Behaviour of pyrene and its decomposition by-products in chlorination

Koji Kosaka, Hiroyuki Koshino, Nobue Yoshida and Shoichi Kunikane

ABSTRACT

The behaviour of pyrene and its decomposition by-products in chlorination and their aryl hydrocarbon receptor (AhR) ligand activities were investigated. In the study on pyrene behaviour in chlorination, the pyrene and chlorine concentrations and the pH range were 0.82–21 $\mu g\,l^{-1}$, 2.0–2.1 $mg\,l^{-1}$ and 3.2–10, respectively. In the pH range of 3.2–5.1, pyrene was decomposed faster in the order of pH 3.2, 3.7 and 5.1. However, when the pH ranged from 5.1 to 10, pyrene was decomposed faster in a pH range of approximately 7.8–8.8. When the effects of the initial pyrene concentration were investigated, a lower pyrene concentration was decomposed faster. Pyrene decomposition in chlorination was inhibited by the presence of 30 mM ethanol, but was enhanced by the presence of 3.0 and 6.0 $mgC\,l^{-1}$ humic acid. Pyrene decomposition by-products in chlorination were also characterized (pyrene, 92 $\mu g\,l^{-1}$; chlorine, 7.9 $mg\,l^{-1}$; pH, 7.1). 1-Chloropyrene, 1,6- and 1,8-dichloropyrenes, and pyrene-4,5-dione were identified as pyrene decomposition by-products. 1-Chloropyrene and pyrene-4,5-dione in chlorination were found to be major by-products at pH 3.2, but minor by-products at pH 7.1 (pyrene, 56–67 $\mu g\,l^{-1}$; chlorine, 3.9–4.0 $mg\,l^{-1}$). The yeast assay revealed that pyrene-4,5-dione was an AhR agonist.

Key words | aryl hydrocarbon receptor, chlorination, decomposition by-products, pyrene

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds generated by the combustion of organic materials, such as petroleum and coal (Edwards 1983). Some PAHs are carcinogenic (IARC 1983). PAHs have also been recognized as agonists of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor (Billiard *et al.* 2002; Sakai *et al.* 2003; Barron *et al.* 2004; Hankinson 2005). After ligand binding, AhR translocates into the nucleus and dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT), resulting in gene expression and toxicity (Hankinson 2005). Thus, the presence of PAHs in environments such as air, soil and vegetation is of concern, and the concentrations of PAHs in these environments have been investigated (Edwards 1983; Menichini 1992). Several PAHs have been found in raw, finished and tap waters (Sorrell *et al.* 1980; Shiraishi *et al.* 1985). In addition, PAHs (e.g. fluoranthene, pyrene and chrysene) have been eluted from new water pipes coated with coal tar epoxy resin (Kunikane *et al.* 2004).

Chlorine is widely used as a disinfectant in water treatment. In the pH range of environmental water, the reactions of inorganic or organic compounds in chlorination are generally expressed by the elementary reaction of hypochlorous acid (HOCl) and the inorganic or organic compounds (Deborde & von Gunten 2008). Similarly, it has been reported that the reaction of PAHs in chlorination are first order with respect to both PAHs and HOCl (Rav-Acha & Blits 1985; Hu *et al.* 2006). However, reports of the second-order reaction rate constants of pyrene,

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a common PAH, with HOCl were different between researcher groups (Rav-Acha & Blits 1985; Hu *et al.* 2006). Also, the decomposition of some PAHs did not follow pseudo-first-order reactions in chlorination when the chlorine concentration was much higher than that of PAHs (Rav-Acha & Blits 1985). These reports suggest that some PAHs could be decomposed by HOCl as well as other reactive species in chlorination. Thus, to understand the behaviour of PAHs in water treatment, it is necessary to investigate the effects of the reaction parameters (e.g. pH and PAH concentration) on PAH decomposition in chlorination. There are many solutes in environmental water. If other reactive species contribute to PAH decomposition in chlorination, the investigation of the effects of coexisting compounds on the PAH decomposition in chlorination is also important.

On the other hand, it is known that harmful disinfection by-products such as trihalomethanes are generated by reactions between chlorine and organic compounds in water (Rook 1974; Symons *et al.* 1975). It has also recently been reported that oestrogen-like compounds (e.g. bisphenol A) or oestrogen itself (i.e. 17β-oestradiol) is rapidly decomposed in chlorination, and some of their by-products also have oestrogenic activities (Hu *et al.* 2002, 2003). Furthermore, the chlorinated and oxygenated derivatives of PAHs have been found after PAH decomposition in chlorination (Oyler *et al.* 1978, 1982; Takahashi *et al.* 1984; Shiraishi *et al.* 1985; Mori *et al.* 1987). Some derivatives have been detected in tap water disinfected with chlorine (Shiraishi *et al.* 1985; Takahashi *et al.* 2007; Kosaka *et al.* 2008). In terms of the toxicity of PAH decomposition by-products in chlorination, some decomposition by-products of fluoranthene and pyrene were found to be mutagenic to *Salmonella typhimurium* TA100 and TA98, independent of the presence of the S9 mix (Takahashi *et al.* 1984; Mori *et al.* 1987). It was also reported that the AhR ligand activities of chlorinated and brominated derivatives of pyrene are greater than that of pyrene (Hu *et al.* 2006).

Here, we investigated the behaviour of pyrene and its decomposition by-products in chlorination. The effects of coexisting compounds on pyrene decomposition in chlorination were also examined. Moreover, we investigated AhR ligand activities of pyrene decomposition by-products.

METHODS

Reagents and solutions

Ultrapure water for the preparations of all solutions was obtained using a Gradient A10 water purification system (Millipore, Bedford, Massachusetts). Pyrene-4,5-dione was purchased from Sigma-Aldrich Library of Rare Chemicals (Bella Fonte, Pennsylvania) and was purified by fractionation with a high-performance liquid chromatograph (HPLC, HP1100, Agilent Technologies, Palo Alto, California) before use. 1-Chloropyrene was synthesized as described in the literature (Mori *et al.* 1986). Its purity, 94.5%, was determined using an HPLC with an ultraviolet (UV) detector. Stock chlorine solution was prepared by sparging chlorine gas generated from commercially available sodium hypochlorite (Wako Pure Chemicals, Osaka, Japan) into sodium hydroxide solution. Pyrene solutions were prepared by adding small amounts (approximately 3–10 mg) of pyrene reagent (Wako Pure Chemicals) into 1–2 l of ultrapure water. After mixing the solution with a magnetic stirrer for 2–3 days in the dark, the solution was filtered (GF/B, Whatman, Florham Park, New Jersey) and the filtrate was used for the experiments. The pyrene concentration in the solution was approximately 80–100 $\mu g\,l^{-1}$, which is close to its reported solubility, 135 $\mu g\,l^{-1}$ (Mackay & Shiu 1977). Humic acid (Aldrich, Bella Fonte) solution was filtered (GF/B, Whatman) and the filtrate was used.

Pyrene chlorination

Three types of experiment on pyrene decomposition in chlorination were conducted in this study: an investigation of pyrene behaviour in chlorination; identification of the decomposition by-products of pyrene in chlorination; and an investigation into the behaviour of pyrene decomposition by-products in chlorination.

To study pyrene behaviour in chlorination, experiments were conducted in Erlenmeyer flasks with caps. The pyrene concentrations ranged from 0.82 to 21 $\mu g\,l^{-1}$, and in many cases were around 1 $\mu g\,l^{-1}$. In this study, pyrene concentrations in the samples were determined using an HPLC with a fluorescence detector (see Analytical Methods)

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without the sample concentration. The pyrene concentration range was chosen on the basis of the analytical instrument's sensitivity. The chlorine concentrations were 2.0–2.1 mg l^{-1}, which were much higher than the pyrene concentrations. According to an Environmental Working Group analysis (Environmental Working Group 2005), pyrene was detected in five communities from tests of 2,982 public water suppliers in 13 US states. The average pyrene concentration in the water in each community was <0.01–0.14 μg l^{-1}, and the maximum concentration was 0.95 μg l^{-1}. The pyrene concentration in surface water was reported to be 0.0022–0.0469 μg l^{-1} (Mori *et al.* 1987). Thus, the pyrene concentration range used in this study was generally higher than that found in actual water treatment conditions. However, it was assumed that in some places pyrene is present at around 1 μg l^{-1}; hence this was used as the initial pyrene concentration in many cases in this study.

The flasks were wrapped with aluminium foil to shield them from light. The reaction was initiated by adding a small amount of chlorine solution to the pyrene solution while using a magnetic stirrer. The volume and pH range of the mixture were 200 ml and 3.2–10 (5 mM phosphate buffer), respectively. In some experiments, 30 mM ethanol or 3.0 and 6.0 mgC l^{-1} humic acid were added as coexisting compounds. Sampling was conducted at selected time intervals, and the reaction was terminated by adding sodium thiosulfate ($Na_2S_2O_3$, Wako Pure Chemicals) solution to the sample solutions. For each experiment, another flask containing 200 ml of 5 mM phosphate buffer at the same pH was prepared and the same amount of chlorine solution was added to the flask. The chlorine concentration in the flask was defined as the initial chlorine concentration in each experiment. The reaction was carried out at 21 ± 1°C in a water bath. After the reactions were completed, the chlorine concentrations in the chlorinated pyrene solution were measured. Except for the presence of humic acid, the residual ratios of the chlorine concentrations were always higher than 0.94. Thus, the chlorine concentration was assumed to be constant during the experiment, except for the presence of humic acid.

For the identification of decomposition by-products of pyrene in chlorination, experiments were conducted in several Erlenmeyer flasks with caps at room temperature (21 ± 2°C). The flasks were wrapped with aluminium foil to shield them from light. Pyrene and chlorine concentrations were 92 μg l^{-1} and 7.9 mg l^{-1}, respectively. The reaction was initiated by adding chlorine solution to each aqueous pyrene solution. The volume and pH of the mixture were 500 ml and 7.1 (4 mM phosphate buffer), respectively. At selected time intervals, $Na_2S_2O_3$ solution was added to one of the chlorinated pyrene solutions to terminate the reaction. The samples were extracted and concentrated by solid phase extraction (SPE) and the concentrated samples were analysed. Also, another flask containing 500 ml of 4 mM phosphate buffer at pH 7.1 was prepared and the same amount of chlorine solution was added to the flask. The chlorine concentration in the flask was defined as the initial chlorine concentration.

For the study of the behaviour of pyrene decomposition by-products in chlorination, experiments were conducted by the same procedure as those of the study of pyrene behaviour in chlorination, as described above. In the experiments, the chlorine and pyrene concentrations were 3.9–4.0 mg l^{-1} and 56–67 μg l^{-1} (280–330 nM), respectively. Note that the pyrene concentration was higher than that in the study of pyrene behaviour in chlorination because the pyrene decomposition by-products were determined directly using an HPLC coupled with an atmospheric pressure photoionization mass spectrometer (LC/APPI/MS) (see Analytical Methods). The pH was 3.1 or 7.1, and the residual ratios of the chlorine concentrations after the reactions were higher than 0.92.

Extraction and concentration of chlorinated pyrene solution

To identify the pyrene decomposition products, the extraction and concentration of the pyrene chlorinated solution were conducted by SPE using an Oasis HLB cartridge (Waters, Milford, Massachusetts). The cartridge was conditioned by passing 10 ml of dichloromethane (CH_2Cl_2, Wako Pure Chemicals), followed by 10 ml of methanol (Wako Pure Chemicals) and 5 ml of ultrapure water through the cartridge. The pH of the sample solution was adjusted to about 3 with nitric acid solution, and 400 ml of the solution was passed through the cartridge at a flow rate of 10 ml min^{-1}. Anthracene (Wako Pure Chemicals) was

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used as an internal standard. After trapping the compounds (i.e. pyrene, its decomposition by-products and anthracene) onto the cartridge, the cartridge was purged with nitrogen gas and eluted with 5 ml of CH_2Cl_2. The effluent was dried under a nitrogen gas stream, and the final volume of the solution was adjusted to 1 ml with methanol. The methanol solution was bombarded with ultrasonic waves for 10 min and used for the analysis.

Analytical methods

For the study of pyrene behaviour in chlorination and the profiles of decomposition by-products, pyrene and 1-chloropyrene concentrations were determined using an HPLC with a fluorescence detector. The excitation and emission wavelengths of the two compounds were 235 and 385 nm, respectively. HPLC separation was carried out isocratically with an Inertsil ODS-3 (250 mm × 4.6 mm, 5 μm, GL Sciences Inc., Tokyo, Japan) column at 30°C using acetonitrile/0.1% acetic acid aqueous solution (85/15 v/v) at a flow rate of 1.0 ml min^{-1}.

The pyrene decomposition by-products were characterized using LC/APPI/MS and nuclear magnetic resonance (NMR) spectrometers. HPLC separation was carried out with a SUPELCOSIL LC-PAH (100 mm × 4.6 mm, 3 μm, Supelco, Bella Fonte) column at 30°C using a gradient of methanol/0.1% formic acid aqueous solution at a flow rate of 0.4 ml min^{-1}. The gradient programme of methanol was as follows: 60% (v/v) for 5 min, a linear increase from 60 to 100% (v/v) over 20 min, and a 100% (v/v) hold for 35 min. Toluene (Kanto Chemical, Tokyo, Japan) was used as a dopant at a flow rate of 0.05 ml min^{-1}. APPI/MS (HP1100 LC/MSD, Agilent Technologies) analysis was carried out in the positive-ion mode, and the conditions were as follows: dry gas flow (nitrogen), 5.0 l min^{-1} at 350°C; nebulizer pressure, 60 psi; capillary voltage, 3,500 V; and fragmentor voltage, 270 V. The NMR spectra recorded in this study were ^{1}H-NMR, ^{13}C-NMR, 1D differential nuclear Overhauser enhancement (NOE), ^{1}H-^{1}H double-quantum filtered correlation spectroscopy (DQF-COSY), ^{1}H-^{1}H total correlation spectroscopy (TOCSY), ^{1}H-^{13}C heteronuclear single-quantum correlation (HSQC), ^{1}H-^{13}C heteronuclear multiple-bond correlation (HMBC) and 2D ^{1}H-^{13}C HSQC-TOCSY spectra. All NMR spectra were measured at 25°C in deuterochloroform ($CDCl_3$, Aldrich) solution using a JNM-ECA600 spectrometer (600 MHz, JEOL, Tokyo, Japan), except for the ^{1}H NMR spectrum of an isolated pyrene-4,5-dione (peak A, see Results and Discussion), which was measured using a JNM-ECP500 spectrometer (500 MHz, JEOL). In some cases, a gas chromatograph/mass spectrometer (GC/MS, HP6890 GC/5973 MSD, Agilent Technologies) was used to supplement the LC/APPI/MS and NMR results.

To evaluate the AhR ligand activities of the pyrene decomposition by-products, a yeast assay was used in this study. The YCM3 strain with human AhR, ARNT and pTXRE5-Z (LacZ) reporter plasmid was used. The yeast assay procedure has been described previously (Miller 1997, 1999). In this procedure, the AhR ligand activities of test compounds were assessed on the basis of β-galactosidase activity.

The chlorine concentrations in the stock chlorine solutions were measured by amperometric titration with phenylarsine oxide (Kanto Chemical) titrant and a titration meter (AT-II, Isomura, Tokyo, Japan) (*Standard Methods* 1998). The chlorine concentrations in the samples were measured colorimetrically with *N,N*-diethyl-*p*-phenylenediamine (Kanto Chemical) (*Standard Methods* 1998). Dissolved organic carbon (DOC) concentration was determined using a total organic carbon analyser (TOC-V CPH, Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION

Pyrene behaviour in chlorination

Figure 1 shows the effects of pH on the profiles of the residual ratios of pyrene in chlorination. Figure 2 shows the relationship between pH and the half-life of pyrene. The initial pyrene concentrations were 0.82–0.97 μg l^{-1} and the chlorine concentrations were 2.0–2.1 mg l^{-1}. The pH range was 3.2–10.

Pyrene decomposition in chlorination was dependent on pH under similar pyrene and chlorine concentrations. In the pH range of 3.2–5.1, pyrene was decomposed faster in the order of pH 3.2, 3.7 and 5.1. Oyler *et al.* (1983) reported that at pH ≤ 4, the decomposition rates of

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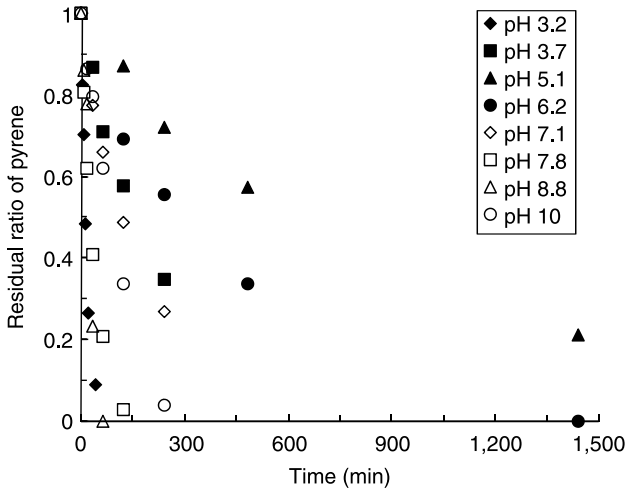

Figure 1 | Effects of pH on the profiles of the residual ratios of pyrene in chlorination. Experimental conditions: initial pyrene concentration, 0.82–0.97 $\mu g\,l^{-1}$; chlorine concentration, 2.0–2.1 $mg\,l^{-1}$; pH, 3.2–10 (5 mM phosphate buffer); temperature, 21 ± 1°C.

fluorene, phenanthrene and fluoranthene in chlorination increased with decreasing pH. Reactions (1) and (2) below were associated with the decompositions.

$$HOCl + H^+ + Cl^- \leftrightarrow Cl_2 + H_2O \quad (1)$$

$$Cl_2 + PAH \rightarrow \text{products} \quad (2)$$

In reactions (1) and (2), Cl_2 is molecular chlorine. The pH dependence of pyrene decomposition in chlorination in this study was consistent with the results of a previous study. Thus, it was suggested that Cl_2 was also

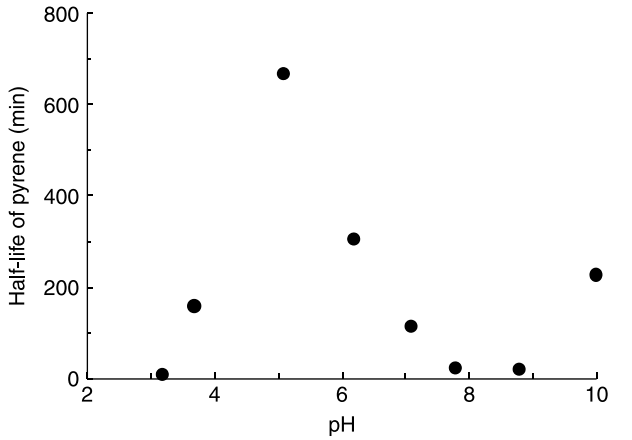

Figure 2 | Relationship between pH and half-life of pyrene in chlorination. Experimental conditions: initial pyrene concentration, 0.82–0.97 $\mu g\,l^{-1}$; chlorine concentration, 2.0–2.1 $mg\,l^{-1}$; pH, 3.2–10 (5 mM phosphate buffer); temperature, 21 ± 1°C.

associated with pyrene decomposition at lower pH. On the other hand, in the pH range of 5.1–10, pyrene was decomposed faster in a pH range of approximately 7.8–8.8. In the pH range of 5.1–7.1, the main species of chlorine is the same (i.e. HOCl, p*K*a = 7.58, Oyler *et al.* 1983), but pyrene was decomposed faster at higher pH. It was reported that when the pH ≥ 5, the reaction rate constant of phenanthrene in chlorination was maximum at around pH 6–8 (Oyler *et al.* 1983). That is, the effects of pH on pyrene decomposition in chlorination shown by this study were similar to those of phenanthrene.

In general, in the pH range of environmental water, the reactions of inorganic and organic compounds with chlorine are expressed by a second-order reaction; that is, first order with respect to both HOCl and inorganic or organic compounds (Deborde & von Gunten 2008). However, from this study and the previous study, it was shown that the decomposition of these two PAHs in chlorination was associated with not only HOCl but also other reactive species. Note that another previous study reported that the reaction of pyrene in chlorination in the pH range of 5.5–9.0 was expressed as first order with respect to both HOCl and pyrene (Hu *et al.* 2006), unlike the results of this study. The pyrene concentration in this study was 0.82–0.97 $\mu g\,l^{-1}$, and that of the previous study was 100 $\mu g\,l^{-1}$. The difference between the two studies is not clear, but the concentration of pyrene affected its decomposition in chlorination as shown below. It was presumed that the decomposition of pyrene by the reactive species in chlorination might be observed at a lower concentration of pyrene. Also, in most cases, the profile of the residual ratios of pyrene could be approximated by a pseudo-first-order reaction, but not at pH 8.8. That is, at pH 8.8, pyrene was slowly decomposed at first and rapidly decomposed later. This suggested that some reactive species can be attributed to pyrene decomposition, and that pyrene was produced from the reaction of HOCl (or hypochlorite ion OCl^-) and pyrene.

Figure 3 shows the effect of pyrene concentration on the profiles of the residual ratios of pyrene in chlorination at pH 7.1. The initial pyrene concentrations were 0.97, 4.6 and 17 $\mu g\,l^{-1}$, and the chlorine concentration was 2.0 $mg\,l^{-1}$. Pyrene was decomposed faster at low concentration. The half-lives of pyrene at 0.97, 4.6 and 17 $\mu g\,l^{-1}$ were

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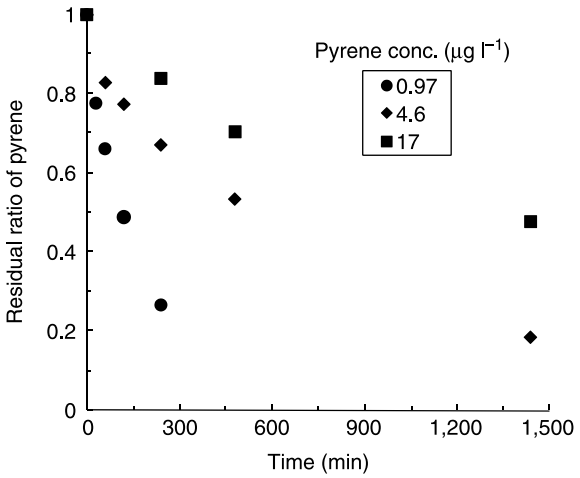

Figure 3 | Effect of initial pyrene concentration on the profiles of the residual ratios of pyrene in chlorination. Experimental conditions: initial pyrene concentration, 0.97–17 $\mu g\,l^{-1}$; chlorine concentration, 2.0 $mg\,l^{-1}$; pH, 7.1 (5 mM phosphate buffer); temperature, 21 ± 1°C.

120, 570 and 1,400 min, respectively. Low concentrations of pyrene were also decomposed faster at pH 3.2 (initial pyrene concentration, 0.89–21 $\mu g\,l^{-1}$; chlorine concentration, 2.0 $mg\,l^{-1}$) (data not shown). Rav-Acha & Blits (1985) reported that the half-life of pyrene in chlorination was 120 min (11 PAHs concentrations including pyrene, 0.1–5 $\mu g\,l^{-1}$; chlorine concentration, 2 $mg\,l^{-1}$; pH, 7; 20°C). On the other hand, Hu *et al.* (2006) reported that the apparent reaction rate constant of pyrene with chlorine at pH 7 was 800 $M^{-1}\,h^{-1}$ (initial pyrene concentration, 100 $\mu g\,l^{-1}$; 20°C), which meant that the half-life of pyrene was 1,800 min at 2 $mg\,l^{-1}$ chlorine. Thus, from the results of this study, it was considered that the initial pyrene concentration was a factor affecting pyrene decomposition in chlorination, and the discrepancy of the results of the two previous studies was due to differences in the initial pyrene concentrations.

The effects of coexisting compounds on pyrene decomposition in chlorination were examined. Some radicals were suggested as the reactive species associated with some PAH decomposition in chlorination (Oyler *et al.* 1983; Rav-Acha & Blits 1985). Thus, ethanol, a known hydroxyl radical (HO·) scavenger, was selected as a coexisting compound. Natural organic matter (NOM) is a major constituent of organic compounds in environmental water. Thus, humic acid, one of the components of NOM, was selected as another coexisting compound. Note that the humic acid used in this study originated from soil, so its effect may be different from that originating from environmental water to some degree.

Figure 4 shows the effects of ethanol on the profiles of the residual ratios of pyrene in chlorination. The initial pyrene concentrations were 0.91 (0.97) and 17 $\mu g\,l^{-1}$, the chlorine concentration was 2.0 $mg\,l^{-1}$ and the ethanol concentration was 30 mM. The chlorine concentration during the experiment was not affected by the presence of ethanol. At 0.91–0.97 $\mu g\,l^{-1}$ pyrene, the residual ratios of pyrene in the presence and absence of ethanol at 240 min were 0.79 and 0.27, respectively. At 17 $\mu g\,l^{-1}$ pyrene, the ratios in the presence and absence of ethanol at 1,440 min were 0.84 and 0.48, respectively. Thus, it was clearly shown that pyrene decomposition was inhibited by ethanol. Pyrene decomposition in chlorination was also inhibited by ethanol at pH 3.2 (data not shown). From the results shown in Figure 4, it was confirmed that pyrene decomposition was associated with reactive species other than chlorine. It was also suggested that HO· might be produced as one of the reactive species.

Figure 5 shows the effects of humic acid on the profiles of the residual ratios of pyrene in chlorination. The initial pyrene concentrations were 0.95–1.2 $\mu g\,l^{-1}$, the chlorine concentration was 2.0 $mg\,l^{-1}$ and humic acid concentrations were 3.0 and 6.0 $mgC\,l^{-1}$. At 30 min, the residual ratio of pyrene in the absence of humic acid was 0.78, and

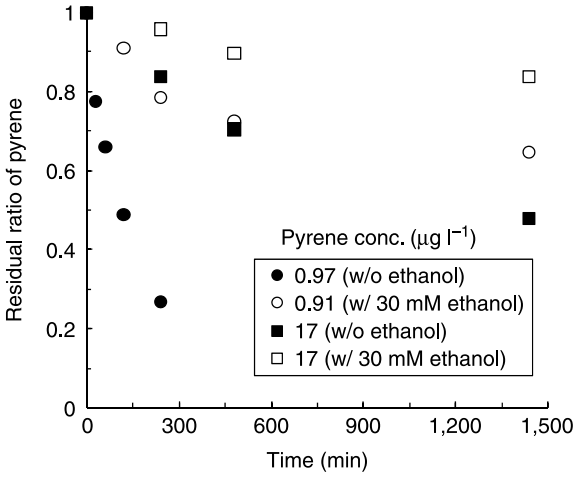

Figure 4 | Effects of ethanol on the profiles of the residual ratios of pyrene in chlorination. Experimental conditions: initial pyrene concentration, 0.91–17 $\mu g\,l^{-1}$; chlorine concentration, 2.0 $mg\,l^{-1}$; ethanol concentration, 30 mM; pH, 7.1 (5 mM phosphate buffer); temperature, 21 ± 1°C.

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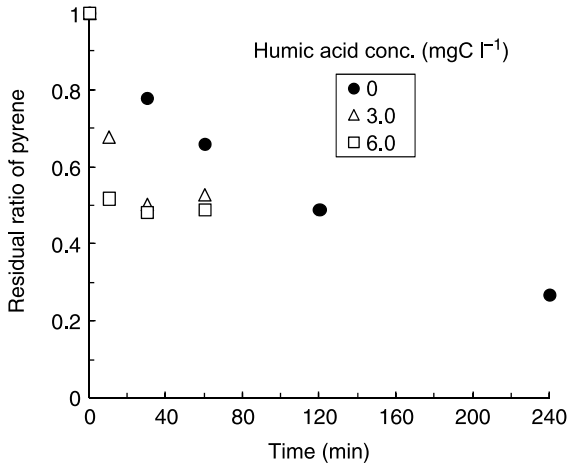

Figure 5 | Effects of humic acid on the profiles of the residual ratios of pyrene in chlorination. Experimental conditions: initial pyrene concentration, 0.95–1.2 $\mu g\,l^{-1}$; chlorine concentration, 2.0 $mg\,l^{-1}$; humic acid concentration, 3.0 and 6.0 $mgC\,l^{-1}$; pH, 7.1 (5 mM phosphate buffer); temperature, 21 ± 1°C.

that in the presence of humic acid was about 0.50. Thus, it was considered that the reactive species associated with pyrene decomposition in chlorination were produced by the reactions of chlorine and humic acid, and pyrene decomposition was enhanced as a result. From 30 to 60 min, the residual ratios of pyrene in the presence of humic acid were not changed. This was because chlorine was decreased by the presence of humic acid (i.e. chlorine concentrations in the presence of 3.0 and 6.0 $mgC\,l^{-1}$ at 60 min were 0.4 and 0.2 $mg\,l^{-1}$, respectively). It was not reported that the reactivity of inorganic or organic compounds in chlorination was inhibited or enhanced by coexisting compounds. Thus, it was considered that pyrene or PAHs have unique characteristics from the point of the reactions in chlorination. The characteristics of NOM including humic acid are different from place to place, so the component of environmental water is dependent on the nature of the environmental water. It was also suggested that during the actual water treatment process, pyrene decomposition in chlorination was dependent on the water treatment plants.

Behaviour of pyrene decomposition by-products in chlorination

Figure 6(a) and (b) shows the LC/APPI/MS chromatograms before chlorination and at 48 h of chlorination, respectively (scan mode: m/z from 150 to 350). The initial pyrene concentration was 92 $\mu g\,l^{-1}$ and the chlorine concentration was 7.9 $mg\,l^{-1}$. Several peaks of the decomposition by-products were observed at 48 h.

Among the peaks of the decomposition by-products, peaks A (17.2 min retention time) and B (35.3 min retention time) were found to correspond to pyrene-4,5-dione and 1-chloropyrene, respectively (Tables 1 and 2). NMR involved analyses of the fractionated peaks A and B. From the results, it was found that pyrene was decomposed by both oxidation and chlorine-substitution reactions in chlorination at neutral pH. It was reported that the decomposition by-products of pyrene other than its chlorinated derivatives might exist from the molar ratios of pyrene: 1-chloropyrene in raw, finished and tap waters (Kosaka *et al.* 2008). That is, the result of this study was in agreement with that of the previous study. Judging from the structure of pyrene-4,5-dione, it was considered that 4-hydroxypyrene was initially generated and transformed into pyrene-4,5-dione by oxidation. However, no peak was observed corresponding to the molecular ion and/or fragment ions of 4-hydroxypyrene in the mass spectra in the chromatograms (e.g. m/z ions 218 $[M]^+$ and 201 $[M\text{-}OH]^+$). 4-Hydroxypyrene has a phenolic functional group, which is an active functional group in chlorination; thus, it was considered that 4-hydroxypyrene was rapidly transformed and pyrene-4,5-dione was detected.

By LC/APPI/MS, NMR and GC/MS analyses, 1,8-dichloropyrene was found as a minor compound in the authentic 1-chloropyrene sample (Tables 1 and 2). The retention time of 1,8-dichloropyrene in the LC/APPI/MS chromatogram was 43.8 min. By the same analyses, 1,6- and 1,8-dichloropyrenes were found as minor compounds in the fraction of peak B (Tables 1 and 2). The molar ratio of 1-chloropyrene: 1,6-dichloropyrene: 1,8-dichloropyrene in the fraction sample was 85:9:6, as determined from NMR spectra. The retention time of 1,6-dichloropyrene in the LC/APPI/MS chromatogram was 42.6 min. The retention time of 1-chloropyrene was shorter than those of 1,6- and 1,8-dichloropyrenes by 7.3 and 8.5 min, respectively; thus, no reason for their contamination in the fraction corresponding to peak B was revealed. However, 1,6- and 1,8-dichloropyrenes were also detected in the sample before fractionation (Figure 6(c), selected ion monitoring (SIM) mode: m/z 200). The monitoring ion (i.e. m/z 200)

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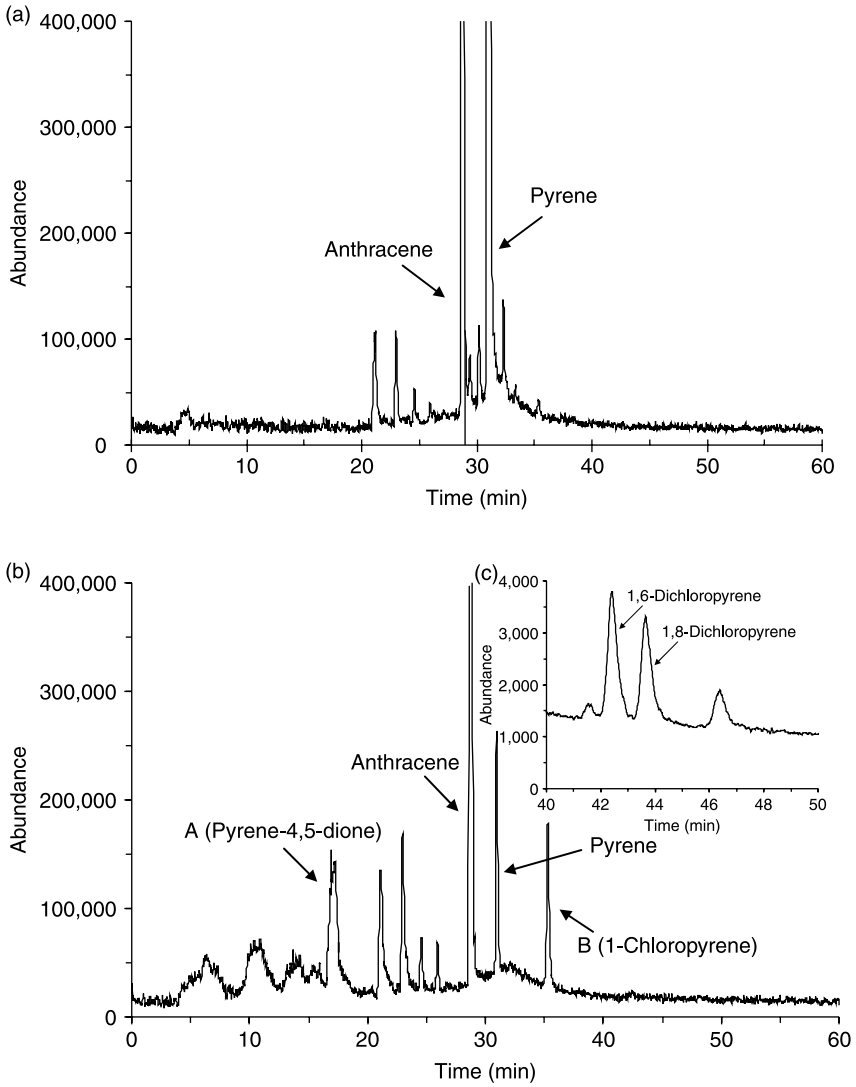

Figure 6 | LC/APPI/MS chromatograms of pyrene (a) before chlorination (scan mode: m/z from 150 to 350), (b) at 48 h chlorination (scan mode: m/z from 150 to 350 and (c) SIM mode: m/z 200). Experimental conditions: initial pyrene concentration, 92 $\mu g\,l^{-1}$; chlorine concentration, 7.9 $mg\,l^{-1}$; pH, 7.1 (4 mM phosphate buffer); temperature, 21 ± 2°C.

corresponds to the fragment ion of 1,6- and 1,8-dichloropyrenes $[M\text{-}2Cl]^+$. Therefore, it was shown that these compounds were generated from pyrene decomposition in chlorination. From the peak areas of the UV absorbance chromatogram, it was shown that the concentrations of 1,6- and 1,8-dichloropyrenes were much lower than that of 1-chloropyrene.

Figure 7(a) and (b) shows the profiles of pyrene, 1-chloropyrene and pyrene-4,5-dione in chlorination at pH 3.2 and 7.1, respectively. The initial pyrene concentrations were 56–67 $\mu g\,l^{-1}$ (280–330 nM) and the chlorine concentrations were 3.9–4.0 $mg\,l^{-1}$. The pyrene concentrations were higher than those mentioned in the previous section. At pH 3.2, the highest percentages of 1-chloropyrene and pyrene-4,5-dione concentrations with respect to the initial pyrene concentration were 35 and 15%, respectively. The mass balance was around 50–60%. On the other hand, at pH 7.1, the highest percentages of 1-chloropyrene and pyrene-4,5-dione concentrations with respect to the initial pyrene concentration were 9.8 and 2.0%, respectively. The mass balance was around 15–20%. Therefore, these two by-products were the major by-products at pH 3.2, but were the minor by-products at pH 7.1. In general, the reactivity of the chlorine-substituted

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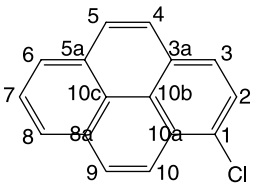
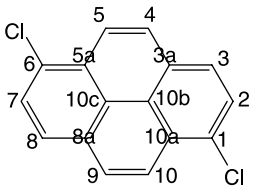
Table 1 | ^{1}H and ^{13}C NMR spectra data for pyrene decomposition by-products in $CDCl_3$

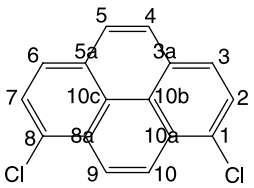
1-Chloropyrene



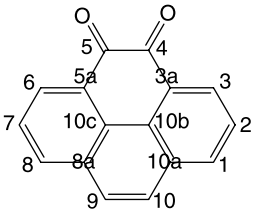
1,6-Dichloropyrene



1,8-Dichloropyrene



Pyrene-4,5-dione



No	^{13}C*	^{1}H*	^{1}H†	^{1}H†	^{1}H†	^{13}C*	^{1}H*	^{1}H‡
1	129.2	–	–	–	–	135.8	8.19 dd(8.1, 1.0)	8.20 dd(7.8, 1.4)
2	126.8	8.05 d(8.1)	8.07–8.03 m	8.10 d(7.7)	8.10 d(7.7)	128.0	7.76 dd(8.1, 7.7)	7.78 dd(7.8, 7.3)
3	125.3	8.09 d(8.1)	8.10 d(8.2)	8.13 d(7.7)	8.14 d(7.7)	130.2	8.50 dd(7.7, 1.0)	8.52 dd(7.3, 1.4)
3a	130.2	–	–	–	–	130.2	–	–
4	127.2	8.03 m	8.07–8.03 m	8.05 s	8.16 d(9.3)	180.5	–	–
5	127.6	8.07 m	8.09 d(9.3)	8.05 s	8.49 d(9.3)	180.5	–	–
5a	131.3	–	–	–	–	130.2	–	–
6	125.7	8.21 d(7.1)	8.22 d(7.1)	8.13 d(7.7)	–	130.2	8.50 dd(7.7, 1.0)	8.52 dd(7.3, 1.4)
7	126.9	8.05 dd(7.1, 7.1)	8.07–8.03 m	8.10 d(7.7)	8.10 d(7.7)	128.0	7.76 dd(8.1, 7.7)	7.78 dd(7.8, 7.3)
8	125.6	8.22 d(7.1)	8.24 d(7.1)	–	8.14 d(7.7)	135.8	8.19 dd(8.1, 1.0)	8.20 dd(7.8, 1.4)
8a	131.1	–	–	–	–	132.1	–	–
9	128.8	8.18 d(9.1)	8.20 d(9.3)	8.59 s	8.16 d(9.3)	127.3	7.86 s	7.88 s
10	123.4	8.47 d(9.1)	8.48 d(9.3)	8.59 s	8.49 d(9.3)	127.3	7.86 s	7.88 s
10a	128.2	–	–	–	–	132.1	–	–
10b	125.8	–	–	–	–	128.5	–	–
10c	124.2	–	–	–	–	128.5	–	–

*Authentic samples.

†Peak B, NMR spectra data of peak B were measured for the mixture of 1-chloropyrene, 1,6- and 1,8-dichloropyrenes, and in the molar ratio = 85:9:6.

‡Peak A.

Coupling constants (J values in Hz) are given in parentheses. Multiplicity is represented by s: singlet, d: doublet, dd: doublet of doublets, and m: multiplet. Solvent signals at 7.26 and 77.0 ppm were used as internal standards for ^{1}H and ^{13}C chemical shifts, respectively.

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Table 2 | LC/APPI/MS and GC/MS mass spectra data of pyrene decomposition by-products

Compound	Molecular ion	m/z of LC/APPI/MS* (relative abundance)	m/z of GC/MS† (relative abundance)
1-Chloropyrene	236	150(10) 151(6) 176(9) 179(5) 198(5) 200(100) 202(36) 203(13)	100(19) 118(11) 200(30) 201(23) 236(100) 237(18) 238(33)
1,6-Dichloropyrene	270	–	135(15) 200(35) 207(27) 235(5) 253(9) 270(100) 271(19) 272(65)
1,8-Dichloropyrene	270	152(12) 163(42) 165(11) 174(17) 176(40) 187(17) 199(17) 200(100) 201(27) 234(7)	135(12) 200(33) 235(5) 270(100) 271(19) 272(66)
Pyrene-4,5-dione	232	151(9) 152(5) 174(20) 175(15) 176(100) 179(23) 203(18)	–

*m/z from 150 to 350.
†m/z from 100 to 350.

compound is lower than that of its precursor compound. Despite this, 1-chloropyrene did not accumulate as much at pH 7.1 compared with pH 3.2. Thus, it was considered that pyrene was not decomposed as much by a chlorine-substituted reaction in chlorination at pH 7.1. On the other hand, pyrene-4,5-dione, the oxidation by-product of pyrene, also did not accumulate at pH 7.1. The reason might be that at pH 7.1, pyrene-4,5-dione was decomposed faster than pyrene; thus, pyrene-4,5-dione was further transformed to other compounds.

Aryl hydrocarbon receptor ligand activities of pyrene and its decomposition by-products

PAHs are AhR agonists and the AhR ligand activities of many PAHs have been evaluated (Billiard *et al.* 2002; Sakai *et al.* 2003; Barron *et al.* 2004; Hankinson 2005). However, information on the ligand activities of PAH decomposition by-products in chlorination is very limited (Hu *et al.* 2006). Figure 8 shows the dose-response curves of the concentrations of the test compounds (i.e. pyrene and pyrene-4,5-dione) versus β-galactosidase activity. It was found that pyrene-4,5-dione was an AhR agonist and its AhR ligand activity was lower than that of pyrene. The pyrene concentration eliciting 20% of the maximum β-galactosidase activity response of pyrene (EC_{Py20}) was 1.5 μM, and the pyrene-4,5-dione concentration equivalent to EC_{Py20} was 3.7 μM. In Figure 8, a strong inhibition of cell growth by pyrene-4,5-dione at 22 μM was observed. Hu *et al.* (2006) reported that 1-chloropyrene and dichloropyrenes (a mixture of 1,6- and 1,8-dichloropyrenes) are AhR agonists and that their AhR ligand activities are greater than that of pyrene. The concentrations of pyrene, 1-chloropyrene, and dichloropyrenes, which were equivalent to 50% of the maximum β-galactosidase activity response of pyrene (EC_{py50}), were 5.6, 1.9 and 0.60 μM,

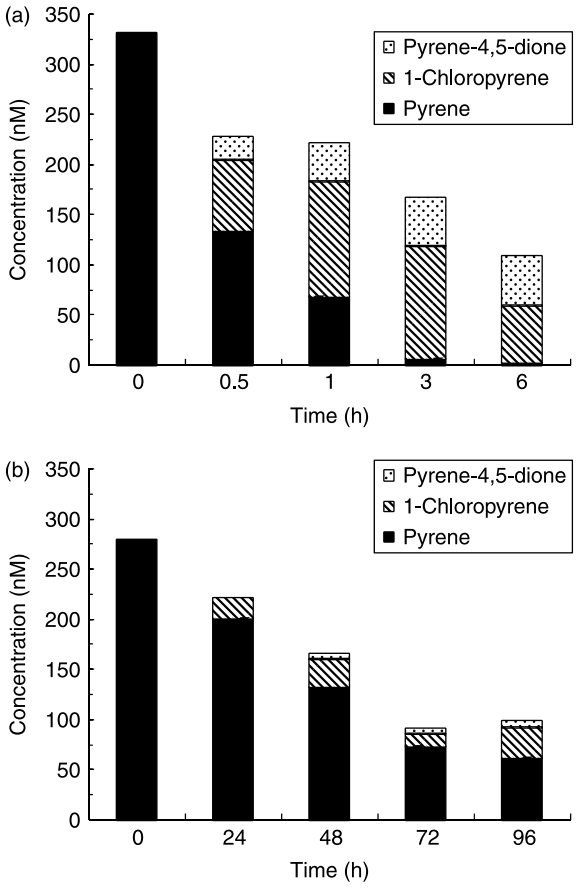

Figure 7 | Profiles of pyrene, 1-chloropyrene and pyrene-4,5-dione concentrations in chlorination. Experimental conditions: initial pyrene concentration, 56–67 μg l^{-1} (280–330 nM); chlorine concentration, 3.9–4.0 mg l^{-1}; pH, (a) 3.2 and (b) 7.1 (5 mM phosphate buffer); temperature, 21 ± 1°C.

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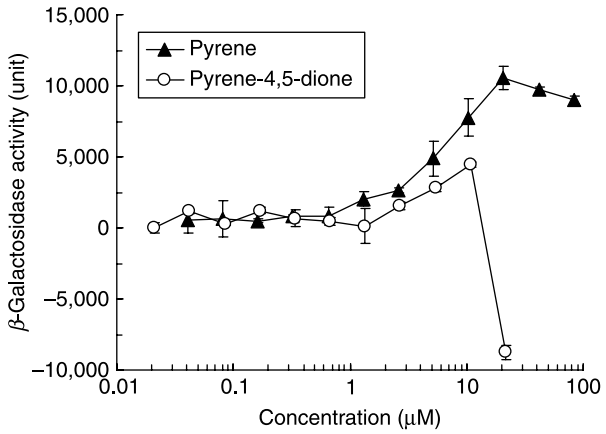

Figure 8 | Dose-response curves of concentrations of test compounds versus β-galactosidase activity.

respectively. These results indicate that the AhR ligand activities of pyrene increased by chlorine-substitution reactions and decreased by oxidation. It was assumed that, compared with the oxygenated derivatives, the chlorinated derivatives of pyrene decomposition products must be considered in terms of AhR ligand activities, particularly the higher chlorinated derivatives.

CONCLUSIONS

1. It was considered that pyrene decomposition in chlorination was associated not only with chlorine but also with other reactive species. The effects of the water quality parameters (i.e. pH and pyrene concentration) on pyrene decomposition in chlorination were unique, compared with those of the usual inorganic and organic compounds. In the pH range of 3.2–5.1, pyrene was decomposed faster in the order of pH 3.2, 3.7 and 5.1, under similar pyrene and chlorine concentrations. On the other hand, in the pH range of 5.1–10, pyrene was decomposed faster in a pH range of approximately 7.8–8.8. When the chlorine concentration was similar, pyrene was decomposed faster in lower pyrene concentrations.
2. Pyrene decomposition in chlorination was affected by coexisting compounds. Decomposition in chlorination was inhibited by the presence of 30 mM ethanol, but was enhanced by the presence of 3.0 and 6.0 $mgCl^{-1}$ humic acid. The component of environmental water is dependent on the environmental source. Thus, it was suggested that during the actual water treatment process, pyrene decomposition in chlorination was dependent on the water treatment plants.
3. 1-Chloropyrene, 1,6- and 1,8-dichloropyrenes, and pyrene-4,5-dione were identified as pyrene decomposition by-products in chlorination. Their profiles in chlorination were dependent on pH. That is, 1-chloropyrene and pyrene-4,5-dione were the major by-products at pH 3.2, but were the minor by-products at pH 7.1.
4. From the yeast assay, it was found that pyrene-4,5-dione was an AhR agonist although its AhR activity was less than that of pyrene. It was assumed that, compared with the oxygenated derivatives, the chlorinated derivatives of pyrene decomposition products must be considered in terms of AhR ligand activities.

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