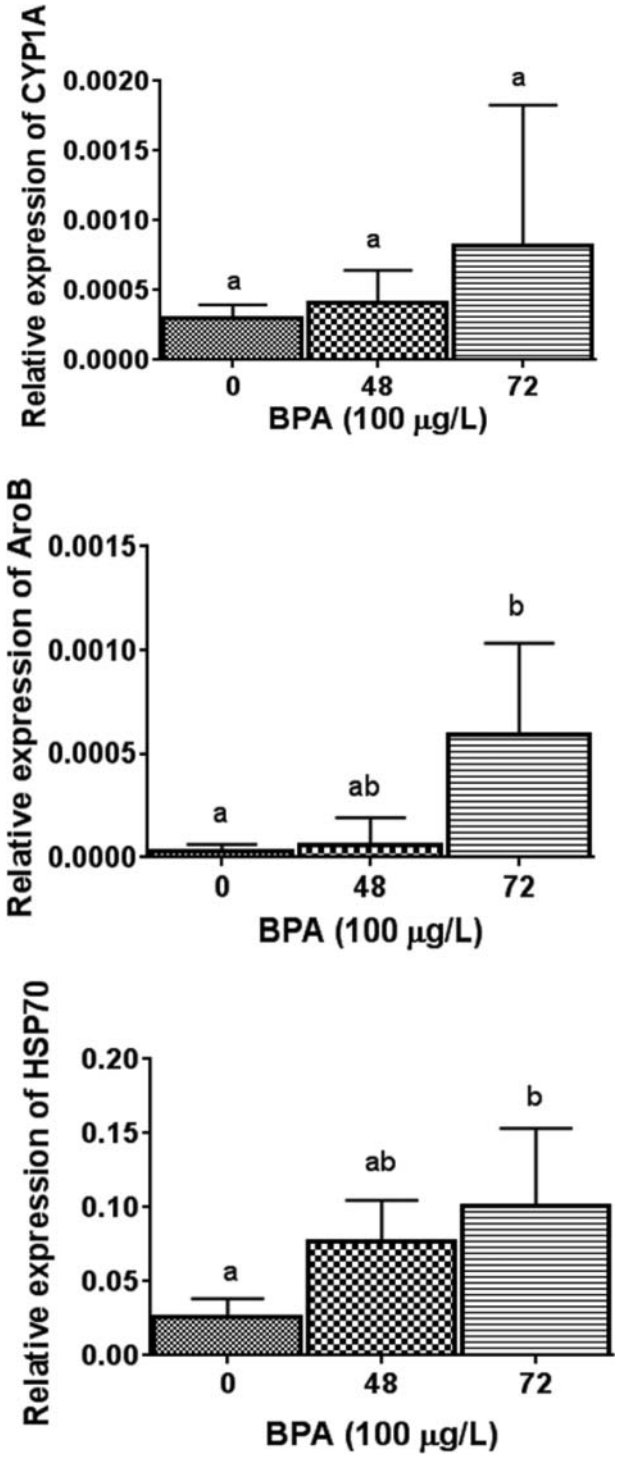

Figure 1. Relative expression levels of CYP1A (a) Aro B (b) HSP-70 (c)genes in zebrafish embryos exposed to 100 μg/L BPA for 48 and 72 hpf. The results are expressed as normalized fold expression levels with respect to the β-actin levels in the same sample. Different letters show significantly different means ($P < 0.05$). All values are means ± SD ($n = 5$).

Dose-related effects of BPA before and after UV-C direct photolysis treatment

Subsequent experiments were carried out to test the dose-related (50, 100, 150, 200 and 250 μg/L) effects of BPA

exposure, before and after UV-C direct photolysis treatment on CYP1A, Aro B and HSP-70 mRNA levels after 72 hpf in zebrafish embryos.

CYP1A transcript

Consistent with the results obtained in the first experiment, exposure of zebrafish embryos at 72 hpf to increasing concentrations of BPA (50, 100, 150, 200 and 250 μg/L) was without a significant effect on CYP1A mRNA level (Fig. 2). Parallel treatment of the embryos with the same concentrations of BPA after UV-C direct photolysis treatment was also without significant effect between treatment groups on CYP1A mRNA levels in 72 hpf zebrafish embryos (Fig. 2). While no changes were observed, using multiple comparison of the means, further statistical analysis by two-way ANOVA revealed significant effect of UV treatment (P = 0.0006), while no significant interaction and effect of dose was found between the two (untreated and treated BPA) groups.

Aro B transcript

Exposure of zebrafish embryos at 72 hpf to increasing concentrations of BPA (50, 100, 150, 200 and 250 μg/L) resulted in significant induction ($P = 0.048$) of Aro B at 100 μg/L (Fig. 3). The observed effect of BPA was biphasic, since doses greater than 100 μg/L did not alter the Aro B transcript level (Fig. 3).

Exposure of zebrafish embryos with UV-C direct photolysis treated BPA did not change Aro B transcript level at any of the concentrations tested, indicating photolytic deactivation of BPA (Fig. 3). Two way ANOVA also revealed significant effect of UV-C direct photolysis treatment on BPA-induced Aro B expression ($P = 0.03$) response.

HSP-70 transcript

Similar to Aro B, exposure of zebrafish embryos at 72 hpf to increasing concentrations of BPA (50, 100, 150, 200 and 250 μg/L) resulted in significant induction (P = 0.0031) of HSP-70 transcript level (Fig. 4). The observed effect of BPA on HSP-70 was complex since significant increase in transcript

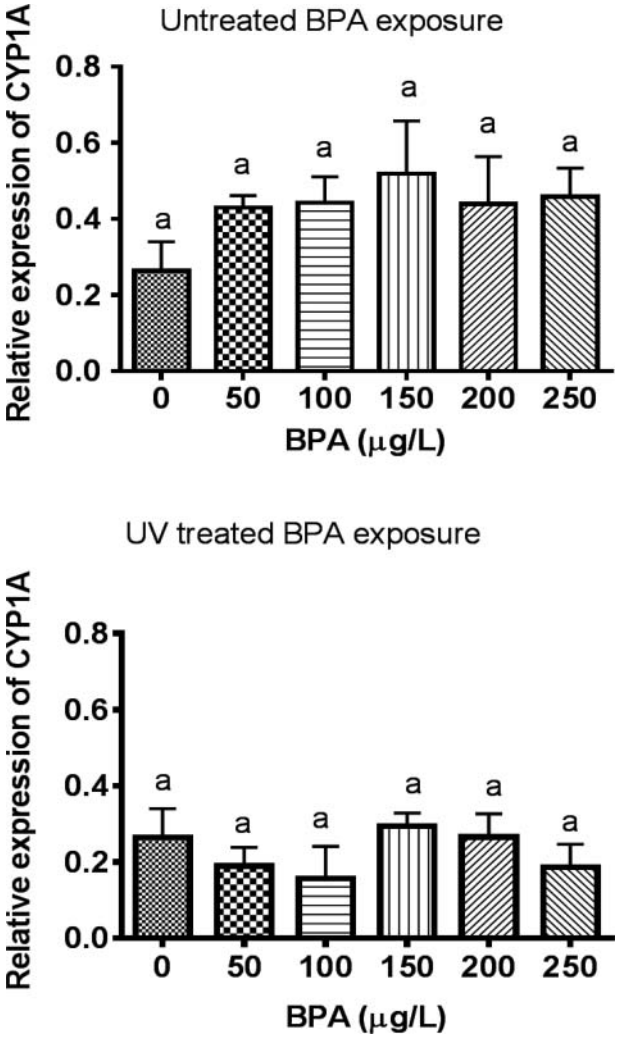

Figure 2. Relative expression levels of CYP1A gene in zebrafish embryos showing control (No BPA) and various concentrations of BPA exposed to zebrafish embryos till 72 hpf (a) CYP1A mRNA levels after BPA exposure, (b) CYP1A mRNA levels after UV treated BPA exposure. The results are expressed as normalized fold expression levels with respect to the β-actin levels in the same sample. Different letters show significantly different means ($P < 0.05$). All values are means ± SD ($n = 5$).

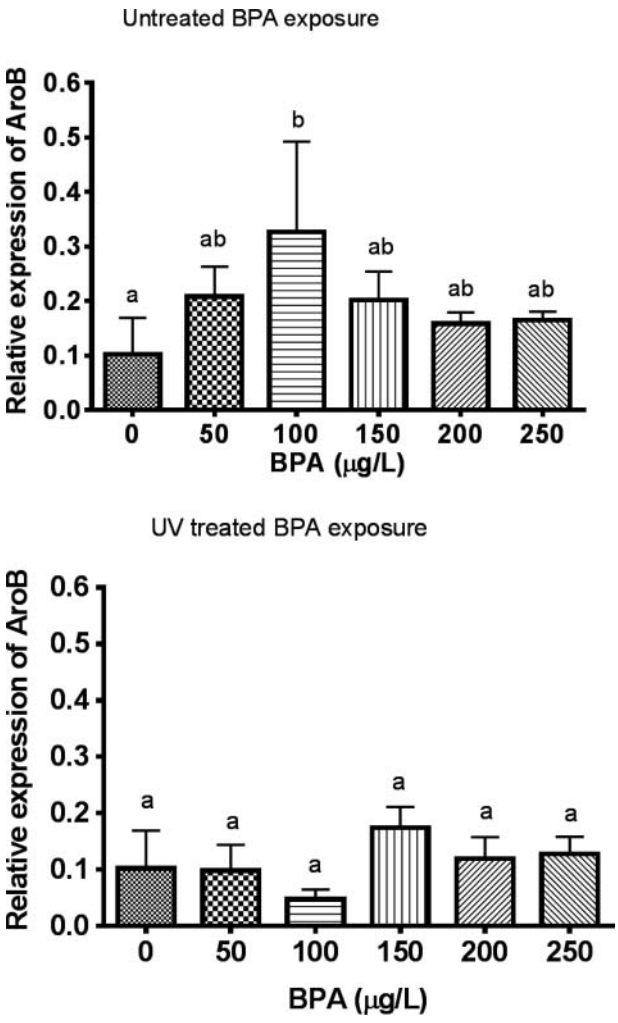

Figure 3. Relative expression levels of Aro B gene in zebrafish showing control (No BPA) and various concentrations of BPA exposed to zebra fish embryos till 72 hpf (a) Aro B mRNA levels after BPA exposure, (b) Aro B mRNA levels after UV treated BPA exposure.The results are expressed as normalized gene expression levels with respect to the β-actin levels in the same sample. Different letters show significantly different means ($P < 0.05$). All values are means ± SD ($n = 5$).

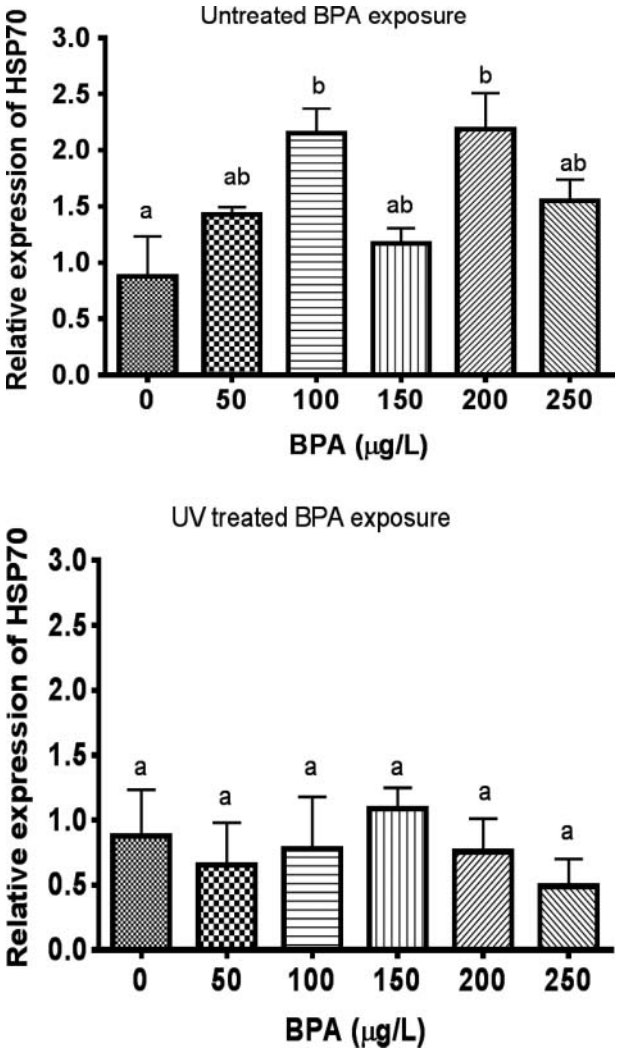

Figure 4. Relative expression of HSP-70 gene in zebrafish showing control (No BPA) and various concentration of BPA exposed to zebrafish embryos till 72 hpf (a) HSP mRNA levels after BPA exposure, (b) HSP mRNA levels after UV treated BPA exposure.The results are expressed as normalized gene expression levels with respect to the β-actin levels in the same sample. Different letters show significantly different means ($P < 0.05$). All values are means $\pm$ SD ($n = 5$).

level was observed following treatment with 100 and 200 μg/L of BPA, but not 150 μg/L. Otherwise similar biphasic effect was observed with no significant response at the lowest and highest doses of BPA test (Fig. 4).

As seen in Aro B, exposure of zebrafish embryos with UV-C direct photolysis treated BPA did not change HSP-70 transcript level at any of the concentrations tested, verifying our observation regarding photolytic deactivation of BPA (Fig. 4).

Discussion

The results presented in this study demonstrate that BPA exposure interferes with the normal expression of Aro B and HSP-70 genes in zebrafish embryos at 72 hpf. The results are consistent with the view that BPA exerts health disruptive effects during the embryonic development in fish and other vertebrates. The observed effects of BPA on Aro B expression are consistent with the reports demonstrating adverse effects of BPA on brain development and behavioral changes mediated by aromatase in the developing zebrafish embryos.[18] Aromatase is important for production of estrogen, which is a key regulator for reproduction. In this context, our results are also consistent with previous studies demonstrating reproductive and behavioral effects of chronic exposure of fish to BPA.[16,18,28,29] Sex differentiation in female is dependent on estrogens, which in turn regulate expression and activity of aromatase via estrogen-response elements located in the promoter region of the aromatase gene.[30] Thus, a contaminant with estrogen-like activity can disrupt normal expression and activity of aromatase, leading to abnormal gonadal development and possible sex reversal as reported previously.[31–33] Aromatase plays an important regulatory role in adults and during the embryonic/neonatal periods. The induction of Aro B upon BPA exposure has also been reported previously by other investigators.[34]

The superfamily of stress related heat-shock proteins includes the constitutive (HSC-70) and inducible (HSP-70) isoforms of heat shock protein 70. These proteins play a role in protection and promotion of the recovery of cells from physiological, pathological, thermal and chemical stress. In the present study, exposure to BPA was found to increase mRNA level for HSP-70 after 72 hpf in zebrafish embryos. Various studies have validated the use of the HSP-70 response as an indicator of stress in fish.[20] The present results of HSP-70 gene induction following exposure to BPA are consistent with previously reported findings.[35,36]

Following time-related study of BPA on CYP1A, Aro B and HSP-70 transcript levels, we investigated dose-related effects of BPA on the expression of the same genes in zebrafish embryos at 72 hpf. The results provide support for the hypothesis that exposure to BPA alter expression of genes coding for proteins involved in estrogen-mediated (Aro B) and stress (HSP-70) pathways in the developing zebrafish embryos. The results from dose-related BPA-induced response indicate a complex pattern of activity. There is evidence for non-monotonic dose-response relationships for BPA, ranging from simple biphasic to more complex response, depending on the end-point investigated (Aro B or HSP-70). This is consistent with a recent study demonstrating non-monotonic effects of BPA in rats.[37] The observed non-monotonic effect of BPA is not surprising, since environmental contaminants do not display the same level of specificity as natural hormones. At higher concentrations, contaminants display an overlap of specificity with different receptor systems, which can result in a complex pattern of physiological or pathological response.

This study was designed in an effort to identify potential biomarkers for testing the efficacy of UV based oxidative processes to convert BPA into inactive by-products to develop an effective remediation procedure. We used dose-related effects of BPA on Aro B and HSP-70 genes as a basis to test biological response to intact and UV based oxidation byproduct of BPA in developing zebrafish embryos. The observed effect of BPA on Cyp1A, however, was not robust enough for testing the efficacy of UV based advanced oxidation treatment in the present study. However, induction of xenobiotic metabolism by CYPs in fish is well established as a useful tool for ecotoxicological assays.[34] Cyp1A and related gene products are involved in the detoxification of pollutants through the aryl hydrocarbon receptor (AhR) mediated pathways during phase I metabolism

of contaminants.[38] However, the observed CP1A response following treatment with BPA was not very clear in the present study. Since the exposure to contaminants that have strong affinity for AhR can cause rapid induction of CYP1A mRNA in fish, making it an ideal biomarker of exposure to certain classes of pollutants.[39] Our results suggest that BPA may be a relatively weak AhR ligand in developing zebrafish embryos. In adult organisms, BPA is known to affect components involved in the maintenance of the redox status by downregulating the expression of antioxidant genes.[40]

The results support the view that UV based advanced oxidation processes can effectively convert BPA into inactive by-products. UV treatment of BPA resulted in a reduction in toxicity by reducing mortality of zebrafish embryos. More studies will be necessary to identify and test the effects of by-products to help with development of effective remediation procedure. The possible degradation mechanism involves attack of the hydroxyl radical produced during the UV photolysis to the benzene ring (hydroxylation), which was then followed by the dissociation of the C—C bond in BPA. The main BPA degradation intermediates were identified to be hydroxylated BPA and hydroquinone.[22]

It should be noted that BPA is not simply an estrogen mimic, and can disrupt physiological functions by altering multiple pathways, including the endocrine and metabolic systems.[29] This implies the need for further investigations of biological effects at lower BPA concentrations close to environmental levels while reconsidering the total allowable concentrations and finally its remediation strategies. Therefore, in this study, changes in biological activity of BPA as a function of UV treatment were evaluated using transcript analysis by QPCR which is a very sensitive quantitative method. UV as an oxidation process was applied to degrade BPA, and the decay of the parent compound along with the formation of degradation products was monitored using HPLC analysis. Bioanalytical assessments of residual biological activity in treated water have been used previously to evaluate the performance of the UV based advanced oxidation process for estrogenic contaminants like BPA in water.[41,42] In a study, phenol, 1,4-dihydroxylbenzene and 1,4-benzoquinone were identified by means of HPLC as intermediate products of the photodegradation of bisphenol A and the disappearance of its estrogenic activity during irradiation of 120 min was reported by Neamtu and Frimmel.[11] The present study applied UV-C exposure in mercury lamp for 90 min and HPLC analysis showed complete mineralization of BPA, causing deactivation of the biological response as shown in BPA-induced Aro B and HSP-70 transcript levels following UV treatment (Figs. 2, 3 and 4). The effectiveness of UV treatment in combination of TiO_2 and other AOPs has been demonstrated previously,[43,44] and some oxidation products generated from incomplete degradation showed potential to increase the estrogenic activity. Feasible applications of treatment technologies are based on complete mineralization of parents and their intermediate compounds without secondary pollution and low operating costs.[45] The present study for BPA degradation, points toward direct UV photolysis as an economical and efficient treatment option that can be tested further for biological effects of individual byproducts formed during degradation of the parent compound. Furthermore, understanding the oxidation processes of BPA with respect to its biological assessment for residual biological activity can add to our fundamental knowledge for clarifying the effects of BPA in the aquatic environment and to come across effective treatment endpoints.

Conclusion

Through this study, we were able to recognize and validate genes sensitive to BPA exposure and degradation during early life periods of zebrafish. These genes may serve as biomarkers or targets for more focused investigations and to determine the effects of other typical compounds and treatment processes for their remediation. The results demonstrate that the induction of Aro B and HSP-70 transcripts may be used to assess the effects of BPA exposure in developing zebrafish embryos. Furthermore, the results suggest that UV photolysis is a cost effective option for BPA degradation in aqueous solutions. This treatment process need to be tested further for the biological activity of the byproducts formed during UV photolysis of parent compound. Further study may be helpful in finding effective and economical treatment for other chemicals to remediate the aquatic environment.

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References

[1] Sharma, V.K.; Anquandah, G.A.K.; Yngard, R.A.; Kim, H.; Fekete, J.; Bouzek, K.; Ray, A. K.; Golovko, D. Nonylphenol, octylphenol, and bisphenol-A in the aquatic environment: A review on occurrence, fate, and treatment. J. Environ. Sci. Heal. A. **2009**, *44*(5),423–442.

[2] Poorahong, S.; Thammakhet, C.; Thavarungkul, P.; Kanatharana, P. Online in-tube microextractor coupled with UV-Vis spectrophotometer for bisphenol A detection. J. Environ. Sci. Heal. A. **2013**, *48*(3), 242–250.

[3] Flint, S.; Markle, T.; Thompson, S.; Wallace, E. Review: Bisphenol Aexposure, effects and policy: A wildlife perspective. J. Environ. Manage. **2012**, *104*, 19–34.

[4] Willhite, C.C.; Ball, G.L.; McLellan, C.J. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. J. Toxicol. Environ. Health B Crit. Rev. **2008**, *11*(2), 69–146.

[5] Karouna-Renier, N.K.; Zehr, J.P. Short term exposures to chronically toxic copper concentrations induced HSP-70 protein in midge larvae (*Chironomus tentans*). Sci. Total Environ. **2003**, *312*(1–3), 267–272.

[6] Perceval, O.; Couillard, Y.; Pinel-Alloul, B.; Giguere, A; Campbell, P. G. Metal induced stress in bivalves living along a gradient of Cd contamination: relating sub-cellular metal distribution to population level responses. Aquat. Toxicol. **2004**, *69*(4), 327–345.

[7] Cajaraville, M.P.; Bebianno, M.J.; Blasco, J.; Porte, C.; Sarasquete, C.; Viarengo, A. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci. Total Environ. **2000**, *247*(2–3), 201–212.

[8] Zhu, X.; Zhu, L.; Duan, Z.; Qi, R.; Li, Y.; Lang, Y. Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to zebrafish (*Danio rerio*) early developmental stage. J. Environ. Sci. Heal. A. **2008**, *43*(3), 278–284.

[9] Ali, S.; Mil, H.G.J.v.; Richardson, M.K. Large-scale assessment of the zebrafish embryo as a possible predictive model in toxicity testing. PLoS ONE. **2011**, *6*(6), e21076.

[10] Pei-Jen-Chen; Rosenfeldt, E.J.; Kullman, S.W.; Hinton, D.E.; Linden, K.G. Biological assessments of a mixture of endocrine disruptors at environmentally relevant concentrations in water following UV/H_2O_2 oxidation. Sci. Total Environ. **2007**, *376*(1–3), 18–26.

[11] Neamtu, M.; Frimmel, F.H. Degradation of endocrine disrupting bisphenol A by 254 nm irradiation in different water matrices and effect on yeast cells. Wat. Res. **2006**, *40*(20), 3745–3750.

[12] Rosenfeldt, E.J.; Chen, P.J.; Kullman, S.; Linden, K.G. Destruction of estrogenic activity in water using UV advanced oxidation. Sci. Total Environ. **2007**, *377*(1), 105–113.

[13] Barney, M.L.; Patil, J.G.; Gunasekera, R.M.; Carter, C.G.Distinct cytochrome P450 aromatase isoforms in the common carp (*Cyprinus carpio*): sexual dimorphism and onset of ontogenic expression. Gen. Comp. Endocrinol. **2008**, *156*(3), 499–508.

[14] Guiguen, Y.; Fostier, A.; Piferrer, F.; Chang, C. F. Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. Gen. Comp. Endocrinol. **2010**, *165*(3), 352–366.

[15] Rasheeda, M.K.; Sridevi, P.; Senthilkumaran, B. Cytochrome P450 aromatases: impact on gonadal development, recrudescence and effect of hCG in the catfish, *Clarias gariepinus*. Gen. Comp. Endocrinol. **2010**, *167*(2), 234–245.

[16] Hatef, A.; Zare, A.; Alavi, S.M.H.; Habibi, H.R.; Linharty, O. Modulations in androgen and estrogen mediating genes and testicular response in male goldfish exposed to bisphenol A. Environ. Toxicol. Chem. **2012**, *31*(9), 2069–2077.

[17] Chung, E.; Gencoa, M.C.; Megrelisa, L.; Rudermana, J.V. Effects of bisphenol A and triclocarban on brain specific expression of aromatase in early zebrafish embryos. PNAS. **2011**, *108*(43), 17732–17737.

[18] Kinch, C.D.; Ibhazehiebo, K.; Jeong, J.; Habibi, H.R.; Kurrasch, D. Low-dose exposure to bisphenol A and replacement bisphenol S induces precocious hypothalamic neurogenesis in embryonic zebrafish. PNAS. **2015**, *112*(5), 1475–1480.

[19] Airaksinen, S.; Jokilehto, T.; Råbergh, C.M.I.; Nikinmaa, M. Heat and cold inducible regulation of HSP-70 expression in zebrafish ZF4 cells. Comp. Biochem. Physiol. Part B. **2003**, *136*(2), 275–282.

[20] Lee, S.; Lee, S.; Park, C.; Choi, J. Expression of heat shock protein and hemoglobin genes in *Chironomus tentans* (*Diptera, chironomidae*) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. Chemosphere. **2006**, *65*(6), 1074–1081.

[21] Whyte, J.J.; Jung, R.E.; Schmitt, C.J.; Tillitt, D.E. Ethoxyresorufin-Odeethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. **2000**, *30*(4), 347–570.

[22] Mehrabani-Zeinabad, M.; Achari, G.; Langford, C.H. Degradation of bisphenol A using advanced oxidation processes. GEN. **2013**, *59*, 01.

[23] Keegan, B.R.; Feldman, J.L.; Lee, D.H.; Koos, D.S.; Ho, R.K.; Stainier, D.Y.; Yelon, D. The elongation factors Pandora/Spt6 and foggy Spt 5 promote transcription in the zebrafish embryo. Development. **2002**, *129*(7), 1623–1632.

[24] Voelker, D.; Vess, C.; Tillmann, M.; Nagel, R.; Otto, G.W.; Geisler, R.; Schirmer, K.; Scholz, S. Differential gene expression as a toxicant sensitive endpoint in zebrafish embryos and larvae. Aquat. Toxicol. **2007**, *81*(4), 355–364.

[25] Sawyer, S.J.; Gerstner, K.A.; Callard, G.V. Real-time PCR analysis of cytochrome P450 aromatase expression in zebrafish: Gene specific tissue distribution, sex differences, developmental programming and estrogen regulation. Gen. Comp. Endocrinol. **2006**, *147*(2), 108–117.

[26] Kreiling, J.A.; Creton, R.; Reinisch, C. Early embryonic exposure to polychlorinated biphenyls disrupts Heat-Shock Protein 70 cognate expression in zebrafish. J. Toxicol. Env. Heal. B. **2007**, *70*(12), 1005–1013.

[27] Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods. **2001**, *25*, 402–408.

[28] Kidd, K.A.; Blanchfield, P.J.; Flick, R.W. Collapse of a fish population after exposure to a synthetic estrogen. Proc. Natl. Acad. Sci. USA. **2007**, *104*(21), 8897–8901.

[29] Jordan, J.; Zare, A.; Jackson, L.J.; Habibi, H.R.; Weljie, A.M. Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. J. Proteome Res. **2012**, *11*(2), 1133–1143.

[30] Fengling, L.; Zhaoxin, L.; Qingyin, W.; Yuxiu, Z. Effects of BPA and E_2 on expression profiles of genes related to hypothalamic pituitary gonadal axis of half-smooth tongue sole *Cynoglossus semilaevis*. Chin. J. Oceanol. Limnol. **2013**, *31*(3), 598–608.

[31] Crisp, T.M.; Clegg, E.D.; Cooper, R.L.; Wood, W.P.; Anderson, D.G.; Baetcke, K.P.; Hoffmann, J.L.; Morrow, M.S.; Rodier, D.J.; Schaeffer, J.E.; Touart, L.W.; Zeeman, M.G.; Patel, Y.M. Environmental endocrine disruption: an effects assessment and analysis. Environ. Health Perspect. **1998**, *106*(Suppl. 1), 11–56.

[32] Jeffries, K.M.; Jackson, L.J.; Ikonomou, M.G.; Habibi, H.R. Presence of natural and anthropogenic organic contaminants and potential fish health impacts along two river gradients in Alberta, Canada. Environ. Toxicol. Chem. **2010**, *29*(10), 2379–2387.

[33] Evans, R.M.; Scholze, M.; Kortenkamp, A. Additive mixture effects of estrogenic chemicals in human cell-based assays can be influenced by inclusion of chemicals with differing effect profiles. PLoS ONE. **2012**, *7*(8), e43606.

[34] Lee, Y.J.; Ryu, H.Y.; Kim, H.K.; Min, C.S.; Lee, J.H.; Kim, E.; Nam, B.H.; Park, J.H.; Jung, J.Y.; Park, E.Y.; Lee, K.H.; Ma, J.Y.; Won, H.S.; Im, M.W.; Leem, J.H.; Hom, Y.C.; Hong, Y.C.; Yoon, H.S. Maternal and fetal exposure to bisphenol A in Korea. Reprod. Toxicol. **2008**, *25*(4), 413–419.

[35] Park, K.; Kwak, I. Expression of stress response HSP-70 gene in asian paddle crabs, *Charybdis japonica*, exposure to endocrine disrupting chemicals, Bisphenol A (BPA) and 4-Nonylphenol (NP). Ocean Sci. J. **2013**, *48*(2), 207–214.

[36] Michail, X.; Kontogiannatos, D.; Syriou, V.; Koutri, A. Bisphenol-A affects the developmental progression and expression of heat-shock protein genes in the moth *Sesamia nonagrioides*. Ecotoxicology. **2012**, *21*(8), 2244–2253.

[37] Liang, Q.; Gao, X.; Chen, Y.; Hong, K.; Wang, H.S. Cellular mechanism of the nonmonotonic dose response of bisphenol A in rat cardiac myocytes. Environ. Health Perspect. **2015**, *122*(6), 601–608.

[38] Holth, T.F.; Nourizadeh-Lillabadi, R.; Blaesbjerg, M.; Grung, M.; Holbech, H.; Petersenf, G.I.; Alestrom, P.; Hylland, K. Differential gene expression and biomarkers in zebrafish (*Danio rerio*) following exposure to produced water components. Aquat. Toxicol. **2008**, *90*(4), 277–291.

[39] Stegeman, J.J.; Hahn, M.E. Biochemistry and molecular biology of monooxygenases: current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species. In *Aquatic Toxicology*; Malins, D.C.; Ostrander, G.K., Eds.; Lewis Publishers: Boca Raton, FL, **1994**; 87–203.

[40] Heinonen, J.; Hanken, J.; Kukkonen, J.V.K.; Holopainen, I.J. Bisphenol A accumulation in the freshwater *Clam Pisidiumamnicum* at low temperature. Arch. Environ. Contam. Toxicol. **2002**, *43*(1), 50–55.

[41] Ishibashi, H.; Watanabe, N.; Matsumura, N.; Hirano, M.; Nagao, Y.; Yoshihara, S.; Arizono, K. Toxicity to early life stages and an estrogenic effect of a bisphenol A metabolite, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene on the medaka (*Oryzias latipes*). Life Sci. **2005**, *77*(21), 2643–2655.

[42] Nomiyama, K.; Tanizaki, T.; Koga, T.; Arizono, K.; Shinohara, R. Oxidative degradation of BPA using TiO_2 in water and transition of estrogenic activity in the degradation pathways. Arch. Environ. Contam. Toxicol. **2007**, *52*(1), 8–15.

[43] Yamaguchi, A.; Ishibashi, H.; Kohra, S.; Arizono, K.; Tominaga, N. Short term effects of endocrine disrupting chemicals on the expression of estrogen responsive genes in male medaka (*Oryzias latipes*). Aquat. Toxicol. **2005**, *72*(3), 239–249.

[44] Rosenfeldt, E.J.; Linden, K.C. Degradation of endocrine disrupting chemicals bisphenol A, ethylestradiol, and estradiol during UV photolysis and advanced oxidation processes. Environ. Sci. Technol. **2004**, *38*(20), 5476–5483.

[45] Pera-Titus, M.; García-Molina, V.; Baños, M. A.; Giménez, J.; Esplugas, S. Degradation of chlorophenols by means of advanced oxidation processes: a general review. Appl. Catal. B: Environ. **2004**, *47*(4), 219–256.