Photodecomposition and Bioconcentration of a Bisphenol A Metabolite in Medaka, *Oryzias latipes*

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Exposure experiments in medaka and photodecomposition tests were performed using a metabolite of bisphenol A [4-methyl-2,4-bis(*p*-hydroxyphenyl)-pent-1-ene; MBP], the solubility limit of which is 42 mg/l of water. Three adult medaka were kept in a 2 l glass beaker at 25 ± 1°C for 4 days. The LC_{50} for 96 hr was > 1000 ppb. The measured average MBP concentration in the breeding water (nominal concentration of 100 ppb) was 49.2 ppb. The average concentration in the whole bodies of medaka after 4 days was 1.92 mg/g-wet body, and the bioconcentration factor (BCF) of MBP was calculated to be 39.0. MBP in water and acetone was decomposed very easily, with about 98% of the MBP being decomposed after several hours under sunlight. MBP was also decomposed after 48 hr of illumination under a white fluorescent lamp.

Key words —— bisphenol A metabolite, bioconcentration factor, photodecomposition

INTRODUCTION

Bisphenol A [BPA; 2,2-bis(4-hidroxyphenyl)propane] is manufactured in large quantities as the main raw material of polycarbonate and epoxy resins and is widely used all over the world. BPA is released into environment, and has been detected not only in river water, sediments, sludge[1,2] and tap water,[3] but also in human biological fluids.[4,5] Numerous researchers have reported that BPA has a weak estrogen effect, as it possesses similar structure and molecular weight as the steroid hormones. Yoshihara *et al*. recently found that the estrogenic activity of 4-methyl-2,4-bis(*p*-hydroxyphenyl)pent-1-ene (MBP), which is an active metabolite of BPA produced after incubation with the rat liver S9 fraction, is much more potent than that of the parent BPA.[6,7] In fact, the estrogenic activity of MBP was several hundred to several thousand times greater than BPA. BPA released into the environment as a result of human activities could be converted to MBP, and if this metabolite disrupts the endocrine systems of living organisms, including humans, the risks of BPA need to be reevaluated. However, data on the behavior of MBP in the environment is required for risk evaluation of BPA and MBP, and thus, we investigated the bioconcentration factor (BCF) and performed photodecomposition testing of MBP.

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MATERIALS AND METHODS

Chemicals —— MBP was synthesized by thermal dissociation following dimerization of BPA.[8] The crude product was subjected to chromatography on a column of silica gel using a 10 : 1 mixture of hexane and ethyl acetate as eluent to give MBP as pale yellow crystals. A sample was recrystallized from hexane and ethyl acetate to give colorless crystals; m.p. = 129–130°C, Anal. Calcd for $C_{18}H_{20}O_2$: C, 80.56; H, 7.51. Found: C, 80.40; H, 7.79. *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) (SPELCO Ltd., U.S.A.) was used as a chemical reagent for silylation. Figure 1 shows the chemical structure and the mass fragment of the silylated struc-

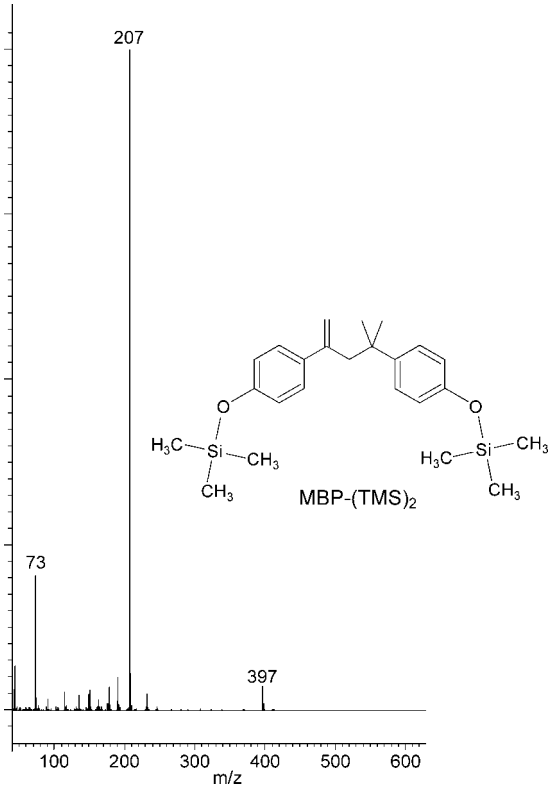

Fig. 1. Chemical Structure and Mass Fragment of Silylated Structure of MBP

Analysis of MBP was possible by GC/MS without silylation. BSTFA was used as a chemical reagent for silylation.

ture of MBP (MBP-TMS). This reaction reduced the polarity of MBP and enabled trace analysis. BPA-d_{16} was used as an internal standard for GC/MS analysis. Other reagents were of analytical-reagent grade for analyzing pesticide residues (Kanto Chemical, Japan) unless otherwise stated.

GC/MS System —— Analysis was conducted on a GC (CP-3800, Varian, U.S.A.) equipped with an ion trap MS detector (Saturn 2000, Varian, U.S.A.) and a capillary column (DB-5ms; length, 30 m; film thickness, 0.25 μm; diameter, 0.25 mm; J & W Scientific, U.S.A.), in splitless mode (10 psi, split ratio = 30) using helium as the carrier gas. Injection volume was 1 μl. The split vent was opened 1 min after starting the analysis. The injection port and ion trap temperatures were set at 300 and 250°C, respectively. Column temperature was initially maintained at 35°C for 1 min and was then increased to 250°C at a rate of 10°C/min. After the column temperature was at 250°C for 10 min, it was again increased to 300°C at a rate of 20°C/min and was maintained at that level for another 5 min. MS analysis was carried out using EI mode with an MS scan speed of 1.2 scans/sec and an MS detector voltage of 1450 V.

Fish and Exposure Conditions —— Six 2-L beakers were prepared for the control and exposure groups. Each beaker maintained three adult Japanese medaka fish (*Oryzias latipes*, d-rR strain). Fish were kept under a constant 14-hr light : 10-hr dark cycle and a temperature of 25 ± 1°C, and were fed commercial feed (freeze dried day-old brine shrimp, Artemia Gold, Nagasaki Rikagaku Co., Nagasaki, Japan) at 2 wt% body weight every morning. During the experimental period, all of the water in the beaker was replaced each morning with dechlorinated tap water. MPB concentration of the tap water was below the detection limit (0.05 ppb). LC_{50} and the solubility of MBP were measured before exposure experiments. The 96 hr-LC_{50} and solubility were over 1000 ppb and 42 ppm, respectively. The exposure concentration for fish was set at 100 ppb. MPB (200 μg) dissolved in 200 μg of dimethyl sulfoxide was added to the beakers of the exposure group. Control fish were exposed to the solvent carrier only.

Measurement of MBP in the Water —— About 80 ml of water was taken out of the beaker and placed in a 100-ml vial, and 1 μg of BPA-d_{16} as an internal standard, 1 ml of 1 M-HCl, and 10 ml of dichloromethane were added. After mixing for 30 min, the dichloromethane was collected and this operation was repeated. The collected dichloromethane solution was mixed with anhydrous sodium sulfate for dehydration. The solution was concentrated to approximately 0.5 ml using a high-purity nitrogen gas flow, and 200 μg of BSTFA was added to the solution for the silylation of MBP and BPA-d_{16}. The sample was analyzed by GC/MS. The peak area ratio of MBP-TMS and BPA-d_{16}-TMS was used for quantitative analysis of MBP.

Measurement of MBP in the Fish —— Nine fish were taken out of the three beakers for analysis after 4 days of exposure. Body weights of all fish were measured. One sample, which substantially deviated from the average, was omitted from the study. Each fish was homogenized with 5 ml methanol and 1 μg BPA-d_{16} was added. Nonpolar impurities in the extract were removed with 20 ml hexane. Target compounds (MBP and BPA-d_{16}) in the remaining solution were extracted with 10 ml dichloromethane. The same operations as described for the water samples were performed after this operation.

Photodecomposition Testing of MBP —— Two types of solution and light source were used for pho-

Table 1. MBP Concentrations in Water and Fish, and BCF

Experimental Group	Water (ppb)				Fish (ppb)[a)]	BCF
	Nominal Concentration	0 hr (n = 2)	24 hr (n = 2)	Average of 0 and 24 hr	96 hr (n = 8)	
Exposure	100	92.2	6.21	49.2	1920	39.0
Control	0	n.d.[b)]	n.d.[b)]	—	n.d.[b)]	—

Every 24 hr during the test period, all of the water in each beaker was changed. MPB concentration in the water was analyzed twice for each group; at 0 and 24 hr after changing of the water. MBP concentration in the fish was analyzed for each group after four days of exposure. *a*) Nano gram-MBP/g-wet body weight, *b*) Not detected.

todecomposition testing of MBP. Filtered and deionized water from Mili-Q water systems (Millipore, Bedford, MA, U.S.A.) was used for the preparation of aqueous MBP solution. To about 1 l of water in a glass beaker was added 200 μg of MBP solution diluted with dimethyl sulfoxide. Twelve glass vials were filled with about 100 ml of the water solutions and sealed with screw caps. The vials were stored in a refrigerator. These were then placed in the sunlight on a clear day. Two vials were taken for analysis of MBP concentration when after the appropriate period of time had passed.

In addition, a 10 ppm MBP solution in acetone was prepared and sealed with screw cap. This solution was tested in sunlight or under a 20-W white fluorescent lamp. The distance between the lamp and the liquid surface was kept constant at 5 cm.

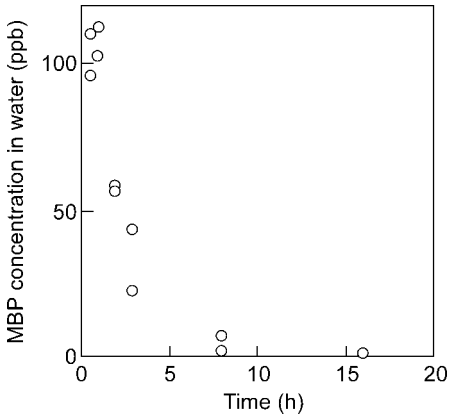

Fig. 2. Relationship between MBP Concentration in Water and Photolysis Period under Sunlight

Initial concentration was 100 ppb. Glass vials, which were filled with an aqueous solution of MBP, were placed in sunlight on a clear day. Two vials were analyzed for MBP concentration after the appropriate period of time had passed.

RESULTS AND DISCUSSION

Bioconcentration Factor in Medaka

The concentrations of MBP in the water containing the 3 breeding adult medaka were 92.2 ppb (n = 2) and 6.21 ppb (n = 2) after 0 hr and 24 hr, respectively (Table 1). The average concentration was 49.2 ppb. MBP concentration in the whole bodies of fish was 1.92 μg/g-wet body (n = 8), and thus BCF was calculated to be 39.0. This value could not be compared with those of other studies as this is the first report of the BCF of MBP in fish.

There are some reports of the BCF of BPA, which has similar chemical structure and properties. Lindholst *et al*. reported that the BCF of BPA ranged from 8.7 to 38.4 in the livers and from 1.7 to 3.6 in the muscles of juvenile rainbow trout.[9)] Heinonen *et al*. and Honkanen *et al*. reported that the BCF was 110–144 and 94–182 in freshwater clams and landlocked salmon, respectively.[10,11)] In spotted halibut, Lee *et al*. reported that the BCF varied between 13 and 38.[12)] The BCF value of MBP was 39.0 in this paper, and this value appears to be similar as those reported for BPA. Thus, MBP was classed as having a low potential for bioaccumulation.[13)]

Photodecomposition Test

Figure 2 shows the relationship between MBP concentration of the water and the photolysis period under the sunlight. MBP concentration in the water decreased by about half for 2–3 hr, and about 99% of the MBP was lost after 16 hr of exposure. Therefore, if MBP was released into the environment from living organisms as a metabolite of BPA, MBP would not persist for a long period of time under sunlight.

Figure 3 shows the relationship between MBP concentration in acetone and the photolysis period under sunlight. MBP was also decomposed after 2–3 hr, even in organic solvent. Figure 4 shows the relationship between MBP concentration in acetone and the photolysis period under the white fluorescent lamp. MBP decomposed more slowly than under sunlight, but almost no MBP was detected after 48 hr. This phenomenon suggests that MBP requires

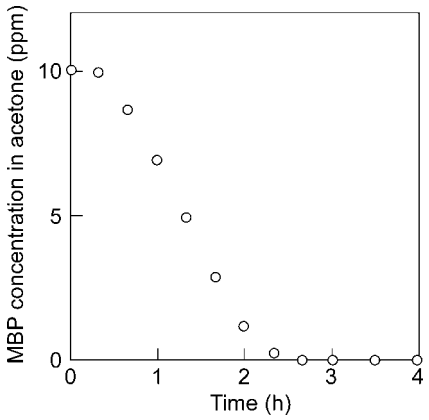

Fig. 3. Relationship between MBP Concentration in Acetone and Photolysis Period under Sunlight

Initial concentration was 10 ppm. Experiment was performed in sunlight on a clear day.

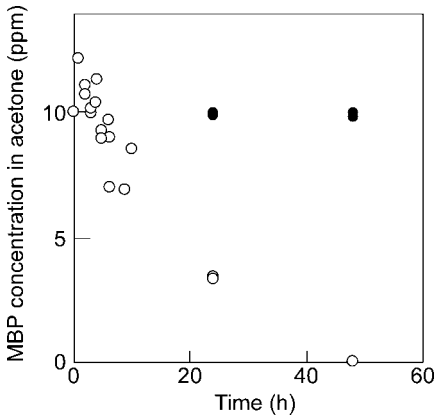

Fig. 4. Relationship between MBP Concentration in Acetone and Photolysis Period (○) under a White Fluorescent Lamp and (●) in the Dark at Room Temperature

Initial concentration was 10 ppm. The solution was tested under a 20-W white fluorescent lamp. The distance between the lamp and the liquid surface was kept constant at 5 cm.

shielding from light when used in a laboratory during an exposure testing or analysis.

Based on bioconcentration testing and photodecomposition testing, MBP can be classified as a low-level group for bioconcentration, while MBP was found to be easily decomposed under sunlight. MBP is not considered to be a serious problem in the environment when compared with persistent organic pollutants, such as PCB or DDT. However, careful follow-up investigations on MBP are required because biological concentration in the food chain does not always occur under sunlight and the estrogenic effects of MBP are extremely potent.

REFERENCES

1) Bolz, U., Hagenmaier, H. and Korner, W. (2001) Phenolic xenoestrogens in surface water, sediments, and sewage sludge from Baden-Wurttemberg, southwest Germany. *Environ. Pollut.*, **115**, 291–301.
2) Fromme, H., Kuchler, T., Otto, T., Pilz, K., Muller, J. and Wenzel, A. (2002) Occurrence of phthalates and bisphenol A and F in the environment. *Water Res.*, **36**, 1429–1438.
3) Kuch, H. M. and Ballschmiter, K. (2001) Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. *Environ. Sci. Technol.*, **35**, 3201–3206.
4) Sajiki, J., Takahashi, K. and Yonekubo, J. (1999) Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. *J. Chromatogr. B*, **736**, 255–261.
5) Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y. and Taketani, Y. (2002) Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Hum. Reprod.*, **17**, 2839–2841.
6) Yoshihara, S., Makishima, M., Suzuki, N. and Ohta, S. (2001) Metabolic activation of bisphenol A by rat liver S9 fraction. *Toxicol. Sci.*, **62**, 221–227.
7) Yoshihara, S., Mizutare, T., Makishima, M., Suzuki, N., Fujimoto, N., Igarashi, K. and Ohta, S. (2004) Potent estrogenic metabolites of bisphenol A and bisphenol B formed by rat liver S9 fraction: Their structures and estrogenic potency. *Toxicol. Sci.*, **78**, 50–59.
8) Dai, S. H., Lin, C. Y., Rao, D. V., Stuber, F. A., Carleton, P. S. and Ulrich, H. (1985) Selective indirect oxidation of phenol to hydroquinone and catechol. *J. Org. Chem.*, **50**, 1722–1725.
9) Lindholst, C., Pedersen, K. L. and Pedersen, S. N. (2000) Estrogenic response of bisphenol A in rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.*, **48**, 87–94.
10) Heinonen, J., Honkanen, J., Kukkonen, J. V. and Holopainen, I. J. (2002) Bisphenol A accumulation in the freshwater Clam *Pisidium amnicum* at low temperatures. *Arch. Environ. Contam. Toxicol.*, **43**, 50–55.
11) Honkanen, J. O., Holopainen, I. J. and Kukkonen, J. V. K. (2004) Bisphenol A induces yolk-sac oedema and other adverse effects in landlocked salmon (*Salmo salar m. sebago*) yolk-sac fry. *Chemosphere*, **55**, 187–196.
12) Lee, H. C., Soyano, K., Ishimatsu, A., Nagae, M., Kohra, S., Ishibashi, Y., Arizono, K. and Takao, Y.

(2004) Bisphenol A and nonylphenol bioconcentration in spotted halibut *Verasper variegates*. *Fish. Sci.*, **70**, 192–194.
13) Kawasaki, M. (1980) Experiences with the test scheme under the chemical control law of Japan: an approach to structure-activity correlations. *Ecotoxicol. Environ. Saf.*, **4**, 444–454.