Environ. Sci. Technol. **2006**, *40,* 487−493

Transformation of Pyrene in Aqueous Chlorination in the Presence and Absence of Bromide Ion: Kinetics, Products, and Their Aryl Hydrocarbon Receptor-Mediated Activities

JIANYING HU,*,† XIAOHUI JIN,† SHOICHI KUNIKANE,‡ YOSHIYASU TERAO,§ AND TAKAKO AIZAWA||

College of Environmental Science, Peking University, Beijing, 100871, China, Department of Water Supply Engineering, National Institute of Public Health, 4−6−1 Shiroganedai, Minato-ku, Tokyo 108, Japan, Institute for Environmental Sciences, University of Shizuoka, 52−1, Yada, Shizuoka 422-8526, Japan, and Yokohama Waterworks Bureau, 522 Kawashima-cho, Hodogaya-ku, 240−0045, Japan

To assess the endocrine-disrupting activity stemming from the presence of pyrene in drinking water, the kinetics of chlorination of pyrene was investigated at room temperature, the products of its aqueous chlorination with and without bromide ion were identified, and their aryl hydrocarbon receptor (AhR)-mediated activities were determined. It was found that the presence of bromide ion greatly promoted the reaction rate of chlorination of pyrene accompanied with the formation of brominated products. While the main product was 1-Cl-pyrene without the addition of bromide ion, di-Br-pyrene and 1-Br-pyrene became the main products in the presence of bromide ion. GC−MS and NMR analysis identified three structures of dibromopyrene in chlorination with the addition of bromide ion as 1,3-di-Br-pyrene, 1,6-di-Br-pyrene, and 1,8-di-Br-pyrene, and their molar ratio was determined to be approximately 0.3:1:1. Finally, 1-Br-pyrene, 1,3-di-Br-pyrene, a mixture of 1,6-di-Br-pyrene and 1,8-di-Br-pyrene (di-Br-pyrene), 1-Cl-pyrene, and a mixture of 1,6-di-Cl-pyrene and 1,8-di-Cl-pyrene (di-Cl-pyrene) were fractionated by HPLC, and their AhR-mediated activities were assessed by a yeast assay. It was found that the effective molar concentrations (or mass concentration) showing half-maximal transcriptional response, EC_{50}, for pyrene, 1-Br-pyrene, 1-Cl-pyrene, di-Cl-pyrene, and di-Br-pyrene were 5632 (1.14), 3089 (0.86), 1942 (0.46), 597.2 (0.21), and 147.3 (0.04) nM (mg/L), respectively.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. Many PAHs are suspected or known potent mutagens and carcinogens that may pose serious human and environmental risks (*1*, *2*) and have been previously recognized as aryl hydrocarbon receptor (AhR) agonists (*3*−*9*). While the principal concern regarding exposure to PAHs has been cancer risk, many papers have been published recently highlighting the health effects due to their endocrine-disrupting activities. PAHs have been reported to have estrogenic and antiestrogenic characteristics (*10*). Mechanistic investigations have clarified that PAHs interfere with estrogen receptor (ER)-mediated signaling by activating the AhR, which mediates a broad spectrum of antiestrogenic responses (*11*−*14*). Some PAHs can be metabolized by cytochromes P4501A1 and P4501A2, which are induced by binding of PAHs to AhR, and their metabolites were reported to induce ER-mediated reporter activity, indicating the potential role for estrogenicity (*15*, *16*). For example, benzo[*a*]anthracene (B[*a*]A), chrysene, and benzo[*a*]pyrene (B[*a*]P) can induce similar ER-reporter activity in MCF-7 cells with 17β-estradiol (E2) at 1000 nM (*15*). Recent studies have found that the estrogenic action of AhR agonists can be induced by a direct interaction between AhR/Arnt (AhR nuclear translocator) and unliganded ER in uterine gene induction and cellular proliferation in the absence of 17β-estradiol (*17*). B[*a*]P at 10 nM can induce the above activity. In addition, several PAHs (e.g., chrysene, benzo[*k*]fluoranthene, and B[*a*]P at 1000 nM) elicited antiandrogenicity by inhibiting the binding of androgen receptor (AR) in nuclear extracts to oligonucleotide probes containing the AR responsive element (*18*).

PAHs are ubiquitous and originate from bacterial reactions and plant fossilization, but they are predominantly synthesized by anthropogenic activities such as the burning of fossil fuels and other combustion sources. The occurrence of PAHs in raw water is due to atmospheric fallout, urban runoff, municipal effluents, industrial effluents, and oil spillage or leakage, which lead to the presence of PAHs in drinking water (*19*−*21*). Although the conventional coagulation process and advanced treatment processes such as ozonation and/or activated carbon before disinfection by chlorine can reduce the concentration of pyrene to a certain extent, PAHs in drinking water stemmed from the coal tar-coated pipes used in public water supply systems (*22*) cannot be also neglected. It was reported that the amounts of fluoranthene and pyrene released from the lacquer coatings on epoxy resin pipes and coal tar-coated pipes that are used in water supply systems were found to be 4.6−13 and 2.4−8.9 μg/L at 23 °C after 16 h of contact time, according to the JWWA K 135 method (*23*). It is well-known that disinfection by chlorination is an essential step in treatment of many water supply systems, and the disinfection process leads to the formation of chlorinated PAHs (*24*−*27*), of which some byproducts of PAHs show strong mutagenicity comparing with the parent PAHs (*28*). While many papers have highlighted the chlorination of PAHs, and the mutagenicity of the chlorinated PAHs has also been investigated, little attention has been paid to the formation of brominated PAHs in the chlorination process. To our knowledge, the formation of brominated PAHs in chlorine-treated water was only reported by Mori et al. (*29*), who mainly focused on the change of mutagenic properties and chemical fate of benzo[*a*]anthracene in chlorine-treated water with and without bromide ion, and no reports about their AhR-mediated activity together with chlorinated PAHs were found. It is well-known that naturally occurring bromide ions in raw water readily change byproduct speciation with enhanced brominated species formation (*30*, *31*). According to a recent nationwide survey, the concentration of bromide ion in US drinking water sources ranged from <5 to 429 μg/L (*31*). Knowledge of the fate of PAHs in chlorination in the presence of bromide ion is needed in order to allow effective

* Corresponding author phone and fax: 86−10−62765520; E-mail: hujy@urban.pku.edu.cn.
† Peking University.
‡ National Institute of Public Health.
§ University of Shizuoka.
|| Yokohama Waterworks Bureau.

10.1021/es0516108 CCC: $33.50
Published on Web 12/03/2005

assessment of the endocrine disruption potential of residual PAHs in drinking water.

When chlorine is used to treat bromide-containing natural waters in drinking water treatment plant, hypobromous acid (HOBr) will be formed as a disinfection byproduct through the reaction $Br^- + HOCl \rightarrow HOBr + Cl^-$. Once formed, hypobromous acid can participate in the oxidation reactions of PAHs, and some bromo derivatives of PAHs would be formed in chlorination process. The investigation on rate constants of reactions of HOBr with phenols showed that HOBr is several orders more reactive than HOCl, so brominated disinfection byproducts are rapidly produced during chlorination (*34*).

Among the numerous PAHs detected in drinking water, pyrene is one of the most common PAHs (*19*). Although pyrene itself is not classified as carcinogenic (*32*), weak, but significant, induction of AhR response in vitro was reported (*5*, *9*). The AhR-mediated activities of its aqueous chlorinated products are still not known. In this study, the kinetics was carried out to critically evaluate the effects of pH and chlorine dose on pyrene reaction rate. The aqueous brominated products of pyrene together with its chlorinated products were identified, and a pathway was proposed on the basis of the GC−MS and ^{1}H NMR methods, as well as quantum chemical modeling analysis. Finally, some brominated and chlorinated byproducts were fractionated and their AhR-mediated activities were also determined in order to examine which byproducts contribute to the AhR-mediated activities in the aqueous chlorinated pyrene solution.

Materials and Methods

Standards and Reagents. A detail description is provided in the Supporting Information.

Kinetics Experiments. All experiments were performed in a water bath to maintain the reaction temperature at 20 °C, under pseudo-first-order kinetics conditions {$[HOCl]_T$($[HOCl] + [OCl^-]$) > 20 × [pyrene]}, and a detailed description is provided in the Supporting Information.

Computational Chemistry. MOPAC (ver.6) was used (CAChe Scientific Inc.) and run on an IBM 600E computer. The PM3 parameter (*14*) served to optimize stable structures. The program was used to obtain optimum geometries and atom highest occupied molecular orbit (HOMO) density. The values of EC_{50} were calculated by software Prism 4 for Windows (GraphPad Software, Inc.)

Identification and Analysis of Byproducts. After chlorination or bromination of pyrene, the products in an aqueous chlorinated solution of pyrene were analyzed by GC−MS and identified by NMR. A detailed description is provided in the Supporting Information.

Fractionation of 1-Cl-pyrene, Di-Cl-pyrene, and Di-Br-pyrene. Because pyrene chlorination byproducts standards are not available (except for 1-Br-pyrene), a fractionation procedure using HPLC was developed. The chlorination procedure was basically similar to the procedure described above. For fractionating the brominated pyrenes, 50 L of solution (1.5 mg/L bromide ion) after 24 h chlorination time was concentrated by SPE as described above. For fractionating the chlorinated pyrenes, 50L of solution (pH 3.0) after 24 h chlorination time was concentrated by SPE as described above. The hexane solutions were dried and redissolved in acetonitrile and then fractionated by HPLC by the same method of the kinetics experiment. Each byproduct fraction was manually collected at the outlet of the UV detector. The extract was then analyzed by GC−MS and NMR.

Synthesis of 1,8-Dibromopyrene and 1,6-Dibromopyrene. A detailed description is provided in the Supporting Information.

Yeast Assay for AhR-Mediated Activity of Products. In this study, the yeast strain YCM3 with human AhR, Arnt, and the LacZ reporter plasmid, pTXRE5-Z, was used to test the AhR-mediated activity induced by the products of pyrene formed during aqueous chlorination with and without the addition of bromide ion. The assay procedure was essentially the same as described in a previous paper (*33*), and a detailed description is provided in the Supporting Information.

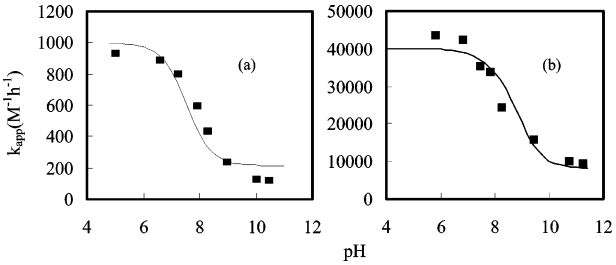

FIGURE 1. Experimental and modeled pH dependence profiles of the second-order rate constant of chlorination of pyrene. (■, experimental data; bold line, nonlinear least-squares regression fit). (a) k_{app}^{Cl}, in the absence of bromide ion; (b) k_{app}^{Br}, in the presence of bromide ion (5 μM).

Results and Discussion

Kinetics of Chlorination of Pyrene. Experiments of pyrene chlorination were conducted in the absence and presence of bromide ions, and the reactions exhibited pseudo-first-order kinetics. It was found that the pseudo-first-order constants (k_{obs}) in the presence of bromide ion were much higher than those in absence of bromide ion, and the constants, k_{obs}^{Br} and k_{obs}^{Cl}, were linear with respect to total concentration of bromine ($[HOBr]_T = [HOBr] + [OBr^-]$) and of chlorine ($[HOCl]_T = [HOCl] + [OCl^-]$), respectively (Figures 1 and 2 of the Supporting Information). Thus, the rates of pyrene disappearance in the absence and presence of bromide ions can be expressed by eqs 1 and 2, respectively.

$$v = -\frac{d[\text{pyrene}]}{dt} = k_{obs}^{Cl}[\text{pyrene}] = k_{app}^{Cl}[HOCl]_T[\text{pyrene}] \quad (1)$$

$$v = -\frac{d[\text{pyrene}]}{dt} = k_{obs}^{Br}[\text{pyrene}] = k_{app}^{Br}[HOBr]_T[\text{pyrene}] \quad (2)$$

where k_{app}^{Cl} and k_{app}^{Br} are second-order kinetic constants and [pyrene] represents the concentration of pyrene. The pH profiles of experimental k_{app}^{Cl} and k_{app}^{Br} are shown in Figure 1. According to following eqs 3−9,

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad K_a^{Cl} \quad (3)$$

$$HOCl + Br^- \rightarrow H^+ + OBr^- + Cl^- \quad (4)$$

$$HOBr \rightleftharpoons H^+ + OBr^- \qquad K_a^{Br} \quad (5)$$

$$\text{pyrene} + HOCl \rightarrow \text{products} \qquad k_1 \quad (6)$$

$$\text{pyrene} + OCl^- \rightarrow \text{products} \qquad k_2 \quad (7)$$

$$\text{pyrene} + HOBr \rightarrow \text{products} \qquad k_3 \quad (8)$$

$$\text{pyrene} + OBr^- \rightarrow \text{products} \qquad k_4 \quad (9)$$

the rate k_{app}^{Cl} and k_{app}^{Br} can be written by eqs 10 and 11 (A

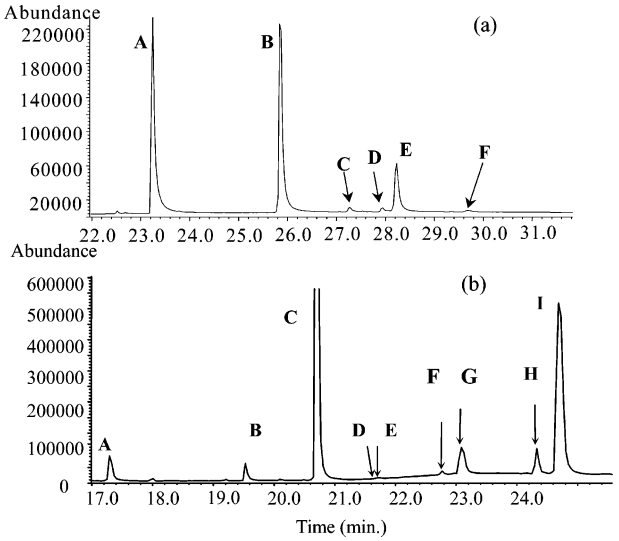

FIGURE 2. GC−MS chromatogram of aqueous chlorinated pyrene. (a) Chlorination in the absence of bromide ion. Reaction time, 65 h; pH 7.22; A, pyrene; B, monochloropyrene; C, monobromopyrene; D, dichloropyrene; E, dichloropyrene; F, monobromomonochloropyrene. (b) Chlorination in the presence of bromide ion (10 μM). Reaction time, 41 h; pH 7.22; A, pyrene; B, monochloropyrene; C, monobromopyrene; D, dichloropyrene; E, dichloropyrene; F, monobromomonochloropyrene; G, monobromomonochloropyrene; H, dibromopyrene; I, dibromopyrene.

detailed description is provided in the Supporting Information), respectively.

$$k_{app}^{Cl} = k_1 \frac{[H^+]}{K_a^{Cl} + [H^+]} + k_2 \frac{K_a^{Cl}}{K_a^{Cl} + [H^+]} \quad (10)$$

$$k_{app}^{Br} = k_3 \frac{[H^+]}{[H^+] + K_a^{Br}} + k_4 \frac{K_a^{Br}}{[H^+] + K_a^{Br}} \quad (11)$$

The rate constants (k_1 and k_2) were determined by a nonlinear least-squares regression of the experimental pH profile of k_{app}^{Cl}, and the values of k_1 and k_2 are 996.01 M^{-1} h^{-1} and 213.15 M^{-1} h^{-1}, respectively. Similarly, in the presence of bromide ion, the k_3 and k_4 are 38682 M^{-1} h^{-1} and 6890.5 M^{-1} h^{-1}, respectively. The values of K_a^{Cl} and K_a^{Br} used for the calculation were $pK_a^{Cl} = 7.54$ and $pK_a^{Br} = 8.89$ (*34*), respectively. Figure 1 represents the experimental and modeled pH profiles for pyrene chlorination in the absence and presence of bromide ion. The correlations between the experimental and modeled values were good on the basis of the fact that their model selection criterions were larger than 3 (Supporting Information).

In addition, the k_{app}^{Cl} at pH 7 for pyrene calculated from the data reported in a previous paper (*26*) was about 729.91 M^{-1} h^{-1}, which is similar to that of our paper (800.00 M^{-1} h^{-1}).

Characterization of Products. Figure 2a shows the GC−MS chromatogram of chlorinated pyrene after 65 h of chlorination. It was found that pyrene reacted slowly with HOCl at a pH of 7.22, and about 20% pyrene residue remained in the solution together with several chlorinated products. According to the mass spectra of three peaks at 25.84, 27.95, and 28.23 min, as shown in Table 1 of the Supporting Information, one monochloropyrene and two dichloropyrenes were identified. While the monochloropyrene was reported to be 1-Cl-pyrene (*27*, *28*), three possible structures for di-Cl-pyrene (1,6-di-Cl-pyrene, 1,8-di-Cl-pyrene, and 1,3-di-Cl-pyrene) were proposed according to the chlorination in organic solvent (*28*), and no further information could be used to identify the precise structures of the di-Cl-pyrenes corresponding with the two peaks at 27.95 and 28.23 min. In addition, 1-Cl-pyrene was found to be more abundant than di-Cl-pyrenes. The above results were in accordance with the generally accepted data for pyrene chlorination (*27*).

It should be noted that in addition to the above products that have been reported in previous papers, however, two unknown peaks were also detected at 27.28 and 29.68 min, although there were only trace concentrations of these products; they were less abundant than mono- and dichloropyrene. Their mass spectra as shown in Table 1 of the Supporting Information indicate that monobromopyrene and Br-Cl-pyrene were formed. The monobromopyrene was deduced to be 1-Br-pyrene on the basis of the fact that the mass spectrum and retention time of monobromopyrene agreed with those of the 1-Br-pyrene standard, which was also identified by NMR: 7.99 (1H, d, $J = 7.5$ Hz), 8.01 (1H, d, $J = 9.2$ Hz), 8.03 (1H, t, $J = 7.4$ Hz), 8.075 (1H, d, $J = 9.2$ Hz), 8.15 (1H, d, $J = 9.2$ Hz) 8.20 (1H, d, $J = 7.4$ Hz), 8.21 (1H, d, $J = 7.4$ Hz), 8.22(1H, d, $J = 7.5$ Hz), 8.42 (1H, d, $J = 9.2$ Hz). However, the structure of Br-Cl-pyrene could not be determined on the basis of the structural information of the MS spectrum. The above results suggest that the trace bromide ion residue in NaOCl solution was incorporated into pyrene and its chlorinated byproducts in the form of organically bound bromine during water chlorination.

The effects of bromide ion on the byproduct profiles in the chlorination process were also investigated, and Figure 2b shows the GC−MS chromatogram of chlorinated pyrene after 24 h of chlorination upon adding bromide ion at a concentration of 10 μM. Compared with the chlorination without the addition of bromide ion, 1-Br-pyrene became the most abundant byproduct which was followed by peak I. From the mass spectra (Supporting Information Table 1) of peaks F, G, H, and I, the peaks F and G were deduced to be the isomers of Cl-Br-pyrene, and the H and I peaks were isomers of di-Br-pyrene. An attempt at identifying the structure of di-Br-pyrene corresponding with peak I at 24.77 min in Figure 2b was made. The di-Br-pyrenes corresponding with peak I at 24.77 min were fractionated by HPLC, and the proton nuclear magnetic resonance (^{1}H NMR) spectrum for this fraction is shown in Figure 3a. From this NMR spectrum, the fraction was speculated to be a mixture that should consist of two or more isomers of di-Br-pyrene on the basis of the GC−MS spectrum of peak I. To further identify their structures, we synthesized 1,6-di-Br-pyrene and 1,8-di-Br-pyrene, and their ^{1}H NMR spectra were shown in Figure 3, parts b and c, respectively. By comparing ^{1}H NMR spectrum of the fraction with those of 1,6-di-Br-pyrene and 1,8-di-Br-pyrene, fortunately, the mixture was identified to consist of 1,6-di-Br-pyrene and 1,8-di-Br-pyrene with approximately equal molar amounts. From the similarity of bromination with chlorination, peak H was speculated to be 1,3-di-Br-pyrene. Because the ratio of GC−MS responses for 1,3-di-Br-pyrene, 1, 6-di-Br-pyrene, and 1,8-di-Br-pyrene was determined to be 1:1.2:2 by analyzing the three di-Br-pyrene, and 1,6-di-Br-pyrene and 1,8-di-Br-pyrene are present in approximately equal molar amounts from the above NMR analytical results, the molar ratio of 1,3-di-Br-pyrene, 1,6-di-Br-pyrene, and 1,8-di-Br-pyrene was determined to be approximately 0.3:1:1 according to their peak area in the GC−MS chromatogram (Figure 2b). To speculate on the structures for other peaks, the atom HOMO density of pyrene and 1-Br-pyrene were calculated using computational chemistry. It has been reported that the mechanism of substitution reactions with PAH proceeds through electrophilic attack of HOCl (*35*). The HOMO density reveals reactive sites based on the distribution of electrons in the highest occupied

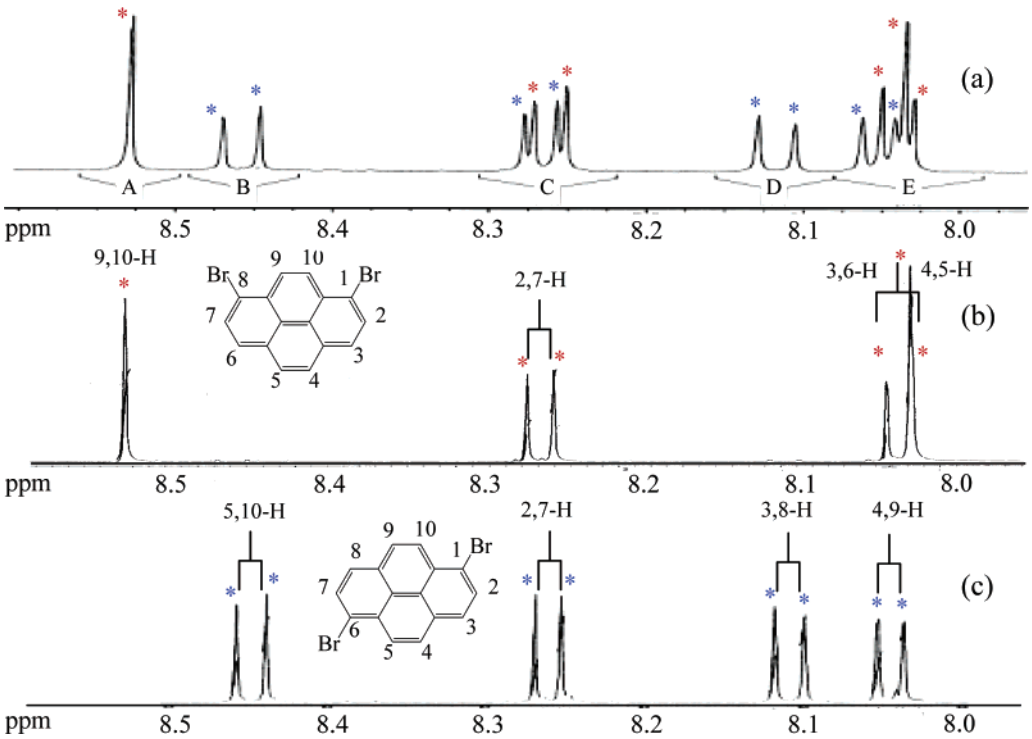

FIGURE 3. ^{1}H NMR spectrum (400 MHz) of samples in $CDCl_3$: (a) fraction corresponding with peak I in Figure 2b, with letters A−E representing the integral value of corresponding peaks [A (1.1937), B (1.0000), C (2.2152), D (1.0258), E (3.4225)]; (b) 1,8-di-Br-pyrene and (c) 1,6-di-Br-pyrene recrystallization standards.

molecular orbit (HOMO) of a PAH, and the regions of the molecule with high values of HOMO density have loosely bound electrons that are reactive to electrophilic attack according to the frontier orbital theory (*36*). From the atom HOMO density of pyrene and 1-Br-pyrene, the bromine substitution reaction primarily occurred at C1 with a HOMO density of 0.119, and 1-Br-pyrene was formed (Supporting Information Figure 3). The 1-Br-pyrene subsequently reacted with hypobromous acid by substitution at C6 or C8 with similar HOMO density (0.119 or 0.118) to form 1,6-di-borompyrene or 1,8-dibromopyrene, which are in competition with attack at C3 with relatively low HOMO density (0.114) to form 1,3-diboromopyrene. Similarly, the structure of Cl-Br-pyrene corresponding with peak F can be speculated to be 1-Cl-3-Br-pyrene, and peak G corresponded with the mixture of 1-Cl-6-Br-pyrene and 1-Cl-8-Br-pyrene. For the structures of di-Cl-pyrene, it was speculated that peak D was 1,3-di-Cl-pyrene and that peak E included 1,6-di-Cl-pyrene and 1,8-di-Cl-pyrene.

It should be noted that oxygenated products of PAHs, which are often reported to be formed during chlorination, were not detected in this study. Mori et al. reported the fate of B[*a*]A in the presence of bromide ion (50 μg/L), and an oxygenated compound, B[*a*]A-7,12-dione, was found to be a predominant byproduct among four byproducts (monochloro-B[*a*]A, monochloro-B[*a*]Br, di-chloro-B[*a*]A, and B[*a*]A-7,12-dione) (*29*). However, we found that the chlorination of B[*a*]A was carried out at a low pH of 4. It is known that pH is an important factor that affects both reaction rate of PAHs and the formation of their byproducts (*27*). Oyler et al. reported the fate of pyrene during the chlorination process using chlorine gas as disinfectant. Oxygenated pyrene was found to be the major byproduct at low pH of 3 or 4, and only trace oxygenated pyrene was observed at moderate pH of 7 or 8 (*27*). In addition, very similar results were also found in the chlorination of pyrene at pH 4, and no oxygenated pyrene was reported at pH of 7 when using sodium hypochlorite solution as disinfectant (*26*). Thus, the condition of pH used in this study may be the main reason oxygenated pyrene was not found in the chlorinated solution in the presence and absence of bromide ion.

Chlorination Pathways of Pyrene. Figure 4 shows the variation in levels of pyrene, 1-Cl-pyrene, the sum of three isomers for di-Cl-pyrene, 1-Br-pyrene, the sum of three isomers of di-Br-pyrene, and the sum of three isomers for Br-Cl-pyrene with reaction time in the absence and presence of bromide ion. For the former reaction conditions (Figure 4a), the concentrations for all byproducts increased with increasing reaction time, and 0.09 μM pyrene still remained even after 65-h reaction. The byproduct with the highest abundance was 1-Cl-pyrene, of which the concentration increased from 0.06 μM after 6-h reaction to 0.33 μM after 65-h reaction. The di-Cl-pyrene is another major byproduct, and the concentration varied from 0.005 μM after 6-h reaction to 0.063 μM after 65-h reaction. In this reaction, trace 1-Br-pyrene and Br-Cl-pyrene were detected because trace bromide ions are residual in raw NaOCl reagent. According to the above results, the pathways of reaction between pyrene and NaOCl are proposed in Figure 5.

For the latter reaction conditions (Figure 4b), pyrene reacted rapidly with HOCl in the presence of bromide ion, and the most abundant was 1-Br-pyrene, followed by di-Br-pyrene, and only a trace of di-Cl-pyrene was detected in aqueous chlorinated solution. The highest concentrations for both 1-Br-pyrene (0.43 μM) and 1-Cl-pyrene (0.027 μM) were found after 1 h of chlorination, and then they decreased with chlorination time together with pyrene, obviously different from those in the chlorination process without the addition of bromide ion. Conversely, the sum of three di-Br-pyrene isomers, the sum of three Br−Cl-pyrene isomers, and the sum of three di-Cl-pyrene isomers showed an increasing trend with reaction time. Taken together, the concentration variation of byproducts with reaction time and the rate constants of pyrene described above, pyrene is first transformed to 1-Br-pyrene and 1-Cl-pyrene, which react further to give di-Br-pyrene, Br-Cl-pyrene, and di-Cl-pyrene.

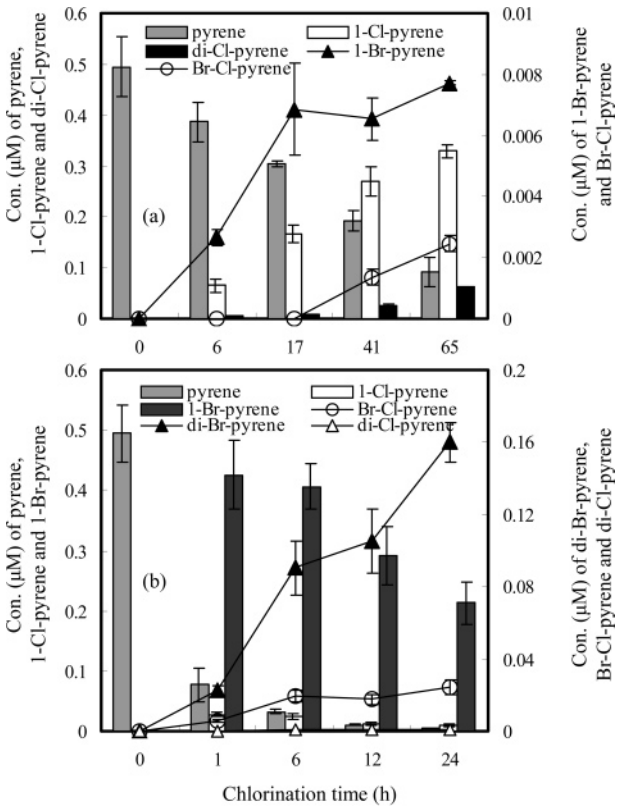

FIGURE 4. Variation of molar concentration of pyrene and its products with chlorination time: (a) $[HOCl]_T = 35\ \mu M$, pH 7.22, [pyrene] = 0.5 μM; (b) $[HOCl]_T = 35\ \mu M$, pH 7.22, [pyrene] = 0.5 μM, $[Br^-] = 10\ \mu M$.

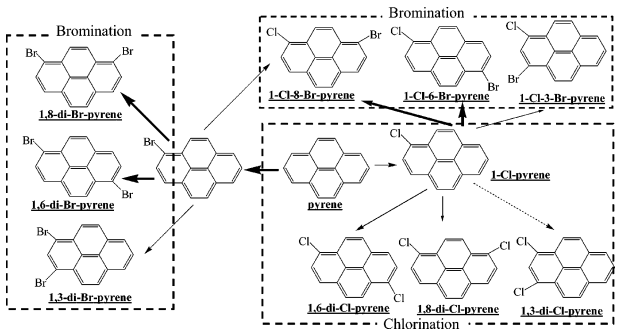

FIGURE 5. Halogenation pathways during chlorination of pyrene in the presence and absence of bromide ion.

It should be noted that the formation of Br-Cl-pyrene could be via bromination of 1-Cl-pyrene or via chlorination of 1-Br-pyrene. The contribution of each pathway depends on several factors, such as the concentrations of HOCl and bromide and pH. Taken together, the chlorination of pyrene in the presence of bromide would lead to the formation of chloro-, bromo- and mixed chlorobromopyrene, and the pathways for the formation of byproducts are shown in Figure 5.

AhR-Mediated Activities. To evaluate the AhR-mediated activities potentially stemming from the presence of pyrene in drinking water, it was necessary to investigate the AhR-mediated activity of these products. A yeast assay based on a functional heterodimeric transcription factor composed of AhR and Arnt proteins was used to assess the AhR-mediated activity of the above chlorinated pyrene solution, by determining the ligand-induced transcriptional activation.

To interpret the β-galactosidase activities of the chlorination products in pyrene solution, 1-Cl-pyrene, 1-Br-pyrene, di-Cl-pyrene (a mixture of 1,6-di-Cl-pyrene and 1,8-Cl-pyrene), and di-Br-pyrene (a mixture of 1,6-di-Br-pyrene and 1,8-di-Br-pyrene) were fractionated by the HPLC method. The dose−response curves of single 1-Cl-pyrene, di-Cl-pyrene, di-Br-pyrene, 1-Br-pyrene, and pyrene were obtained (Figure 6). It was found that 1-Br-pyrene and 1-Cl-pyrene elicited similar AhR-mediated activity, which was slightly greater than that of pyrene, and the effective concentrations showing half-maximal transcriptional response (EC_{50}), which represent the concentrations required to achieve half-maximal β-galactosidase activity for 1-Br-pyrene, 1-Cl-pyrene, and pyrene, were about 3089, 1942, and 5632 nM, respectively. On the other hand, for di-Br-pyrene and di-Cl-pyrene, it was found that the maximal β-galactosidase activities induced by di-Br-pyrene and di-Cl-pyrene were lower than those of 1-Br-pyrene, 1-Cl-pyrene, and pyrene, their EC_{50} values being 147.3 and 597.2 nM, respectively, which were significantly lower concentrations than those of 1-Br-pyrene, 1-Cl-pyrene, and pyrene. For reference, the EC_{50} for the typical AhR agonists hexachlorobenzene and benzo[*a*]pyrene were 200 and 40 nM, as determined by the same yeast system as in this study (*37*). The above results demonstrate that bromide ions greatly affected the product speciation resulting from aqueous chlorination of pyrene, and the AhR-mediated potency of byproducts, especially di-Br-pyrene and di-Cl-pyrene, become high compared with pyrene in the in vitro system. However, to assess the likely environmental exposure risks of these products, their AhR-mediated potency in in vivo systems requires further investigation, because the accessibility of AhR receptors to these byproducts within in vivo systems is more complex than in the in vitro system used in this study. Because the physical and chemical properties will largely affect the exposure risk of chemicals, we estimated the physical and chemical properties for these byproducts as shown in Table 1. Generally the EC_{10} are slightly higher than their aqueous solubility, which it seem did not relate the measured AhR potency to likely environmental exposure risks. However, it should be noted that their bioaccumulation will lead to higher exposure concentrations and even biomagnification, espe-

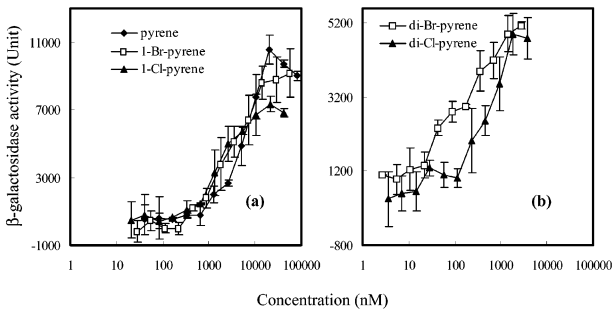

FIGURE 6. Dose−response curves of (a) pyrene, 1-Br-pyrene, and 1-Cl-pyrene and (b) di-Br-pyrene and di-Cl-pyrene.

TABLE 1. Physicochemical Properties of Pyrene and Its Byproducts[a]

compd	log K_{ow}	Sw (mg/L)	BCF	EC_{10} (mg/L)
pyrene	5.17 ± 0.17	0.115	1288	0.113
1-Br-pyrene	5.94 ± 0.28	0.011	3356	0.136
di-Br-pyrene	6.7 ± 0.40	0.001	8635	0.0019
1-Cl-pyrene	5.77 ± 0.21	0.017	2717	0.037
di-Cl-pyrene	6.36 ± 0.24	0.003	5658	0.0046

[a] The values of log K_{ow} were calculated by ACD logP ver.1.0 (Advanced Chemistry Development Inc., Toronto, Canada); Sw values were estimated using log Sw = −1.49 log K_{ow} + 1.46 (*38*); BCF were estimated using log BCF = 0.54 log K_{ow} + 0.32 (*39*); EC_{10} values were calculated by Benchmark Dose Software (BMDS ver. 1.3.2).

cially for the di-Br-pyrene and di-Cl-pyrene, which would be expected according to their log K_{ow} and BCF values (5.17−6.7).

Acknowledgments

Financial support by the National High Technology Research and Development Program [2002AA601140-6], the Japanese Governmental Research Fund (Ministry of Health & Welfare), and the National Natural Science Foundation of China [40021101] is gratefully acknowledged.

Supporting Information Available

Text, figures, and tables addressing (1) methods for kinetics experiment and HPLC analysis, disinfection byproducts identification and analysis by GC−MS and NMR, synthesis of 1,8-dibromopyrene and 1,6-dibromopyrene, and yeast assay for AhR-mediated activity of products; (2) details about kinetics in the presence and absence of bromide ion; (3) figures demonstrating atom HOMO density of pyrene and 1-Br-pyrene; and (4) calculation of model selection criterion (MSC). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) National Recommended Water Quality Criteria: 2002. Health and Ecological Criteria Division, Office of Science and Technology. US EPA 822-R-02-047. 2002.

(2) Environmental Health Criteria 202. *Selected Nonheterocyclic Polycyclic Aromatic Hydrocarbons*; World Health Organization/International Program on Chemical Safety: Geneva, 1998.

(3) Bigelow, S. W.; Nebert, D. W. The Ah regulatory gene product. Survey of nineteen polycyclic aromatic compounds' and fifteen benzo[*a*]pyrene metabolites' capacity to bind to the cytosolic receptor. *Toxicol. Lett.* **1982**, *10*, 109−118.

(4) Jones, J. M.; Anderson, J. W. Relative potencies of PAHs and PCBs based on the response of human cells. *Environ. Toxicol. Pharm.* **1999**, *7*, 19−26.

(5) Machala, M.; Vondracek, J.; Blaha, L.; Ciganek, M.; Neca, J. V. Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using in vitro reporter gene assay. *Mutat. Res.* **2001**, *497*, 49−62.

(6) Piskorska-Pliszczynska, J.; Keys, B.; Safe, S.; Newman, M. S. The cytosolic receptor binding affinities and AHH induction potencies of 29 polynuclear aromatic hydrocarbons. *Toxicol. Lett.* **1986**, *34*, 67−74.

(7) Ziccardi, M. H.; Gardner, I. A.; Denison, M. S. Application of the luciferase recombinant cell culture bioassay system for the analysis of polycyclic aromatic hydrocarbons. *Environ. Toxicol. Chem.* **2002**, *21*, 2027−2033.

(8) Willett, K. L.; Gardinali, P. R.; Sericano, J. L.; Wade, T. L.; Safe, S. H. Characterization of the H4IIE rat hepatoma cell bioassay for evaluation of environmental samples containing polycyclic polynuclear aromatic hydrocarbons. *Arch. Environ. Cantam. Toxicol.* **1997**, *32*, 442−448.

(9) Fent, K.; Batscher, R. Cytochrome O4501A induction potencies of polycyclic aromatic hydrocarbons in a fish hepatoma cell line: Demonstration of additive interactions. *Environ. Toxicol. Chem.* **2000**, *19*, 2047−2058.

(10) Santodonato, J. Review of the estrogenic and antiestrogenic activity of polycyclic aromatic hydrocarbons: Relationship to carcinogenicity. *Chemosphere* **1997**, *34*, 835−848.

(11) Arcaro, K. F.; O'Keefe, P. W.; Yang, Y.; Clayton, W.; Gierthy, J. F. Antiestrogenicity of environmental polycyclic aromatic hydrocarbons in human breast cancer cells. *Toxicology* **1999**, *133*, 115−127.

(12) Chaloupka, K.; Krishnan, V.; Safe, S. Polynuclear aromatic hydrocarbon carcinogens as antiestrogens in MCF-7 human breast cancer cells: Role of the Ah receptor. *Carcinogenesis* **1992**, *13*, 2233−2239.

(13) Navas, J. M.; Segner, H. Antiestrogenic activity of anthropogenic and natural chemicals. *Environ. Sci. Pollut. Res.* **1998**, *5* (2), 75−82.

(14) Safe, S. Molecular biology of the Ah receptor and its role in carcinogenesis. *Toxicol. Lett.* **2001**, *120*, 1−7.

(15) Gozgit, J. M.; Nestor, K. M.; Fasco, M. J.; Pentecost, B. T.; Arcaro, K. F. Differential action of polycyclic aromatic hydrocarbons on endogenous estrogen-responsive genes and on a transfected estrogen-responsive reporter in MCF-7 cells. *Toxicol. Appl. Pharm.* **2004**, *196*, 58−67.

(16) Charles, G. D.; Bartels, M. J.; Zacharewski, T. R.; Gollapudi, B. B.; Freshour, N. L.; Carney, E. W. Activity of benzo[*a*]pyrene and its hydroxylated metabolites in an estrogen receptor-a reporter gene assay. *Toxicol. Sci.* **2000**, *55*, 320−326.

(17) Ohtake, F.; Takeyama, K.; Matsumoto, T.; Kitagawa, H.; Yamamoto, Y.; Nohara, K.; Tohyama, C.; Krust, A.; Mimurak, J.; Chambon, P.; Yanagisawa, J.; Fujii-Kuriyamak, Y.; Kato, S. Modulation of oestrogen receptor signaling by association with the activated dioxin receptor. *Nature* **2003**, *423*, 545−550.

(18) Kizu, R.; Okamura, K.; Toriba, A.; Kakishima, H.; Mizokami, A.; Burnstein, K. L.; Hayakawa, K. A role of aryl hydrocarbon receptor in the antiandrogenic effects of polycyclic aromatic hydrocarbons in LNCaP human prostate carcinoma cells. *Arch. Toxicol.* **2003**, *77*, 335−343.

(19) Manoli, E.; Samara, C. Polycyclic aromatic hydrocarbons in natural waters: Sources, occurrence and analysis. *Trends Anal. Chem.* **1999**, *18*, 417−427.

(20) Shiraishi, H.; Pilkington, N. H.; Otsuki, A.; Fuwa, K. Occurrence of chlorinated polynuclear aromatic hydrocarbons in tap water. *Environ. Sci. Technol.* **1985**, *19*, 585−590.

(21) Kveseth, K.; Sortlant, B.; Bokn, T. Polycyclic aromatic hydrocarbons in sewage, mussels and tap water. *Chemosphere* **1982**, *11*, 623−639.

(22) Alben, K. Coal tar coatings of storage tanks, a source of contamination of the potable water supply. *Environ. Sci. Technol.* **1980**, *14*, 468−470.

(23) Kunikane, S. *Human Exposure to Endocrine Disrupting Compounds from Tap Water*; Report of Ministry of Health and Welfare of Japan, March 2003.

(24) Onodera, S.; Igarashi, K.; Fukuda, A.; Ouchi, J.; Suzuki, S. Chemical changes of organic compounds in chlorinated water. XVI. Gas chromatographic−mass spectrometric studies of reactions of tricyclic aromatic hydrocarbons with hypochlorite in dilute aqueous solution. *J. Chromatogr. A* **1989**, *466*, 233−249.

(25) Oyler, A. R.; Liukkonen, R. J.; Lukasewycz, M. T.; Heikkila, K. E.; Cox, D. A.; Carlson, R. M., Chlorine disinfection chemistry of aromatic-compounds−polynuclear aromatic-hydrocarbons−Rates, products, and mechanisms. *Environ. Sci. Technol.* **1983**, *17*, 334−342.

(26) Mori, Y.; Goto, S.; Onodera, S.; Naito, S.; Matsushita, H. Aqueous chlorination of tetracyclic aromatic hydrocarbons: Reactivity and product distribution. *Chemosphere* **1991**, *22*, 495−501.

(27) Oyler, A. R.; Liukkonen, R. J.; Lukasewycz, M. K.; Cox, D. A.; Peake, D. A.; Carlson, R. M. Implications of treating water containing polynuclear aromatic hydrocarbons with chlorine: A gas chromatographic mass spectrometric study. *Environ. Health Persp.* **1982**, *46*, 73−86.

(28) Colmsjo, A.; Rannug, A.; Rannug, U. Some chloro derivatives of polynuclear aromatic hydrocarbons are potent mutagens in *Salmonella typhimurium*. *Mutat. Res.* **1984**, *135*, 21−29.

(29) Mori, Y.; Goto, S.; Onodera, S.; Naito, S.; Matsushita, H.; Takitani, S. Change in mutagenic properties and chemical fate of benz-[*a*]anthracene in chlorine-treated water with and without bromide ion. *Chemosphere* **1993**, *27*, 2155−2162.

(30) Nikolaou, A. D.; Golfinopoulos, S. K.; Lekkas, T. D. Formation of organic byproducts during chlorination of natural waters. *J. Environ. Monit.* **2002**, *4* (6), 910−916.

(31) Cowman, G. A.; Singer, P. C. Effect of bromide ion on haloacetic acid speciation resulting from chlorination and chloramination of aquatic humic substances. *Environ. Sci. Technol.* **1996**, *30*, 16−24.

(32) Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). Agency for Toxic Substances and Disease Registry: Atlanta, GA. http://www.atsdr.cdc.gov/toxprofiles/tp69.html.

(33) Miller III, C. A. A human aryl hydrocarbon receptor signaling pathway constructed in yeast displays additive responses to ligand mixtures. *Toxicol. Appl. Pharm.* **1999**, *160*, 297−303.

(34) Gallard, H.; Pellizzari, F.; Croue, J. P.; Legube, B. Rate constants of reactions of bromine with phenols in aqueous solution. *Water Res.* **2003**, *37*, 2883−2892.

(35) Draley, J. E.; Chairman, S. In *Aqueous Chemistry of Chlorine*; Jolley, R. L., Ed.; Ann Arbor Science: Ann Arbor, MI, 1975; p 18.

(36) Fukui, K. In *Nobel Lectures in Chemistry 1981*−1990; Malmstrom, B. M., Ed.; World Scientific Publishing Co.: River Edge, NJ, 1993; pp 9−26.
(37) Miller, C. A., III. Expression of the human aryl hydrocarbon receptor complex in yeast. *J. Biol. Chem.* **1997**, *272* (52), 32824−32829.
(38) Chiou, C. T.; Freed, V. H.; Schmedding, D. W.; Kohnert, R. L. Partition coefficients and bioaccumulation of selected organic chemicals. *Environ. Sci. Technol.* **1977**, *11*, 475−478.
(39) Neely, W. B.; Branson, D. R.; Blau, G. E. Partition coefficients to measure bioconcentration potential of organic chemicals in fish. *Environ. Sci. Technol.* **1974**, *8*, 1113−1115.

Received for review August 13, 2005. Revised manuscript received October 25, 2005. Accepted November 4, 2005.

ES0516108